Oxygen Stimulation of Apparent Photosynthesis in Flaveria linearis

Received for publication December 26, 1985

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ABSTRACT

A plant was found in the C3-C4 intermediate species, Flaveria linearis, in which apparent photosynthesis is stimulated by atmospheric O2 concentrations. A survey of 44 selfed progeny of the plant showed that the O2 stimulation of apparent photosynthesis was passed on to the progeny. When leaves equilibrated at 210 milliliters per liter O2 were transferred to 20 milliliters per liter O2; apparent photosynthesis was initially stimulated, but gradually declined so that at 30 to 40 minutes the rate was only about 80 to 85% of that at 210 milliliters per liter O2. Switching from 20 to 210 milliliters per liter caused the opposite transition in apparent photosynthesis. All other plants of F. linearis reached steady rates within 5 minutes after switching O2 that were 20 to 24% lower in 210 than in 20 milliliters per liter O2. At low intercellular CO2 concentrations and low irradiances, O2 inhibition of apparent photosynthesis of the aberrant plant was similar to that in normal plants, but at an irradiance of 2 millimoles quanta per square meter per second and near 300 micromoles per liter CO2 apparent photosynthesis was consistently higher at 210 than at 20 milliliters per liter O2. In morphology and leaf anatomy, the aberrant plant is like the normal plants in F. linearis. The stimulation of apparent photosynthesis at air levels of O2 in the aberrant plant is similar to other literature reports on observations with C5 plants at high CO2 concentrations, high irradiance and/or low temperatures, and may be related to limitation of photosynthesis by triose phosphate utilization.

Inhibition of AP2 by O2 is a well documented phenomenon in C5 plants and results from competition between O2 and CO2 for RuBP during CO2 assimilation plus the oxidation of glycolate to yield CO2 in the photosynthetic cycle (6). The extent of inhibition of AP by atmospheric O2 concentration is about 30% in C5 species when tested near atmospheric levels of CO2 (2). Some species classified as C5-C4 intermediates are less sensitive, their AP being inhibited by about 20% at atmospheric levels of O2 (3, 11, 14). Flaveria linearis is one of the C5-C4 species found to have lower O2 inhibition than C3 species (14).

Because CO2 and O2 compete for reaction with RuBP, AP of leaves of C5 species are generally O2-insensitive at high CO2 concentrations (1, 5, 18), but in some cases AP has been slightly higher at 210 ml L-1 compared to 20 ml L-1 at lower O2 levels (5, 8, 12, 15, 18, 20, 21). It has been suggested that O2 is required to sustain a high rate of photophosphorylation to support AP at high CO2 concentrations (5). Sharkey (17, 18) recently postulated that O2-insensitivity of AP may result from AP being limited by TPU. If AP at 210 ml L-1 O2 is limited by utilization of triose phosphates rather than by substrate levels (RuBP or CO2) then it is predicted that reducing O2 around the leaf would not increase AP. According to Sharkey (17, 18), shortage of Pi may be involved in the limitation because of accumulation of phosphate in carbon cycle intermediates. It has been observed that when Pi is sequestered by infiltrating mannose into spinach leaf discs O2 sensitivity of AP is lost (10). The shortage of Pi may be exacerbated by low O2 since under conditions of O2-insensitivity of AP, lowering O2 concentration has been found to raise the levels of RuBP and 3-P-glyceraldehyde (TD Sharkey, personal communication). Therefore, under conditions of TPU limitation, O2 may have no effect or may actually stimulate AP because less Pi is tied up in phosphorylated intermediates and phosphoglycolate produced by reaction of O2 and RuBP is also a source of Pi.

O2-insensitivity or stimulation of AP has been observed only at low temperatures (8, 12), at CO2 levels above atmospheric (12, 17, 18, 20, 21), or at concentrations near atmospheric in plants under stress (18). In this paper we report the O2 response of AP in a plant of the species, F. linearis, in which AP is stimulated by 210 ml L-1 O2 at atmospheric CO2 concentrations, and under nonstress growth conditions.

MATERIALS AND METHODS

Plant Material. Seeds of Flaveria linearis were kindly supplied by Dr. M. S. B. Ku, Washington State University. When several plants grown from these seed were tested for O2 inhibition of AP at 320 μl L-1 CO2 all exhibited about 20% inhibition by 210 ml L-1 O2 compared to 20 ml L-1, except one plant designated 8-4-9, in which AP was either O2-insensitive or was lower at 20 ml L-1 than 210 ml L-1 O2. This plant was propagated vegetatively and grown in 3-L pots filled with a 1:1:1 (by volume) mixture of peat:soil:perlite. Other plants from the same seed lot were handled in the same way. In addition, for some experiments plants of the C4 species Flaveria trinervia (14) and the C5 species Panicum boliviense (4) were grown under the same conditions for comparison to F. linearis. Plants were grown in a greenhouse in which temperatures ranged from a maximum of 30 to 35°C during the d to a minimum of about 25°C at night. They were grown during summer when maximum irradiance at midday was 1.5 to 2.2 mmol quanta m-2 s-1 PAR. Plants were fertilized twice weekly with full strength Hoagland solution.

