RESEARCH ARTICLE

Abscisic Acid Induces Rapid Reductions in Mesophyll Conductance to Carbon Dioxide

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Abstract

The rate of photosynthesis (A) of plants exposed to water deficit is a function of stomatal (g_s) and mesophyll (g_m) conductance determining the availability of CO_2 at the site of carboxylation within the chloroplast. Mesophyll conductance often represents the greatest impediment to photosynthetic uptake of CO_2, and a crucial determinant of the photosynthetic effects of drought. Abscisic acid (ABA) plays a fundamental role in signalling and co-ordination of plant responses to drought; however, the effect of ABA on g_m is not well-defined. Rose, cherry, olive and poplar were exposed to exogenous ABA and their leaf gas exchange parameters recorded over a four hour period. Application with ABA induced reductions in values of A, g_s and g_m in all four species. Reduced g_m occurred within one hour of ABA treatment in three of the four analysed species; indicating that the effect of ABA on g_m occurs on a shorter timescale than previously considered. These declines in g_m values associated with ABA were not the result of physical changes in leaf properties due to altered turgor affecting movement of CO_2, or caused by a reduction in the sub-stomatal concentration of CO_2 (C_i). Increased [ABA] likely induces biochemical changes in the properties of the interface between the sub-stomatal air-space and mesophyll layer through the actions of cooporins to regulate the transport of CO_2. The results of this study provide further evidence that g_m is highly responsive to fluctuations in the external environment, and stress signals such as ABA induce co-ordinated modifications of both g_s and g_m in the regulation of photosynthesis.

Introduction

The rate of photosynthesis (A) in drought stressed plants is frequently constrained by the availability of carbon dioxide at the site of carboxylation. The concentration of CO_2 within the

chloroplast envelope (C_c) is determined by stomatal (g_s) and mesophyll (g_m) conductance [1–3]. During water deficit, levels of ABA within the leaf are often enhanced by the transport of ABA from the roots to the shoots [4] and the conversion of 'fixed' glycosylated ABA stored within the vacuole to 'free' ABA in the cytosol [5, 6]. Stomatal conductance is negatively correlated to the concentration of ABA in the xylem [7] and leaf [8]. The effect of ABA on stomatal closure may be further enhanced as apoplastic ABA concentrates at the sites of evaporation close to the stomatal pores [9], and pH changes on a cellular level facilitate the movement of ABA to the guard cells [10]. Generally, stomatal and mesophyll conductance operate in tandem, showing marked reductions to drought [2, 11, 12]. It was shown that this tight coordination was controlled in an ultradian fashion in *Eucalyptus citriodora* plants subjected to different degrees of water deficit [13]. However, despite transport of CO_2 across the mesophyll layer representing the largest resistance step in the uptake of CO_2 for photosynthesis and a critical component in the drought stress response of plants, the response of g_m to ABA is not clear.

Mesophyll conductance is determined by physical [14–16] and biochemical [17, 18] factors. Any reduction in leaf turgor could alter physical resistances, such as apoplastic space and the porosity of the cell wall encountered by CO_2 during transport from the sub-stomatal air-space to the chloroplast [9, 19, 20]. It is therefore necessary to distinguish between the physical influence of changes in leaf turgor experienced during drought, and any direct biochemical effects of ABA on g_m. Relatively few studies have investigated the effect of ABA on g_m, and have produced contrasting results; possibly due to the experimental approaches utilised to modify [ABA], either through increased endogenous production of ABA or exogenous application e. g. [21, 22], and the methods employed to measure g_m e.g. [23, 24, 25]. Exogenous application of ABA to hydroponically grown soybean (*Glycine max* L.) and tobacco (*Nicotiana tabaccum* L.) resulted in reduced g_s and g_m values over the course of ten days [3]. Identical reductions of both g_s and g_m were observed in cut-leaves of drought stressed and well-watered wild-type and ABA deficient mutants of tobacco (*Nicotiana plumbaginifolia*) two hours after exposure to a solution containing ABA [9]. The addition of ABA to the nutrient solution of 'sand-grown' sunflowers (*Helianthus annuus* L.) also reduced canopy-level g_m after three days [26]. In contrast, perlite grown sunflower plants supplied with an exogenous ABA solution showed reduced g_s, but no alteration of g_m after three days [25]. Furthermore, no difference was observed in g_m values of wild-type and ABA insensitive mutants of *Arabidopsis thaliana* [27].

