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Gas-exchange response of grapevine leaves under fluctuating light*)

by

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Summary: Gas exchange responses to lightflecks of different frequency and duration were studied in mature grapevine leaves. Light-response curves under intermittent light showed lower carbon exchange rate (CER) than continuous light at non-saturating PFD levels ($10\text{--}500\ \mu\text{mol m}^{-2}\text{s}^{-1}$). White-dark and white-green alternating light of equal bright and shade periods at 0.33–5.33 Hz systematically resulted in improved light utilization as compared to high continuous light. Transpiration (T) was slightly more limited than the CER under intermittent light, resulting in higher water use efficiency. No differences in carboxylation efficiency were found by comparing the high continuous light treatment to white-dark lightflecks at 0.33 and 2.67 Hz. Our results show that, under short-term alternating light, CER of the grapevine leaf fully responds to the mean PFD level resulting from light oscillations.

Key words: light, photosynthesis, transpiration, lightflecks, Vitis vinifera.

Introduction

The leaves in the interior of a canopy are normally subjected to a diffuse light regime of green-enriched radiation for most of the season (ROSS 1981). However, these leaves, or portions thereof, can experience on clear windless days long-term sunflecks as a result of canopy gaps and varying solar elevation. Under windy conditions, the internal leaves can be subject to short-term alternating periods of sun and shade whose frequency and duration may also depend upon canopy structure and density, type of supporting trellis, shoot and leaf morphology and size, and cloud movement. Under these circumstances, a large fraction of CO_2 assimilation can occur under transient light conditions (PEARCY 1988).

Although the naturally variable light regime of a canopy and the related mechanisms of CO_2 assimilation are difficult to investigate due to spatial variation of sunflecks at foliage level, this matter has recently been reviewed (PEARCY 1990). On clear days, 30–60 % of the daily carbon gain by understory plants in tropical forest can be attributed to the utilization of sunflecks (PEARCY and CALKIN 1983; PEARCY 1988; CHAZDON 1988). There are several studies showing that, during flashing light, the efficiency of light utilization by leaves for photosynthesis (quantum yield) can be higher than observed under high continuous light (POLLARD 1970; GALLEGOS *et al.* 1980; PEARCY *et al.* 1985; STITT 1986). An increase in quantum yield has also been reported in studies on apple and grapevine (KRIEDEMANN *et al.* 1973; LAKSO and BARNES 1978). Of the light-dark intervals tested, KRIEDEMANN *et al.* highlighted that a dark interval of 1.8 s was required for maximum utilization of 0.05 s flashes in Sultana leaves.

The present study investigated the effects of the relative duration of direct (high) and filtered (low) radiation periods and the increasing frequency of high and low light periods of equal duration on gas exchange of grapevine leaves under laboratory conditions. The resulting

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data form a basis for future research aimed at field evaluation of the characteristics and the importance of naturally occurring sunflecks in grapevine canopies of various design and structure.

Materials and methods

Plant material and light environment: The experiments were carried out on plants of cvs Sangiovese and Chardonnay (*Vitis vinifera* L.) grown from two-bud hardwood cuttings rooted in heated bench in a greenhouse. At the beginning of leaf formation (mid-February) the plants were transferred to 2.5 l pots containing a mixture of sand and perlite (1:1 v/v) and fertilized weekly. Plants were grown at a photon flux density (PFD) of 500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ provided by natural and supplemental lighting. After 7 weeks, 10 plants per cultivar, having 6-8 fully expanded leaves, were selected and transferred to laboratory growth bench with 12 h photoperiod at a PFD of 500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and a temperature of 20 ± 2 °C.

The experiments were begun after 2 d of acclimation in the new environment and the artificial lighting was provided by an Osram 2000 W metal halide bulb; the heat of the lamp was removed by means of a water filter.

F i r s t e x p e r i m e n t: Lightflecks of varying duration were produced by passing the direct light of the lamp through a 30 cm diameter perspex disk equipped with interchangeable shutters. The disk was rotated at a constant rate of 6 s per revolution and a combination of decreasing direct/filtered radiation ratios were achieved using the shutters represented in Fig. 1 a. They were made of transparent film and completely light-opaque, gray, 0.15 mm thick cardboard.

