Modélisation dynamique de la réponse stomatique

Introduction

Ce second chapitre fait office d'introduction méthodologique au modèle dynamique utilisé durant l'ensemble de ces travaux de thèse afin de décrire la réponse temporelle des stomates à des stimuli environnementaux.

Les stomates sont des pores répartis sur l'ensemble de la surface foliaires des végétaux. En fonction des espèces, les stomates sont situés sur les faces adaxiales et abaxiales des feuilles, certaines plantes disposant de stomates sur ces deux faces tandis que d'autres n'en possèdent que sur l'une d'entre elles, à l'instar des deux espèces de chênes faisant l'objet de ce travail de thèse dont les feuilles sont pourvues de stomates uniquement sur la face abaxiale. Ceux-ci jouent un rôle essentiel dans le contrôle des échanges gazeux foliaires des plantes en régulant à la fois les pertes en eau par transpiration et l'entrée du CO_2 atmosphérique nécessaire à l'assimilation par photosynthèse. Par ailleurs, la surface foliaire restante est recouverte d'une fine couche cuticulaire relativement imperméable. De fait, les stomates constituent une interface privilégiée entre les plantes et leur atmosphère à travers laquelle la majorité des échanges gazeux sont réalisés (Taiz & Zeiger, 2006). Indirectement, les stomates jouent également un rôle de refroidissement des feuilles à travers la transpiration.

A chaque instant les stomates sont caractérisés par un certain degré d'ouverture régulé par la différence de pression de turgescence entre les cellules de garde accompagnant les stomates et les cellules épidermiques, résultant de facteurs à la fois internes et externes à la plante / de l'état physiologique de la plante en réponse à son environnement. Ce degré d'ouverture permet la circulation des gaz au sein de la cavité sous stomatique et leur diffusion à travers la plante se traduisant en conductance stomatique pour l'eau et le CO_2. Ainsi, un fort degré d'ouverture favorisera la diffusion des gaz et donc une conductance stomatique plus élevée, à l'inverse, une faible ouverture des stomates limitera les échanges entre la plante et son atmosphère. Le degré d'ouverture des stomates ainsi que la conductance stomatique qui en résulte sont sensibles à de multiples influences environnementales. La réponse des stomates au microclimat local (statut hydrique de la plante, la quantité de lumière ainsi que la qualité du spectre lumineux, humidité de l'air, température, [CO_2] atmosphérique) a donc été extensivement étudiée et les réponses à ces facteurs, finement décrites (Jarvis, 1976 **;** Jones, 1992, 1998 **;** Monteith, 1995). Cependant, le développement d'approches intégratives complètes intégrant la réponse stomatique à de multiples facteurs environnementaux demeure entravé par la complexité de leurs interactions ainsi que des possibles couplages entre ces

différents facteurs, auxquels s'additionne le fait que l'ensemble des mécanismes impliqués dans la captation, la transduction et la traduction des stimuli environnementaux en une réponse stomatique finale n'aient pas encore été totalement élucidés. Ainsi, régulée aussi bien par des signaux intrinsèques que par les interactions que la plante entretient avec son environnement, la pression de turgescence du complexe stomatique fluctue constamment au cours du cycle journalier des végétaux résultant en mouvements d'ouvertures et de fermetures d'amplitudes et de rapidités variées.

Description de la dynamique de réponse stomatique (g_s)

