

STOMATAL OPENING AT ELEVATED TEMPERATURE: AN UNDERESTIMATED REGULATORY MECHANISM?*

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Summary. Climate models predict more frequent and more severe extreme events (e.g. heat waves, extended drought periods) in Europe during the next decades. The response of plants to elevated temperature is a key issue in this context. Stomatal regulation is not only relevant for the diffusion of CO₂ from the ambient air into the leaves, but it plays also an important role for the control of transpiration and leaf cooling. The regulation of stomatal aperture by the water status (hydroactive and hydropassive feed-back) and by internal CO₂ availability (CO₂ feed-back) are well documented in the literature, while the response of the stomates to elevated temperature was far less considered in the past. Photosynthesis is negatively affected by elevated temperature, but the water loss via transpiration may still be high. In the experiments reported here, bean leaf segments were incubated in darkness floating on water in the range from 20 to 50°C and then analyzed immediately by taking a photograph with a digital microscope. Stomatal aperture was measured on these pictures in order to quantify stomatal opening. After the incubation for 30 min, the opening was 0.66, 2.76 and 4.28 μm at 23, 30 and 35°C respectively. This opening at elevated temperature was fully reversible. Abscisic acid (0.1 μM) in the incubation medium shifted the temperature for stomatal opening to higher values. It can be concluded that elevated temperature stimulates stomatal opening regardless of the CO₂

* Dedicated to Professor Klimentina Demirevska on the occasion of her 60th birthday

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assimilation status and that there is a trade-off between leaf cooling on one hand and limiting water loss during drought periods on the other hand.

Key words: Drought, Global change, Heat, Leaf temperature, Regulation, Stomates

Abbreviations: ABA – abscisic acid; PAR – photosynthetic active radiation; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39)

INTRODUCTION

Climate models predict for the next decades besides an increasing average temperature in Europe increased temperature variability and as a consequence more frequent and more severe heat waves (Schär et al., 2004). Heat influences leaf metabolism in multiple ways (Demirevska-Kepova and Feller, 2004; Sharkey, 2005; Hikosaka et al., 2006). During the past decade, Rubisco activase properties and Rubisco activation state were identified as key aspects in this context (Feller et al., 1998; Crafts-Brandner and Salvucci, 2000; Portis, 2003; Haldimann and Feller, 2004, 2005). Rubisco activase was found to be far more sensitive to heat than Rubisco (Eckardt and Portis, 1997). Rubisco becomes spontaneously inactivated and must be permanently reactivated by Rubisco activase (Salvucci, 1993; Spreitzer and Salvucci, 2002). Reversible effects of elevated leaf temperature on Rubisco activase and on Rubisco are schematically shown on Figure 1. At elevated temperature, the reversible inhibition of photosynthesis is closely correlated with Rubisco activation (Law et al., 1999; Haldimann and Feller, 2004, 2005). Yamori et al. (2006) reported recently that Rubisco activation state plays an important role in limiting photosynthesis at high temperature, while other factors are more relevant at low temperature.

Leaf temperatures may exceed in the field the air temperature by as much as 10°C (Moore et al., 1998; Haldimann et al., 2005). Temperatures of leaves from several plant species are documented in Table 1. Depending on the plant species, the physiological status, the temperature level and the duration of the elevated temperature, heat effects can reversibly (Feller et al., 1998; Haldimann and Feller, 2004, 2005) or also irreversibly affect the photosynthetic apparatus in leaf segments (Herrmann and Feller, 1998; Demirevska-Kepova et al., 2005) as well as in attached leaves in the field (Haldimann et al., 2005).

A series of factors involved in the control of stomatal opening are known (Zeiger, 1983; Moore et al., 1998; Roelfsema and Hedrich, 2005). The different factors may interact in a complex manner (Talbot et al., 2006). While the effects of the water status of the internal CO₂ concentration and of the illumination on stomatal aperture are well documented, our knowledge concerning temperature effects are quite limited (Zeiger, 1983; Lu et al., 2000). The aim of the work reported here was to char-