The light response curve under intermittent light registered using this set of shutters was compared to the light response curve under continuous light, which was produced by progressively reducing the direct saturating light by layering stainless-steel screens over the top of the cuvette. In both cases the initial saturating PFD level was gradually reduced, and each successive step required 5-10 min to reach a steady state. Measurements were performed on leaves exposed for ≥ 1 h to saturating light to ensure full induction.

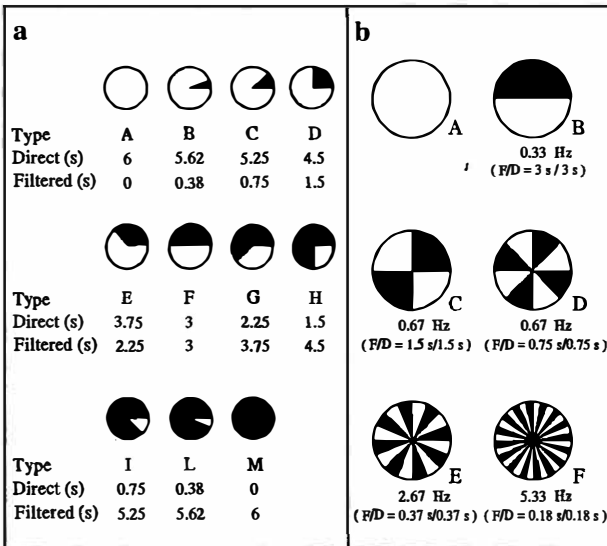


Fig. 1: a Type of shutters and combinations of white (direct)/dark (filtered) periods used to trace light-response curve under intermittent lighting. b Shutters used for inducing increasing frequency of white-dark and white-green alternating light with periods of equal duration. D = direct; F = filtered.

Second experiment: The effects of a 1:1 direct/filtered radiation ratio at increasing frequencies of lightflecks on gas exchange were evaluated. The shutters were prepared according to Fig. 1 b and, to simulate light transmitted through the foliage, new shutters were also made by replacing the cardboard sectors with a green, 0.10 mm thick film that absorbed about 74 % of direct radiation. Spectral properties of the light filtered through the cardboard and the green film as compared to a grapevine leaf are shown in Tab. 1. Light spectra were determined by a LiCor 1800 spectroradiometer. In a preliminary test only the cardboard A, B, C, D and E shutters were used, while in the second both materials and an additional F shutter were employed. In the latter case, comparable PFDs at each frequency for the two materials were achieved by adjusting the distance of the cuvette from the light source.

Table 1

Light quantity and quality of the Osram lamp and different shading materials. Proportions are based on lamp PFD.

	PFD ($\mu\text{mol m}^{-2}\text{s}^{-1}$) (400-700nm)	Percentage of light in the wavebands		
		Blue (400-480nm)	Green (480-580nm)	Red (600-700nm)
Lamp	829	24	35	19
Cardboard	13	0.3	0.5	0.3
Green film	210	2	19	0.8
Leaf	73	1	4	2

Third experiment: Designed to test the limitations to photosynthesis due to stomata and non-stomatal contributions, a final experiment considered intercellular CO_2 concentration (C_i) response curves of CER, which were constructed by measuring on recently fully expanded Sangiovese leaves exposed to intermittent light of 0.33 and 2.67 Hz (3 s/3 s and 0.375 s/0.375 s D/F, respectively) via B and E cardboard shutters and to high continuous light; seven C_i concentrations were tested by mixing increasing fractions of decarbonated air with bottled air containing CO_2 at a concentration of 1000 $\mu\text{l l}^{-1}$.

Gas exchange and light measurements: In each experiment, leaf carbon exchange rate (CER) and transpiration flux (T) were measured on the youngest, fully expanded leaves by an IRGA-based, steady state gas exchange system. A water jacketed cuvette, having a volume of 60,000 mm^3 , sampled a leaf surface of about 700 mm^2 ; the gas flux passing through the cuvette was mixed by a small fan and the leaf temperature was measured by a copper-constantan thin wire thermocouple touching the abaxial leaf surface. Bottled air containing 350 $\mu\text{l l}^{-1}$ of CO_2 was passed through the cuvette. The leaf temperature and the ambient (cuvette) CO_2 concentration (C_a) and its dew point were kept constant at 25 ± 0.2 °C, 320 ± 10 $\mu\text{l l}^{-1}$, and 9 ± 1 °C, respectively.

