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Photosynthesis in the growing leaf of *Phaseolus vulgaris*

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Many investigators have been interested in the dependence of the rate of photosynthesis on the quantity of assimilatory pigments. According to Willstätter and Stoll (1918) the rate of CO_2 assimilation is not limited by the amount of chlorophyll in leaves, but by another factor (or factors). This view is based mainly on the fact that the rate of photosynthesis shown by leaves of aurea (yellow) varieties is not lower than that of normal leaves. This, however, is a statement which is not confirmed by some results of recent research. For instance, Šesták (1963) found a linear relation between the rate of photosynthesis and the chlorophyll content in the leaves arranged in different positions on the stem of *Nicotiana sanderae* and *Brassica oleracea*. Gabrielsen (1948) showed that chlorophyll is a factor limiting the photosynthesis only in the leaves with a low pigment content (less than 4—5 mg per 1 dcm^2) under the condition that the leaves are illuminated by weak light.

It has been previously shown (Więckowski 1959, 1960 a, b, 1961), that in the bean the expansion of the leaf area and the biosynthesis of the assimilatory pigments do not run paralelly. In the initial phase of the leaf growth the rate of biosynthesis of the assimilatory pigments exceeds the rate of the processes which are responsible for the expansion of the leaf area. In a later phase of growth the situation is reversed. In results, characteristic changes in the chlorophyll and carotenoids concentrations are observed during the period of leaf growth; the pigments concentrations per unit of leaf area or per unit of fresh weight increase during the first 3 days and decrease during the next 4—5 days.

In connection with these facts there arises the question whether the changes in the concentration of assimilatory pigments occurring in growing bean leaves are able to modify the rate of photosynthesis, in other words, whether the activity of the CO_2 assimilation apparatus changes during the period of the leaf growth.

This paper summarizes the results of research undertaken with the aim to give an answer to this question.

MATERIAL AND METHODS

Plant cultivation. The experiments were carried out on the primary leaves of *Phaseolus vulgaris* var. Bronowicka. The plants were allowed to grow in controlled conditions: continuous and constant illumination, about 24 000 $ergs/cm^2 \cdot sec.$; 29°C; Knop's liquid nutrient solution (for more details see Więckowski, 1959)

Chlorophyll determination. The chlorophyll content in 80% aceton was estimated by Arnon's (1949) modification of the generally known Mackinney's method. A Uvispek Hilger spectrophotometer equipped with a glass prism was used for this determination. For other details see Więckowski (1959).

Gas exchange estimation. The gas exchanges were estimated by the manometric method. However, the estimation of the respiratory and photosynthetic rates of the whole leaf required an adaptation of this method, especially a modification of the size of the vessels which were made of plexiglass and were 10,4 cm in diameter. The vessel chamber was divided by two plexiglass plates with numerous holes (7 mm in diameter) into three compartments (*a*, *b*, *c* fig. 1). Owing to the size of the vessel it was possible to introduce a whole mature leaf (or several young leaves) into the upper compartment, whereas the bottom of the vessel (compartment c) was filled with the solution of Warburg's carbonate buffer no 9 (25 ml). The distance between the buffer solution and the leaf was about 3 mm. The high value of the surface tension of the buffer solution is a factor preventing its contact with the leaf when the apparatus is in motion. The small distance between the leaf and the buffer surface and the continuous oscilations of the buffer mennisci in the holes provided favourable conditions for the gas exchange between the leaf and the liquid phase.

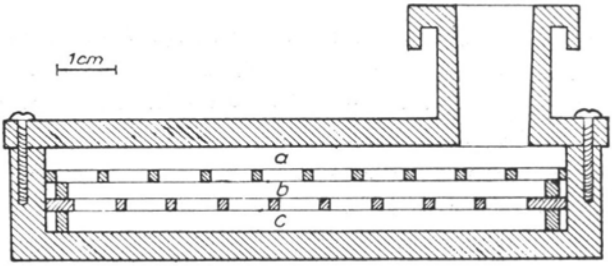

Fig. 1. Vertical section of the vessel used in manometric measurements.

