MEDEDELINGEN VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN, NEDERLAND **62** (12) 1-9 (1962).

PRELIMINARY NOTE ON PHOTOSYNTHESIS IN GREEN PLUM FRUITS

by

Q. P. VAN DER MEER and E. C. WASSINK

(Laboratory of Plant Physiological Research, Agricultural University, Wageningen, Netherlands, 221st Communication; 81st Comm. on Photosynthesis).

(Received 14.11.'62)

1. Introduction

From a practical point of view the problem of photosynthesis in fruits is of some interest, e.g. with regard to the question whether light can influence the well-known, and much studied CO₂-output in picked fruits. Studies on CO₂ production of fruit have received much attention in the I.B.V.T. (Institute for Research on Storage and Processing of Horticultural Produce, Wageningen, Netherlands). Attention was especially directed towards possible relations between storage quality, and CO₂-output after picking. Several fruits gradually change colour from green towards yellow during maturation. Also this makes a look into the possible capacity for photosynthesis worth while, in connection with the question whether light might compensate carbon loss during early storage periods.

Aside of these and several other related practical questions, the study of photosynthesis in fruits has a considerable physiological interest, especially also to contribute to the understanding of factors affecting the growth of fruits. Most fruits are definitely green before they are mature, several of them are more or less green in their interior, some of them stay so up to or even after they have become mature. Most fruits, during growth, have a considerable organic acid content. Recent developments in the field of photosynthesis (see e.g. [1]) enable fruitful attempts at understanding of photosynthetic metabolism types connected with large shifts in acids, known for long as Crassulacean acid metabolism (see, e.g. [2]), and it seems probable that these play a role in green fruit light metabolism also.

The present paper is concerned with purely physiological questions, not with applied problems. Therefore, we have chosen as object a type of fruit which is very green to a considerable depth of tissue, and stays so even after being mature.

An aspect of the study of gas exchange in fruits that should be kept in

mind is their structure of 'compact organs' which may lead to considerably delayed shifts in gas exchange, e.g., by transfer from dark into light, or by change in the surrounding gas phase. Especially CO₂ which is very 'soluble' in the fruit juice, and probably hindered in rapid diffusion by the thickness of the tissue layer and the relative impermeability of the skin and moreover underlies the effect of various buffer capacities, may show considerably delayed exchange, and may build up considerably enhanced (or decreased) internal concentrations. This problem has been dealt with in our laboratory in the case of potatoes (3); in our present study we have not yet obtained detailed data about it; we hope, however, to have taken sufficiently care of it by prolonged experimental periods, so as to have produced reliable data.

2. LITERATURE

Photosynthesis of fruits has not yet been very extensively studied. KURS-SANOV (4) as one of the first workers in this field, measured photosynthesis in unripe apples, cucumber, and peas. He reports considerable photosynthesis especially when supply of assimilates from the leaves for some reason decreases, e.g. by detachment. His view is that photosynthesis of fruits is low as long as they are at the plant but that the light may be important for internal conversions.

More recently, Kidd and West (5) studied gas exchange of unripe apples in daylight. Compared with photosynthesis of apple leaves, measured elsewhere, the intensity found in the fruits was very low.

Wolf (6) observed that, again in unripe apples, CO₂-production of picked specimens increased considerably in the first hours if the fruits had been in strong light before. Wolf suggests connections with the Crassulacean acid metabolism.

BEAN and Todd (7) studied CO₂-fixation in oranges in dark. The juice cells fix considerable quantities both in light and dark. In the green skin, malic acid increases upon CO₂-fixation. Also these authors lay a connection with Crassulacean acid metabolism.

TODD, BEAN and PROBST (8) did not find a definite correlation between photosynthesis and chlorophyll content in lemons, oranges, and cucumbers. Contrary to Kurssanov's observations, they observed a gradual decrease in photosynthesis after picking.

We did not find any report on photosynthesis in plums.

3. Material and methods

Our investigation was made with picked fruits of the green plum variety 'Reine Claude verte', the greengages. They were taken from a tree of about 20 years old with a rather good crop and good foliage. The observations were made during a period between the last week of June and the end of August, 1960. During this period the size of the fruits increased from about 23 to about 33 mm diameter, and the proportion surface/volume decreased about $1^{1/2}$ times. At the end of the growth season large fruits showed bursts even before they were completely mature, probably owing to large rainfall.