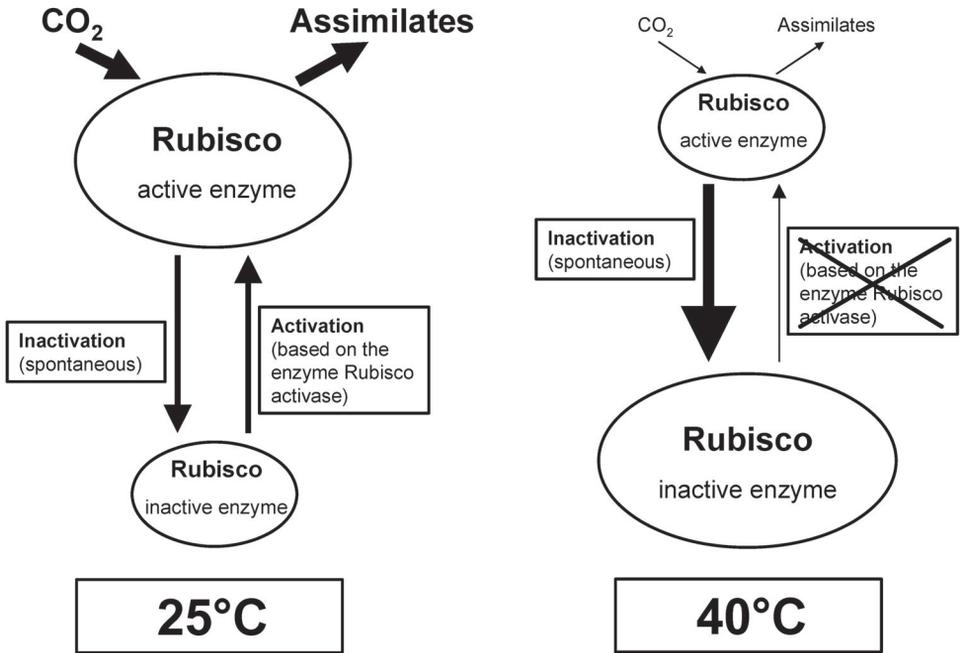


Figure 1. Effects of heat on Rubisco activase, Rubisco activation state and Calvin cycle activity. Heat affects the activation state of Rubisco and as a consequence the activity of the Calvin cycle in two ways: a faster inactivation of active Rubisco is accompanied by a slower reactivation by the heat-sensitive enzyme Rubisco activate (Saluki and Crafts-Brandner, 2004).

acterize the response of stomates to elevated temperature in a model system and to relate these findings to leaf temperatures observed in the field.

MATERIALS AND METHODS

Bean plants (*Phaseolus vulgaris* L.) for the investigation of stomatal opening under controlled conditions were grown hydroponically as reported previously (Hildbrand et al. 1994). Primary leaves were used for the experiments 2-4 weeks after germination. For the analyses of stomatal aperture, leaf segments were incubated floating in darkness on water at the temperature indicated. The collected samples were immediately placed on a slide and a picture was taken with a digital microscope. Stomatal opening was afterwards determined on the pictures (all stomates on one picture were averaged). At least 4 separately incubated leaf segments were analyzed for each treatment.

Air temperature in the field was determined with a classical mercury thermometer kept in the shadow close to the leaves analyzed. Leaf temperature was measured

Table 1: Leaf temperatures in the field. Rather hot days from three different years (2002, 2003 and 2006) were considered (means \pm SD from 6 to 10 measurements). The air temperature was determined with a classical thermometer in the shadow near the leaves to be analyzed. n.a.: not available.