The disparity in responses of g_m to ABA may be related to interspecific differences in drought stress physiology. For example, concomitant reductions in both g_s and g_m are frequently observed in response to water-deficit e.g. [2]. However, in drought tolerant plants, persistent long-term water deficit may result in an enhancement of g_m in comparison to the early stages of drought, whilst g_s remains low [28, 29]. One other potential explanation is the methodology employed to gauge g_m. It is not possible to measure g_m directly, and all approaches involve a number of assumptions and potential uncertainties [23]. Mesophyll conductance can be calculated through simultaneous measurement of leaf gas exchange and chlorophyll fluorescence parameters (the variable J method) [30, 31], determination of leaf gas exchange and carbon isotope discrimination [32, 33] or analysis of the curve of the photosynthetic response to increased [CO_2] in the internal sub-stomatal air-space (C_i) [34]. It has been suggested that stomatal closure during drought stress may alter rates of photosynthetic 'recapture' of CO_2 released during photorespiration, affecting the measurement of levels of respiration in the light (R_d) and thus the determination of g_m using the variable J method [23, 35]. However, observations of g_m sensitivity and insensitivity to ABA have been recorded using both the variable J method [3, 9, 25] and the carbon isotopic discrimination approach [9, 25, 26]. This may suggest that it is not possible to fully account for the disparity in g_m responses to ABA due to uncertainties with the methodological approach utilised to characterise g_m. Furthermore,

analysis of the effects of drought on g_m levels in rice (*Oryza sativa*) using both the variable J method and analysis of carbon isotope discrimination in recently synthesised sugars method indicated that the variable J technique was equally effective as the carbon isotopic discrimination approach in gauging g_m at low levels of water availability, but crucially produced more robust results under well-watered control conditions [2].

Abscisic acid plays a fundamental role in the drought stress response of plants. Given the wide-range of g_m responses to ABA reported in the literature over different timescales, we investigated g_m sensitivity to exogenous ABA application in four commercially important woody species. To characterise the direct biochemical response of g_m to ABA, measurements were taken over a short time period (four hours) to minimise any physical effects that may be associated with leaf water status that could affect the movement of CO_2 from the sub-stomatal air-space to the chloroplast envelope. This study aimed to: i) investigate the effect of exogenous ABA on stomatal and mesophyll conductance over a four hour time-course; ii) characterise any interspecific variations in the g_m response of the four species to ABA; iii) assess diffusive constraints imposed by g_s and g_m following ABA treatment that determine rates of photosynthesis.

Materials and Methods

Plant material, growing conditions and ABA treatment

Cherry (*Prunus avium* L.), black poplar (*Populus nigra* L.), olive (*Olea europaea* L.) and rose (*Rosoideae rosa*, hybrid tea rose "Camp David") were grown in a greenhouse at the National Research Council, Monterotondo, Rome, Italy. The plants were approximately one year-old and grown in comparatively large 6 dm^3 pots, where they did not experience root-restriction, containing a sand-perlite mixture (1:3) under natural sunlight and photoperiod from June to August. The respective daily maximum and minimum air temperatures were 38 and 20°C. To avoid any water and nutrient limitation, the saplings were watered every other day to pot water capacity and fertilised once a week, with Hoagland nutrient solution to supply nutrients at free access rates [36].

The evening prior to measurement the plants were watered to pot water capacity. The next morning branches were cut under distilled water; control treatment branches remained in distilled water, and *cis-trans* ABA (99% purity, Sigma) was added to the water of branches subject to ABA treatment. An ABA solution of 10^{-4} M concentration was used. Simultaneous measurement of gas exchange and chlorophyll fluorescence was then performed every hour over a four hour period between 08:00 and 12:00 in a well ventilated air-conditioned room at 25°C with control and ABA-treated shoots placed under a metal halide light emitting 800 µmol m^{-2} s^{-1} PPFD.

