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Morphology and Photosynthetic Efficiency of Tobacco Leaves That Received End-of-Day Red or Far Red Light during Development[1]

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ABSTRACT

Shaded leaves in plant canopies receive a higher proportion of far red relative to red light than is received by unshaded leaves. Brief end-of-day irradiations with red or far red light, acting through the phytochrome system, reversibly control morphological development of tobacco plants. Leaves that received far red light for 5 minutes at the end of each day during development were longer and narrower than those that received end-of-day red light. The far red treated leaves weighed less, had fewer stomata, and had less chlorophyll per unit area of leaf. Net CO_2 assimilation rates did not differ significantly between red- and far red-treated leaves on an area basis; however, the far red-treated leaves assimilated significantly more CO_2 on a leaf weight basis.

Many investigators have reported that shading influences plant growth and development. Reduced light intensity is an obvious consequence of shading; however, shaded leaves in plant canopies receive light of different spectral distribution as well as lower intensity than that received by unshaded leaves (12). There is a pronounced increase in the ratio of FR[2] light relative to R light received by the shaded leaves (12).

Brief end-of-day irradiations with R and FR, acting through the phytochrome system, reversibly control the morphological development and chemical composition of tobacco (12, 13, 18) and other plants (6, 7). We postulated that morphological differences in the primary photosynthetic tissue, the leaf, may influence the photosynthetic efficiency of those leaves (12). Thus, the objective of this study was to determine photosynthetic rates in leaves that were morphologically different as a result of end-of-day phytochrome manipulation.

MATERIALS AND METHODS

Plant Materials. Tobacco (*Nicotiana tabacum* L. cv. Burley 21) seedlings were started in expanded peat pellets at 28 C under 14-hr photoperiods with illumination of 1600 ft-c from cool-white fluorescent lamps. Eight-week-old seedlings were transplanted to 2-liter pots containing a soil-perlite (2:1, v/v) mixture and transferred to controlled environment chambers for conditioning and treatment. The seedlings were subirrigated, as needed, with half-strength Hoagland's nutrient solution No. 1 (10) during the starting, conditioning, and treatment periods. They were conditioned to the growth chamber environments for 7 days before R and FR treatments were started. At the end of the conditioning period, all leaves longer than 5 cm were removed from each plant. Thus, the leaves used in this study included only those that developed during the 18-day treatment period.

Treatments. During the conditioning and treatment periods, plants received 8-hr daily illumination periods at an intensity of 2200 ft-c from cool-white VHO fluorescent lamps. Day and night temperatures were maintained at 25 C. At the end of each daily illumination period, for 18 consecutive days, plants were irradiated for 5 min with R or FR light. All plants were kept in the same controlled environment chamber, except during the daily R and FR irradiations. The intensities of R and FR were 360 $\mu w/cm^2$ over the wavelength bands of 600 to 700 and 700 to 770 nm, respectively. The R radiation unit consisted of two layers of red cellophane under a bank of cool-white fluorescent lamps, whereas the FR unit consisted of two layers of red and two of dark blue cellophane under internal reflector, incandescent filament lamps. At the end of the 16-hr dark period following the 18th treatment, recently expanded leaves were collected from some plants for chlorophyll determinations. Other plants were transferred in light-tight boxes from the controlled environment chamber to a laboratory for CO_2 assimilation studies.

Chlorophyll. Chlorophyll *a*, *b*, and totals were determined according to the method of Arnon (1).

Laminar Mass/Area. Weights per unit area of leaf lamina were calculated from leaf plugs obtained with a cork borer. Each plug had an area of 0.5 cm^2. Ten plugs were taken from each of two leaves from each of six plants that received end-of-day R and from six that received FR.

Photosynthesis. Plants were studied individually, and the order was alternated between treatments (*i.e.*, R, FR, R, FR) to eliminate bias due to difference in time lapse since the last end-of-day phytochrome manipulation. CO_2 assimilation rates were measured in recently expanded attached leaves. One leaf per plant was enclosed in a transparent, double-walled chamber that was maintained at approximately 27 C by circulating water between the walls.