Up to the end of July, the epidermis had intact stomata; thereafter they gradually changed into lenticels. The bursts, referred to above, mostly originated from lenticels and mostly extended through several layers of cells. Microscopic sections showed that below the epidermis 2 to 4 layers of cells do not contain plastids. Below these some 10 layers of cells follow containing most of the chlorophyll. At least after the beginning of July, these layers showed several interruptions. The number of layers of cells containing chlorophyll gradually decreased to about 4. Cells situated further in the interior of the fruit also contained plastids, however smaller ones, and probably they were not chloroplasts.

The measurements were mainly concerned with CO₂-consumption and production; the heat-conductivity method (diaferometer or katharometer) was used. An outline of the equipment is given in fig. 1. Several fruits were in a

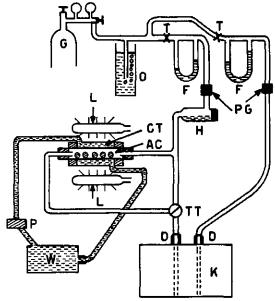


Fig. 1. Outline of the apparatus.
G: gas flask, O: overflow, T: tap, F: flow meter, PG: precision gauge, H: humidifying tube, AC: assimilation chamber (with 5 fruits), TT: three-way tap, D: drying tube, K: katharometer, W: water bath, P: water pump, CT: cooling tube, L: lamp.

glass cylinder, provided with a water mantle, the temperature of which was adjusted. The temperature of the fruits was measured with a thermocouple. Light was provided by two high pressure mercury tubes (450 Watt each) at opposite sides of the assimilation chamber, about 9 cm from the centre of the cilinder. Different light intensities were obtained by different layers of metal gauze, each absorbing about 50% of the light. The intensity of the unweakened light at the level of the fruits was about 2.105 ergs/cm2sec. The gas phase was air, or air enriched with 6% CO2, the relative humidity was kept high throughout.

Some experiments were made with peeled fruits to obtain an idea about the localization of photosynthesis.

The main aim of this paper is to show that photosynthesis in these green fruits can be definitely demonstrated, and to report on some quantitative data about its magnitude.

4. RESULTS AND THEIR DISCUSSION

Since it proved impossible to prevent the temperature of the fruits from rising rather considerably during strong illumination, an experiment was carried out with a lifeless object, viz., a piece of foam plastic of 3 g in which 35 g water, saturated with 5% CO₂ in air, was soaked up. The weight of this object is comparable to that of an average lot of fruits as used normally in our investigation. The object was placed in the assimilation chamber and subjected to a rise in temperature, comparable to that reached in some experiments. As expected, a rise in temperature resulted in CO₂-evolution from the object, owing to a decrease in solubility of the gas (fig. 2).

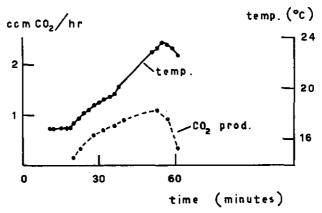


Fig. 2. CO₂-production in air with 5% CO₂ by 3 grams of foam plastic containing 35 grams of water.

CO₂-evolution from the fruits in darkness was found to decrease rather considerably during the period of the investigation (fig. 3), both in air and in air enriched with 6% CO₂. In general, the values observed in the latter gas mixture were somewhat below those recorded in air. At the end of the season, the gradual decline of the values was replaced by a rather sudden increase which is unexplained. Two possible explanations of this increase present themselves: firstly it may represent the beginning of the 'climacterium' in the ripening stage; secondly it may be connected with the occurrence of small bursts in the skin, listed above (p. 2), resulting in an increased supply of oxygen to the interior, and increased CO₂-production. The general decrease, observed throughout the season, is not obviously explained either. It may represent a general decline in activity connected with ageing of tissues (see e.g. [9]), or have something to do with the fact that the surface/volume re-

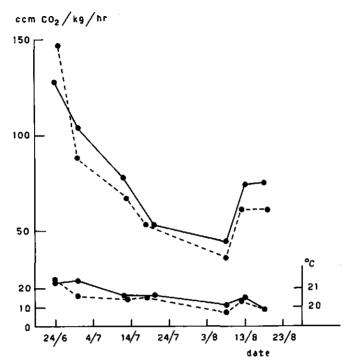


Fig. 3. CO₂-production of greengages in the dark during the last two months before picking time, ——: in air, ---: in air with 6% CO₂.