Gas exchange and fluorescence measurements

Leaf gas exchange and fluorescence parameters of the central leaf section were simultaneously measured using a LI-6400-40 leaf chamber fluorometer (Li-Cor, Inc., Nebraska, USA) equipped with a 2 cm^2 cuvette. One branch for the ABA and control treatments was taken from each of four plants, with the youngest fully expanded leaf from four branches measured for each treatment. The measurements were made at a saturating photon flux density (PPFD) of 1600 µmol m^{-2}s^{-1} measured using the internal quantum sensor within the leaf chamber and leaf temperature of 25°C for all four species. Leaves were exposed to a contaminant and pollutant free flux of synthetic air, composed of a mixture of nitrogen (80%), O_2 (20%) and CO_2 (385 ppm). The relative humidity of the air flow (500 µmol s-1) was maintained at 40–50%. To reduce diffusion leaks through the chamber gasket [37], a supplementary external chamber gasket composed of the same polymer foam was added to create an interspace between the two

gaskets (i.e. a double-gasket design with a 5 mm space separating the internal and external gaskets). Then the CO_2 and H_2O gradients between the in-chamber air and pre-chamber air were minimized by feeding the IRGA exhaust air into the interspace between the chamber and the pre-chamber gaskets [38].

The variable J method has proven to be effective in determining g_m in both well-watered and drought stressed rice varieties [2], we therefore chose this approach to assess the effect of ABA on g_m in the four plant species. Mesophyll conductance was calculated using the variable J method involving simultaneous measurements of gas-exchange and chlorophyll fluorescence parameters as described by Harley et al. (30) and Loreto et al. (31) (Eqs 1 and 2):

$$g_m = \frac{A}{C_i - \frac{\Gamma * [J_F + 8*(A+R_d)]}{J_F - 4*(A+R_d)}} \tag{1}$$

where the electron transport rate (J_F) is calculated from fluorescence [39]:

$$J_F = PPFD * \frac{\Delta F}{F_m} * \alpha * \beta \tag{2}$$

where F_m is the fluorescence maximum and the partitioning factor (β) between photosystems I and II was considered to be 0.5 and leaf absorbance (α) (0.85) [40].

The CO_2 compensation point to photorespiration (Γ^*) was measured on individual attached leaves of intact plants by increasing C_i at four different levels of photosynthetically active radiation [41, 42]. Levels of respiration in the light (R_d) were analysed using the Kok method [43, 44]; and respiration in the dark (R_n) was measured by switching off the light in the cuvette, when CO_2 release from the leaf had become stable for approximately five to ten minutes this was recorded and considered to represent R_n [45]. Values of Γ^*, R_d and R_n used in the calculation of g_m utilising the variable J method are given in Table 1. Total conductance to CO_2 (g_{tot}) was calculated as:

$$g_{tot} = [g_s * g_m]/[g_s + g_m] \tag{3}$$

Leaf water status

Immediately following the gas exchange measurements, each leaf was detached and weighed to determine leaf fresh mass (F_M). The leaves were then placed in a plastic bag and with the cut-end submerged in distilled water and allowed to rehydrate in darkness at 5°C for 18 hours. After rehydration the leaves were dried using paper towels to remove any water on their surfaces, and then the leaves were weighed to determine the saturated mass (S_M). Leaves were then dried at 80°C for 48 hours to measure dry mass (D_M). The relative water content (RWC) of each leaf was then calculated as follows [46]:

$$RWC = [F_M - D_M]/[S_M - D_M] \tag{4}$$

Table 1. Values of the CO_2 compensation point to photorespiration (Γ^*), respiration in the light (R_d) and respiration in the dark (R_n) used to calculate g_m levels of the four plant species using the variable J method [30, 31]. ± indicates one standard deviation.

| Species	R_n (µmol m^{-2}s^{-1})	R_d (µmol m^{-2}s^{-1})	Γ^* (µmol mol-1)
Cherry	1.97 ± 0.28	1.40 ± 0.13	46.6 ± 2.77
Olive	1.72 ± 0.10	1.28 ± 0.09	58.4 ± 2.59
Poplar	1.80 ± 0.09	1.06 ± 0.12	45.5 ± 3.72
Rose	1.94 ± 0.20	1.24 ± 0.10	55.3 ± 3.59

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Statistical analyses

Statistical analyses were performed using SPSS 20 (IBM, New York, USA). A one-way ANOVA with LSD *post-hoc* test was used to assess differences in variance between samples subjected to control conditions and ABA treatment. A significant difference between treatments was assumed to occur at a *P*-value <0.05. Linear regression was used to investigate potential relationships between conductance to CO_2 and *A* in the four plant species under control conditions and ABA treatment.