Plant species	Date (Time)	Temperature (°C)		
		Air	Leaf (sun)	Leaf (shadow)
<i>Solanum tuberosum</i>	July 27, 2002 (14:00) ^a	25.5	28.0 \pm 1.5	24.8 \pm 1.2
<i>Solanum tuberosum</i>	July 28, 2002 (15:30) ^a	29.5	31.2 \pm 2.3	25.1 \pm 1.1
<i>Helianthus annuus</i>	July 27, 2002 (14:00) ^a	25.5	23.0 \pm 0.9	21.2 \pm 1.5
<i>Helianthus annuus</i>	July 28, 2002 (15:30) ^a	29.5	22.8 \pm 1.6	20.0 \pm 1.1
<i>Helianthus annuus</i>	July 20, 2003 (14:20) ^b	32.0	28.5 \pm 1.3	25.5 \pm 1.3
<i>Fagus sylvatica</i>	July 27, 2002 (14:00) ^a	25.5	29.0 \pm 1.2	24.7 \pm 1.4
<i>Fagus sylvatica</i>	July 28, 2002 (15:30) ^a	29.5	32.1 \pm 1.0	25.0 \pm 0.7
<i>Corylus avellana</i>	July 27, 2002 (14:00) ^a	25.5	37.5 \pm 2.0	26.6 \pm 0.7
<i>Corylus avellana</i>	July 28, 2002 (15:30) ^a	29.5	40.0 \pm 2.0	28.7 \pm 0.5
<i>Corylus avellana</i>	July 30, 2003 (10:40) ^b	28.0	41.3 \pm 0.2	23.2 \pm 0.2
<i>Corylus avellana</i>	July 20, 2006 (15:10) ^c	n.a.	35.9 \pm 1.4	27.2 \pm 0.5
<i>Solidago canadensis</i>	August 6, 2003 (11:05) ^b	30.5	34.9 \pm 1.7	29.0 \pm 0.4
<i>Solidago canadensis</i>	August 6, 2003 (13:05) ^b	37.0	41.6 \pm 0.8	34.7 \pm 0.3
<i>Rubus fruticosus</i>	August 6, 2003 (11:10) ^b	31.5	32.2 \pm 1.2	26.7 \pm 1.3
<i>Rubus fruticosus</i>	August 6, 2003 (13:10) ^b	38.0	40.7 \pm 2.2	32.5 \pm 2.0
<i>Fraxinus excelsior</i>	August 6, 2003 (11:10) ^b	31.5	34.7 \pm 0.8	28.0 \pm 0.3
<i>Fraxinus excelsior</i>	August 6, 2003 (13:10) ^b	38.0	41.0 \pm 1.5	32.9 \pm 0.2
<i>Cornus sanguinea</i>	August 6, 2003 (11:15) ^b	31.5	35.2 \pm 1.2	29.2 \pm 0.4
<i>Cornus sanguinea</i>	August 6, 2003 (13:15) ^b	38.0	41.5 \pm 1.4	34.2 \pm 1.4
<i>Salix alba</i>	August 6, 2003 (11:30) ^b	31.5	32.5 \pm 1.6	28.4 \pm 0.6
<i>Salix alba</i>	August 6, 2003 (13:30) ^b	38.0	36.0 \pm 1.1	31.7 \pm 0.3
<i>Acer pseudoplatanus</i>	August 6, 2003 (14:50) ^b	40.5	41.2 \pm 0.4	36.7 \pm 0.2
<i>Acer pseudoplatanus</i>	July 25, 2006 (14:40) ^c	n.a.	42.6 \pm 0.4	32.7 \pm 0.2
<i>Acer pseudoplatanus</i>	July 27, 2006 (10:50) ^c	24.0	35.6 \pm 0.7	25.6 \pm 0.4
<i>Citrus sinensis</i>	July 25, 2006 (11:30) ^c	n.a.	40.9 \pm 1.4	30.0 \pm 0.1

^a: Measurements from a hill in Bremgarten near Bern, Switzerland

^b: Measurements from the Inforama "Rütti" in Zollikofen near Bern, Switzerland

^c: Measurements from the Botanical Garden in Bern, Switzerland

with an infrared thermometer (avoiding shading of the leaf before and during the measurement) and for selected samples also confirmed with infrared thermography (using an infrared camera).

RESULTS

Leaf temperatures may vary in a wide range (Table 1). The temperature of leaves in the shadow was in general several degrees per Celsius below the air temperature (cooling by transpiration), while the temperature of fully sun-exposed leaves was often considerably higher than the air temperature and reached values above 40°C. Furthermore, leaf temperature depended on air convection (Table 2). Leaves exposed to wind had a considerably lower temperature than comparable leaves protected from wind. Changes in leaf temperature in an apple tree during a day are documented on Figure 2. During the early afternoon, the temperature in sun-exposed leaves reached values near 40°C, while the values for leaves in the shadow were 4 to 8°C lower. The measurements mentioned above document that leaf temperature easily reaches values above 40°C and depends on a series of factors such as air temperature, plant species, illumination of the leaves and air convection.

Leaf temperatures and their effect on stomatal opening was not a key issue during the past years. These effects were therefore considered in more detail for the investigations reported here. It was possible to analyze the aperture of the stomates on photographs from whole leaf segments taken immediately after the treatments with a digital camera mounted on a microscope. Some of these pictures are shown on Figure 3 and demonstrate the opening of the stomates after heating the leaf segment from 23°C to 45°C. This effect was reversible, since the stomates closed again

Table 2: Effect of wind on leaf temperatures in the field. Leaves (sun-exposed and in the shadow) from two *Quercus robur* plants from the same location were considered. One plant was exposed to wind, while the other was quite well protected from wind by a small hill. Measurements were taken in Bremgarten near Bern, Switzerland (means±SD from 10 measurements).

