

Development of Photosynthesis in Cotton Seedlings, *Gossypium hirsutum* L.<sup>1</sup>G. L. Richardson<sup>2</sup>

## ABSTRACT

An infrared gas analyzer was used to follow CO<sub>2</sub> exchange (dark respiration and net photosynthesis) through the first 248 hr of germination of cotton seeds. The following events were noted: (a) dark respiration was first detectable 6 hr after placing the dry seed in water (planting); (b) the photosynthetic compensation point was reached 86 hr after planting and 24 hr after emergence and occurred during the unfolding of the cotyledonary leaves; (c) maximum rates of net photosynthesis (33 mg CO<sub>2</sub>/dm<sup>2</sup>/hr at 64.8 kilolux with 500 ppm CO<sub>2</sub>) were attained by 200 hr after planting and remained at this level during the remainder of the test period (48 hr); and (d) photosynthetic rates increased as light and CO<sub>2</sub> concentrations were increased. The fully expanded cotyledonary leaf stage (200 to 248 hr after planting) of the cotton seedling is suitable for photosynthetic studies because of the stability and uniformity of the photosynthetic rate.

General Electric Cool Beam lamps were found to be equally as effective as sunlight in providing energy for fixation of CO<sub>2</sub>.

Dark respiration rates, determined throughout the experiment, increased from 0 to a maximum by 120 to 130 hr after planting and remained constant for the next 24 hr.

**A** KNOWLEDGE of when developing seedling plants such as cotton first become photosynthetically self-sufficient should be useful to the plant scientist or the farmer. Since environment is so critical in the early stages of development, information of this type should be helpful in optimizing the environmental conditions. Plant competition for light, moisture, nutrients, and space might be more precisely controlled for the benefit of the developing seedlings. The principal objective of the experiments was to determine when seedling cotton plants become photosynthetically self-sufficient.

The present experiments were preliminary to a search for a simple and early test for potential yield of varieties or lines of the genus, *Gossypium*. It was hoped that the seedling stage might be suitable for testing photosynthetic efficiency as a measure of potential yield. Another objective, therefore, was to trace the development of photosynthetic activity of cotton seedlings in hopes of finding a period of stable CO<sub>2</sub> exchange rates.

Since one of the most critical aspects of measuring plant response is the selection and preparation of the plant material to be used, especially where comparative results are desired, a technique to help insure the use of comparable plant material and test conditions would be advantageous. Pretreatment conditions undoubtedly are important in CO<sub>2</sub> exchange studies and may account for some of the conflicting results obtained, especially where environmental conditions were seemingly controlled during the actual rate measurements. Many factors in a plant's development tend to make comparative results difficult to

obtain, such as age of the plant, physiological activity of the leaf (in the case of individual leaf studies), nutritional status, light energy interception, insect and disease attacks, root development, etc. Greater uniformity of light intensity can be easily maintained when all of the leaves are oriented in the same plane with respect to the light source. Distance from the light source, in the case of artificial light, is also of paramount importance. The use of seedling plants developing at uniform rates may help overcome some of these difficulties. The availability of a relatively new lamp as a source of energy for CO<sub>2</sub> fixation prompted a comparison of it with natural sunlight.

## LITERATURE REVIEW

Most of the studies on photosynthesis of developing leaves have been concerned with the effect of age of the leaf on CO<sub>2</sub> exchange. Smillie (13), working with peas, found that the rate of photosynthetic activity increased to a maximum before full leaf expansion and then declined slowly. The maximum rate of photosynthesis occurred when the leaves were 9 days old. Similar results were obtained by Briggs (2), Sacki (8), Singh and Lai (11), and Sesták and Catsky (9), working with several plant species.

Irving (6) traced the development of photosynthetic activity of barley seedlings and concluded that photosynthesis did not occur until the seedlings had attained "a full grass-green colour." This occurred on the third day after the leaf protruded through the sheath. His barley seedlings never reached the photosynthetic compensation point under the conditions maintained in his experiments (air with very low CO<sub>2</sub> content).