Results

Following abscission of the cuttings, the RWC of the leaves was recorded at each hourly measurement interval to assess any alteration in leaf water content that may have influenced g_m via a change in turgor. The RWC of control cherry leaves remained within the range of 88.7–94.1% throughout the measurement period (Fig 1a). However, after two hours the mean RWC of leaves exposed to exogenous ABA increased from 90.9% to 95.7%; a significant difference between the RWC values of the control and ABA treated leaves only became apparent after four hours of exposure (one-way ANOVA LSD *post-hoc* test, $F_{1,6} = 25.954$; $P = 0.00223$). The RWC of rose leaves exhibited a similar pattern, with the mean RWC of ABA treated leaves exhibiting an increase from 94.6% to 97.2% after two hours. However, RWC parameters of control leaves also showed a slight increase during the measurement period over a range of 92.2 to 96.7%. Statistically significant differences were observed in the RWC values of control and ABA treated rose leaves after two hours (one-way ANOVA LSD *post-hoc* test, $F_{1,6} = 9.416$; $P = 0.0220$) and this persisted for the remainder of the measurement period (Fig 1d). The RWC of poplar leaves showed no significant decrease between the control and ABA treatments, although RWC rose slightly by 1.6–3.4% over the four hour measurement period (Fig 1c). The RWC values of olive leaves displayed no significant change during the four hour measurement period, and no significant treatment effect associated with the application of ABA (Fig 1b).

Exposure to exogenous ABA induced consistent declines in *A* in all four of the species studied. The control plants showed no declines in *A* that may have been associated with branch abscission (Fig 2). These reductions in *A* following exposure to ABA corresponded to identical declines in g_s and g_m. However, the rate of reduction in g_s and g_m values differed between the four species. Cherry exhibited a consistent decline in *A*, g_s and g_m throughout the measurement period following ABA treatment (Fig 2a–2c). Olive and poplar displayed rapid reductions in g_s
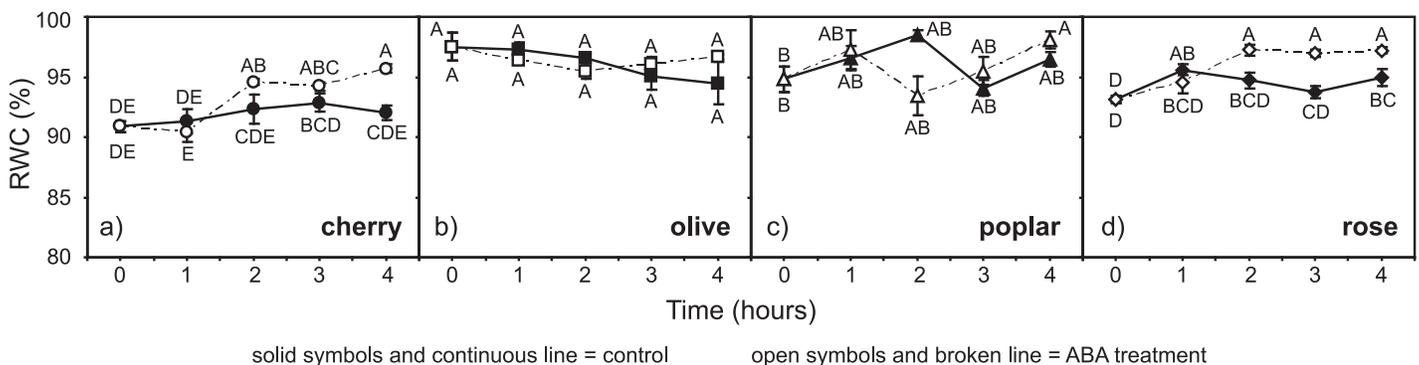
Fig 1. Relative water content values of control and ABA treated leaves of cherry (a), olive (b), poplar (c) and rose (d) over the four hour measurement period. Solid symbols and continuous line indicate control samples; open symbols and broken line indicate ABA treatment. Error bars indicate one standard error either side of mean. Different letters indicate significant differences between datasets based upon one-way ANOVA and LSD *post-hoc* test.

