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STOMATAL RESPONSES TO LIGHT IN
XANTHIUM STRUMARIUM AND OTHER SPECIES

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STOMATAL RESPONSES TO LIGHT IN *XANTHIUM STRUMARIUM* AND OTHER SPECIES

By

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ABSTRACT

STOMATAL RESPONSES TO LIGHT IN
XANTHIUM STRUMARIUM AND OTHER SPECIES

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The stomatal response to light was investigated with detached leaves in an attempt to answer the following questions:

1. To what degree do stomata respond to light indirectly via the effect of photosynthesis on the concentration of CO_2 inside the leaf?
2. How do factors other than light influence the stomatal response to light?
3. What are the photoreceptors involved in the stomatal response to light?

Stomatal conductance was monitored by measuring the humidification of air that had passed over a leaf and the water vapor concentration difference between the inside and outside of the leaf.

It was shown that stomata of leaves of *Xanthium strumarium* responded to light when photosynthesis was eliminated by the electron transport inhibitor cyanazine (2-chloro-4-(1-cyano-1-methylethyl amino)-6-ethyl amino-s-triazine). An analysis of slopes of curves relating stomatal conductance to light intensity and intercellular CO_2 concentration indicates that the response to light of stomata of *X. strumarium*, *Phaseolus vulgaris*, *Perilla frutescens*, and *Gossypium hirsutum* is for the most part not mediated by changes in the intercellular CO_2 concentration. However, in *Zea mays* at low irradiance, the light-dependent depletion of CO_2 inside the leaf provided more than one-half of the opening stimulus. At high irradiance in *Z. mays*, the intercellular CO_2

concentration did not vary with changes in irradiance and since it is known that the stomatal response to CO_2 is not strong enough to keep the intercellular CO_2 concentration constant, it is concluded that the stomata were responding directly to light.

Although the stomatal response to light generally did not depend on the stomatal response to CO_2, the stomata of all of the species studied here did open when the CO_2 concentration was lowered. However, in P. frutescens, P. vulgaris, and X. strumarium the stomatal response to CO_2 was diminished or absent at high irradiance. In X. strumarium, it was demonstrated that the direct stomatal response to light was diminished at high CO_2 concentrations. Abscisic acid, humidity, and leaf temperature affected the stomatal response to light in such a way that the various curves were coincident when they were plotted as a percentage of the conductance at the highest irradiance.

Experiments with leaves illuminated on either the adaxial or abaxial surface indicate that the photoreceptor pigment for the direct stomatal response to light is in the epidermis, presumably in the guard cells.

An action spectrum of stomatal opening in X. strumarium showed that blue light was very effective, while red light was one-tenth to one-fifth as effective as blue light, and green light was hardly effective at all. The responses of stomata and CO_2 assimilation to red light had similar action spectra and were both eliminated by cyanazine. This evidence shows that chlorophyll is the pigment responsible for the red light response of stomata. The red light response was not, however, mediated by photosynthesis-dependent changes in the intercellular CO_2 concentration, since the ambient CO_2 concentration was manipulated to keep the internal CO_2 concentration constant.

The blue light response of stomata was only slightly reduced by cyanazine. This indicates that a photoreceptor pigment other than chlorophyll is also involved in the stomatal response to light.

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KEY TO SYMBOLS

A	assimilation rate (usual units are μmol m^{-2} sec^{-1})
ABA	abscisic acid
C_3	plants in which the first identifiable product of photosynthesis is 3-phosphoglyceric acid (All plants reported herein except Zea mays are C_3 plants.)
C_4	plants in which the first identifiable product of photosynthesis is a four carbon dicarboxylic acid (Of the plants reported herein, only Zea mays is a C_4 plant.)
c_a	ambient CO_2 concentration (usual units are $\mu\ell$ ℓ^{-1})
c_i	CO_2 concentration in the intercellular spaces of the leaf (usual units are $\mu\ell$ ℓ^{-1})
cyanazine	2-chloro-4-(1-cyano-1-methylethyl amino)-6-ethyl amino-s-triazine
DCMU	3-(3,4-dichlorophenyl)-1,1 dimethylurea
g	conductance to water vapor, inverse of the more common but less appropriate resistance to water vapor (usual units are cmol m^{-2} sec^{-1})
I	irradiance or quantum flux (usual units are w m^{-2} for white light and μE m^{-2} sec^{-1} for monochromatic light)

PEP	phosphoenolpyruvate
r	resistance to water vapor (usual units are m^2 sec $cmol^{-1}$)
RH	relative humidity
RuBP	ribulose bisphosphate
VPD	vapor pressure deficit (usual units are ml water vapor/l air)
λ	wavelength of light (usual units are nm)
$(\partial x/\partial y)_z$	the slope of the curve of x versus y with z held constant

INTRODUCTION

Stomata regulate gas exchange between leaves and their environment. More water vapor diffuses through the stomata than any other gas; on the order of 1000 times more water vapor is lost through the stomata than is carbon dioxide taken up over the life of the plant. Although this water loss is usually seen as a deleterious consequence of stomatal opening for carbon dioxide uptake, it plays an important role in dissipating the energy received from the sun. Two other important gases diffusing through the stomata are carbon dioxide and oxygen. During photosynthesis, CO_2 diffuses into the leaf and O_2 diffuses out. The CO_2 concentration difference between the inside and the outside of the leaf can never be greater than 320 $\mu\ell\ \ell^{-1}$, since this is the CO_2 concentration in the air. Since oxygen diffuses faster than CO_2 (1.17 times faster theoretically), the O_2 concentration difference between the inside and outside of the leaf is less than the CO_2 concentration difference. This concentration difference is insignificant when compared to the concentration of O_2 in the air (2×10^5 $\mu\ell\ \ell^{-1}$) and so stomatal movements do not exert large effects on the O_2 concentration. Since water vapor and CO_2 are the most important gases diffusing through the stomata, it is reasonable to expect that stomatal function will be related to plant water status and photosynthesis in some way.

Two reasons to study gas exchange and stomata are the following: first, to develop a basic understanding of stomata and how they move,

and second, to understand how stomatal responses to various stimuli allow plants to be successful in diverse environments. This information can be used to determine what, if any, limitations stomata impose on photosynthesis and plant growth. It may also help plant breeders to fit crop plants to the environment, for example, to determine which plants would best take advantage of an irrigated field and which plants would be able to survive a dry land farming situation. There may also be room for improvement by selecting stomatal behavior that is best suited to a large economic yield from crop plants, rather than the stomatal behavior best suited to propagation, which natural selection would have favored.

The Stomatal Mechanism

Stomata open by filling with salts of potassium which increase the osmotic pressure inside the guard cells (11,37). The resultant increase in turgor pressure allows the guard cells to push the surrounding epidermal cells apart, leaving a hole between the two guard cells. Malate and chloride serve as anions and, in epidermal strips of *Vicia faba*, the proportion of potassium ions balanced by malate depends on the availability of chloride (40,47). The processes initiating the increase in osmotic pressure that leads to stomatal opening are unknown at this time.

Stomatal Responses to Light and Other Environmental Factors

Stomata open in response to an increase in irradiance, an increase (or decrease {27}) in leaf temperature, a decrease in the water vapor pressure deficit, or a decrease in the intercellular CO_2 concentration. Studying stomatal responses to environmental factors is difficult

because changing one factor often causes changes in other factors. For example, an increase in the irradiance falling on a leaf can also cause at least three other environmental factors to change. They are: increased leaf temperature because of the increased heat load, increased water vapor pressure deficit because of the increased leaf temperature, and finally decreased intercellular CO_2 concentration because of increased CO_2 assimilation. After the stomata have responded to these changes in their environment, these factors may again be different. For example, if the stomata respond to light to a large degree, the intercellular CO_2 concentration could eventually be higher than before the increase in irradiance.

Stomatal responses to environmental variables often show interactions. For example, Hall and Kaufmann (8) found that stomata responded more strongly to humidity when the intercellular CO_2 concentration was above 200 $\mu\ell\ \ell^{-1}$ than when it was below 100 $\mu\ell\ \ell^{-1}$. Raschke (36) found that stomata of Xanthium strumarium in light of 340 w m^{-2} did not close in response to an increase in the CO_2 concentration unless the leaves had been fed abscisic acid. Similarly, he found that the stomata did not close after feeding the leaves abscisic acid unless there was CO_2 in the air. Heath and Russell (10) and Gaastra (7) found that stomata responded less to CO_2 at high irradiance than in darkness.

The stomatal response to light can be very complex. In 1932, Scarth (41) proposed that stomata respond indirectly to light. According to Scarth, an increase in irradiance causes an increase in the rate of CO_2 assimilation, which lowers the concentration of CO_2 in the intercellular spaces. Stomata respond to the lowered intercellular CO_2 concentration by opening. There is some evidence that stomata of Zea mays can respond

to light in this way (39), and this view has been favored by recent reviewers (24,35). This mechanism for stomatal response to light is feasible in C_4 plants where the CO_2 concentration drop across the stomata is large and the stomatal conductance is usually small. However, it cannot be very important in C_3 plants, which often have a CO_2 concentration drop of 30 $\mu\ell\ \ell^{-1}$ or even less (6) and very large stomatal conductances.

Heath and Russell (10) suggested that there might be "An indirect effect not operating by the reduction of the internal carbon dioxide in the guard cells, transmitted . . . from the mesophyll cells by some agent (chemical or electrical) as yet unknown" (p.290). Wong (54) also suggested that some type of messenger travels from the mesophyll to the epidermis such that the photosynthetic rate controls the stomatal conductance. The evidence in favor of this view is primarily the observation that stomatal conductance responds to many environmental factors in the same way as does photosynthesis. The photosynthetic and stomatal responses are so similar that the intercellular CO_2 concentration remains constant when environmental influences such as light are changed over a wide range.

The simplest explanation for stomatal responses to light, however, is that light absorbed in the guard cells themselves results in stomatal opening. The fact that stomata of epidermes stripped from the mesophyll can respond to light indicates that this mechanism of stomatal response to light occurs in at least some situations.

Wong et al. (53) measured stomatal responses to light and CO_2 in Eucalyptus pauciflora. They found that the stomatal conductance and the assimilation rate responded to changes in irradiance (in the range

of 0.25 to 2 mE m^{-2} sec^{-1}) in such a way that the intercellular CO_2 concentration remained constant. In this case, despite the stomatal sensitivity to CO_2 that they observed, the indirect stomatal response to light mediated by CO_2 played no role in the overall stomatal response to light.

Effect of Light Quality on Stomatal Opening

Stomata respond primarily to blue light (23). Voskresenskaya and Polyakov (50) found that when blue light was added to red light, the photosynthetic rate gradually went up until it was 2 to 3 times greater than in red light alone, even though the added blue light had an intensity of 1% that of the red. The enhancement of the CO_2 assimilation rate by blue light was correlated with an enhancement of transpiration, which was interpreted as an indication that the blue light effect was caused by stomatal opening.

The action spectrum of stomatal opening determined by Liebig (21) showed a relative effectiveness of blue:green:red of 168:42:100. Since she had calculated the absorption of a mixture of chlophyll a and b to be similar, she concluded that chlorophyll was the only pigment involved in light reception leading to stomatal opening. Heath (9) recalculated Liebig's data taking into account that a quantum of blue light has more energy than a quantum of red light and found that the relative quantum efficiency for stomatal opening was blue:green:red, 234:50:100. This relationship he felt was indicative of the participation of carotenoids or xanthophylls as well as chlorophyll.

Karvé (17) criticized Liebig's work and that of many other investigators because they used the "steady state" method. For this method,

the leaf is put into the light of known intensity and quality and the stomatal response is monitored until it no longer changes; a reading is then taken. Karvé's criticism was that this method does not take into account the power (i.e. dose) received by the leaf. He believed that the reading of the stomatal response to light should be taken after a certain time in the light, regardless of whether the stomata were still opening or not. In this way, the dose received by the leaf at each wavelength would be the same. However, some of the implicit assumptions of this view are unfounded. For example, there is no a priori reason to believe that the rate of stomatal opening is related to or controlled by the dose of light received by the stomata. If light is a signal for stomatal opening rather than an energy source, then the rate of stomatal opening need not depend on the light intensity or its effectiveness in causing stomatal opening. Karvé's action spectrum, nonetheless, was very similar to that of Liebig.

Kuiper (20) determined an action spectrum for the maintenance of stomatal opening with epidermal strips of Senecio odoris. He found that blue light was more than twice as effective as red light in maintaining stomatal opening.

Hsiao et al. (12) determined the action spectrum for Rb+ (as a tracer for K+) uptake as well as for stomatal opening. Stomatal opening and Rb+ uptake were well correlated and at low irradiance responded only to blue light. At a higher irradiance, some activity in the red became apparent.

Ogawa et al. (29) found the action spectrum for malate formation in sonicated epidermal strips to be similar to the action spectra already described except that the peak in the blue was about 10 times higher

than the peak in the red. Ogawa et al. (29) showed that a small amount of blue light added to a background of red light was much more effective than either the red light or the blue light alone. They postulated that there must be two photoreactions involved in the stomatal response to light: one mediated by chlorophyll and accounting for the red peak and part of the blue peak, and a separate blue light photoreaction. They measured the action spectrum of the blue light photoreaction by giving small amounts of blue light in a background of red light. In this way, the peak in the blue was not distorted by the fact that the proposed chlorophyll reaction would absorb some wavelengths of blue light but not others. The resultant action spectrum had two peaks, one at 460 nm and one at 380 nm, which they interpreted as evidence that a flavin is involved in the blue light response of stomata.

Brogårdh (2) also was able to show that stomatal responses to red and blue light are fundamentally different. He found that blue light caused a large, rapid increase in transpiration that quickly fell, i.e. an overshoot. Red light caused only a slow increase in transpiration rate. Johnsson et al. (14) found that this response was limited to grasses. Skaar and Johnsson (44) determined an action spectrum for the blue light induced transpiration response in Avena and found a broad peak of activity around 446 nm. Pretreatments (2) or a continuous background (44) of red light enhanced the blue light response.

In summary, most action spectra determined since 1940 have been in basic agreement that blue light is 2 to 10 times more effective in causing stomatal opening than red and that green light has very little, if any, effect. Although there is no direct evidence for it, most authors assume that the red peak is caused by the absorption of light

by chlorophyll and some (Liebig and Kuiper) believe that photosynthesis is sufficient to account for the entire action spectrum. Ogawa et al. (29) and Brogårdh (2) showed, by virtue of their observed "synergistic" action of red and blue light, that the blue and red photoreactions are fundamentally different. As a result, the overall action spectrum of stomatal opening probably does not correspond to any single photoreceptor absorption spectrum. What is needed is the determination of the action spectra of the separate photoreactions as Ogawa et al. (29) did for the blue light reaction.

Possible Effects of Light on Guard Cells

Most investigators assume that red light affects stomata via photosynthesis. For example, Brogårdh (2) proposed that the only effect of red light was indirect and mediated by changes in the intercellular CO_2 concentration. Clearly this view is wrong since three separate investigators found that red light was active in stomatal opening using strips of epidermis removed from the mesophyll (12,20,29). It is interesting to note, however, that the activity of red light relative to the activity of blue light was usually higher in intact leaves than in stripped epidermis. For example, Heath's (9) recalculation of Liebig's (21) data (intact leaves) gives a ratio of 0.4 to 1 while Ogawa et al. (29) (epidermis only) found a ratio of about 0.1 to 1. Part of the activity found for red light in intact leaves may very well be a CO_2-mediated effect.

It was been proposed that the effect of red light on guard cells is via photosynthesis in the guard cells. Guard cells contain chloroplasts while, in most species, the other epidermal cells do not. Guard cells of Paphiopedilum spp., however, do not contain chlorophyll (26) and

still respond to light. Von Mohl (25) suggested in 1856 that photosynthesis in the guard cells could produce the osmotica necessary for stomatal opening. Lloyd showed in 1908 (22) that stomata responded to light in CO_2-free air. He also found that the starch content in the guard cells decreased in the light and increased in the dark in air containing CO_2. This pattern is opposite to that which one would expect for a photosynthetic organ and opposite that which he found in the underlying mesophyll tissue of the leaves he was studying. From his experiments, Lloyd concluded that there was a stomatal response to light, but that photosynthesis occurring in the guard cells was of minor significance in stomatal function. Shaw and Maclachlan (43) found that the rate of carbon dioxide fixation was too low to account for the osmotic pressure increase necessary for stomatal opening. More recently, it has been shown that guard cells do not fix carbon dioxide by the reductive pentose pathway; they lack the ability to convert ribose-5-phosphate to RuBP (38). The CO_2 fixation that is always found associated with guard cells is carboxylation of PEP by PEP carboxylase (30,45,52) with the ultimate formation of malate and aspartate (31,38,42,51). The rate of CO_2 fixation by guard cells is usually (but not always {42}) stimulated by light (38,43,51).

Many attempts to determine whether or not chlorophyll is necessary for stomatal opening have involved the use of variegeted leaves, but as Virgin (48) pointed out, "so-called non-chlorophyllous parts of variegated plants contain small amounts of chlorophyll pigments" (p.184). Virgin (48) did find an albino mutant of barley that was devoid of chlorophyll and found no stomatal response to light. He also found (49) that stomata of etiolated leaves of wheat did not begin to respond to

light until they had been exposed to light for 2 to 3 hours, at which time chlorophyll was beginning to form in some of the treatments. However, stomatal responsiveness to light was not correlated with the chlorophyll content as he measured it.

Kuiper (20) found that stomata of epidermal strips floating on a solution of 10^{-5}M DCMU, a photosynthetic electron transport inhibitor, closed. Allaway and Mansfield (1) found that stomata of leaves fed CMU, which acts in the same way as DCMU, closed slightly but reopened when CO_2-free air was passed over the leaf. They criticized Kuiper's interpretation that photophosphorylation was involved in the stomatal response to light, since they felt that the mode of action of inhibitors of photosynthesis was to raise the intercellular CO_2 concentration. However, Kuiper had used epidermal strips and so the closure he observed could not have been the result of an increased intercellular CO_2 concentration. Perhaps of more importance is the fact that Kuiper used an incandescent lamp for illumination, which should provide primarily red light. Humble and Hsiao (13) showed that stomata of Vicia faba epidermal strips closed only slightly in the presence of DCMU in a nitrogen atmosphere. However, when Cl-CCP, an uncoupler of phosphorylation, and DCMU were both present, the stomata closed. From these experiments they concluded that "the energy derived from photosynthetic cyclic electron flow can be sufficient and possibly necessary for K+ uptake and stomatal opening in the light" (p.487). This conclusion, however, is no longer certain in view of the facts that there are probably two separate photoreactions responsible for the stomatal response to light and that Cl-CCP upcouples oxidative phosphorylation as well as photophosphorylation.