Date (Time)	Wind	Temperature (°C)		
		Air	Leaf (sun)	Leaf (shadow)
July 27, 2002 (14:00)	no wind	25.5	37.8±4.8	26.4±1.7
July 27, 2002 (14:00)	slightly windy	25.5	25.5±2.0	21.6±1.3
July 28, 2002 (15:30)	no wind	29.5	41.0±1.2	31.9±1.9
July 28, 2002 (15:30)	slightly windy	29.5	32.3±1.9	25.0±0.7

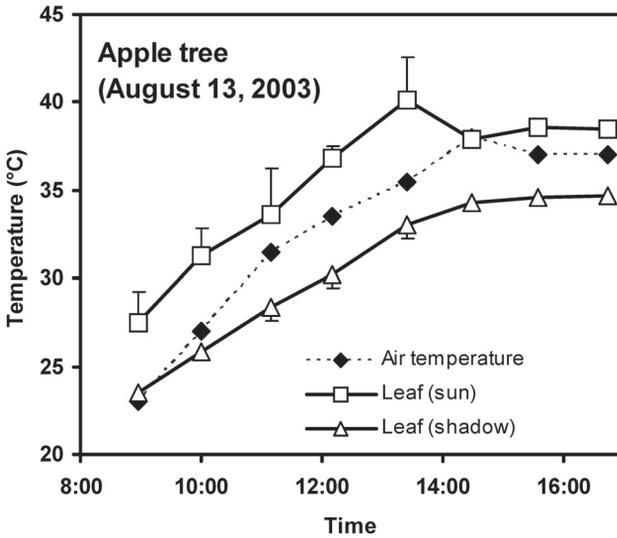


Figure 2. Air temperature and temperatures in sun-exposed and shadowed leaves of an apple tree during a sunny summer day.

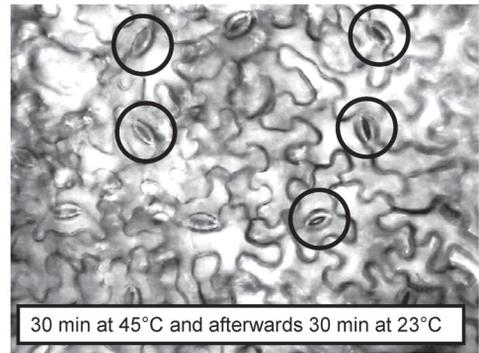
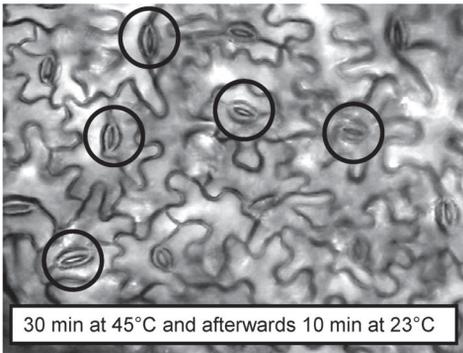
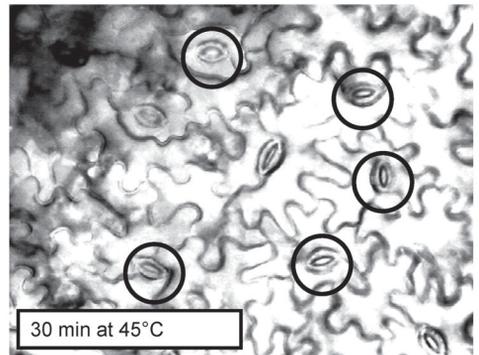
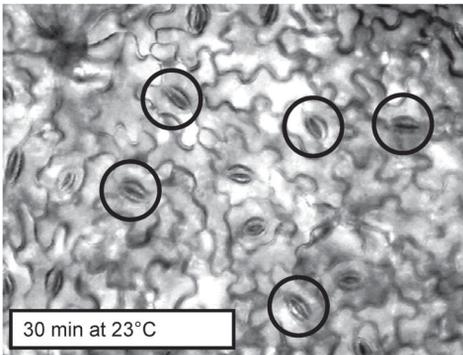
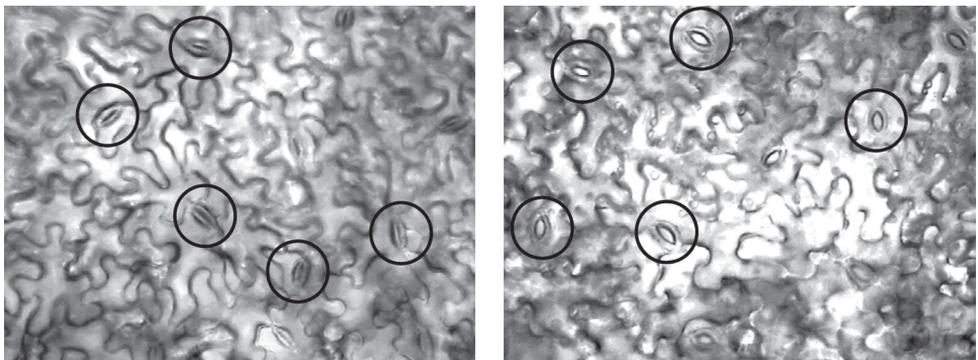


