Action Spectra for Guard Cell Rb\(^+\) Uptake and Stomatal Opening in *Vicia faba*

Theodore C. Hsiao and W. G. Allaway
Department of Environmental Biology, Research School of Biological Sciences, Australian National University, Canberra 2601, Australia

L. T. Evans
Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra 2601, Australia

ABSTRACT

Abaxial epidermal strips, containing guard cells as the only viable cells, were prepared from leaves of *Vicia faba* following a period in darkness, and floated, under CO\(_2\)-free air, on 2 mM RbCl + 0.1 mM CaCl\(_2\) labeled with Rb\(^+\). Under white light (high pressure mercury vapor lamp), stomatal opening in these strips approached its maximum at less than 0.02 calorie per square centimeter per minute. Under light of different wavelengths, 20 nanometers apart, and at a low quantum flux density of \(7 \times 10^4\) quanta per square centimeter per second, Rb\(^+\) uptake and stomatal opening were activated only in the blue and long ultraviolet regions, with a peak at 420 to 460 nanometers. The action spectrum suggests that the underlying process is not photosynthesis. At higher quantum flux density (\(38 \times 10^4\) quanta per square centimeter per second), uptake and opening also responded to red (600–680 nanometers) and somewhat to green light, with a minimum at 540 to 560 nanometers, indicating a possible involvement of the photosynthetic process. This light-induced opening appeared not to be mediated by a lowering of CO\(_2\) concentration, since CO\(_2\)-free air was used in all treatments and controls. Stomatal opening paralleled Rb\(^+\) uptake in all cases. This constitutes further evidence for the potassium transport hypothesis of stomatal movement.

In the abaxial surface of leaf discs under air of normal CO\(_2\) concentration, stomatal opening in white light approached its maximum at an intensity similar to that for epidermal strips. At both quantum flux densities, the action spectra for opening in leaf discs were very similar to those for epidermal strips. Thus, these light-linked processes for stomatal opening are likely to be the same in leaves as in epidermal strips.

There is considerable evidence that a mechanism underlying stomatal opening is the uptake of K\(^+\) by guard cells (8, 13) in osmotically significant amounts (4). Opening, induced either by light (7, 11, 12, 29) or by CO\(_2\)-free air (7), is associated with large increases in guard cell K\(^+\). On the other hand, closing in darkness is associated with a loss of K\(^+\) (11, 29). With stomata in isolated epidermal strips of *Vicia faba*, exogenous K\(^+\) must be supplied to obtain substantial opening in response to light (7, 10). Therefore, opening appears to be the result of K\(^+\) uptake, not the cause. In this role, K\(^+\) can be replaced by Rb\(^+\), but other monovalent cations are ineffective (10) unless supplied in concentrations much higher than expected to occur in the leaf apoplast (10, 33).

The dependence of a light-driven process on wavelength can be indicative of the pigment and mechanism involved. Action spectra for stomata have been studied previously. To date, however, such data are scanty for opening without the complication introduced by the underlying mesophyll. Also, there are no data on the dependence on wavelength of K\(^+\) or Rb\(^+\) uptake associated with opening in light.

Most earlier studies of the effects of wavelength on stomatal opening indicated that blue light was most effective and green least, with red in between (21). Since leaf pieces were often used, the light effect might have been indirect, mediated through the mesophyll. For example, light would increase photosynthesis by mesophyll cells, and the resultant lowering of intercellular CO\(_2\) could induce stomata to open. The earlier data were also limited by the use, in most cases, of broad spectral bands or only few wavelengths (21). In a more comprehensive investigation, Kuiper (18) studied the spectrum in detail with epidermal strips of *Senecio odoris*. However, stomata in his strips did not open in response to light, and so he was limited to examining the effect of light in preventing closing. He concluded that the action spectrum showed that photosynthesis of guard cell chloroplasts was responsible for preventing closing.