Gas Exchange Measurements. Measurements of AP and transpiration were made in acrylic plastic chambers 13 cm long, 7 cm wide, and 7.5 cm deep. The chambers had a removable top which was held in place by bolts and wing nuts. Soft closed-cell plastic gaskets provided an effective seal when leaves were enclosed. The chamber was separated into two compartments by

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1 Supported by state and Hatch funds allocated to the Georgia Agricultural Experiment Station and the United States Department of Agriculture, Agricultural Research Service, under Grant 5901-0410-8-0181-0 from the Competitive Research Grants Office.

2 Abbreviations: AP, apparent photosynthesis; RuBP, ribulose bisphosphate; TPU, triose phosphate utilization; c3, intercellular CO2 concentration.
an acrylic plastic partition which had a 2.5 cm diameter hole in each end. The upper compartment was 1 cm deep and the lower was 6.2 cm deep. A fan and a cooling coil was installed in the bottom gas compartment, and air was circulated over the coil, through the hole in the opposite end of the partition, and past the leaf enclosed in the upper compartment. Water was circulated through the cooling coil from a temperature controlled bath. A fine wire thermocouple was pressed against the lower side of the enclosed leaf. Four similar chambers were arranged in a circle under a 1500 W multivapor lamp and a switching system was used to sequentially monitor chambers for exhaust gas and temperature for 5 min periods. Thus, four leaves could be measured in one experiment.

Prepared gas mixtures were humidified to a dewpoint of 11 to 13°C (constant to within 0.3°C for a given experiment) by passing first through a flask containing water and then through a water cooled condenser. Transpiration raised the dewpoint in the chamber to 17 to 24°C. Water vapor and CO₂ differentials in intake and exhaust gas were measured with a dewpoint hygrometer and an IR gas analyzer, respectively. Water vapor and CO₂ exchange were calculated from flow rates and concentration differentials in intake and exhaust air. Leaf temperature was maintained at 30°C and incident PAR was 2 mmol quanta m⁻² s⁻¹, except in the experiment involving light response. All AP measurements were made on the youngest fully expanded leaf on vegetative shoots.

**Variation in O₂ Sensitivity of AP within the Genotype.** *F. linearis* 84-9 was self-pollinated by dusting the inflorescence with pollen collected from several 84-9 plants, using a small, soft bristle brush. Seeds produced were germinated and plants were cultured as in the previous section, except the pots were smaller (1L). Forty-four plants were tested for O₂ inhibition of AP when they had grown to a height of 10 to 20 cm.

Testing was done by measuring AP at 20 and 210 ml L⁻¹ O₂. Measurements were made at CO₂ concentrations and dewpoints in the leaf chamber which ranged from 295 to 315 μL L⁻¹ and 17 to 21°C, respectively. For a given leaf, CO₂ concentrations in the chamber never differed more than 10 μL L⁻¹ for comparison of AP at 20 and 210 ml L⁻¹ O₂ and the differences were usually less than 5 μL L⁻¹. Leaf area in the chamber ranged from 2.5 to 5.0 cm². The measurements of AP were repeated on three separate leaves of each plant.

**Time Course of AP after Changes in O₂ Concentration.** Compressed gas mixtures were prepared with mixtures containing 335 μL L⁻¹ CO₂, and 20 or 210 ml L⁻¹ O₂, with the balance N₂. One of the chambers described under the gas exchange measurement section was used, and AP was monitored continuously. The leaf chamber was flushed at a rate of 0.4 L min⁻¹ with 20 or 210 ml L⁻¹ O₂ until steady state AP was attained. The gas stream was then quickly changed with a switching valve to the other O₂ concentration and AP was followed for 25 min in the first set of measurements and 40 min in others with a constant flow rate. Responses of a plant produced from seed of 84-9 (designated 84-9s), and one from rooted cuttings of *F. linearis* (84-5) were compared. Measurements were made on three leaves of each plant after switching from 20 to 210 ml L⁻¹ and three leaves of 84-9s and two of 84-5 after switching from 20 to 210 ml L⁻¹ O₂. Rates of AP were calculated at 5 min intervals after switching O₂ concentrations. The chamber was fully flushed at 2 to 3 min after switching.