La modélisation constitue un outil particulièrement adapté et efficace à des fins prédictives, de simulation et d'intégration à des échelles diverses. De fait, l'assimilation du carbone atmosphérique par photosynthèse ainsi que les pertes hydriques par transpiration, respectivement estimées à travers la conductance pour le CO_2 et H_2O, s'inscrivent parmi les fonctions physiologiques ayant été massivement sujettes à modélisation au cours des dernières décennies (Damour et al., 2010). Les échanges gazeux entre les plantes et l'atmosphère ont ainsi été modélisés à des échelles et temporalités diverses. En écologie, certaines approches consistent à décrire la réponse globale d'un système végétal. Néanmoins, cette échelle d'approche ne permet généralement pas d'appréhender les mécanismes impliqués dans la réponse stomatique aux conditions environnementales. A une échelle réduite, de nombreux modèles intégrés uniquement au niveau de la plante ou de la feuille ont été proposés (Damour et al., 2010). La majorité de ces modèles sont empiriques, basés sur les corrélations statistiques entre les facteurs environnementaux, internes aux plantes et la conductance stomatique (g_s). Par ailleurs, seule une faible proportion de ces modèles adopte des approches fondamentalement mécanistiques. La littérature dispose ainsi de nombreux modèles semi empiriques reposant sur des hypothèses physiologiques et intégrant un nombre variable de facteurs influant sur le degré d'ouverture stomatique. La majorité des modèles développés sont des modèles dits « steady states » n'intégrant pas le délai de réponse des stomates à un changement environnemental. La réponse temporelle des stomates à un changement environnemental a longtemps été négligée malgré quelques travaux préliminaires (Kirschbaum et al., 1988). Ce n'est que récemment que des modèles décrivant plus finement la dynamique de réponse stomatique ont été proposés (Damour et al., 2010 ; Vialet-Chabrand et al., 2013b). En effet, établir clairement l'ensemble des mécanismes impliqués dans la réponse des stomates à leur environnement, comprendre l'ensemble des couplages et interactions des différents stimuli et intégrer la vitesse de réponse à laquelle les plantes répondent à ces stimuli pourrait alors revêtir une importance majeure dans un contexte de changements climatiques afin d'anticiper la manière dont les stomates intégreront les changements en [CO_2], température, humidité de l'air et du sol.

Impacts de la dynamique de réponse stomatique sur les échanges gazeux foliaires

Les stomates contrôlent les échanges gazeux entre la feuille et l'environnement. En conséquence, les ajustements de l'ouverture stomatique aux changements de conditions environnementales et des facteurs internes à la plante déterminent la diffusion de CO_2 au sein de la feuille ainsi que la perte d'eau par transpiration (Lawson, 2018). De fait, la régulation des flux gazeux à travers la feuille est essentielle au maintien d'une température foliaire viable ainsi qu'à l'assimilation du carbone atmosphérique nécessaire à la production de biomasse tout en conservant une balance hydrique fonctionnelle au sein de la plante. La relation entre l'assimilation et la conductance stomatique est démontrée de longue date (Wong et al., 1979). Néanmoins, la réponse stomatique est généralement plus lente que la réponse photosynthétique (Lawson & Blatt, 2014 **;** Lawson & Vialet-Chabrand, 2018). Il en résulte une réponse asynchrone des deux composantes de l'efficience d'utilisation de l'eau intrinsèque ayant potentiellement pour conséquence l'induction d'une efficience transitoire suboptimale (Matthews et al., 2017 **;** McAusland et al., 2016). La caractérisation de la dynamique de réponse fait depuis peu l'objet d'une grande attention, néanmoins l'ensemble des mécanismes physiologiques impliqués ainsi que les facteurs influençant la réponse temporelle des stomates restent à être finement élucidés.