This paper reports on the action spectrum for Rb\(^+\) uptake into guard cells and the associated stomatal opening in *Vicia epidermal strips, and on the action spectrum for stomatal opening in leaf discs.

MATERIALS AND METHODS

Plant Material. *Vicia faba* L. (cv. Early Long Pod) was grown in a greenhouse in solution culture. Supplementary tungsten lights were used in the winter. Leaves fourth through sixth from the shoot apex were harvested, kept in darkness to close stomata, and then used for preparing epidermal strips or leaf discs. Strips about 10 mm\(^2\) in area were taken from between major veins of the abaxial (lower) leaf surface in the following way. The epidermis was cut with a U-shaped template.
made of a strip of band razor blade; one point of a pair of needle forceps was inserted between the mesophyll and epidermis along the uncut side; and the epidermis was gripped, stripped off, and floated on 0.1 mm CaCl₂ in the dark for 8 min or longer. The strips were then rolled gently between a Tygon-sleeved roller and a Perspex board, and replaced on 0.1 mm CaCl₂ in darkness. The only viable cells in these rolled strips were guard cells. Details of the rolling procedure are given elsewhere (1), together with data demonstrating that only the viable cells contained K⁺ stainable with colbalininitrile. Leaf discs (1.3 cm diameter) were taken with a circular punch made from a strip of band razor blade. In all experiments, a replicate always consisted of strips taken from the same leaflet or of discs from leaflets of the same leaf. One strip (or disc) was used in each treatment within a replicate.

**Light.** Light of various wave bands was obtained from a spectrograph, the light source being a 2500-w air-cooled Xenon arc lamp, arc length about 5 mm, housed in a Zeiss Xenosol III projection unit. The beam emerging from the aperture slit was projected by a front-aluminized concave mirror onto a Bausch and Lomb diffraction grating mounted face down on the ceiling. The grating had a ruled area of 206 × 206 mm, with 1200 grooves per mm blazed at 410 nm. From the grating the beam was projected to the plane of the wavelength stations, 2.7 m below. Linear dispersion at this level was 3.83 mm/nm, and with a slit 2.5 cm wide the flux density of the beam was up to 0.063 cal cm⁻² min⁻¹. Black walls, ceiling, and baffles reduced background light to below 28 μcal cm⁻² min⁻¹. The aperture slits used were either 1.2 or 2.5 cm wide; the respective half-band widths were less than 40 nm and about 65 nm. With still narrower slits, which would have reduced bandwidth and given sharper peaks to the action spectra, adequate flux density could not be obtained at the extremes of the spectrum. An orange filter removed second order wavelengths from the red end of the spectrum. The wavelength stations were checked daily with interference filters and a spectroradiometer. Neutral density filters (exposed photographic negatives) were used at each wavelength station. 20 nm apart, to establish the same quantum flux density for all stations in each action spectrum (within ±3%). These filters were fixed on the clear Perspex top of rectangular boxes with open bottom and blackened sides, which covered the samples. The station at the 360 nm position, though nominally designated as such, actually received light averaging longer than 360 nm and of a narrower wave band than the other stations because the Perspex of the covering box was effectively opaque to light below 365 nm. The flux density at each station under the box was measured with a Kipp compensated thermopile and microvoltmeter.

White light, from high pressure mercury vapor lamps (Philips HPLR, 400 w), was filtered through several centimeters of water. The ultraviolet component was largely filtered out by the clear Perspex top of the boxes. Intensity was varied by altering the distance from the source and using fine metal screens and was measured under the box with a silicon photocell and millivoltmeter which had been calibrated with the same light source against an Eppley pyranometer.

**Atmosphere and Temperature.** The boxes, one at each wavelength station, rested on a blackened Perspex sheet. The atmosphere within was controlled by pumping air in through tubes in the sheet and letting the air escape between the box edges and sheet. The flow rate through each box was sufficient for two to four renewals of air per minute (box volume about 85 ml) and was checked for each box each day. The air was drawn from outside the laboratory and passed through a soda lime tower to remove CO₂ and then bubbled through water. The soda lime tower was bypassed to obtain normal air (ambient content of CO₂).