Figure 3. Stomatal aperture in bean leaf segments exposed to elevated temperature in darkness. Leaf segments were incubated floating on water kept at the temperature indicated in darkness. After the incubation, the segments were placed on slide and the picture was taken immediately (<10 seconds) without further preparation.

Table 3: Stomatal opening at elevated temperature in darkness. Bean leaf segments were incubated floating on H₂O at the temperature indicated. Means±SE of 4 independent samples are shown for the opening of the stomates (μ m).

Temperature (°C)	Time of incubation (min)		
	10	20	30
23	0.21±0.09	0.26±0.26	0.66±0.21
30	0.46±0.17	1.85±0.33	2.76±0.64
35	1.90±0.87	3.19±0.45	4.28±0.49
40	1.24±0.36	3.05±0.41	4.26±0.34
45	2.92±0.56	4.23±0.22	4.47±0.22

gradually after transferring the samples from 45°C back to 23°C. The opening of the stomates in darkness as a function of the incubation temperature was quantified by measuring the stomatal aperture on the pictures taken. These results are summarized in Table 3. At 23°C, the stomatal openings were in the range of 0.2 to 0.7 μ m. A slow opening reaching 2.76 μ m after 30 min was observed at 30°C, while at higher temperatures the opening occurred more rapidly. At 45°C, the opening was at 2.92 μ m after 10 min and at 4.47 μ m after 30 min. The reversibility of this effect was



+ 0.1 μ M ABA

-ABA (control)

30 min at 40°C

Figure 4. Influence of abscisic acid (ABA) on stomatal aperture at elevated temperature. Bean leaf segments were incubated in presence and absence of 0.1 μ M ABA in darkness and analyzed afterwards as indicated in the legend to Fig. 3.

tested in these samples. It became evident that the heat-induced opening was fully reversible (within 30 min).

An interesting interaction between the incubation temperature and ABA in the incubation medium was observed (Fig. 4). The addition of 0.1 μM ABA to the incubation medium caused a shift of the stomatal opening towards higher temperatures. While the stomates were open ($> 4 \mu\text{m}$) after 30 min on water at 40°C, they were essentially closed on 0.1 μM ABA at the same temperature. However, the stomates also opened on ABA when the temperature was further increased to 45°C and 47°C (data not shown). As mentioned above for the incubations on water, the opening was also reversible in the leaf segments incubated in 0.1 μM ABA. These results suggest an antagonistic interaction between heat and drought.

In the field, the illumination of a leaf or of a part of a leaf may change rapidly as a consequence of altered shadowing by other plant parts or of a changed distribution of clouds. Artificial shadowing was used to mimic this situation (Fig. 5). A difference of about 10°C between permanently sun-exposed leaves and permanently shadowed leaves was observed. The transfer from sun to shadow or vice versa led to a rapid temperature change during the first minute after altering the illumination. Air convection and transpiration contribute to the cooling of the previously illuminated leaves, while the energy absorbed from the sunlight leads to a rapid increase in the temperature of a previously shadowed leaf.

Sometimes (but not always) a peak in the leaf temperature was observed in the field after the transition from shadow to full sunlight. In order to investigate this aspect in more detail, such experiments were performed with hydroponically grown bean plants in the laboratory (Fig. 6). The leaf temperature in darkness was at 23°C to 25°C. After exposure of these plants to a halogen lamp, the leaf temperature increased within 2 min to 38°C, declined afterwards again to 33°C (after 11 min) and increased to a next peak (38°C after 25 min). This oscillation was most likely caused by opening and closure of the stomates. The leaf temperature of plants permanently exposed to the same light was stable between 33°C and 34°C. A similar oscillation was detected when plants kept previously in darkness were exposed to an infrared lamp heating the leaves (but with a low PAR). In this case the temperature reached in the peak values above 40°C and declined then again before increasing to the next (smaller) peak. The leaf temperature of plants permanently exposed to the infrared lamp was around 38°C. It must be borne in mind, that warming in the leaves starts immediately after the transfer of the plants from darkness to light, while it may take several minutes to adjust the aperture of the stomates to the new conditions (Table 3).

