Revisiting Blackman's limiting conditions for photosynthesis: influence of temperature on photosynthetic rate of leaf disks

Abstract

Among other factors, ambient temperature has been reported to significantly influence the apparent photosynthetic rate of plants. In this paper, we demonstrate the influence of ambient temperature on apparent photosynthetic rate using a leaf disk model system. Our results indicate that apparent photosynthetic rate increases with temperature. We discuss the possible reasons for the observed pattern and discuss the implications that the relation may have for plant growth and productivity.

Introduction

Among several environmental variables, temperature has been known to strongly influence photosynthetic rate of plants (Blackman, 1905). Classically the effects of temperature on photosynthetic rates follow a parabolic relationship, with rates being low at extremely low and high temperatures and high at moderate temperatures (Rabinowitch and Govindjee, 1969). Consequent to this relationship, plant growth and productivity is often limited by extremes of temperature, with either too low or high temperatures affecting the growth and productivity of plants (Rabinowitch and Govindjee, 1969; Govindjee, 1975). Obviously it appears that extremes of temperature could adversely affect the apparent photosynthetic rate of leaves. Further, while tissue respiration rates may remain unaltered under extreme temperatures, decrease in the photosynthetic rates can lead to a negative carbon balance in the plant (Hipkins, 1987). Under such conditions, the growth and productivity and consequently the adaptability of plants would be jeopardized. These arguments pre-suppose that plant species in nature have an optimum temperature at which their photosynthetic rates are maximized. Extreme temperatures either too low or too high depress the photosynthetic rates. In recent years, there has been renewed interest in evaluating plant responses to elevated temperature owing to the green house effect.

In this paper we explicitly examine the hypothesis that within a limited range of temperatures, the photosynthetic rates of leaves would scale positively with temperature. We discuss the results in the light of existing knowledge on the role of temperature in influencing apparent photosynthetic rates in species and how such relation could have implications for plant growth and productivity.

Materials and Methods

Rationale of the experiment

The experiments were performed on spinach leaf disks. Leaf disks contain spongy mesophyll layer of cells, which largely comprise of large air spaces. By depleting the air spaces under vacuum, the leaf disks sink in water. However, under sufficiently lighted conditions when the leaf disks photosynthesize, the air spaces are refilled with oxygen (a product of photosynthetic oxygen evolution) and tend to float again. The rate at which the leaf disks begin to float can be used as a surrogate measure of the photosynthetic rate of the leaf disks. Thus leaf disks that fail to float are those in which photosynthetic oxygen evolution has not occurred (and hence in which photosynthesis is absent). On the other hand and keeping everything else constant, it can be inferred that leaf disks that float slowly are those were the photosynthetic rates are relatively slow compared to disks that float rapidly.

Preparation of leaf disks for incubation

Leaf disks were made using a hole puncher. About 60 leaf disks were prepared from 2 or 3 fresh leaves of spinach at each effort. With the help of a surgical syringe, air was removed from the spongy tissue of the leaf disks by creating vacum and replaced with sodium bicarbonate solution (0.2 percent w/v). After this process, most of the disks sink to the bottom of the syringe, indicating that in these disks the air spaces were successfully evacuated and replaced with bicarbonate solution. Disks that remained afloat were discarded. The sunken disks were transferred to fresh sodium bicarbonate solution and maintained in dark.

Treatment details

The study comprised of three temperature treatments, namely, one below room temperature (7^{O}C), one above room temperature (36^{O} C) and yet another at room temperature (28^{O} C). Temperature regimes (28^{O} C and 36^{O} C) were provided by maintaining the Petri dishes in incubators with the respective temperatures pre-set; temperature of 7^{O}C was maintained by incubating the Petri dishes in temperature controlled refrigerated incubators. All treatments were conducted under light from incandescent bulbs. For each treatment, four trials were conducted. Each trial consisted of one Petri dish containing 20 leaf disks. Using a forceps, skillfully and rapidly, twenty disks were transferred from the sunken disks maintained in sodium bicarbonate solution in dark. Care was taken to randomise the allocation of leaf disks into each of the treatment blocks in order to avoid all systematic biases in the experiment. After equilibration for 10 sec, the lamps were turned on and the experiment begun.

