Radiation use and stomatal behaviour of three tropical forage legumes

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Abstract

Three promising tropical forage legumes, *Cratylia argentea*, *Flemingia macrophylla* and *Desmodium velutinum* were evaluated in a tropical monsoon environment in Hainan Province, China. *D. velutinum* had higher net photosynthetic rate (\(P_N\)) and water use efficiency (WUE) than the other two species, and photosynthetic performance was consistent with their stomatal conductance (\(g_s\)). Responses in \(g_s\) to increasing photosynthetically active radiation (PAR) showed two phases. Light was the main trigger for opening stomata at low light intensity (0–400 \(\mu\)mol quanta/m\(^2\)/s), as was a reduction in intercellular CO\(_2\) (\(C_i\)). Indirect actions contributed more to stomatal behaviour than the direct effect of light at intermediate to intense light intensity (400–2000 \(\mu\)mol quanta/m\(^2\)/s), with water status being the major factor that controlled stomatal behaviour. *D. velutinum* was more affected by vapour pressure deficit (VPD) feedback regulation in intense light, and less by \(C_i\) at low light intensity than were the other two species.

Introduction

*Cratylia argentea*, *Flemingia macrophylla* and *Desmodium velutinum* are promising forage legumes adapted to acid soils with high levels of exchangeable aluminium in tropical areas of China (Yi et al. 1998). Resource use efficiency is a critical element in determining forage yield (Steduto and Albrizio 2005), and physiological responses to environmental changes should be included in early evaluation, particularly with respect to CO\(_2\) and water exchange (González-Rodríguez et al. 2005). It is clear that stomata play a key role in this regard (Hetherington and Woodward 2003).

Stomata allow CO\(_2\) and water exchange, and their partial opening or closure regulates the relative balance between CO\(_2\) absorption and water loss (Larcher 1995; Medrano et al. 2002; Talbott et al. 2003). Stomata are sensitive to numerous environmental signals such as humidity (Talbott et al. 2003), CO\(_2\) (Morison 1987; Assmann 1999), drought (Medrano et al. 2002), temperature (Shimono et al. 2004), nutrition (Saneoka et al. 2004), salinity (Downton et al. 1985) and especially the intensity (Takashi et al. 2001) and quality (Kim et al. 2004) of light. However, apart from the direct effect, light also initiates two important negative feedback loops, regulating stomatal conductance of CO\(_2\) and water (Larcher 1995; Hetherington and Woodward 2003). While a reduction of intercellular CO\(_2\) (\(C_i\)), due to photosynthetic assimilation, stimulates stomatal opening (Morison 1987; Assmann 1999), the light-induced vapour pressure deficit (VPD) stimulates stomatal closure to reduce water loss (Oren et al. 1999).

To date, ecological research has concentrated on investigations of direct relationships, while indirect interactions have often been overlooked (Krivtsov 2004). However, indirect effects are fundamental to the complexity of living systems, and provide challenges to predicting the impacts of environmental change. As a result, studies
Wang Li, Yang Yunfei, Liu Jinxiang and Ma Fang have increasingly considered both direct and indirect relationships (Wootton 2002). Resource use efficiency of *Cratylia argentea*, *Flemingia macrophylla* and *Desmodium velutinum* was examined at irradiances for photosynthetic saturation. To improve yield of these forage legumes, physiological responses to changes in light intensity were considered, and path analysis was used to determine the causal effects of the variables on stomatal behaviour. In particular, the research focused on the partitioning of absorbed radiation energy between CO$_2$ and water feedback regulation processes.

Materials and methods

Study area

An experiment was conducted at the Tropical Pasture Research Center (19°30′ N, 109°30′ E), Chinese Academy of Tropical Agricultural Sciences, Hainan Province, which is situated in a tropical monsoon climatic region. Mean annual temperature is 23.9°C with a mean temperature of 7.1°C in February and 38.4°C in July. Mean annual rainfall is 1815 mm, most falling between June and October. Annual evapotranspiration is 1628 mm. Soils in this area are oxisols (Liu et al. 2002).

Plant materials

The tropical legume forages, *Cratylia argentea*, *Flemingia macrophylla* and *Desmodium velutinum*, are deep-rooted shrubs, and are tolerant of both drought and acid soil conditions (Yi et al. 1998). They were introduced from South America to China in 1997. Sample plants were annual individuals, growing naturally in the field, with no disturbance, competition or nutrient stress.