The stomatal response to blue light is clearly not mediated by changes in the CO_2 concentration in the intercellular spaces (23). Two effects of blue light that may be operating in the guard cells are the blue light stimulation of respiration (15,18,19) and the blue light stimulation of PEP carboxylase activity (15,16,28). Both of these responses have action spectra (15,18,32) similar to that determined by Ogawa et al. (29) for the blue light response of stomata.

MATERIALS AND METHODS

Analysis of Slopes

In order to separate and measure stomatal responses to light, I used the method described by Farquhar et al. (6), stated briefly here. For the purposes of this investigation, I say that stomatal conductance (g) is a function of irradiance (I) and intercellular CO_2 concentration (c_i) in order to separate responses mediated by CO_2 from those that are not. Other factors known to affect stomatal conductance, such as leaf temperature and humidity, were held nearly constant and so are not considered in this analysis. Therefore the following can be written:

$$g=g(c_i,I) \tag{1}$$

Differentiating equation 1 gives:

$$dg=\left(\frac{\partial g}{\partial c_i}\right)_I dc_i + \left(\frac{\partial g}{\partial I}\right)_{c_i} dI \tag{2}$$

The partial differentials in equation 2 can be evaluated by determining the slopes of the curves representing the relationships between stomatal conductance and intercellular CO_2 concentration or light intensity. Since the intercellular CO_2 concentration is a function of assimilation and stomatal conductance, we can write:

$$dc_i=\left(\frac{\partial c_i}{\partial A}\right)_g dA + \left(\frac{\partial c_i}{\partial g}\right)_A dg \tag{3}$$

Assimilation is a function of irradiance and intercellular CO_2 concentration if temperature, etc. is held constant so:

$$dA=\left(\frac{\partial A}{\partial c_i}\right)_I dc_i + \left(\frac{\partial A}{\partial I}\right)_{c_i} dI \tag{4}$$

By substituting equation 4 into equation 3 and the result into equation 2, we derive the following:

$$\frac{dg}{dI} = \frac{\overbrace{\left(\frac{\partial g}{\partial I}\right)_{c_i}\left\{1-\left(\frac{\partial A}{\partial c_i}\right)_I\left(\frac{\partial c_i}{\partial A}\right)_g\right\}}^{\text{direct response}} + \overbrace{\left(\frac{\partial g}{\partial c_i}\right)_I\left(\frac{\partial c_i}{\partial A}\right)_g\left(\frac{\partial A}{\partial I}\right)_{c_i}}^{\text{indirect response}}}{1-\left(\frac{\partial A}{\partial c_i}\right)_I\left(\frac{\partial c_i}{\partial A}\right)_g-\left(\frac{\partial g}{\partial c_i}\right)_I\left(\frac{\partial c_i}{\partial g}\right)_A} \qquad (5)$$

Equation 5 shows the response of stomatal conductance to light as the sum of two terms. The first term describes the effects of light that are not mediated by CO_2 (including mesophyll-dependent and mesophyll-independent effects) and will be referred to as the direct stomatal response to light. The second term describes the indirect effect of light on stomata mediated by changes in the intercellular CO_2 concentration.

In a similar way, the response of assimilation to light can be derived to show the direct response of assimilation to light and the effect of the increased availability of CO_2 caused by the direct stomatal response to light:

$$\frac{dA}{dI} = \frac{\overbrace{\left(\frac{\partial A}{\partial I}\right)_{c_i}\left\{1-\left(\frac{\partial g}{\partial c_i}\right)_I\left(\frac{\partial c_i}{\partial g}\right)_A\right\}}^{\text{direct response}} + \overbrace{\left(\frac{\partial A}{\partial c_i}\right)_I\left(\frac{\partial c_i}{\partial g}\right)_A\left(\frac{\partial g}{\partial I}\right)_{c_i}}^{\text{indirect response}}}{1-\left(\frac{\partial A}{\partial c_i}\right)_I\left(\frac{\partial c_i}{\partial A}\right)_g-\left(\frac{\partial g}{\partial c_i}\right)_I\left(\frac{\partial c_i}{\partial g}\right)_A} \qquad (6)$$

Most of the partials in equations 5 and 6 are easy to determine, but the partial $(\partial g/\partial I)_{c_i}$ is not. Since a change in irradiance affects assimilation, c_i may vary as irradiance is changed. Two approaches were used to overcome this problem. First, an inhibitor of photosynthesis that did not affect stomatal function was added to the

transpiration stream. When the net exchange of CO_2 fell to zero, changes in irradiance no longer affected the concentration of CO_2 in the leaf. The second approach was to determine the way that stomatal conductance varied with c_i at various irradiances, then to choose one c_i and determine what the conductance would have been at each irradiance. A graph of conductance versus irradiance at constant c_i can then be constructed.

Calculations were done at two or four arbitrarily chosen light levels. For calculations made when a photosynthesis inhibitor was present, an intercellular CO_2 concentration of 250 $\mu\ell\ \ell^{-1}$ was chosen as being representative for C_3 plants. In other calculations, c_i was determined from a plot of c_i versus ambient CO_2 concentration, assuming an ambient CO_2 concentration of 320 $\mu\ell\ \ell^{-1}$.

It was not possible to use this approach to separate mesophyll-dependent from mesophyll-independent stomatal responses to light, since we must begin with the following equation:

$$dg = \left(\frac{\partial g}{\partial c_i}\right)_{I,A} dc_i + \left(\frac{\partial g}{\partial I}\right)_{c_i,A} dI + \left(\frac{\partial g}{\partial A}\right)_{I,c_i} dA$$

The first two partial differentials can be evaluated when photosynthesis is eliminated by feeding an inhibitor, but there is no satisfactory way to evaluate the third partial differential.

Gas Analysis

The gas analysis system consisted of five URAS II (Hartmann und Braun, Frankfurt a.M., W. Germany) gas analyzers, five specially constructed temperature-controlled leaf chambers, a digital voltmeter, and a minicomputer.

Lab-compressed air was passed through two columns of soda lime to remove the CO_2. After the air was humidified, the CO_2 concentration was

adjusted by allowing 1 or 5% CO_2 to flow into the air through capillaries of varying resistance. The air was then passed through a glass condenser. The temperature of the condenser was maintained by a constant temperature water bath and was measured with a thermocouple. The air stream was split and the air stream passing over the leaf was adjusted to 50 l hr^{-1} over each surface. The leaves were mounted in aluminum chambers through which water was pumped to control the air temperature. The petioles of the leaves dipped into beakers containing water or solutions to be fed to the leaves. The chambers allowed 2.44 cm^2 leaf area to be exposed to the air stream. A small copper-constantan thermocouple was pressed against the nonilluminated surface of the leaf. Those parts of the leaves not covered by the chambers were trimmed off to prevent excessive water loss (34). The molar fluxes of CO_2 and H_2O for both the upper and lower leaf surfaces were measured with four gas analyzers used as differential analyzers to increase the sensitivity. For X. strumarium, P. frutescens, and G. hirsutum, the temperature of the leaf chamber was kept at 23 C. For Z. mays, it was 27 C and for P. vulgaris it was 21 C.

Assimilation and evaporation rates, conductance (stomatal and boundary layer together), and intercellular CO_2 concentration were calculated by computer. The intercellular CO_2 concentration (c_i) was calculated using the following equation:

$$c_i = c_a - 1.6A\ r$$

where c_a is the CO_2 concentration in the air passing over the leaf, A is the assimilation rate, and r is the resistance to water vapor loss of the stomata and the boundary layer. The factor 1.6 is the ratio of diffusivities of water vapor and CO_2 in the air. The units used for gas exchange parameters are consistent with those of Cowan (3) and Farquhar et al. (6).

Light

White light was provided by an Osram XBF 6000 w water-cooled xenon arc lamp shining through a Corning no. 4600 infrared-absorbing glass filter. The irradiance was reduced with neutral density Plexiglas filters (no. 800 and 838, Rohm und Haas, Darmstadt, Germany). Irradiance was monitored with a silicon cell in the same plane as the leaf chambers that had been calibrated with an Eppley pyranometer. White light measurements are reported in w m^{-2} but can be converted to quantum flux by the conversion factor 4.5 μE sec^{-1}=1 w. This conversion factor can only be considered approximate since iron oxide from the cooling water accumulated on the lamp over the course of the day and changed the spectral distribution of the light.

Monochromatic light was produced by air-cooled xenon arc lamps and focused through band pass filters to select about a 150 nm band of light. This light passed through a water-cooled interference filter, then into a box from which all other light was excluded (33). The interference filters had a half-band width of 20 nm except for the experiment reported in Figure 13, for which a different set of interference filters with a half-band width of 12 nm was used. Type DAL (20 nm half-band width) or DIL (12 nm half-band width) tandem filters made by Schott, Mainz, W. Germany were used. Two 2.5 kw lamps were set up to shine into one box. Inside, the leaf chamber was mounted on an optical bench and could be positioned under either lamp. With this setup, the wavelength of the light shining on the leaf could be changed in less than three seconds by sliding the chamber from one lamp to the other. A 6.5 kw lamp was available when very high intensities were needed. The leaf chamber used for monochromatic light work was fitted with a beam splitter

(a microscope slide fixed at a 45 degree angle to the light beam) which reflected about 10% of the light to two silicon cells. These silicon cells were calibrated by the following method: An intermediate pair of silicon cells was calibrated against an Eppley thermopile (model D-3, Eppley Lab., Newport, RI), then put into the leaf chamber. The silicon cells of the beam splitter were then calibrated against the intermediate pair of silicon cells so that the signal from the beam splitter silicon cells could be converted directly into quantum flux inside the leaf chamber. This calibration was done for each wavelength of light used.

Plants

Xanthium strumarium L. (Chicago strain) and Perilla frutescens (L.) Britt. (red-leaved Perilla) were grown in a gravel-soil mixture in a greenhouse. The natural photoperiod was extended to 20 hours with 0.3 w m^{-2} light from Sylvania Gro-lux fluorescent tubes. Temperature maxima were between 23 C and 29 C; the relative humidity was between 70 and 80%. For X. strumarium, the fifth or sixth leaf from the apex of 6 to 12 week old plants was used. For P. frutescens, the youngest fully expanded leaf pair from one month old plants was used for analysis.

X. strumarium plants used for the monochromatic light experiments were grown in a growth chamber in an attempt to produce uniform leaves. A 20 cm X 2.5 cm wick was placed in a pot so that about 15 cm was in contact with the soil and 5 cm dipped into a gravel bed containing distilled water. The temperature of the growth chamber was 24 C/20 C, day/night, daylength was 20 hours, and the highest of three levels of irradiance was 230 w m^{-2}. The RH was 75%.

Zea mays L. cv. Michigan 500 was grown in a soil-perlite mixture in a growth chamber. The temperature was 30 C/20 C, day/night; the RH was

about 75%. The day length was 12 hours. The highest of three levels of irradiance was 230 w m^{-2} between 400 and 700 nm. The fifth leaf (by emergence) of 3 to 4 week old plants was used for analysis. Gossypium hirsutum L. cv. Acala SJ-1 was grown in a soil-perlite mixture in a growth chamber. The temperature was 32 C/22 C, day/night; the RH was about 75%. The highest of three levels of irradiance was 230 w m^{-2} and day length was 20 hours. The fifth leaf from the apex of 1 to 2 month old plants was used for analysis. Phaseolus vulgaris L. cv. Montcalm was grown in a gravel-vermiculite mixture in a growth chamber. The temperature was 23 C/20 C, day/night; the RH was about 80%. Irradiance was 180 w m^{-2} between 400 and 700 nm. The primary leaves of plants 10 to 15 days old were used for analysis.

Procedure

For experiments where cyanazine was used as an inhibitor of photosynthesis, the leaves were put into the gas analysis chambers in the morning and cyanazine was added to the irrigation stream. The CO_2 concentration was held at 250 $\mu\ell\ \ell^{-1}$ and the light level was about 280 w m^{-2} until the stomata were open and photosynthesis was reduced enough that the intercellular CO_2 concentration was within 10 $\mu\ell\ \ell^{-1}$ of the CO_2 concentration in the air (usually about 11:00 a.m., 2 hours after the light was turned on). Except where noted, stomatal behavior was followed as the CO_2 concentration was increased from 0 or light was decreased from about 280 w m^{-2}. This procedure was followed for experiments described by Figures 1, 2, 4-6, 8, and 10 to eliminate the small effect of hysteresis on the results (see Figure 9).

RESULTS

Measurement of Direct and Indirect Stomatal Responses to White Light

The photosynthesis inhibitor cyanazine was added to the irrigation water of leaves of Xanthium strumarium and Gossypium hirsutum. A concentration of 10^{-5}M cyanazine reduced photosynthesis to the compensation point so that there was no measurable CO_2 exchange. Under these conditions, the intercellular CO_2 concentration is equal to the CO_2 concentration in the air around the leaf and a change in the irradiance does not cause a change in the intercellular CO_2 concentration. Stomata of X. strumarium were apparently unaffected by cyanazine, while stomata of G. hirsutum became more sensitive to CO_2 (Figure 1). Atrazine and DCMU were also tested on X. strumarium leaves and found to be suitable. (This method of analyzing stomatal responses to light could not be used on leaves of Zea mays or Commelina communis because a high concentration of cyanazine was required to inhibit photosynthesis within 2 hours. As a result, respiration was stimulated by the end of the experiment, so that the intercellular CO_2 concentration was no longer independent of changes in stomatal conductance.) The total stomatal response to light for X. strumarium and G. hirsutum was calculated for irradiances of 50 and 300 w m^{-2} (Table 1). For these calculations, an intercellular CO_2 concentration of 250 $\mu\ell\ \ell^{-1}$ was chosen (an average value for C_3 plants). In X. strumarium, the direct stomatal response to light accounted for 79% of the total response at low irradiance and 55% at high light, while in G. hirsutum nearly 90% of the response to light was a direct response

Figure 1. Effect of cyanazine on stomatal response to CO_2.

Detached, trimmed leaves were put into 10 ml beakers containing 10^{-5}M cyanazine. The light intensity was 290 w/m^2. Each point is the sum of the conductance of the adaxial and abaxial leaf surfaces.

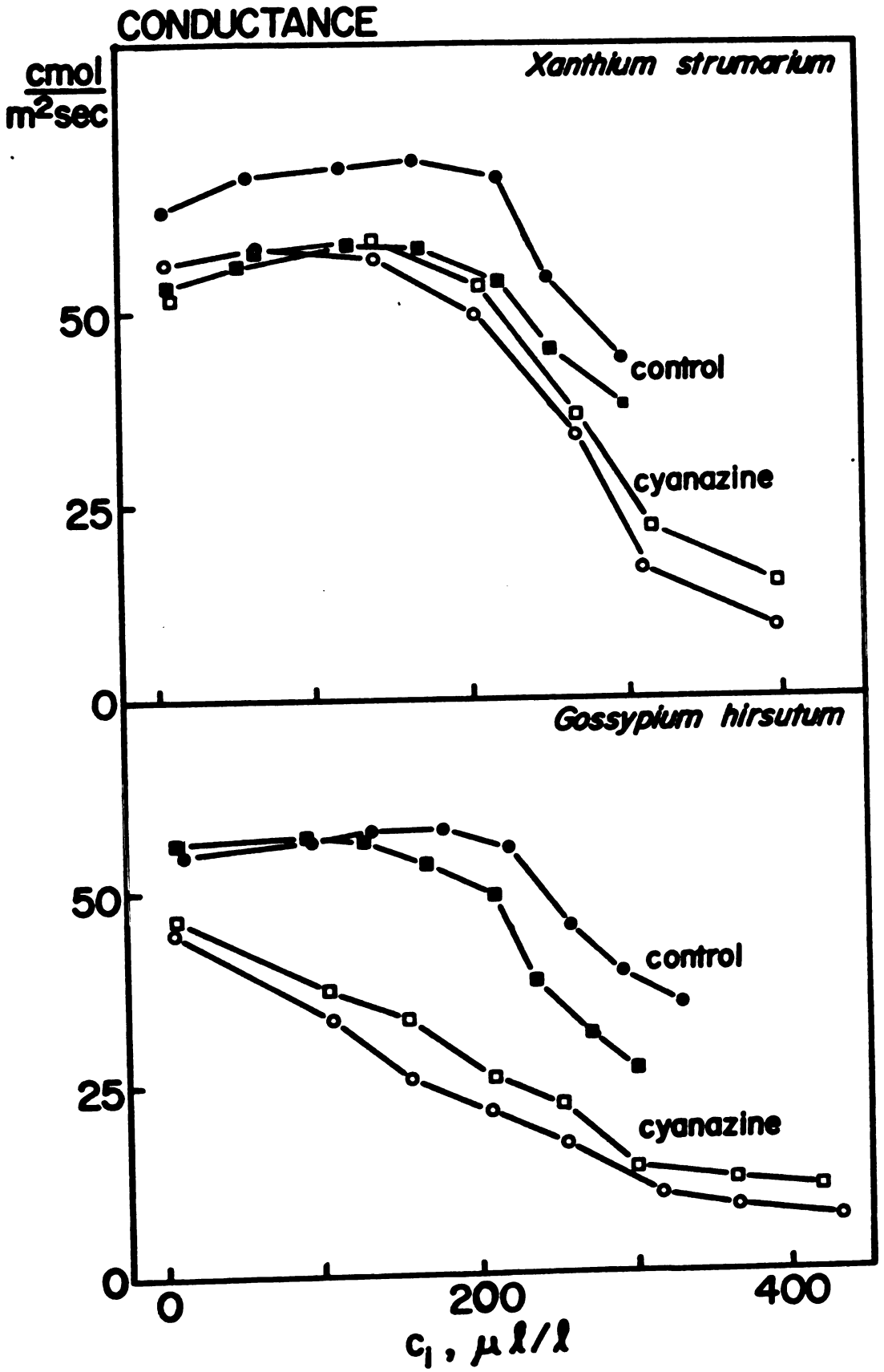

CONDUCTANCE
cmol
m²sec
Xanthium strumarium
50
25
0
control
cyanazine
Gossypium hirsutum
50
25
0
control
cyanazine
0
200
400
c_l , µl/l

Table 1. Effect of light on stomatal conductance and assimilation using cyanazine treated leaves.