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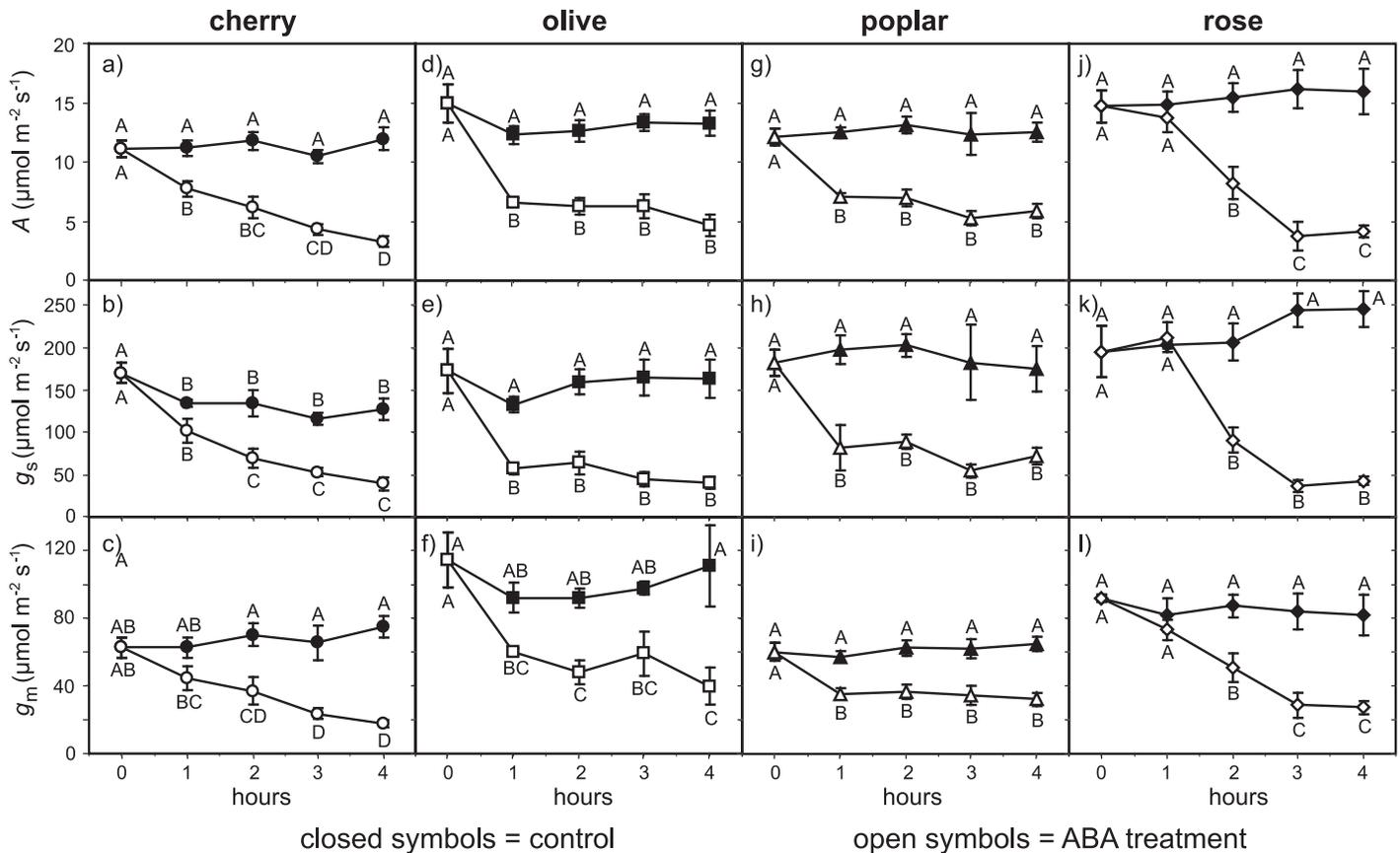
Fig 2. Time course A, g_s **and** g_m **responses of cherry (a-c), olive (d-f), poplar (g-i) and rose (j-l) cuttings to application of exogenous ABA solution.** Solid symbols indicate control samples; open symbols indicate ABA treatment. Error bars indicate one standard error either side of mean. Different letters indicate significant differences between datasets based upon one-way ANOVA and LSD *post-hoc* test.

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(olive: -66.7%; poplar– 54.8%) and g_m (olive: -47.3%; poplar– 40.9%) in the first hour after exposure to exogenous ABA, before maintaining relatively stable g_s and g_m values for the remainder of the measurement period. In contrast, rose does not show a decline in levels of A, g_s and g_m in the first hour after ABA treatment, before exhibiting a rapid decline in the second hour (A: -44.2%; g_s: -53.5%; g_m: -44.5%) then stabilising in the third and fourth hour (Fig 2j–2l). The lower levels of conductance to CO_2 and A following four hours of ABA treatment led to decreases in C_i of 8.4 to 28.0% in all four plant species. However, this reduced C_i was statistically significant only in poplar and rose (Fig 3).