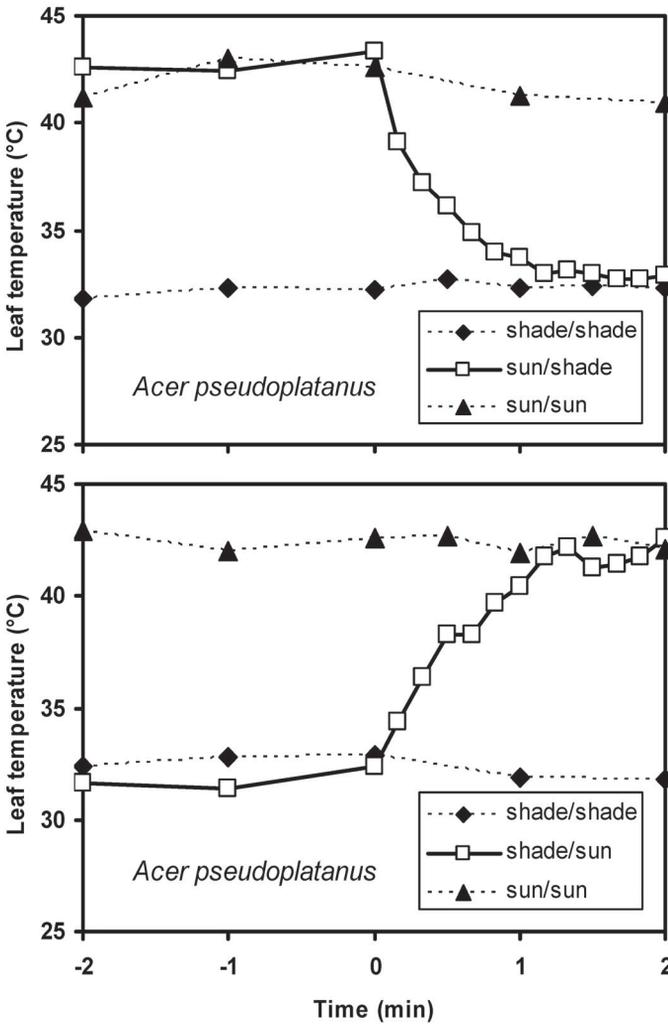


Figure 5. Effects of the exposure to sunlight on the temperature in leaves of *Acer pseudoplatanus* in the field. Fully sun-exposed leaves (sun/sun), leaves artificially shadowed with a cardboard and the transition between these two treatments at 0 min (sun/shade and shade/sun) were analyzed.

DISCUSSION

Factors affecting stomatal opening and stomatal closure are summarized on Figure 7. Heat must be considered as a relevant factor in this context. There might be antagonistic signals coming on one hand from heat (stimulating stomatal opening) and on the other hand from limited water availability (causing a stomatal closure). This is especially important during heat waves, which are often characterized by high air

