Photosynthesis and Stomatal Behaviour

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Contents

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Abstract In order for plants to use water efficiently, stomata must ensure an appropriate balance between CO_2 demands for photosynthesis and water loss through transpiration. To achieve this, stomatal conductance (*gs*) often correlates with mesophyll photosynthetic rates. However, the underlying mechanisms and signals that promote this relationship are currently unknown. Stomata and photosynthesis respond to a number of environmental cues; however, the dynamics and magnitude of these responses are not identical, with stomatal responses generally an order of magnitude slower than mesophyll photosynthesis. The resulting disconnection between stomatal conductance and photosynthetic rate means that under naturally fluctuating environmental conditions water use efficiency (WUE) can be far from optimal. Manipulation of stomatal behaviour provides an obvious mechanism for producing plants with improved WUE; however, before such an approach is possible we must first understand the hierarchy of stomatal responses to varying environmental parameters, the mechanisms behind these complex signalling pathways, and how stomatal behaviour is tuned to mesophyll photosynthetic rates or capacity.

1 Introduction

Plants require sufficient CO_2 to enter the leaf for photosynthesis while conserving water to avoid dehydration and metabolic disruption. As the leaf is almost impermeable to water and CO_2, almost all of the water transpired as well as the CO_2 absorbed pass through stomatal pores (Cowan and Troughton 1971; Jones 1992) and therefore stomata are essential to plant water and carbon status. Additionally stomata also play key roles in nutrient uptake and evaporative cooling of the leaf tissue (Morison 2003). The transpirational loss of water through stomata is unavoidable because of the plant's need to expose internal cell surfaces to the external air surrounding the leaf for photosynthetic CO_2 uptake. Only about 2% of the water taken up by a plant is used for biochemical reactions, with the remaining 98% being lost by evaporation from the cell surfaces (Morison 2003). The rate of diffusion of gases into or out of the leaf from/to the surrounding environment depends upon the concentration gradient and the resistance of diffusion along the pathway (see Weyers and Meidner 1990). For water loss from the mesophyll cells inside the leaf, the major

pathway is therefore from the mesophyll cell wall through the substomatal cavity to the stomatal pore and then out through the layer of air immediately surrounding the leaf (boundary layer) to the mixed air stream. The pathway for CO_2 uptake is essentially the same but in reverse, with an additional resistance component represented by the entry into the mesophyll cell chloroplasts. The resistance of the stomatal pathway depends on the geometry of the pores as well as their density (or frequency). Although the pore area may represent only a small fraction (between 0.5 and 3%) of the total leaf area when fully open, evaporation rates can be equivalent to 50% of that of a wet surface of the same area due to edge effects of diffusion (Willmer and Fricker 1996). Modelling stomatal conductance based on pore geometry, Weyers and Lawson (1997) illustrated that the main determinant of g_s was pore aperture, while stomatal density makes a smaller contribution, although greater than that of depth or length. Stomata and their function therefore, play a central role in determining the amount of carbon gained per unit water lost, known as plant "water use efficiency (WUE)".

Crop breeders and scientists are under growing pressure to produce crop plants with increased yields (or even sustained yield) and greater water and nutrient use efficiency to meet the increasing demand for food and sustainable fuel for the ever-expanding global population. Future plants must also have the ability to maintain production in predicted future climates of increased atmospheric CO_2 concentration, increased temperature and reduced water availability. The regulatory role of stomatal control over water loss and CO_2 uptake for photosynthesis makes these cells an obvious target for manipulation for improving WUE in future crops plants. However, before such an approach is possible, we must first be able to understand the complex signalling and response pathways and mechanisms that enable stomata to respond to environmental stimuli and to mesophyll demands for CO_2 and the interaction and hierarchy of responses that obviously exist in guard cells.

1.1 Stomatal Function, Plant Productivity and Water Use Efficiency

In order for stomata to function effectively (maximising CO_2 uptake while minimising water loss), they respond to changes in external conditions and internal signals (Raschke 1978). Environmental factors can influence stomatal behaviour either directly or indirectly (Wong et al. 1979). Direct factors are those affecting the guard cells themselves, while indirect effects are those affecting stomatal behaviour by influencing the photosynthesis of the mesophyll (Willmer and Fricker 1996). Typically, stomatal pores in C_3 and C_4 plants open with light the extent of which has been shown to be wavelength specific (see Kuiper 1964), low CO_2 concentrations and high humidity (linked with temperature and the driving force of water loss), while closure is induced under conditions of darkness, high CO_2 concentration

(see Sect. 2.1), low humidity and high temperatures (see reviews by Assmann 1993; Willmer and Fricker 1996; Outlaw 2003; Vavasseur and Raghavendra 2005; Shimazaki et al. 2007). There are exceptions to these typical stomatal responses dependent upon the photosynthetic mechanisms employed (discussed in further detail in Sect. 3).

Changes in stomatal aperture are driven by changes in turgor pressure of guard cells (Heath 1938) due to the accumulation of ions and/or solutes (Imamura 1943; Fujino 1967; Outlaw 1983), which increases the osmotic potential and lowers the water potential of guard cells (Weyers and Meidner 1990; Willmer and Fricker 1996). Stomatal conductance (g_s) to water and CO_2 is dependent upon stomatal characters such as density (number of stomata per unit leaf area) and stomatal aperture (pore width), both of which are influenced by environmental conditions surrounding the leaf, including gas concentrations, relative humidity and temperature. The number, size and distribution of stomata vary both between and within species (Tichà 1982) and are often dependent upon environmental growth conditions (Weyers et al. 1997; Weyers and Lawson 1997). Anatomical features of stomata and their influence on gas exchange and photosynthesis will be considered in Sect. 4. Considerable heterogeneity in stomatal characters, behaviour and function has also been demonstrated at several levels, from spatial patterns on individual leaves to whole leaves within plants, and also species differences (Lawson 1997; Weyers and Lawson 1997). An understanding of the nature of stomatal heterogeneity and its origins may be important as this could provide plants with some functional advantages or disadvantages with respect to photosynthesis or WUE (e.g. stomatal patchiness; Mott and Peak 2007).

To improve WUE, two approaches are immediately obvious. The first is to improve photosynthetic carbon assimilation/growth rate and yield without the need for increased water loss by the plant. Numerous studies have altered expression of targeted plant enzymes to identify their control on carbon assimilation. Such studies have targeted Calvin cycle enzymes (Harrison et al. 1998; Haake et al. 1999; Henkes et al. 2001; Raines 2003; Lefebvre et al. 2005; Suzuki et al. 2007) and also photorespiratory enzymes (Wingler et al. 1999). Single enzyme transformations have demonstrated that enhanced photosynthetic rates are possible, for example, increased. Sedoheptulose-1,7-bisphosphatase activity resulted in tobacco plants with improvements in carbon assimilation by 6–12% (Lefebvre et al. 2005). The inclusion of thermostable Rubisco activase improved photosynthetic rates in Arabidopsis (Kurek et al. 2007); however, increasing Rubisco content in rice plants showed no enhancement in photosynthetic rate (Suzuki et al. 2009).

The potential outcomes of targeting more than one enzyme have recently been modelled by Zhu et al. (2007). The second approach is to breed plants with an altered stomatal conductance (Jones 1976, 1977). Increased stomatal conductance could remove stomatal limitation on photosynthesis and increase carbon uptake, while reduced stomatal conductance (particularly under water stress conditions) could increase water use but at the expense of carbon gain. However, before contemplating the potential of altering stomatal conductance to increased WUE, the extent to which stomata limit photosynthesis under the plant natural growth

environmental must first be determined (Jones 1987). It has been reported for several C_3 species that removal of stomatal limitation increases photosynthetic rates by only 10–20% and that in C_4 species this is even less (Farquhar and Sharkey 1982; Jones 1985). Under drought stress conditions metabolic limitation of photosynthesis may be much greater than that caused by reduced stomatal aperture (Lawlor 2002). A third less-obvious approach for increasing WUE which would include selecting plants with stomata that respond more rapidly to the fluctuating environmental conditions that plants experience in the field environment could be envisaged. However, before such an approach is attempted, we must first understand the mechanisms and response(s) of stomata to varying environmental factors and the combination of such changes, and the mechanisms by which stomatal conductance relates to mesophyll photosynthesis. In the following sections we will examine anatomical and physiological aspects of stomatal function in relation to photosynthetic carbon assimilation. It is worth noting that commercially grown wheat varieties with increased WUE have been identified using an innovative isotope discrimination screen ($\Delta^{13}C$ and $\delta^{18}O$), which provides information on photosynthesis, transpiration and stomatal behaviour (see Rebetzke et al. 2006; Condon et al. 2007; Ripullone et al. 2008). In another case, mutants of the ERECTA gene (which "regulates transpiration efficiency") were isolated in *Arabidopsis* using a similar carbon isotope screening technique (Masle et al. 2005).

2 Stomata Responses to Environmental Parameters

Environmental variables such as light, [CO_2] and temperature are often considered to have the greatest influence on photosynthetic rates, as well as direct and indirect impacts on stomatal behaviour. Despite CO_2 concentration in nature remaining relatively stable, stomata are often assessed relative to [CO_2] because internal CO_2 concentration (C_i), which is ultimately controlled by external CO_2 concentration and photosynthetic carbon assimilation rates, is often believed to provide a key controlling mechanism linking stomatal behaviour with mesophyll photosynthetic rates (see Mott 2009). We have not attempted to review the vast amount of literature currently available on these areas of research but refer readers to two recent comprehensive reviews examining stomatal responses to CO_2 (Vavasseur and Raghavendra 2005) and light (Shimazaki et al. 2007).

2.1 Stomatal Responses to CO_2 Concentration

It is well-accepted that stomatal conductance responds to CO_2, a necessary requirement if plants are to optimise water use with carbon demand. In short-term responses, stomata generally reduce aperture with increased CO_2 concentration

and open under lowered CO_2 concentrations (in both darkness and light). The signalling and response mechanisms that underlie stomatal responses to CO_2 have proven difficult to identify until recently. Hashimoto et al. (2006) demonstrated that stomata of the Arabidopsis *high leaf temperature 1* mutants (*hrt1-1* and *hrt1-2*) have reduced ability to control stomatal movements in response to altering CO_2 concentrations, indicating that HT1 kinase is an important regulator of stomatal responses to CO_2. Several reports have also postulated an interactive role between ABA and stomatal CO_2 responses (for details see Vavasseur and Raghavendra 2005). Long-term exposure to elevated CO_2 concentrations may result in (1) physiological acclimation of stomata, for example reduced sensitivity to CO_2 concentration compared with those observed under short-term fluctuations (e.g. Berryman et al. 1994; Xu et al. 1994; Lodge et al. 2001; Maherali et al. 2002); (2) changes in anatomy (Woodward 1987; McElwain and Chaloner 1995; McElwain et al. 1995); and (3) attenuated responses to environmental parameters other than [CO_2] (Ainsworth and Rogers 2007). Since the first reports of decreasing aperture with elevated CO_2 concentration (Freudenberger 1940; Heath and Russell 1954), numerous researchers and laboratories have studied the effects of both short- and long-term responses of stomatal aperture to increasing and decreasing CO_2 concentrations (e.g. Raschke 1972) and illustrated a variety of responses that appear to be species dependent (e.g. Maherali et al. 2002) or independent (e.g. Morison 1985) of the plants photosynthetic pathway or biochemistry. Different stomatal responses to CO_2 have been reported for the same species from different laboratories; for example, Tuba et al. (1994) reported little change in *Triticum aestivum* stomatal sensitivity when grown at elevated CO_2 concentration, but a lack of sensitivity could not be confirmed in later studies by Šantrůček and Sage (1996). Stomatal responses to CO_2 do, however, appear to be governed by growth conditions. For example *Vicia faba* plants grown under ambient CO_2 concentrations in a growth chamber had enhanced CO_2 responses compared with plants grown in glass house conditions, which was reported to be an intrinsic property of the guard cells (Frechilla et al. 2002), although combined effects of differing light and humidity could also explain these results. Additionally, plants grown under well watered conditions or in environments with low evaporative demands have been shown to have reduced sensitivity to CO_2 (Mansfield and Atkinson 1990; Mansfield et al. 1990; Mansfield 1994). It is believed that CO_2 sensing occurs within the guard cells themselves, as CO_2 responses have been demonstrated in epidermal peels (Fitzisimons and Weyers 1986) and it is generally accepted that guard cells sense C_i rather than atmospheric CO_2 (C_a) (Mott 1988). However, recent work on transgenic plants may provide evidence that stomata respond to C_a rather than C_i as stomata closed in response to increases in C_a and at ambient C_a stomatal conductance was similar in both wild type (WT) and transgenic plants despite elevated C_i concentrations due to reduced photosynthetic rates (von Caemmerer et al. 2004; Baroli et al. 2008). Stomatal sensitivity to C_a has recently been the focus of an evolutionary study on WUE (see Brodribb et al. 2009) (see below).

2.2 Stomatal Responses to Light

The relationship between light, photosynthesis and stomatal conductance is often discussed in association with the complex interactions with C_i. This is fuelled by the fact that guard cells have two light response components. The first of these light response components is the photosynthesis-independent blue light response, which is associated with rapid opening and is sensed by the guard cells (Zeiger et al. 2002; Kinoshita and Shimazaki 1999; Shimazaki et al. 2007) while the second is thought to be a photosynthetic mediated response (often termed the red light response), which saturates at rates similar to mesophyll photosynthesis (see Sharkey and Raschke 1981). The red light response is often believed to be a C_i response operating through mesophyll consumption of CO_2 (e.g. see Roelfsema et al. 2002). Support for a C_i driven red light response comes from experiments conducted on albino leaves or leaves treated with an inhibitor of carotenoid synthesis, in which stomata responded to blue light, but failed to open under red illumination (Roelfsema et al. 2006). Additional support for a CO_2-induced red light response comes from mutant plants that lack a CO_2 response; these mutants respond to blue light but show no response to red light (Hashimoto et al. 2006; Marten et al. 2008). However, several studies have argued against a direct C_i stomatal-driven response to red light; for example stomata were shown to respond to red light even when C_i was held constant (Messinger et al. 2006; Lawson et al. 2008; Wang et al. 2008). Sharkey and Raschke (1981) confirmed the work of Wong et al. (1979) and reported that stomatal responses to C_i were too low to account for the large changes observed under light. The site of perception for the stomatal red light response is controversial, with some studies suggesting the involvement of guard cell photosynthesis (see Lawson 2009) while others suggest a mesophyll driven signal (Mott 2009). The influence of such mesophyll driven signals on stomatal behaviour has implications for the correlation between stomatal function and photosynthetic capacity (Sect. 3).

The blue light response of stomata has been reported to have faster dynamics than the red light response (although this could be species dependent) and is believed to be involved in rapid dawn opening (Zeiger et al. 1985) and in response to sunflecks (Kirschbaum et al. 1988). However, in tobacco leaves subjected to step increases in red or red and blue light, no difference in rate of stomatal opening was observed (Fig. 1). Only the intensity of the irradiation influenced the rates of opening, and interestingly the final conductance achieved was closely related to the initial rate of opening (Fig. 1). Lawson et al. (2008) reported a similar correlation between red light induced rate of opening and final conductance in plants with differing photosynthetic capacity owing to differing activities of the Calvin cycle enzyme sedoheptulose-1,7-bisphosphatase (SBPase). These data demonstrate the importance of the dynamics of stomatal responses on the final conductance obtained and highlight the potential of manipulating stomatal response in order to control g_s and WUE.

