

metabolism was lowered drastically at 2° C. These experiments will be reported fully at a later date.

SUMMARY

The effects of the monovalent cations K, Na, NH₄, Rb, Li, and Cs and of the divalent cations Ca, Mg, and Ba upon the absorption of carrier-free Cs¹³⁷ by excised barley roots has been investigated.

In the concentration range of 0 to 0.10 meq per liter Na, Li, Ca, Ba, and Mg had essentially no depressant effect upon Cs¹³⁷ uptake whereas K, Rb, NH₄, and Cs were markedly effective.

At higher concentrations all ions investigated inhibited the absorption of Cs¹³⁷.

The uptake of carrier-free Cs¹³⁷ was found to be strongly temperature dependent and is therefore largely the result of metabolic absorption rather than of diffusion and adsorption phenomena. In spite of this the behavior of the ions studied in inhibiting up-

take of Cs¹³⁷ parallels their behavior in respect to competition for exchange sites upon soil colloids. A possible reason for this is discussed.

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PHOTOSYNTHESIS & RESPIRATION IN DEVELOPING FRUITS¹. II. COMPARATIVE RATES AT VARIOUS STAGES OF DEVELOPMENT

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Surface layers of the young fruits of many plants contain chlorophyll and apparently are capable of carrying on photosynthesis as was shown by the use of C¹⁴O₂ in the previous paper of this series (3). It would be of some interest to know to what extent photosynthesis in the fruit might contribute to the growth of the fruit. Little quantitative work has been done on photosynthesis in green fruits. Kursanov (8) made measurements of the photosynthetic rates of apple, cucumber, and pea fruits in sunlight. His data seemed to show the rather surprising effect that photosynthesis in the fruit was markedly increased when the fruit was detached from the plant or the interchange between the plant and the fruit was altered by severing the phloem. It has been estimated from experiments where developing barley ears were shaded that as much as 30% of the assimilate may be produced by that organ itself (1, 10). However, developing flowers and fruits do put heavy de-

mands on the carbohydrate supply of a plant, as noted for cotton (5).

It seemed desirable to extend the available data on photosynthesis to other fruits. This report presents data for photosynthesis and respiratory rates in lemons, oranges, and avocados of varying ages. For simplicity, these experiments were carried out with detached fruits. Kursanov's suggestion that detaching the fruit leads to drastic metabolic changes made it mandatory to determine if such changes actually occurred in our experiments. Data obtained under better controlled conditions than those used by Kursanov are presented here to demonstrate that marked changes in the photosynthetic or respiratory behavior of the fruit do not occur when the fruit is detached from the plant.

METHODS & MATERIALS

For experiments where detached fruits were used, the fruits were contained in large, wide-mouthed Warburg vessels (75 cm³) immersed in a water bath at 25° C. They were illuminated either from below with fluorescent lamps (approx 1,000 ft-c) or from above with sunlight (approx 10,000 ft-c).

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Under these conditions, the photosynthetic rate values were obtained from the formula $PS = A(r-p)$, where PS is photosynthetic rate in CO_2 assimilation per hour, A is the air flow rate factor, r is the measured CO_2 content of the effluent air during respiration in the dark and p is the CO_2 content of the exit air during photosynthesis. Respiration rate (CO_2 evolved/hr) was similarly obtained from $R = A(r-a)$ where A and r are the same as above and a is the CO_2 content of the input air.

Air was passed over each fruit at an accurately determined rate of flow. The rate of flow used for various experiments was as follows:

Oranges, lemons, and avocados (data reported in figures 1, 2, 3) about five liters per hour. Oranges in sunlight and in artificial light (table I, fig 6) about 18 to 23 liters per hour. Oranges in dark (table I & fig 6) about five liters per hour. Cucumbers (table I & fig 5) about 12 liters per hour. The air leaving the flask was passed through a drying tube into a Liston-Becker Infra-red CO_2 Analyzer Model 15 to measure the CO_2 content of the air. The rate of flow was adjusted so that CO_2 measurements were within a readable range on the analyzer. During photosynthesis the content of the air passing out of the flasks containing the fruit was not allowed to fall below 0.02% CO_2 .