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[2] Abbreviations: FR: far red light; R: red light; P: phytochrome.

Table I. *Configuration of Leaves That Received Red or Far Red for 5 min at the End of Each Day during Development*

End-of-Day Radiation	Leaf Shape			Laminar Mass/Area
	Length	Width	Length/width	
	cm			*mg/cm²*
Red	32.8	18.2	1.8	22.3
Far red	36.5	16.5	2.2	18.6
	*	*	*	*

* Indicates that values differ at the 5% level of significance.

Table II. *Distribution and Size of Stomata on Lamina of Leaves That Received Red or Far Red for 5 min at the End of Each Day during Development*

End-of-Day Radiation	Stomata Distribution[1]			Mean Stomate Size[2]	
	Upper	Lower	(Upper + lower)	Upper	Lower
	No./cm² of surface		*No./mg (fresh wt)*	*μ*	
Red	5,110	12,510	790	19.6	20.8
Far red	4,060	10,520	784	19.5	20.3
	*	*	NS	NS	NS

[1] Values are based on means for 60 microscope fields counted at 400× magnification.

[2] Values are mean pore lengths for 60 stomates measured at 1200× magnification.

* indicates that values differ at the 5% level of significance. NS indicates that differences are not statistically significant at the 5% level.

The light source was a bank of internal reflector, incandescent filament lamps partly submerged in a transparent-bottom tank of running tap water. Different light intensities within the chamber were obtained by adding or removing neutral screens between the light source and the leaf chamber.

CO_2 assimilation rates were determined by comparing CO_2 concentrations of the air at the entrance and exit ports of the chamber with an infrared CO_2 analysis system (15). Leaves were allowed to equilibrate at each light intensity before CO_2 assimilation rates were recorded.

Stomatal Distribution. Leaf surface replicas were made by covering portions of the upper and lower leaf surfaces with several thin layers of colorless fingernail polish after the CO_2 studies were completed. The replicas were removed from the leaves and taped to microscope slides. Stomata per unit area were determined under 400× magnification. Ten areas were counted from both upper and lower surfaces of one leaf from each of six plants representing each end-of-day treatment. Pore lengths were measured on 10 stomata from each surface of the six leaves from each end-of-day treatment. Stomate lengths were measured under 1200× magnification. No attempts were made to measure pore widths.

RESULTS

Leaf Morphology. Leaves developed on plants that received FR at the end of each daily light period were longer, but narrower than those developed on plants that received end-of-day R (Table I). Further, end-of-day R significantly increased lamina mass/area.

End-of-day FR resulted in development of fewer stomata per unit area of leaf lamina (Table II). However, the number of stomata per unit weight of leaf lamina was not affected significantly by end-of-day radiation. Within each end-of-day treatment, stomata on the lower surface were slightly larger than those on the upper surface (Table II). However, mean stomate size was not affected significantly by end-of-day radiation.

Chlorophyll Content. Concentration of chlorophyll per unit area of lamina was significantly greater after R than after FR end-of-day radiation treatment (Table III). There was no significant difference in chlorophyll *a* concentration per gram of lamina associated with end-of-day radiation; however, the concentration of chlorophyll *b* was greater after R than after FR end-of-day treatments.

Photosynthetic Rate. Net CO_2 assimilation rates of leaves that received R and FR did not differ significantly on a leaf area basis; however, over the range of light intensities used in our experiment, net CO_2 uptake was significantly greater on a weight basis for leaves that had received FR light at the end of each day during development (Table IV).