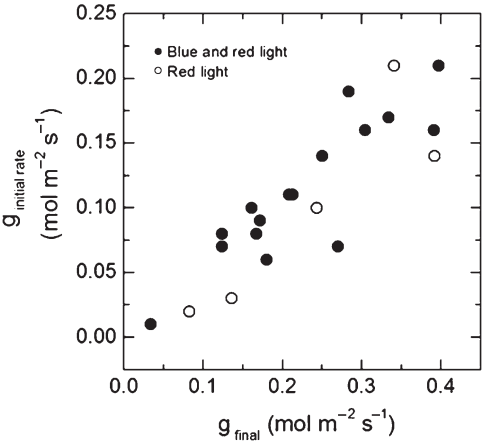

Fig. 1 Relationship between final stomatal conductance and initial rate of opening in tobacco in response to either red or blue light. Leaves were acclimated to 100 μmol m^{-2} s^{-1} followed by step changes in irradiance at different intensities. Open symbol shows stomatal responses to red light, and close symbol blue light

2.3 Temperature Response of Stomata

Variations in leaf and air temperature can alter the rate of transpiration, which will have a direct effect on plant WUE (Šantrůček and Sage 1996), plant productivity (Morison 1993) and crop yield (Lu et al. 2000). In species that lack CO_2 concentrating mechanisms, increasing temperature can cause indirect effects due to metabolic increases in photorespiration and respiration relative to photosynthetic rates, bringing about a rise in internal CO_2 concentration which, if stomata are sensitive to C_i, will bring about stomatal closure (Ball et al. 1987; Willmer and Fricker 1996). Stomatal responses to temperature are variable, depending upon the species and growth conditions (Sage and Sharkey 1987; Aphalo and Jarvis 1991). The effect of temperature on stomata is complicated by the consequential change in leaf−air vapour pressure deficit (VPD) (Hall et al. 1976; Šantrůček and Sage 1996; Willmer and Fricker 1996). At constant VPD, some species show increased g_s with an increasing temperature (Šantrůček and Sage 1996), while others show no change (Aphalo and Jarvis 1991). As temperature increases, g_s will generally decline as a result of increased VPD, although both C_3 (Sage and Sharkey 1987; Kudoyarova et al. 2007) and C_4 (Dwyer et al. 2007) species have been reported to increase g_s with temperature despite increasing VPD. These observations have been attributed to an overriding effect of temperature on g_s that is independent of VPD (Sage and Sharkey 1987; Dwyer et al. 2007) or increased hydraulic conductance in well watered soils (Kudoyarova et al. 2007). Temperature dependent increases in g_s have been attributed to increase metabolic activity in guard cell respiration and increase proton pumping, independent of mesophyll photosynthesis (Lu et al. 2000). Understanding the impact of temperature and the combined effect of temperature, CO_2 and humidity on stomatal behaviour is critical to understanding optimal stomatal conductance and plant WUE. Additionally, long term growth under elevated temperature and/or CO_2 concentration may result in

stomatal acclimation, which could counterbalance the expected future increases in WUE as demonstrated and predicted from short-term studies (Šantrůček and Sage 1996).

2.4 Stomatal Responses Under Fluctuating Environmental Conditions

Stomatal responses to environmental signals such as light, CO_2 and VPD have been extensively studied under steady state conditions, but rarely do they vary in isolation under natural conditions (Lawson and Morison 2004). Fewer studies have examined dynamic stomatal behaviour in a natural environment, where multiple signals must be integrated and speed of response can be critical to both daily carbon gain and WUE (Raschke 1970; Kirschbaum et al. 1988; Knapp 1993; Tinoco-Ojanguren and Pearcy 1993; Cardon et al. 1994; Allen and Pearcy 2000; Lawson and Morison 2004; Noe and Giersch 2004). The disconnection in the time response of stomatal opening and photosynthetic response was examined in detail by Pearcy and co-workers, who studied photosynthetic behaviour to sunflecks in plant canopies and demonstrated that stomatal opening in response to a light signal continued long after the signal had ceased (Kirschbaum et al. 1988; Pearcy 1990). An example of stomatal opening in response to light in Fig. 2 shows the difference in the rate of increase in stomatal conductance and CO_2 assimilation rate and highlights the dilemma faced by plants in coordinating the two processes. The duration and intensity of sun (or shade) flecks influence the response of both g_s and A, as illustrated in Fig. 3. After a period of low light, an increase in irradiance does not result in an immediate increase in A, but shows an initial increase followed by a delay before maximum A is achieved (see Fig. 3a). This lag period is due to both mesophyll induction (involving light regulation of enzymes and metabolite pools) as shown in Fig. 3a and, depending on the duration of the sun fleck, changes in stomatal aperture (Fig. 3b). Although the increase of g_s in response to a light increase during sun flecks is faster than the decreasing response to a drop in light, stomatal movements can take up to tens of minutes and can "overshoot" – continuing to open after the fleck has passed (Kirschbaum et al. 1988; Tinoco-Ojanguren and Pearcy 1993). Most work has indicated that the main control of assimilation during the first 10 min of induction is with the biochemical control of assimilation rate and that stomata do not cause a major limitation (Barradas and Jones 1996; Fig. 3a). However, in sun flecks greater than 10 min, stomatal aperture can limit assimilation rate (Fig. 3b).

The effect(s) of shade flecks on g_s and A are less well studied. Decreased light intensity for a period of 5 min or less greatly decreases the A rate and has little impact on stomatal behaviour; therefore, on restoration of the light, identical A rates can be achieved prior to the shade fleck (Fig. 3c). However, during sunflecks longer than about 8 min, g_s will decrease and continue to decrease for a further 5–8 min

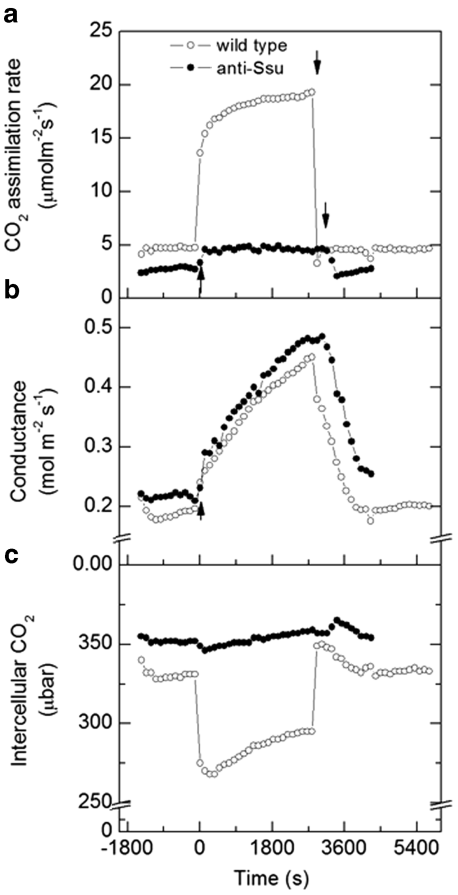

Fig. 2 Examples of changes in (**a**) CO_2 assimilation rate, (**b**) leaf conductance and (**c**) intercellular CO_2 with time after a step change in irradiance from 100 to 1,000 μmol quanta m^{-2} s^{-1}. for wild type (*open circle*) and transgenic tobacco with reduced Rubisco (anti-Ssu, *closed circles*). Ambient CO_2 and water vapour were maintained at 380 μmol mol^{-1} and 23 mmol mol^{-1}. Leaf temperature was maintained at 25°C. Arrows indicate when light was increased from 100 to 1,000 μmol quanta m^{-2} s^{-1} and returned to 100 μmol quanta m^{-2} s^{-1}. (Figure reproduced from von Caemmerer et al. (2004) by permission of Society of Experimental Biology (http://www.sebiology.org) & Oxford University Press)

after the shade fleck has been removed, which restricts restoration of *A* for about 15 min (Fig. 3d). The examples used here illustrate the potential fluctuating changes in stomatal behaviour relative to *A*, the responses of which are dependent not only on the duration but also on the intensity of the flecks, proportional change in irradiance relative to the starting irradiance (see, Lawson 1997), as well as the species studied. When scaled up to the plant or crop level, the impact of the disproportional change in g_s and *A* can result in significant decreases in plant WUE (see Sect. 6). Increasing stomatal response times to fluctuating environmental parameters could potentially decrease lag times and reduce the amount of time stomata restrict carbon assimilation. Additionally, such improvements would reduce the time periods in which plants lose water unnecessarily because of a greater stomatal conductance than is required for the potential carbon gain at that time (e.g. during a shade fleck).

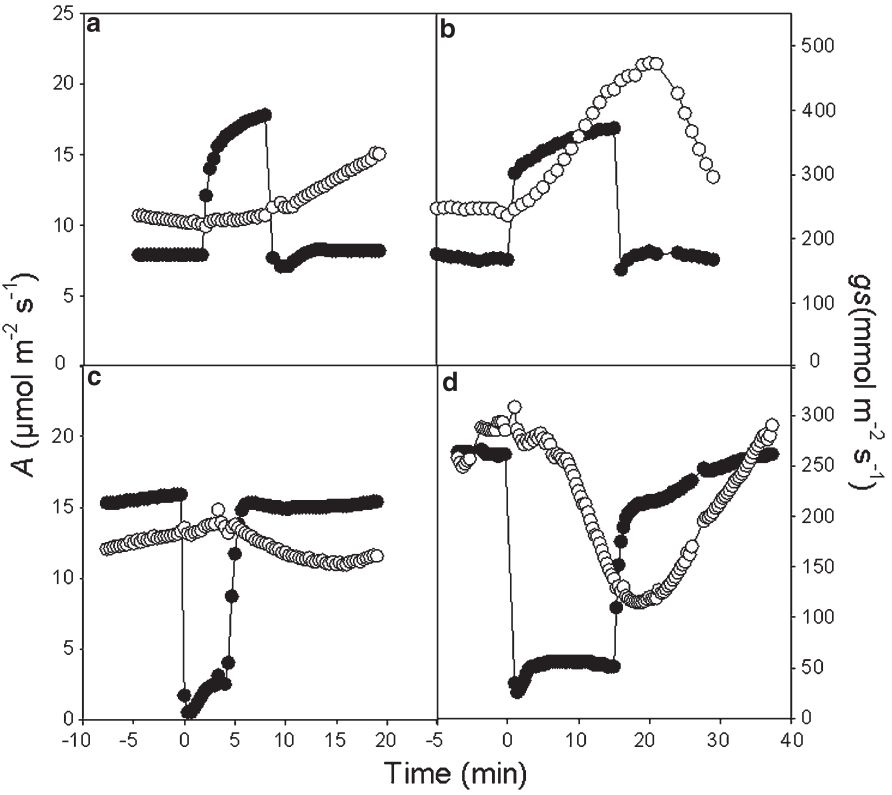

Fig. 3 The effect of sun and shade flecks on CO_2 assimilation rate and stomatal conductance. (**a**) and (**b**) *Sun flecks*: PPFD was increased from 230 to 615 μmol m^{-2} s^{-1} at time zero for (**a**) 5 min, (**b**) 15 min, (**c**) and (**d**) *Shade flecks*: PPFD was decreased from 615 μmol m^{-2} s^{-1} at time zero. Measurements of assimilation rate (*solid circles*) and stomatal conductance (*open circles*) made every 5 s with cuvette CO_2 maintained at 357 μmol mol^{-1}, temperature 25°C and VPD 1.34 kPa

Although under controlled environmental conditions, within-leaf patterns of photosynthesis often correlate with stomatal behaviour (Weyers et al. 1997), in natural fluctuating environments, there is significantly greater within-leaf variation in stomatal conductance and photosynthetic rates, the patterns of which often show no correlation (Weyers and Lawson 1997). The observed variation and lack of correlation between stomatal conductance and assimilation rate over individual leaves of *Phaseolus vulgaris* were attributed to the differences in the induction times for stomata and photosynthesis to respond and keep track of variable conditions (Lawson and Weyers 1999). The rate of stomatal opening and closing reflects the kinetics of multiple processes such as signal transduction, water movement, the establishment of osmotic potential, as well as the influence of the mechanical properties of guard cells and is therefore a complex kinetic function (Kirschbaum et al. 1988; Cardon et al. 1994; Franks and Farquhar 2007). It is clear that the kinetics of stomatal responses (and resulting g_s) to certain environmental

parameters are not consistent across species (see Hetherington and Woodward 2003; Sharkey and Raschke 1981) and that direct and indirect responses are significantly influenced by the type of photosynthetic pathway (Huxman and Monson 2003).3

2.5 Night Time Stomatal Conductance

It is generally accepted that stomata close in response to darkness, although significant night time stomatal conductances, which can result in 5–30% of the daily water loss (Snyder et al. 2003), have been observed in a range of species (see Donovan et al. 1999; Daley and Phillips 2006; Caird et al. 2007), including C_3 and C_4 photosynthetic types. Rates of up to 90% of day time conductances have been reported, although rates of water loss depend on VPD, which is generally much lower during the night (Caird et al. 2007). As night time stomatal conductance allows water loss with no carbon gain, and with reduced leaf cooling requirements, the benefit for the plant is at present still unclear (Caird et al. 2007). It has been suggested that night time stomatal conductance could enhance nutrient uptake (Donovan et al. 2001; Caird et al. 2007) or oxygen delivery to respiring cells (Daley and Phillips 2006), repair xylem embolism (Rogiers et al. 2009) and/or could enhance early morning/predawn stomatal opening that has the potential to maximise carbon uptake when temperature and VPD are lower (Caird et al. 2007). However, it appears that rates of night time conductance are correlated with day time stomatal conductance. Night time stomatal conductance could not only have significant impacts on plant water status and WUE, but also has important implications for pollutant uptake (e.g. ozone, Grulke et al. 2004) and isotope signatures for determining environmental impacts on transpiration rates (Barbour et al. 2005).

3 Stomatal Interactions with Photosynthesis

3.1 Photosynthetic Pathways and Stomatal Function

Most reviews on stomatal function are C_3-centric, most likely as a result of the bias toward stomatal research on C_3 plants, as they tend to have larger stomata to work with. In this section, we briefly examine some of the key differences in stomatal behaviour between C_3, C_4 and Crassulation Acid Metabolism (CAM) plants. The global distribution of C_3, C_4 and CAM plants is unequal, driven by global variation in environmental variables. C_3 plants are the most widely distributed plants, dominating the temperate regions of the world and covering an estimated 87.4 million km^2 compared with 18.8 million km^2 for C_4 species (Still et al. 2003).