Epidermal strips or leaf discs were floated on solutions during experiments. The temperature of the solution under different treatments was monitored with thermocouples and partly controlled by cooling in the case of treatments receiving high radiant energy. During an experiment the temperature might increase as much as 3 or 4 °C with time, but differences among treatments at any one time were almost always within 1.5 °C. The range of experimental temperatures was generally between 21 and 26 °C.

**Rb⁺ Uptake and Stomatal Opening in Strips.** "Rb⁺" uptake and stomatal aperture were determined on the same strip, after exposure for 3 hr to selected conditions while floating, cuticle up, on 2 mm RbCl-0.1 mm CaCl₂. Stomata in _Vicia_ strips (11) or leaf discs (3) reach steady state aperture in 3 hr or less under white light. The solution was labeled with "RbCl to a specific radioactivity of 8 to 20 × 10⁶ cpm/μmole. Strips were transferred from 0.1 mm CaCl₂ to glass vials (1.5 cm diameter) containing about 0.2 ml of solution and placed immediately under light or darkness in CO₂-free air. After 3 hr the strips were removed, washed (for a total of about 5 min) through a series of eight beakers of nonradioactive 2 mm RbCl-0.1 mm CaCl₂ to remove exchangeable "Rb⁺", and mounted in oil for microscopic measurements.

At 40× magnification, the mean length and width of the strip were estimated with an ocular micrometer and used to calculate area. With the iris diaphragm closed down, the strip was scanned to confirm that there were no intact epidermal cells, which present a bulging appearance, in contrast to the flattened and wrinkled look of broken cells (1). Dead guard cells, judged largely by disorganized cellular content and excessive Brownian motion (1), were counted at 400× magnification in four fields. One field was selected randomly from each quadrant of the strip. Aperture was measured with an ocular micrometer at 1000× with oil immersion on 20 stomata formed by live guard cells (four groups of five each, at random) on each strip. The measurements took 7 to 8 min. Stomatal aperture in epidermal strips in immersion oil does not change for at least 30 min (5). "Rb⁺" did not leak from strips into the oil during measurement (20 oil samples checked all gave counts the same as background). For counting, the strip was removed from the microscope slide and placed in the center of a planchet. Radioactivity was measured with a gas flow counter. The slight amount of oil adhering to the strip did not affect counting efficiency. Aliquots of the radioactive RbCl-CaCl₂ were dried down in the center of other planchetts for use as standards.

The mean number of stomata per field (400X) was calculated from determinations made on several strips in each replicate. The proportion of dead guard cells, calculated for individual strips on the basis of this mean, usually ranged from one to several per cent but infrequently ran as high as 20%. Dead guard cells were assumed to contain no radioactivity. Uptake by each strip was corrected so that the value is based on 100% live guard cells.

When opening alone was studied, strips were treated similarly except that "Rb⁺" was omitted from the solution and desorption (to remove exchangeable "Rb⁺") was not carried out.

**Stomatal Opening in Leaf Discs.** The study with leaf discs was done also on stomata on the abaxial surface. Leaf discs were always floated, abaxial surface up, on 0.1 mm CaCl₂ during treatment. Thus, only this surface received the specified quantity and quality of light. For direct stomatal measurement, immersion oil was placed on the surface and the aperture of 20 stomata (five each in four areas) determined at
1000× using the oil immersion lens without a cover slip and very bright illumination. The aperture stayed constant for 30 min or longer in strips in oil. In contrast, in leaf discs under oil, the aperture began to change in about 15 min, probably because the oil did not penetrate readily to the underside of the epidermis. The measurement on each disc took only 5 or 6 min.