Derivations of dg/dI and dA/dI are given in the Materials and Methods section. All partial derivatives not involving photosynthesis used in the calculations were determined with leaves fed cyanazine. The intercellular CO_2 concentration was 250 µℓ/ℓ. Data for X. strumarium were derived in part from Figures 5 and 8.

Species	I	dg/dI	direct stomatal response to light	dA/dI	effect of direct stomatal response to light
	w/m^2	$\frac{mmol}{w\ sec}$	% of dg/dI	$\frac{mmol}{w\ sec}$	% of dA/dI
Xanthium	300	67	55	62	3
strumarium	50	610	79	100	14
Gossypium	300	60	87	18	13
hirsutum	50	250	89	54	13

at both light levels. (The increased sensitivity of G. hirsutum stomata to CO_2 caused by cyanazine results in an overestimation of the indirect stomatal response to light.) The proportion of the assimilation response to light caused by the direct stomatal response to light never exceeded 14%, indicating that photosynthesis was not greatly limited by stomatal restriction of the CO_2 supply in either species once stomata had opened.

Since it generally required 1 day to evaluate the stomatal response to CO_2 at one irradiance, the plant material had to behave consistently for 4 days in order for the graphic analysis method to be useful. (Experiments with C. communis and Amaranthus powelli failed because the day to day variations in stomatal responses were too large.) The intercellular CO_2 concentrations used for these calculations were determined from graphs of intercellular versus ambient CO_2 concentration for each irradiance, given an ambient CO_2 concentration of 320 $\mu\ell\ \ell^{-1}$. Table 2 shows the stomatal responses to light at four light levels for four plant species. The results for Perilla frutescens and Phaseolus vulgaris were similar to those in Table 1 in that most of the stomatal response to light was caused by the direct response to light and the proportion of the assimilation response to light caused by the direct stomatal response to light was small. At high irradiance, the proportion of the increase in assimilation caused by the stomatal response to light in P. frutescens and Z. mays was 100%, but this only occurred because assimilation was saturated with respect to light and does not mean that stomata had a large effect on photosynthesis. In Z. mays at low irradiance the indirect stomatal response to light was stronger than the direct response. (The calculations could not be done for Z. mays in darkness since the stomatal conductance was so small that gas exchange

Table 2. Effect of light on stomatal conductance and assimilation of leaves.

Derivations of dg/dI and dA/dI are given in the Materials and Methods section. The intercellular CO_2 concentrations corresponded to those that would occur in an ambient CO_2 concentration of 320 µl/l and are listed in Table 4. The data were derived in part from Figures 3 and 4.

Species	I	dg/dI	direct stomatal response to light	dA/dI	effect of direct stomatal response to light
	w/m^2	$\frac{mmol}{w\ sec}$	% of dg/dI	$\frac{mmol}{w\ sec}$	% of dA/dI
Perilla frutescens	285	2.8	100	0.06	100
	155	46.4	94	17.4	4
	45	127	82	29.0	3
	0	163	79	29.5	0
Phaseolus vulgaris	280	66.4	99	6.2	37
	160	100	92	25.7	15
	45	133	65	53.2	2
	0	112	66	63.6	0
Zea mays	620	2.5	100	0.1	100
	265	25.3	81	10.9	13
	145	60.2	49	51.9	3
	50	489	31	113	0
Xanthium strumarium	265	0.24	0	14.5	0
	155	53.0	95	34.3	2
	45	231	96	60.4	0
	0	2230	98	99.0	0
Xanthium strumarium + 10^{-6}M (±)-ABA	285	29.1	73	16.6	4
	150	90.6	76	43.5	2
	45	249	78	87.6	1
	0	191	97	56.0	0

could not be measured accurately.) However, as the irradiance increased so did the relative importance of the direct effect of light on the stomata. The X. strumarium plants used for these experiments were less sensitive to CO_2 than were those used for the experiments reported in Table 1. This was reflected in the large proportion of the light response caused directly. At the highest irradiance the stomatal response to light was saturated and so the apparent complete indirect response to light is meaningless. The addition of a low concentration of ABA to the irrigation water sensitized the stomata to CO_2, which increased the relative importance of the indirect response to light mediated by CO_2.

The partial differentials used for evaluations presented in Table 2 are given in Table 3. The partial differential $(\partial g/\partial c_i)_I$ describes the stomatal sensitivity to CO_2. For the C_3 plants, increased irradiance caused decreased stomatal sensitivity to CO_2 (excluding values obtained in darkness). For the C_4 plant Z. mays, however, the stomatal sensitivity to CO_2 was constant over the range of 145 to 620 w m^{-2}. When X. strumarium was fed a low concentration of ABA, the stomatal sensitivity to CO_2 was greatly increased. Abscisic acid did not cause large changes in any other partial differential, indicating that the only effect of ABA on the light response of stomata was via its action on the CO_2 sensitivity of stomata.

The intercellular CO_2 concentration used in the calculations listed in Tables 2 and 3 are given in Table 4 along with the assimilation rates and stomatal conductances. As the irradiance increased, the intercellular CO_2 concentration calculated for X. strumarium decreased. This was not true, however, for the other species. For example, for

Table 3. Values for partial differentials.

The first partial differentials were evaluated according to the following equations: $(\partial c_i/\partial g)_A = 1.6A/g^2$ and $(\partial c_i/\partial A)_g = -1.6/g$. All of the other partial differentials were evaluated by determining the slopes of the curves representing the relationships described by the partial differentials. The intercellular CO_2 concentrations, assimilation rates, and stomatal conductance are listed in Table 4.

Species	I	$(\partial c_i/\partial g)_A$	$(\partial g/\partial c_i)_I$	$(\partial g/\partial I)_{c_i}$
	w/m^2	$\frac{\mu m^2 \text{ sec}}{mol}$	$\frac{mol}{m^2 \text{ sec}}$	$\frac{mmol}{w \text{ sec}}$
Perilla frutescens	285	163	-50	2.8
	155	145	-200	45
	45	252	-550	118
	0	-473	-175	118
Phaseolus vulgaris	280	128	-300	68
	160	218	-530	102
	45	378	-630	106
	0	-1094	-105	66
Zea mays	620	470	-1150	3.8
	265	605	-1120	33
	145	898	-1150	58
	50	-1333	-450	60
Xanthium strumarium	265	60	-62	0
	155	48	-280	51
	45	19	-400	224
	0	-53	-330	2140
Xanthium strumarium + 10^{-6}M (±)-ABA	285	83	-1800	24
	150	81	-1550	77
	45	149	-650	212
	0	-272	-30	183

Table 3 continued

Species	I	$(\partial c_i/\partial A)_g$	$(\partial A/\partial c_i)_I$	$(\partial A/\partial I)_{c_i}$
	w/m^2	$\frac{m^2\ sec}{mol}$	$\frac{mmol}{m^2\ sec}$	$\frac{\mu mol}{w\ sec}$
Perilla frutescens	285	-7.4	15	0
	155	-7.8	11	180
	45	-16.8	3	300
	0	-61.5	0	300
Phaseolus vulgaris	280	-4.9	32	40
	160	-7.3	22	250
	45	-17.4	3	540
	0	-50	0	640
Zea mays	620	-6.8	9	0
	265	-7.8	13	100
	145	-10.8	7	520
	50	-26.7	0	1130
Xanthium strumarium	265	-2.7	39	160
	155	-2.8	25	360
	45	-3.6	4	610
	0	-14.5	0	990
Xanthium strumarium + 10^{-6}M (±)-ABA	285	-3.2	48	180
	150	-3.7	18	450
	45	-10.7	2	890
	0	-38.1	0	560

Table 4. Intercellular CO_2 concentration, stomatal conductance, and assimilation rate at four light levels.

Some values were obtained by interpolation, so that all values correspond to those that would occur in a constant ambient CO_2 concentration of 320 µℓ/ℓ.

Species	I w/m^2	c_i µℓ/ℓ	g $cmol/m^2$ sec	A $\mu mol/m^2$ sec
Perilla frutescens	285	285	21.5	4.7
	155	293	20.5	3.8
	45	293	9.5	1.4
	0	326	2.6	-0.2
Phaseolus vulgaris	280	284	32.4	8.4
	160	278	22.0	6.6
	45	278	9.2	2.0
	0	356	3.2	-0.7
Zea mays	620	215	23.4	16.1
	265	198	20.5	15.9
	145	198	14.8	12.3
	50	236	6.0	3.0
Xanthium strumarium	265	288	58.6	12.8
	155	293	57.5	10.0
	45	312	44.7	2.4
	0	326	11.0	-0.4
Xanthium strumarium + 10^{-6}M (±)-ABA	285	277	50.5	13.2
	150	288	43.2	9.4
	45	303	15.0	2.1
	0	333	4.2	-0.3

P. frutescens, at 155 w m^{-2} the intercellular CO_2 concentration was the same as at 45 w m^{-2}, and in both P. vulgaris and Z. mays the intercellular CO_2 concentration was higher at the highest irradiance than at an intermediate light intensity.

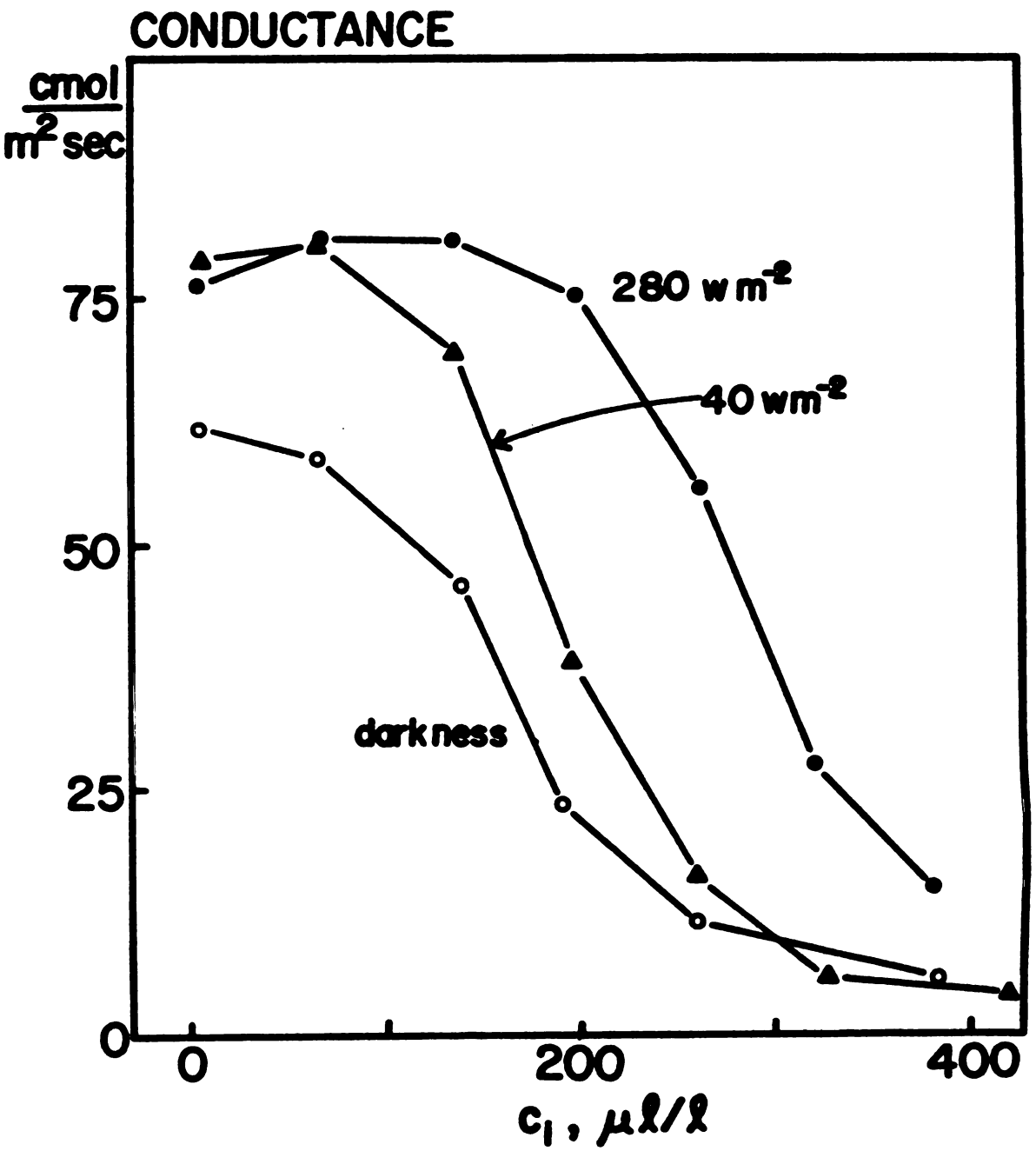
The intercellular CO_2 concentrations in Table 4 were obtained by interpolation between data points on a graph of intercellular CO_2 concentration versus ambient CO_2 concentration. The intercellular CO_2 concentrations calculated directly from the assimilation rates and stomatal conductances given in Table 4 differ from those listed by as much as 9 $\mu\ell\ \ell^{-1}$ (P. vulgaris at 45 w m^{-2}). However, twelve of the values differ by 2 $\mu\ell\ \ell^{-1}$ or less. The intercellular CO_2 concentrations listed in Table 4 are more conservative than those obtained by calculations based on the assimilation rates and stomatal conductances; the intercellular CO_2 concentration at the highest irradiance is 6 and 17 $\mu\ell\ \ell^{-1}$ higher than at an intermediate irradiance for P. vulgaris and Z. mays respectively, rather than 7 and 23 $\mu\ell\ \ell^{-1}$. Nevertheless, the uncertainty in the determination of intercellular CO_2 concentration indicates that the increase in intercellular CO_2 concentration with an increase in irradiance may not be significant in P. vulgaris, but certainly is in Z. mays.

Interaction between Stomatal Responses to White Light and CO_2

The response of stomata of greenhouse-grown X. strumarium to CO_2 varies, although most of the time they are insensitive to CO_2 in the light. Stomata of leaves used for the experiments with cyanazine (done in the fall of 1978) were sensitive to CO_2. Even so, light obviously reduced the stomatal response to CO_2, especially when the CO_2 concentration was between 150 and 250 $\mu\ell\ \ell^{-1}$ (Figure 2). Stomata of leaves

Figure 2. Stomatal response to CO_2 of leaves of Xanthium strumarium fed cyanazine at three irradiance levels.

Detached, trimmed leaves were put into 10 ml beakers containing 10^{-5}M cyanazine. Each curve represents the average of the sum of the conductance of the abaxial and adaxial leaf surfaces for four leaves.


CONDUCTANCE
cmol
m² sec
75
50
25
0
0
200
400
280 w m⁻²
40 w m⁻²
darkness
c_i , µl/l

used in later experiments (February 1979, Table 2) were insensitive to CO_2 in the light, in agreement with earlier observations (34), but in darkness or low light intensities, the stomata did respond to CO_2 (Figure 3). The pattern of decreasing stomatal response to CO_2 with increasing irradiance was also observed in P. frutescens and P. vulgaris (Figure 4). Stomata of Poa pratensis cv. Merion and Triticum aestivum cv. Genesee were also found to be insensitive to CO_2 in strong light (data not shown).

In Figure 5, the response to light of stomata of X. strumarium leaves fed cyanazine is shown at three concentrations of CO_2. The initial slope of the curve representing the relationship between conductance and irradiance was steepest in CO_2-free air. Similar results were obtained with G. hirsutum. These experiments could not be performed with other species since the inhibitor of photosynthesis could not be used.

Effect of ABA, Humidity, and Leaf Temperature on the Stomatal Response to White Light

Stomatal conductance at light saturation was lower in ABA-treated leaves than in control leaves of X. strumarium fed cyanazine (inset Figure 6), but the pattern of the relationship between conductance and irradiance was not affected. The normalized curves representing control, 10^{-7}, and 10^{-6}M (±)-ABA-fed leaves were indistinguishable from each other (Figure 6).

Changing the leaf temperature or the humidity affected the stomatal response to light in a similar manner. Lowering the leaf temperature from 22C to 15.5C or raising the vapor pressure deficit from 8 ml l^{-1} to 17.5 ml l^{-1} decreased the conductance at light saturation by about half, but the normalized curves were virtually indistinguishable from

Figure 3. Stomatal response to CO_2 of Xanthium strumarium (without cyanazine) at four irradiance levels.

Detached, trimmed leaves were used. Each curve represents the average of the sum of the conductance of the abaxial and adaxial leaf surfaces for four leaves.