Data collection

Physiological variables were measured in an open infrared gas analysis system (IRGAs) with a ‘LiCor LI-6400 Portable Photosynthesis System’ (LI-COR Company, Lincoln, USA). A 6400–02 LED (red+blue) light source was used to control the intensity of photosynthetically active radiation (PAR) inside the leaf chamber. Light intensities were: 0, 20, 50, 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 µmol quanta/m$^2$/s, and values of physiological parameters, including net photosynthetic rate ($P_N$), transpiration rate ($E$), vapour pressure deficit (VPD), intercellular CO$_2$ ($C_i$) and stomatal conductance ($g_s$) were logged automatically. The minimum time used to equilibrate stomatal conductance was ≥15 min for each light intensity (i.e., within a 1% coefficient of variation). Environmental conditions for the forage legumes were controlled by this instrument with an air-flow of 500 µmol/s, CO$_2$ concentration of 370 µmol/mol, relative humidity (RH) of 50% and air temperature of 30°C.

Five individual plants of each species were randomly selected and measured in mid-November, when plants were at the reproductive stage. The third, fully expanded and intact leaf from the top of each plant was measured. Measurements were taken at approximately the same time of the day.

Data analysis

Water use efficiency (WUE) is defined as the ratio of net assimilation to water loss as follows:

$$WUE = \frac{P_N}{E}$$  

[1]

Path analysis can be used to partition the direct and indirect effects of PAR, $C_i$ and VPD on stomatal behaviour. The simple recursive path diagrams represent the cause-and-effect relationship (Figure 1).

![Figure 1. Path diagram representing the cause-and-effect relationship between the direct variable [photosynthetically active radiation (PAR)], two indirect variables [intercellular CO$_2$ ($C_i$) and vapour pressure deficit (VPD)], and the response variable [stomatal conductance ($g_s$)].](image)

Standardised partial-regression coefficients of the following expressions [2], [3] and [4] were used to estimate path coefficients for PAR ($P_{Y1}$), $C_i$ ($P_{Y2}$) and VPD ($P_{Y3}$) to $g_s$, as well as for PAR to $C_i$ ($P_{21}$) and VPD ($P_{31}$). The data for PAR were transformed logarithmically to avoid large variances:
Radiation use and stomatal behaviour

\[ g_s = b_1 + b_{y1} \text{PAR} + b_{y2} C_i + b_{y3} \text{VPD} \]  
[2]

\[ C_i = b_2 + b_{21} \text{PAR} \]  
[3]

\[ \text{VPD} = b_3 + b_{31} \text{PAR} \]  
[4]

where \( b_i \) is a constant and \( b_{ij} \) is a partial-regression coefficient. Indirect effects were determined by multiplying the correlation coefficients \( (r_{ij}) \) by their respective path coefficients, \( r_{12} P_{y2} \) and \( r_{13} P_{y3} \), representing the indirect effect of PAR to \( g_s \) via \( C_i \) and VPD, respectively. Residuals were estimated to evaluate the degree of determination for the path analysis (Li 1975). Fitting of models and path analysis for the three legume species were performed using SPSS 10.0 for Windows (SPSS Inc., Chicago).

Results

Comparison of physiological characteristics

While \( P_N \), WUE and \( g_s \) were significantly (\( P < 0.01 \)) lower in \( C. argentea \) and \( F. macrophylla \) than in \( D. velutinum \), \( E \), VPD and \( C_i \) did not differ among the species (Table 1). The efficiency of carbon assimilation and water use in the various species was consistent with their stomatal conductance.

Physiological responses to light intensity

Responses of \( g_s \) to increasing PAR showed two distinct phases in the three forage legumes. At low light intensity (0–400 \( \mu \)mol quanta/m\(^2\)/s), \( g_s \) increased linearly with photo flux density. At intermediate and intense light intensity (400–2000 \( \mu \)mol quanta/m\(^2\)/s), \( g_s \) changed much more slowly and decreased gradually at irradiances well below full sunlight (Figure 2A). \( D. velutinum \) had a much higher \( g_s \) than \( C. argentea \) and \( F. macrophylla \), which lead to a steeper increase in response to irradiation at low light intensity and a smaller decrease at high light intensities.

