Kok Effect and the Quantum Yield of Photosynthesis[1]

LIGHT PARTIALLY INHIBITS DARK RESPIRATION

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ABSTRACT

The linear response of photosynthesis to light at low photon flux densities is known to change abruptly in the vicinity of the light compensation point so that the quantum yield seems to decrease as radiation increases. We studied this 'Kok effect' in attached sunflower (*Helianthus annuus* L. cv IS894) leaves using gas exchange techniques. The effect was present even though respiration was constant in the dark. It was observed at a similar photon flux density (7 to 11 micromole photons per square meter per second absorbed photosynthetically active radiation) despite a wide range of light compensation points as well as rates of photosynthesis. The effect was not apparent when photorespiration was inhibited at low pO₂ (1 kilopascal), but this result was complicated because dark respiration was quite O₂-sensitive and was partially suppressed under these conditions. The Kok effect was observed at saturating pCO₂ and, therefore, could not be explained by a change in photorespiration. Instead, the magnitude of the effect varied as dark respiration varied in a single leaf, and was minimized when dark respiration was minimized, indicating that a partial suppression of dark respiration by light is responsible. Quantum yields measured at photon flux densities between 0 and 7 to 11 micromole photons per square meter per second, therefore, represent the combined yields of photosynthesis and of the suppression of a component of dark respiration by light. This leads to an overestimate of the quantum yield of photosynthesis. In view of these results, quantum yields of photosynthesis must be measured (a) when respiration is constant in the dark, and (b) when dark respiration has been inhibited either at low pO₂ to eliminate most of the light-induced suppression of dark respiration or at photon flux densities above that required to saturate the light-induced suppression of dark respiration. Significant errors in quantum yields of photosynthesis can result in leaves exhibiting this respiratory behavior if these principles are not followed.

The response of photosynthesis to light is of fundamental importance for understanding the photochemical efficiency of the process. Typically, the response is linear at low levels of radiation and saturates at high levels. However, the linear response is often complicated by a sudden change in the vicinity of the light compensation point so that the efficiency, *i.e.* the quantum yield, seems to decrease abruptly as radiation increases. This change, first observed by Kok (19) in *Chlorella* and termed the 'Kok effect,' has been reported for several species of algae

(12) and higher plants (8, 9, 17, 18, 33).

An understanding of the Kok effect is essential for measuring the quantum yield of photosynthesis. However, interpretation of the effect is complicated by the difficulties of quantifying photosynthesis at low radiation levels where the rate of 'dark' respiration can have a large effect on net CO₂ or O₂ exchange. Despite numerous physiological and biochemical studies, the extent to which dark respiratory activity continues during illumination remains unresolved (15). Thus, the original suggestion that the Kok effect is caused by a progressive, light-induced inhibition of dark respiration (19–21) has not been verified. More recently, studies with higher plants suggested that there may be a decrease in the proportion of photorespiration to photosynthesis below a threshold level of radiation (8, 17, 18). This suggestion arose from the absence of the Kok effect when photorespiratory CO₂ evolution was minimal, first, in plants with the C₄ pathway of photosynthesis, and second, in plants with the C₃ pathway of photosynthesis exposed to low partial pressures of O₂. However, the observation by Van der Veen (33) of an apparent Kok effect in tobacco exposed to 3600 Pa (36,000 μbar) CO₂, where photorespiratory activity would almost certainly be negligible (due to saturation of the carboxylase activity of RuBP carboxylase-oxygenase), indicates that this suggestion may be premature.

The interpretation of these relationships is further complicated by temporal changes in the rate of dark respiration. These changes caused Emerson (11) and Heath (16) to question the reality of the Kok effect. Pretreatment and measurement conditions that ensure a stable rate of respiration in the dark seem essential to resolve the question of whether an inhibition of dark respiration or a change in photorespiration is responsible for the Kok effect. These precautions were taken by Kok (20), but appear to have been applied less rigorously in studies of higher plants.

In this report, we studied the Kok effect in attached sunflower leaves while checking that respiration was constant in the dark, and we varied conditions known to affect photorespiration and dark respiration during the measurements of photosynthesis. We show that the Kok effect is attributable to a change in dark respiration caused by light, as originally proposed (19–21), and not to a change in photorespiration. Significant errors in measurements of quantum yield can result from these effects on dark respiration.

MATERIALS AND METHODS

Three- to 4-week-old sunflower (*Helianthus annuus* L. cv IS894) plants were grown from seed in a soil-peat-perlite mix (2:1:1) in a controlled environment (day/night temperature, 30/20 ± 1°C; day/night RH, 85/95 ± 5%; photon flux density, 900 μmol photons m⁻² s⁻¹ supplied by cool-white fluorescent lamps; photoperiod, 14 h). Plants were watered twice daily, and nutrients

SHARP *ET AL.*

were supplied twice weekly.