**Light Response of AP.** Measurements of AP were made at irradiances of 0.2, 0.45, 0.8, and 2.0 mmol quanta m⁻² s⁻¹. Irradiance was varied by placing plastic shade screens over the leaf chamber. At each irradiance AP was measured at 20 and 210 ml L⁻¹ O₂, on three leaves of *F. linearis* (84-5) and three leaves each of two plants grown from seed of 84-9 (84-9s). Concentrations of CO₂ in the leaf chamber were maintained in the range of 310 to 340 μL L⁻¹ (the higher concentrations at the lowest irradiance) and dewpoint was kept in the range of 17 to 22°C. For two leaves of each plant the irradiance was changed stepwise from highest to lowest and in the third from lowest to highest. The sequence did not appear to influence the response.

**Response of AP to CO₂ Concentrations.** The response of AP to CO₂ concentration was determined by measurements at 20 ml L⁻¹ O₂ and CO₂ concentrations entering the leaf chamber of 83, 125, 207, 335, and 500 μL L⁻¹. Measurements at 210 ml L⁻¹ O₂ were made with gases containing 83, 113, 210, 328, and 487 μL L⁻¹ CO₂. Concentrations of CO₂ leaving the chamber were assumed to represent that surrounding the leaf, since the air was well stirred in the chamber. CO₂ was calculated from leaf conductance derived from transpiration. Dewpoints in the leaf chamber ranged from 17 to 22°C during the experiment, and for a given leaf the variation was 2°C or less.

Response of AP to CO₂ was determined for *F. linearis* (plants 84-9, 84-5, 84-7, and 84-8), *F. trinervia* (Ca), and the C₃ species *Panicum laxum* (4). Response curves were established for three separate leaves of each plant, except *F. linearis* 84-9, for which five leaves were measured. Since the response was similar for *F. linearis* 84-5, 84-7, and 84-8 the data for these were averaged.

**RESULTS**

The influence of 210 ml L⁻¹ O₂ on AP in progeny of *Flaveria linearis* 84-9 ranged from 12.7% inhibition to 27.3% stimulation (data not shown). Of the 44 plants tested, however, only three had O₂ inhibition of AP, with values of 3.7, 5.8, and 12.7%. Response of steady state AP to O₂ for none of the plants approached the 20 to 24% O₂ inhibition in other *F. linearis* plants (Fig. 1). There was a range of AP at 210 ml L⁻¹ O₂ from 13.4 to 27.4 mmol CO₂ m⁻² s⁻¹, but AP rate was not related to O₂ response.

The stimulation of AP by O₂ in plant 84-9s was not immediate upon switching from 20 to 210 ml L⁻¹ or vice versa. There was an initial (5 min after switching) inhibition of AP in two or three leaves upon changing from 20 to 210 ml L⁻¹ O₂ and AP was initially stimulated upon changing from 210 to 20 ml L⁻¹ O₂ (Fig. 1). Inhibition of AP by 210 ml L⁻¹ O₂ and stimulation by 20 ml L⁻¹ disappeared with time after switching, rapidly for about 20 min and more slowly for an additional 20 min. After 40 min, stimulation of AP by 210 ml L⁻¹ O₂ was 20 to 25%. In contrast to plant 84-9s, AP of *F. linearis* 84-5 was inhibited 20 to 24% by 210 ml L⁻¹ O₂ and stimulated 27 to 30% by low O₂ and the O₂ effect was constant from 5 to 40 min after changing.

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**Fig. 1.** Percent change in AP at various times after switching from 210 to 20 ml L⁻¹ O₂ (— — —) and from 20 to 210 ml L⁻¹ O₂ (——) for *F. linearis* 84-5 (○, ●, △) and selfed progeny of 84-9 (□, △, ▲). Measurements were made at 2.0 mmol quanta m⁻² s⁻¹, 30°C and 335 μL L⁻¹ CO₂. Different symbols represent separate leaves.
O₂ concentrations. This degree of O₂ inhibition at atmospheric CO₂ levels is consistent with other data for C₃-C₄ species (4, 11).

The stimulation of AP by O₂ occurred only at high irradiances. At the lowest irradiance AP of all three F. linearis plants was inhibited about 30%, a value typical of C₃ species (Fig. 2A). Upon raising the irradiance to 2 mmol quanta m⁻² s⁻¹, AP was inhibited 19.5% by O₂ in plant 84-5, whereas, AP of the two 84-9s plants was stimulated by 20 to 25%. The influence of O₂ on AP in the 84-9s plants changed steadily with increasing irradiance, whereas in 84-5 inhibition of AP remained nearly constant at 30% until irradiance was raised above 0.8 mmol quanta m⁻² s⁻¹.

