PROGRESS REPORT

DOE GRANT # DE-FG03-90ER20011
"Sensory Transduction of the CO_2 Response of Guard Cells"
July 1, 2000 to June 30, 2003

SUMMARY OF PUBLICATIONS

Full Papers:

1. Zeiger, E. 2000. Sensory transduction of blue light in guard cells. Trends in Plant Science: 183-184.
2. Frechilla, S; Talbott, L. D.; Bogomolni, R. A.; Zeiger, E. 2000. Reversal of blue light-stimulated stomatal opening by green light. Plant and Cell Physiology. 41: 171-176.
3. Ulloa, M.; Cantrell, R. G.; Percy, R. G.; Zeiger, E.; Lu, Z. 2000. QTL analysis of stomatal conductance and relationship to lint yield in an interspecific cotton. The Journal of Cotton Science 4:10-18.
4. Lu, Z.; Quinones, M. A.; Zeiger, E. 2000. Temperature dependence of guard cell respiration and stomatal conductance co-segregate in an F2 population of Pima cotton. Australian Journal of Plant Physiology 27: 457-462.
5. Frechilla, S.; Talbott, L. D.; Zeiger E. 2002. The CO_2 response of *Vicia* guard cells acclimates to growth environment. Journal of Experimental Botany. 53: 545-550.
6. Talbott, L. D.; Nikolova, G.; Ortiz, A.; Shmayevich, I.; Zeiger, E. 2002. Green Reversal of blue light-stimulated opening is found in a diversity of plant species. American Journal of Botany. 89: 366-368.
7. Zeiger, E. 2002. The guard cell chloroplast: A perspective for the 21st century. New Phytologist 153: 415-424.
8. Talbott, L. D.; Zhu, J.; Han, S. W.; Zeiger, E. 2002. Phytochrome and blue light-mediated stomatal opening in the orchid, *Paphiopedilum*. Plant and Cell Physiology. 43: 639-646.
9. Talbott, L. D.; Raveh, E.; Zeiger, E. Relative humidity is a key factor in the acclimation of the stomatal response to CO_2. Submitted to the Journal of Experimental Botany.

Textbook:

Taiz, L; Zeiger, E. Plant physiology, Third edition. Sinauer Associates, Sunderland, Massachusetts, USA. (2002) in press

Conference Presentations:

1. Gruszecki, W.; Zhu, J.; Talbott, L.D.; Zeiger, E. 2000. Spectroscopic evidence for a pair of interconverting isomers of zeaxanthin that mediate blue light-stimulated opening and its reversal by green light. American Society of Plant Physiologists meeting July 15-19, San Diego, CA.
2. Zeiger, E.; Gruszecki, W.; Frechilla, S.; Talbott, L.D.; Zhu, J. 2000. Spectroscopic and physiological evidence for a pair of interconverting isomers of zeaxanthin that mediate blue light-stimulated stomatal opening and its reversal by green light. American Society of Plant Physiologists meeting July 15-19, San Diego, CA.
3. Zeiger, E. The coleoptile chloroplast. Invited presentation to the annual meeting of the Society for Experimental Biology, U.K., Exeter, 27-31 March, 2000

4. Talbott, L.D.; Zhu, J.; Han, S. W.; Zeiger, E. 2001. Zeaxanthin and phytochrome-mediated stomatal opening in an orchid. American Society of Plant Physiologists meeting July 21-25, Providence, RI.
5. Zeiger, E. 2001. The guard cell chloroplast: a perspective for the 21th century. Invited presentation to "Stomata 2001", Birmingham, July 26-29, 2001.
6. Zeiger. E. 2001. Blue-green reversibility of stomatal movements. Invited presentation to symposium on "Plant Morphogenesis", Univ. of Tokyo, November 25-27, 2001.

SCIENTIFIC CONTRIBUTIONS.