For experiments in which fruits remained attached to the plant, individual fruits were inserted into a container of suitable size and a seal was made around the stem with modeling clay. Air was passed through the container and analyzed as described above. The plants were maintained in air at $20^\circ \pm 0.5^\circ\text{C}$. Light was supplied with a bank of fluorescent lamps (approx 1,000 ft-c).

Surface areas of avocado and lemon fruits, which are ellipsoidal, were calculated using the tables compiled by Turrell (9). For oranges, the displacement volume was determined and the surface area calculated with the assumption that the orange was a perfect sphere. Areas of large cucumber fruits were determined by peeling the fruit and measuring the area of the peel with a planimeter.

The fruits used were: Eureka lemon (*Citrus limon*, Burm. f.); Valencia orange (*C. sinensis*, Osbeck); Hass avocado (*Persea americana*, Mill.); Heinz Pickling cucumbers (*Cucumis sativus*, L.).

Chlorophyll content of the fruit surface was determined by extracting the peel with acetone, centrifuging, and diluting to a known volume. The optical density of the supernatant liquid was measured at 665, 644, and 624 $m\mu$ with a Beckman Model DU Spectrophotometer and the chlorophyll a concentration was calculated from this information using the formula of Koski (7).

RESULTS & DISCUSSION

CONTRIBUTION OF PHOTOSYNTHESIS TO DEVELOPING GREEN FRUIT. Lemons could be obtained in widely varying sizes for single experiments for

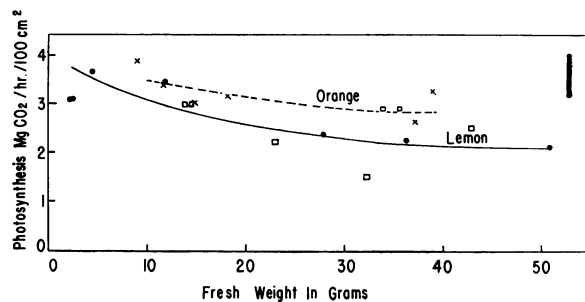
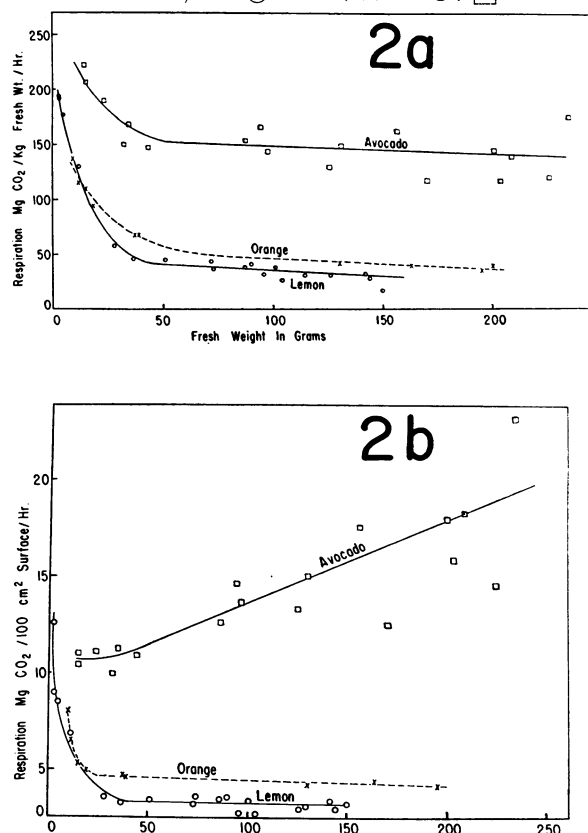


FIG. 1. Relationship of photosynthesis to fruit size. \circ Lemon, \times orange, \square avocado.

simultaneous comparison of photosynthetic and respiratory activity at different ages, hence data are more extensive for lemons than for the other fruits. It is apparent (fig 1) that photosynthetic activity of the lemon fruit declines gradually with an increase in fruit size. The photosynthetic rates for oranges and avocados appear to follow similar trends for the ranges examined.