Experiments on spectral response required a large number of determinations, which were done expeditiously with a mass flow porometer similar to that of Spanner and Heath (30), using a total head of 10 cm of water. The values of standard resistances of the porometer were calculated from the applied pressure heads and the rates of air flow across the resistances, measured with accurate flow meters. Data are presented as conductance in cm sec⁻¹ bar⁻¹ (cm² of air per cm² of porometer cup area per second per bar). Only the abaxial stomata were of concern. Therefore, at the end of treatment an area of the adaxial (upper) epidermis sufficient to accommodate the porometer cup was quickly stripped, and the resistance of the abaxial epidermis plus the mesophyll (the latter should constitute negligible resistance) was determined. This process required less than 3 min for each leaf disc.

Treatment Pattern. One strip or disc was taken randomly from every replicate for the determination of initial stomatal aperture before treatment began. To ensure consistency, all strips or discs in any one replicate were measured by one person. Each treatment within the replicate was therefore started at a different time. Three replicates were carried concurrently through each experiment on spectral response. The starting sequence for each replicate was determined using first a table of random numbers and then adjusting to avoid bunching of the same treatment of all three replicates in the same time period. About 3 hr were needed to complete the starting sequence.

Variability in Experimental Material. The considerable variability in the plant material hindered efforts to obtain precise data. Variability was at all levels: from stoma to stoma, area to area on a leaflet, leaf to leaf, experiment to experiment, and season to season. It was greater with strips than with leaf discs. The variability between experiments is readily seen for both strips and discs when data for a treatment common to several figures are compared (e.g., “white light” of Figs. 3A, 4A, 4B). The variability within experiments is evident in the high coefficient of variation found for the 20 aperture measurements made per treatment replicate (e.g., range 7–23% in one experiment in Fig. 4A). In consequence, large numbers of measurements were required for meaningful comparisons; for example, each data point for aperture in Figure 4A represents a mean of 360 stomata in strips from 18 leaflets.

RESULTS

Conductance as Related to Aperture in Leaf Discs. The conductance of the leaf discs with the adaxial epidermis removed, as measured with the mass flow porometer, was curvilinearly dependent on stomatal aperture on the abaxial surface (Fig. 1). The relationship given was for measurements on the same area of the disc. The use of separate areas of the same disc, one for porometry and one for the microscope measurement, gave greater scattering of the points (data not shown).

Opening at Different Quantum Flux Densities. Under white light (high pressure mercury vapor lamp) and CO₂-free air, the opening response of stomata in epidermal strips approached its maximum at a quantum flux density of less than 0.02 cal cm⁻² min⁻¹ (Fig. 2A). This corresponded to less than 80 × 10⁶ quanta (of mixed wavelengths) cm⁻² sec⁻¹ based on the spectral distribution of the light source. Stomata on strips were moderately open (4 to 7 μ) at the start of treatments (initial aperture), as a result of the release of back pressure on guard cells when the surrounding epidermal cells were broken (1). All strips were taken from leaflets with closed stomata, as routinely tested with a mass flow porometer. In leaf discs under normal air, the quantum flux density required for maximal stomatal opening (Fig. 2B) was about the same as that for epidermal strips. The magnitude of the response to light was considerably greater with leaf discs than with strips, averaging about 9 versus 3 μ. Fischer (5) earlier found light response to be about equal in Vicia strips and discs. The present smaller response in strips was not the result of rolling to break epidermal cells. Stomata in unrolled strips showed an equally small opening in light. Responses of stomata in strips to a range of quantum flux densities were compared at blue, green, and red wavelengths in the same experiments. Limited data (Fig. 3A) suggest that saturation may have been reached at the highest quantum levels used for the three wavelengths. This is not at all certain, however, because of the limited number of quantum levels used for each wavelength and the variability in the data. Similar data for stomata in leaf discs under normal air (Fig. 3B), with the possible exception of those for the blue, did not suggest saturation.