The relative large internal volume of the cuvette, the moderate gas flux in the system and the large volume of the IRGA cells resulted in an intentionally slow response time of the system so as to attain a smooth output signal from the IRGA, which was unaffected by the transients induced by the light variation.

CER, T, and sub-stomatal CO_2 concentration (C_i) were calculated after VON CAEMMERER and FARQUHAR (1981). Water use efficiency (WUE), i.e. the amount of fixed carbon per amount of water transpired (CER/T), was also calculated.

In each experiment, PFD was measured by a selenium photocell attached to the side of the cuvette. Within each steady state, mean PFD was calculated over single readings recorded every 0.1 s by a CR21X Campbell data-logger.

Results

Light response curves: Fig. 2 shows the light response curves under continuous light and white-dark intermittent light (first experiment). Photosynthetic rates were higher under continuous light at non-saturating light intensities ($10\text{--}500 \mu\text{mol m}^{-2}\text{s}^{-1}$). The light saturation point for CO_2 uptake was reached at approximately $700 \mu\text{mol m}^{-2}\text{s}^{-1}$ for continuous light and at higher irradiance (about $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$) for intermittent light.

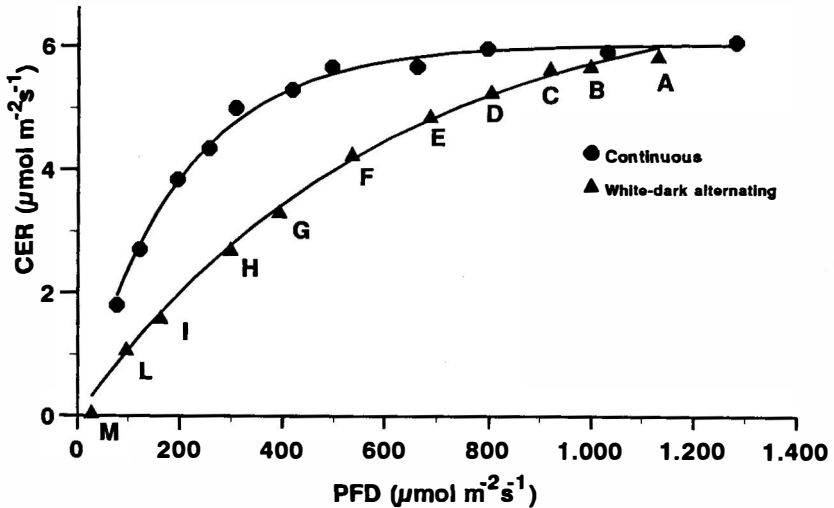


Fig. 2: Light response curves under continuous light and white-dark alternating light (first experiment). Letters correspond to type of shutters used. Each plotted value is the mean of 8 and 10 replicates, respectively. Non linear model was: $y = a - (1 - \text{EXP}(-bx))$. Regression equations were: $y = 6.04 - (1 - \text{EXP}(-0.00509x))$, $R^2 = 0.993$ (continuous); $y = 7.055 - (1 - \text{EXP}(-0.00167x))$, $R^2 = 0.997$ (alternating). The b coefficients of fitted equations differ for t test, $P < 0.01$.

Table 2

Mean PFD values and calculated photon yield for increasing frequencies of white-dark and white-green alternating light compared to high and low continuous light.

Lightfleck frequency (Hz)	Measured PFD ^a ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Photon Yield	
		(mmol CO_2 / $\mu\text{mol quanta}$) ^b	Relative ^d (%)
White-dark			
0.33 (3s) ^c	534 ± 25	10.7	162
0.67 (1.5s)	530 ± 25	11.4	174
1.33 (0.75s)	531 ± 27	12.2	185
2.67 (0.375s)	545 ± 29	11.8	180
5.33 (0.1875s)	525 ± 26	13.0	198
High light	1070 ± 34	6.6	100
Low light	25 ± 4	12.8	194
White-green			
0.33 (3s) ^c	545 ± 28	12.1	173
0.67 (1.5s)	541 ± 27	12.2	177
1.33 (0.75s)	548 ± 27	12.3	179
2.67 (0.375s)	552 ± 26	12.5	181
5.33 (0.1875s)	553 ± 25	12.4	180
High light	1039 ± 38	6.9	100
Low light	260 ± 14	20.3	294

^a Mean ± SE. ^b Calculated from mean CER and PFD values. ^c Relative duration (seconds) of white and dark or green cycles. ^d Based on value recorded under high continuous light.