La lumière est l'un des paramètres environnementaux impactant à la fois l'assimilation photosynthétique du carbone (A_n) et la conductance stomatique (g_s). Dûe à la couverture nuageuse ainsi qu'à l'ombrage au sein de la canopée causé par la superposition des feuilles ou par les plantes voisines, la quantité de lumière atteignant une feuille fluctue au cours de la journée. Les feuilles sont ainsi soumises à des phases d'illumination ou d'obscurité (respectivement nommés « sunfleck » et « shadefleck » au sein de la littérature) dont les durées varient de quelques secondes à quelques heures (Lawson & Vialet-Chabrand, 2018). Des variations inter et intra-spécifiques considérables de la rapidité et de l'amplitude de réponse de la conductance stomatique en réponse à un changement de lumière ont été observées (Cardon et al., 1994 ; Elliot-Kingston et al., 2016 ; McAusland et al., 2016). Celles-ci dépendant aussi bien du type des cellules de garde (Hetherington & Woodward, 2003 ; McAusland et al., 2016) que des conditions de croissance (Qu et al., 2016 ; Hepworth et al., 2018) ou encore de l'intensité du stimulus environnemental (Elliot-Kingston et al., 2016 ; Hepworth et al., 2018). Par ailleurs, l'énergie lumineuse perçue par la plante est également susceptible de générer des fluctuations rapides mais néanmoins importantes de la température foliaire et par extension du déficit de pression de vapeur d'eau entre la feuille et l'atmosphère, deux stimuli environnementaux auxquels les stomates sont également sensibles. L'intensité ainsi que la durée des signaux lumineux influencent les réponses temporelles de l'assimilation, la conductance stomatique et l'efficience d'utilisation de l'eau qui en résulte (Vialet-Chabrand et al., 2016). Ce faisant, un changement abrupt de lumière

constitue un ensemble de stimuli complexe, impliquant des voies de signalisation multiples. L'amplitude de réponse stomatique entre deux steady-state ainsi que la rapidité de cette réponse a des implications sur la limitation qu'exerce la conductance stomatique sur l'assimilation et l'efficience d'utilisation de l'eau qui en résulte (Kaiser et al., 2015 **;** figure 5).

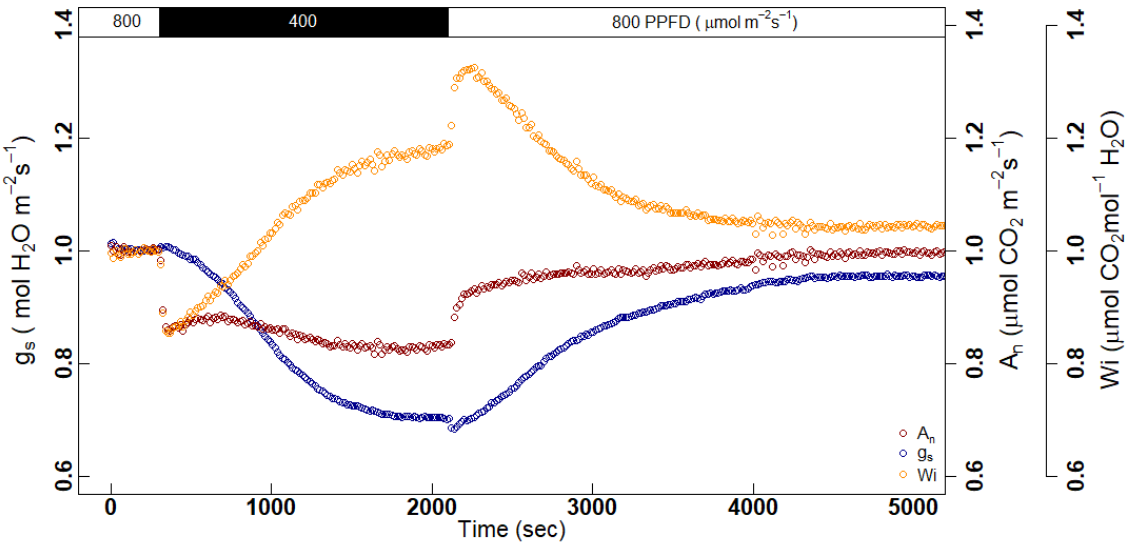

Figure 5 : Visualisation de la dynamique de réponse de l'assimilation en carbone (A_n : courbe rouge), de la conductance stomatique (g_s : courbe bleue) et d'efficience d'utilisation de l'eau intrinsèque qui en résulte (Wi : courbe jaune) à la suite de changements de lumière chez un jeune chêne sessile. Les valeurs ont été normalisées sur la base du steady-state initial.