Singh and Lai (11) found maximum rates of "real" assimilation of 2.94 and 1.84 mg CO<sub>2</sub>/dm<sup>2</sup>/hr for first leaves of 7-day old wheat and flax seedlings, respectively, when measured under high CO<sub>2</sub> concentrations (3,300 to 3,800 ppm). Muramoto, et al. (7) measured mean rates of net photosynthesis of cotyledons of 35 to 50 mg CO<sub>2</sub>/dm<sup>2</sup>/hr for several American cottons in air containing approximately 300 ppm CO<sub>2</sub>. In a later study, El-Sharkawy, et al. (4) found maximum rates of 18 to 49 mg CO<sub>2</sub>/dm<sup>2</sup>/hr. Ibragimov (5) measured photosynthesis rates of cotyledonary leaves of three cotton varieties and found a range of 8.7 to 17.8 mg CO<sub>2</sub>/dm<sup>2</sup>/hr.

Woodstock and Feeley (14) found that early rates of respiration (2 to 3 days after water was applied to the seed) were correlated with subsequent rates of seedling growth of corn. Smillie (13) determined that CO<sub>2</sub> evolution (dark respiration) decreased continuously during leaf expansion of peas. However, very little information is available on the development of respiratory activity during germination and emergence of cotton seedlings.

Plant scientists have long recognized the difficulties in obtaining suitable plant material for comparing rates of CO<sub>2</sub> exchange. Recently, El-Sharkawy et al. (4) suggested that light intensity, air temperature, and soil moisture may be responsible for conditioning cot-

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ton leaves for higher rates of photosynthesis. They found almost a 100% variability in rates of photosynthesis between single leaves from two cotton plants that were identical in appearance. They surmised that the large difference must have been associated with soil moisture conditions. The literature indicates that variability in response seems to be positively associated with age of the plant, which can be explained on the basis of the environmental differences that continue to occur.

## METHODS

One seed of cotton, *Gossypium hirsutum* L., Var. Acala 44-10, was planted in each of forty 100-ml beakers containing 140 g of acid-washed sand. Thirty-five ml of distilled water were added and the beakers were placed in a germinator in the dark at temperatures of 30 C during the day and 24 C at night. Every few hours the beakers were transferred to a clear plexiglas chamber fitted with a black, light-tight cloth for measurement of rates of  $\text{CO}_2$  evolution in the dark. The cylindrical chamber had a volume of 967 ml and air was circulated through it at the rate of approximately 11 liters/min. As soon as the cotyledons reached the surface of the sand, the beakers were transferred to a light source of 32.4 kilolux with a 14-hr photoperiod. Periodically, they were carefully sealed in the plexiglas chamber under 64.8 kilolux illumination and air temperature of  $30 \pm .25$  C to measure development of photosynthetic activity. Checks for leaks in the closed system were made by releasing a fine jet of  $\text{CO}_2$  at strategic points and observing the deflection of the potentiometer needle of the  $\text{CO}_2$  analyzer. Air temperature in the chamber was controlled by circulating cold water through copper coils placed inside the chamber, with a Thermo-cap relay (Niagara Electron Laboratories, Andover, N.Y.) to energize the water pump in a Forma-Temp, Jr. refrigerated bath manufactured by Forma Scientific, Marietta, Ohio. Distilled water was added twice daily as needed to replenish the moisture to its original level in the beaker.

The Beckman, Model #15A, infrared gas analyzer was used to measure rates of  $\text{CO}_2$  exchange in a closed system, similar to that used by Decker (3). Two sources of light were used: sunlight and 150-watt, Cool Beam lamps, manufactured by General Electric Company, Cleveland, Ohio. The spectral distribution curves and details of construction and application of the lamps were presented by Beesley, et al. (1). Light intensity was measured with a Weston illumination meter and spectral distribution was checked with an ISCO Model SR spectroradiometer, manufactured by Instrument Specialties Company, Inc., Lincoln, Neb. Light intensity was adjusted downward by placing layers of black metal cloth between the light source and the chamber. Black ink was sometimes added to a 10-cm thick water barrier located between the light source and the chamber to make small adjustments in light intensity. Total light energy reaching the leaves was easily reproduced in these studies, since the two cotyledonary leaves were equidistant from and perpendicular to the light source.