Contributions of photosynthesis to the growth of the fruit must be examined in conjunction with the

FIG. 2. Relationship of respiration to fruit size. 2a, Respiration/kg fresh weight/hr and 2b, Respiration/100 cm^2 fruit surface/hr. \circ Lemon, \times orange, \square avocado.



respiratory requirements. Plotted on the usual basis of the CO_2 output per unit weight of fruit (fig 2a), a steep decline occurs during the early growth of the lemon and a slightly lower decline occurs during the growth of avocados and oranges. Bain (2) obtained a similar result for respiration in developing Valencia orange fruit. When respiration is plotted as a function of the surface for lemons and oranges (fig 2b) it becomes apparent that respiration is quite constant, per unit of surface, during the later stages of growth. This would indicate that respiration in the larger citrus fruits is, in some manner, more a function of surface than of the total mass of the fruit. This idea fits well with other data which indicates that the outer flavedo tissues of the citrus fruits carry on respiration at rates many times higher than the underlying tissues (6). Avocados, on the other hand, show a positive slope for the surface plots during most of the growth period, indicating that the surface is not the restricting factor in the respiration of avocado tissues and that the mass of the fruit is of more importance. Presumably, during the early stages of growth, all tissues of the citrus fruits are also active in respiration, giving rise to the bimodal curves. The break in the curve would correspond approxi-

mately to the transition from the cell division stage to the cell expansion stage.

Figure 3 clearly shows that the ratio of photosynthesis/respiration for lemons and oranges increased during the early growth stages because of the very rapid decrease in respiration with the slower fall of photosynthesis. Thus for the citrus fruits, photosynthesis may actually be offsetting a greater percentage of the respiratory loss of CO_2 in the large fruit than in the very small fruit. This is of particular interest since the surface area will increase only as the square of the radius while the mass of the fruit will be proportional to the cube; therefore the contribution of photosynthesis per unit of weight in the large fruit might have been expected to be considerably less than in the small fruit. In the later stages of development, the ratio seems nearly constant although it would be expected to drop sharply with the loss of chlorophyll accompanying ripening. It should be noted that photosynthesis, in no case, reached the compensation point. Since the photosynthesis measurements made in the above experiments were made with fluorescent lights of relatively low intensity, in one experiment the rate was determined using sunlight. For fruit weighing 52 grams, the photosynthetic/respiratory ratio reached

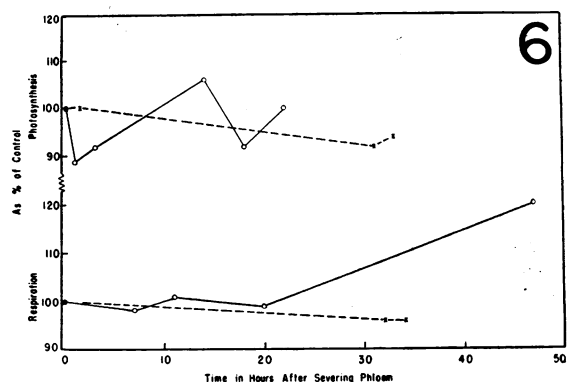
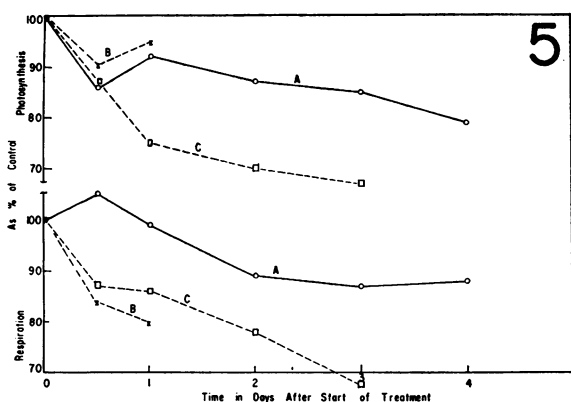
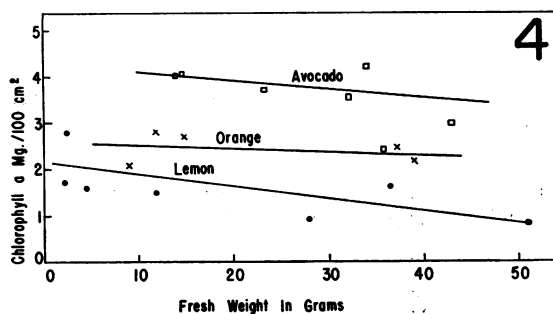
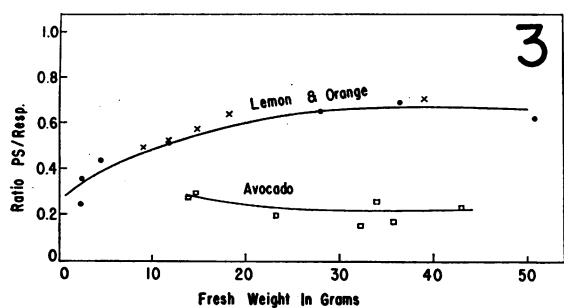