The CO_2 response of stomata from *Vicia faba* undergoes a reversible acclimation to growth environment. (ref 5 above)

Stomata from well watered *Vicia faba* have a higher CO_2 sensitivity when grown under growth chamber conditions than when grown under greenhouse conditions. The differential sensitivity can be demonstrated in the intact leaf by placing both growth chamber or greenhouse-grown plants in either environment and altering ambient CO_2 concentration. In these experiments growth chamber-grown leaves consistantly show a high sensitivity, and green house-grown leaves a low sensitivity, indicating that an acclimation response rather than an instantaneous change in an environmental factor is responsible for the observed difference in the CO_2 response (Fig. 1). The acclimation can also be demonstrated in isolated stomata of detached epidermis, indicating that the difference in CO_2 sensitivity is an intrinsic property of the guard cell. Transfer of plants from the growth chamber to the greenhouse and vice versa, showed that the stomatal response to CO_2 acclimates to growth conditions with a characteristic time course (Fig. 2). Stomata from growth chamber-grown leaves transferred to a greenhouse lost their CO_2 sensitivity within 2 to 3 days, whereas stomata from greenhouse-grown leaves transferred to a growth chamber acquired a high CO_2 sensitivity in 5 to 7 days.

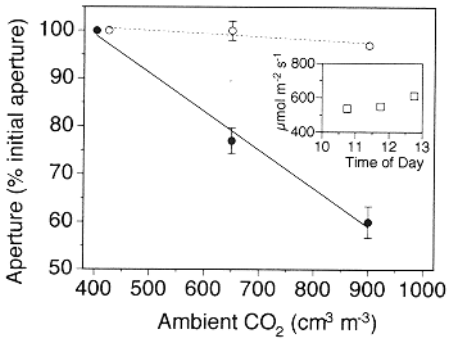

Fig. 1 Aperture change of stomata from greenhouse (o) and growth chamber (o) plants in response to CO_2.

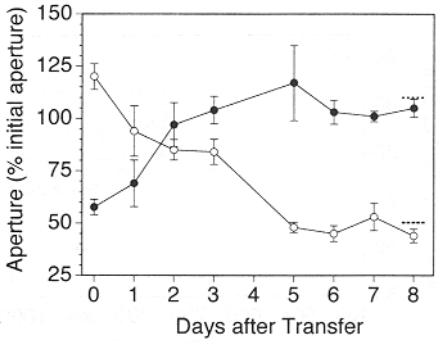

Fig. 2 Acclimation of the stomatal response to CO_2 in plants transferred from greenhouse to growth chamber conditions (o) or vise versa (o).

Relative humidity is a key environmental factor in the acclimation of the stomatal response to CO_2. (ref 9 above)

Manipulation of light quality in a greenhouse environment failed to affect the stomatal response to CO_2. On the other hand, an increase in greenhouse relative humidity, achieved by misting, to

levels prevailing in the growth chamber environment resulted in an increase in the stomatal CO_2 sensitivity to that characteristic of growth chamber-grown plants (Fig. 3). Experiments in which plants were transferred between misted and non-misted areas of the same greenhouse showed that the stomatal response to CO_2 underwent a shift between high and low sensitivity with the same acclimation time course of that found in the growth chamber/greenhouse transfer experiments. Similarly, experiments manipulating growth chamber relative humidity were found to be equally effective in stimulating acclimation of the stomatal CO_2 response. This study thus identifies relative humidity as the key factor triggering the acclimation of the CO_2 response in growth chamber and greenhouse conditions.

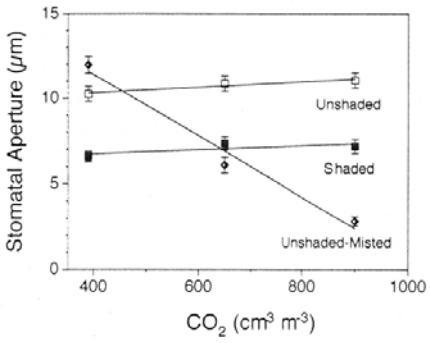

Fig. 3 The CO_2 response of stomata grown under various conditions in a greenhouse.