Plants with the C_4 photosynthetic pathway appeared about 8 million years ago and tend to dominate in warmer, dryer habitats at 21–23° latitudes (Sage et al. 1999), representing less than 4% of all plant species. CAM plants occurred earlier in evolution in the carboniferous and differ greatly in stomata behavioural patterns, opening in darkness and remaining closed during the light period (Osmond 1978; Black and Osmond 2003). In CAM plants, stomata open at night for net CO_2 uptake catalysed by phosphoenolpyruvate carboxylase (PEPC), which is stored in the vacuole as malic acid. During the light period, decarboxylation of vacuole acids releases CO_2 behind closed stomata, enabling high CO_2 concentration to develop for refixation by Rubisco (Cockburn et al. 1979). Stomata remain closed during the light period, except during late afternoon (termed phase IV), when reduced acid concentration and optimal conditions (namely water supply) permit stomata to reopen to perform C_3 photosynthetic CO_2 fixation. Until recently, stomatal behaviour in CAM plant has been associated with changing C_i concentration due to the action of PEPC at night and decarboxylation during the day (Cockburn et al. 1979; Spalding et al. 1979); however, von Caemmerer and Griffiths (2009) demonstrated that stomata of Kalanchoe species did not respond when C_i was manipulated. Stomata of a particular CAM species, *Tillandsia recurvata*, have been shown to be sensitive and directly responsive to changes in ambient air humidity during nocturnal CO_2 fixation. Changes in stomatal aperture were shown to be a direct response to changes in humidity and not to bulk tissue water conditions of the leaves (Lange and Medina 1979). The induction of CAM in the facultative CAM plant *Mesembryanthemum crystallinum* has been shown to abolish the stomatal blue light response, which is apparent when the plant is in C_3 mode (Mawson and Zaugg 1994), a response that has been linked to the a lack of light-induced formation of zeaxanthin (Tallman et al. 1997). Zeaxanthin has previously been linked with blue light responses in guard cells (Zeiger and Zhu 1998; Frechilla et al. 1999). Stomata of *Portulacaria afra* have been shown to have typical blue and red light responses when in C_3 mode, both of which become undetectable in CAM mode (Lee and Assmann 1992). However, red light response was lacking in *M. crystallinum* even in C_3 mode (Mawson and Zaugg 1994). Studies on the rate of opening in response to a decrease in CO_2 concentration in the CAM plants *Kalenchoe daigremontiana* and *Kalanchoe pinnata* showed that the rate of opening in the dark was as rapid as in the light during Phase IV of the CAM cycle, where CO_2 is fixed primarily via C_3 photosynthesis, indicating that energy can be sourced via mitochondrial respiration or stored carbohydrate (von Caemmerer and Griffiths 2009). The fact that stomata and photosynthesis are temporally segregated in CAM plants provides an ideal opportunity to use such systems to examine stomatal responses in isolation from mesophyll C_3 metabolite production or demands (von Caemmerer and Griffiths 2009).

Stomatal function and behaviour in C_4 plants are more similar to those of C_3 plants than of CAM plants, although differences in magnitude and sensitivity to light and CO_2 have been reported between C_3 and C_4 species (Huxman and Monson 2003; Maherali et al. 2002). For example, several studies have demonstrated that stomata of C_4 plants have greater sensitivity to C_i than C_3 species

(Dubbe et al. 1978; Sharkey and Raschke 1981; Ramos and Hall 1982), although Morison and Gifford (1983) reported similar sensitivities. It would, however, be interesting to see if such reported differences in sensitivity hold true if stomatal response was measured as a function of C_a rather than C_i. C_3 species have also been reported to be less prone to closure than C_4 when light was decreased or CO_2 increased (Akita and Moss 1972). Stomatal acclimation to CO_2 responses have been observed in C_3 forbs but not in C_3 or C_4 grasses (Maherali et al. 2002). In response to severe drought, reduced stomatal aperture could limit photosynthesis to a greater degree in C_4 plants than in C_3, due to the fact that C_4 photosynthesis operates closer to the C_i inflection point and there is a steep photosynthetic response with increasing C_i at low C_i (see Ghannoum 2009). Additionally, C_3 and C_4 plants have been reported to differ in their diurnal periodicity of opening, with maximum g_s reported for C_3 plants at 10 am, while for C_4 plants the maximum is at 12 noon (Das and Santakurmari 1977), suggesting that low light levels and low temperature promote stomatal opening in C_3 plants compared with C_4.

Dissimilar sensitivity could be due to different sensory or signalling mechanisms or differences in anatomical features. The more evolutionary advanced dumb-bell shaped guard cells (often found in grasses and C_4 species) are able to open more rapidly compared with kidney shaped guard cells (Hetherington and Woodward 2003; Franks and Farquhar 2007). The speed of stomatal opening in grasses is believed to have evolved to increase photosynthesis and WUE (Grantz and Assmann 1991; Huxman and Monson 2003). The physical explanation for the reported increased speed and efficiency is due the dumb-bell shape of the guard cells enabling a greater magnitude of change in stomatal aperture with relatively small changes in turgor (Raschke 1975) which "maximise the potential of stomata to track changes in environmental conditions" (Hetherington and Woodward 2003). Both opening and closing movements of stomata are active energy consuming steps and according to Assmann and Zeiger (1987) ATP synthesis by either mitochondria or chloroplasts could accommodate the energetic requirements of stomatal opening. The fact that dumb-bell type guard cells require small changes in guard cell turgor would also imply smaller energy requirements (Hetherington and Woodward 2003).

Differences in anatomical features have also been observed between C_4 plants, which have reportedly a high stomatal density on the upper leaf surface (relative to the lower leaf surface) compared with C_3 plants (e.g. Das and Santakurmari 1977). Environmental growth conditions also influence stomatal responses; for example, sun and shade leaves differ in their response to light (Turner 1979) and also stomata on upper and lower surfaces will differ in their response to light, mostly likely due to the fact that their growth microenvironments are different (Pemadasa 1981). Additionally, we often quote that the stomata of C_3 (and C_4) plants are closed in darkness; however, as discussed above, night time stomatal conductance has been observed in a diverse range of both C_3 and C_4 species (see Caird et al. 2007).

3.2 Correlation Between Stomatal Conductance and Photosynthetic Capacity

Across species and under a variety of growth conditions, plants regulate their transpiration and photosynthetic rates in parallel, maintaining a balance between stomatal and photosynthetic capacity. This results in the conservation of the ratio of C_i to C_a (Wong et al. 1979, 1985; Hetherington and Woodward 2003). This empirical direct correlation between photosynthesis and stomatal conductance was central to initial models of stomatal control of photosynthesis (Farquhar and Wong 1984; Ball et al. 1987) and has been carried over to more recent models (Dewar 2002; Buckley et al. 2003). However, the underlying regulatory mechanisms are still unclear.

3.2.1 Evidence for and Against a Mesophyll Driven Signal

Guard cells may respond to photosynthetic demand by direct sensing of C_i or C_i/C_a (Ball and Berry 1982; Mott 1988; Roelfsema et al. 2002). Contrary to the predictions of the above mentioned models, transgenic plants with impairments in different steps of the photosynthetic process maintain normal stomatal conductances, resulting in elevated C_is and bringing into question the postulated C_i control of stomatal movement (for review see von Caemmerer et al. 2004). Alternatively, it has been proposed that guard cells sense the metabolic status of the mesophyll via a diffusible factor that is a product of photosynthetic activity in the mesophyll (Wong et al. 1979; Mott et al. 2008) and that stomatal aperture would be inversely proportional to the pool size of such diffusible factor (Farquhar and Wong 1984). Possible metabolites include ATP, NADPH, or ribulose bisphosphate (RuBP), the concentration of which depends strongly on the balance between chloroplast electron transport and the carboxylation reaction catalysed by Rubisco (Messinger et al. 2006).

The first suggestion of a mesophyll driven signal on stomatal behaviour was proposed by Heath and Russell in 1954. These researchers separated an indirect C_i effect from a direct light effect on stomatal behaviour and suggested that there was an indirect effect transmitted from either the epidermal cells or the mesophyll cells by a chemical or an electrical signal (Heath and Russell 1954). The influence of mesophyll on stomatal behaviour was illustrated by Lee and Bowling (1992) who demonstrated different behaviour patterns in isolated epidermis of *Commelina communis* compared with intact leaves. In the same study, the authors reported that epidermis incubated with mesophyll cells showed greater opening responses that those incubated without mesophyll cells or when mesophyll cells were kept in the dark. These results suggested that a product of photosynthesis (which they named stomatin) aided stomatal opening, although the compound was never identified. They ruled out D-glucose, sucrose, malic acid and ATP (Lee and Bowling 1992). Guard cell membrane hyperpolarization by light and CO_2 in intact leaves,

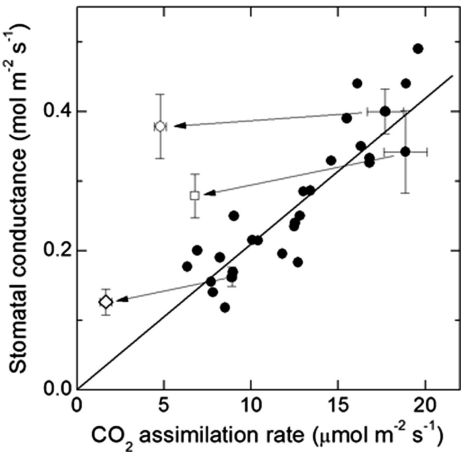

Fig. 4 Relationship between stomatal conductance and CO_2 assimilation rate in wild type and transgenic tobacco plants impaired in photosynthesis by a decrease in Rubisco function (anti-Ssu plants). Data are redrawn from (Baroli et al. 2008; www.plantphysiol.org: Copyright American Society of Plant Biologists) and plants were grown under elevated CO_2 in environmentally controlled chambers and conductance and photosynthesis measurements were performed under ambient CO_2. *Filled circles*, wild type; *open diamond*, mean ± SE ($n = 4$) from low light (LL) grown anti-Ssu plants; *open square*, mean ± SE ($n = 4$) of medium light (ML)-grown anti-Ssu plants, *open circle*, mean ± SE ($n = 5$) from ML-grown anti-Ssu plants assayed in red/blue light (redrawn from von Caemmerer et al. 2004; www.jxb.oxfordjournals.org: Copyright Society of Experimental Biology). *Arrows* link data from anti-Ssu plants with the mean ± SE of 4–5 wild type plants grown and assayed under identical conditions at the same time. The *solid line* represents linear regression fit of all wild type data ($y = 0.0217$ (± 0.00069) $\times x$, $R = 0.90$). Each data point not showing error bars corresponds to an individual plant. Error bars represent SE

but not in isolated epidermis or leaves treated with photosynthetic inhibitors, provided further evidence for a mesophyll driven signal (Lee and Bowling 1993). In a viewpoint paper, Lee and Bowling (1995) collated evidence for mesophyll influence on stomatal opening and concluded that the stomatal mechanism was controlled by command and operations and that "command originates in the mesophyll cells and operation in the guard cells". However, from studies with transgenic plants with impaired photosynthesis, it is clear that the rate of opening is not influenced by the current rate of photosynthesis (Fig. 4) ((von Caemmerer et al. 2004; Baroli et al. 2008).

A recent publication by Mott et al. (2008) has revised the potential for a mesophyll driven signal. Isolated epidermal peels grafted onto mesophyll (from the same species or another leaf) showed rapid reversible responses to light and CO_2. Mott and coworkers put forward two working hypotheses to explain the findings: (1) something produced by the mesophyll sensitises the guard cells to light and CO_2; (2) stomata respond to a signal generated in the mesophyll in response to light and CO_2. Their findings supported the second hypothesis.

3.3 Involvement of Guard Cell Photosynthesis in Stomatal Responses

The fact that guard cells contain functional chloroplasts (Willmer and Fricker 1996; Lawson et al. 2003), carry out linear electron transport (Hipkins et al. 1983; Shimazaki and Zeiger 1985; Willmer and Fricker 2006; Lawson et al. 2002), possess many of the main Calvin cycle enzymes (Zemel and Gepstein 1985; Shimazaki and Zeiger 1985; Gotow et al. 1988; Shimazaki et al. 1989) and guard and mesophyll cell photosynthetic efficiency correlate closely (Lawson et al. 2002, 2003) has provided attractive but controversial alternative sensory and/or regulatory mechanisms allowing stomatal behaviour to track mesophyll photosynthetic performance and explain the close correlation between mesophyll photosynthesis and stomatal conductance (Lawson 2009). Guard cell chloroplasts could contribute to stomatal behaviour in several different ways. Electron transport in guard cells, coupled to the production of ATP and/or reductants, is required for osmoregulation (Schwartz and Zeiger 1984; Shimazaki and Zeiger 1985; Tominaga et al. 2001). ATP produced through either linear (Shimazaki and Zeiger 1985; Lawson et al. 2002, 2003) or cyclic electron transport (Lurrie 1977) could provide sufficient ATP to drive ion exchange during stomatal opening. Although the exact mechanisms of ATP export from the chloroplast for use in the cytosol are unclear, specific ATP transporters have recently been identified that may provide an explanation for this mechanism (for detail see Weber and Fischer 2007). Tominaga et al. (2001) conducted experiments on epidermal peels of *Commelina benghalensis* under red light with and without inhibitors of oxidative phosphorylation (oligomycin) and photosystem II (DCMU) and demonstrated that under red light ATP produced during photophosphorylation was utilised by the plasma membrane H^+-ATPase for H^+ pumping. Several independent studies have demonstrated the use of ATP and NADPH from photosynthetic electron transport for the reduction of oxaloacetate (OAA, from starch breakdown or imported) and 3-phosphoglycerate (3-PGA, from guard cell CO_2-fixation or imported from the cytosol) which is subsequently exported to the cytosol via a 3-PGA-triose phosphate shuttle (Shimazaki et al. 1989; Ritte and Raschke 2003).

Guard cell chloroplasts also act as a starch storage facility which provides a second mechanism by which guard cell chloroplasts could contribute to coordinate stomatal movements. Carbon skeletons produced from starch breakdown are used to produce malate via PEPC and CO_2 fixation within the guard cells (Willmer and Dittrich 1974; Raschke and Dittrich 1977; Schnabl et al. 1982; Willmer 1983; Outlaw 1990). As alluded above, OAA (provided from starch breakdown) is reduced to malate (Rao and Anderson 1983; Scheibe et al. 1990) and malate accumulation has been correlated with stomatal aperture (Allaway 1973; Pearson 1973; Pearson and Milthorpe 1974; Vavasseur and Raghavendra 2005). The third and controversial mechanism concerns CO_2 fixation by the Calvin cycle within guard cell chloroplasts, and the use of end products in stomatal movements (see Outlaw 2003; Lawson 2009). Although the presence of Calvin cycle enzymes has

been clearly demonstrated (Zemel and Gepstein 1985; Shimazaki and Zeiger 1985; Gotow et al. 1988; Shimazaki 1989), numerous reports have questioned the functional capacity of the Calvin cycle for producing osmotica (Outlaw et al. 1979, 1982; Outlaw 1982, 1987, 1989; Tarczynski et al. 1989). On the other hand, several studies have reported that the Calvin cycle represents a major sink for the products of photosynthetic electron transport (Cardon and Berry 1992; Lawson et al. 2002, 2003) and incorporation of $^{14}CO_2$ into 3-PGA (Gotow et al. 1988). Photosynthetic dependence of sucrose accumulation has also been demonstrated in epidermal peels of *V. faba* exposed to red illumination (Poffenroth et al. 1992). The reported contribution of osmotica produced for carbon reduction within the guard cells for stomatal opening ranges from 2% (Reckmann et al. 1990) suggesting that rates are too low for any functional significance (Outlaw 1989) to 40% (Poffenroth et al. 1992) representing a significant source of osmotica for guard cell function. Guard cell chloroplast function in stomatal movements provides an attractive hypothesis for several reasons: firstly, the guard cell chloroplasts are ideally and conveniently located (as such mechanisms would be required to be in proximity to the stomatal guard cells); secondly, the functional role of these highly conserved guard cell chloroplasts remains elusive; and finally, a mechanism for stomatal movements and behaviour linked to the guard cell chloroplasts would provide an ideal link between stomatal behaviour and the tight correlation observed with mesophyll photosynthetic rates, with both being governed by chloroplast performance.