Photosynthetic rates of the four species were largely determined by conductance to CO_2 (Fig 4). Under control conditions, rose generally exhibited the highest levels of A, g_s and g_m, while cherry displayed the lowest. However, after four hours of ABA treatment, rose and cherry showed identical photosynthetic rates and levels of conductance to CO_2. The leaves treated with exogenous ABA exhibited lower levels of g_s, g_m and g_{tot} that correspond to lower A. The relationship between A and conductance to CO_2 was most robust when g_s and g_m were combined to determine g_{tot} (Fig 4c).

Discussion

Abscisic acid plays a fundamental role in plant responses to water-deficit. The effect of ABA on g_m is poorly characterised in comparison to the influence of ABA on stomatal behaviour e.g. [4,
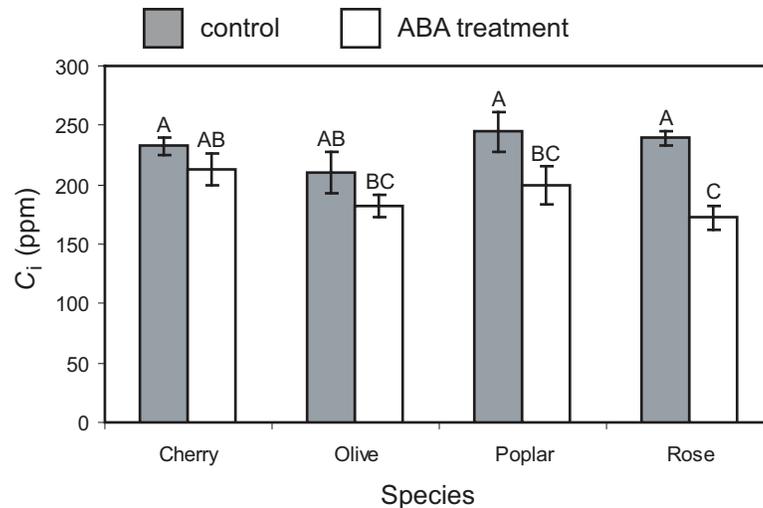
Fig 3. Internal sub-stomatal concentrations of CO$_2$ (C_i) of control and ABA treated cuttings after four hours. Grey indicates control; white indicates ABA treatment. Error bars indicate one standard error either side of mean. * indicates significant difference between control and ABA treatment values using a one-way ANOVA (cherry, $F_{1,6} = 1.501$, $P = 0.266$; olive, $F_{1,6} = 2.020$, $P = 0.205$; poplar, $F_{1,6} = 8.998$, $P = 0.0240$; rose, $F_{1,6} = 34.991$, $P = 0.00104$). Different letters indicate significant differences between datasets based upon one-way ANOVA and LSD *post-hoc* test.

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47, 48]. This study demonstrated that exogenous ABA produced a rapid reduction in g_m values within two hours of application in all of the four species studied. These declines in g_m corresponded to reductions in g_s, analogous to co-ordination of g_s and g_m observed during drought [2, 12, 49] and as circadian pattern [13]. The reductions in g_s following ABA application are indicative of stomatal sensitivity to ABA and active physiological stomatal behaviour in all four of the plant species studied [50, 51]. Photosynthesis was closely related to conductance to CO$_2$ in the control and ABA treated cuttings (Fig 4), suggesting that ABA determined A through its