In contrast to most C₃ species and to plant 84-5 in Figure 2, AP in the 84-9s plants was saturated at lower irradiances with 20 than with 210 ml L⁻¹ O₂ (Fig. 2B). Because of this, AP became higher at 210 than 20 ml L⁻¹ O₂ at about 1.1 mmol quanta m⁻² s⁻¹ in one 84-9s plant and about 0.65 mmol quanta m⁻² s⁻¹ in the other. Stimulation of AP by O₂ occurred at a lower irradiance in the plant that had the lower maximum AP rate.

The response of AP to CO₂ was similar in 84-9 and other F. linearis plants at low cᵢ, but at cᵢ above 100 μl L⁻¹ the divergence is remarkable (Fig. 3). Whereas for normal F. linearis plants AP was a linear function of cᵢ up to about 200 μl L⁻¹ and with some curvature to about 325 μl L⁻¹, AP at 210 ml L⁻¹ O₂ in 84-9 was CO₂ saturated at about 225 μl L⁻¹. At 20 ml L⁻¹ O₂ there was a sharp break in the response of AP at a cᵢ of 100 μl L⁻¹ and a decrease in AP with further increases in cᵢ. The maximum AP at 20 ml L⁻¹ O₂ was only about 50% as high for plant 84-9 as for the other F. linearis plants. Data (not shown) for F. trinervia (C₄) and F. laxum (C₃) were similar to that previously published for C₄ and C₃ species (2). The C₄ species showed no O₂ sensitivity at any CO₂ concentrations and AP saturated at about 100 μl L⁻¹ cᵢ. In F. laxum AP was inhibited about 35% at 200 ml L⁻¹ O₂ at a cᵢ (200 μl L⁻¹) in approximate equilibrium with atmospheric CO₂.

**DISCUSSION**

The plant Flaveria linearis 84-9 has an O₂ sensitivity of AP different from other plants in the species and also unique among plants reported in the literature. This unique response is most clearly demonstrated as a stimulation of AP by O₂ at atmospheric levels of CO₂, high irradiance, and near optimum temperature. This is in contrast to all other species reported, which under these conditions show either O₂ inhibition of AP as in C₃ and C₃-C₄ species or a lack of O₂ sensitivity characteristic of C₄ species, CAM species during nocturnal CO₂ assimilation, and some algae (2, 4, 6, 11, 12).

In characteristics other than CO₂ exchange plant 84-9 appears similar to other F. linearis plants with which we have compared it. Floral and foliar characteristics are identical to those of other plants from the same seed source. Leaf anatomy and CO₂ compensation concentrations are not distinguishable from other F. linearis plants (data not shown).

Plant 84-9 is easily distinguishable in the magnitude of AP and especially its response to CO₂, irradiance, and O₂. In the test of 84-9 progeny the average AP at 210 ml L⁻¹ O₂ was 21.6 ± 2.3 μmol CO₂ m⁻² s⁻¹ which along with data in Figures 2B and 3 indicate that this plant had about 70 to 80% of the AP of other F. linearis plants. In plant 84-9, AP was saturated at lower cᵢ and irradiance than other F. linearis plants. The lower AP and lower CO₂ and irradiance levels required for saturation, along with O₂ stimulation of AP, may indicate TPU limitation of AP suggested by Sharkey (17, 18).

A stimulation of AP by O₂ has been reported in some C₃ species under conditions of high CO₂, high irradiance, and/or low temperature (5, 8, 12, 14, 15, 17, 18). However, no reports of O₂ stimulation of AP have been made for temperatures as high as 30°C with cᵢ as low as 200 μl L⁻¹. In addition, inhibition of AP by exposure to low O₂ has in several cases been transitory, with recovery requiring only a few minutes (5, 15, 20). Response of AP of 84-9 to CO₂ and O₂ concentrations most closely resembles the results of Cornic and Louason (8) for measurements conducted with C₃ species at 5°C. They observed stimulation of AP by 210 ml L⁻¹ O₂ at CO₂ concentrations of 200 to 400 μl L⁻¹ or higher. In most other reports, much higher CO₂ concentrations were required (5, 15, 18, 21). Some of the variation in conditions required for O₂ stimulation of AP is undoubtedly due to the way plants were grown and experimental protocol. The stimulation in 84-9, however, is not due to growth or experimental conditions, since other plants under the same conditions did not show O₂ insensitivity or stimulation of AP.