To measure the photosynthetic rate the leaves were illuminated by 250 W incandescent lamps. The author used neutral filters (blackened metallic nets) and modified the distance between the source of light and the leaves in order to obtain light of different intensity. Since the neck joining the vessel with the manometer was situated eccentrically on the cover (fig. 1) it was possible to illuminate the leaves from above.

The measurement of the respiratory rate was made in darkness.

The experiments were repeated 12 times; the mean value and the standard deviations were than calculated.

Measurement of light intensity. Light intensities were measured by Kipp thermopile in the range 375—700 mμ by using GG—18 and RG-8 filters.

RESULTS

In our conditions the length of the growth period of leaves was limited to about 7 days. The maximum chlorophyll concentration in leaves is attained on the third day; the difference between the maximum and minimum values of the concentrations during the growth period examined may reach 50% (Więckowski 1959,

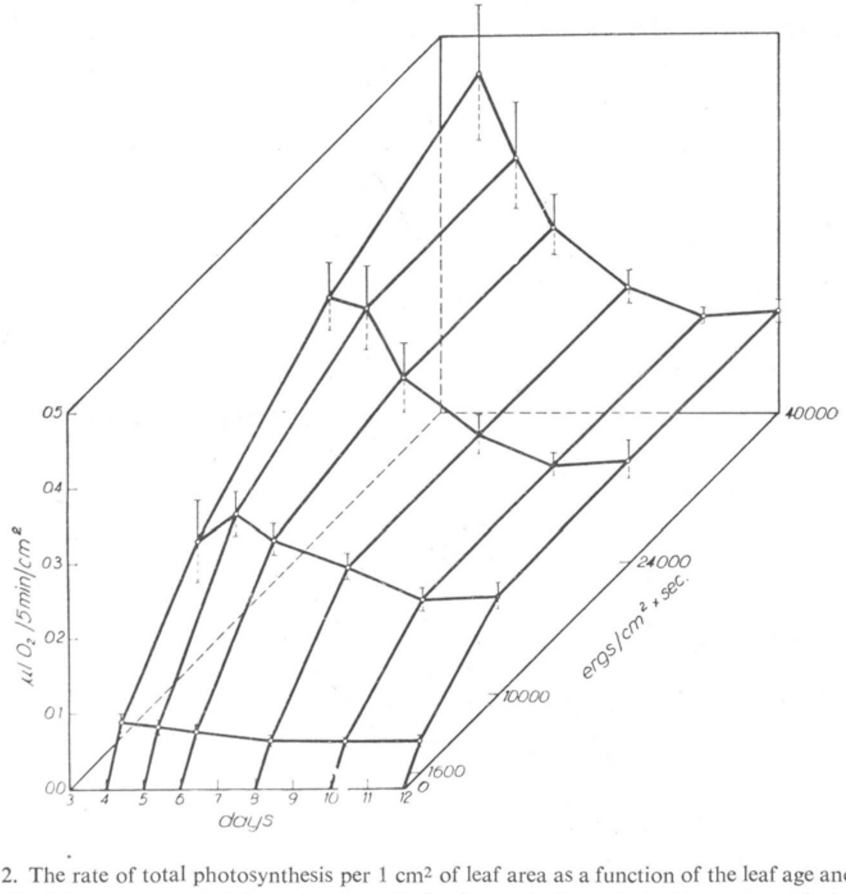

Fig. 2. The rate of total photosynthesis per 1 cm² of leaf area as a function of the leaf age and light intensity (Time was counted from the day of placeing seeds for germination — see Więckowski, 1959).

1960a). The changes in the rate of CO_2 assimilation which takes place during the growth are shown in fig. 2, the effects of different light intensities are also indicated there. It follows from the graph that at any light intensity the younger the leaf the greater the total photosynthesis. The younger the leaf, however, the higher is the light intensity which is required to assure the saturation of the leaf with luminous energy. In the last stage of growth the light saturation intensity is about 10 000 ergs/cm^2. sec., whereas in earlier stages it exceeds 40 000 ergs/cm^2. sec.. In the plants illuminated with the light of high intensity (40 000 ergs/cm^2. sec.) the assimilation rate decreases during the growth of the leaf , but remains on a constant level as soon as the leaf has ceased to grow. The intensity of photosynthesis decreases about three times during the period of the leaf growth. In lower light intensities a marked decline of the assimilation rate is observed from the moment the chlorophyll concentration has attained its maximum value. In earlier stages the increase of the rate of photosynthesis, if any, is insignificant.