Light frequency: Since the preliminary test responses were similar to those of the second experiment, only the latter's results are reported. With cardboard shutters the lowest frequency of lightflecks (0.33 Hz) resulted in 76 % CER of high continuous light; thereafter CER increased along with the frequency of lightflecks, and maximum rates (92 % of assimilation under high continuous light) were recorded at 5.33 Hz (Fig. 3). As the measured mean PFDs were similar under intermittent light regime, the variations in photon yield were essentially those of CER (Tab. 2), although the highest photon yield at 5.33 Hz is partially attributable to a slightly lower measured PFD. Since transpiration flux was generally more limited than CER under intermittent light, WUE also slightly increased along with the frequency of lightflecks (Fig. 3). The use of the green film shutters showed a non-significant increase of CER along with increasing frequency, since the lowest frequency (0.33 Hz) already had a photosynthetic rate only 9 % lower than that of high continuous light. Regardless of the quality of lightflecks, WUE in these tests showed a slight, occasionally significant, tendency to increase along with increasing lightfleck frequency.

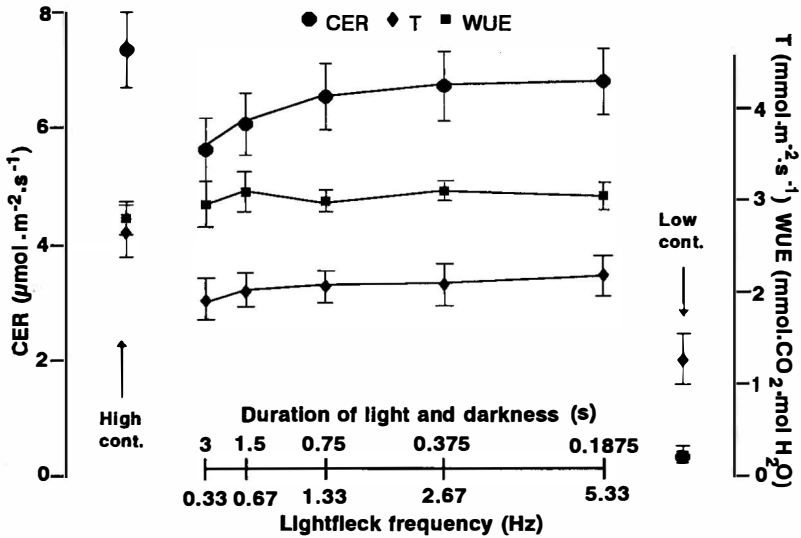


Fig. 3: Carbon exchange rate (CER), transpiration flux (T) and water use efficiency (WUE) recorded for leaves subjected to increasing frequencies of white-dark alternating light as compared to high and low continuous light (2nd experiment). Each plotted value is the mean of 6 replicates. Vertical bars indicate SE.

CER / C_i curves: The response of CER to increasing leaves of C_i evaluated for high continuous light and white-dark alternating light at 0.33 and 2.67 Hz, showed no statistical differences among regression coefficients calculated for initial slopes. Similarly, final slopes did not differ among treatments.

Discussion

The comparison between the light-response curves under continuous and white-dark intermittent radiation (Fig. 2) shows a marked reduction in the utilization of low (non-saturating) PFD levels under alternating light. KRIEDEMANN *et al.* (1973) highlighted similar photosynthetic rates at weak radiation for continuous and white-dark intermittent radiation and conversely, an improved utilization of intense radiation (above 25-50 W/m²) under conditions of alternating light. The discrepancy between these findings seems to be related to the characteristics of intermittent light regimes, since the latter authors used constant 0.05 s

light/0.2 s dark intervals of varying intensities to produce different radiation levels. Additionally, the photosynthetic rates presented by these authors were adjusted to the duration of each light period.