Rates of  $\text{CO}_2$  exchange under illumination were measured at three levels of  $\text{CO}_2$ : 20, 300, and 500 ppm. A mixture of 80% nitrogen and 20% oxygen was introduced into the system to adjust the  $\text{CO}_2$  concentration to near 0 for measuring rates of  $\text{CO}_2$  evolution at 20 ppm. Leaf area was determined daily after seedling emergence by "printing" both cotyledonary leaves on photographic paper similar to the procedure used by Shibles and MacDonald (10).

Relative humidity was maintained at or near saturation in the closed system, although no measurements were made.

A minimum of 10 seedlings that had emerged and oriented their leaves horizontally at approximately the same time, had about the same leaf area, and otherwise appeared equally vigorous were used for testing.

## RESULTS AND DISCUSSION

**Dark respiration.** Measurable amounts of  $\text{CO}_2$  were released through respiration by the seeds within 6 hr after water was applied to the seeds (Fig. 1). Release of  $\text{CO}_2$  remained relatively constant for the next 6 hr, at which time the expanding seedling broke open the seed coat. The resulting increase in  $\text{CO}_2$  evolution

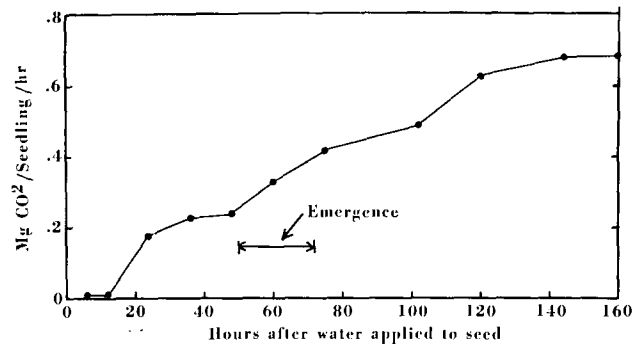


Fig. 1. Dark respiration of cotton seedlings at 300 ppm  $\text{CO}_2$ .

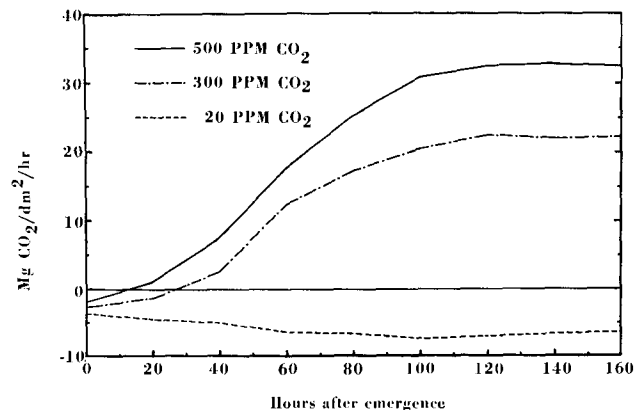


Fig. 2. Effect of  $\text{CO}_2$  concentration on net photosynthesis of developing cotton seedlings.

was probably aided by the unrestricted release of  $\text{CO}_2$  into the surrounding atmosphere. The large air spaces between the 2 mm sand particles undoubtedly allowed adequate gaseous exchange for relatively accurate and fast measurement of  $\text{CO}_2$  evolution by the developing seedlings.

Dark respiration tended to stabilize for approximately 24 hr prior to emergence of the seedlings and then accelerated until about 120 hr after initial water application.  $\text{CO}_2$  evolution remained relatively constant for the next 24 hr.

**Net Photosynthesis.** The seedlings became photosynthetically self-sufficient (reached the compensation point) in air containing 300 ppm  $\text{CO}_2$  within 24 hr after emergence and within 12 hr at 500 ppm  $\text{CO}_2$  (Fig. 2). The cotyledonary leaves were approximately half unfolded when the seedlings were able to carry on enough photosynthesis to maintain themselves under ambient  $\text{CO}_2$  levels. The color of the leaves at this developmental stage indicated that the chlorophyll content was much below maximum, although the amount of chlorophyll present was not determined.

Photosynthetic activity was detected as soon as the cotyledons started intercepting light energy, undoubtedly coinciding somewhat with chloroplast development and chlorophyll synthesis. At emergence, the seedlings were responding significantly to changes in  $\text{CO}_2$  concentration of the surrounding air. A few preliminary measurements revealed that they were also responding to varying levels of light intensity.

