

QUANTUM EFFICIENCY AND THE KOK EFFECT IN WHOLE PLANT PHOTOSYNTHESIS

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Photosynthesis begins the food chain of life. The conversion of light energy into the chemical potential energy of reduced carbon compounds by higher plant chloroplasts is among the most important and complex photochemical processes in nature. Fundamental to understanding photosynthesis is the determination of the relationship between absorbed radiation and the rate of photosynthesis driven by that radiation. It is prudent, therefore, that the plant physiologist has endeavored to describe accurately the light response curve of photosynthesis.

In darkness, the enzymes of the oxidative pentose phosphate cycle are operative. In the transition from dark to light, some light energy is utilized for the reductive deactivation of oxidative enzymes and for activation of the reductive enzymes of the Calvin-Bassham cycle (1). It is not clear whether there exists a finite threshold light intensity for photosynthetic CO₂ reduction.

The light compensation point of photosynthesis (LCP) is the light intensity at zero net flux of CO₂, where the rate of photosynthetic CO₂ uptake just compensates the rate of respiratory CO₂ evolution. For leaves exposed to the sun, net photosynthesis becomes positive and then negative each dawn and dusk, respectively, as the irradiance level passes through the LCP. The LCP is an arbitrary parameter which is dependent primarily upon the rate of *dark* respiration which occurs in the light. Several factors including species, carbohydrate status of the leaf, temperature, and the light environment of the leaf during development affect the rate of dark respiration and, hence, the LCP. Consequently, no single irradiance level can be used as a benchmark to indicate the amount of light required to observe net CO₂ uptake by a leaf. At higher light intensities, net CO₂ uptake becomes increasingly positive until at light saturation there is no response of photosynthesis to increasing light. Under certain conditions, further increasing the light intensity causes damage to the photosynthetic apparatus, or photoinhibition.

Light is absorbed by plant pigments in discrete packets, termed quanta (or photons), the energy of which are dependent upon the frequency of the waves in which the

quanta travel. Quanta of appropriate energy, 400-700 nm wavelength, are capable of functioning in photosynthesis. The quantum yield (Φ) for any light-driven process is a measure of the effectiveness of absorbed radiation in producing particular physical and chemical changes.

$$\Phi = \frac{\text{number of molecules undergoing process}}{\text{number of quanta absorbed}}$$

The concept emerged from the second basic law of photochemistry, also known as Einstein's law of photochemical equivalency, which states that a single quantum of light can excite a single molecule. In photosynthesis, a single quantum can excite a single chlorophyll molecule to release an electron, and quantum yields of unity have been observed for the primary photooxidation of photosynthetic pigments (2). However, Einstein's principle applies only to the primary photochemical act, while empirical quantum yields for secondary reactions are usually much less than unity.

This is certainly true in photosynthesis where there are many reactions subsequent to the initial photoact. Losses in efficiency and branches along the pathway render somewhat indirect the relationship between absorbed quanta and the final product, CO₂ reduced to the level of glucose. Nevertheless, the maximum quantum yield has important mechanistic implications. It is not surprising, therefore, that the efficiency with which chloroplasts transduce light into chemical energy has received more experimental and theoretical effort and probably has generated more controversy than any other aspect of photosynthesis research. Indeed, investigations of the quantum yield led to much of our present understanding of the physical nature and energetic pathways of the photosynthetic apparatus.

The Maximum Quantum Yield. It has been recognized for over a century that, as with most energy consuming processes, the energy conversion efficiency and quantum yield of photosynthesis increase as radiation (or energy consumption) decreases. Thus, the maximum quantum yield for O₂ evolution or CO₂ uptake is observed at very low photon flux densities where the relationship between the rate of net photosynthesis and absorbed radiation is

linear. Accordingly, the quantum yield of photosynthesis is generally taken as the slope of this linear relationship. Despite the seeming simplicity of the measurement, however, the value of the maximum quantum yield was debated for several decades.

Without question, much of the confusion has been directly or indirectly due to the complexity of the relationship between light absorption and the eventual uptake and reduction of CO₂. In addition, many early studies of quantum efficiency were based on measurements of O₂ evolution or CO₂ uptake by unicellular algae, and interpretation of the results was complicated by the difficulties of quantifying the rate of photosynthesis at low photon flux densities where, as noted above, the rate of dark respiration has a large effect on net O₂ or CO₂ exchange. Despite numerous biochemical and physiological studies, the extent to which dark respiratory activity continues in the light has remained unresolved to this day (3).