Figure 2. Responses in: (A) stomatal conductance (\( g_s \)); (B) vapour pressure deficit (VPD); and (C) intercellular CO\(_2\) (\( C_i \)) of three tropical legumes to increasing photosynthetically active radiation, PAR. \( Ca = Cratylia argentea \); \( Fm = Flemingia macrophylla \); and \( Dv = Desmodium velutinum \). VPD\(_{Ca}\) = 0.0008PAR + 0.493; VPD\(_{Fm}\) = 0.0008PAR + 0.3896; VPD\(_{Dv}\) = 0.0007PAR + 0.8857; \( C_i\)\(_{Ca}\) = 215.3633(1 + 0.7883EXP(–0.2217PAR/215.3633)); \( C_i\)\(_{Fm}\) = 211.8136(1 + 0.7551EXP(–0.2448PAR/211.8136)) and \( C_i\)\(_{Dv}\) = 225.7932(1 + 0.6331EXP(–0.3534PAR/225.7932)).
There was a positive, linear relationship between VPD and PAR in all species. Slopes for fitted regression lines in *C. argentea*, *F. macrophylla* and *D. velutinum*, were 0.0008, 0.0008 and 0.0007 with intercepts of 0.49, 0.39 and 0.89 kPa. While there was less increase in VPD with increasing light intensity in *D. velutinum*, VPD was higher in *D. velutinum* than in the other two species throughout (Figure 2B).

Ci of all species decreased exponentially with increasing light intensity, but the rate differed among the three species (Figure 2C). The initial slopes of curves were 0.79, 0.76 and 0.63 in *C. argentea*, *F. macrophylla* and *D. velutinum*, respectively, with the Ci of *D. velutinum* decreasing more slowly with increasing light intensity than those of the other two species.

**Stomatal conductance as regulated by key physiological parameters**

Path coefficients between PAR, Ci and VPD and stomatal conductance reflected the effect of light (inducement or inhibition), CO₂ and H₂O feedback control on stomatal behaviour (Figure 3).

The path coefficients derived from PAR and Ci (or VPD) accounted for most of the variation in gs as indicated by a small residual effect (0.121–0.161). This suggests that contribution by additional traits to stomatal behaviour would be small (Table 2).

Light acts both directly and indirectly via Ci feedback regulation at low light intensity (0–400 μmol quanta/m²/s). Path coefficients indicate that the indirect effect via VPD is not significant, and the effect of PAR is a direct effect rather than an indirect effect via Ci. Light had its strongest impact in *D. velutinum* and the smallest in *C. argentea*. In contrast, the strongest indirect action of Ci was found in *C. argentea* and the smallest in *D. velutinum* (Table 2; Figure 3A). This corresponds with their falling rate of Ci (Figure 2C).

Light and severe VPD force stomata of these three tropical legumes to close in intermediate and intense light (400–2000 μmol quanta/m²/s). The indirect effect of VPD is the dominant effect rather than the direct effect of light. The strongest inhibition of light was found in *F. macrophylla* and the smallest in *D. velutinum*. In contrast, the strongest indirect action of VPD was found in...
D. velutinum and the smallest in F. macrophylla (Table 2; Figure 3B), a finding consistent with their magnitude of VPD (Table 1; Figure 2B). In this process, the indirect effects via C_i were not significant for the three tropical species.

Discussion

Among the three tested forage legumes, D. velutinum displayed the highest P_N and WUE, and these photosynthetic parameters (measured at steady state and at light saturation) were closely related to stomatal conductance. D. velutinum also had the highest g_s of the three species, resulting in the severest water loss. As a result, stomatal behaviour of D. velutinum was more affected by the VPD feedback system in intense light. In contrast, the smaller g_s of C. argentea and F. macrophylla reduced water loss, but also would limit CO_2 absorption for photosynthesis at low light. As a result, stomatal behaviour of these species was more affected by the CO_2 feedback system at low light.

The path analysis results in the current study support the hypothesis that, apart from direct responses to PAR, two negative feedback loops serve to maintain stomatal conductance of water vapour and CO_2 in an appropriate range (Larcher 1995; Hetherington and Woodward 2003). At low light intensity (0–400 µmol quanta/m²/s), the main trigger for opening stomata is the direct effect of light, while the reduction of C_i due to substantial photosynthesis stimulates stomatal opening at the same time. At intermediate and high light intensities, light is no longer the primary limiting factor, and stomata respond to a shortage of water in the three tropical legumes by closing.

In intense light, the reduction in g_s protects plants from suffering water deficit and is beneficial for plants in regions with high radiation, high temperature and high evaporation. However, stomatal closure will result in heat stress (Valladares and Pearcy 1997) and more severe photoinhibition (Cornic 1994) on leaves under the intense light environment of tropical regions.