Fig. 3 shows that the younger the leaf the higher the rate of CO_2 assimilation per 1 mg of chlorophyll. At any light intensity this rate decreases concomi-

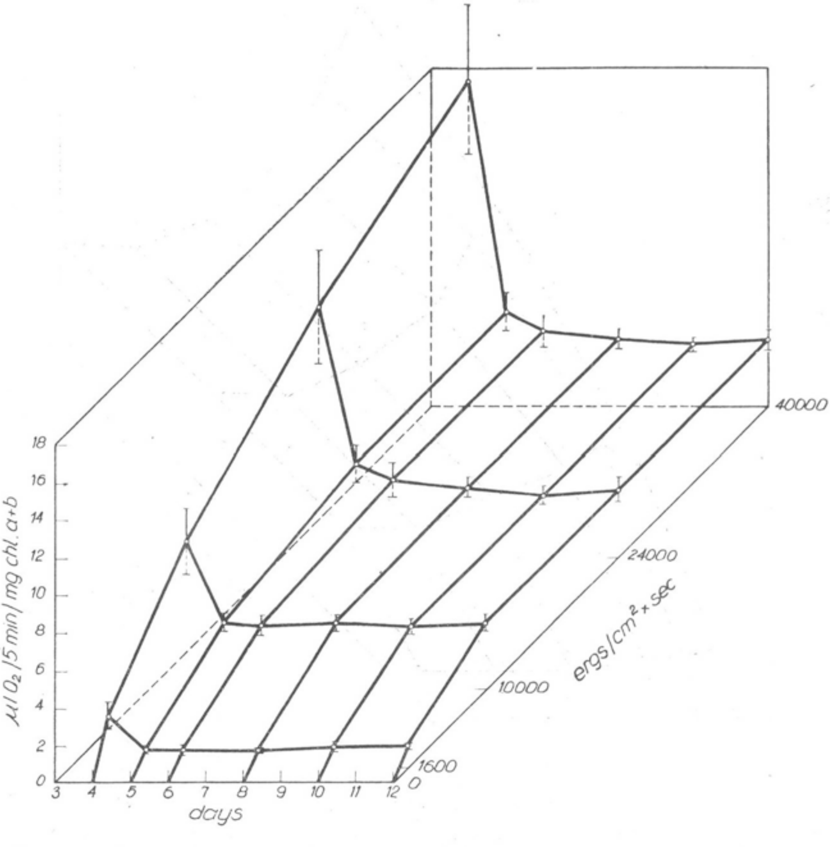

Fig. 3. The rate of total photosynthesis per 1 mg of chlorophyll as a function of the leaf age and light intensity.

tantly with the development of the leaves: the decline is more rapid in the initial than in the final phase. The results indicate that on one hand the photosynthetic apparatus is more active in young leaves, but on the other that very high light intensities are required to assure the full development of its photosynthetic capacity. In our research even a light intensity of 40 000 ergs/cm^2. sec. was too low to obtain the maximum assimilation rate of young leaf, whereas some days later about 10 000 ergs/cm^2. sec. were already sufficient to attain this aim.

During the leaf growth the respiration rate decreases about 10 times (fig. 4). This decline, which is rapid during the first 1—2 days proceeds more slowly in the following few days.

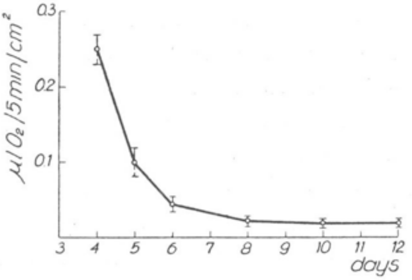

Fig. 4. Changes in the respiratory rate during the leaf growth.