Observation and data analysis

For each trial in each of the various treatments, the number of leaf disks floating at every two minute interval, for 10 minutes was recorded. From this basic data set, we computed the following additional parameters: a) the average time to float, taken as the median value between two successive time intervals of observation, b) the total number of leaf disks floating at each interval of observation, c) the number of new leaf disks that floated in every successive interval of observation.

From the above observation and computations, we further computed the average time taken for leaf disks to float in each trial. This was calculated as the sum of the product of the new disks floating at each interval of time and the average time it took to float, normalized with the total number of disks found floating. We then computed the average time taken for the leaf disks to float, considering the total number of disks that finally was found floating.

The average time taken for leaf disk to float in each trial was used as an estimate of time it took to photosynthesize 2µl of oxygen (the average amount of oxygen gas in each floating disk). Accordingly, we calculated the amount of photosynthetic oxygen produced for every minute and expressed it as µl oxygen/min. This was expressed as the average photosynthetic rate of the system. The mean of the average photosynthetic rate obtained for the four trials for each of the treatment provided an estimate of the overall photosynthetic rate of the system. Finally we calculated the standard deviation and standard error of mean for each of the estimate.

Results

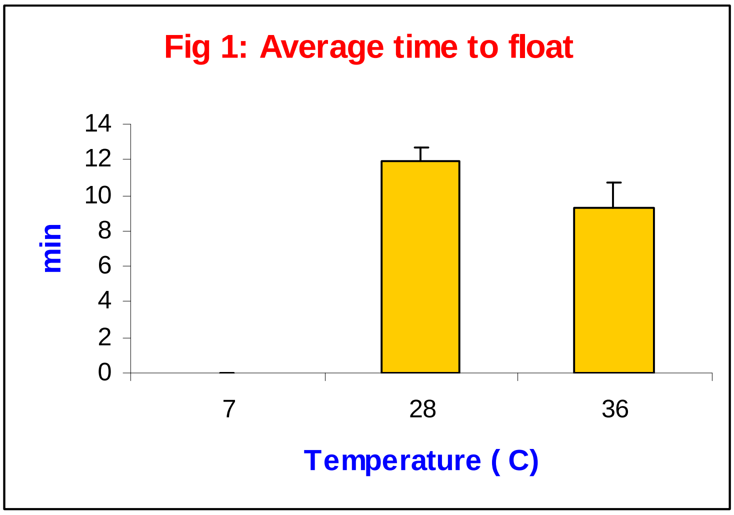
Based on the experiments performed, we present key results pertaining to the effect of temperature on the photosynthetic rate of leaf disks. Table 1 provides results on the average photosynthetic rate, percent floaters and the overall average photosynthetic rate of leaf disks exposed to different temperature regimes. At low temperature (7^{O} C), everything else remaining constant, none of the leaf disks floated indicating that none of the leaf disks photosynthesized.

Table 1: Average photosynthetic rate, percent floaters and overall average photosynthetic rate of leaf disks at different temperatures

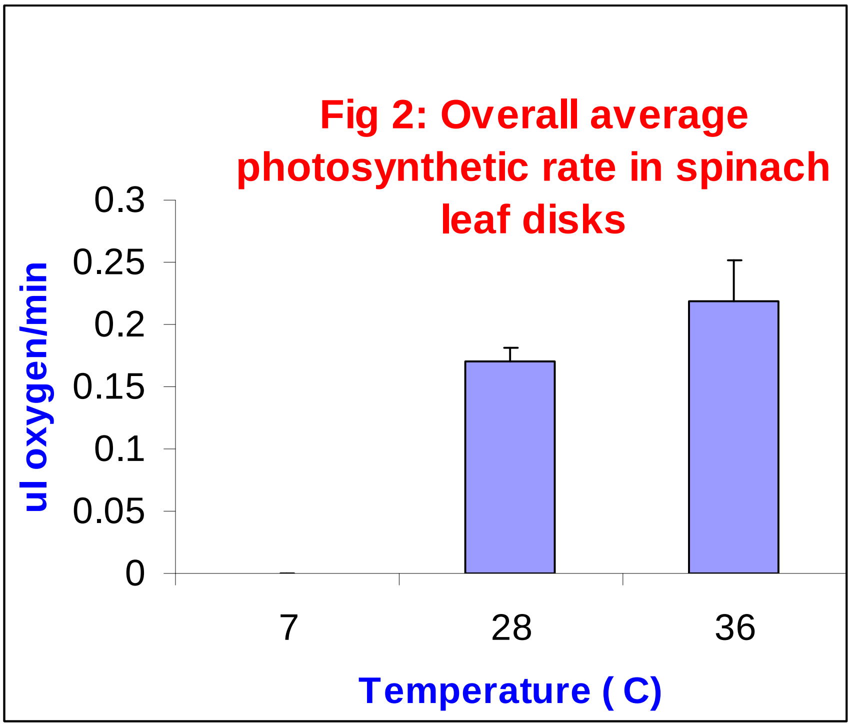
Treatment Temperature © degree C	Average photsynrate ul oxygen/min	Percent floaters	Overall avg photsynrate ul oxygen/min
7	0	0	0
28	0.164	100	0.164
	0.168	100	0.168