Sur la base de modélisations, une réponse synchrone de A_n et g_s induirait une augmentation théorique de l'efficience d'utilisation de l'eau intrinsèque en raison d'un meilleur couplage de ses deux composantes (Lawson & Blatt, 2014). Un faible stimulus lumineux n'implique pas nécessairement un changement de conductance stomatique, bien qu'il puisse influencer l'assimilation (Lawson et al., 2012). Des changements plus intenses et de longue durée peuvent en revanche induire une réponse d'ouverture ou de fermeture stomatique à la forme sigmoïdale plus ou moins rapide. La rapidité de réponse aura de larges implications sur la relation qu'entretiennent la conductance stomatique et l'assimilation. Ainsi, la disposition des plantes à répondre rapidement à leur environnement semble essentielle à l'optimisation des échanges gazeux et ce plus particulièrement chez les plantes soumises à des conditions de stress hydrique pour lesquelles l'adoption d'un comportement stomatique plus rapide s'avérerait avantageux en termes d'efficience d'utilisation de l'eau (figure 6).

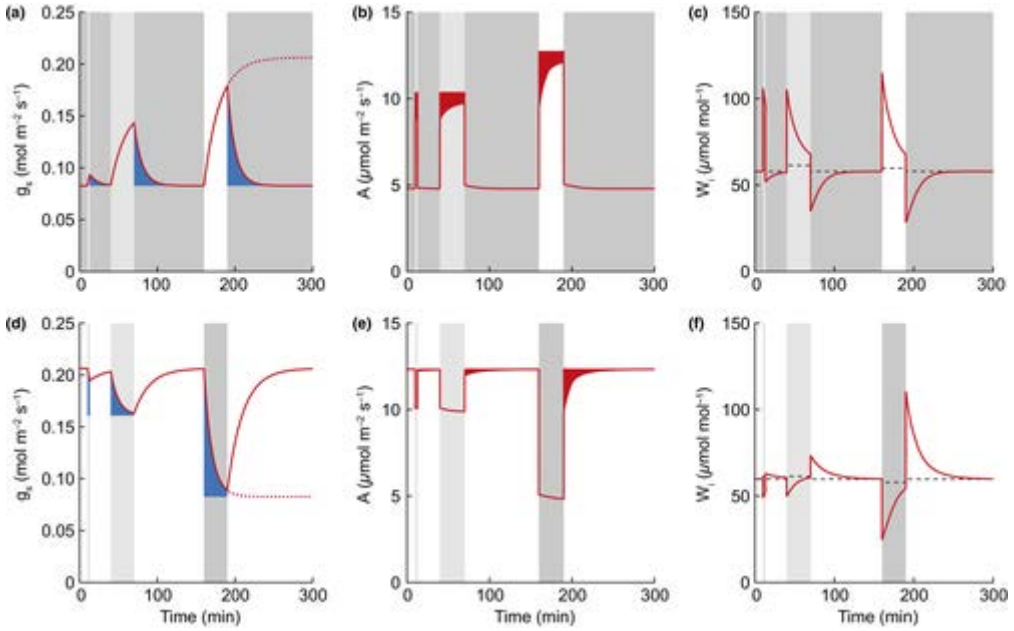

Figure 6 : Représentation schématique de la réponse temporelle de la conductance stomatique, de l'assimilation en carbone et de l'efficience d'utilisation de l'eau intrinsèque (Wi) suite à des phases d'illumination (a-c) ou d'obscurcissement (d-f) de différentes durées et intensités. Les portions sous fond blanc représentent les phases lumineuses et les portions sous fond gris, les phases d'obscurité. Les zones bleues illustrent les pertes non nécessaires d'eau (par rapport à A) en raison d'une réponse de la conductance stomatique asynchrone et lente. Les zones rouges illustrent la limitation de A par la conductance stomatique lors de l'ouverture stomatique. Les lignes pointillées noires (c,f) schématisent Wi dans les cas théoriques pour lesquels la réponse de g_s est instantanée suite au changement de lumière (d'après Lawson & Vialet-chabrand 2018).