CER in the first experiment (Fig. 2) was more limited along with increasing duration of the dark period. With shade intervals exceeding light intervals in duration, hysteresis effects on CER at a sudden increase of PFD can be expected. It has been shown that a leaf exposed to low light for a certain period of time shows, when subjected to a sudden increase in PFD, a gradual rise in CO₂ assimilation (PEARCY 1990). This is the well-known induction requirement for photosynthesis, which results from slow light regulation of photosynthetic enzymes and light-driven stomatal opening (RABINOVITCH 1957; WALKER 1981; EDWARDS and WALKER 1983). From a practical standpoint, it is worth noting that a low irradiance uniformly spread over the leaf achieves higher photosynthesis than a photon flux of similar magnitude resulting from a combination of high and low radiation. This would suggest that internal leaves of a canopy experiencing a diffuse low light regime may provide more photosynthates than leaves located in deep shade which receive occasional saturating sunflecks.

The data reported in Fig. 3 show that at least 76 % of maximum CER recorded under high continuous light occurred during intermittent white-dark lighting. As the PFD under alternating light was reduced by approximately 50 % as compared to high continuous light, increased photosynthetic efficiency under alternating light is confirmed, as reported for apple (LAKSO and BARNES 1978) and grapevine (KRIEDEMANN *et al.* 1973) in white-dark cycles of 0.5 s and 1 s, respectively. The mechanisms underlying this enhanced assimilation might be the equilibrium concentration of intermediates during the "light reaction" of photosynthesis, which places no limitation on the subsequent dark reaction, and, more specifically, a post-illumination CO₂ fixation. In this latter connection, it has been shown that, with short-term lightflecks, assimilation may continue at a relatively high rate for some time after the lightfleck as a result of utilization of metabolite pools built up during it (PEARCY *et al.* 1985).

The data in the present study also show that an increased photosynthetic efficiency occurred with more frequent bright and shade periods of equal duration (Tab. 2). Generally, alternating light of 0.75 s light/dark achieved a photosynthetic efficiency close to maximum and only slight improvement could be induced by further increasing the frequency. Interestingly, KRIEDEMANN *et al.* (1973) have pointed out that maximum effectiveness coincided with light/dark cycles between 0.8 s/1.2 s and 0.6 s/1.4 s. It would thus appear that an intervening light period < 1 s followed by a dark period of equal or slightly longer duration may represent an effective combination for the utilization of alternating light by grapevine leaves.

Even though green radiation is known to be absorbed and utilized by leaves to a lesser degree than white light (HOLMES 1981), alternating white/green cycles still resulted in higher photon yield than high continuous light (Tab. 2). Yet this parameter was less responsive to increasing lightfleck frequency when compared to the pattern shown under white-dark intermittence (Tab. 2). Since this comparison was carried out at similar PFDs, this finding must take account of the difference in intensity of each bright and shade period of the two light regimes depending upon different light transmittance of the cardboard and the green film. In any case the grapevine leaf shows the ability to detect this difference.

Given the overall results recorded with lightflecks of varying frequency and quality (Fig. 3, Tab. 2), the mean photosynthetic rate of grapevine leaves under each regime of flashing light is typically higher than the rate calculated from the mean of steady state measurements in continuous high and low light. This information is somewhat inherent to the shape of the light response curve under continuous light (Fig. 2), fitted to a negative exponential equation, although it is highly significant that we can predict the photosynthetic rate under short-term alternating light on the basis of these results. Reliable estimates of the photosynthetic rates can be made by taking the mean of the two PFD levels associated with the alternating high and low light and extrapolating the predicted rate from a light-response curve determined for a specific cultivar.

With light/dark cycles of equal duration (Fig. 3), WUE was generally higher than under high continuous light since transpiration was more limited than photosynthesis. The WUE response to intermittent light observed in the present study would suggest that at the tested frequencies a slow stomatal reopening during each light period contributed the most to the measured steady-state response of transpiration. Thus the hypothesis of lower WUE as a result of a delayed stomatal closure during the low-light periods of an intermittent light regime, when assimilation is already strongly limited by light (PEARCY 1988, 1990), is here not confirmed.