Spectral Response of Rb⁺ Uptake and Opening in Epidermal Strips. The opening in strips were studied in detail always under CO₂-free air. Preliminary experiments showed that the opening response to light was reduced under normal air as reported by Fischer (5). It was not feasible to delineate the spectral dependence of stomatal opening for strips in normal air with this reduced response and the inherent variability of the plant material. Three control treatments, also under CO₂-free air, were included in each experiment in the spectrograph. These were: white light (high pressure mercury vapor lamp, 0.25 cal cm⁻² min⁻¹), dark (under a completely opaque box), and background light (under a box with clear Perspex top placed near the spectrograph beam, outside the area directly lit by the beam).

The spectral dependence of Rb⁺ uptake and stomatal open-

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**Fig. 1.** Conductance to mass flow of air as related to mean aperture of stomata on the abaxial leaf surface of Vicia. Leaf discs from the same group of plants were placed under different light intensities to obtain a range of apertures. Adaxial epidermis was quickly removed, and conductance of the abaxial epidermis (and the adhering mesophyll) was estimated with a porometer. Immediately afterwards 20 apertures within the area covered originally by the porometer cup were measured under oil. Data points are for single discs and represent two experiments conducted within a week.
Plant flux

Upon opening, the ground


ACTION SPECTRA FOR STOMATA OF VICTIA

85

μcal cm⁻² min⁻¹, the limit of detectability for the measuring instrument.

Spectral response at the high quantum flux density was different (Fig. 4B). In addition to the peak in the blue region, estimated Rb⁺ uptake showed a clear response in the red region, with a broad maximum apparently at 620 to 680 nm. Response in the green region was slight, with the minimum apparently at 560 nm. Again, the opening corresponded very closely with Rb⁺ uptake for all treatments. To emphasize the correspondence between opening and Rb⁺ uptake, different scales for uptake are used in Figures 4A and 4B. The reason for the difference between the low and high quantum flux density data in the amount of Rb⁺ taken up per unit of aperture increase is not known. Since uptake is based on per unit of strip area, the difference could possibly be due to dissimilar stomatal frequencies, guard cell sizes, or cell wall elastic properties of the two groups of plant material. Uptake and opening in white light were abnormally small in the series of experiments presented in Figure 4B (compare with Figs. 2A, 3A, and 4A). We can offer no good explanation for the anomaly. The majority of the experiments at low quantum flux density were conducted with plants grown during the summer and autumn, whereas the majority of experiments at the high quantum flux density were carried out during the winter months of low natural light.

FIG. 2. Abaxial stomatal opening in Vicia as dependent on quantum flux density of white light. Exposure was for 3 hr. Quantum flux density was calculated from the intensity (cal cm⁻² min⁻¹). The factor used was 1.1 x 10⁶ quanta cal⁻¹, which was based on the spectral energy distribution of the light measured in the range of 360 to 720 nm. Vertical bars represent twice the standard error of the mean. A: Epidermal strips under CO₂-free air. Data are means of two experiments, each consisting of six replicates. Mean initial aperture was 4.3 ± 0.4 μ. B: Leaf discs under normal air with abaxial epidermis facing the light. Conductance was measured with porometers, and results from experiment 1 () and 2 (○), each consisting of five replicates, are shown.

Fig. 3. Abaxial stomatal opening in Vicia as dependent on quantum flux density at 440, 560, and 660 nm. Exposure was for 3 hr in the spectograph. Vertical bars represent twice the standard error of the mean and horizontal bars represent ranges of quantum flux density. A: Epidermal strips under CO₂-free air. Apertures and quantum flux densities are means of six experiments, each consisting of four to six replicates. Initial aperture averaged 6.6 ± 0.3 μ. B: Leaf discs under normal air. Openings were measured with porometers and are given as mean conductances for two experiments, each consisting of five replicates. Initial conductance averaged 85 ± 10 cm sec⁻¹ bar⁻¹.
error of the Rb\(^+\) uptake was

Data was presented in Figure 5A, but about the same in data of Figure 5B. There was a slight opening in 3 hr under normal air and darkness. This may reflect the endogenous diurnal patterns of opening (31).