DISCUSSION

The results presented in this paper indicate that the photosynthetic rate of a unit of leaf area is higher in the young bean leaf and decreases gradually with its age. We suppose that photosynthesis is much lower in the earliest stages of leaf development, i.e. when the leaves are still partly shaded by cotyledons and seed coats. We also suppose that the length of the periods with different photosynthetic rate depends on the species and conditions of plant cultivation.

If we assume these suppositions, the results presented are on the whole in agreement with the results of many other investigators. For instance, Irving (1910) and Briggs (1920) proved that young leaves of *Vicia Faba*, *Avena sativa* and *Phaseolus vulgaris* are capable of chlorophyll synthesis but during the initial phase they have no ability to assimilate CO_2. The photosynthetic ability increases during the leaf development. It cannot be excluded that the inactive phase to CO_2 assimilation also occurs in our case but in the earliest stages of leaf development. It is also possible that different experimental conditions made these differences very marked. In Briggs' experiments the rate of leaf respiration decreased from 1.2 to 0.8 units during three days of experimenting. In our case the respiration of the leaves decreased about 7 times during the first three days. This fact indicates that the changes of the metabolic activity of our plant material were much greater than those in Briggs' experiments.

Smillie (1962) also investigated the photosynthetic rate of pea leaves of different age. In those experiments the leaf growth took place between the fifth and sixteenth day after seed germination. This author has established that the photosynthesis attained its maximum in the leaf on the nineth day. Smillie linked the changes of photosynthetic rate of growing leaf with similar changes in the activity of photosynthetic enzymes.

In the pea leaf the maximum of chlorophyll concentration occurs on the twelfth day (Smillie and Krotkov, 1961). The authors did not calculate the rate of photosynthesis per 1 mg of chlorophyll but a comparison of the data in fig 1 (Smillie, 1962) with those in fig. 4 (Smillie and Krotkov, 1961) indicates that the maximum of photosynthesis per mg of chlorophyll does not occur on the twelfth day when the chlorophyll concentration attains its maximum but between the seventh and nineth day. This comparison indicates that the rate of photosynthesis per 1 mg of chlorophyll also changes during the development of the pea leaf; it is higher in the young leaf although not in the youngest.

Below the factors are listed which may be responsible for the high activity of the photosynthetic apparatus in a young leaf:

1. a relatively greater amount of an active form of chlorophyll,

2. a higher activity of photosynthetic enzymes. Smillie (1962) found that the activity of such enzymes as ribulose 1,5 diphosphate carboxylase and photosynthetic pyridine nucleotide reductase change parallely to the changes of photosynthetic rate,

3. It is also possible that this high activity is connected with the high activity of general metabolism, of which the very intensive respiration is an exponent. Although many experiments on the interdependence between photosynthesis and respiration have shown that photosynthesis or illumination modify unsignificantly, if at all, the rate of respiration (see: James 1953; Brown 1953 and others), this does not mean that respiration is without any influence on photosynthesis. The following facts indicate the connection of these processes: a, below the compensation point the photosynthetic efficiency is higher (Kok 1951), b. certain ecological observations also support this interpretation, e.g. Müller and Nielsen (1965) showed that the course of many anabolic and catabolic processes including photosynthesis and respiration are much higher in plants grown in the tropical than in the temperate climate.

SUMMARY

The rate of photosynthesis was determined in the growing primary leaves of *Phaseolus vulgaris*. var. Bronowicka.

It was established that the photosynthetic rate per unit of leaf area is higher in the young than in older leaves, but in a young leaf greater light intensity is necessary to obtain the saturation point.

The rate of photosynthesis per 1 mg of chlorophyll (a+b) is also higher in a young leaf.

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Fotosynteza w rosnącym liściu Phaseolus vulgaris

Streszczenie

Zbadano zmiany natężenia fotosyntezy w rosnącym liściu fasoli (*Phaseolus vulgaris* odm. Bronowicka).

Natężenie fotosyntezy, w przeliczeniu na jednostkę powierzchni lub na 1 mg chlorofilu $a + b$, spada w miarę wzrostu liścia; im młodszy liść tym konieczna jest jednak wyższa intensywność światła dla uzyskania punktu wysycenia.

Przedyskutowano także przypuszczalne przyczyny wyższej sprawności aparatu asymilacyjnego we wczesnych fazach wzrostu.