Beginning in the 1920's, Warburg et al (4) propounded that the efficiency of quantum conversion was nearly 90%, with a minimum quantum requirement (= reciprocal of quantum yield) for O₂ evolution of 3 to 4. However, Emerson found that when anomalous behavior of O₂ and CO₂ during the dark to light transition was avoided, a quantum requirement of 8 to 12 was observed (5). Partially in an effort to avoid such problems, in later studies, quantum yields of partial reactions of photosynthesis have been determined using isolated chloroplasts, thylakoid membranes, and even fractions thereof referred to as reaction centers. From this work, there is now a wide acceptance of a minimum quantum requirement of >8 (maximum quantum yield of <0.125). Quantum yield measurements made under monochromatic and dichromatic light led to recognition of the "Emerson enhancement effect" (6), a more than additive increase in quantum efficiency observed when certain wavelengths of light are used. These kinds of experiments were fundamental to the development of the present concept of two cooperating photosystems (Photosystem I and Photosystem II) that move reducing power from oxidized water to ATP and NADPH.

The Kok Effect. While investigations of quantum efficiency using more defined systems frequently eliminate many of the variables which hamper studies on intact systems, they are usually restricted to measurements of short duration due to the instability of the preparation, and, of course, must contend with potential isolation artifacts. On the other hand, gas exchange measurements using intact systems are non-intrusive and offer the plant physiologist an *in situ* assay of the photochemical efficiency of the complete photosynthetic process. Nevertheless, one of the earliest controversies regarding quantum yields of intact systems has remained unresolved. In 1948, Kok reported an apparent anomaly in the response of photosynthesis to very low levels of radiation (7). In what is now known as the Kok effect, the typical linear response is complicated by a sudden change in the vicinity of the LCP so that the quantum yield appears to decrease abruptly as radiation increases. Kok observed the phenomenon first in *Chlorella*, and it has since been reported (and often discounted) in a range of species including several higher plants.

An understanding of the Kok effect is essential for measuring accurately the quantum yield of photosyn-

thesis. We recently reinvestigated the Kok effect and found the phenomenon to be present in attached leaves of sunflower plants (8). Curve a in Fig 1 shows the response of net photosynthesis to low flux densities of absorbed radiation when measured under normal atmospheric conditions. The two-slope relationship is virtually the same as that reported by Kok for *Chlorella* (7). The quantum yield (slope) below the LCP is substantially higher than that above the LCP. Why does the quantum efficiency change and which is the true quantum yield?

Several mechanisms have been suggested since Kok's original investigation. Emerson (9) and Heath (10) suggested that the Kok effect was an artifact caused by temporal changes in the rate of dark respiration during the measurements of photosynthesis. We demonstrated that this was not the case in our experiments in two ways. First, we determined that the rate of dark respiration was very stable from 2 to 10 hours after the end of a photoperiod and subsequently made measurements of photosynthesis at low photon flux densities during that period. Secondly, the rate of respiration in the dark was measured intermittently during the several hours required for determination of the quantum yield (inset of Fig 1), and was virtually constant.

Franck (11) speculated that the amount of energy required per molecule of photosynthetic O₂ evolved was less when respiration exceeds photosynthesis than above the LCP, perhaps because intermediates of oxidative metabolism could substitute for CO₂ as substrates for

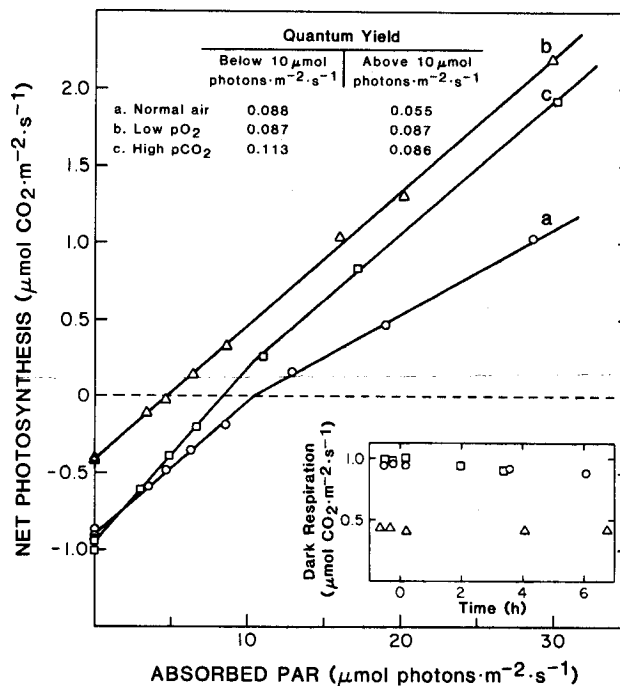


Fig 1 Net photosynthesis of attached sunflower leaves at limiting flux densities of absorbed photosynthetically active radiation (PAR). Absorbed radiation was determined by subtracting transmitted and reflected radiation from that incident at the leaf surface. Quantum yields for CO₂ uptake were calculated from the slope of the photosynthesis-flux density relationship. Respiration in the dark was checked for stability at the beginning and end of the photosynthesis measurements (inset). The air temperature was 20°C. Measurement conditions: a, 33 Pa pCO₂, 21 kPa pO₂; b, 33 Pa pCO₂, 1 kPa pO₂; c, 205 Pa pCO₂, 21 kPa pO₂.