Gas exchange measurements were made with a closed compensating gas analysis system described by Martin *et al.* (23). All measurements were made on single, attached leaves that were almost fully expanded. Rates of respiration in the dark and of net photosynthesis at low photon flux densities were determined from the times required for the leaf to change the partial pressure of CO_2 (pCO_2) inside the assimilation chamber by 2 Pa (20 μbar). The partial pressure of O_2 (pO_2) was monitored with a YSI 5331 O_2 probe (Yellow Springs Instrument Co., Inc., Yellow Springs, OH)[3] suspended in the chamber air. The pCO_2 and pO_2 were varied for different experiments. The internal pCO_2 (int pCO_2) was computed from measurements of net photosynthesis (P), transpiration (T), water vapor concentration difference between the leaf ($[H_2O]_{int}$) and ambient atmosphere ($[H_2O]_{ext}$), and the external pCO_2 (ext pCO_2):

$$\text{int } pCO_2 = \text{ext } pCO_2 - (P/T)\frac{D_{H_2O}}{D_{CO_2}}([H_2O]_{int} - [H_2O]_{ext})$$

where D_{H_2O} and D_{CO_2} are the diffusion coefficients for water vapor and CO_2 in still air. The validity of this expression has recently been experimentally confirmed (31).

All photosynthesis measurements were made under white light. The incident photon flux density was varied with neutral density filters which established a radiation field that was uniform to ±5% across the leaf. Absorbed radiation was determined for each leaf by subtracting transmitted and reflected radiation from that incident at the leaf surface. Radiation measurements were made with a LI-190S Quantum Sensor (Lambda Instruments, Lincoln, NE). Reflected radiation was measured 1 cm above the leaf surface at an angle of 45° from the perpendicular and transmitted radiation was measured normal to the plane immediately under the leaf. These measurements were made in several positions for each leaf. At the beginning and end of each experiment the leaf image was traced for the gravimetric determination of leaf area.

Measurements of net photosynthesis at low photon flux densities are reported only where the rate of respiration in the dark was similar at the beginning and end of the experiment; in most cases, an intermediate check of the stability of the rate of respiration in the dark was also made. Unless otherwise stated, measurements were made at an air temperature of 20°C and near saturating humidity. The variation in leaf temperature with photon flux density was less than 0.1°C. At each photon flux density, sufficient time was allowed (up to 45 min) to establish a constant photosynthetic rate. Quantum yields (mol $CO_2 \cdot$ mol photons absorbed PAR^{-1}) were calculated from the slope of the linear relation between the rate of net photosynthesis and absorbed radiation. Results are reported for replicated experiments.

Leaf water potential was determined by isopiestic thermocouple psychrometry as previously described (5, 7).

RESULTS

Time- and O_2-Dependence of Respiration in the Dark. We observed that dark respiration often varied substantially in sunflower leaves and, because it was essential to make photosynthesis measurements when the rate of respiration was constant in the dark, we investigated the source of variability. Figure 1 shows that the rate of CO_2 efflux in the dark by an attached sunflower leaf decreased rapidly during the first 2 h following a 14-h photoperiod. Afterward, the rate of CO_2 efflux remained rela-

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[3] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture or the University of Illinois and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
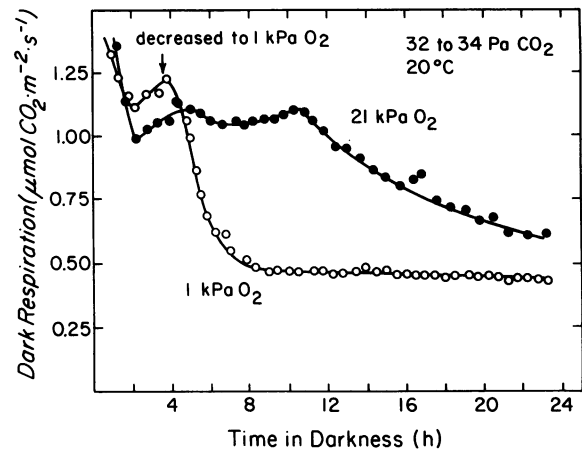

FIG. 1. Time course of dark respiration in attached sunflower leaves following a 14-h photoperiod. The pO_2 was either maintained at 21 kPa (●) or decreased to 1 kPa after 4 h (O). The pCO_2 was maintained between 32 and 34 Pa, and the air temperature was 20°C.

tively stable for approximately 9 h and then slowly decreased. This behavior was invariably observed when the leaf was maintained at a pO_2 of 21 kPa (21%). In contrast, lowering the pO_2 to 1 kPa (1%) 4 h after the 14-h photoperiod resulted in a marked decrease in the rate of CO_2 efflux to a low stable rate (Fig. 1). The depressive effect of low pO_2 on the rate of dark CO_2 efflux was completely reversible upon reexposure to 21 kPa pO_2 (Fig. 2A), and had a threshold of approximately 8 kPa pO_2 (Fig. 2B). At both 21 kPa and 1 kPa pO_2, intermittent irradiation at low photon flux densities (below 60 μmol photons m² s⁻¹ PAR) did not disturb the rate of respiration in the dark.