○ cherry   □ olive   △ poplar   ◇ rose   closed symbols = control   open symbols = ABA
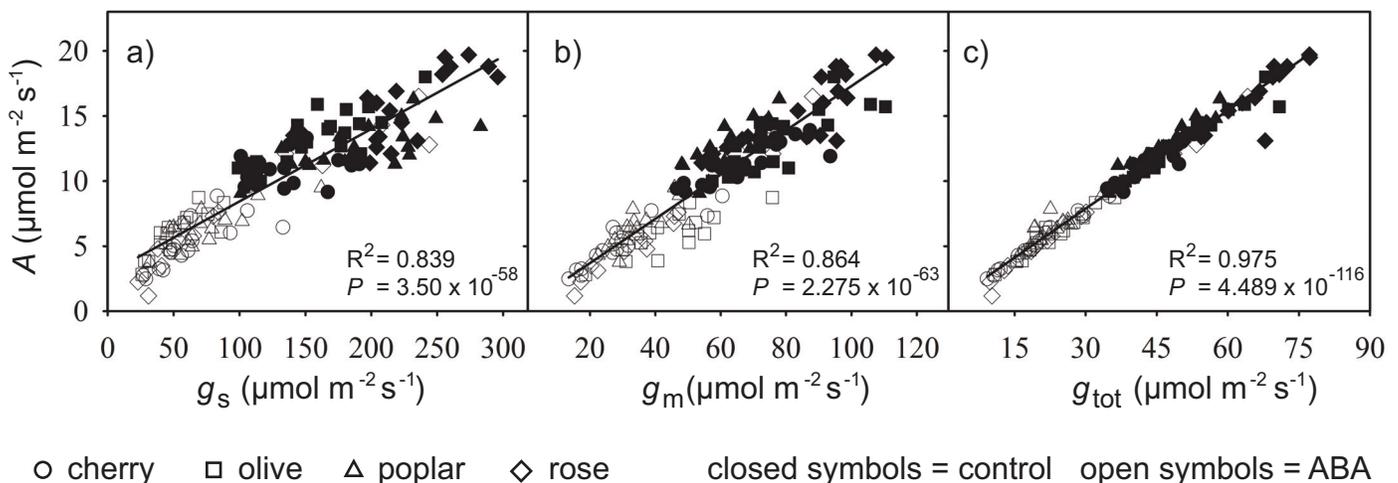
Fig 4. Relationship of A to g_s (a), g_m (b), and g_{tot} (c) in control (closed symbols) and ABA treated (open symbols) cuttings of cherry (circle symbol), olive (square symbol), poplar (triangle symbol) and rose (diamond symbol) after four hours of treatment. Line indicates best fit determined by linear regression: stomatal conductance ($F_{1,142} = 740.620$; $R^2 = 0.839$; $P = 3.350 \times 10{-58}$), mesophyll conductance ($F_{1,142} = 901.489$; $R^2 = 0.864$; $P = 2.275 \times 10{-63}$), and; total conductance to CO$_2$ ($F_{1,142} = 5618.153$; $R^2 = 0.975$; $P = 4.488 \times 10{-116}$).

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action upon g_s and g_m. The speed of response of g_m, g_s and A to ABA application varied between the four species, possibly due to differences in water transport e.g. [52] affecting the uptake and movement of ABA, or biochemical differences in the effect of ABA signalling between the plants e.g. [53, 54].

Mesophyll conductance is composed of physical and chemical components that determine the movement of CO_2 from the internal sub-stomatal air-space to inside the chloroplast envelope [55]. Increased RWC in ABA treated cherry and rose (Fig 1), possibly due to stomatal closure reducing transpirative water-loss from the leaf, may have altered the physical properties of the leaf and thus affected g_m indirectly. However, in the evening prior to abscission the plants were watered to pot capacity, and all of the leaves exhibited high RWC values that correspond to full leaf turgor [56], likely suggesting that the proportionally small percentage increases of 2–3% in RWC following ABA application did not induce significant alteration of leaf structural properties. It is noteworthy that significant differences in the RWC of control and ABA treated leaves of cherry only became apparent after two hours, whereas g_m values in ABA treated leaves declined significantly within one hour (Fig 2c); suggesting that the initial declines in following ABA application were solely the result of biochemical changes, and not due to alteration of the physical properties of leaves associated with a change in foliar water status.

The effect of ABA on g_m observed in this study was rapid, occurring within one hour of exposure in three of the four species studied. Short-term fluctuations in g_m have also been recorded in response to $[CO_2]$ [37], salinity [1, 57], light quality [58, 59], light intensity [37] and temperature [18], and have been ascribed to the action of carbonic anhydrase and cooporins that transport CO_2 across the plasma membrane [17, 60–65]. The conversion of gaseous CO_2 to aqueous carbonic acid (H_2CO_3) represents one of the largest resistance steps encountered in photosynthetic CO_2-uptake [66]. The results of this study may suggest that ABA acts to reduce the activity of cooporins involved in the transport of CO_2. Cooporins belong to a group of proteins known as aquaporins, that also facilitate the movement of water across membranes [67]. As foliar [ABA] increased, the activity of aquaporins have been shown to decline during drought stress [68, 69], and induce stomatal closure through increased guard cell permeability and reduced water movement across the membrane [70]. Exogenous application of ABA to fully hydrated leaves achieved similar results [71]. However, increased [ABA] may also enhance the expression of certain aquaporins to enhance drought tolerance through increased water transport [72].