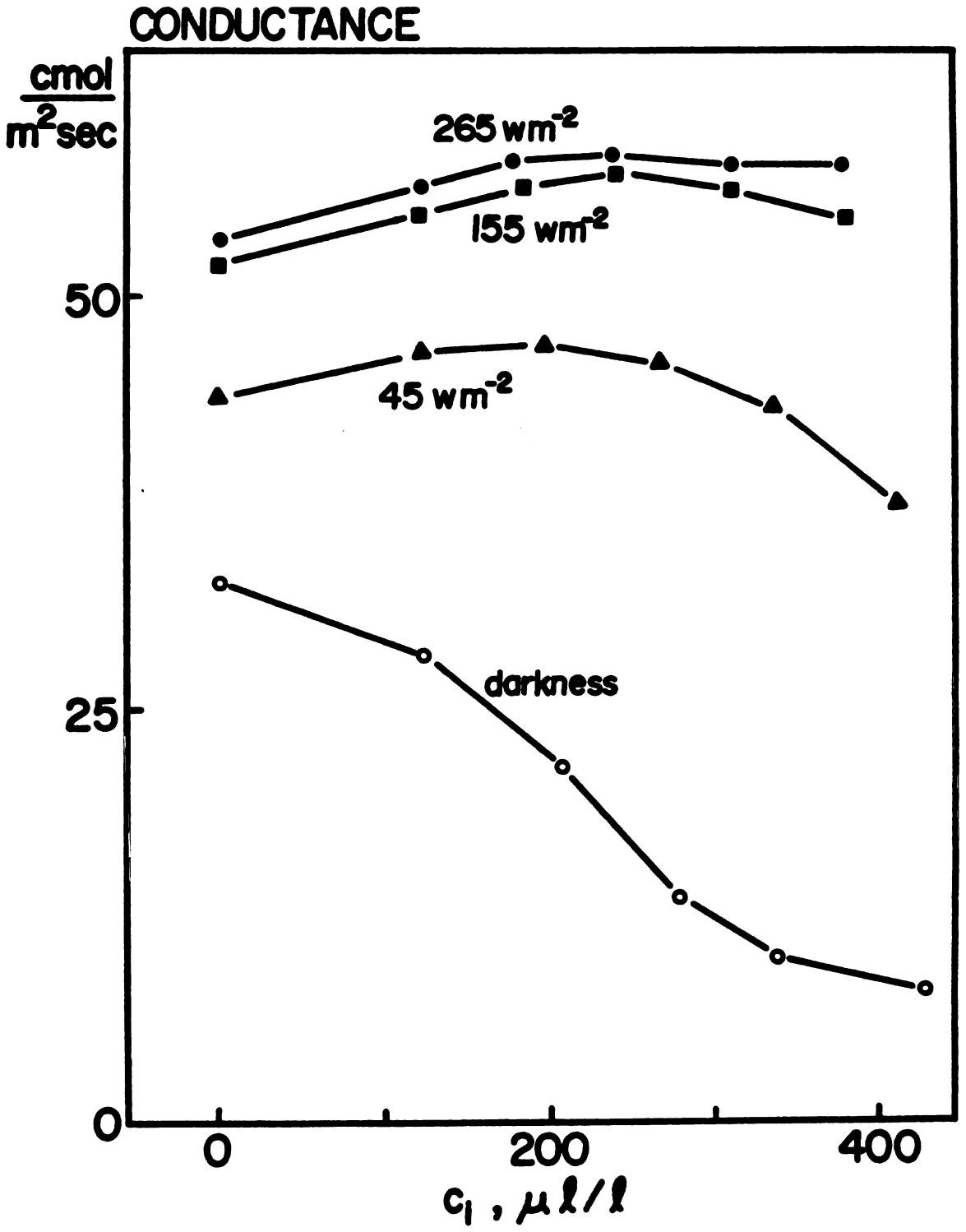

CONDUCTANCE
cmol
m²sec
265 wm⁻²
155 wm⁻²
45 wm⁻²
darkness
50
25
0
0
200
400
c_i , µl/l

Figure 4. Stomatal response to CO_2 at various irradiance levels for Perilla frutescens, Phaseolus vulgaris, Gossypium hirsutum, and Zea mays. Detached, trimmed leaves were used. Cyanazine was present only with Gossypium hirsutum. Each curve represents the average of the sum of the conductance of the abaxial and adaxial leaf surfaces for four leaves. □=620 w/m^2; ●=265-285 w/m^2; ■=145-160 w/m^2; ▲=45-50 w/m^2; ○=darkness.

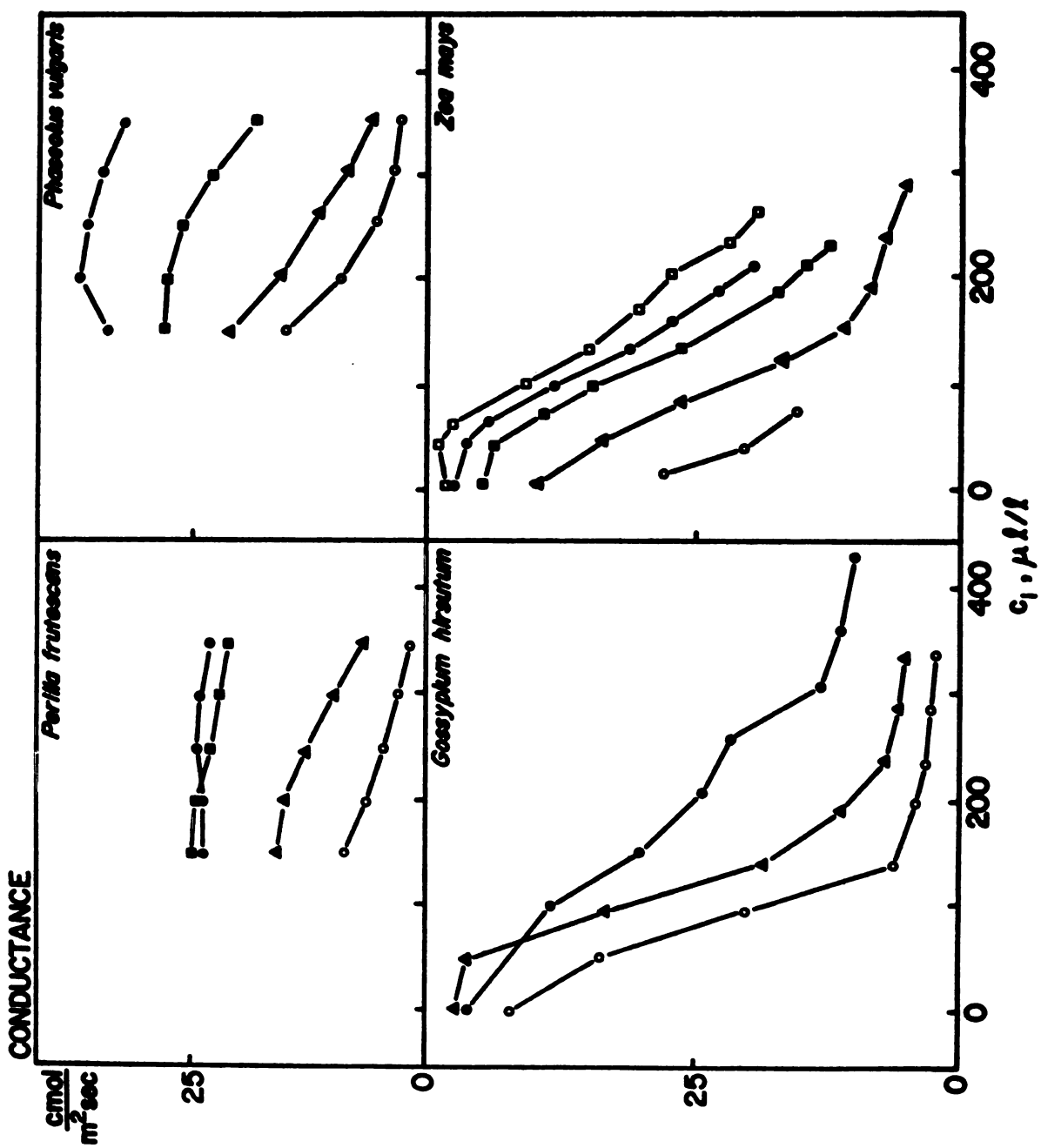

CONDUCTANCE
cmol/m²sec
25
0
Perilla frutescens
Phaseolus vulgaris
Gossypium hirsutum
Zea mays
0
200
400
c_i , µl/l

Figure 5. Stomatal response to light of leaves of Xanthium strumarium fed cyanazine at three concentrations of CO_2.

Detached, trimmed leaves were put into 10 ml beakers with 10^{-5}M cyanazine. Each curve is the average of the sum of the conductance of the abaxial and adaxial leaf surfaces for four leaves.

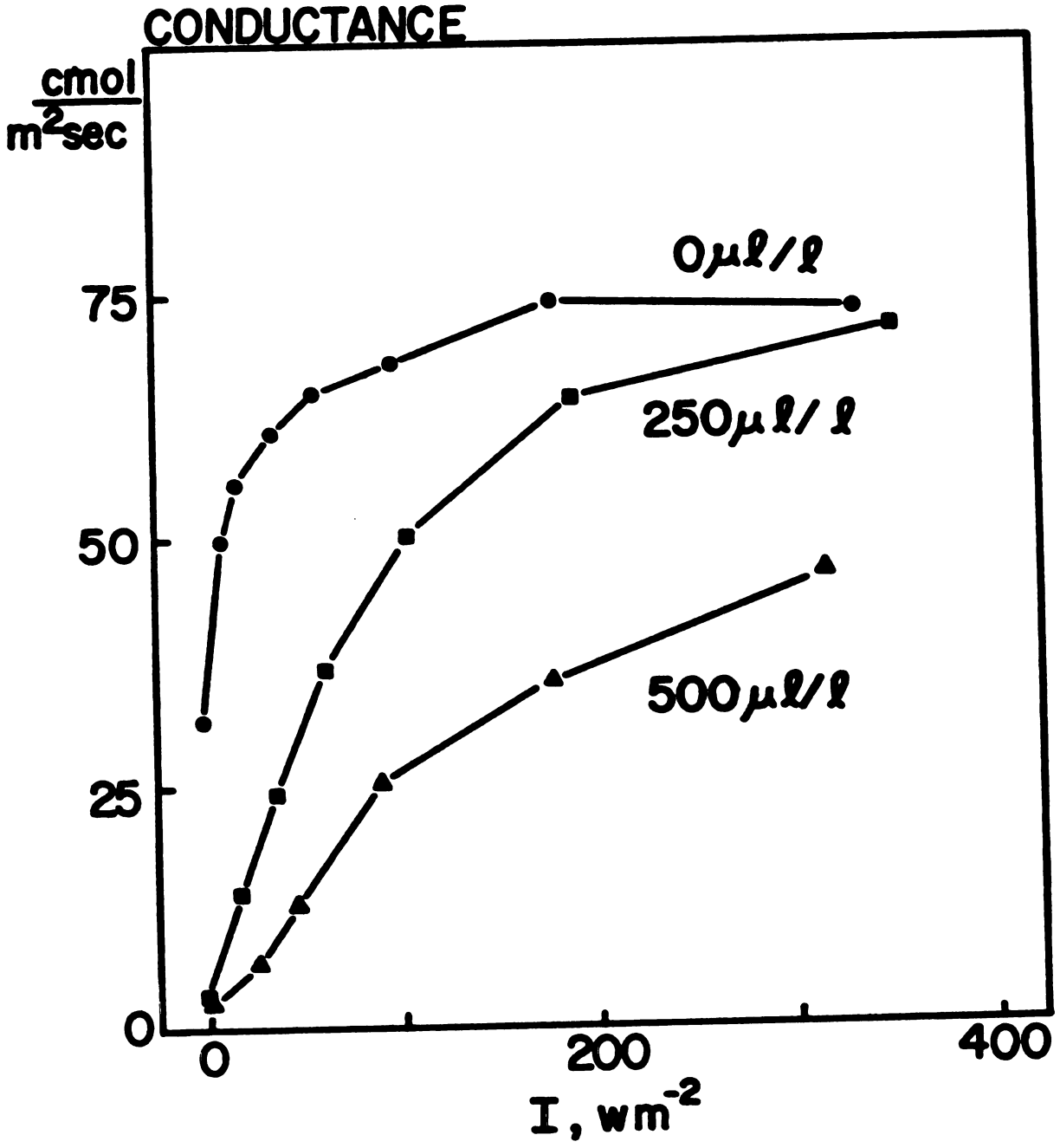

CONDUCTANCE
cmol
m²sec
0µl/l
250µl/l
500µl/l
75
50
25
0
0
200
400
I, wm⁻²

Figure 6. Stomatal response to light of leaves of Xanthium strumarium fed cyanazine and abscisic acid.

Detached, trimmed leaves were put into 10 ml beakers with 10^{-5}M cyanazine and the indicated concentration of abscisic acid (ABA). Each point is the sum of the conductance of the adaxial and abaxial leaf surfaces for one leaf. The data are presented as percentages of the conductance measured at the highest light intensity; the absolute data are shown in the inset. The CO_2 concentration in the ambient air and inside the leaf was 100 µℓ/ℓ.

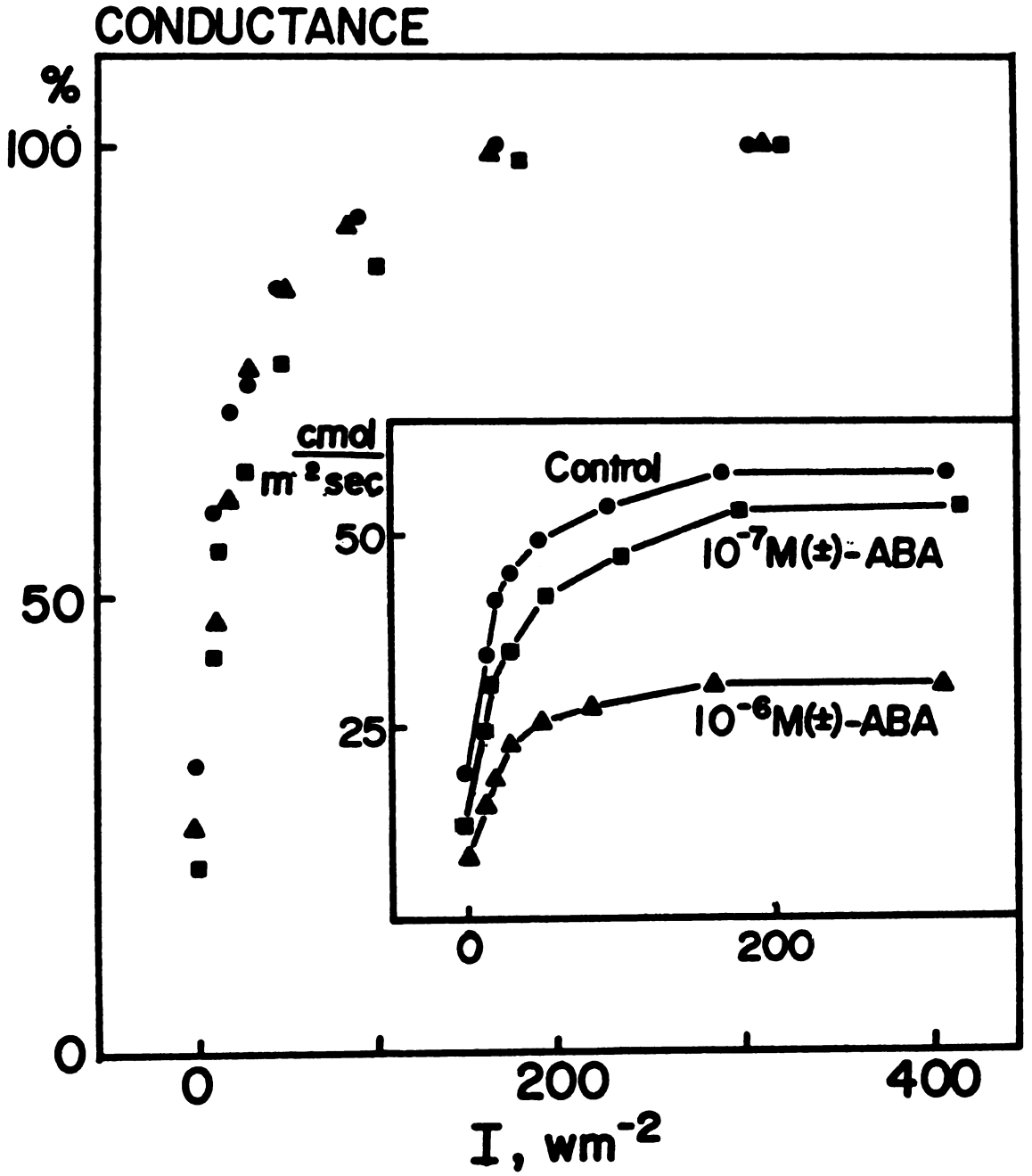
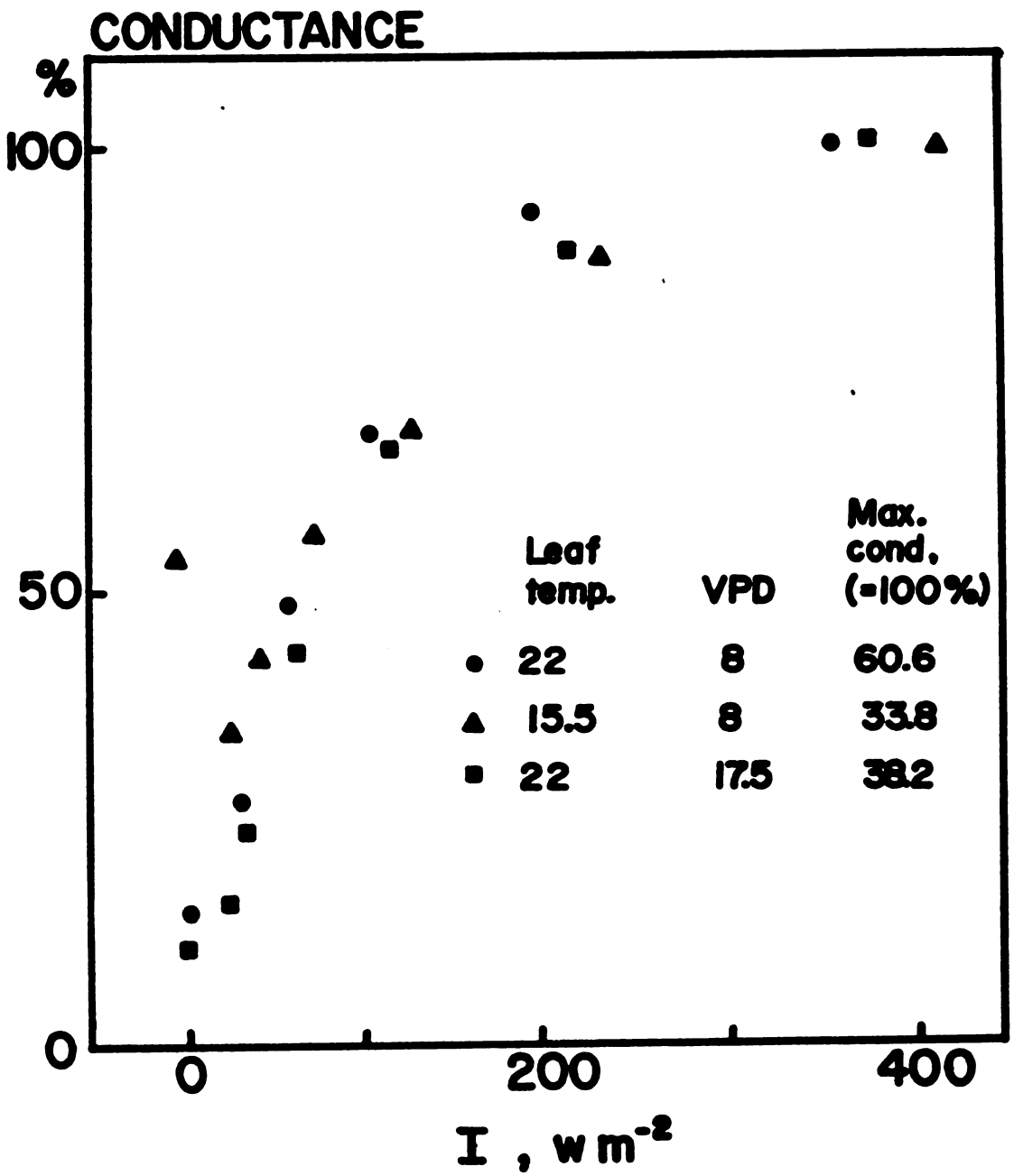

CONDUCTANCE
%
100
50
0
0
200
400
I, wm-2
cmol
m-2sec
50
25
0
200
Control
10-7M(±)-ABA
10-6M(±)-ABA

Figure 7. Stomatal response to light of leaves of Xanthium strumarium at various humidity and leaf temperature combinations.