The seedlings continued increasing their rate of assimilation as the leaves unfolded and expanded to

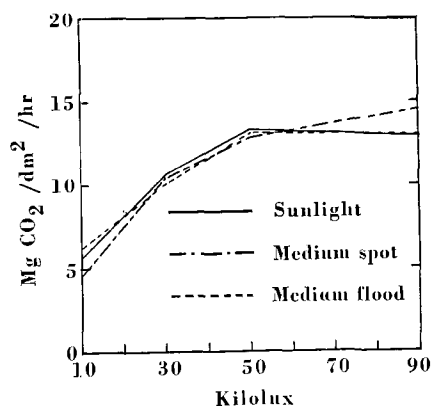


Fig. 3. Effect of light source and intensity on net photosynthesis of fully expanded cotyledonary leaves of cotton.

full size, which occurred at approximately 120 hr after emergence. Thereafter, the rates of net photosynthesis stabilized for a period of at least 40 hr (end of the experiment). No attempt was made to accurately measure net photosynthesis after 160 hr after emergence because of the initiation of growth of the new leaf buds and the resultant difficulty in accurately measuring the photosynthetic area involved. The seedling plants, however, responded similarly to leaves of older cotton plants as reported by other workers (4, 7). A preliminary test on attached leaves of older plants, using the same technique except that a plastic leaf chamber was used, revealed that the seedlings responded similarly to secondary leaves of older cotton plants. The stability of the response to all three levels of CO<sub>2</sub> indicated that the period of at least 40 hr after full leaf expansion may be suitable for precisely measuring responses to relatively small changes in environmental conditions.

The fully expanded leaves responded positively to increases in light intensity and CO<sub>2</sub> concentration and reached a maximum of 33 mg CO<sub>2</sub>/dm<sup>2</sup>/hr at 64.8 kilolux with 500 ppm CO<sub>2</sub>. At 300 ppm, the maximum rate was 22 mg CO<sub>2</sub>/dm<sup>2</sup>/hr, which is somewhat slower than those reported by Muramoto et al. (7), but faster than those obtained by Slatyer and Bierhuizen (12) for older cotton leaves.

**Light source.** The Cool Beam lamps were as effective as natural sunlight in providing energy for fixation of CO<sub>2</sub> (Fig. 3). Light intensities up to 108 kilolux were used successfully as long as no sudden and drastic increases in intensity were imposed on the seedlings. Figure 4 shows the spectral energy distribution of the Cool Beam lamp (Medium Spot) compared to sunlight, both at light intensities of 108 kilolux. The lamps produced less energy than sunlight at the shorter wavelengths—up to 535 nanometers (millimicrons)—but produced more at the longer wavelengths. When integrated over the entire visible spectrum, sunlight produced 37,845 microwatts/cm<sup>2</sup> and the Cool Beam lamp 54,438. Both light sources were first adjusted to 108 kilolux on the Weston illumina-

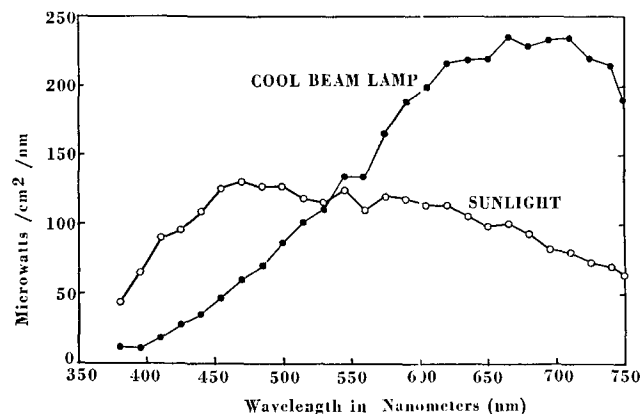


Fig. 4. Spectral energy distribution of sunlight and Cool Beam (Medium Spot) lamp at 108 kilolux light intensity.

tion meter and then checked with the spectroradiometer. Thus, the lamps appeared to be a suitable source of light energy for measurements of photosynthetic responses, especially where the results are to be related to sunlight.

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