Sharkey (17, 18) proposed that in addition to the limitation of AP by RuBP carboxylase activity and RuBP regeneration, AP could also be limited by TPU. This limitation was postulated to
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occur when the capacity of a leaf to convert triose phosphates to starch or sucrose is reached. Under this limitation P1 would be accumulated in carbon cycle intermediates and Pi in the chloroplast would drop below the level required for photophosphorylation. Limitation by TPU is most likely to occur at high irradiance and CO2 concentrations when synthesis of triose phosphates is high or under stress conditions when utilization of photosynthetic products is low. Under these conditions, reducing O2 concentration would not stimulate AP because the rate is limited by utilization of photosynthetic products rather than by substrate levels or activation of RuBP carboxylase.

As shown by Sharkey (18) for plants with O2-insensitivity, photorespiration was probably occurring in plant 84-9 under conditions of O2 stimulation of AP. This is indicated by the initial inhibition of AP when leaves equilibrated at 20 ml L-1 O2 were switched to 210 ml L-1 O2. Switching from 210 to 20 ml L-1 O2 also caused an initial stimulation of AP. In fact, the rate of change in AP from 5 to 10 min after switching O2 levels (Fig. 1) indicates that the initial effect of switching may have been similar to that in the other F. linearis. So RuBP oxygenation rates in plant 84-9 may be similar to those in other F. linearis at high as well as low CO2 concentrations.

Higher photophosphorylation and consequently greater RuBP synthesis has been suggested as the cause of higher AP when O2 is increased (5, 15). Since O2 stimulation in plant 84-9 occurs at CO2 levels where RuBP limitation is most likely, this suggestion implies that AP would be increased at 210 ml L-1 O2 due to increased RuBP pool size. However, evidence that RuBP levels in leaves are higher at 210 than 20 ml L-1 O2 is not available and most published data show the opposite. In spinach protoplasts (7) and soybean and bean leaves (1, 9) RuBP levels decreased as O2 was increased. In wheat leaves O2 had little effect on RuBP concentration at 350 and 1400 μl L-1 CO2, but at 100 μl L-1 CO2 RuBP was decreased in 210 compared to 1 ml L-1 O2 (16). Thus, it appears that RuBP levels are similar or higher at low O2 concentrations even under conditions such as high irradiance and high CO2 in which AP would be expected to be insensitive to or stimulated by O2.

The changes in AP with time after switching O2 concentrations in Figure 1 may reflect changes in activation of RuBP carboxylase. Sharkey et al. (19) have shown that under conditions of TPU limitation in bean, RuBP carboxylase is at a higher state of activation under atmospheric O2 than at low levels. Changes in AP with time after switching O2 in Figure 1 are similar to the time required for changes in activation of RuBP carboxylase in wheat leaves after raising or lowering O2 concentrations (16).

The difference in O2 response between 84-9 and other F. linearis plants is undoubtedly genetic, and possibly results from mutation of some gene or genes coding for enzymes involved in CO2 metabolism. The variation in O2 response of AP among the self-pollinated progeny from 84-9 may be genetic variation, but none of the plants had O2 inhibition approaching that of the other F. linearis plants and variability among the replicate measurements of the progeny was high. Further studies of inheritance of this trait will be conducted using hybrids between 84-9 and other F. linearis plants as well as other Flaveria species.

The significance of occurrence of a plant with O2 stimulation of AP in a species with C3-C4 photosynthesis is not known. Perhaps it is an extreme case of the reduced O2 inhibition usually observed in C3-C4 species and the mechanism for reducing O2 sensitivity may be common for 84-9 and other C3-C4 plants.

Except for two experiments, one each with species of Panicum and Flaveria (13, 14) AP has not been examined in plants of this photosynthetic type at CO2 concentrations above atmospheric. In both of these studies, however, AP was inhibited by O2 in the C3-C4 species at the highest CO2 levels used (about 400 μl L-1 c). In C3-C4 Flaveria species the inhibition of AP at 350 μl L-1 c appeared to be greater than in the C3-C4 species Lycopersicon esculentum (13). So, if O2 response of AP in the plant 84-9 results from the same mechanism as the reduced O2 inhibition of AP in other C3-C4 plants, it is quantitatively a substantially different response.

The mechanism of O2 stimulation of AP is still far from clear, as are its implications under physiological conditions. The plant described in this report should be a valuable one for study of O2 sensitivity of AP since O2 stimulates AP under temperature, light, CO2, and O2 conditions commonly encountered in nature.

LITERATURE CITED