Under natural conditions, an acclimation of the stomatal response to CO_2 triggered by air relative humidity would optimize leaf gas exchange in dense leaf canopies. Within dense, sheltered canopies, leaves would experience low light, relatively low ambient CO_2 concentration, and elevated relative humidity. Under these conditions, light stimulated stomatal opening would be low, possibly limiting photosynthesis because of inadequate CO_2 uptake. An acclimation that enhances the stomatal sensitivity to CO_2 via high air relative humidity, would increase stomatal conductance and maximize CO_2 uptake and photosynthetic efficiency. In a low wind-speed, closed canopy environment, the high stomatal conductance would not entail high transpiration rates because of the prevailing low VPD.

In pilot experiments testing whether contrasting air relative humidity values within the canopy would alter stomatal CO_2 sensitivity in field-grown plants, two plots of *Vicia* were planted in the botanical garden at UCLA. One plot was left open with good airflow through the canopy. The east, north and west side of the other plot was enclosed with plastic sheeting, limiting airflow through the canopy. Monitoring of relative humidity showed wide variation in humidity within the canopies depending on ambient relative humidity, wind speed and direction. However, relative humidity within the canopy of the unsheltered plot was rarely more than 10% above ambient values while relative humidity within the enclosed sheltered frequently exceed ambient values by 30%. Stomatal CO_2 sensitivity in these two plots was tested by measuring the aperture change of stomata in isolated peels in response to changes in ambient CO_2. Stomata of leaves taken from inside the canopy of the sheltered plot responded to a 900 cm m^{-3} change in ambient CO_2 with a 2 μm change in aperture. Apertures of stomata from leaves taken from inside the unsheltered canopy, however, showed little if any aperture change over the same CO_2 range (Fig. 4). This difference in response patterns is the same as that previously seen in stomata taken from greenhouse and growth chamber-grown plants.

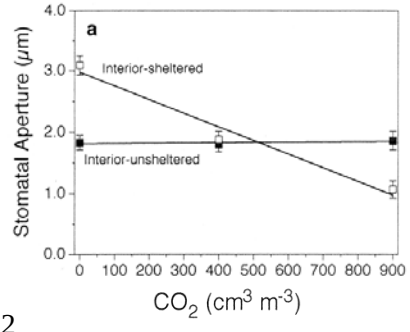

Fig. 4 Stomatal CO_2 response of leaves growing in sheltered and unsheltered field plots.

Stomatal CO_2 sensitivity was also measured in leaves taken from the outer surface of the south (unsheltered) side of the canopy in the sheltered plot. These leaves were exposed to ambient

levels of relative humidity, as well as higher levels of incident radiation. Apertures in isolated stomata from these leaves were insensitive to CO_2 over the 0-900 cm m^{-3} range tested. Thus the same difference in CO_2 sensitivity found between sheltered and unsheltered plots could also be found between sheltered and unsheltered leaves within the same plot. These results provide support for the hypothesis that relative humidity-driven acclimation of the stomatal CO_2 response may optimize leaf gas exchange within dense canopies.

Blue light-stimulated stomatal opening is reversed by green light. (refs 2 and 6 above)

Blue light-specific stomatal opening has been linked to both light-CO_2 sensing by guard cells and to red light enhancement of stomatal opening through the action of the carotenoid zeaxanthin, a proposed blue light photoreceptor (see refs 1 and 7 above). Blue light-specific stomatal opening was reversed by green light in a dose-dependent fashion. Under continuous illumination, blue light opening was completely reversed by a 2:1 ratio of green to blue light illumination. Experiments in which stomata received sequential pulses of blue and green light showed that stomatal opening responded according to the color of the last pulse given, in a manner analogous to the red, far red reversibility of phytochrome-mediated responses (Fig. 5). An action spectrum for the green reversibility showed a maximum at 540 nm and minor peaks at 490 and 580 nm. This action spectrum does not resemble the absorption spectrum of either phytochrome or chlorophyll, but does resemble the action spectrum for blue light-specific opening, red shifted by about 90 nm. This suggests that the blue green reversibility of stomatal opening could result from the photoisomerization of a pair of interconvertible isomers of zeaxanthin, a physiologically inactive blue absorbing form and a physiologically active green absorbing form. Green reversibility of blue light-specific opening was found in a survey of 8 species over a wide range of genera, including dicots, monocots, legumes and grasses. The discovery of green reversibility provides a important tool for probing the extent of blue light-specific activity involved in specific responses such as CO_2 stimulated opening.