The Kok Effect. When the leaf was irradiated at several low photon flux densities while respiration in the dark was stable, photosynthesis was linearly related to the flux density of absorbed PAR, but there was a break in the relationship at the light compensation point at approximately 10 μmol photons m⁻² s⁻¹ (Fig. 3A). This Kok effect occurred despite the stable rate of respiration in the dark throughout the experiment (Fig. 3A, inset). As a result, the quantum yield of CO_2 uptake appeared to decrease from 0.088 to 0.055 above the light compensation point. Below the light compensation point the data extrapolate to the steady state rate of respiration in the dark. Similar experiments established that the observed relation between photosynthesis and absorbed PAR was independent of the order in which the photosynthesis measurements were made. Moreover, the stability of the rate of respiration in the dark indicates that the Kok effect was not merely an artifact of temporal changes in dark respiration, although the possible involvement of light-induced changes in dark respiration cannot be excluded. The quantum yield of 0.055 above the light compensation point is comparable to quantum yields measured over a comparatively wide range of higher photon flux densities in C_3 species at the same atmospheric and radiation conditions (10). In contrast, the quantum yield of 0.088 measured below the light compensation point compares with quantum yields for sunflower and other C_3 species under conditions where photorespiratory CO_2 evolution was minimal (10, 24). The data therefore suggested that photorespiratory activity might be inhibited below the light compensation point. To test this possibility, we repeated the measurements of photosynthesis at an air temperature of 31.5°C (Fig. 3B). Dark respiration was increased at the increased temperature, and net photosynthesis was depressed accordingly (cf. Figs. 3A and 3B). The Kok effect was observed at a photon flux density that was well below the light compensation point under these conditions (Fig. 3B). At the increased temperature, fluctuations in dark respira-
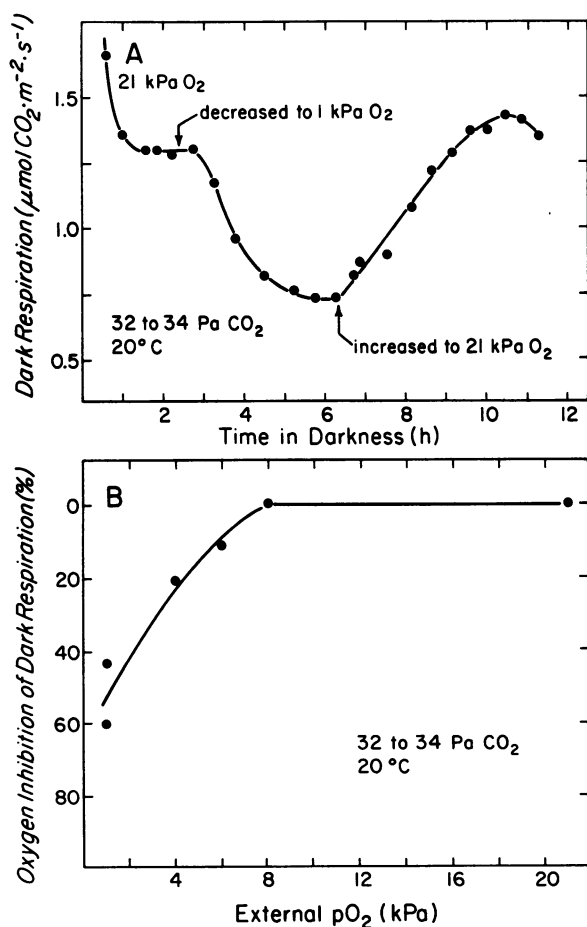
FIG. 2. The O_2 dependence of dark respiration in attached sunflower leaves. Following a 14-h photoperiod, leaves were maintained at 21 kPa pO_2 until dark respiration was stable. A range of low pO_2 were then imposed. After dark respiration had stabilized, the pO_2 was returned to 21 kPa. The pCO_2 was maintained between 32 and 34 Pa, and the air temperature was 20°C. A, Time course of dark respiration in a leaf exposed to 1 kPa pO_2; B, the inhibition of dark respiration at low pO_2 as a percentage of the preceding rate at 21 kPa pO_2. Each datum is from a different leaf. In each case, the inhibition of dark respiration at low pO_2 was fully reversible.
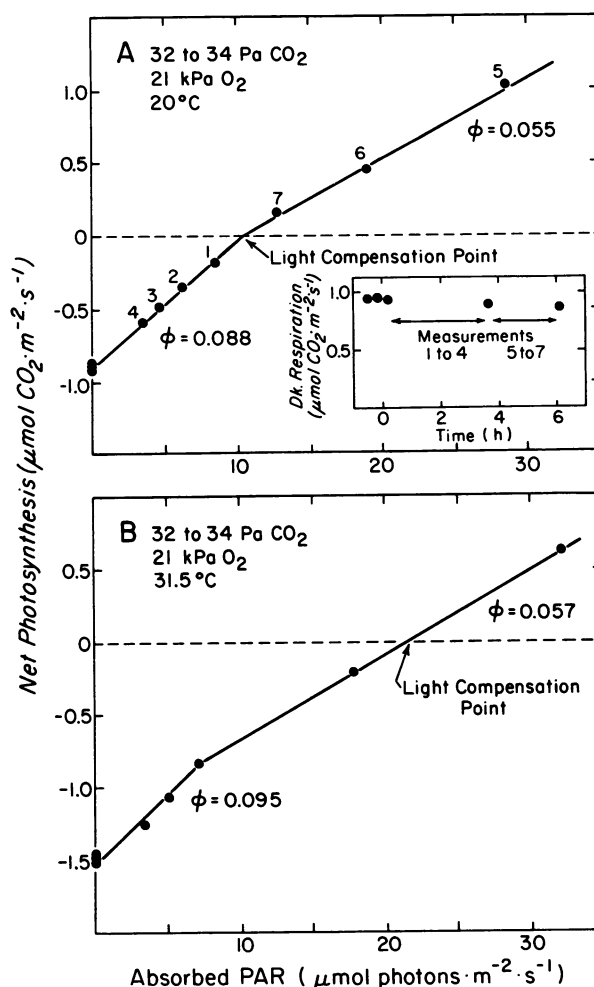