FIG. 3. Relationship of the ratio photosynthesis/respiration to fruit size. \circ Lemon, \times orange, \square avocado.

FIG. 4. Relationship of chlorophyll content to fruit size. \circ Lemon, \times orange, \square avocado.

FIG. 5. Changes in photosynthesis and respiration of cucumber fruit as the result of treatments affecting food supply to the fruit. A, Phloem severed. B, Completely severed. C, Leaves removed from vine.

FIG. 6. Effect of severing the phloem connecting citrus fruits to the tree on the subsequent rates of photosynthesis and respiration. \circ -- \circ Exp 1; \times -- \times Exp 2.

0.99 for sunlight (actual photosynthesis was 3.38 mg CO₂/hr/100 cm²) vs. 0.46 in fluorescent light. Thus even with fruit exposed to sunlight and air containing about 0.03 % of CO₂, the contribution of photosynthesis could not do more than supply enough energy to replace that used in respiration while the plant was exposed to the light.

The ratio of photosynthesis to respiration for avocados (fig 3) was considerably lower than for citrus and the only trend observed was that with the smaller fruit there was a slight increase in this ratio. The respiratory rate of these fruit being higher in relation to the rate of photosynthesis would indicate that the contribution of photosynthesis to fruit growth would be of even less significance than for citrus.

The chlorophyll contents of the fruit used for the photosynthesis and respiratory measurements are given in figure 4. The lemon and avocado fruit had a higher chlorophyll a content in the smaller fruit while the orange remained constant over the range sampled. There was not a good correlation between photosynthetic activity and chlorophyll content.

TABLE I
PHOTOSYNTHESIS & RESPIRATION AT 20° C OF ORANGE
& CUCUMBER FRUITS WHILE STILL ATTACHED
TO PLANT

PLANT MATERIAL	WT/ FRUIT g	SURFACE /FRUIT cm ²	RESP mg. CO ₂ /kg fr wt/hr	PHOTO. mg. CO ₂ /hr/ 100 cm ²	RATIO PHOTO/ RESP
Orange fruit	43.14	68.4	40.0	2.50	1.04
	62.05	98.5	38.6	0.66	0.31
Cucumber fruit	2.0	7.0	99.0	2.00	0.71
	6.0	18.0	81.3	1.99	0.73
	22.0	42.0	56.6	1.66	0.56
	26.2	47.0	43.0	2.40	1.00
	61.0	93.0	109.9	4.53	0.63
	67.0	101.0	84.4	5.49	0.65
	90.9	127.0	44.0	1.86	0.59
124.8	160.0	41.0	1.95	0.61	

PHOTOSYNTHETIC & RESPIRATORY EFFECTS AS RESULT OF INTERRUPTING FRUIT'S FOOD SUPPLY: Kursanov (8) reported that cucumbers apparently did not photosynthesize at all when still attached to a normal plant, but when leaves were either covered with bags, or the fruit removed from the plant, photosynthesis in the fruit rose sharply from the initial level of zero uptake of carbon dioxide to a value as high as 5.3 mg CO₂/100 cm²/hr. In re-examining these results by measuring photosynthesis in attached and detached oranges and cucumbers we found photosynthetic rates ranging from 1.7 to 5.5 mg CO₂/100 cm²/hr for cucumbers attached to normal plants (table I). Oranges were also found to carry on significant photosynthesis while still attached to

normal plants (table I). The results here should not be directly compared with the detached fruit experiments reported in figure 1 since they were done at different temperatures, but the photosynthesis/respiration ratios are in similar ranges (fig 3 & table I), suggesting that there is no essential difference between attached and detached oranges.