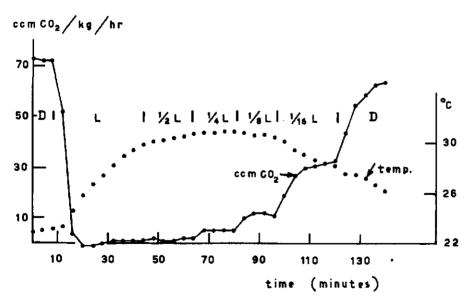


Fig. 4. CO_2 -production of greengages at different light intensities in air. D: dark, L: light $(2.10^5 \text{ergs/cm}^2\text{sec.})$.

lation of the fruits decreases with their increase in size, which will unfavorably affect their gas exchange. A slight indication in this direction may be obtained from the observed increase of CO₂-output in connection with the occurrence of skin bursts, as mentioned above. Probably, both ageing of the tissue, and the change in surface/volume factor play a part, since the decrease in the latter factor only was about 1.5 over the experimental period, while the decrease in CO₂-output was about 3-fold.

A first indication of the occurrence of photosynthesis was obtained from the experiment, represented in fig. 4, showing CO₂-production of fruits exposed to different light intensities in air, not enriched in CO₂. Notwithstanding an unavoidable increase in fruit temperature, CO₂-output strongly decreased, to about zero, in strong light, and again increased somewhat upon weakening of the light by gauze filters.

Photosynthesis is more definitely demonstrated in fig. 5, representing a

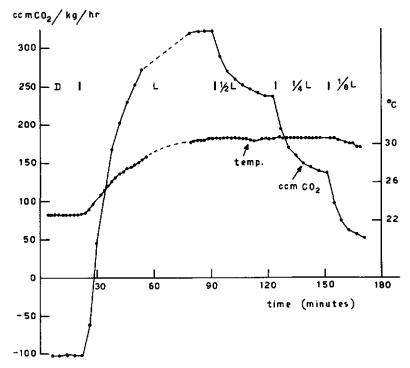


Fig. 5. Photosynthesis of greengages at various light intensities in air with 6% CO₂. D: dark, L: light (2.10 ergs/cm²sec.).

similar experiment in air with 6% CO₂. Positive values (uptake of CO₂) are very obvious here, and a stronger reaction upon weakening of the light is observed.

Fig. 6 shows the data of fig. 5 (final levels), corrected for dark output, in relation to light intensity. Obviously, light saturation is reached only at very high light intensities under the conditions of these experiments.

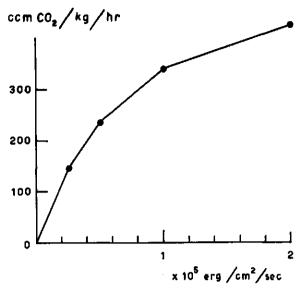


Fig. 6. Photosynthesis of greengages in relation to light intensity (30°C, air with 6% CO₂).

The results of the two experiments discussed (see figs. 4 and 5) together indicate that, in normal air, photosynthesis at high light intensities is strongly limited by insufficient CO₂-supply. (It remains possible that this is less so in fruits under natural conditions, attached to the tree; we have no data hereabout.)

In order to obtain a preliminary idea of the distribution of photosynthesis among the various tissues, some observations were made on peeled fruits and their peelings. This type of experiment, of course, is liable to much criticism owing to the introduction of large wounds and the disturbancy of the prevailing conditions for gas exchange. Moreover, the data obtained still are somewhat incomplete (Table 1). The data are given as observed (CO₂-output

Table 1. CO_2 -production and consumption in air +6% CO_2 by peelings and peeled plums separately, in ccm/kg/hr.