**Spectral Response of Two Other Species.** Spectral responses of stomatal opening of several other species were examined on a very limited basis. Stripping the epidermis was often difficult. Incomplete data were obtained for adaxial stomata in spinach leaf discs and for stomata in abaxial epidermal strips of Commelina communis. Commelina strips showed little or no opening response to light on 2 to 10 mM KCl or on 10 mM NaCl. Reasonable opening in light required floating them on 67 mM KCl-67 mM potassium phosphate at pH 6.8, the medium used by Willmer and Mansfield (32). Both spinach

In all experiments depicted in Figure 4, some Rb\(^+\) uptake and opening occurred in strips kept in the dark for 3 hr (compare "initial" with "dark"), apparently in response to CO\(_2\)-free air. The desorption procedure used was apparently effective in removing adsorbed \(^{86}\)Rb\(^+\). Strips put through the procedure after they were floated on the radioactive solution in the dark for 3 to 5 min retained very little radioactivity. The retained activity for all replicates averaged 2.1 \(\mu\)eq mm\(^{-2}\) in four experiments depicted in Figure 4A and 1.9 \(\mu\)eq mm\(^{-2}\) in two experiments shown in Figure 4B.

**Spectral Response of Opening in Leaf Discs.** Stomatal opening in discs was studied under normal air. Data for the low quantum flux density (Fig. 5A) showed pronounced opening in the blue region, peaking at 420 to 460 nm. There was no response in the green region around 560 nm, but the data do not rule out a very slight response in the red region. At the high quantum flux density (Fig. 5B), the responses to red and green were substantial, with the minimum at 540 to 560 nm. In leaf discs under normal air, openings at both quantum flux densities were less in blue light than in the white light control, with the difference being larger at the low quantum flux density. Opening was greater in background light than in "dark" in data presented in Figure 5A, but about the same in data of Figure 5B.

Fig. 4. Spectral dependence at equal quantum flux density of Rb\(^+\) uptake and stomatal opening in *Vicia* epidermal strips. Exposure was for 3 hr under CO\(_2\)-free air. Estimated uptake is based on mm\(^2\) of epidermal area. Vertical bars represent twice the standard error of the mean for uptake or aperture of a particular treatment. Data in A and B were obtained in separate experiments at different times and should not be compared with each other on strict quantitative terms. A: Low quantum flux density (7.8 \(\times\) 10\(^4\) quanta cm\(^{-2}\) sec\(^{-1}\) except for 360 and 720 nm, which received 5.9 \(\times\) 10\(^4\) and 6.6 \(\times\) 10\(^4\) quanta cm\(^{-2}\) sec\(^{-1}\), respectively). Data are means of six experiments, each consisting of three replicates. Mean initial aperture was 6.8 \(\pm\) 0.3 \(\mu\). B: High quantum flux density (38 \(\times\) 10\(^4\) quanta cm\(^{-2}\) sec\(^{-1}\)). Data are means of five experiments, each consisting of three replicates. Mean initial aperture was 5.1 \(\pm\) 0.3 \(\mu\).

Fig. 5. Spectral dependence at equal quantum flux density of stomatal opening in *Vicia* leaf discs. Exposure was for 3 hr under normal air and opening in the abaxial epidermis was measured with porometers. Experiments represented in A and B were conducted separately. Data are means of two experiments, each consisting of six replicates. Vertical bars represent twice the standard error of the mean. A: Low quantum flux density (7 \(\times\) 10\(^4\) quanta cm\(^{-2}\) sec\(^{-1}\)). B: High quantum flux density (38 \(\times\) 10\(^4\) quanta cm\(^{-2}\) sec\(^{-1}\) except for 380 nm, which received 32.5 \(\times\) 10\(^4\) quanta cm\(^{-2}\) sec\(^{-1}\)).
stomata in discs and Commelina stomata in strips responded markedly to blue light of low quantum flux density (respectively $7 \times 10^4$ and $4.3 \times 10^4$ quanta cm$^{-2}$ sec$^{-1}$), with blue peaks at about the same position as for *Vicia*.