DISCUSSION

CO_2 assimilation by a leaf in response to various intensities of light depends on a number of environmental factors, including CO_2 concentration of the air, air flow rate, temperature, water stress, prior mineral nutrition, physiological age, and light intensity preconditioning (5). Several investigators have studied sun- and shade-adapted plants (2, 3, 16). We used controlled environment chambers and brief exposures to R

Table III. *Chlorophyll Content of Leaf Lamina That Received Red or Far Red for 5 min at the End of Each Day during Development*

End-of-Day Radiation	Chlorophyll					
	a	*b*	Total	*a*	*b*	Total
	mg/dm²			*mg/g (fresh wt.)*		
Red	2.004	1.072	3.076	0.899	0.481	1.380
Far red	1.649	0.837	2.486	0.884	0.449	1.333
	*	*	*	NS	*	*

* indicates that values differ at the 5% level of significance. NS indicates differences are not statistically significant at the 5% level.

Table IV. *Net CO_2 Assimilation Rates of Leaves That Received Red or Far Red for 5 min at the End of Each Day during Development*

End-of-Day Radiation	Net CO_2 Assimilation Rates per hour at Light Intensities (ft-c) of:					
	260	430	780	1200	2300	3900
	mg CO_2/dm²					
Red	3.1	5.5	8.4	11.9	14.9	15.3
Far red	3.3	5.3	7.9	11.8	14.8	14.9
	NS	NS	NS	NS	NS	NS
	mg CO_2/g (fresh wt.)					
Red	1.4	2.5	3.8	5.4	6.7	6.9
Far red	1.8	3.0	4.4	6.6	8.2	8.3
	NS	NS	NS	*	*	*

* indicates that values differ at the 5% level of significance. NS indicates that differences are not statistically significant at the 5% level.

or FR at the end of each day to study the effect of spectral distribution of light (one consequence of shading by other plants) without significantly altering the total light energy received per unit area of leaf surface during growth and development of the tested leaves.

Our results show that spectral distribution of light, specifically the amount of R relative to FR, received during leaf development can influence the morphology (Tables I and II) and photosynthetic efficiency of those leaves (Table IV). The controlling mechanism is believed to involve the phytochrome system because of the R-FR photoreversible control of developmental patterns (4). Further, the amount of phytochrome in the FR-absorbing form (Pfr) relative to the total amount of phytochrome (P), particularly at the beginning of a dark period, appears to play a critical role in signalling photosynthate distribution and developmental patterns (6, 12).

It is not clear, however, whether a low Pfr/P ratio (the consequence of irradiation with FR) triggers a chain of metabolic events leading to shade-adapted development or whether the events occur because the Pfr/P ratio is too low to signal a chain of events leading to sun-adapted development. Whichever the case, some effects of FR are similar to those of exogenous GA. The thin leaves, light color, and somewhat elongated internodes of plants treated with GA (17) and end-of-day FR (12) (see also Tables I and II of this report) suggest that both treatments may involve the same metabolic pathway. Both GA- and FR-treated plants have reduced total chlorophyll, and the reduction of chlorophyll *b* is greater than the reduction of chlorophyll *a* (17) (see also Table III of this report). Possibly end-of-day FR, through its influence via the phytochrome system during the dark period, may initiate shifts in the balance of naturally occurring growth regulators such that the imbalance tips in favor of gibberellins much the same as when exogenous GA is added to a plant.

In our work, the rate of CO_2 assimilation on a leaf area basis (Table IV) was about the same as that found for tobacco in an earlier report (9). Nevertheless, our FR-treated leaves were thinner and photosynthetically more efficient than the R-treated leaves on a weight basis. This result is similar to the finding that shade-adapted plants were thinner and more efficient than sun-adapted plants in assimilation of CO_2 at low light intensities (11).

Our work suggests that shading, through its effect on the ratio of FR to R received by developing leaves in plant canopies, can influence morphological development of the plant and photosynthetic efficiency of those leaves. Thus, it appears that the phytochrome system is involved in signalling metabolic events leading to adaptive morphological development such that the plant is better suited to compete with other plants in its growth environment. The developmental response to phytochrome manipulation at the end of a white light period may be an adaptive aspect of the so-called "high energy phenomena" of photomorphogenesis (8, 14).

Finally, our work suggests that short term photosynthetic studies with various leaves in a plant canopy can be influenced by the spectral distribution of the light that existed during leaf development, as well as by the preconditioning factors pointed out earlier in this discussion.

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