Detached, trimmed leaves were used. The ambient CO_2 concentration was 320 μℓ/ℓ (cyanazine was not present). Data are plotted as a percentage of the conductance at the highest light intensity. Each point is the average of the sum of the conductance of the abaxial and adaxial leaf surfaces for four leaves. The units are: leaf temperature-degrees C, vapor pressure deficit (VPD)-ml H_2O/l air, conductance-cmol/m^2 sec.


CONDUCTANCE
%
100
50
0
0
200
400
I , w m⁻²
Leaf temp.
VPD
Max. cond. (=100%)
22
8
60.6
15.5
8
33.8
22
17.5
38.2

each other (Figure 7).

Inverted Leaf Experiments

Stomatal conductance of X. strumarium leaves fed cyanazine decreased as the irradiance decreased (Figure 8). The relationship between stomatal conductance and light was similar when the light was increased or decreased and stomata were opening or closing (Figure 9). Surprisingly, the stomata of the upper (adaxial) epidermis required a higher irradiance for saturation than did those of the lower (abaxial) epidermis (Figure 8), despite the fact that the stomata on the lower surface were shaded by the mesophyll. When leaves of X. strumarium were put into the chambers upside down, the stomata on the abaxial surface (now directly illuminated) began to open at a much lower irradiance than when the leaf was in the normal orientation (Figure 10). The adaxial stomata required much more light to open when the leaf was inverted than when it was in the normal orientation. Identical results were obtained with leaves not fed cyanazine. Based on the saturation light intensity, it can be calculated that the abaxial stomata were about 20 times more sensitive to light than were the adaxial stomata. Absorptivity of light by the leaf did show a slight dependence on orientation, but it was considered insignificant in this context (see Appendix).

Effect of Light Quality on Stomatal Conductance and Assimilation

Stomatal conductance and assimilation changed when a leaf was irradiated first with blue light and when with red light of equal quantum flux density. Stomata were substantially more open in blue light than in red light, despite a lower assimilation rate and a much higher

Figure 8. Stomatal response to light of leaves of Xanthium strumarium fed cyanazine showing differences between adaxial and abaxial responses.

Detached, trimmed leaves were put into a 10 ml beaker with 10^{-5}M cyanazine. The CO_2 concentration in the air (and inside the leaf) was 270 μℓ/ℓ. Each curve represents the stomatal behavior of one leaf.

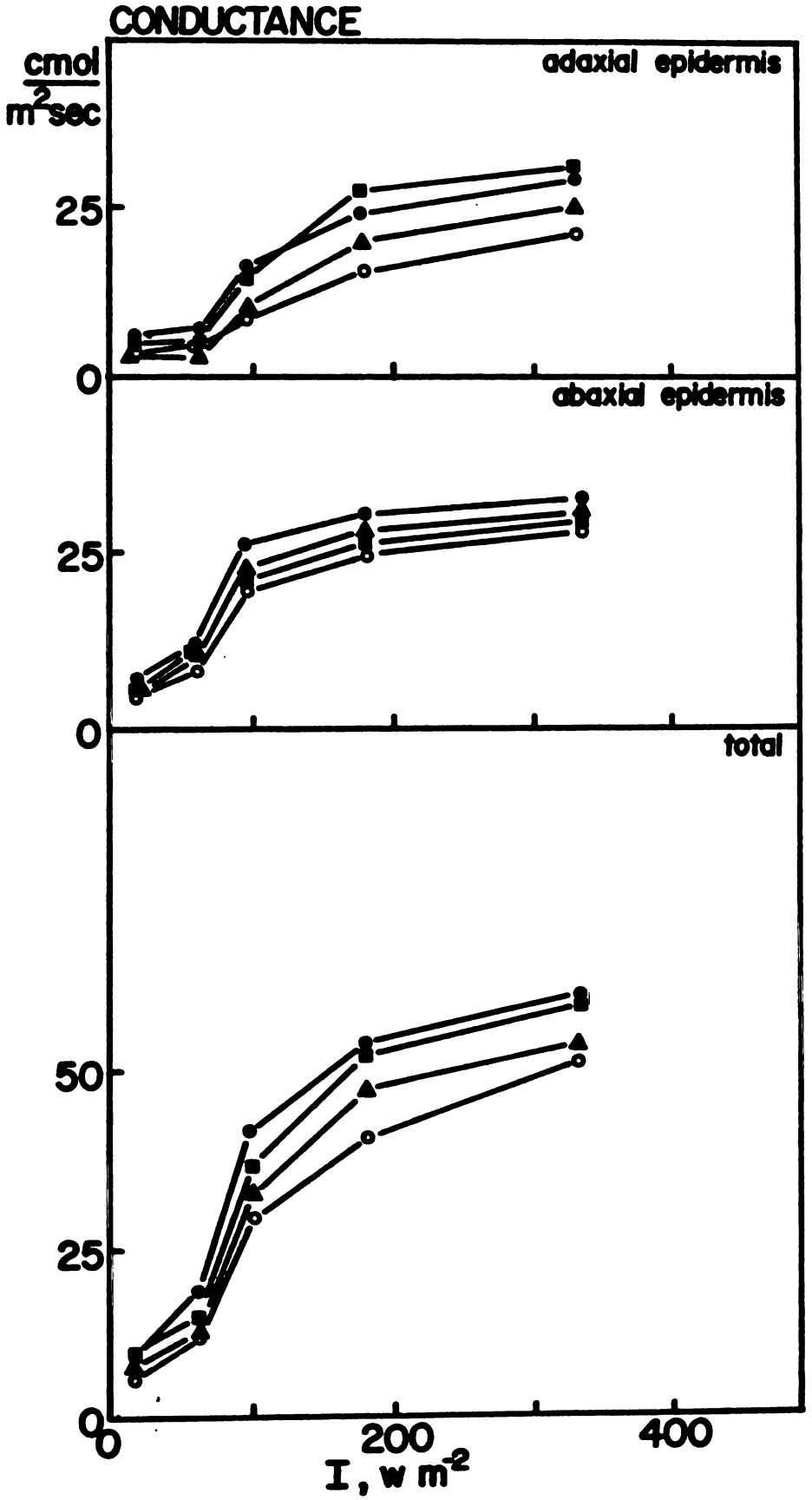

CONDUCTANCE
cmol
m²sec
adaxial epidermis
25
0
abaxial epidermis
25
0
total
50
25
0
0
200
400
I, w m⁻²

Figure 9. Stomatal response to light of leaves of Xanthium strumarium as light was increased and then decreased.

Detached, trimmed leaves were used. The CO_2 concentration inside the leaf was held constant at 290 μℓ/ℓ (±10μℓ/ℓ) by adjustments of the CO_2 concentration in the ambient air. Each point is the average of the sum of the conductance of the abaxial and adaxial leaf surfaces for four leaves.

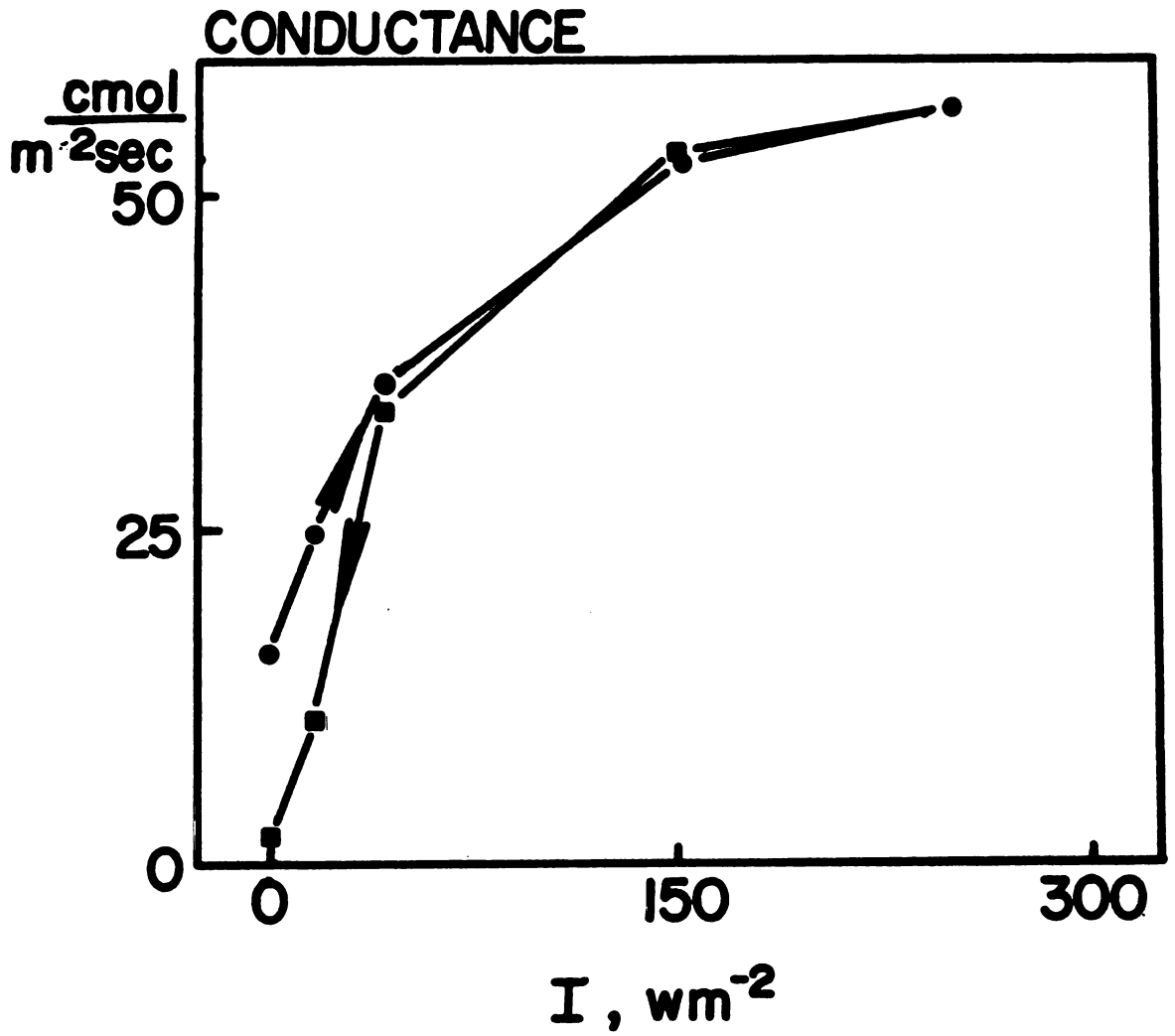
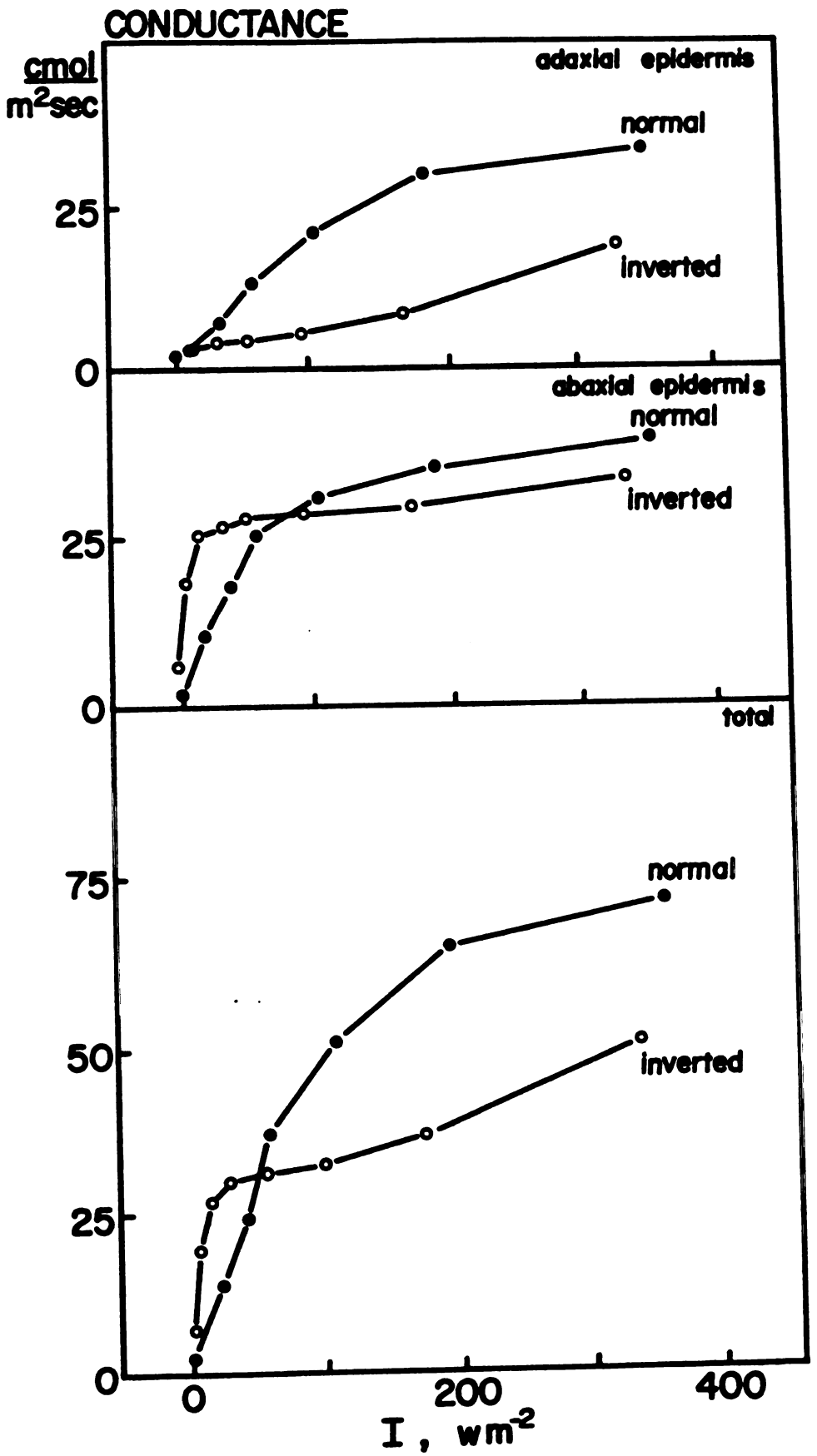

CONDUCTANCE
cmol
m⁻²sec
50
25
0
0
150
300
I , wm⁻²

Figure 10. Stomatal response to light of leaves of Xanthium strumarium in the normal or inverted orientation.

Detached, trimmed leaves were put into 10 ml beakers containing 10^{-5}M cyanazine. The leaf blade was positioned so that the light was shining on either the adaxial surface (normal) or abaxial surface (inverted). The CO_2 concentration in the air and inside the leaf was 255 µℓ/ℓ.


CONDUCTANCE
cmol
m²sec
adaxial epidermis
normal
inverted
25
0
abaxial epidermis
normal
inverted
25
0
total
normal
75
inverted
50
25
0
0
200
400
I , wm⁻²

intercellular CO_2 concentration (Table 5). This result is consistent with the results presented above, which indicate that the stomatal response to light is for the most part a direct response to light and is not mediated by changes in CO_2 concentration or by hypothetical changes in the supply of assimilates to the guard cells.

Since it had been determined that stomata on the abaxial (lower) surface of leaves of X. strumarium were much more sensitive to light than were the stomata on the adaxial surface (Figure 10), all subsequent work with monochromatic light was performed with the leaf inverted so that the abaxial surface was directly illuminated. Conductance values reported are for the directly illuminated abaxial surface only, while the assimilation rates reported are the sums from both sides of the leaves.

An action spectrum for stomatal opening was constructed using the "steady state" method (i.e. a leaf was exposed to one quantum flux at a particular wavelength until the conductance no longer changed. In general, the stomatal response to a new quantum flux took 30 minutes.) The stomatal response to increasing quantum flux is shown for 14 wavelengths of light in Figure 11. There were three responses that could be distinguished (Figures 16, 17). At low quantum flux, the stomata responded weakly, if at all, to light. At intermediate quantum fluxes, the stomata responded linearly to the logarithm of the quantum flux. At very high quantum fluxes, the stomata were once again insensitive to changes in the quantum flux. A linear regression was performed for the three or four points that fell in the linear response range for each wavelength (except at 711 nm for which only two points could be used). No correlation coefficient was less than 0.98 and six correlation

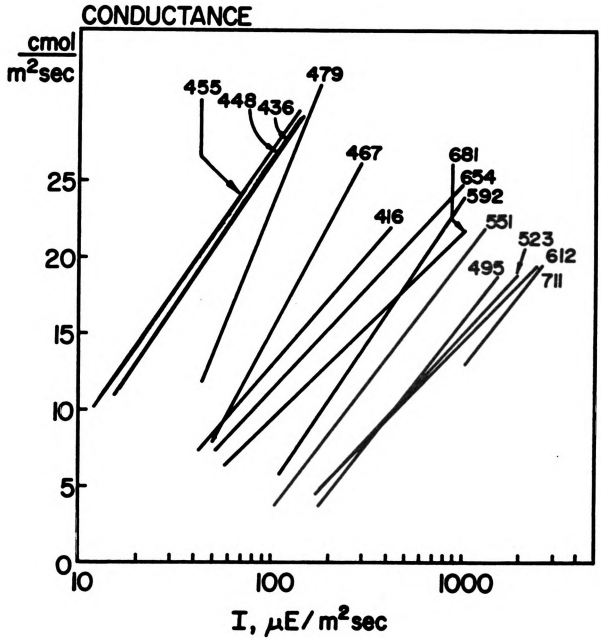
Table 5. Effect of red or blue light on assimilation rate, intercellular CO_2 concentration, and stomatal conductance in leaves of Xanthium strumarium.