Since the initial slopes of CER versus C_i did not differ, the efficiency of carboxylation (i.e. the amount and/or activity of Rubisco) did not seem to be strongly affected by the imposed light regimes; beyond the initial linear portion, when C_i is above $150 \mu\text{mol mol}^{-1}$, a very moderate inhibition of photosynthesis occurred regardless of treatment.

The gas-exchange of grapevine leaves subjected to an alternating light regime thus shows a dependency upon the timing and duration of lightflecks. When the latter are frequent and of short duration, certain mechanisms set in substantially to increase the carbon gain. However, when the light period during a sunfleck alternates with a shade period of increasing length, the induction requirement which produces hysteresis will likely become increasingly limiting.

These results can greatly help in estimating the photosynthetic response of canopies characterized by the rapid fluctuations in PFD which typically occur under windy conditions. In a traditional trellis, KRIEDEMANN *et al.* (1973) were unsuccessful in attempting to correlate the frequency of sunflecks to wind speed. However, some of the modern training systems for grapes without foliage wires and free shoots could have the effect of creating more consistent relationships between wind speed and patterns of sunflecks. Research in this direction is already in progress.

References

- CHAZDON, R. L.; 1988: Sunflecks and their importance to forest understory plants. *Adv. Ecol. Res.* **18**, 1-63.
- EDWARDS, G.; WALKER, D. A.; 1983: C_3 and C_4 : Mechanisms, and Cellular and Environmental Regulation of Photosynthesis. Univ. of California Press, Berkeley.
- GALLEGOS, C. L.; HORNBERGER, G. M.; KELLEY, M. G.; 1980: Photosynthesis-light relationships of a mixed culture of phytoplankton in fluctuating light. *Limnol. Oceanogr.* **25**, 1082-1092.
- HOLMES, M. G.; 1981: Spectral distribution of radiation in plant canopies. In: SMITH, H. (Ed.): *Plants and the Daylight Spectrum*, 147-158. Academic Press, New York.
- KRIEDEMANN, P. E.; TOROKFALVY, E.; SMART, R. E.; 1973: Natural occurrence and photosynthetic utilization of sunflecks in grapevine leaves. *Photosynthetica* **7**, 18-27.
- LAJKO, A. N.; BARNES, J. E.; 1978: Apple leaf photosynthesis in alternating light. *HortScience* **13**, 473-474.
- PEARCY, R. W.; 1988: Photosynthetic utilization of lightflecks by understory plants. *Austral. J. Plant Physiol.* **15**, 223-238.
- ; 1990: Sunflecks and photosynthesis in plant canopies. *Ann. Rev. Plant Physiol.* **41**, 421-453.
- ; CALKIN, H.; 1983: Carbon dioxide exchange of C_3 and C_4 tree species in the understory of Hawaiian forest. *Oecologia* **58**, 26-32.
- ; OSTERYOUNG, K.; CALKIN, H. W.; 1985: Photosynthetic responses to dynamic light environments by Hawaiian trees. The time course of CO_2 uptake and carbon gain during sunflecks. *Plant Physiol.* **79**, 896-902.
- POLLARD, D. F. W.; 1970: The effect of rapidly changing light on the rate of photosynthesis in bigtooth aspen (*Populus grandidentata*). *Can. J. Bot.* **48**, 823-829.
- RABINOVITCH, E. I. (Ed.); 1957: *Photosynthesis and Related Processes*. Vol. **II**, Part 2. Interscience Publ., New York.
- ROSS, J.; 1981: The radiation regime and architecture. Ser. Ed. HELMUT LIETH. The Hague, Boston.
- STITT, M.; 1986: Limitation of photosynthesis by carbon metabolism. I. Evidence for excess electron transport capacity in leaves carrying out photosynthesis in saturating light and CO_2 . *Plant Physiol.* **81**, 1115-1122.
- VON CAEMMERER, S.; FARQUHAR, G. D.; 1981: Some relationships between the biochemistry of photosynthesis and the gas-exchange of leaves. *Planta* **153**, 376-387.
- WALKER, D. A.; 1981: Photosynthetic induction. In: AKOYONOGLOU, G. (Ed.): *Proc. 5th Intern. Congr. Photosynthesis*, Vol. **IV**, 189-202. Balaban Intern. Sci. Ser., Philadelphia.