The comparative effects of severing the phloem of the fruit stem, removing the leaves from the plant, or completely severing the fruit are illustrated in figure 5 for cucumbers and in figure 6 for oranges. Each fruit was compared with a normal fruit attached to a normal plant over a period of time following the treatment. For all treatments cucumbers show a relatively steady drop in both photosynthesis and respiration following treatment while oranges show little or no significant changes in either factor during our experimental period. Other workers (4) have found that there is generally a gradual decrease in respiration of citrus fruits after detaching them from the tree.

From this data we may conclude that in cucumbers and oranges, photosynthesis and respiration occur at comparable rates in the fruits attached to normal plants or in freshly detached fruits. After detaching, the rates of photosynthesis and respiration appear to decline slowly.

SUMMARY & CONCLUSIONS

Measurements were made on rates of photosynthesis and respiration in green orange, lemon, and avocado fruits of various ages and sizes, ranging from 2.5 g to over 200 g. Photosynthetic activity, per unit of surface declined somewhat during growth in lemons and oranges although little or no change was noted for avocados. Respiration in citrus drops rapidly during early growth causing an increase in the photosynthesis/respiration ratio in the larger fruit. In avocados there was little change in this ratio and the ratio was considerably smaller than for citrus. In no case did the photosynthetic rate of the various fruits exceed the compensation point and in fluorescent light, the rate of photosynthesis was well below the compensation point. It is concluded that the contribution of photosynthesis within a fruit towards its own development is small.

The respiratory rate calculated on a surface basis remained almost constant for citrus fruit in the range of 30 to 200 g while the respiration of avocados per unit of surface showed a marked increase with an increase in fruit size. This would indicate that respiration in the larger citrus fruits is more a function of surface than of the total mass of the fruit, while the reverse is true for avocados.

The effect on photosynthesis of interrupting food supply to the fruit was also examined. Using cucumbers and oranges, photosynthesis was measured with fruit attached to normal plants, attached to normal plants but with phloem of the fruit stem severed, attached to defoliated plants, or with the fruit com-

pletely detached. In contrast with an earlier report that similar treatments gave rise to large increases in photosynthetic rates in cucumbers, little or no stimulation of respiration or photosynthesis was produced by any of these treatments. Instead, there appears to be a gradual decline in both photosynthesis and respiration.

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INFLUENCE OF COBALT ON NITROGEN FIXATION BY MEDICAGO¹

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Reisenauer (6) has discussed the reasons for exploring the possibility that cobalt may be implicated in symbiotic nitrogen fixation. Data were also presented which demonstrated the essential role of cobalt in the functioning of the *Rhizobium-alfalfa* (*Medicago sativa* L.) system in nitrogen fixation.

The present paper presents additional data obtained during the early winter season (Oct.-Dec. 1959) under somewhat more favorable greenhouse conditions and with some other modifications. As a consequence yield differences reflecting efficiency of nitrogen fixation with and without cobalt supplements are considerably greater than those reported earlier for summer (May-July 1959) experiments, as well as those obtained by Ahmend and Evans with *Glycine max* Merr. (1). Also, numerous requests for more detailed information on preparation of growth media sufficiently low in cobalt to permit observations of direct positive influence of the element on nitrogen fixation make it seem desirable to elaborate in detail on our experimental procedures in this paper.

MATERIALS & METHODS

WATER SUPPLY: All water used in cleaning glassware, including solution storage bottles and culture vessels, in preparing nutrient media, and in making up water during the growth period was of high purity as determined by the dithizone test for heavy metals. Distilled water prepared by the usual procedures in an all block-tin system was again distilled from a still provided with a borosilicate glass head and condenser. Water was stored in borosilicate glass bottles.

GLASSWARE: All glassware, of borosilicate glass, was rigorously cleaned by washing with 0.1 M tri-sodium ethylenediamine tetraacetic acid (EDTA) solution, rinsing with redistilled water, followed by washing with 1 M HNO_3 , and rinsing again with redistilled water. Culture vessels were 4-liter beakers with covers of plaster of paris coated with paraffin.

PURIFICATION OF SALTS USED IN NUTRIENT SOLUTIONS. Earlier experiments designed to test the cobalt requirements of tomatoes indicated that cobalt could be successfully removed from salt solutions by alkaline sulfide precipitation with copper as carrier (3). However, further study of the procedure

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