FIG. 3. Net photosynthesis at limiting flux densities of absorbed PAR. A, The numbers against the data indicate the order in which the measurements were made. Respiration in the dark was checked for stability at the middle and end of the photosynthesis measurements (A, inset). Quantum yields (ϕ) are represented by the slope of the photosynthesis-flux density relationship. Measurement conditions: A, 32 to 34 Pa pCO_2, 21 kPa pO_2, 20°C air temperature; B, 32 to 34 Pa pCO_2, 21 kPa pO_2, 31.5°C air temperature.

tion were more significant than at 20°C and, therefore, fewer measurements of photosynthesis were made between checks of the rate of respiration in the dark. All of these checks are shown at the Y intercept of Figure 3B, and show that respiration in the dark was virtually constant throughout the experiment. It is clear that the Kok effect was not associated with the light compensation point but, rather, occurred at an independent, low photon flux density.

Influence of Photorespiration on the Kok Effect. We investigated the possibility that a change in photorespiration was responsible for the Kok effect by examining the influence of conditions where photorespiratory CO_2 evolution was minimal. Rates of net photosynthesis were measured as in Figure 3A except that the pO_2 was decreased to 1 kPa, where photorespiration is negligible because of the suppression of the oxygenase activity of RuBP carboxylase-oxygenase. Figure 4A shows that at 1 kPa pO_2 the Kok effect was not present. There was no evidence of a break in the linear relation between net photosynthesis and absorbed PAR, and the measurements of photosynthesis extrapolate to the steady-state rate of respiration in the dark. The quantum yield was 0.087, which compares with 0.086

when the photon flux density was very low at 21 kPa pO_2 (Fig. 3A). These data, therefore, suggest the loss of photorespiratory activity at very low photon flux densities. However, this experiment was complicated by a depression of dark respiration (Fig. 1) that also occurred when photorespiration was inhibited at 1 kPa pO_2. Thus, dark respiration was much lower than is usual for these leaves (compare dark respiration rates in Figs. 1–3 with those in Fig. 4A).