**DISCUSSION**

Stomatal opening showed a striking correspondence with estimated Rb$^+$ uptake in all cases. This close correspondence held not only across the spectrum (Fig. 4) but also for various controls. Opening in “dark” above that of “initial” was well correlated with uptake. So was opening in background light above that in “dark.” Even the anomalously small opening in “white light” shown in Figure 4B was related to a similarly small uptake.

For discussion, it is assumed that Rb$^+$ and K$^+$ are transported similarly into guard cells and act similarly in stomatal movement. In a previous study, stomatal opening in strips on Rb$^+$ was indistinguishable from that on K$^+$ (10). Preliminary experiments established that the relationship between stomatal aperture and uptake was similar for *K* and *R*, as expected from data of Fischer (6). In higher plant systems, the transport of Rb$^+$ generally resembles closely that of K$^+$ provided that Ca$^{2+}$ is present to maintain the integrity of the plasmalemma (19).

Rb$^+$ uptake in this study was estimated from the radioactivity retained in the strips. The estimation should reflect net uptake closely since previous results showed little or no exchange between Rb$^+$ in *Vicia* strips and K$^+$ in the external solution in 30 min (7), and only a minor exchange in 2 hr (11). Further, earlier work (11) showed a pronounced increase in stable K$^+$ in guard cells of *Vicia* strips on KCl after a light treatment, indicating a net gain of K$^+$. In this study, uptake should be only by guard cells since the strips contained no intact epidermal cells. Intact epidermal cells of *Vicia* contain considerable stable K$^+$ whereas broken ones contain none (1). Guard cells in *Vicia* strips which were obviously dead also do not stain for K$^+$ (1). It has been pointed out (7, 12) that the K$^+$ taken up by guard cells, along with a counter ion, would be sufficient to account for the measured increase in solute concentration responsible for bringing about water uptake and stomatal opening. Uptake of Rb$^+$ per unit of stomatal opening in the present experiments was within the range of values found previously with *Vicia* strips (7, 11).

The action spectra at low quantum flux density show marked effectiveness in the blue region but little or no action in the red. The result is in general agreement with the blue light effect described for whole leaves by others (21, 27). Additionally, the data indicate for the first time that the blue light effect on opening is mediated through the same basic process of K$^+$ (or Rb$^+$) uptake as is opening in other light. We observed substantial action in the ultraviolet region down to below 380 nm (Fig. 4A), in agreement with Mouraviev's report (24) of action at 390 nm. In apparent disagreement are data of Kuiper (18), suggesting that ultraviolet light was ineffective in preventing the closing of stomata in *Senecio*. The data of McCree (22) indicate that near ultraviolet light can sustain photosynthesis in leaves of many species. Photosynthetically linked light stimulation of ion uptake is known for several nonstomatal systems (28), including some in higher plants (14, 20, 25). However, the ineffectiveness of red light in our action spectra for stomatal opening and uptake of Rb$^+$ by guard cells at low quantum flux density suggests that these responses are mediated by a blue-absorbing pigment rather than by photosynthesis. Another system with a comparable action spectrum is the enhancement of respiration by light in *Chlorella* (17); in this the maximal effect of 455 nm is approached at a quantum flux density of only $1 \times 10^4$ quanta cm$^{-2}$ sec$^{-1}$ (16). Comparable action spectra have also been described for other physiological processes, for example, phototropism (2). The work of Kuiper (18) showed that blue light was more effective than red but did not reveal a spectrum with action confined to blue light at a quantum flux density almost identical to our low one (his curve for 1.14 $\times 10^4$ einstein cm$^{-2}$ sec$^{-1}$). The reason for the discrepancy is not known. Kuiper used epidermal strips from *Senecio*, but our data on spinach and Commelina and work on other species (21) suggest that the blue light effect may be a widespread phenomenon. Kuiper studied the prevention of closing, whereas we examined the opening process.