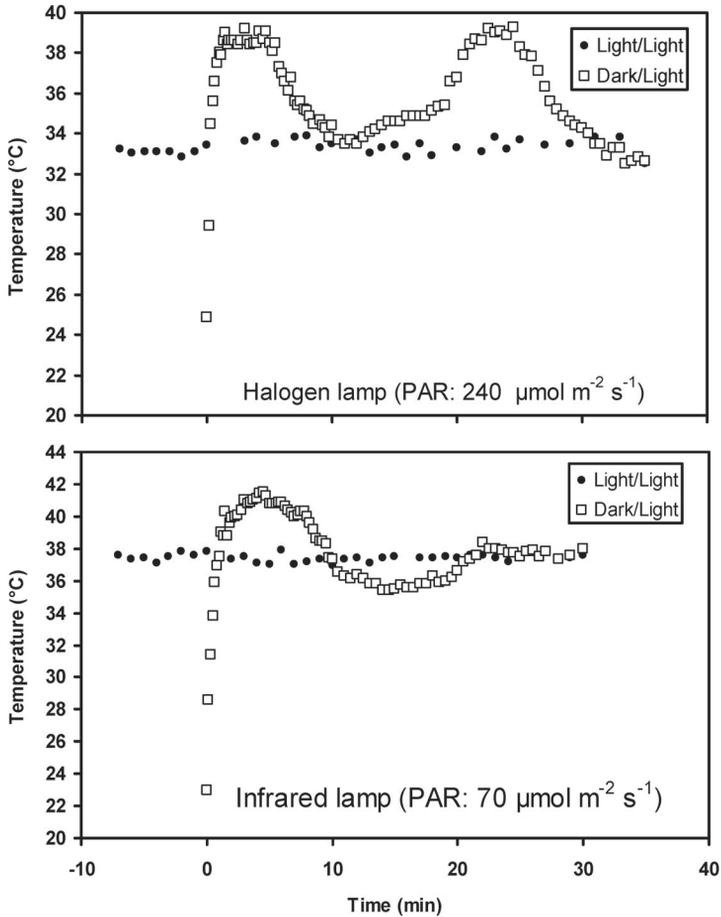


Figure 6. Temperature in primary leaves of *Phaseolus vulgaris* exposed to a halogen lamp or an infrared lamp. Plants kept in darkness for at least 30 min were exposed to artificial light (deriving from a halogen lamp with a high PAR or from an infrared lamp generating heat with a low PAR; Dark/Light) were compared with plants illuminated permanently with the same lamp (Light/Light).

temperatures combined with drought. As long as sufficient water is available for the plants in the soil, stomatal opening and transpiration may be high allowing an efficient cooling of the leaf even in the shadow where net photosynthesis is low. In full sunlight, the low activation state of Rubisco at elevated temperature caused by a faster inactivation of Rubisco and a slower reactivation by the thermosensitive enzyme Rubisco activase leads to a metabolic limitation of photosynthesis, while the electron transport chain may still be functioning. Data from various groups suggest that stomatal opening is under such conditions most likely not limiting net photosynthesis. However, the aperture of the stomates is an important response to heat and may allow an efficient cooling of the leaf and as a consequence also the protec-

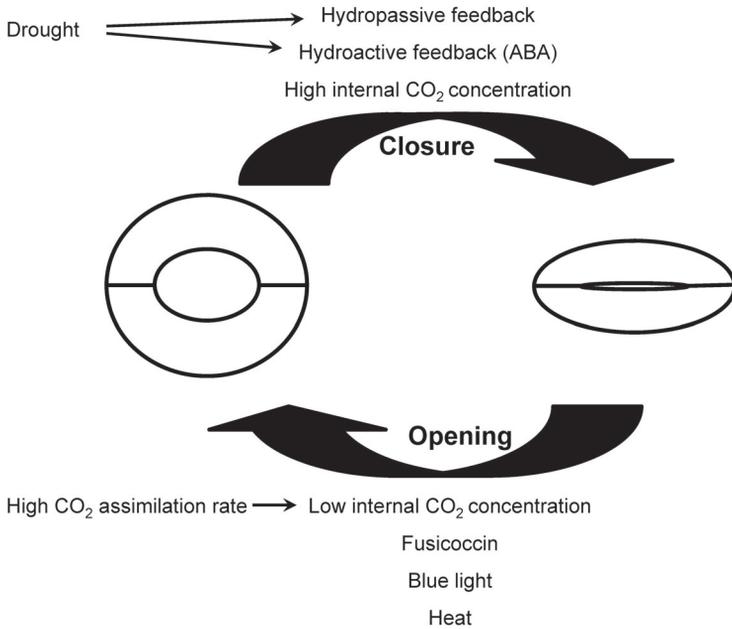


Figure 7. Regulation of stomatal aperture by endogenous and exogenous factors including heat.

tion of the photosynthetic apparatus. Therefore, the response of the guard cells to elevated temperature and the interference of the heat effect with other regulatory mechanisms (e.g. response to drought) is an important point to be considered for the overall stress tolerance of plant in the field.

The results presented in this paper refer mainly to investigations with a model plant (*Phaseolus vulgaris*) and to changes in stomatal opening in darkness. It became evident that stomates open in darkness with increasing temperature, although in the system used (leaf segments floating on water) no leaf cooling by transpiration was possible. It is a challenge for future experiments to elucidate the response of stomates to heat in the light and to consider the response of the stomates to heat in different plant species in the field.

Acknowledgements

I thank Iwona Anders, Marco Schnyder, Thomas von Känel, Anita Zumsteg and Anita Langenegger for contributing to the analyses of leaf temperature and stomatal aperture. The work was supported by the SCOPES project “DILPA” (Swiss National Science Foundation) and by the NCCR “Climate” (Swiss National Science Foundation).