Physiological activity involves highly interactive processes, and plants can adapt to the changing circumstances by complex physiological modulations (Mackenzie et al. 1998). Although results from this experiment have revealed an aspect of whole-plant physiology, it is still far from being predictive under natural conditions.

Table 1. Differences in net photosynthetic rate (P_N), transpiration rate (E), water use efficiency (WUE), stomatal conductance (g_s), vapour pressure deficit (VPD) and intercellular CO_2 (C_i) among three tropical legume forages at irradiances for photosynthetic saturation (1862 ±120 µmol quanta/m²/s) (Means of 5 replications ± standard deviations).

<table>
<thead>
<tr>
<th>Species</th>
<th>P_N (µmol CO_2/m²/s)</th>
<th>E (mmol H_2O/m²²/s)</th>
<th>WUE (µmol CO_2/mmol H_2O)</th>
<th>g_s (mol/m²/s)</th>
<th>VPD (kPa)</th>
<th>C_i (µmol CO_2/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. argentea</td>
<td>19.7±0.73 A¹</td>
<td>8.93±0.51</td>
<td>2.20±0.05 A</td>
<td>0.42±0.04 A</td>
<td>1.74±0.25</td>
<td>249.6±12.85</td>
</tr>
<tr>
<td>F. macrophylla</td>
<td>19.6±0.41 A</td>
<td>8.27±0.49</td>
<td>2.38±0.14 A</td>
<td>0.47±0.04 A</td>
<td>1.68±0.31</td>
<td>238.6±13.95</td>
</tr>
<tr>
<td>D. velutinum</td>
<td>23.63±1.03 B</td>
<td>8.68±0.60</td>
<td>2.73±0.08 B</td>
<td>0.65±0.02 B</td>
<td>1.94±0.18</td>
<td>238.5±8.62</td>
</tr>
<tr>
<td>F</td>
<td>44.77**</td>
<td>1.88</td>
<td>36.46**</td>
<td>0.61±0.74**</td>
<td>1.431</td>
<td>1.41</td>
</tr>
<tr>
<td>P</td>
<td>0.000</td>
<td>0.195</td>
<td>0.000</td>
<td>0.000</td>
<td>0.277</td>
<td>0.283</td>
</tr>
</tbody>
</table>

¹ Values within columns followed by different letters differ (P<0.01).

Table 2. Correlation and path coefficients for direct and indirect effects between photosynthetically active radiation (PAR), intercellular CO_2 (C_i) and vapour pressure deficit (VPD), and stomatal conductance (g_s) for three tropical forage legumes.

<table>
<thead>
<tr>
<th>Species</th>
<th>PAR (µmol quanta/m²²/s)</th>
<th>Correlation coefficient (r₁₁) ²</th>
<th>Residual effect</th>
<th>Direct effect</th>
<th>Indirect effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VPD (kPa)</td>
</tr>
<tr>
<td>C. argentea</td>
<td>0–400</td>
<td>0.963**</td>
<td>0.139</td>
<td>0.659</td>
<td>ns</td>
</tr>
<tr>
<td>F. macrophylla</td>
<td>0–400</td>
<td>0.974**</td>
<td>0.128</td>
<td>0.696</td>
<td>ns</td>
</tr>
<tr>
<td>D. velutinum</td>
<td>0–400</td>
<td>0.974**</td>
<td>0.133</td>
<td>0.716</td>
<td>ns</td>
</tr>
<tr>
<td>C. argentea</td>
<td>400–2000</td>
<td>−0.981**</td>
<td>0.121</td>
<td>−0.387</td>
<td>−0.594</td>
</tr>
<tr>
<td>F. macrophylla</td>
<td>400–2000</td>
<td>−0.973**</td>
<td>0.143</td>
<td>−0.397</td>
<td>−0.576</td>
</tr>
<tr>
<td>D. velutinum</td>
<td>400–2000</td>
<td>−0.972**</td>
<td>0.161</td>
<td>−0.263</td>
<td>−0.710</td>
</tr>
</tbody>
</table>

² r₁₁ is the correlation coefficient between PAR and g_s.
and other parameters and interactions have to be considered further. Specifically, studies should scale up from leaves to canopies. Such results will be useful for the management of forage crops. For example, modification of the environment during the reproductive stage of forage legumes may be used to favour growth or reproduction.

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References


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