However, work on photosynthetic transgenic plants with impaired carboxylation (von Caemmerer et al. 2004) or RuBP regeneration (Lawson et al. 2008) has shown that despite similar decreases in photosynthesis in the mesophyll as well as in the guard cells, stomata can achieve equivalent or even greater g_s than wild type plants. These data clearly demonstrate that guard cell photosynthesis is not essential for stomatal function, but may play a subtle role in stomatal opening rate and final conductance under red light conditions (Lawson et al. 2008).

3.4 Sucrose as Signal Between Photosynthesis and Stomatal Behaviour

Reports suggesting relatively low levels of Calvin cycle activity and sucrose production in guard cell chloroplasts led to the alternative suggestion that sucrose produced by mesophyll photosynthesis could provide osmotica for stomatal movements, and thereby link stomatal behaviour with photosynthetic rates (Lu et al. 1995, 1997; Ritte et al. 1999; Outlaw and De Vleighere-He 2001; Kang et al. 2007a). Hite et al. (1993) showed that guard cells could act as carbon sinks, importing sucrose via plasma membrane transporters (Stadler et al. 2003). Sucrose import into the guard cells not only provides an osmoticum for stomatal opening but can also provide a "transpiration-linked, photosynthetic-dependent passive mechanism for modulation of stomatal aperture size" (Kang et al. 2007a). The guard

cell apoplastic sucrose can also exert an osmotic effect, which can drive stomatal closure, acting as a possible signal between mesophyll assimilation rate and transpiration (Kang et al. 2007a). Under photosynthetic conditions, sucrose accumulation in the guard cell apoplast driven by transpiration is sufficient to diminish stomatal aperture. The guard cell apoplastic sucrose is a product of recent mesophyll photosynthesis (Lu et al. 1997); Outlaw and co-workers have hypothesised that its concentration is correlated with the rate of photosynthesis as well as transpiration (Outlaw and De Vleighere-He 2001) providing a signal that prevents further stomatal opening when photosynthesis and transpiration are high (Kang et al. 2007a). However, such a mechanism may only be possible in apoplastic phloem loaders (Kang et al. 2007b). It is also difficult to see how this mechanism works in maintaining high photosynthetic rates, as under the hypothesis proposed, limited CO_2 supply as a result of preventing stomatal opening would potentially increase mesophyll demand for CO_2.

3.5 ROS Signalling in Stomata and Relationship with Photosynthesis

Reactive oxygen species (ROS) result from the incomplete reduction of molecular oxygen. They include superoxide, hydrogen peroxide, singlet oxygen and the hydroxyl radical. Besides being unavoidable toxic by-products of metabolism and environmental stress, ROS play a central role as messengers in the signal transduction chain leading to the acclimation response to stress (Van Breusegem et al. 2008; Pfannschmidt et al. 2009). In particular, hydrogen peroxide has been shown to mediate ABA-induced stomatal closure and it is thought that the source of H_2O_2 is the guard cells themselves (reviewed in Wang and Song 2008). MAP kinases have recently been implicated in the down stream function of ROS activity to regulate guard cell ABA signalling positively (Jammes et al. 2009). Chloroplasts are considered to be the primary sources of ROS in plant cells (Asada 2006) and ROS production in the chloroplasts of guard cells has been observed in response to ozone treatment (Joo et al. 2005). However, whether and to what extent chloroplast-generated ROS contribute directly to the signal transduction that leads to stomatal movements has not been defined (Wang and Song 2008). In fact, H_2O_2 generated at the guard cell plasma membrane by the action of NADPH oxidase has emerged as a major player in ABA-mediated signal transduction in guard cells. H_2O_2 is required to initiate stomatal closure (Wang and Song 2008). In Arabidopsis, mutations in two of the ten NADPH oxidase subunits, AtrbohD and AtrbohF, abolish ABA-induced ROS production and stomatal closure and the effect of the mutations is cancelled by the exogenous addition of H_2O_2 (Kwak et al. 2003). The signal transduction cascade involving ABA and ROS has been shown to include cytosolic Ca^{2+} transients, G proteins, protein kinases and phosphatases, phosphatidic acid and transcription factors (Wang and Song 2008; Pham and Desikan 2009; Cho et al. 2009; Zhang et al. 2009). In rice, a zinc-finger transcription factor named DST

(for *drought and salt tolerance*), which accumulates H_2O_2, has been recently shown to negatively regulate stomatal closure by direct modulation of the genes related to H_2O_2 homeostasis (including peroxidases), with plants lacking DST exhibiting increased stomatal closure and reduced stomatal density, resulting in enhanced tolerance to drought (Huang et al. 2009).

Scavenging and prevention of excess production of ROS are integral features of cellular metabolism (Niyogi 2000). ROS are detoxified in plant cells by a combination of enzymatic reactions involving superoxide dismutases, ascorbate peroxidases, catalases and glutathione peroxidases, as well as small antioxidant molecules, such as ascorbate and glutathione. Detoxification of ROS is essential for normal stomatal movements. An Arabidopsis mutant lacking a glutathione peroxidase (AtGPX3) exhibits enhanced production of H_2O_2 in guard cells and reduced WUE, whereas plants overexpressing AtGPX3 showed increased WUE and were less sensitive to water stress (Miao et al. 2006). Ascorbate peroxidase (APX1) deficient Arabidopsis plants, which show reduced photosynthetic rates compared to wild type plants, also show abnormal stomatal conductances, in particular with respect to light-to dark transitions, although their response to exogenous ABA applications appears normal (Pnueli et al. 2003).

3.6 Role for Respiration

Although the relationship between g_s and A is key to examining WUE, the effect of respiration (by either guard or mesophyll cells) may play an important role in contributing to stomatal sensory or signalling mechanisms in response to changing environmental parameters (Lawson 2009). Guard cells are known to contain numerous mitochondria (Willmer and Fricker 1996; Vavasseur and Raghavendra 2005) and high metabolic fluxes through the catabolic pathway have been reported (Hampp et al. 1982). Raghavendra and colleagues have suggested that ATP produced through oxidative phosphorylation is important for stomatal movements (Raghavendra and Vani 1989; Parvathi and Raghavendra 1997). The application of inhibitors of oxidative phosphorylation such as KCN has revealed an impaired stomatal response to light induced opening (Schwartz and Zeiger 1984). Transgenic tomato plants with reductions in mitochondrial fumarate hydratase (fumarase) activity (Nunes-Nesi et al. 2007) and malate dehydrogenase (Nunes-Nesi et al. 2005) show reductions in stomatal aperture and CO_2 limitation of photosynthesis (Nunes-Nesi et al. 2007). Additionally, Lu et al. (2000) demonstrated a positive correlation between stomatal conductance and guard cell respiration rates in Pima cotton. A role for respiratory ATP in stomatal opening in CAM plants has also recently been demonstrated (von Caemmerer and Griffiths 2009). It has been suggested that both the photosynthetic and respiratory pathways in guard cells are important in stomatal function (Asai et al. 2000) and that the relative importance of each pathway maybe altered if either one is restricted (see Parvathi and Raghavendra 1997).

4 Environmental Control of Stomatal Development and the Role of Photosynthesis

The developmental control underpinning the coordination between stomatal conductance and photosynthesis is not well understood. Developmental changes during the life span of the leaf have been shown to be independent of the photosynthetic capacity of the plant. For example, Jiang and Rodermel (1995) showed that stomatal conductance followed similar developmental changes with leaf age in antisense small subunit Rubisco (anti-Ssu) plants and wild type tobacco plants despite their different photosynthetic rates. Similarly, it was shown that, despite the very different photosynthetic capacities, the leaf and stomatal developmental response to growth light environment in anti-SSu and wild type plants was similar, resulting in fewer stomata per leaf area in leaves developed under low compared to high light in both genotypes (Baroli et al. 2008).

4.1 The Genetic Pathway of Stomatal Development

Recent work in Arabidopsis has substantially advanced our understanding of the genetics of stomatal differentiation (see Nadeau 2009; Bergmann and Sack 2007 and references therein). The process appears to be highly regulated and involves a series of cell divisions which are asymmetrical and oriented so as to ensure correct stomatal spacing by preventing the formation of adjacent stomata. The stomatal developmental pathway begins when a protodermal cell in the epidermis of the unfolding leaf converts to a meristemoid mother cell, which then undergoes an asymmetric cell division producing a small meristemoid cell and a larger sister cell. The meristemoid cell can undergo several self-renewing asymmetric divisions before differentiating into a guard mother cell, which then divides symmetrically and further differentiates to form the pair of mature guard cells that surround the stoma. The asymmetry of cell divisions in the stomatal lineage was shown recently to be determined by the intracellular distribution of the product of the BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) gene (Dong et al. 2009). Cell–cell signalling in response to positional cues during stomatal development is mediated by the putative cell surface receptors TOO MANY MOUTHS (TMM) and members of the ERECTA family of leucine-rich repeat containing receptor kinases (ER, ERL1 and ERL2). These are proposed to interact with the small secretory peptides EPIDERMAL PATTERNING FACTORS (EPF) 1 and 2, produced by stomatal precursors, which may act as mobile positional signals (Hara et al. 2007; Hunt and Gray 2009). Activation of the receptors stimulates a mitogen-activated protein kinase (MAPK) cascade starting with the MAPKKK YODA (YDA), which in turn activates MKK4/MKK5 and MPK3/MPK6 (Wang et al., 2007a, b). This MAPK cascade negatively regulates stomatal development,

and it may target three structurally related transcription factors belonging to the basic helix-loop-helix (bHLH) family SPEECHLESS (SPCH), which acts as a positive regulator of the initial asymmetric cell division of the meristemoid, along with MUTE and FAMA, which control meristemoid differentiation and guard cell morphogenesis, respectively. In addition, MYB transcription factors (MYB124, MYB88 and FOUR LIPS) are also involved in the final fate transitions of the stomatal differentiation pathway.

The importance of identifying the pathways regulating stomatal differentiation in order to develop plants with greater water use efficiencies has recently been reviewed by Wang et al. (2007a, b). However, changes in anatomical stomatal characters do not always result in changes in stomatal conductance and/or increase WUE (Lawson and Morison 2004; Lawson 2009). This has been demonstrated in Arabidopsis plants over-expressing STOMATAL DENSITY AND DISTRUIBTUION 1 (SDD1) gene, which conveyed a 40% reduction in stomatal density, while the sdd1-1 mutants (Berger and Altmann 2000) increased density by 300% relative to the wild type. Yet both plants showed similar stomatal conductance and assimilation rates as WT plants. Decreases in density were compensated for by greater apertures and *vice versa* (Bussis et al. 2006).

4.2 Interaction Between Stomatal Development Genes and Environmental Signals

While considerable knowledge exists on the effect of environmental factors such as light intensity and CO_2 concentration on the signalling mechanisms determining stomatal pore aperture, very little is known about their effects on the modulation of stomatal development. Moreover, it is not clear whether the stomatal lineage cells perceive the environmental stimuli directly (Casson and Gray 2008). However, links are starting to be established between the stomatal developmental genes and other known regulators of gene expression in response to environmental conditions. The bHLH-leucine zipper transcription factor SCREAM1 (SCRM1) has been shown recently to interact directly with and specify the sequential actions of SPCH, MUTE and FAMA (Kanaoka et al. 2008). SCRM1 was previously identified as INDUCER OF CBF EXPRESSION 1 (ICE1), a regulator of the expression of cold-induced genes. Another point of environmental regulation of stomatal patterning may be the MAP kinase cascade (Wang et al. 2007a, b), as its components MKK4, MKK5, MAPK3 and MAPK6 have previously been shown to play a role in the environmental stress response, with MAPK3 and MAPK6 directly involved in osmotic stress (Nakagami et al. 2005). Recent work with photoreceptor mutants in Arabidopsis has placed the YDA gene downstream of a developmental cascade involving the master regulator of photomorphogenesis COP1, the red light photoreceptors phytochrome A and phytochrome B and the blue-light photoreceptor cryptochrome, indicating that the three photoreceptors act additively to promote stomatal development in response to light quality (Kang et al. 2009).

4.3 Systemic Signals and Control of Stomatal Density in Response to the Environment

The stomatal density (the number of pores per unit area) and the stomatal index (number of guard cells relative to total epidermal cells) on the leaf epidermis are regulated during leaf expansion by humidity, temperature, CO_2 concentration and light intensity and once determined they remain unchanged for the lifetime of the leaf (Casson and Gray 2008). Light intensity and CO_2 concentration are the two most studied environmental variables with respect to their effects on stomatal differentiation. In general, both stomatal density and index are higher in plants grown in full sunlight or at high light intensities than in plants grown in shade (Willmer and Fricker 1996; Schoch et al. 1984; Boccalandro et al. 2009; Casson et al. 2009). Although exceptions have been recorded (notably, in free-air CO_2 enrichment (FACE) experiments, see Ainsworth and Rogers 2007) many species acclimate to increases in CO_2 levels by decreasing their stomatal density. This has been observed to occur during the past two centuries in industrial Europe, under laboratory conditions and through plant evolution (Woodward 1987; Hetherington and Woodward 2003), so much so that stomatal density is used as a proxy of paleo-CO_2 levels (Chaloner and McElwain 1997). Because the response to environmental conditions that controls stomatal patterning occurs before leaf expansion, this acclimation process must require the integration of signals at the whole plant level. In fact, experiments in which developing and mature leaves were subjected to different light intensities and CO_2 concentrations have demonstrated that the stomatal pattern of developing leaves is influenced by the conditions experienced by the mature leaves (Schoch et al. 1980; Lake et al. 2001; Thomas et al. 2004; Driscoll et al. 2006; Miyazawa et al. 2006). In Arabidopsis, growth of mature leaves at elevated CO_2 resulted in a 20–30% decrease (relative to control plants grown at ambient CO_2) in stomatal index in developing leaves that were exposed to ambient CO_2 levels. Conversely, in reciprocal experiments where mature leaves were exposed to ambient CO_2 and the developing leaves to elevated CO_2, the stomatal index was increased in developing leaves (Lake et al. 2001). Similar results have been reported for light intensity in *Chenopodium album* (Yano and Terashima 2001).

So far, only the HIGH CARBON DIOXIDE (HIC) gene of Arabidopsis has been identified as having a role in modulating changes in stomatal index in response to elevated CO_2 (Gray et al. 2000). When exposed to elevated levels of CO_2, HIC mutant plants showed a significant increase in stomatal index, whereas the parental ecotype showed a small decrease. HIC is expressed in guard cells and shares high homology with the Arabidopsis KCS1 gene, a 3-ketoacyl CoA synthase involved in the production of very long chain fatty acids found in the cuticular waxes. The mechanism by which HIC affects stomatal patterning in response to CO_2 is unknown. It is possible that a cuticular wax or an intermediate is a signalling compound that influences stomatal development. Alternatively, HIC may indirectly affect stomatal patterning in response to CO_2 by altering the permeability to water, CO_2, or another signalling compound within the epidermis.