We also tested the effect of low rates of photorespiration when photosynthesis was CO_2 saturated, which had the advantage that photorespiration could be inhibited without affecting dark respiration. Photosynthesis in sunflower was CO_2 saturated above an internal pCO_2 of 150 Pa (equivalent to an external pCO_2 of 204 Pa), as shown from the lack of further increase of photosynthesis when the internal pCO_2 was raised to 200 Pa (Fig. 4B, inset). Furthermore, at an internal pCO_2 above 150 Pa, decreasing the pO_2 from 21 kPa to between 1 and 2 kPa also caused no increase in net photosynthesis, as expected if photorespiration was inhibited by the high pCO_2. This test of CO_2 saturation (Fig. 4B, inset) was made at a comparatively high photon flux density (630 μmol photons m^{-2} s^{-1}) to ensure that the decrease in the
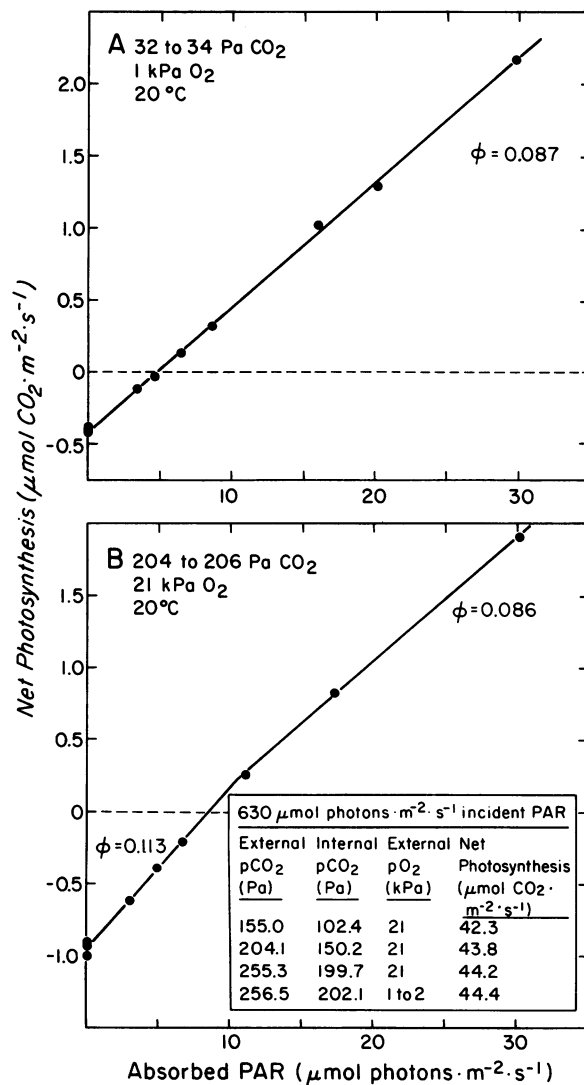
FIG. 4. Net photosynthesis at limiting flux densities of absorbed PAR. Respiration in the dark was checked for stability at the middle and end of the photosynthesis measurements. Quantum yields (ϕ) are represented by the slope of the photosynthesis-flux density relationship. Measurement conditions: A, 32 to 34 Pa pCO_2, 1 kPa pO_2, 20°C air temperature; B, 204 to 206 Pa pCO_2, 21 kPa pO_2, 20°C air temperature. The inset in B shows that photosynthesis was CO_2 saturated above an internal pCO_2 of 150 Pa (see text).

rate of dark respiration at low pO_2 (Fig. 1) was of little consequence to the rate of net photosynthesis.

At low photon flux densities, internal pCO_2 differed only slightly from external pCO_2, as expected (data not shown). Rates of net photosynthesis were therefore measured as in Figure 3A except that photorespiration was inhibited by maintaining a saturating external pCO_2 of 204 to 206 Pa. Figure 4B shows that the Kok effect was clearly apparent under these conditions. An abrupt increase in quantum yield occurred below approximately 10 μmol photons m^{-2} s^{-1} absorbed PAR. Thus, the loss of photorespiratory activity at low photon flux densities cannot account for the Kok effect. Moreover, the similar magnitude of the Kok effect under photorespiratory conditions (quantum yield changed 0.033, Fig. 3A) and under conditions of minimal photorespiratory CO_2 evolution (quantum yield changed 0.027, Fig. 4B) is indicative of the constancy of the phenomenon causing the effect regardless of photorespiration. This suggests that the

oxygenase activity of RuBP carboxylase-oxygenase and the associated photorespiratory activity has no involvement in the Kok effect.