photosynthetic reducing power. Consistent with the hypothesis is the fact that some photosynthetic reducing power is used for reactions other than RuBP carboxylation, such as nitrate reduction. However, when the rate of respiration was varied in attached sunflower leaves by varying the leaf temperature, we found that the flux density of absorbed radiation at which the Kok effect occurred was similar (approx $10 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) despite a wide range of light compensation points and rates of net photosynthesis. Thus, the occurrence of the Kok effect in close proximity to the LCP is merely coincidental.

Another school of thought has argued that the Kok effect in higher plants is due to a differential effect of low photon flux densities on photosynthesis and photorespiration. This suggestion arose from the absence of the Kok effect when photorespiratory CO_2 evolution was minimal, first, in plants with the C4 pathway of photosynthesis, and second, in plants with the C3 pathway exposed to low partial pressures of O_2 ($p\text{O}_2$) which suppress photorespiration (12, 13). When this possibility was investigated in sunflower (a C3 species), we also found that the Kok effect was absent at low $p\text{O}_2$ (curve b in Fig 1). The quantum yield above $10 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ increased to that observed at lower photon flux densities under ambient conditions. However, when photorespiration was inhibited by saturating partial pressures of CO_2 ($p\text{CO}_2$) instead of low $p\text{O}_2$ (curve c in Fig 1), the Kok effect was again evident. This result is not reconcilable with photorespiratory activity being involved.

How then can the Kok effect be explained? The high quantum yield of 0.113 which we observed below $10 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ at saturating $p\text{CO}_2$ suggests a quantum efficiency of near 100%. Since this is unlikely, it is probable that some response additional to photosynthesis was contributing to the rate of net CO_2 uptake in this range of photon flux densities. We investigated Kok's original hypothesis that the effect was due to a light-induced inhibition of dark respiration by measuring quantum yields at different stable rates of dark respiration induced by varying leaf carbohydrate status. [This approach avoided possible interpretational difficulties arising from effects of temperature on quantum yield (see Fig 2 below).] We observed that the magnitude of the Kok effect varied as the rate of dark respiration varied in a single leaf, and was minimized when dark respiration was minimized (Table 1).

Table 1. Apparent quantum yields for photosynthesis of attached sunflower leaves at photon flux densities below the Kok effect. Quantum yields were determined on the same leaf after various lengths of dark pretreatments to deplete leaf carbohydrate and, thereby, the rate of dark respiration.

Dark Respiration ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$)	Quantum yield below $10 \mu\text{mol photons m}^{-2}\text{s}^{-1}$
1.84	0.106
0.54	0.078
0.35	0.072

The implication of these results is that the Kok effect is attributable to a progressive inhibition of dark respiration up to a photon flux density of about $10 \mu\text{mol photons m}^{-2}\text{s}^{-1}$. The decrease in respiratory CO_2 evolution with increasing radiation results in a progressive increase in the rate of net CO_2 uptake, in addition to that resulting from increasing CO_2 fixation. Thus, the high apparent quantum yield which is measured at extremely low photon flux densities is attributable to the true quantum yield of photosynthesis plus the quantum yield of the partial suppression of dark respiration by light. It follows that at higher photon flux densities where the light-induced suppression of dark respiration is saturated, the slope of the relationship between net CO_2 uptake and absorbed radiation should indicate the true quantum yield of photosynthesis.

The absence of the Kok effect in sunflower at low $p\text{O}_2$ would seem to have resulted from the substantial decrease in the rate of dark respiration that occurred under these conditions (Fig 1), rather than from the concurrent suppression of photorespiration. The threshold for O_2 inhibition of dark respiration that we observed was considerably higher than that generally reported for intact leaves [see (8)]. The explanation for the apparent absence of the Kok effect in C4 plants in normal air remains open to investigation.