Opening at the high quantum flux density was different in that it showed clear action in red light and some action in the green region (Figs. 4B and 5B), an action spectrum more nearly resembling that for leaf photosynthesis (22), although still showing a very high blue peak. The spectrum differs significantly from that reported for prevention of stomatal closing in *Senecio* (18) in that in our curve the trough in the green region is shallower and the fall-off less steep at the ultraviolet and far red ends of the spectrum. At the higher quantum flux density, either a photosynthesis-linked process becomes effective or, perhaps less likely, an unknown process with photoreceptor in the red region comes into play and the spectrum results from superposition of those for the blue- and red-absorbing processes. Data on *Vicia* strips obtained with inhibitors of photosynthesis and far red light have been interpreted to mean that photosystem I can provide the energy necessary for K$^+$ uptake and stomatal opening under some conditions (11).

Somewhat unexpectedly, the action spectra for opening in leaf discs under normal air closely resemble those in strips under CO$_2$-free air under both the low and high quantum fluxes. Additionally, saturation curves under white light were about the same for strips in CO$_2$-free air and discs in normal air and differed from the saturation curves for leaf photosynthesis (9) in that much less light is required to saturate opening. These results point to the possibility that even in leaf discs under normal air the light response we observed was not the result of changes in CO$_2$ levels via photosynthesis, but of light-activated changes in the distribution of K$^+$.

Changes in CO$_2$ concentration in the leaf have been considered by Meidner and Mansfield (23) to play a key role in stomatal movement, although they also pointed out that movement can be effected independently of CO$_2$. Raschke (26) suggested that light, aside from lowering the CO$_2$ concentration, supplies energy to guard cells for opening. In our study there can be little doubt that, under low light, opening in strips is independent of CO$_2$ concentration changes. The shape of the action spectrum indicates a nonphotosynthetic process. Additionally, the strips were always kept under CO$_2$-free air during treatment and the major possible sink and source of CO$_2$, the leaf mesophyll, had been removed. Further, the very low light intensity used (Figs. 2A and 4A) was probably close to the light compensation point for CO$_2$ assimilation. Under the high light level, the opening in strips (Figs. 2A and 4B) is probably also not mediated by a lowering of CO$_2$ since CO$_2$-free atmosphere was again maintained. Although changes in CO$_2$ are not considered to be the basis for the opening response to light in this study, we do not imply that such changes cannot elicit stomatal movement in our system. Earlier work has demonstrated an opening effect by lowering of CO$_2$ independent of light in *Vicia* strips and discs (5). In the present study, as mentioned above, placing strips under normal air instead of CO$_2$-free air appeared to reduce the magnitude of light response. Opening in the dark...
above the "initial" value (Figs. 2A, 3A, and 4) presumably was largely a response to CO₂-free air. Also, stomata in leaf discs in the dark opened substantially under CO₂-free air as compared with normal air. Obviously, both the light- and CO₂-mediated processes are functional, and the relative contribution of each to opening would depend on the environmental conditions employed and on the plant species. The possibility that CO₂ fixation may compete with ion transport for photosynthetically produced ATP has been suggested for *Elodea densa* (15) and *Hydrodictyon affricum* (28). If such a system were to operate in guard cells, it could explain the reduction of the light response of *Vicia* stomata in the presence of CO₂. The well known lowering of intercellular space CO₂ in leaves by photosynthesis would ordinarily ensure an accentuated stomatal opening response to light.

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**LITERATURE CITED**