		0.165	100	0.165
		0.187	100	0.187
Mean		0.171		0.171
SD		0.010801234		0.0108
	36	0.234	100	0.234
		0.253	100	0.253
		0.177	100	0.177
		0.212	100	0.212
Mean		0.219		0.219
SD		0.03262923		0.0326

On the other hand, with increase in temperature, both at 28^{O} C and 36^{O} C, all disks floated (Table 1). The average time to float decreased from 11.87±0.85 min at 28^{O} C to 9.28±1.458 min at 36^{O} C (Fig 1).



At elevated temperature of 36^{O} C, there was a greater rate of oxygen evolution compared to that at 28^{O} C (Fig 2). The average overall photosynthetic rate (ul oxygen/min) at 36^{O} C was 0.219 ±0.0326 compared to 0.171±0.0108 at 28^{O} C .



Discussion

About a hundred years ago, Blackman from the University of Cambridge, UK, demonstrated that the rate of photosynthesis in plants is strongly influenced by three factors, namely, carbon dioxide concentration, light intensity and temperature (Blackman, 1905). A hundred years since, and after innumerable number of studies, the basic findings of Blackman has been so well recognized that the three variables are often referred to as the "limiting factors" for photosynthesis in plants. Crop physiologists, agronomists, ecologists, crop modelers and a score of others interested in plants have endlessly toyed with these variables in their pursuits to understand plant responses to extreme climatic conditions to, in their efforts to design and breed plants with higher productivity. Obviously the implications of understanding the role of these "limiting factors" have important implications for crop improvement and in crop modeling.

Results obtained in this study using a simple leaf disk model system, clearly validate the hypothesis that photosynthetic rates are strongly influenced by ambient temperature conditions. The observed results are best explained by the sensitivity of photochemical and biochemical events involved in photosynthesis to temperature (Rabinowitch and Govindjee, 1969; Govindjee, 1975). Especially, the effect of temperature on photosynthesis will depend upon how stable and active the key enzyme, RUBP carboxylase is with changes in ambient temperature (Ellis, 1979).

At low temperatures (such as at 7^{O} C), the rate of carboxylating reaction is slow or even absent, because of extremely low rates of molecular collisions of the enzyme with the substrate. Besides the lowered chemical kinetics, low temperatures are also known to change the chloroplast membrane fluidity leading to a poor photochemical function. On the other hand, at higher temperatures (such as at 28^{O} C and 36^{O} C), both because of a higher rate of molecular collision frequency (of enzyme with substrate) as well as due to greater chloroplast membrane and enzyme stability, the photosynthetic yields are higher (Rabinowitch and Govindjee, 1969; Govindjee, 1975).

In summary, our studies using the flotation technique, confirm the effect of temperature on photosynthetic rate, an observation first made over a hundred years ago by Blackman. The implications of these results are manifold, especially considering the consequences on plant growth and productivity. For example, the simple demonstration here indicates why on an average, the tropical forests are more productive than are temperate forests. In other words, temperature as a limiting factor might therefore play an important role in species distribution and abundance. The results also imply that keeping everything else constant, increase in temperature such as due to global warming, can actually lead to a higher biomass productivity of a species.

References

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