Quantum Yields of C3 and C4 Plants. We noted above the increase in the quantum yield of the C3 sunflower when photorespiration is suppressed at low $p\text{O}_2$ or high $p\text{CO}_2$. Ehleringer (14, 15) has investigated extensively the response of quantum yield to various photorespiratory conditions. From measurements on a wide range of species, including C3 and various subpathways of C4 metabolism, he showed that, despite small differences within each group, the quantum yields of C3 and C4 plants are similar at leaf temperatures of 25 to 30C despite the lack of photorespiration in the C4 species. Soon after the discovery of the C4 photosynthetic pathway, it was suggested that the maximum quantum yield might be less for C4 species than for C3 species since it can be calculated that 2 to 3 additional ATP/ CO_2 fixed are required (16). Apparently, at these temperatures, the lack of photorespiration in C4 species is offset by their higher energy requirement for CO_2 reduction. However, the temperature responses of the quantum yield in C3 and C4 species are quite different. The quantum yield of C3 species is greatest at low temperatures and least at high temperatures, whereas there is little response to temperature in the C4 species (Fig 2). This difference is probably due primarily to the greater temperature sensitivity of the oxygenase activity compared to the carboxylase activity of RuBP carboxylase-oxygenase (17). It has been argued that the lower quantum yield of the C4 pathway at lower temperatures is causally related to the predominance of C3 species in cooler habitats (18).

Quantum Yield and the Environment. Photosynthesis is inhibited by a range of unfavorable environmental conditions. Quantum yield measurements of intact leaves, as an *in situ* assay of chloroplast function, have proved extremely useful in separating stomatal and non-stomatal limitations under such conditions. For example,

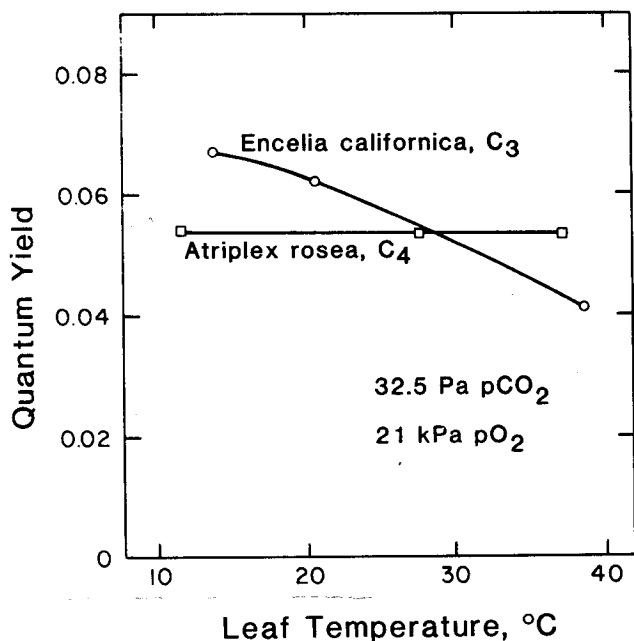


Fig 2 Typical response of the quantum yield for CO₂ uptake to temperature for leaves of C₃ and C₄ species in normal air (32.5 Pa pCO₂, 21 kPa pO₂). [Reproduced from (14), with permission.]

whereas for many years it was believed that the inhibition of photosynthesis by water deficits was largely due to stomatal closure, recent evidence, including measurements of quantum yield, suggests that loss in chloroplast activity can be the more limiting factor (19). Furthermore, in species where photosynthesis has been shown to acclimate to suboptimal environments, the sensitivity of the quantum yield to those environments has been similarly decreased (19, 20, 21). In addition to the sensitivity to water deficits, the quantum yield is also inhibited at both high (20) and low (22) temperatures, although, interestingly, inhibition at chilling temperatures appears to occur only when plants are exposed simultaneously to high photon flux densities (22). In contrast, the loss of quantum yield at low water potentials is independent of photon flux density and, thus, appears to be a direct effect of dehydration on chloroplast function (23).

While the results of these experiments clearly show that lesions in the photosynthetic apparatus occur in extreme environments, and that there is a plasticity among and within species in the sensitivity of the photosynthetic apparatus to environmental conditions, it is important to recognize the limitations to interpreting quantum yield measurements. As we pointed out earlier, the quantum yield for photosynthesis relates two widely

separated partial reactions of photosynthesis, the absorption of light and the reduction of CO₂. The quantum yield is measured under light limiting conditions, where, by definition, the limitations to photosynthesis are clearly different from those under light saturating conditions. Lesions which occur energetically upstream of the rate limitation at high photon flux densities will not be apparent at light saturation, but, nevertheless, may be reflected directly in the quantum yield. Furthermore, lesions might occur which limit the rate of light saturated photosynthesis but which are not severe enough to be observed at the low rates of photosynthesis which occur during a quantum yield determination. The above mentioned interactive effect of chilling and high light on the quantum yield provides a good example of the latter possibility. While chilling in darkness does not reduce the quantum yield in tomato, the rate of light saturated photosynthesis is greatly reduced (24). Consequently, the lack of an inhibition of quantum yield does not necessarily reflect robust chloroplasts. Nevertheless, assays of quantum yield provide direct evidence of impaired chloroplast photochemistry, and, thereby, are an elegant probe of the effects of environmental stress on chloroplast function.

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