It is unclear whether alterations in g_m are a by-product of the g_s response, or whether the two processes are linked by co-ordinated physiological signalling [73]. Instantaneous increases in C_i applied to plants grown under optimal conditions induce reductions in g_m, as the limiting effect of CO_2 availability on A declines at higher C_i [37]. This would suggest that if the g_m responses observed following ABA treatment in the present study were solely the result of reduced C_i due to stomatal closure, an increase in g_m might be expected. However, reduced g_m was recorded in all four species after application of exogenous ABA; furthermore, only two of the species exhibited significant declines in C_i after ABA treatment (Fig 3). Application of the same concentration of ABA used in this study to the roots of sunflower did not affect g_m but did reduce g_s. The relationship between g_m and C_i in ABA treated sunflower was also identical to control plants; showing a positive correlation between g_m and C_i at concentrations above 200 ppm [25]. However, detached leaves of wild-type tobacco plants when treated with exogenous ABA exhibited a positive g_m—C_i relationship at sub-ambient C_i, whilst the control counterparts showed a negative g_m—C_i relationship [73]. Furthermore, g_m response to C_i has been shown to occur on a shorter timescale than g_s response to C_i in tobacco, wheat and in both wild-type and mutant *Arabidopsis thaliana* that lacked the capacity for stomatal closure [74]. This may suggest that the concomitant declines in g_s and g_m observed in this study following ABA treatment are the result of a shared signalling mechanism regulating rates of stomatal and

mesophyll conductance to CO_2 [73] in addition to any subsequent effects on g_m associated with stomatal closure [73–75], respiration [23] or photorespiration [24].

It is noteworthy that the effect of ABA on g_m observed in the present study occurs more rapidly than previously reported e.g. [3, 9, 25, 26]. Increased foliar [ABA] following drought stress induces stomatal closure to restrict water-loss from the internal leaf [76]. It would initially appear incongruous to reduce transport of CO_2 across the mesophyll layer in concert with decreased g_s to minimise water-loss from the internal leaf [73]; as a high g_m to g_s ratio would permit the maintenance of a degree of CO_2 uptake and enhanced water use efficiency during drought [27]. However, the rapid declines in g_m after application with exogenous ABA may indicate a selective advantage of reduced conductance to CO_2 across the mesophyll during episodes of water-deficit. A decline in the activity of cooporins responsible for the transport of CO_2 may reduce energy consumption [77]. Higher cellular [78] and apoplastic [79] ABA may also be associated with changes in pH that alter membrane properties through the action of proteins such as aquaporins [80], thus affecting g_m [17]. Nonetheless, it is presently unclear as to the nature of the mechanisms responsible for such rapid alterations in g_m following ABA treatment or their functional significance. Further analysis of the expression of cooporin protein RNA may elucidate the biochemistry underlying this response e.g. [81]. Application with exogenous ABA induced declines in g_m within one hour in three of the species studied (and within two hours in the remaining species, rose); this would suggest that ABA has a clear effect on g_m, and the interaction of ABA and g_m plays an important role in plant drought stress response.

Conclusion

The results of this study show consistent reductions in A, g_s and g_m values of all four plants following exposure to exogenous ABA. Photosynthesis in the plant species was positively related to the availability of CO_2 within the chloroplast envelope (Fig 4). The observed declines in g_m occurred on a shorter timescale than those reported in previous studies; suggesting that ABA serves to induce rapid reductions in g_m following exposure to water deficit. These falls in g_m values occurred prior to any significant alteration in RWC; suggesting that they are not associated with physical effects of increased turgor affecting the permeability of cell membranes, and are instead the result of alteration to the biochemical properties of the interface between the mesophyll and the internal sub-stomatal air-space. The reductions in g_m recorded in the present study are unlikely to be the by-product of stomatal closure causing a decrease in C_i, as plants exposed to ABA exhibited reduced g_m rather than increased conductance to counter lower availability of CO_2 in the sub-stomatal air-space. The effect of ABA on g_m is likely through the diminished activity of cooporins and carbonic anhydrase, whether these responses act on a dose-dependent basis or occur over different timescales is currently unclear. However, the findings of the present study indicate that ABA functions by inducing rapid reductions in g_m that are associated with concomitant declines in g_s as part of a co-ordinated gas exchange response to water deficit.

Acknowledgments

Author Contributions

Conceived and designed the experiments: MC. Performed the experiments: SZ SW TM MC GS. Analyzed the data: MH MC. Contributed reagents/materials/analysis tools: MC. Wrote the paper: MH MC.

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