Influence of Dark Respiration on the Kok Effect. It is noteworthy that at saturating pCO_2 where photorespiratory CO_2 evolution was minimal (Fig. 4B), the quantum yield increased from 0.086 to 0.113 below 10 μmol photons m^{-2} s^{-1} absorbed PAR. The higher value of 0.113 is higher than generally observed and approaches the theoretical maximum for photosynthesis (0.125). It seemed possible that this high value reflected some additional effect that caused an apparent quantum yield higher than the true quantum yield of CO_2 uptake. Because the Kok effect was not present at low pO_2 where dark respiration was substantially inhibited (Fig. 4A), we investigated the possibility that a progressive, light-induced depression of dark respiration between 0 and 10 μmol photons m^{-2} s^{-1} absorbed PAR was responsible for an artifactually high quantum yield in this range of photon flux densities. If this is correct, comparatively low rates of dark respiration should, in absolute terms, be subject to less inhibition by low photon flux densities and, accordingly, be associated with decreased quantum yields. To test this possibility, we measured the quantum yield below 10 μmol photons m^{-2} s^{-1} absorbed PAR in one leaf displaying three different rates of respiration in the dark at 21 kPa pO_2. These stable rates were obtained first during the elevated phase of dark respiration shown in Figure 1, then after 24 h when the intermediate rate of dark respiration had been attained (Fig. 1), and again after 48 h following a further, slow decrease in dark respiration. A positive correlation between the quantum yield and the rate of dark respiration was obtained (Fig. 5A). At a steady rate of respiration in the dark of 0.92 μmol CO_2 m^{-2} s^{-1} the quantum yield was 0.106, whereas at rates of respiration in the dark of 0.53 and 0.35 μmol CO_2 m^{-2} s^{-1}, quantum yields were 0.078 and 0.072, respectively. Using a similar experimental design, the quantum yield above 10 μmol photons m^{-2} s^{-1} absorbed PAR was found to be independent of the steady rate of respiration in the dark (Fig. 5B), which would be expected if the rate of dark respiratory activity was constant at higher photon flux densities.

The Kok Effect at Low Leaf Water Potential. It has been reported that, in sunflower, low leaf water potentials inhibit the quantum yield of photosynthesis (24) and the rate of dark respiration (6). Therefore, it was possible to test whether the Kok effect is changed when photosynthetic and dark respiratory activities are altered by conditions completely different from those described in Figures 1 to 5. Figure 6 shows that at a leaf water potential of −2.5 MPa, the Kok effect was clearly apparent at a flux density of absorbed PAR similar to that at high leaf water potential (cf. Fig. 3A). A large inhibition of quantum yield occurred at photon flux densities above those of the Kok effect. The rate of dark respiration was also low in this leaf.

DISCUSSION

These results show that the Kok effect exists in higher plants despite previous assertions that it is an artifactual result of temporal changes in the rate of dark respiration during measurements of photosynthesis (11, 16). The effect was present even though pretreatment and measurement conditions minimized variability in the rate of dark respiration in attached sunflower leaves. It occurs at very low photon flux densities that, in this and in numerous previous studies (9, 17, 18, 21, 33), are at or in close proximity to the light compensation point. However, our data with different temperatures show that the occurrence of the Kok effect at the light compensation point is merely coincidental. Kok also made this observation in his early studies with *Chlorella* (20). Therefore, explanations of the Kok effect cannot be based on the change from net CO_2 efflux to net CO_2 influx nor on changes in internal pCO_2 or the $CO_2:O_2$ ratio that occur around
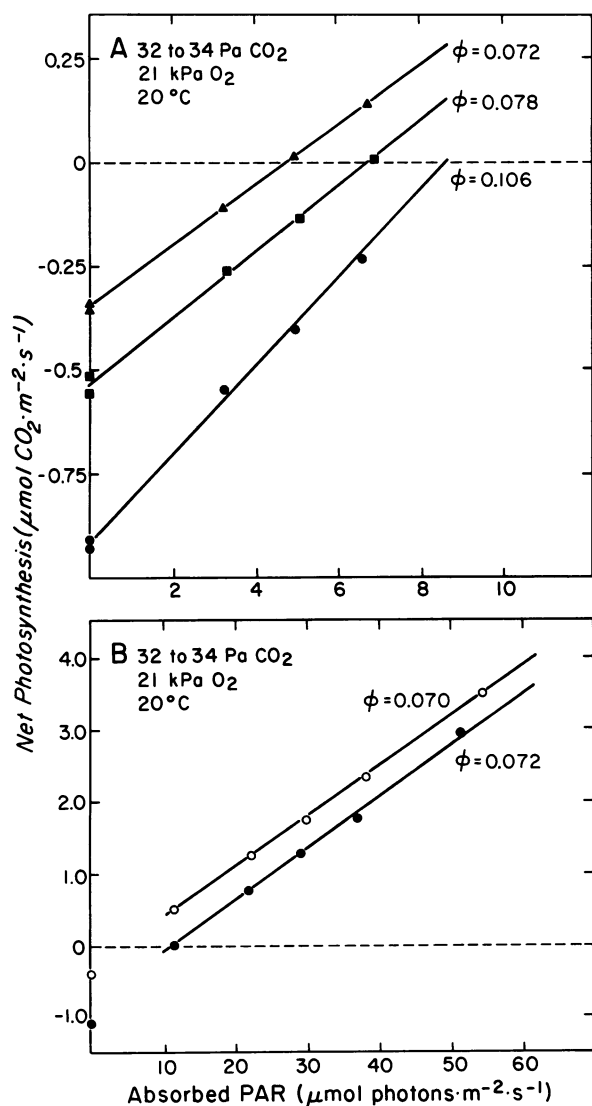
FIG. 5. Net photosynthesis at limiting flux densities of absorbed PAR at different rates of dark respiration. The data in A and B are each from a single leaf. A, Flux densities below 10 μmol photons m^{-2} s^{-1}; B, flux densities above 10 μmol photons m^{-2} s^{-1}. Respiration in the dark was checked for stability at the end of each series of photosynthesis measurements. Quantum yields (ϕ) are represented by the slope of the photosynthesis-flux density relationship. Measurement conditions: 32 to 34 Pa pCO_2, 21 kPa pO_2, 20°C air temperature.