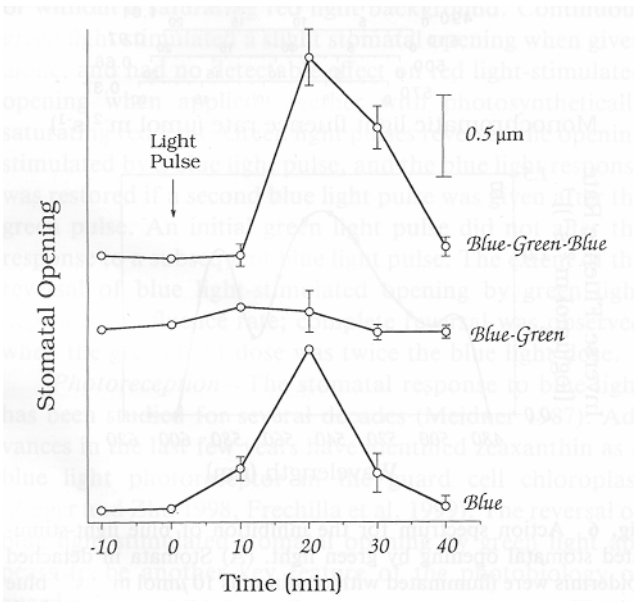

Fig. 5 Stomatal opening in response to sequential pulses of blue and green light.

Phytochrome-mediated stomatal opening in Paphiopedilum orchid. (ref 8 above)

Guard cells of the orchid genus, *Paphiopedilum* lack developed chloroplasts and detectable chlorophyll *a* autofluorescence. *Paphiopedilum* stomata lack a photosynthesis-dependent opening response but have a blue light-specific opening and respond to changes in ambient CO_2 concentration. Low fluence rate green and red light elicited stomatal opening in *Paphiopedilum* and this opening was reversed by far red light, indicating the presence of a phytochrome-mediated opening response. Phytochrome-dependent, red light-stimulated opening was largest under low fluence rates and decreased to near zero as fluence rate increased. The recently discovered green light reversibility of blue light-specific stomatal opening (see above) was used to probe the properties of the blue light response of *Paphiopedilum* stomata. Blue light-

stimulated opening was completely reversed by green light in the presence of far red light. Red light enhanced the blue light response of *Paphiopedilum* guard cells when given as a pretreatment or together with blue light. Analysis of guard cell pigments showed that guard cells have small amounts of chlorophyll *a* and *b*, zeaxanthin, violaxanthin, antheraxanthin and lutein. Zeaxanthin content increased in response to blue light or ascorbate and declined in the dark or under illumination in the presence of dithiothreitol, indicating the presence of an active xanthophyll cycle. Thus *Paphiopedilum* stomata possess both a blue light-mediated opening response with characteristics similar to the zeaxanthin model of blue light/CO_2 sensing, and a novel phytochrome-mediated opening response.

Genetic analysis of stomatal opening response in cotton and identification of loci involved in sensory transduction pathway. (paper refs 3 and 4 above)

Advanced lines of cotton show higher stomatal conductance than less advanced lines. This elevated conductance is associated with higher yields and increased stomatal opening in response to high temperatures. The increased conductance is also associated with a stronger temperature dependence of guard cell respiration rates in these advanced lines. Crosses between primitive and advanced lines showed co-segregation of these traits in F2 populations, indicating genetic control of a signal transduction chain involved in the regulation of stomatal opening. Plants with high and low conductance were identified in a segregating population and DNA was isolated and used to produce a linkage map with 199 DNA sequence markers. Genetic analysis of the replicated F3 families permitted identification of qualitative trait loci (QTL) influencing stomatal conductance. Two putative QTLs for stomatal conductance were identified on two cotton linkage groups. These findings define a valuable experimental system for further characterization of the genetics of stomatal conductance and the sensory transduction mechanisms involved in stomatal opening responses.

Taiz, L; Zeiger, E. Plant Physiology, Third edition.

Publication of the third edition of a plant physiology textbook provided us with a unique opportunity to make new information from DOE-sponsored research in our laboratory available for the education of undergraduate students in the United States and abroad.