Incident quantum flux was 740 $\mu E/m^2$ sec for both red and blue light. The CO_2 concentration was 305 $\mu\ell/\ell$.

Light	λ	Assimilation rate	Intercellular CO_2 concentration	Stomatal conductance
	nm	$\mu mol/m^2$ sec	$\mu\ell/\ell$	$cmol/m^2$ sec
red	681	11.6	255	35.9
blue	436	9.9	273	52.9

Figure 11. Stomatal response to light quality of leaves of Xanthium strumarium.

Detached, trimmed leaves were positioned in the leaf chamber so that the abaxial leaf surface was directly illuminated. The lines are linear regressions of the three or four points that fell on the linear response portion of the curve. The extent of the line indicates the range over which the stomata responded linearly to log changes in irradiance. Each line was derived from data for one leaf. There was no response up to 1000 $\mu E/m^2$ sec at 749 and 731 nm and up to 100 $\mu E/m^2$ sec (limit of the light source) at 386 and 372 nm.

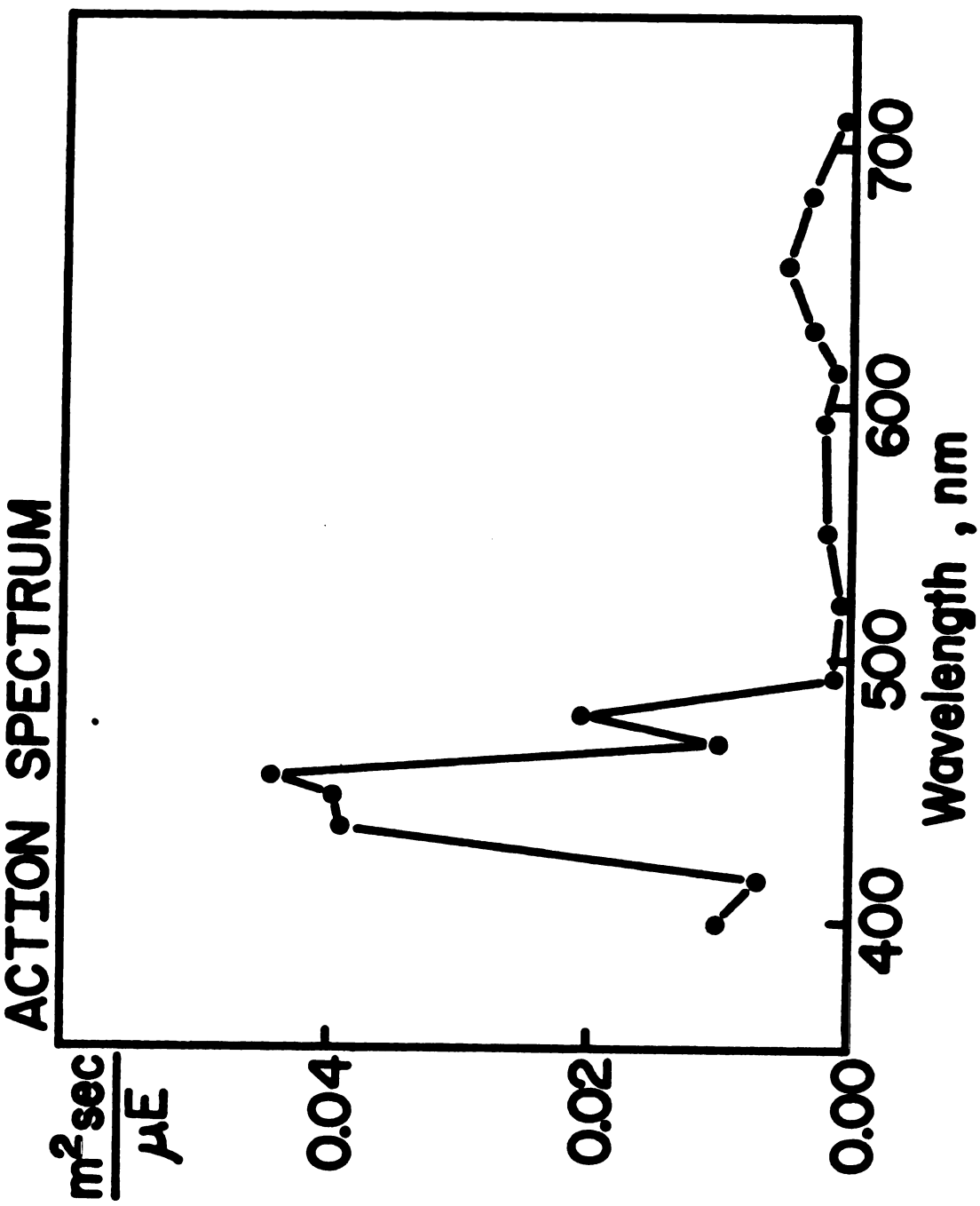

CONDUCTANCE
cmol
m²sec
455
448
436
479
467
681
654
592
416
551
523
612
495
711
25
20
15
10
5
0
10
100
1000
I, μE/m²sec

coefficients were equal to or greater than 0.998. The length of the line in Figure 11 indicates the range over which the stomatal response was linear with logarithmic changes in quantum flux. The slopes of the lines in blue light were greater than the slopes in red light. The average slope of all lines between 400 and 500 nm was 19.4±3.7 cmol m^{-2} sec^{-1} per decade change in quantum flux, while the average slope of all the lines between 600 and 700 nm was 12.8±0.8 cmol m^{-2} sec^{-1} per decade. Using data from Figure 11, an action spectrum was constructed by plotting the inverse of the quantum flux required to produce a conductance of 15 cmol m^{-2} sec^{-1} versus wavelength (Figure 12). This method of construction for the action spectrum deemphasizes the peak of activity in red light, since the slopes of the fluence response curves are less in the red light than in the blue. Another action spectrum was constructed (not shown) by extrapolating the regression lines to the conductance value obtained in the dark for the individual leaf and plotting the inverse of the quantum flux thus obtained versus wavelength. In that case, the red peak was 20% of the blue peak, rather than 10% as in Figure 12.

Many investigators have hypothesized that chlorophyll is involved in the stomatal response to light. While chlorophyll cannot be the only pigment involved, it could be the pigment responsible for the activity of red light in causing stomatal opening. To test this, a leaf was put into the gas analysis chamber and the steady state conductance in a quantum flux of 300 μE m^{-2} sec^{-1} at each of 5 wavelengths of red light was determined at the long wavelength end of the chlorophyll absorption spectrum where interference by accessory pigments should be minimal. For two leaves, the wavelength was increased from the shortest to the

Figure 12. Action spectrum of stomatal opening.

The data were derived from Figure 11 by reading the quantum flux required to give a stomatal conductance of 15 cmol m^{-2} sec^{-1} and plotting the inverse of that quantum flux versus wavelength.


ACTION SPECTRUM
m² sec / μE
0.04
0.02
0.00
400
500
600
700
Wavelength , nm

longest and for two additional leaves the wavelength was decreased from the longest to the shortest. For each leaf, the shape of the curve relating conductance to wavelength was the same, although the conductance was variable from leaf to leaf. In each of the four leaves, the conductance at 691 nm oscillated with a period of about 25 minutes and decreasing amplitude. The results, averaged from the four leaves, are shown in Figure 13. The intercellular CO_2 concentration was controlled at about 250 $\mu\ell\ \ell^{-1}$ and the measured average concentration was 253±8 $\mu\ell\ \ell^{-1}$ for all wavelengths except 711 nm, for which the intercellular CO_2 concentration was 282 $\mu\ell\ \ell^{-1}$, providing evidence that the stomatal response to red light shown in Figure 13 was not the result of changes in the intercellular CO_2 concentration. The stomatal response to a change in wavelength of red light was nearly identical to the response of assimilation. Figure 14 shows additional evidence that the stomatal response to red light does not depend on changes in the intercellular CO_2 concentration. Stomata of a leaf in 674 nm light (300 $\mu E\ m^{-2}\ sec^{-1}$) closed when the light was turned off, even though the ambient CO_2 concentration was lowered enough to maintain the intercellular CO_2 concentration constant. (The intercellular CO_2 concentration determined after a large change in the ambient CO_2 concentration is unreliable so there are no lines connecting the value obtained at 13:43.)

The stomatal response to red light was sensitive to DCMU and cyanazine. Figure 15 shows the results of one of three similar experiments. Detached leaves were put into 10 ml beakers containing 5×10^{-5}M cyanazine (as in Figure 15) or DCMU (as in Table 6). When these leaves were put into 455 nm light (650 $\mu E\ m^{-2}\ sec^{-1}$), both conductance and assimilation increased rapidly until the photosynthesis inhibitor began to reduce

Figure 13. Stomatal conductance and assimilation rate in various wavelengths of red light.

Detached, trimmed leaves were positioned in the gas analysis chamber such that the abaxial surface was directly illuminated. The data are the average of four leaves. Stomatal conductance data are for the directly illuminated abaxial surface only, while the assimilation data are for both leaf surfaces. The CO_2 concentration varied to keep the intercellular CO_2 concentration constant at 250 $\mu\ell/\ell$ and the quantum flux was 300 $\mu E/m^2$ sec at each wavelength. Assimilation (squares) is plotted against the left scale and stomatal conductance is plotted against the right scale. The average stomatal conductance in darkness was 4.6 $cmol/m^2$ sec. The interference filters used had a half-band width of 12 nm and the one-tenth band width was 20 nm.

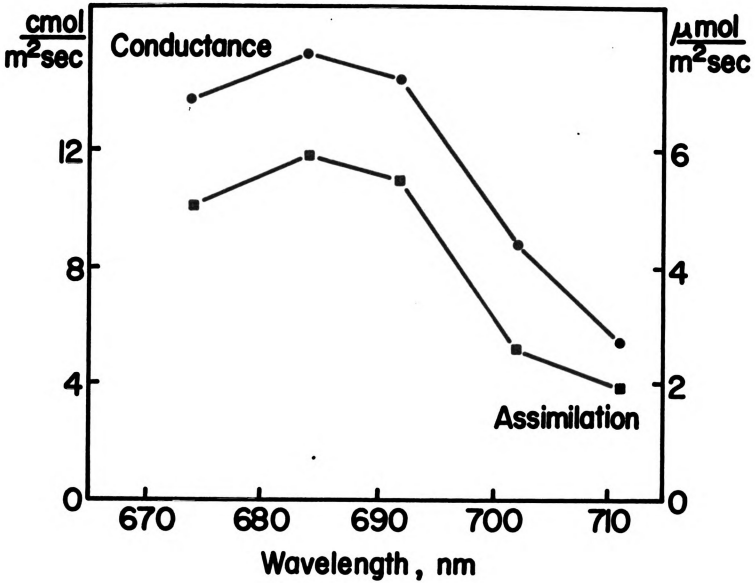

cmol / m²sec
Conductance
μmol / m²sec
12
8
4
0
6
4
2
0
Assimilation
670
680
690
700
710
Wavelength, nm

Figure 14. Stomatal closure in response to turning off the light (674 nm) with the intercellular CO_2 concentration held constant.

The data are for one of the leaves used in the experiment described in Figure 13.

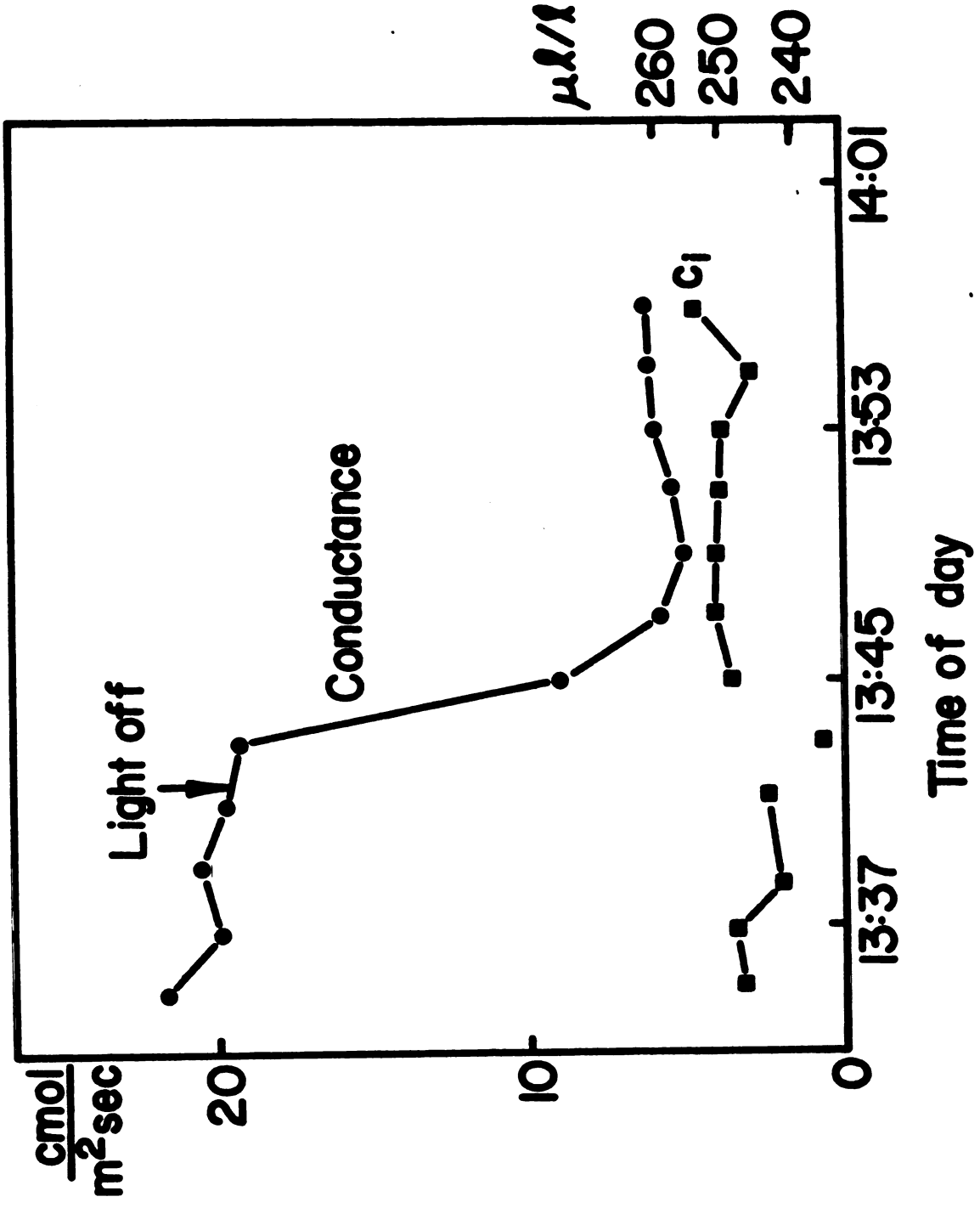
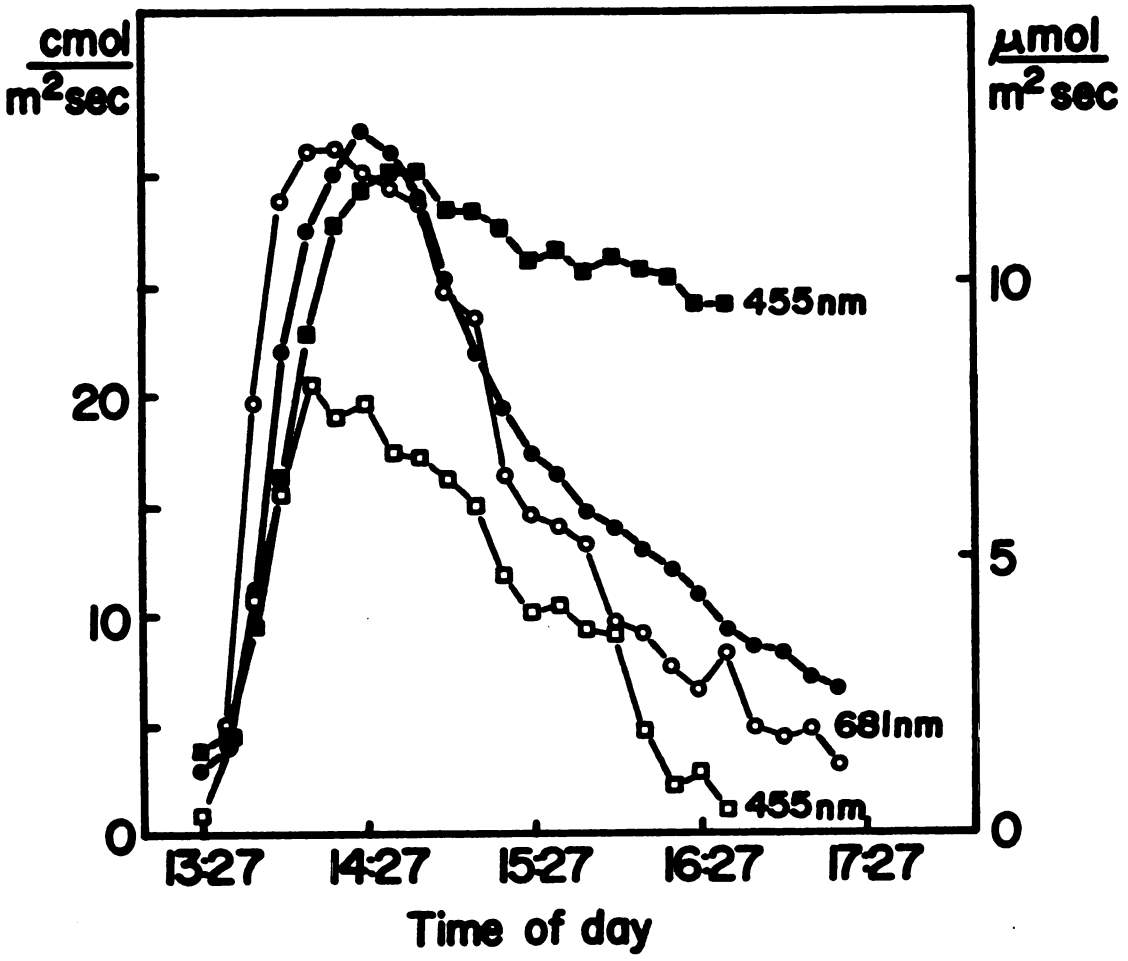

cmol / m²sec
20
10
0
Light off
Conductance
Ci
μl/l
260
250
240
13:37
13:45
13:53
14:01
Time of day

Figure 15. Stomatal conductance and assimilation of Xanthium strumarium leaves fed cyanazine and placed in red or blue light.