the light compensation point.

Related reports (8, 17, 18) involving photorespiration also seem unable to account for the Kok effect. These authors observed that the effect was apparently absent both in C$_4$ plants and in C$_3$ plants at low pO_2. At present, the explanation for the absence of the Kok effect in C$_4$ plants remains open to conjecture. Our data verified that in the C$_3$ sunflower, the Kok effect was not apparent at low pO_2 (1 kPa). However, this result could have been caused by the combined effects of the inhibition of photorespiration and the suppression of dark respiration at low pO_2. That is, if dark respiration had not been suppressed by the low pO_2 (Figs. 1 and 2), the Kok effect might still have been observed. When we conducted a similar experiment but using saturating pCO_2 instead of low pO_2, we could inhibit photorespiration but not dark respiration and the Kok effect was observed. Thus, changes in the oxygenase activity of RuBP carboxylase-oxygenase and the associated photorespiratory activity cannot account for
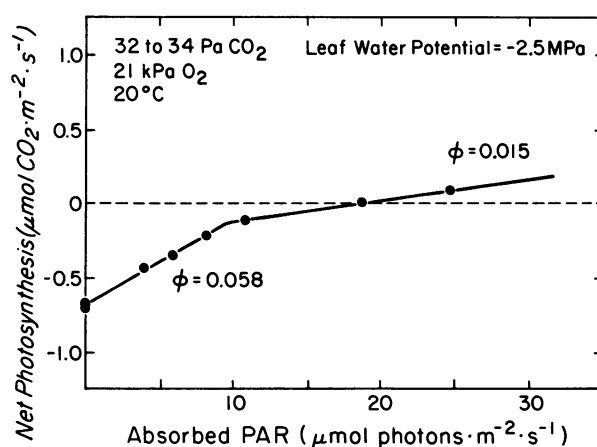

FIG. 6. Net photosynthesis at limiting flux densities of absorbed PAR at a leaf water potential of −2.5 MPa. The low water potential was imposed by withholding water from the soil for 3 d. Respiration in the dark was checked for stability at the middle and end of the photosynthesis measurements. Leaf water potential was determined immediately following the final respiration measurement. Quantum yields (ϕ) are represented by the slope of the photosynthesis-flux density relationship. Measurement conditions: 32 to 34 Pa pCO_2, 21 kPa pO_2, 20°C air temperature.

the Kok effect.

We observed instead that the flux density of absorbed PAR at which the Kok effect occurred was similar (7 to 11 μmol photons m^{-2} s^{-1}) despite a wide range of light compensation points as well as rates of photosynthesis. The magnitude of the Kok effect varied as dark respiratory activity varied in a single leaf, and was minimized when dark respiration was minimized. Thus, our data can only be explained by a progressive inhibition of a portion of dark respiratory activity between 0 and approximately 10 μmol photons m^{-2} s^{-1} of absorbed PAR. This, concomitantly with photosynthesis, gives rise to the Kok effect. The high apparent quantum yield between 0 and 10 μmol photons m^{-2} s^{-1} of absorbed PAR is then attributable to the quantum yield of photosynthesis plus the quantum yield of the partial suppression of dark respiration by light. The apparent loss of the Kok effect in rice leaves when dark respiration was greatly decreased at low temperature (18) supports our interpretation that dark respiratory activity is involved.

We wish to emphasize that the design of these experiments allowed the comparison of quantum yields measured on the same leaf at different dark respiration rates but at the same temperature, and thereby avoided possible interpretational difficulties arising from effects of temperature on quantum yield (10).

The suppression of dark respiration by light is likely to be partial rather than complete. Photosynthesis at low PAR (to approximately 10 μmol photons m^{-2} s^{-1}) always extrapolated to the rate of respiration measured in the dark (Figs. 3–6). Applying the same principle to photosynthesis measured at higher PAR, extrapolation to 0 PAR should give the rate of dark respiration in the light after light-induced suppression. This rate was 0.29 to 0.42 μmol CO_2 m^{-2} s^{-1} less than the directly measured dark rates, which varied between 0.69 and 1.10 μmol CO_2 m^{-2} s^{-1} in leaves that had not been exposed to prolonged dark or low pO_2.