The nature of the stomatal differentiation signal that is transported from mature leaves to developing leaves is also not clear. Carbohydrate accumulation and sugar signalling are involved in plant development and cell cycle control (Rolland et al. 2006). However, the fact that increased light intensity and elevated CO_2 both enhance rates of photosynthesis and sugar content of mature leaves, but exert opposite effects on the stomatal density of new leaves, argues against a photosynthetic nature of the signal. Consistent with this is the observation that the levels of sugars increased in mature and developing leaves when they were exposed to high CO_2 and decreased with shade treatment (Coupe et al. 2006). Based on the linear correlation between carbon isotope discrimination values and stomatal density found in cowpeas subjected to different CO_2, water and phosphorus environments, Sekiya and Yano (2008) proposed that the C_i/C_a ratio may be involved in the systemic signalling that determines stomatal density. However, transgenic tobacco plants with decreased Rubisco content show a normal response of stomatal density to light intensity despite maintaining a high C_i/C_a (Baroli et al. 2008), suggesting that, at least when light intensity is the environmental stimulus, the C_i/C_a ratio would not be part of the signalling mechanism. In experiments in which CO_2 concentration, irradiance and water pressure deficit were varied in mature leaves independently of the conditions around the developing leaves, the stomatal index of the developing leaves was positively and highly correlated with the stomatal conductance of the mature leaves and independent of their net photosynthesis (Miyazawa et al. 2006). This result suggests that it is not the carbohydrate production but the transpiration rates of mature leaves that could influence stomatal development, independently of photosynthesis, by controlling the delivery rates of hormones, such as cytokinins and abscisic acid, that are transported through the xylem from the roots to the expanding leaves. Boonman et al. (2007) have recently shown that cytokinin delivery through the xylem is dependent on transpiration rates in Arabidopsis and tobacco and they suggest that cytokinin import rate into the leaf could be a signal for photosynthetic acclimation to environmental variables such as light intensity.

4.4 Hydraulic Conductance Correlates with Stomatal Behaviour

Stomatal density and size are not the only anatomic features of the leaf that can exert control over stomatal behaviour. Hydraulic conductivity, which is a measure of the efficiency of water transport within leaves, has been shown to correlate with maximum stomatal conductance (Brodribb and Holbrook 2004; Brodribb et al. 2005; Nardini and Salleo 2005; Brodribb and Jordan 2008) as well as stomatal sensitivity to perturbations in VPD (Franks and Farquhar 1999). Evidence exists for a hydraulic influence on both long-term adaptation of maximum conductance as well as short-term stomatal responses (Brodribb and Jordan 2008). It has been suggested that species with a high hydraulic conductance might be more

buffered and therefore respond less to changes in VPD (Franks and Farquhar 1999). However, there is considerable species–species variation in the physiological influence that plant hydraulic conductance exerts over leaf conductance (Sperry 2000; Meinzer 2002). A strong correlation exists between maximum leaf hydraulic conductance and leaf anatomical characters including vein density, stomatal pore area index and palisade thickness (Aasamaa et al. 2001; Sack and Frole 2006). These observations indicate that hydraulic efficiency is highly adapted (Brodribb and Holbrook 2004), with implications for evolutionary coordination between gas exchange and hydraulic capacity (Franks and Farquhar 1999; Brodribb and Field 2000). The relationship between anatomical characters and hydraulic conductivity could provide a means for paleo-reconstruction of stomatal behaviour/sensitivity in past climatic environments (Brodribb and Jordan 2008).

5 Stomatal Manipulation to Improve Water Use Efficiency

Since altering stomatal anatomical features such as density and size may not necessarily always result in plants with increased WUE, modifications in stomatal behavioural characteristics may provide an alternative strategy (Nilson and Assmann 2007). To exploit such an opportunity, we first need to gain a solid understanding of stomatal metabolism for engineering drought resistance in future crop plants. Several studies have demonstrated the potential of such an approach by examining plants with increased or decreased amounts of a single enzyme and shown an alteration in stomatal conductance. *Zea mays* plants with increased amount of NADP-malic enzyme demonstrated signs of drought resistance with decreased stomatal conductance (Laporte et al. 2002). Increased drought resistance has also been demonstrated in Arabidopsis plants with alterations in guard cell membrane transporters (Klein et al. 2004), calcium dependent protein kinases (Ma and Wu 2007), aquaporin genes (Cui et al. 2008) and ABA biosynthesis or ABA sensitivity (Jakab et al. 2005; Wang et al. 2005; Yang et al. 2005). Hu et al (2006) established drought tolerance in rice over-expressing SNAC1 (STRESS-RESPONSIVE NAC 1, which encodes a NAC transcription factor). NAC genes play important roles in developmental processes, auxin signalling, defense and abiotic stress responses. Recently, a previously unknown zinc finger protein DST (drought and salt tolerance) has been identified as a regulator of stomatal closure through modulation of H_2O_2 signalling pathways in guard cells (Huang et al. 2009), providing possible new avenues of enhanced drought and salt tolerance in rice. Understanding the mechanisms involved in stomatal sensing/signalling pathways in response to changing CO_2 may play a fundamental role in plant WUE as illustrated in a recent study exploring the evolution of stomatal sensitivity to CO_2 (Brodribb et al. 2009). Plant species with reduced CO_2 sensitivity (such as ferns) had a reduced WUE compared to CO_2 sensitive angiosperms, suggesting that atmospheric CO_2

concentration is an evolutionary driving force for optimising WUE (Brodribb et al. 2009). However, in order to increase WUE, a combination of alterations in stomatal function and photosynthetic capacity may be required. For example, increased WUE in the *Erecta* mutants was not only because of modifications to cell expansion and division and stomatal density but also because of alterations in leaf diffusive properties and mesophyll photosynthetic capacity (Masle et al. 2005).

It is also worth bearing in mind that any modifications in stomatal (or photosynthetic) capacity and behaviour would require testing in a field situation, with fluctuating environmental conditions, predation and competition. For example, ABA over-sensitive Arabidopsis mutants with reduced g_s could not compete for water with WT plants (Basco et al. 2008). Although such findings have implications for screening protocols, they may not be so critical for monocultures of crops.

The potential to identify genes involved in stomatal responses to various environmental stresses/factors has recently been explored using various "-omic" approaches (Leonhardt et al. 2004; Coupe et al. 2006). Using microarray technologies, Leonhardt et al (2004) showed reductions in guard cell metabolism when Arabidopsis leaves were sprayed with ABA. Such findings agreed with earlier studies that reported decreased PEPC transcripts under drought conditions (Kopka et al. 1997). A functional proteomic study in Arabidopsis guard cells protoplasts by Zhao et al. (2008) identified new proteins and signalling pathways required for ABA responses in guard cells. Analysis of functional groups of genes revealed only a 1.9% higher representation of photosynthetic genes in mesophyll and guard cells. Transcriptomics analysis has also been used to identify transcriptional factors that are necessary for mediating stomatal movement in response to light (Gray 2005; Casson and Gray 2008) and water deficit (Cominelli et al. 2005). Such information is critical in helping to determine the link between mesophyll photosynthesis and guard cell behaviour. For example, a comparative proteomic study between mesophyll and guard cells in *Brassica napus* revealed functional differentiation between the two cell types. Expression patterns from guard cells were enriched with proteins involved in respiration, transport, transcription, cell structure and signalling, while proteins involved in photosynthesis, starch synthesis and defense mechanisms were more prevalent in mesophyll cells (Zhu et al. 2009). Although such approaches are invaluable in the information they provide on stomatal signalling and response mechanisms, to date most studies have relied upon the use of guard cell protoplasts. There are two main concerns with the use of protoplasts; firstly, the isolation procedure will inevitable result in cellular damage, resulting in the production of ROS, which are known to be involved in signal transduction pathways and alteration of gene expression (Galvez-Valdivieso et al. 2009). Secondly, the isolation of guard cells from the mesophyll will immediately remove the potential of any mesophyll-driven signalling event. A third (most likely less important) point is the use of bulk samples removing possible identification of heterogeneity between guard cells, as it is well established that considerable variation in stomatal behaviour is apparent over individual leaves (Weyers and Lawson 1997).

6 Scaling-Up: From Leaf to Canopy

In this chapter, we have focused on stomatal and photosynthetic responses at the leaf level, reporting results mainly obtained from leaf cuvette gas exchange measurements. An appreciation of the dynamic role of stomata in photosynthetic physiology and WUE at the canopy/crop level (or beyond) is essential for a mechanistic understanding of ecosystem carbon and water fluxes and can be predicted with consideration of models and scaling processes. At the same time, modelling and attempts to scale-up must also pay due regard to the intricacy and variation often found at smaller scales (Weyers et al. 1997). A full review of scaling measurements from the leaf to canopy level is beyond the scope of this chapter and its authors; however, in this section we briefly highlight some of the problems and complexities associated with scaling processes.

Under constant environmental conditions, leaves are tightly coupled with the atmosphere and transpiration is proportional to g_s; however, in the open air the relationship becomes more complex because of additional resistance pathways between stomatal and the bulk atmosphere that feedback on ecosystem evapotranspiration (Bernacchi et al. 2007). Carbon and water fluxes from a vegetation canopy can be predicted using leaf scale parameters by "bottom-up" scaling, using the parallel sum of individual leaf measurements within a canopy structure and taking into account the microenvironment surrounding leaves (Jarvis 1993). A prerequisite for such approaches is knowledge of the variation of parameters throughout the canopy, making such models impractical for regional or global applications (Kruijt et al. 1997). Alternatively, canopy fluxes can be measured directly using eddy covariance techniques and are characterised as "big leaf" models using a "top-down" approach (Kruijt et al. 1997). The degree of influence of leaf or canopy conductance on evaporation to the bulk atmosphere depends on boundary layer conductance and efficiencies of heat and mass transfer between the canopy "surface" and bulk atmosphere (Jarvis and McNaughton 1986; McNaughton and Jarvis 1991). Coupling between stomatal behaviour and the bulk atmosphere depends upon the relative size and structure of the boundary layer, and as boundary layer influences become large, stomatal influences become less important (Jarvis and McNaughton 1986). The influence of stomatal conductance on transpiration gained at one scale can be used to predict what may happen at another scale only if the coupling between saturation deficit at the leaf surface and that of the air outside the leaf boundary layer is known and similar at the two scales (Jarvis and McNaughton 1986). As scale increases, the importance of stomatal behaviour on flux parameters tends to decrease; for example, if the ratio of stomatal conductance to boundary layer conductance is large, only crude models of stomatal responses to environmental variables are required; however, better models are needed if this ratio is small. However, when water stress causes stomatal closure, the importance of leaf conductance increases and more reliable estimates of stomatal responses to changing environmental parameters are required (McNaughton and Jarvis 1991). Vegetation type and canopy structure may also significantly impact on large

scale predictive models of transpiration, photosynthesis and WUE. Irrespective of whether the canopy is constructed of a single species or mixed population, significant variation in stomatal behaviour and transpiration will exist between leaves and between plants that make up the canopy, leading to local differences in saturation deficit and coupling between leaf surfaces and the bulk atmosphere. The impact of changing environmental conditions on stomatal behaviour in different plant species will also contribute to local differences. For example, decreases in soil water status will reduce stomatal conductance which will differentially influence photosynthesis and transpiration in C3 and C4 species (Jarvis and McNaughton 1986). Recently, Bernacchi et al. (2007) demonstrated close coupling of stomatal conductance with ecosystem evapotranspiration in a soybean crop grown in the field under elevated CO_2. Experiments conducted over four growing seasons demonstrated an average decrease in g_s by 10% with elevated CO_2 which correlated with an 8.6% decrease in evapotranspiration, clearly demonstrating a close coupling of stomatal conductance with ecosystem evapotranspiration, which was not driven by changes in growth. Such findings demonstrated that despite system feedbacks, changing g_s of upper canopy leaves can modify the transfer of water vapour to the bulk atmosphere, as well as illustrate the importance of stomatal responses to changing climatic conditions on ecosystem evapotranspiration (Bernacchi et al. 2007). The response of g_s to elevated CO_2 is a critical parameter for large scale ecosystem models of photosynthesis and transpiration, as many of the modelling approaches incorporate the Ball et al. (1987) model of stomatal conductance in which stomatal conductance is dependent on the sensitivity of g_s to CO_2 concentration, assimilation rate and relative humidity which would alter with any acclamatory response (Ainsworth and Rogers 2007). However, it is clear from field based FACE experiments that CO_2 effects on stomatal conductance are very much species specific and dependent on the photosynthetic pathways used (Ainsworth and Rogers 2007).

The continual fluctuation in input energy into a canopy via sun and shade flecks also impacts on the accuracy of scaling models, via spatial heterogeneity in stomatal conductance and rate of photosynthesis. The lack of detailed spatial resolution results in many "big-leaf" models overestimating canopy photosynthetic rates. By fractionating the canopy into sun and shade-lit proportions, De Pury and Farquhar (1997) were able to scale photosynthesis from leaves to canopies avoiding the errors associated with big-leaf models.

There are many other considerations and complexities that we have not covered, including the influence of scaling on time and fluctuating dynamics of leaf level measurements and the errors associated with these measurements (Buckley et al. 2003). Temporal and spatial variations and fluctuations in environmental variables will influence the minute-to-minute responses of stomata and photosynthesis with varying degrees of spatial resolution and each of these will have an error associated with them. Such spatial–temporal regulation at the leaf level also has implications for measurement collection protocols and sample sizes.

7 Conclusion

A detailed understanding of stomatal behaviour in relation to photosynthesis is essential to understand the impacts of changing climate on plant performance and WUE. In this chapter, we have examined stomatal behaviour in response to several key environmental parameters, and highlighted the differences observed between different photosynthetic pathways. We have tried to emphasise the role of stomata in determining mesophyll CO_2 assimilation rates and the impact of fluctuating environmental parameters on both photosynthesis and stomatal behaviour. Although we often consider stomatal function to be key to controlling CO_2 and H_2O fluxes, the importance and impact of anatomical features such as stomatal density and size should not be overlooked. Increasing developments and advancements in modern techniques such as proteomics and transcriptomics are providing novel and exciting approaches in the quest to understand the complex and plastic responses that are apparent in stomatal function. By combining modern approaches with traditional plant physiological procedures and employing a holistic approach, we are becoming closer to determining the link that correlates stomata function with photosynthesis which will improve our ability to model and predict ecosystem-level responses to carbon and water fluxes.