Detached, trimmed leaves were positioned in the leaf chamber so that the abaxial surface was directly illuminated. The ends of the petioles were in 10 ml beakers containing 5 X 10^{-5}M cyanazine. Assimilation rates (open symbols, left scale) and stomatal conductance (solid symbols, right scale) rose initially in response to the light (650 $\mu E/m^2$ sec at both wavelengths). The CO_2 concentration in the air was 320 $\mu\ell/\ell$. The stomatal conductance data are for the directly illuminated abaxial surface only, while the assimilation rate data are for both surfaces.


cmol
m²sec
μmol
m²sec
20
10
0
10
5
0
455nm
681nm
455nm
13·27
14·27
15·27
16·27
17·27
Time of day

the photosynthetic rate. The stomatal conductance fell slightly; this may have been a CO_2 effect, since the intercellular CO_2 concentration rose by 30 $\mu\ell\ \ell^{-1}$ as the photosynthetic rate fell. In light of 681 nm (same quantum flux), the conductance and assimilation rate both fell as the inhibitor of photosynthesis began to act. Table 6 contains data from a similar experiment, this time with DCMU as the photosynthesis inhibitor. Since ten times more quanta of red light than of blue were required to produce a conductance of 15 cmol m^{-2} sec^{-1} (Figure 12), the experiment included one treatment in which the quantum flux of blue light was one-tenth that of the red light. After 6 hours in the light, the two leaves in blue light had large stomatal conductances, while the leaf in red light had a very low stomatal conductance. Because the stomata closed in red light but remained open in blue light, the amount of DCMU taken up during the course of the experiments (as judged by the water lost by the leaves) was 50% higher for the blue light treatments than for the red light treatment. After the stomata were closed by DCMU in red light, they still responded to blue light, though not as strongly as during the first opening movement.

Some investigators, notably Brogårdh (2), believe that the stomatal response to red light depends on changes in the concentration of CO_2, while the blue light response is independent of CO_2. To test the effect of CO_2 on the stomatal response to red or blue light, a fluence response curve was determined in high or low CO_2 in red or blue light. The results, shown in Figure 16, indicate that CO_2 affected the stomatal response to blue light in a similar manner as it did the response to red light. For both red and blue light, leaves in the high concentration of CO_2 required more light for the same stomatal conductance and the

Table 6. Effect of DCMU on stomatal conductance and assimilation in red and blue light.

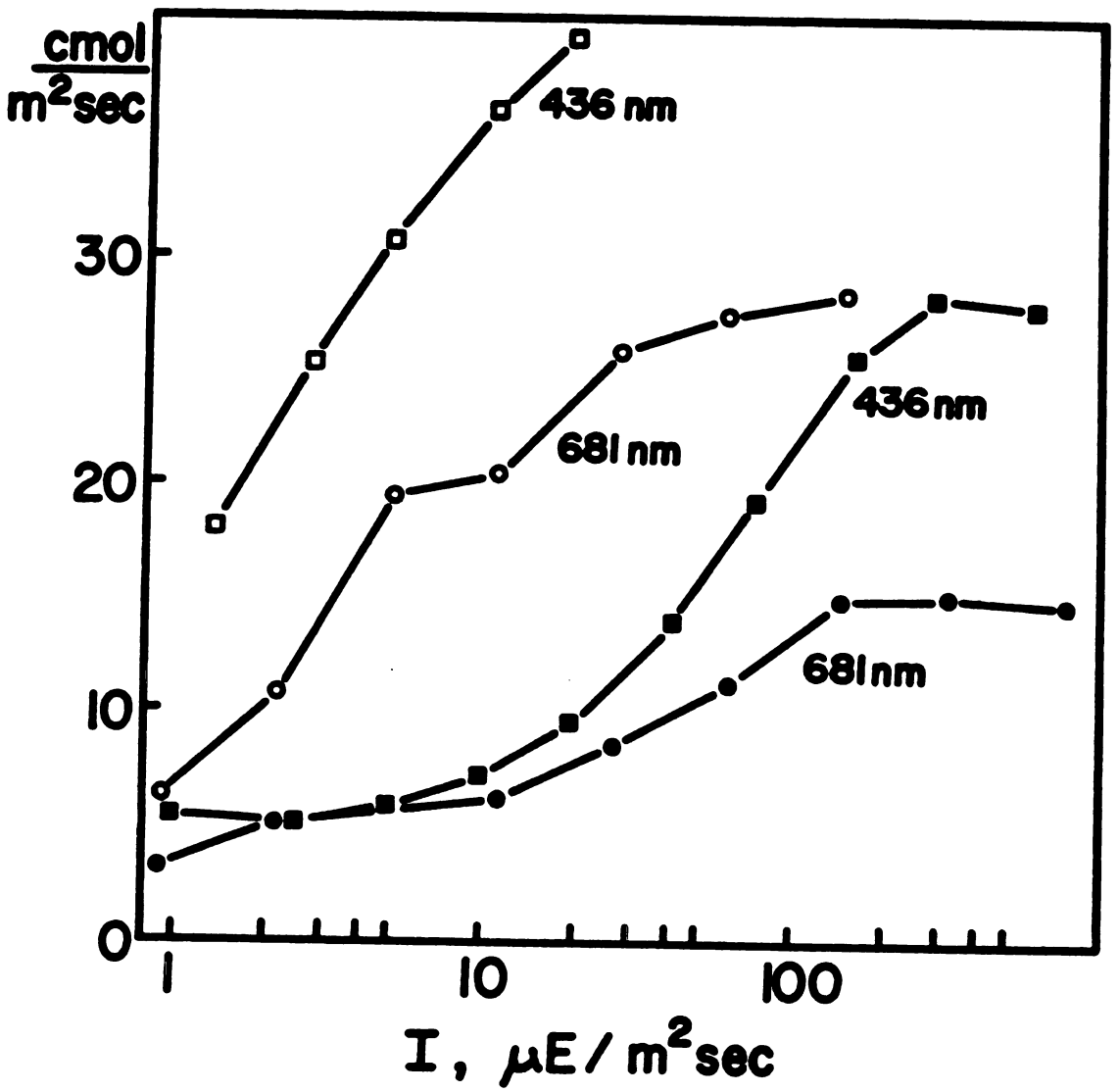
The dose was calculated by integrating the stripchart record of water loss for the 6 hour duration of the experiment and multiplying the result by the concentration of DCMU in the irrigation water (5 X 10^{-5}M).

Light	λ	Quantum flux	Stomatal conductance	Assimilation rate	Dose
	nm	μE/m^2 sec	cmol/m^2 sec	μmol/m^2 sec	nmol
blue	455	650	33.2	0.21	36.5
blue	455	65	33.6	0.33	36.3
red	684	650	7.8	1.99	23.0
*blue	455	650	16.8	1.97	--

*Same leaf as used for 684 nm. This line shows that when stomata in red light are closed by DCMU, they can still respond to blue light.

Figure 16. Effect of CO_2 on the stomatal response to red or blue light.

Detached, trimmed leaves were positioned in the gas analysis chamber so that the abaxial surface was directly illuminated. The conductance reported was for the directly illuminated abaxial surface. The open symbols are at low CO_2 concentration (110 µℓ/ℓ for blue, 130 µℓ/ℓ for red) and the filled symbols are for high CO_2 concentration (510 µℓ/ℓ for blue, 580 µℓ/ℓ for red).

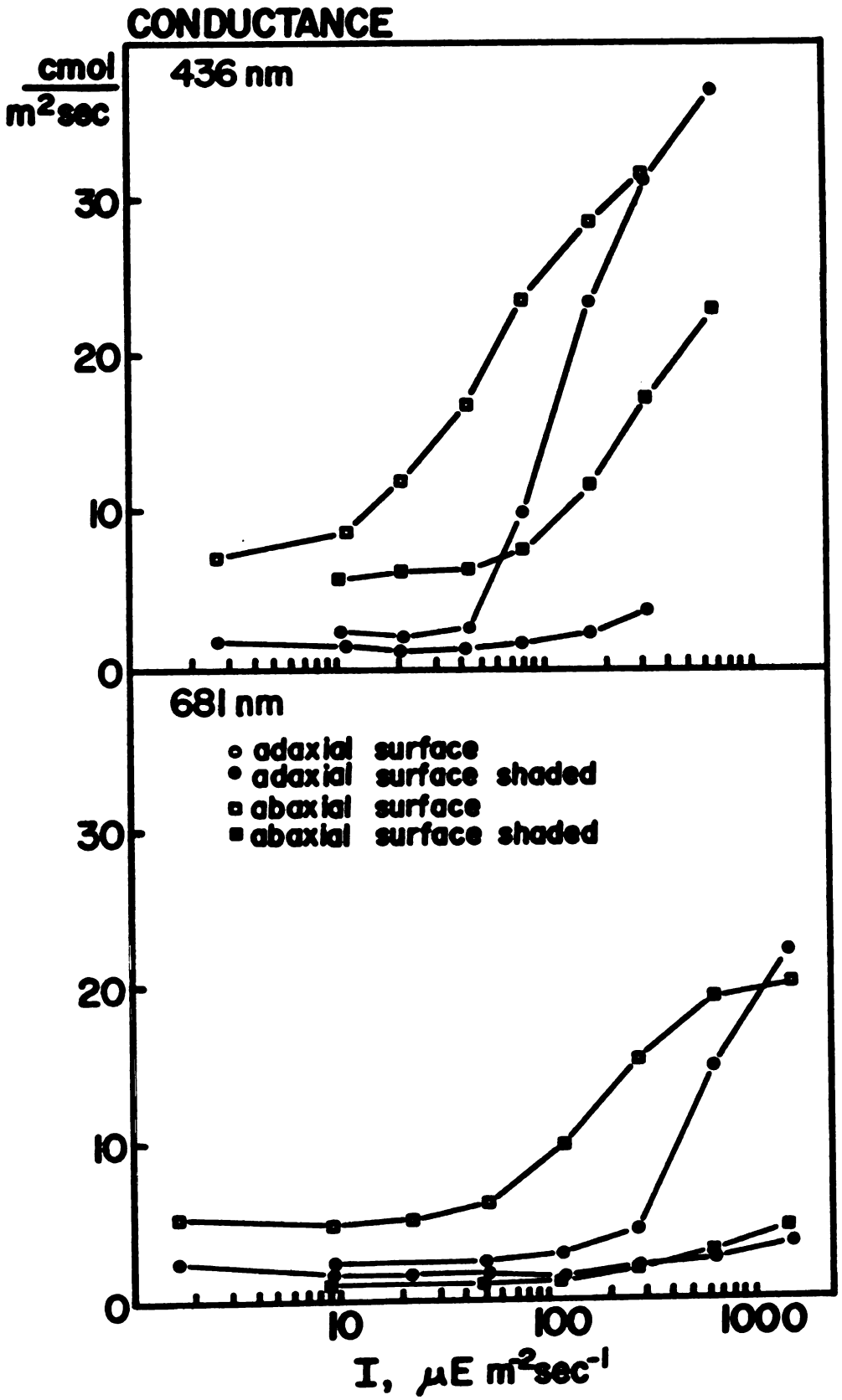

cmol
m²sec
436 nm
681 nm
436 nm
681 nm
30
20
10
0
1
10
100
I, μE/m²sec

maximum conductance attained was lower.

To test whether the stomatal response to red light was mesophyll-dependent or mesophyll-independent, the inverted leaf experiment shown in Figure 10 was repeated with monochromatic blue or red light. Figure 17 shows that when the abaxial surface was illuminated directly it required much less light than when it was shaded by the mesophyll in both red and blue light.

Figure 17. Stomatal response to monochromatic blue or red light in the normal or inverted orientation.

Detached, trimmed leaves were put into 10 ml beakers containing water. The leaf blade was positioned so that light was shining on the adaxial surface (abaxial shaded by the mesophyll) or on the abaxial (adaxial shaded). The filters had half bandwidths of 20 nm. The average transmittance in blue light was 1.7% and in red light it was 3.9% for the Xanthium strumarium leaves used in this experiment.


CONDUCTANCE
cmol
m²sec
436 nm
30
20
10
0
681 nm
adaxial surface
adaxial surface shaded
abaxial surface
abaxial surface shaded
30
20
10
0
10
100
1000
I, μE m⁻²sec⁻¹

DISCUSSION

Direct Versus Indirect Effects of Light on Stomata

Stomatal opening in response to light was almost entirely a direct response (not mediated by CO_2) in all species studied here. The species studied were C_3 plants and one C_4 plant. Nobel and Hartsock (27) found that stomata of the CAM plant Agave deserti responded to light but not to CO_2 when the plant was well watered and operating as a C_3 plant. Wong et al. (53) measured the stomatal response to light in Eucalyptus pauciflora and found that 70% of the stomatal response to light was a direct response. Based on this evidence, we conclude that stomata of most well watered plants respond primarily directly to light (i.e. the response is not mediated by changes in the intercellular CO_2 concentration). The only exception to this is Zea mays at low irradiance (Table 2 and reference 39).

The intercellular CO_2 concentration is determined by the flux of CO_2 through the stomata and the resistance to that flux (assuming the external CO_2 concentration remains constant). As long as the stomatal response to light matches the response of assimilation to light, there is no change in the intercellular CO_2 concentration. In these cases, the stomatal sensitivity to CO_2 plays no role in stomatal responses to light. For example, for Perilla frutescens, at 155 w m^{-2} the intercellular CO_2 concentration was the same as at 45 w m^{-2}, even though the stomatal conductance was more than two times greater (Table 4). Since it is known that the stomatal response to CO_2 is not strong enough to keep the

intercellular CO_2 concentration constant, the increase in conductance from 9.5 to 20.5 cmol m^{-2} sec^{-1} is a direct response to light. From Table 2 it would be expected that between 82 and 94% of the stomatal response to light would be a direct response, but instead it is observed that all of the response is accounted for by the direct response to light. This discrepancy comes about because the values in Table 2 are differentials and are valid only over an infinitesimal light range and cannot be used to predict what the integrated response will be. In fact, the direct stomatal response to light is often strong enough to eliminate the reduction of the intercellular CO_2 concentration, which is the signal for the indirect stomatal response to light. In Zea mays and Phaseolus vulgaris, the stomatal conductance increased more than the assimilation rate at high irradiance (Table 4), causing a higher intercellular CO_2 concentration at the highest irradiance than at intermediate light intensities. In these cases, stomatal sensitivity to CO_2 should have caused stomatal closure, but the direct stomatal response to light completely overcame this closing stimulus. This behavior had been postulated by Cowan and Farquhar (4) if stomata function to minimize the amount of water lost for a given amount of carbon dioxide taken up. In P. vulgaris, the increase was only 6 $\mu\ell$ ℓ^{-1} and the stomata were insensitive to CO_2, so this rise in intercellular CO_2 concentration was probably unimportant. However, in Z. mays the increase was 17 $\mu\ell$ ℓ^{-1} and the stomata were sensitive to CO_2. At constant irradiance, such an increase in intercellular CO_2 concentration would reduce stomatal conductance by 2 to 3 cmol m^{-2} sec^{-1}.

Mesophyll-Dependent Versus Mesophyll-Independent Effects of Light on Stomata

Although it is not possible to quantitatively separate mesophyll-dependent from mesophyll-independent effects of light on stomata, three pieces of evidence lead to the conclusion that, at least in X. strumarium, the direct stomatal response to light is primarily mesophyll-independent. First, in leaves in which photosynthetic electron transport was blocked by cyanazine, the stomata still responded to CO_2. Second, a higher irradiance was required for stomatal opening when the surface being measured was shaded by the mesophyll than when illuminated directly (Figures 10,17). Similar results were obtained by Turner (46) with Nicotiana and Sorghum. If the rate of photosynthesis in the underlying mesophyll plays a large role in stomatal regulation, one would expect little difference in the stomatal response to light when the leaf was illuminated from above or below. Third, blue light was more effective in causing stomatal opening than red light, while the photosynthetic rate was greater in the red (Table 5). The large number of studies using epidermal strips to study stomatal opening in response to light also speaks in favor of a mesophyll-independent light response.

Interactions between Stomatal Responses to Light and CO_2, ABA, Leaf Temperature, and Vapor Pressure Deficit

High irradiance caused the stomata to become insensitive to CO_2 in X. strumarium, P. vulgaris, and P. frutescens. In G. hirsutum, the response to CO_2 was smallest at high irradiance. Gaastra (7) found several years ago that stomata of turnip responded less to CO_2 in high light than in low, and Heath and Russell (10) found that stomata of wheat became insensitive to CO_2 at high light intensities. Thus, in all C_3 plants studied, light lessens the stomatal response to CO_2 and I know

of no counter examples. In a similar way, the response to light of stomata of X. strumarium and G. hirsutum depended inversely on the CO_2 concentration. Z. mays, on the other hand, did not fit this pattern. The slope of the curve representing the relationship between stomatal conductance and CO_2 was independent of the irradiance, although the curve was displaced along the CO_2 concentration scale by changing the irradiance (Figure 4).