References

- Crafts-Brandner, S.J., M.E. Salvucci, 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO₂, Proc. Natl. Acad. Sci. USA, 97, 13430-13435.
- Demirevska-Kepova, K., U. Feller, 2004. Heat sensitivity of Rubisco, Rubisco activase and Rubisco binding protein in higher plants, Acta Physiol. Plant., 26, 103-114.
- Demirevska-Kepova, K., R. Hölzer, L. Simova-Stoilova, U. Feller, 2005. Heat stress effects on Rubisco, Rubisco binding protein and Rubisco activase in wheat leaves, Biol. Plant., 49, 521-525.
- Eckardt, N.A., A.R. Portis, 1997. Heat denaturation profiles of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and Rubisco activase and the inability of Rubisco activase to restore activity of heat-denatured Rubisco, Plant Physiol., 113, 243-248.
- Feller, U., S.J. Crafts-Brandner, M.E. Salvucci, 1998. Moderately high temperatures inhibit ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. Plant Physiol., 116, 539-546.
- Haldimann, P., U. Feller, 2004. Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase, Plant Cell Environ., 27, 1169-1183.
- Haldimann, P., U. Feller, 2005. Growth at moderately elevated temperature alters the physiological response of the photosynthetic apparatus to heat stress in pea (*Pisum sativum* L.) leaves, Plant Cell Environ., 28, 302-317.
- Haldimann, P., A. Gallü, U. Feller, 2005. Impact of drought and heat stress on the photosynthetic apparatus of *Quercus pubescens*: a field study. In: Photosynthesis: Fundamental Aspects to Global perspectives, Eds. A. van der Est, D. Bruce, International Society of Photosynthesis, 601-603.
- Herrmann, B., U. Feller, 1998. CO₂, light and temperature influence senescence and protein degradation in wheat leaf segments, Physiol. Plant., 103, 320-326.
- Hikosaka, K., K. Ishikawa, A. Borjigidai, O. Muller, Y. Onoda, 2006. Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate, J. Exp. Bot., 57, 291-302.
- Hildbrand, M., A. Fischer, U. Feller, 1994. Protein catabolism in bean leaf discs: accumulation of a soluble fragment of ribulose-1,5-bisphosphate carboxylase/oxygenase under oxygen deficiency, J. Exp. Bot., 45, 1197-1204.
- Law, R.D., S.J. Crafts-Brandner, 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase, Plant Physiol., 120, 173-181.
- Lu, Z.M., M.A. Quinones, E. Zeiger, 2000. Temperature dependence of guard cell respiration and stomatal conductance co-segregate in an F-2 population of Pima cotton, Aust. J. Plant Physiol., 27, 457-462.
- Moore, R., W.D. Clark, D.S. Vodopich, 1998. Botany, second edition. WCB/McGraw-Hill, New York.

- Portis, A.R., 2003. Rubisco activase – Rubisco’s catalytic chaperone. *Photosynth. Res.*, 75, 11-27.
- Roelfsema, M.R.G., R. Hedrich, 2005. In the light of stomatal opening: new insights into ‘the Watergate’, *New Phytol.*, 167, 665-691.
- Salvucci, M.E., 1993. Covalent modification of a highly reactive and essential lysine residue of ribulose-1,5-bisphosphate carboxylase – oxygenase activase, *Plant Physiol.*, 103, 501-508.
- Salvucci, M.E., S.J. Crafts-Brandner, 2004. Mechanisms for deactivation of Rubisco under moderate heat stress, *Phasiol. Plant.*, 122, 513-519.
- Schär; C., P.L. Vidale, D. Lüthi, C. Frei, C. Häberli, M.A. Liniger, C. Appenzeller, 2004. The role of increasing temperature variability in European summer haetwaves, *Nature*, 427, 1189-1202.
- Sharkey, T.D., 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant Cell Environ.*, 28, 269-277.
- Spreitzer, R.J., M.E. Salvucci, 2002. Rubisco: structure, regulatory interactions, and possibilities for a better enzyme, *Annu. Rev. Plant Biol.*, 53, 449-475.
- Talbott, L.D., J.W. Hammad, L.C. Harn, V.H. Nguyen, J. Patel, E. Zeiger, 2006. Reversal by green light of blue light-stimulated stomatal opening in intact, attached leaves of *Arabidopsis* operates only in the potassium-dependent, morning phase of movement, *Plant Cell Physiol.*, 47, 332-339.
- Yamori, W., K. Suzuki, K. Noguchi, M. Nakai, I. Terashima, 2006. Effects of Rubisco kinetics and Rubisco activation state on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures, *Plant Cell Environ.*, 29, 1659-1670.
- Zeiger, E., 1983. The biology of stomatal guard cells, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 34, 441-475.