References

Aasamaa K, Sober A, Rahi M (2001) Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. Aust J Plant Physiol 28:765–774

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: molecular mechanisms and environmental interactions. Plant Cell Environ 30:258–270

Akita S, Moss DN (1972) Differential stomatal response between C_3 and C_4 species to atmospheric CO_2 concentration and light. Crop Sci 12:789–793

Allaway WD (1973) Accumulation of malate in guard cells of *Vicia faba* during stomatal opening. Planta 110:63–70

Allen MT, Pearcy RW (2000) Stomatal behaviour and photosynthetic performance under dynamic light regimes in a seasonally dry tropical rain forest. Oecologia 122:470–478

Aphalo PJ, Jarvis PG (1991) Do stomata respond to relative humidity? Plant Cell Environ 14:127–132

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Asai N, Nakajima N, Tamaoki M, Kamada H, Kondo N (2000) Role of malate synthesis mediated by phosphoenolpyruvate carboxylase in guard cell in the regulation of stomatal movements. Plant Cell Physiol 41:10–15

Assmann SM, Zeiger E (1987) Guard cell bioenergetics. In: Zeiger E, Farquhar G, Cowan IR (eds) Stomatal function. Stanford University press, Stanford, pp 163–194

Assmann SM (1993) Signal transductions in guard cells. Annu Rev Cell Biol 9:345–375

Ball JT, Berry JA (1982) The C_i/C_a ratio: a basis for predicting stomatal control of photosynthesis, vol 81. Carnegie Institute of Washington Yearbook, Washington, pp 88–92

Ball TJ, Woodrow IE, Berry JA (1987) A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In: Biggins J (ed) Progress in photosynthesis research. Martinus-Nijhoff, Dordrecht, pp 221–224

Barbour MM, Cernusak LA, Whitehead D, Griffin KL, Turnbull MH, Tissue DT, Farquhar GD (2005) Nocturnal stomatal conductance and implications for modeling $\delta^{18}O$ of leaf-respired CO_2 in temperate tree species. Funct Plant Biol 32:1107–1121

Baroli I, Price GD, Badger MR, von Caemmerer S (2008) The contribution of photosynthesis to the red light response of stomatal conductance. Plant Physiol 146:737–747

Barradas VL, Jones HG (1996) Responses of CO_2 assimilation to changes in irradiance: laboratory and field data and a model for beans (*Phaseolus vulgaris* L.). J Exp Bot 47:639–645

Basco R, Janda T, Galiba G, Papp I (2008) Restricted transpiration may not result in improved drought tolerance in a competitive environment for water. Plant Sci 174:200–204

Berger D, Altmann T (2000) A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in *Arabidopsis thaliana*. Genes Dev 14:1119–1131

Bergmann DC, Sack FD (2007) Stomatal development. Annu Rev Plant Biol 58:163–81

Bernacchi CJ, Kimball BA, Quarles DR, Long SP, Ort DR (2007) Decreases in stomatal conductance of soybean under open-air elevation of [CO_2] are closely coupled with decreases in ecosystem evapotranspiration. Plant Physiol 143:134–144

Berryman CA, Eamus D, Duff GA (1994) Stomatal responses to a range of variables in two tropical tree species grown with CO_2 enrichment. J Exp Bot 45:539–546

Black CC, Osmond CB (2003) Crassulacean acid metabolism photosynthesis: 'Working the night shift'. Photosynth Res 76:329–341

Boccalandro HE, Rugnone ML, Moreno JE, Ploschuk EL, Serna L, Yanovsky MJ, Casal JJ (2009) Phytochrome B enhances photosynthesis at the expense of water-use efficiency in Arabidopsis. Plant Physiol 150:1083–1092

Boonman A, Prinsen E, Gilmer F, Schurr U, Peeters AJ, Voesenek LACJ, Pons TL (2007) Cytokinin import rate as a signal for photosynthetic acclimation to canopy light gradients. Plant Physiol 143:1841–1852

Brodribb TJ, Field TS (2000) Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. Plant Cell Environ 23:1381–1388

Brodribb T, Holbrook N (2004) Stomatal protection against hydraulic failure: a comparison of coexisting ferns and angiosperms. New Phytol 162:663–670

Brodribb T, Holbrook NM, Zwieniecki MA, Palma B (2005) Leaf hydraulic capacity in ferns, conifers and angiosperms: impacts on photosynthetic maxima. New Phytol 165:839–846

Brodribb T, Jordan G (2008) Internal coordination between hydraulics and stomatal control in leaves. Plant Cell Environ 31:1557–1564

Brodribb TJ, McAdam SMA, Jordan GJ, Field TS (2009) Evolution of stomatal responsiveness to CO_2 and optimization of water-use efficiency among land plants. New Phytol 183:839–847

Buckley TN, Mott KA, Farquhar GD (2003) A hydromechanical and biochemical model of stomatal conductance. Plant Cell Environ 26:1767–1785

Bussis D, von Groll U, Fisahn J, Altmann T (2006) Stomatal aperture can compensate altered stomatal density in *Arabidopsis thaliana* at growth light conditions. Funct Plant Biol 33:1037–1043

Caird MA, Richards JH, Donovan LA (2007) Nighttime stomatal conductance and transpiration in C_3 and C_4 plants. Plant Physiol 143:4–10

Cardon ZG, Berry JA (1992) Effects of O_2 and CO_2 concentration on the steady-state fluorescence yield of single guard cell pairs in intact leaf discs of *Tradescantia albiflora*. Plant Physiol 99:1238–1244

Cardon ZG, Berry JA, Woodrow IE (1994) Dependence of the extent and direction of average stomatal response in *Zea mays* L. and *Phaseolus vulgaris* L. on the frequency of fluctuations in environmental stimuli. Plant Physiol 105:1007–1013

Casson S, Gray JE (2008) Influence of environmental factors on stomatal development. New Phytol 178:9–23

Casson SA, Franklin KS, Gray JE, Grierson CS, Whitelam GC, Hetherington AM (2009) Phytochrome B and PIF4 regulate stomatal development in response to light quantity. Curr Biol 19:229–234

Chaloner WG, McElwain J (1997) The fossil plant record and global climatic change. Rev Palaeobot Palynol 95:73–82

Cho D, Shin D, Jeon BW, Kwak JM (2009) ROS-mediated ABA signalling. J Plant Biol 52:102–113

Cockburn W, Ting IP, Steinberg LV (1979) Relationships between stomatal behaviour and internal carbon dioxide concentration in CAM plants. Plant Physiol 63:1029–1032

Cominelli E, Galbiati M, Vavasseur A, Conti L, Sala T, Vuylsteke M, Leonhardt N, Dellaporta SL, Tonelli C (2005) A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. Curr Biol 15:1196–1200

Condon AG, Reynold MP, Rebetzke GJ, Ginkel M, Richards RR, Farquhar GD (2007) Using stomatal aperture-related traits to select for high yield potential in bread wheat. In: Buck HT, Nisi JE, Salomon N (eds) Developments in plant breeding 11 – wheat production in stressed environments, vol 12. Springer, Berlin, Heidelberg, New York, pp 612–624

Coupe SA, Palmer BG, Lake JA, Overy SA, Oxborough K, Woodward FI, Gray JE, Quick WP (2006) Systemic signaling of environmental cues in *Arabidopsis* leaves. J Exp Bot 57:329–341

Cowan IR, Troughton JH (1971) The relative role of stomata in transpiration and assimilation. Planta 97:325–336

Cui XH, Hai FS, Chen H, Chen J, Wang XC (2008) Expression of the *Vicia faba* VfPIP1 gene in *Arabidopsis thaliana* plants improves their drought resistance. J Plant Res 121:207–214

Daley MJ, Phillips NG (2006) Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. Tree Physiol 26:411–419

Das VSR, Santakurmari (1977) Stomatal characteristics of some dicotyledonous plants in relation to the 4 carbon and 3 carbon pathways of photosynthesis. Plant Cell Physiol 18:935–938

De Pury DGG, Farquhar GD (1997) Simple scaling of photosynthesis from leaves to canopies without the errors of big-leaf models. Plant Cell Environ 20:537–557

Dewar RC (2002) The Ball–Berry–Leuning and Tardieu–Davies stomatal models: synthesis and extension within a spatially aggregated picture of guard cell function. Plant Cell Environ 25:1383–1398

Dong J, MacAlister CA, Bergmann DC (2009) BASL controls asymmetric cell division in Arabidopsis. Cell 137:1320–1330

Donovan LA, Grisé DJ, West JB, Pappert RA, Alder NN, Richards JH (1999) Predawn disequilibrium between plant and soil water potentials in two cold-desert shrubs. Oecologia 120:209–217

Donovan LA, Linton MJ, Richards JH (2001) Predawn plant water potential does not necessarily equilibrate with soil water potential under well watered conditions. Oecologia 129:328–335

Driscoll SP, Prins A, Olmos E, Kunert KJ, Foyer CH (2006) Specification of adaxial and abaxial stomata, epidermal structure and photosynthesis to CO_2 enrichment in maize leaves. J Exp Bot 57:381–390

Dubbe DR, Farquhar GD, Raschke K (1978) Effect of abscisic acid on the gain of the feedback loop involving carbon dioxide and stomata. Plant Physiol 62:413–417

Dwyer SA, Ghannoum O, Nicotra A, von Caemmerer S (2007) High temperature acclimation of C_4 photosynthesis is linked to changes in photosynthetic biochemistry. Plant Cell Environ 30:53–66

Farquhar DG, Sharkey TD (1982) Stomatal conductance and photosynthesis. Ann Rev Plant Physiol 33:317–345

Farquhar GD, Wong CS (1984) An empirical model of stomatal conductance. Aust J Plant Physiol 11:191–210

Franks PJ, Farquhar GD (1999) A relationship between humidity response, growth form and photosynthetic operating point I C_3 plants. Plant Cell Environ 22:1337–1349

Franks PJ, Farquhar GD (2007) The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiol 143:78–87

Fitzisimons PJ, Weyers JDB (1986) Volume changes of *Commelina communis* L. guard cell protoplasts in response to K^+, light and CO_2. Physiol Plant 66:463–468

Frechilla S, Talbott LD, Zeiger E (2002) The CO_2 response of *Vicia faba* guard cells acclimates to growth environment. J Exp Bot 53:545–550

Frechilla S, Zhu J, Talbott LD, Zeiger E (1999) Stomata from *npq1*, a Zeaxanthin-less Arabidopsis mutant, lack a specific response to blue light. Plant Cell Physiol 40:949–954

Freudenberger H (1940) Die Reaktion der Schliesszellen auf Kohlensäure and Sauerstoffentzug. Protoplasma 35:15–54

Fujino M (1967) Role of adenosine triphosphate and adenosine triphosphatase in stomatal movements. Sci Bull Fac Educ Nagasaki Until 18:1–47

Galvez-Valdivieso G, Fryer MJ, Lawson T, Slattery K, Truman W, Smirnoff N, Asami T, Davies WJH, Jones AM, Baker NR, Mullineaux PM (2009) The high light response in Arabidopsis involves ABA signaling between vascular and bundle sheath cells. Plant Cell 21:2143–2162

Ghannoum O (2009) C_4 photosynthesis and water stress. Ann Bot 103:635–644

Grantz DA, Assmann SM (1991) Stomatal response to blue-light – water-use efficiency in sugarcane and soybean. Plant Cell Environ 14:683–690

Gray JE (2005) Guard cells: transcription factors regulate stomatal movements. Curr Biol 15:593–595

Gray JE, Holroyd GH, van der Lee FM, Bahrami AR, Sijmons PC, Woodward FI, Schuch W, Hetherington AM (2000) The HIC signalling pathway links CO_2 perception to stomatal development. Nature 408:713–716

Grulke NE, Alonso R, Nguyen T, Cascio C, Dobrowolski W (2004) Stomata open at night in pole-sized and mature ponderosa pine: implications for O_3 exposure metrics. Tree Physiol 24:1001–1010

Gotow K, Taylor S, Zeiger E (1988) Photosynthetic carbon fixation in guard cell protoplasts of *Vicia faba* L. Evidence from radiolabel experiments. Plant Physiol 86:700–705

Haake V, Geiger M, Walch-Liu P, Engels C, Zrenner R, Stitt M (1999) Changes in aldolase activity in wild-type potato plants are important for acclimation to growth irradiance and carbon dioxide concentration, because plastid aldolase exerts control over the ambient rate of photosynthesis across a range of growth conditions. Plant J 17:479–489

Hall AE, Schulze ED, Lange OL (1976) Current perspectives of steady state stomatal responses to environments. In: Lange OL, Kappen L, Schulze ED (eds) Water and plant life: ecological studies, vol 19. Springer, Berlin, Heidelberg, New York, pp 169–188

Hampp R, Outlaw WH Jr, Tarczynski MC (1982) Profile of basic carbon pathways in guard cells and other leaf cells of *Vicia faba* L. Plant Physiol 70:1582–1585

Hara K, Kajita R, Torii KU, Bergmann DC, Kakimoto T (2007) The secretory peptide gene *EPF1* enforces the stomatal one-cell-spacing rule. Genes Dev 21:1720–1725

Harrison EP, Willingham NM, Lloyd JC, Raines CA (1998) Reduced sedoheptulose-1,7-bisphosphatase levels in transgenic tobacco lead to decreased photosynthetic capacity and altered carbohydrate accumulation. Planta 20:27–36

Hashimoto M, Negi J, Young J, Israelsson M, Schroeder JI, Iba K (2006) *Arabidopsis* HT1 kinase controls stomatal movements in response to CO_2. Nat Cell Biol 8:391–397

Heath OVS (1938) An experimental investigation of the mechanism of stomatal movement, with some preliminary observations upon the response of guard cells to shock. New Phytol 37:385–395

Heath OVS, Russell J (1954) Studies in stomatal behaviour IV. An investigation of the light response of wheat stomata with the attempted elimination of control by the mesophyll. J Exp Bot 5:269–292

Henkes S, Sonnewald U, Badur R, Flachmann R, Stitt M (2001) A small decrease of plastid transketolase activity in antisense tobacco transformants has dramatic effects on photosynthesis and phenylpropanoid metabolism. Plant Cell 13:535–551

Hetherington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. Nature 424:901–908

Hipkins ME, Fitzimons PJ, Weyers JDB (1983) The primary processes of photosystem II in purified guard-cell and mesophyll-cell protoplasts from *Commelina communis* L. Planta 159:554–560

Hite DRC, Outlaw WH Jr, Tarczynski MC (1993) Elevated levels of both sucrose-phosphate synthase and sucrose synthase in *Vicia* guard cells indicate cell-specific carbohydrate interconversion. Plant Physiol 101:1217–1221

Hu H, Dai M, YaoJ XB, Li X, Zhang Q, Xiong L (2006) Over-expressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc Natl Acad Sci USA 103:12987–12992

Huang XY, Chao DY, Gao JP, Zhu MZ, Shi M, Lin HX (2009) A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. Genes Dev 23:1805–1817

Hunt L, Gray JE (2009) The signaling peptide EPF2 controls asymmetric cell divisions during stomatal development. Curr Biol 19:864–869

Huxman TE, Monson RK (2003) Stomatal responses of C_3, C_3–C_4, and C_4 *Flaveria* species to light and intercellular CO_2 concentration: implications for the evolution of stomatal behaviour. Plant Cell Environ 26:313–322

Imamura S (1943) Untersuchungen uber den Mechanismus der Turgorschwankung der Spaltöffnungesschließzellen. Jpn J Bot 12:82–88

Jakab G, Ton J, Flors V, Zimmerli L, Metraux J-P, Mauch-Mani B (2005) Enhancing *Arabidopsis* salt and drought stress tolerance by chemical priming for its abscisic acid response. Plant Physiol 139:267–274

Jammes F, Song C, Shin D, Munemasa S, Takeda K, Gu D, Cho D, Lee S, Giordo R, Sritubitim S, Leonhardt N, Elis B, Murata Y, Kwak M (2009) MAP kinases MPK9 and MPK12 are preferentially expressed in guard cells and positively regulate ROS-mediate ABA signaling. Proc Natl Acad Sci USA 106:20520–20525

Jarvis PG (1993) Prospects for bottom-up models. In: Ehleringer J, Field C (eds) Scaling physiological processes; leaf to globe. Academic, New York, pp 115–116

Jarvis PG, McNaughton KG (1986) Stomatal control of transpiration: scaling up from leaf to region. Adv Ecol Res 15:1–49