If water is not limiting, a high stomatal sensitivity to CO_2 will be a liability for a C_3 plant, in which the photosynthetic rate does not saturate with respect to CO_2 until well above the ambient CO_2 concentration of 320 to 340 $\mu\ell\ \ell^{-1}$ (in other words, any increase in stomatal conductance would increase the photosynthetic rate by increasing the CO_2 supply). Consequently, for a well watered C_3 plant, the stomata should open very widely and allow the intercellular CO_2 concentration to be as close as possible to the ambient CO_2 concentration. In X. strumarium, P. vulgaris, and P. frutescens the stomata were insensitive to CO_2 (Figures 3 and 4) and the intercellular CO_2 concentration was close to ambient (Table 4). Photosynthesis of Z. mays, however, saturates between 100 and 200 $\mu\ell\ \ell^{-1}$ intercellular CO_2 (6, and data from this investigation not presented). In this case, a high sensitivity to CO_2 is not a liability as long as the intercellular CO_2 concentration does not fall below the saturation point for photosynthesis. Again, this is the behavior observed (Figure 4 and Table 4).

If, however, water is in limited supply, a large stomatal conductance will be a liability. Since stomatal closure will limit transpiration more than photosynthesis in C_3 plants at least at high conductances (37), the stomata of water-stressed plants should close. A high stomatal

sensitivity to CO_2 assures that stomatal conductance does not greatly exceed that needed for photosynthesis. This allows a judicious use of water and only a small reduction in the rate of photosynthesis. It has already been shown that ABA causes stomata to become more sensitive to CO_2 (5,36). It was therefore not surprising to find that the addition of ABA to the transpiration stream increased the proportion of the stomatal response to light mediated by CO_2 (Table 2). If the photosynthetic apparatus becomes impaired (as can occur during water stress) the intercellular CO_2 concentration will rise, causing stomatal closure and a decrease in stomatal sensitivity to light. On the other hand, if the photosynthetic apparatus is not inhibited, if the stomata are restricting the supply of CO_2 to a great degree, then the intercellular CO_2 concentration will fall, which in turn will cause stomatal opening and an increase in the stomatal sensitivity to light. If leaf temperature is reduced, the need for CO_2 will be diminished by the falling photosynthetic rate and so stomatal closure will not limit photosynthesis. If the vapor pressure deficit is high, stomata will be less open than when it is low, so that water loss will not be excessive. Apparently these complex interactions between environmental and physiological factors allow stomata to control gas exchange in a way that is appropriate for the particular state of the plant (e.g. during periods of optimal or suboptimal water supply, and during periods with conditions favorable to or not favorable to photosynthesis).

Effects of Light, CO_2, and ABA on Guard Cells

Light causes stomata to open, whereas CO_2 causes stomata to close. If the irradiance is high enough, the stomata require a greater closing stimulus than is provided by the 320 to 340 $\mu\ell\ \ell^{-1}$ CO_2 normally found in

the atmosphere so that the stomata appear to be insensitive to CO_2. Stomatal insensitivity to CO_2 can occur at an intensity one-half that of full sunlight (Figure 3). Abscisic acid alters the relationship between light and CO_2 so that stomata respond to CO_2 in the light. Since the saturation irradiance is the same with or without ABA (Figure 6) and stomatal conductance in the dark is reduced by ABA (Table 3), the effect of ABA must be to add to the closing stimulus of CO_2 either by enhancing the effectiveness of CO_2 or by providing a separate closing stimulus.

Effect of Light Quality on Stomatal Opening

Stomatal conductance is greater in blue light than in red (Table 5). Previously, it has been shown that gas exchange is enhanced by blue light (2,23,50) and that uptake of Rb+ into guard cells, production of malate by guard cells, and swelling of guard cell protoplasts are stimulated by blue light (12,29,55). Of special interest in Table 5 are the findings that the rate of assimilation fell slightly, and the intercellular CO_2 concentration rose by almost 20 $\mu\ell\ \ell^{-1}$, two signals believed to cause stomatal closure. Thus, the results with monochromatic light corroborate the results with white light that indicated that light has a large direct effect on stomatal conductance.

What are the photoreceptor pigments involved in the stomatal response to light? The results of Brogårdh (2) and Ogawa et al. (29) indicate that there are at least two pigments involved, one that absorbs blue light (Ogawa et al. {29} suggested a flavin) and one that absorbs red light, which several investigators have hypothesized is chlorophyll (2, 12,20,21). Two methods were employed to determine if chlorophyll is

indeed responsible for the red light response: action spectra of stomatal opening were determined and inhibitors of photosynthetic electron transport were given to leaves.

Action spectra

One method of determining the photoreceptor involved in response to light is to measure the relative effectiveness of light of various wavelengths in producing the action of interest and comparing the action spectrum obtained with the absorption spectrum of the presumed photoreceptor pigment. Usually, an action spectrum is produced by irradiating with light of various wavelengths but equal quantum flux and determining the action at each wavelength. If the quantum flux is too low, then some wavelengths of light will appear to have no effect, either because the response is too small to be measured or because some threshold quantum flux was not exceeded. If the quantum flux is too high, the response to light may be saturated over some portion of the spectrum; peaks will be broad and the action spectrum may be distorted. To overcome these problems, Hsiao et al. (12) used two different quantum fluxes (78 μE m^{-2} sec^{-1} and 380 μE m^{-2} sec^{-1}). At the low quantum flux, they saw no activity of red light in causing stomatal opening or Rb+ transport. At the higher quantum flux they observed that red light was quite effective in causing stomatal opening and Rb+ transport, though not as effective as blue light. They interpreted their results as indicating two different photoreactions, one effective at low quantum flux and one effective at high quantum flux. There is, however, no need to invoke two photoreactions based on their evidence alone. The low quantum flux may have been below the threshold for light activity in the red but not in the blue. Action spectra similar to those of Hsiao et al. (12) can

be constructed from the data in Figure 11 of this thesis by reading the stomatal conductance at either 78 or 380 $\mu E\ m^{-2}\ sec^{-1}$. At the low irradiance, only blue light was active, i.e. the stomata responded very little to red light. At the high irradiance, the stomata responded strongly to red light and the blue light response was saturated, resulting in an overestimation of the red peak of activity relative to the blue one.

A preferable method for constructing an action spectrum is to determine fluence response curves at each wavelength of interest and then determine the quantum flux required to produce a standard response. The disadvantage of this method is that it requires a different leaf for each wavelength of light investigated and so the accuracy of the action spectrum will be limited by variability of the plant material.

A preliminary action spectrum covering the range from 399 to 711 nm was determined by selecting a conductance of 15 $cmol\ m^{-2}\ sec^{-1}$ as the standard response (Figure 12). However, it was observed that the standard response selected to determine the action spectrum from the fluence response curves can affect the shape of the action spectrum. An alternative to the response used to determine the action spectrum in Figure 12 is to extrapolate the linear regression to the initial conductance for that leaf in the dark. The action spectrum based on these "threshold" quantum fluxes (not shown) was different from that shown in Figure 12 in that the shoulder at 478 nm was almost nonexistent and the height of the red peak relative to the blue peak was 0.2 instead of 0.1. These differences are the result of the fact that the slopes of the fluence curves were variable and generally greater in the blue than in the red. This observation is consistent with the hypothesis that the

stomatal response to light is mediated by chlorophyll (shown in this thesis) and a pigment absorbing blue light, possibly a flavin (29).

A more detailed action spectrum with filters of 12 nm half-band width (compared to 20 nm half-band width filters used for Figure 12) was determined between 674 and 711 nm where only chlorophyll should be absorbing light. Five wavelengths of light, all at 300 $\mu E\ m^{-2}\ sec^{-1}$, were used and the results of four leaves were averaged. Since the least effective wavelength (711 nm) produced a measurable increase in stomatal conductance over the dark value, and the highest conductance attained was lower than the maximum attainable stomatal conductance (Figure 11), this action spectrum avoids the potential errors pointed out above for this type of action spectrum. The effect of CO_2 on the stomatal response to light was eliminated by maintaining the intercellular CO_2 concentration constant. The action spectrum for stomatal opening in the red was very similar to the action spectrum for CO_2 assimilation, indicating that the same photoreceptor probably accounts for both processes.

Inhibitors

Both DCMU and cyanazine had little or no effect on stomatal conductance of X. strumarium leaves in white light or blue light. In red light, however, both inhibitors caused stomatal closure. This result may explain why Kuiper (20) found that stomata of epidermal strips floated on solutions of DCMU closed, since he used an incandescent lamp, which produces primarily red light. The experiment of Allaway and Mansfield (1) does not show that the effect of DCMU is only the result of a change in the concentration of CO_2 inside the leaf, because they did not keep the concentration of CO_2 inside the leaf constant, but rather used CO_2-free air, which independently causes stomatal opening.

The stomatal opening that occurs in response to CO_2-free air has no significance in stomatal function, since plants are never exposed to CO_2-free air. It is my opinion that the closure of stomata in red light caused by the inhibitors of photosynthetic electron transport and the strong similarity of the action spectra for photosynthesis and stomatal opening in red light constitute conclusive proof that chlorophyll is one of two or more photoreceptors involved in the stomatal response to light.

Wong (54) has suggested that photosynthesis in the underlying mesophyll tissue can affect the stomatal response to light. The inverted leaf experiment (Figure 17) shows, however, that the absorption of light that causes stomatal opening occurs near the surface of the leaf. In blue light, it can be seen that it requires about 10 times more light to cause stomatal opening on the abaxial epidermis when it is shaded than when it is direct illuminated. Since the average transmittance of the leaves in blue light is 0.02, stomatal opening will require 50 times more light when the leaf is inverted than when it is in the normal orientation. One possible reason for this discrepancy is that the average transmittance is quite different from the transmittance of the leaf above some of the guard cells (39). Additional evidence that the pigment absorbing red light is located in the guard cells comes from the work of Hsiao et al. (12), Kuiper (20), and Ogawa et al. (29), all of whom found that stomatal activities were stimulated by red light in isolated epidermes.

The photosynthetic electron transport system may provide NADPH for the reduction of oxaloacetate to malate or ATP for pumping K+ ions, but I speculate that the most important function is related to information,

(The stomata open very nicely in total darkness in CO_2-free air, so stomata do not need the light for energy.) Environmental influences which affect photosynthetic electron transport in the mesophyll will also affect the photosynthetic electron transport in the guard cells, and as a result the stomatal response to light may depend to some degree on the same environmental influences as photosynthesis in the mesophyll. It is my opinion that this is the function of the chlorophyll-mediated stomatal response to light and that this is why stomata and photosynthesis often respond in a similar way to various environmental influences.

SUMMARY

Stomatal responses to light were investigated and found to be composed of three separate responses. They are: a stomatal response to changes in the intercellular CO_2 concentration, a direct stomatal response to light mediated by some blue light photoreceptor, and a direct stomatal response to light mediated by chlorophyll.

The CO_2-mediated indirect stomatal response to light can be important at low irradiances, since the stomata respond to changes in the intercellular CO_2 concentration and the intercellular CO_2 concentration changes with variations in irradiance at low irradiance. However, at high irradiance the intercellular CO_2 concentration does not change greatly with variations in irradiance and in the C_3 species studied, the stomata are not sensitive to CO_2. This interaction between responses to light and CO_2 is similar to that reported for ABA and CO_2 (35). Leaf temperature, water vapor pressure deficit, and ABA did not interact with the stomatal response to light.

Stomata were most sensitive to blue light. Quantum fluxes of 1 μE m^{-2} sec^{-1} or less caused measurable increases in stomatal conductance. Inhibitors of photosynthetic electron transport caused only a slight stomatal closure in leaves in blue light.

The stomatal response to red light had the same action spectrum as did photosynthesis. The effect of red light was eliminated by the addition of the inhibitors of photosynthetic electron flow, DCMU or cyanazine, to the transpiration stream. These two pieces of evidence

lead to the conclusion that chlorophyll is involved in the direct stomatal response to light. Since chlorophyll also absorbs blue light, some of the activity of blue light in causing stomatal opening is probably the result of the chlorophyll-mediated response. It is speculated that the role of the chlorophyll-mediated response may be to keep the stomatal response to light matched to the response of assimilation to light, since environmental influences on electron transport in the mesophyll would also affect the chlorophyll-mediated stomatal response to light.

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APPENDIX

APPENDIX

Absorption of Light by Leaves

The lower surface of *Xanthium strumarium* leaves has a higher reflectance than does the upper surface. As a consequence, the amount of light absorbed depends on the orientation of the leaf with respect to the light source. Surprisingly, the transmittance was found to be the same when the leaf was illuminated from the top or the bottom (Table 1-A). This suggests that the lower epidermis reflects light back up through the leaf when the leaf is illuminated from above. A model of light absorption was developed that predicts equal transmittance when the leaf is illuminated from above and below; this can be used to evaluate the effect of a highly reflective lower leaf surface. Figure 1-A shows 5 paths of light that can be accounted for by this model. Path 4 is usually very small and is lumped with path 3 in the mathematical treatment.

The transmittance of light through the leaf (T) is equal to the incident light (I) minus the reflected light (R) and the absorbed light (A):

$$T=I-R-A \tag{1}$$

The amount of light absorbed by the leaf (light paths 2 and 3 of Figure 1) is given by:

$$A=(I-Ir_u)a+(I-r_u-(I-Ir_u)a)r_l \tag{2}$$

where r_u and r_l are the reflectances of the upper and lower surfaces respectively and a is the absorptivity of the mesophyll. Setting I equal to 1 and substituting equation 2 into equation 1 we obtain:

$$T=1-a+ar_u-r_l+r_ur_l+ar_l-ar_ur_l-r_u \tag{3}$$

From equation 3 it can be seen that the transmission of light is independent of the orientation of the light source, since r_u and r_l could be

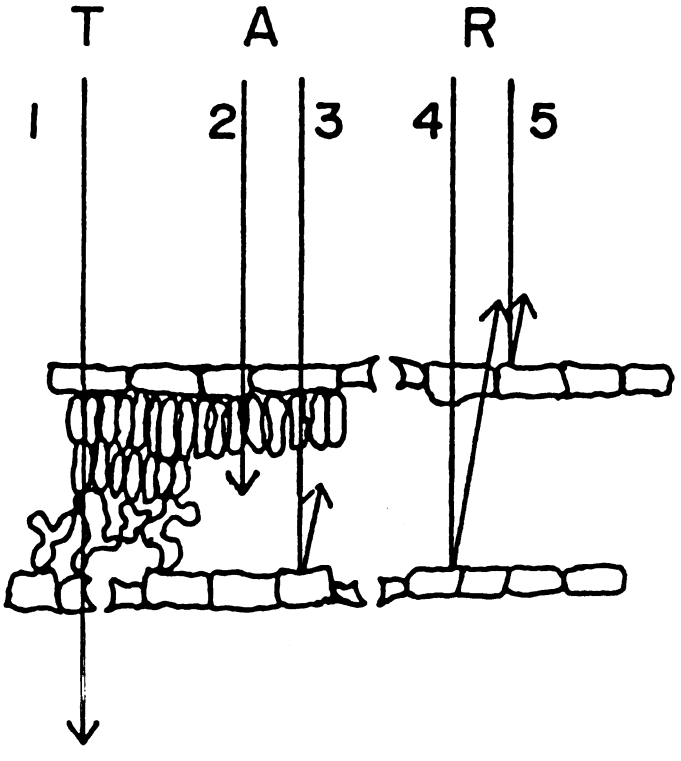
Table 1-A. Transmissivity, reflectivity, and absorptivity of leaves.

Transmissivity, reflectivity, and absorptivity of light of a leaf of Xanthium strumarium measured at two wavelengths of light. Measurements were made with the light shining on the upper surface (normal) or the lower surface (inverted). All measurements were made with a Zeiss PMQ II spectrophotometer with an integrating sphere. Absorptivity was calculated using equation 1 in the text.

	436 nm		681 nm	
	normal	inverted	normal	inverted
Transmissivity (%)	2.6	2.7	5.5	5.6
Reflectivity (%)	5.2	7.5	6.0	9.9
Absorptivity (%)	92.2	89.9	88.5	84.5

Figure 1-A. Paths of light through leaves.

The model of light absorption developed in the text can account for these five paths of light through leaves. Since light path 4 was determined to be very small, it is lumped with light path 3 in the analysis.


T
A
R
1
2
3
4
5

interchanged without changing the equation.

As the reflectance of the lower surface of the leaf increases, light path 3 of Figure 1 also increases. To evaluate this effect on the total absorption of light, it is necessary to solve for a (T, r_u, and r_l can be measured directly).

$$a=(1-r_l-r_u+r_ur_l-T)/(1-r_u-r_l+r_ur_l) \quad (4)$$

Light path 3 (LP3) is given by:

$$LP3=(I-Ir_u-(I-Ir_u)a)r_l \quad (5)$$

By arbitrarily setting the reflectivity of the lower surface equal to that of the upper surface and subtracting that result from the result of equation 5, we can determine the extra absorbance caused by the higher reflectivity of the lower surface. These calculations were done with data from Xanthium strumarium and Populus alba, a tree whose leaves have a hirsute lower surface with a high reflectivity. The reflectivity of the upper and lower surface and the transmissivity of each leaf was measured with a Ziess PMQ II spectrophotometer with an integrating sphere. Measurements were recorded every 10 nanometers from 400 to 700 nanometers. The averaged results are presented in Table 2-A. The effect of the reflective lower surface was small in the blue and the red because nearly all of the light was absorbed the first time it passed through the leaf. However, a fair amount of green light was reflected from the lower surface of the leaf. At 540 nm, 7% of the incident light was reflected from the lower surface of leaves of Populus alba. As a result, the relative intensities of light at various wavelengths is important. The average was calculated for two light distributions. First, it was assumed that there was an equal number of quanta at each wavelength and second, to simulate the light distribution in the understory, it was

assumed that the light had the same distribution as the transmission spectrum of the leaf being studied. This was done by multiplying each value by the transmissivity at that wavelength and dividing by the average transmissivity. The data in Table 2-A indicate that the effect of the reflective lower surface of Xanthium strumarium leaves is insignificant in terms of light absorption, but for Populus alba the effect can be significant, especially for leaves in the understory.

Table 2-A. Light absorbed by leaves after reflection from the lower surface.

Calculations were made assuming an equal quantum flux at all wavelengths (labeled "normal light distribution") and assuming that the incident light had first passed through an identical leaf (labeled "understory light distribution"). See text for more detail on how these calculations were made.

	Absorption (%)	
	Light distribution	
	Normal	Understory
Populus alba	2.1	4.9
Xanthium strumarium	0.3	0.6