1 Date	2 Process	3 Peel	4 Temp. (°C)	5 Flesh	6 Temp. (°C)	7 Intact fruit	8 Temp. (°C)	9 Peel + flesh
6-7	Respiration Photosynthesis	261 724	20.0 22.4	116 54	20.0 23.7	75 396	19.0 21.9	164 277
14-7	Respiration Photosynthesis	368 885	20.5 24.6	124 57	20.5 23.4	68.5	23.1	181 252
21-7	Respiration	-	_		_	45	_	120

or intake), so that photosynthesis values are not corrected for respiration. As might be expected, peeling strongly increases CO₂-production per unit fresh weight (cf. col⁵. 7 and 9), photosynthesis is much less affected. Both CO₂-output in dark, and photosynthesis are much larger in the peeling (the outer layers) than in the underlying tissue (col⁵. 3 and 5); the difference is especially large for photosynthesis which appears practically localized in the outer layers containing most of the chlorophyll (cf. above) at least in this part of the season.

We have indications that CO₂-uptake in light, like CO₂-output in dark, strongly decreases (even 6-fold or more) during the season. This may be connected in part with the observation that the chlorophyll-containing layer becomes thinner during the season. The effect of season upon photosynthesis may have been somewhat exaggerated by the fact that in the beginning of our experiments we were less successful in preventing temperature rise in the fruit than later on (fig. 7). However, an effect of season nevertheless seems certain.

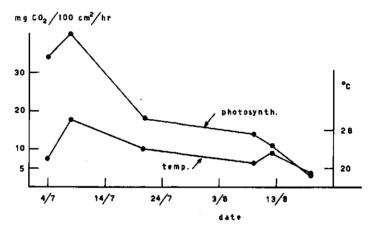


Fig. 7. Photosynthesis of greengages in air with 6% CO₂ during the last 2 months before picking time.

Some preliminary observations on the existence of Crassulacean acid metabolism remained inconclusive. However, they were made late in the season, and will have to be repeated. Observations of changes in CO₂-exchange upon changes from light to darkness and vice versa, and upon changes in CO₂-content of gas phase could be interpreted as consistent with expectations based on the occurrence of Crassulacean acid metabolism, but did not seem conclusive as such, the more so, since in estimations of a similar type no changes in pH were observed. The observation listed above as to the strong relation of CO₂-uptake in light with the CO₂-content of the gas phase (figs. 4 and 5), contain an indication as to relative unimportance of Crassulacean acid metabolism. However, in these experiments, no precautions were taken to preconditioning the fruits so as to bring out most clearly the features of the acid metabolism. Thus, the interesting question as to the possible role of the connection between photosynthesis and acid metabolism in these fruits has to be left undecided for the moment. We hope to have an opportunity to collect more definite observations in the near future.

5. Summary

Observations on CO₂-exchange with the diaferometer (or katharometer) method indicated the existence of photosynthesis in detached green plum fruits

of the variety Reine Claude ('greengages') during their growing season. In normal air, photosynthesis in the detached fruits was found to be CO₂-limited and does not go much further than to compensation of respiration; in CO₂enriched air (6% CO₂), a strongly positive, light intensity dependent photosynthesis is observed. Both CO₂-output in the dark, and CO₂-uptake at high light intensities in CO₂-enriched air, rather strongly decreased during the period of observation (end of June to end of August).

Preliminary observations as to the existence of a Crassulacean type acid metabolism in these fruits remained inconclusive so far, and will be continued.

6. References

- CALVIN, M., 3me Congrès international de Biochimie, Bruxelles, 1955, Conférences et Rapports, pp.211-225, Liège, 1956.
- 2. Thomas, M. in: Carbon dioxide fixation in photosynthesis, pp. 72-93, Cambridge (U.K.).
- 3. Spierings, F. H., Harris, G. P. and Wassink, E. C., Mededelingen van de Landbouwhogeschool Wageningen/Nederland 52, 93-104 (1952).
- 4. Kurssanov, A. L., Planta 22, 240-250 (1934).
- Kidd, F. and West, C., New Phytol. 46, 274-275 (1947).
 Wolf, J., Planta 50, 576-578 (1958).
- 7. BEAN, R. C. and TODD, G. W., Plant Physiol. 35, 425-429 (1960).
- 8. TODD, G. W., BEAN, R. C. and PROBST, B., Plant Physiol. 36, 69-73 (1961).
 9. WASSINK, E. C., Rec. trav. botan. néerl. 31, 583-690 (1934).