Jiang CZ, Rodermel SR (1995) Regulation of photosynthesis during leaf development in *RbcS* antisense DNA mutants of tobacco. Plant Physiol 107:215–224

Jones HG (1976) Crop characteristics and the ratio between assimilation and transpiration. J Appl Ecol 13:605–622

Jones HG (1977) Transpiration in barley lines with differing stomatal frequencies. J Exp Bot 23:162–168

Jones HG (1985) Partitioning stomatal and non-stomatal limitation to photosynthesis. Plant Cell Environ 8:95–104

Jones HG (1987) Breeding for stomatal characters. In: Zeiger E, Farquhar GD, Cowan IR (eds) Stomatal function. Stanford University Press, Stanford, pp 431–443

Jones HG (1992) Plants and microclimate: a quantative approach to environmental plant physiology. Cambridge University Press, Cambridge

Joo JH, Wang S, Chen JG, Jones AM, Fedoroff NV (2005) Different signaling and cell death roles of heterotrimeric G protein alpha and beta subunits in the Arabidopsis oxidative stress response to ozone. Plant Cell 17:957–970

Kanaoka MM, Pillitteri LJ, Fujii H, Yoshida Y, Bogenschutz NL, Takabayashi J, Zhu J-K, Torii K (2008) SCREAM/ICE1 and SCREAM2 specify three cell-state transitional steps leading to Arabidopsis stomatal differentiation. Plant Cell 20:1775–1785

Kang Y, Lian HL, Wang FF, Huang JR, Yang HQ (2009) Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in Arabidopsis. Plant Cell 21: 2624–2641

Kang Y, Outlaw WH Jr, Anderson PC, Fiore GB (2007a) Guard cell apoplastic sucrose concentration – a link between leaf photosynthesis and stomatal aperture size in apoplastic phloem loader *Vicia faba* L. Plant Cell Environ 30:551–558

Kang Y, Outlaw WH Jr, Fiore GB, Riddle KA (2007b) Guard cell apoplastic photosynthate accumulation corresponds to a phloem-loading mechanisms. J Exp Bot 58:4061–4070

Klein M, Geiser M, Suh SJ, Kolukisaoglu HU, Azevedo L, Plaza S, Curtis MD, Richter A, Weder B, Schulz B, Martinoia E (2004) Disruption of AtMRP4, a guard cell plasma membrane ABCC-type ABC transporter, leads to deregulation of stomatal opening and increased drought susceptibility. Plant J 39:219–236

Kirschbaum MUF, Gross LJ, Pearcy RW (1988) Observed and modelled stomatal responses to dynamic light environments in the shade plant *Alocasia macrorrhiza*. Plant Cell Environ 11:111–121

Kinoshita T, Shimazaki K (1999) Blue light activates the plasma membrane H+-ATPase by phosphorylation of the C-terminus in stomatal guard cells. EMBO J 18:5548–5558

Knapp AK (1993) Gas-exchange dynamics in C_3 and C_4 grasses – consequences of differences in stomatal conductance. Ecology 74:113–123

Kopka J, Provart NJ, Muller-Rober B (1997) Potato guard cells respond to drying soil by a complex change in the expression of genes related to carbon metabolism and turgor regulation. Plant J 11:871–882

Kruijt B, Ongeri S, Jarvis PG (1997) Scaling of PAR absorption, photosynthesis and transpiration from leaves to canopy. In: van Gardingen P, Foody G, Curran P (eds) Scaling up, society for experimental biology seminar series. Cambridge University Press, Cambridge, pp 79–104

Kudoyarova GR, Veselov DS, Faizov RG, Veselova SV, Ivanov EA, Farkhutdinov RG (2007) Stomata response to changes in temperature and humidity in wheat cultivars grown under contrasting climatic conditions. Russ J Plant Physiol 54:46–49

Kuiper PJC (1964) Dependence upon wavelength of stomatal movement in epidermal tissue of *Senecio odoris*. Plant Physiol 39:952–955

Kurek I, Chang TK, Bertain SM, Madrigal A, Liu L, Lassner MW, Zhu GH (2007) Enhanced thermostability of Arabidopsis Rubisco activase improves photosynthesis and growth rates under moderate heat stress. Plant Cell 19:3230–3241

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JDG, Schroeder JI (2003) NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO J 22:2623–2633

Lake JA, Quick WP, Beerling DJ, Woodward FI (2001) Plant development: signals from mature to new leaves. Nature 411:154

Lange OL, Medina E (1979) Stomata of the CAM plant *Tillandsia recurvata* respond directly to humidity. Oecologia 40:357–363

Laporte MM, Shen B, Tarczynski C (2002) Engineering for drought avoidance: expression of maize NADP-malic enzyme in tobacco results in altered stomatal function. J Exp Bot 53:699–705

Lawlor DW (2002) Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. Ann Bot 89:871–885

Lawson T (1997) Heterogeneity in stomatal characteristics, PhD thesis, Dundee

Lawson T (2009) Guard cell photosynthesis and stomatal function. New Phytol 181:13–34

Lawson T, Lefebvre S, Baker NR, Morison JIL, Raines C (2008) Reductions in mesophyll and guard cell photosynthesis impact on the control of stomatal responses to light and CO_2. J Exp Bot 59:3609–3619

Lawson T, Morison JIL (2004) Stomatal function and physiology. In: Hemsley AR, Poole I (eds) The evolution of plant physiology; from whole plants to ecosystem. Elsevier Academic, Cambridge, pp 217–242

Lawson T, Oxborough K, Morison JIL, Baker NR (2002) Responses of photosynthetic electron transport in stomatal guard cells and mesophyll cells in intact leaves to light, CO_2, and humidity. Plant Physiol 128:52–62

Lawson T, Oxborough K, Morison JIL, Baker NR (2003) The response of guard cell photosynthesis to CO_2, O_2, light and water stress in a range of species are similar. J Exp Bot 54:1734–1752

Lawson T, Weyers JDB (1999) Spatial and temporal variation in gas exchange over the lower surface of *Phaseolus vulgaris* primary leaves. J Exp Bot 50:1381–1391

Lee DM, Assmann SM (1992) Stomatal responses to light in the facultative Crassulacean acid metabolism species, *Portulacaria afra*. Plant Physiol 85:35–42

Lee J-S, Bowling DJF (1992) Effect of the mesophyll on stomatal opening in *Commelina communis*. J Exp Bot 43:951–957

Lee J-S, Bowling DJF (1993) The effect of a mesophyll factor on the swelling of guard cell protoplasts of *Commelina communis* L. J Plant Physiol 142:203–207

Lee J-S, Bowling DJF (1995) Influence of the mesophyll on stomatal opening. Aust J Plant Physiol 22:357–363

Lefebvre S, Lawson T, Zakhleniuk OV, Lloyd JC, Raines CA (2005) Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. Plant Physiol 138:451–460

Leonhardt N, Kwak JM, Robert N, Waner D, Leonhardt G, Schroeder J (2004) Microarray expression analyses of *Arabidopsis* guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. Plant Cell 16:596–615

Lodge RJ, Dijkstra P, Drake BG, Morison JIL (2001) Stomatal acclimation to increased CO_2 in a Florida scrub oak species *Quercus myrtifolia* Willd. Plant Cell Environ 24:77–88

Lu P, Outlaw WH Jr, Smith BD, Freed GA (1997) A new mechanism for the regulation of stomatal aperture size in intact leaves: accumulation of mesophyll-derived sucrose in guard cell walls of *Vicia faba*. Plant Physiol 114:109–118

Lu P, Zhang SQ, Outlaw WH Jr, Riddle KA (1995) Sucrose: a solute that accumulates in the guard-cell- apoplast and guard cell symplast of open stomata. FEBS Lett 326:180–184

Lu Z, Quiñones MA, Zeiger E (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

Lurrie S (1977) Photochemical properties of guard cell chloroplasts. Plant Sci Let 10:219–223

Ma SY, Wu WH (2007) AtcPK23 functions in Arabidopsis responses to drought and salt stresses. Plant Mol Biol 65:511–518

Maherali H, Reid CD, Polley HW, Johnson HB, Jackson RB (2002) Stomatal acclimation over a sub-ambient to elevated CO_2 gradient in a C_3/C_4 grassland. Plant Cell Environ 25:557–566

Mansfield TA (1994) Some aspects of stomatal physiology relevant to plants cultured *in vitro*. In: Lumsden PJ, Nicholas JR, Davies WJ (eds) Physiology, growth and development of plants in culture. Kluwer, Dordrecht, pp 120–131

Mansfield TA, Hetherington AM, Atkinson CJ (1990) Some current aspects of stomatal physiology. Annu Rev Plant Physiol Plant Mol Biol 41:55–75

Mansfield TA, Atkinson CJ (1990) Stomatal behaviour in water stressed plants. In: Alscher RG, Cumming JR (eds) Stress responses in plants: adaptation and acclimation mechanisms. Wiley-Liss, New York, pp 241–264

Marten H, Hyun T, Gomi K, Seo S, Hedrich R, Roelfsema MRG (2008) Silencing of *Nt*MPK4 impairs CO_2-induced stomatal closure, activation of anion channels and cytosolic Ca^{2+} signals in *Nicotiana tabacum* guard cells. Plant J 55:698–708

Masle J, Gilmour SR, Farquhar GD (2005) The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*. Nature 436:866–870

Mawson BT, Zaugg MW (1994) Modulation of light-dependent stomatal opening in isolated epidermis following induction of crassulacean acid metabolism in *Mesembryanthemum crystallinum* L. Plant Physiol 144:740–746

McElwain JC, Chaloner WG (1995) Stomatal density and index of fossil plants track atmospheric carbon dioxide in the palaeozoic. Ann Bot 76:389–395

McElwain JC, Mitchell FJG, Jones MB (1995) Relationship of stomatal density and index of *Salix cinerea* to atmospheric carbon dioxide concentration of the Holocene. Holocene 5:216–219

Meinzer FC (2002) Co-ordination of vapour and liquid phase water transport properties in plants. Plant Cell Environ 25:265–274
Messinger SM, Buckley TN, Mott KA (2006) Evidence for involvement of photosynthetic processes in the stomatal response to CO_2. Plant Physiol 140:771–778
Miao Y, Lv D, Wang P, Wang X-C, Chen J, Wang XC, Chen J, Miao C, Song CP (2006) An Arabidopsis glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. Plant Cell 18:2749–2766
Miyazawa S-I, Livingston NJ, Turpin DH (2006) Stomatal development in new leaves is related to the stomatal conductance of mature leaves in poplar (*Populus trichocarpa* x *P. deltoides*). J Exp Bot 57:373–380
McNaughton KG, Jarvis PG (1991) Effects of spatial scale on stomatal control of transpiration. Agricult Forest Meteorol 54:279–301
Morison JIL (1985) Intercellular CO_2 concentration and stomatal response to CO_2. In: Zeiger E, Cowan IR, Farquhar GD (eds) Stomatal function. Stanford University Press, Stanford
Morison JIL (1993) Response of plants to CO_2 under water limited conditions. Vegetation 105:193–209
Morison JIL (2003) Plant water use, stomatal control. In: Stewart BA, Howell TA (eds) Encyclopedia of water science. Marcel Dekker, New York, pp 680–685
Morison JIL, Gifford RM (1983) Stomatal sensitivity to carbon dioxide and humidity: a comparison of two C_3 and two C_4 grass species. Plant Physiol 71:789–796
Mott KA (1988) Do stomata respond to CO_2 concentrations other than intercellular? Plant Physiol 86:200–203
Mott KA (2009) Opinion: stomatal responses to light and CO_2 depend on the mesophyll. Plant Cell Environ 32:1479–1486
Mott KA, Peak D (2007) Stomatal patchiness and task-performing networks. Ann Bot 99:219–226
Mott KA, Sibbernsen ED, Shope JC (2008) The role of the mesophyll in stomatal responses to light and CO_2. Plant Cell Environ 31:1299–1306
Nadeau JA (2009) Stomatal development: new signals and fate determinants. Curr Opin Plant Biol 12:29–35
Nakagami H, Pitzschke A, Hirt H (2005) Emerging MAP kinase pathways in plant stress signalling. Trends Plant Sci 10:339–346
Nardini A, Salleo S (2005) Water stress-induced modifications on leaf hydraulic architecture in sunflower: co-ordination with gas exchange. J Exp Bot 56:3093–3101
Nilson SE, Assmann SM (2007) The control of transpiration. Insights from Arabidopsis. Plant Physiol 143:19–27
Niyogi KK (2000) Safety valves for photosynthesis. Curr Opin Plant Biol 3:455–460
Noe SM, Giersch C (2004) A simple dynamic model of photosynthesis in oak leaves: coupling leaf conductance and photosynthetic carbon fixation by a variable intracellular CO_2 pool. Funct Plant Biol 31:1195–1204
Nunes-Nesi A, Carrari F, Gibon Y, Sulpice R, Lytovchenko A, Fisahn J, Graham J, Ratcliffe RG, Sweetlove LJ, Fernie AR (2007) Deficiency of mitochondrial fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function. Plant J 50:1093–1106
Nunes-Nesi A, Carrari F, Lytovchenko A, Smith AM, Loureiro ME, Ratcliffe RG, Sweetlove LJ, Fernie AR (2005) Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. Plant Physiol 137:611–622
Osmond CB (1978) Crassulacean acid metabolism: a curiosity in context. Annu Rev Plant Physiol 29:397–414
Outlaw WH Jr (1982) Carbon metabolism in guard cells. Recent Adv Phytochem 17:185–222
Outlaw WH Jr (1983) Current concepts on the role of potassium in stomatal movements. Physiol Plant 59:302–311
Outlaw WH Jr (1987) A minireview: comparative biochemistry of photosynthesis in palisade cells, spongy cells and guard cells of C_3 leaves. In: Biggins J (ed) Progress in photosynthesis research, vol IV, 5. Martinus Nijhoff, Dordrecht, pp 265–272