A light-induced suppression of dark respiration was the original explanation of the Kok effect suggested by Kok in studies with *Chlorella* (19–21). Indeed, a correlation similar to ours between the quantum yield at very low photon flux densities and the rate of dark respiration has also been reported (19).

At high photon flux densities the quantum yield was shown to be independent of the steady rate of respiration in the dark. Assuming our interpretation of the data is correct, this indicates

that saturation of the light-induced suppression of dark respiratory activity occurs above approximately 10 μmol photons m^{-2} s^{-1} of absorbed PAR. When compared on the same leaf, the light-saturated suppression of dark respiration was generally less than the low pO_2 suppression of dark respiration. It is therefore possible that at low pO_2 the Kok effect was absent because the component of dark respiration that is suppressed during illumination was encompassed by the comparatively large component of 'O_2-sensitive' dark respiration. Interestingly, in the previous reports of the absence of the Kok effect at low pO_2 (8, 18), the corresponding rate of dark respiration was also lower than that observed under atmospheric conditions, perhaps indicating that similar respiratory O_2-sensitivity existed in the experimental material.

The O_2-sensitivity of dark respiration that we observed indicates a substantial component of respiratory activity with a low O_2-affinity. The Cyt path of plant respiration has a high affinity for O_2 and, accordingly, decreases in pO_2 from atmospheric to 1 kPa or less have generally had little or no effect on the rate of dark respiration (4, 13, 14, 29). By contrast, in sunflower leaves a progressive and reversible inhibition of dark respiratory activity was evident below a threshold pO_2 of approximately 8 kPa (Fig. 2). However, it should be noted that the O_2 inhibition of respiration occurred over a period of hours, whereas in some previous studies where decreases in pO_2 have reportedly had no influence on dark respiration, much shorter periods at low pO_2 were used (*e.g.* 4).

It is noteworthy that in a number of studies where activity of the cyanide-resistant, alternative path of respiration has been observed, the respiratory affinity for O_2 was considerably lower than that for Cyt oxidase *in situ* (22, 32). Indeed, Azcón-Bieto *et al.* (3) have recently reported that leaves of wheat which exhibited an elevated rate of dark respiration similar to that in sunflower following a period at high photon flux density showed a large cyanide-resistant component of respiration. However, in contrast with sunflower, it was reported that decreasing the pO_2 to 3 kPa had no influence on dark respiratory activity (2).

The evidence that the Kok effect is indicative of a light-induced partial suppression of dark respiration is in accord with studies suggesting that pathways of dark respiratory metabolism continue to operate in illuminated leaves, albeit at substantially decreased rates (1, 2, 15, 25, 26). While continued dark respiration in the light is of clear significance to the growth of plants (34), the implications for the estimation of gross photosynthesis by the correction of net photosynthetic rates for the loss of CO_2 by respiratory activity have received little attention. Whereas at high rates of photosynthesis the required correction for dark respiration may be of little consequence, under conditions of low photosynthetic activity the converse is true. The results of this study reinforce the assertion of Radmer and Kok (30) that particular caution is required when quantum yields are estimated from the linear relation of photosynthesis and absorbed radiation determined of necessity at low photon flux densities and at correspondingly low rates of photosynthesis. Quantum yields are frequently determined from net photosynthesis measurements without correcting for dark respiration, with the underlying assumption that the rate of dark respiratory activity is the same at each photon flux density. Our data show that where the Kok effect is present, this procedure is clearly not applicable at very low flux densities because the quantum yield represents the combined yields of photosynthesis and of the suppression of dark respiration. Furthermore, where the rate of dark respiration after illumination is substantially different from that before illumination, the convention has been to correct measurements of photosynthesis for the drift in dark respiration by interpolation between measurements of respiration in the dark. However, if dark respiration is partially suppressed by illumination, such

corrections will be in error because the magnitude of the drift in respiration during the measurements of photosynthesis will be overestimated.

We know of no method for adequately correcting these measurements for dark respiration. Several investigators have measured quantum yields at photon flux densities that were high enough to avoid most of the problems of not correcting for dark respiration (*e.g.* 10, 27, 28). However, under conditions of low photosynthetic activity, for example at low leaf water potentials (Fig. 6), changes in dark respiration will have a large effect on measurements of photosynthesis. Therefore, the only way to obtain accurate quantum yields in species displaying the Kok effect is to make measurements only when respiration in the dark is constant and (a) at low pO_2 where the Kok effect can be shown to be absent, or (b) at photon flux densities above that required to saturate the light-induced suppression of dark respiration.

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