Outlaw WH Jr (1989) Critical examination of the quantitative evidence for and against photosynthetic CO_2 fixation in guard cells. Physiol Plant 77:275–281
Outlaw WH Jr (1990) Kinetic properties of guard-cell phosphoenolpyruvate carboxylase. Biochem Physiol Pflanz 186:317–325
Outlaw WH Jr (2003) Integration of cellular and physiological functions of guard cells. Crit Rev Plant Sci 22:503–529
Outlaw WH Jr, De Vleighere-He X (2001) Transpiration rate: an importance factor in controlling sucrose content of the guard cell apoplast of broad bean. Plant Physiol 126:1717–1724
Outlaw WH Jr, Manchester J, Di Camelli CA, Randall DD, Rapp B, Veither GM (1979) Photosynthetic carbon reduction pathway is absent in chloroplasts of *Vicia faba* guard cells. Proc Natl Acad Sci USA 76:6371–6375
Outlaw WH Jr, Tarczynski MC, Anderson LC (1982) Taxonomic survey for the presence of ribulose-1,5-bisphosphate carboxylase activity in guard cells. Plant Physiol 70:1218–1220
Parvathi K, Raghavendra AS (1997) Both Rubisco and phosphoenolpyruvate carboxylase are beneficial for stomatal function in epidermal strips of *Commelina benghalensis*. Plant Sci 124:153–157
Pearcy RW (1990) Sunflecks and photosynthesis in plant canopies. Annu Rev Plant Physiol Plant Mol Biol 41:421–453
Pearson CJ (1973) Daily changes in stomatal aperture and in carbohydrates and malate within epidermis and mesophyll of leaves of *Commelina cyanea* and *Vicia faba*. Aust J Biol Sci 26:1035–1044
Pearson CJ, Milthorpe FL (1974) Structure, carbon dioxide fixation and metabolism of stomata. Aust J Plant Physiol 1:221–236
Pemadasa MA (1981) Photocontrol of stomatal movements. Biol Rev Camb Phil Soc 56:551–559
Pfannschmidt T, Bräutigam K, Wagner R, Dietzel L, Schröter Y, Steiner S, Nykytenko A (2009) Potential regulation of gene expression in photosynthetic cells by redox and energy state: approaches towards better understanding. Ann Bot 103:599–607
Pham J, Desikan R (2009) ROS signalling in stomata. In: Rio LA, Puppo A (eds) Reactive oxygen species in plant signaling. Springer, Berlin, Heidelberg, New York, pp 55–71
Pnueli L, Liang H, Rozenberg M, Mittler R (2003) Growth suppression, altered stomatal responses, and augmented induction of heat shock proteins in cytosolic ascorbate peroxidase (Apx1)-deficient Arabidopsis plants. Plant J 34:187–203
Poffenroth M, Green DB, Tallman G (1992) Sugar concentrations in guard cells of *Vicia faba* illuminated with red or blue light: analysis by high performance liquid chromatography. Plant Physiol 98:1460–1471
Raghavendra AS, Vani T (1989) Respiration in guard cells: pattern and possible role in stomatal function. J Plant Physiol 135:3–8
Rao M, Anderson LE (1983) Light and stomatal metabolism. 1. Possible involvement of light-modulation of enzymes in stomatal movement. Plant Physiol 71:456–459
Raines CA (2003) The Calvin cycle revisited. Photosynth Res 75:1–10
Ramos C, Hall AE (1982) Relationships between leaf conductance, intercellular CO_2 partial pressure and CO_2 uptake rate in two C_3 and two C_4 plant species. Photosynthetica 16:343–355
Raschke K (1970) Temperature dependencies and apparent activation energies of stomatal opening and closing. Planta 95:1–17
Raschke K (1978) Movements using turgor mechanisms. In: Haupt W, Feinleib ME (eds) Encyclopedia of plant physiology, physiology of movements. Springer, Berlin, Heidelberg, New York, pp 387–441
Raschke K (1972) Saturation kinetics of the velocity of stomatal closing to CO_2. Plant Physiol 49:229–234
Raschke K (1975) Stomatal action. Annu Rev Plant Physiol 26:309–340
Raschke K, Dittrich P (1977) [^{14}C] carbon dioxide fixation by isolated leaf epidermis with stomata closed or open. Planta 134:69–75
Rebetzke GJ, Richards RA, Condon AG, Farquhar GD (2006) Inheritance of carbon isotope discrimination in bread wheat (*Triticum aestivum* L.). Euphytica 150:97–106

Reckmann U, Scheibe R, Raschke K (1990) Rubisco activity in guard cell compared with the solute requirement for stomatal opening. Plant Physiol 92:246–253

Ripullone F, Matsuo N, Stuart-Williams H, Wong SC, Borghetti M, Tani M, Farquhar GD (2008) Environmental effects on oxygen isotope enrichment of leaf water in cotton leaves. Plant Physiol 146:729–736

Ritte G, Raschke K (2003) Metabolite export of isolated guard cell chloroplasts of *Vicia faba*. New Phytol 159:195–202

Ritte G, Rosenfeld J, Rohrig K, Raschke K (1999) Rates of sugar uptake by guard cell protoplasts of *Pisum satvum* L. related to the solute requirements for stomatal opening. Plant Physiol 121:647–655

Roelfsema MRG, Hanstein S, Fell HH, Hedrich R (2002) CO_2 provides an intermediate link in the red light response of guard cells. Plant J 32:65–75

Roelfsema MRG, Konrad KR, Marten H, Psaras GK, Hartung W, Hedrich H (2006) Guard cells in albino leaf patches do not respond to photosynthetically active radiation, but are sensitive to blue light, CO_2 and abscisic acid. Plant Cell Environ 29:1595–1605

Rogiers SY, Greer DH, Hutton RJ, Landsberg JJ (2009) Does night time transpiration contribute to anisohydric behaviour in a *Vitus vinifera* cultivar? J Exp Bot 60:3763–3763

Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol 57:675–709

Sack L, Frole K (2006) Leaf structural diversity is related to hydraulic capacity in tropical rainforest trees. Ecology 87:483–491

Sage RF, Sharkey TD (1987) The effect of temperature on the occurrence of O_2 and CO_2 insensitive photosynthesis in field grown plants. Plant Physiol 84:658–664

Sage RF, Wedin DA, Li M (1999) The biogeography of C_4 photosynthesis: patterns and controlling factors. In: Sage RF, Monson RK (eds) C_4 biology. Academic, San Diego, pp 313–374

Šantrůček J, Sage RF (1996) Acclimation of stomatal conductance to a CO_2-enriched atmosphere and elevated temperature in *Chenopodium album*. Aust J Plant Physiol 23:467–478

Scheibe R, Reckmann U, Hedrich R, Raschke K (1990) Malate dehydrogenases in guard cells of *Pitsum sativum*. Plant Physiol 93:1358–1364

Schnabl H, Elbert C, Krammer G (1982) The regulation of the starch-malate balances during volume changes of guard cell protoplasts. J Exp Bot 33:996–1003

Schoch PG, Zinsou C, Sibi M (1980) Dependence of the stomatal index on environmental factors during stomatal differentiation in leaves of *Vigna sinensis* L. I Effect of light intensity. J Exp Bot 31:1211–1216

Schoch PG, Jacques R, Lecharny A, Sibi M (1984) Dependence of the stomatal index on environmental factors during stomatal differentiation in leaves of *Vigna sinensis* L.II Effect of different light quality. J Exp Bot 35:1405–1409

Schwartz A, Zeiger E (1984) Metabolic energy for stomatal opening. Role of photophosphorylation and oxidative phosphorylation. Planta 161:129–136

Sekiya N, Yano K (2008) Stomatal density of cowpea correlates with carbon isotope discrimination in different phosphorus, water and CO_2 environments. New Phytol 179:799–807

Sharkey TD, Raschke K (1981) Separation and measurement of direct and indirect effects of light on stomata. Plant Physiol 68:33–40

Shimazaki K (1989) Ribulose bisphosphate carboxylase activity and photosynthetic O_2 evolution rate in *Vicia* guard cell protoplasts. Plant Physiol 91:459–463

Shimazaki K-I, Doi M, Assmann SM, Kinoshita T (2007) Light regulation of stomatal movements. Annu Rev Plant Biol 58:219–247

Shimazaki K, Zeiger E (1985) Cyclic and non-cyclic phosphorylation in isolated guard cell protoplasts from *Vicia faba* L. Plant Physiol 78:211–214

Shimazaki K, Terada J, Tanaka K, Kondo N (1989) Calvin–Benson cycle enzymes in guard-cell protoplasts from *Vicia faba* L. Plant Physiol 90:1057–1064

Snyder KA, Richards JH, Donovan LA (2003) Night-time conductance in C_3 and C_4 species: do plants lose water at night? J. Exp Bot 54:861–865

Spalding MH, Stumpf DK, Ku MSB, Burris RH, Edwards GE (1979) Crassulacean acid metabolism and diurnal variations of internal CO_2 and O_2 concentrations in *Sedum praealtum*. Aust J Plant Physiol 8:557–567

Sperry JS (2000) Hydraulic constraints on plant gas exchange. Agr Forest Meteorol 104:13–23

Stadler R, Buttner M, Ache P, Hedrich R, Ivashikina N, Melzer M, Shearson SM, Smith SM, Sauer N (2003) Diurnal and light-regulated expression of AtsSTP1 in guard cells of Arabidopsis. Plant Physiol 133:528–537

Still CJ, Berry JA, Collatz GJ, DeFries RS (2003) Global distribution of C_3 and C_4 vegetation: carbon cycle implications. Global Biogeochem Cycles 17:1006

Suzuki Y, Miyamoto T, Yoshizawa R, Mae T, Makino A (2009) Rubisco content and photosynthesis of leaves at different positions in transgenic rice with an over-expression of RBCS. Plant Cell Environ 32:417–427

Tallman G, Zhu J, Mawson BT, Amodeo G, Nouhi Z, Levy K, Zeiger E (1997) Induction of CAM in *Mesembryanthemum crystallinum* abolishes the stomatal response to blue light and light-dependent Zeaxanthin formation in guard cell chloroplasts. Plant Cell Physiol 38:236–242

Tarczynski MC, Outlaw WH Jr, Arold N, Neuhoff V, Hampp R (1989) Electrophoretic assay for ribulose 1,5-bisphosphate carboxylase/oxygenase in guard cells and other leaf cells of *Vicia faba* L. Plant Physiol 89:1088–1093

Thomas PW, Woodward FI, Quick WP (2004) Systemic irradiance signalling in tobacco. New Phytol 161:193–198

Tichà I (1982) Photosynthetic characteristics during ontogenesis of leaves. 7. Stomatal density and sizes. Photosynthetica 16:375–471

Tinoco-Ojanguren C, Pearcy RW (1993) Stomatal dynamics and its importance to carbon gain in 2 rain forest Piper species.1. VPD Effects on the transient stomatal response to light flecks. Oecologia 94:388–394

Tominaga M, Kinoshita T, Shimazaki K (2001) Guard-cell chloroplasts provide ATP required for H^+ pumping in the plasma membrane and stomatal opening. Plant Cell Physiol 42:795–802

Tuba Z, Szente K, Koch J (1994) Response of photosynthesis, stomatal conductance, water use efficiency and production to long-term elevated CO_2 in winter wheat. J Plant Physiol 144:661–668

Turner NC (1979) Differences in response of adaxial and abaxial stomata to environmental variables. In: Sen DS, Chawan DD, Bansal RP (eds) Structure function and ecology of the stomata. Bishen Singh Mahendra Pal Singh, Dehra Dun, pp 320–329

Van Breusegem F, Bailey-Serres J, Mittler R (2008) Unraveling the tapestry of networks involving reactive oxygen species in plants. Plant Physiol 147:978–984

Vavasseur A, Raghavendra AS (2005) Guard cell metabolism and CO_2 sensing. New Phytol 165:665–682

von Caemmerer S, Griffiths H (2009) Stomatal responses to CO_2 during a diel Crassulacean acid metabolism cycle in *Kalanchoe daigremontiana* and *Kalanchoe pinnata*. Plant Cell Environ 32:567–576

von Caemmerer S, Lawson T, Oxborough K, Baker NR, Andrews TJ, Raines CA (2004) Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of Rubisco. J Exp Bot 55:1157–1166

Wang Y, Chen X, Xiang C-B (2007a) Stomatal density and bio-water saving. J Integr Plant Biol 49:1435–1444

Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S (2007b) Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in Arabidopsis. Plant Cell 19:63–73

Wang P, Song CP (2008) Guard-cell signalling for hydrogen peroxide and abscisic acid. New Phytol 178:703–718

Wang Y, Ying JF, Kuzma M, Chalifoux M, Sample A, McArthur C, Uchacz T, Sarvas C, Wan JX, Dennis DT, McCourt P, Huang YF (2005) Molecular tailoring of farnesylation for plant drought tolerance and yield protection. Plant J 43:413–424

Wang Y, Noguchi KO, Terashima I (2008) Distinct light responses of adaxial and abaxial stomata in leaves of *Helianthus annuus* L. Plant Cell Environ 31:1307–1315

Weber PM, Fischer K (2007) Making the connections – the crucial role of metabolite transporters at the interface between chloroplast and cytosol. FEBS Lett 581:2215–2222

Weyers JDB, Lawson T (1997) Heterogeneity in stomatal characteristics. Adv Bot Res 26:317–352

Weyers JDB, Lawson T, Peng Z (1997) Variation in stomatal characteristics at the whole leaf level. In: van Gardingen P, Foody G, Curran P (eds) Scaling up. Society for experimental biology seminar series. Cambridge University Press, Cambridge, pp 129–149

Weyers JDB, Meidner H (1990) Methods in stomatal research. Longman Scientific and Technical, Harlow

Willmer CM (1983) Phosphoenolpyruvate carboxylase activity and stomatal operation. Physiol Veg 21:943–953

Willmer CM, Dittrich P (1974) Carbon dioxide fixation by epidermal and mesophyll tissues of *Tulipa* and *Commelina*. Planta 117:123–132

Willmer CM, Fricker M (1996) Stomata, 2nd edn. Chapman and Hall, London

Wingler A, Quick WP, Bungard RA, Bailey KJ, Lea PJ, Leegood RC (1999) The role of photorespiration during drought stress: an analysis utilizing barley mutants with reduced activities of photorespiratory enzymes. Plant Cell Environ 22:361–373

Wong SC, Cowan IR, Farquhar GD (1979) Stomatal conductance correlates with photosynthetic capacity. Nature 282:424–426

Wong SC, Cowan IR, Farquhar GD (1985) Leaf conductance in relation to rate of CO_2 assimilation II. Effects of short-term exposures to different photon flux densities. Plant Physiol 78:826–829

Woodward FI (1987) Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. Nature 327:617–618

Xu DQ, Terashima K, Crang RFE, Chen XM, Hesketh JD (1994) Stomatal and non-stomatal acclimation to a CO_2-enriched atmosphere. Biotronics 23:1–9

Yang CY, Chen YC, Jauh GY, Wang CS (2005) A lily ASR protein involves abscisic acid signaling and confers drought and salt resistance in *Arabidopsis*. Plant Physiol 139:836–846

Yano S, Terashima I (2001) Separate localization of light signal perception for sun or shade type chloroplast and palisade tissue differentiation in *Chenopodium album*. Plant Cell Physiol 42:1303–1310

Zeiger E, Iino M, Ogawa T (1985) The blue light response of stomata: pulse kinetics and some mechanistic implications. Photochem Photobiol 42:759–763

Zeiger E, Talbott LD, Frechilla S, Srivastava A, Zhu J (2002) The guard cell chloroplast: a perspective for the twenty-first century. New Phytol 153:415–424

Zeiger E, Zhu JX (1998) Role of zeaxanthin in blue light photoreception and the modulation of light-CO_2 interactions in guard cells. J Exp Bot 49:433–442

Zemel E, Gepstein S (1985) Immunological evidence for the presence of ribulose bisphosphate carboxylase in guard cell chloroplasts. Plant Physiol 78:586–590

Zhao Z, Zhang W, Stanley BA, Assmann SM (2008) Functional proteomics of *Arabidopsis thaliana* guard cells uncovers new stomatal signaling pathways. Plant Cell 20:3210–3226

Zhang Y, Zhu H, Zhang Q, Li M, Yan M, Wang R, Wang L, Welti R, Zhang W, Wang X (2009) Phospholipase Dal1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in Arabidopsis. Plant Cell 21:2357–2377

Zhu M, Dai S, McClung S, Yan X, Chen S (2009) Functional differentiation of *Brassica napus* guard cells and mesophyll cells revealed by comparative proteomics. Mol Cell Proteom 8:752–766

Zhu XG, de Sturler E, Long SP (2007) Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. Plant Physiol 145:513–526