Facteurs influençant la dynamique

Parmi les facteurs susceptibles d'influencer la rapidité de réponse stomatique à un stimulus environnemental, la morphologie stomatique a souvent été proposée. En outre, les différentes morphologies et les propriétés mécaniques qui en découlent sont susceptibles d'expliquer les différences de dynamique entre différentes espèces et écotypes. Ainsi, chez les fougères, la réduction des avantages mécaniques des cellules épidermiques par rapport aux cellules de garde serait à l'origine de mouvements plus rapides par leurs stomates (Franks & Farquhar, 2007).

La réduction de la taille des cellules de garde entourant le pore stomatique a pour effet d'augmenter le ratio de la surface membranaire par rapport au volume de la cellule. En partant du principe que le nombre de canaux de transport par unité de surface demeure constant, une diminution de la taille des cellules de garde devrait théoriquement accélérer proportionnellement le flux de composés osmotiques par unité de volume. Il en résulterait ainsi une réponse stomatique plus rapide (Schlüter et al., 2003**;** Tanaka et al., 2013**;** Franks and Farquhar, 2007; Drake et al., 2013; Lawson & Blatt, 2014**).** Une série de modèles intégrant la morphologie des cellules de garde et du complexe stomatique (Doheny-Adams et al., 2012 ; Dow et al., 2014 **;** Lehman & Or, 2015) ainsi que l'activité de transport membranaire (Chen et al., 2012**;** Wong et al., 2012**;** Lawson & Blatt, 2014) tendent à conforter l'influence de l'anatomie stomatique sur la vitesse de réponse. Cependant ces relations ne

seraient pas systématiquement valides lorsque l'on considère une large gamme d'espèces **(**Elliott-Kingston et al., 2016 ; McAusland et al., 2016). Ainsi Elliot-Kingston et al., (2016) dans une étude examinant la diversité de la rapidité de fermeture stomatique parmi des espèces aux écotypes variés ne fut pas en mesure d'établir cette relation entre morphologie stomatique et rapidité de réponse. McAusland et al., (2016) parvinrent au même constat parmi une gamme d'espèces de grande culture disposant de stomates de type elliptiques. Néanmoins, un ensemble de traits sont susceptibles d'influencer la rapidité de réponse indépendamment de la taille ou de la densité stomatique, tels que le nombre de cellules subsidiaires impliquées dans les échanges osmotiques, l'expression génique des canaux hydriques ainsi que des transporteurs des composés osmotiques ou encore l'activité biochimique au sein des cellules de garde **(**Farquhar et al., 2007**).** De récents travaux ont par ailleurs mis en évidence un impact des niveaux de transcription des facteurs nécessaires au développement des cellules subsidiaires sur la dynamique de réponse stomatique ainsi que la conductance stomatique **(**Raissig et al., 2017).

Par ailleurs, la mise en place de l'architecture stomatique au cours du développement de la feuille influencerait le comportement des stomates. La majorité des espèces végétales suivent en effet la règle du « one-cell spacing » au cours du développement épidermique, règle suivant laquelle lors de leur mise en place les stomates sont séparés les uns des autres par au moins une cellule épidermique (Geisler et al., 2000 ; Peterson et al., 2010; Pillitteri & Dong, 2013). Il existe cependant un certain nombre d'espèces s'étant affranchies de ces schémas de développement, à l'image du genre Begonia (Nebauer, 1967 **;** Papanatsiou et al., 2017**).** Une telle architecture stomatique chez Begonia est interprétée comme étant une adaptation à une niche écologique imposant une faible demande évaporative. Outre les schémas d'architectures, la structure interne des stomates pourrait également jouer un rôle dans les mouvements stomatiques à travers l'organisation des filaments d'actine (Eisinger et al., 2012) ainsi que les propriétés physiques de la paroi cellulaire (Woolfenden et al., 2017).

Ainsi, l'anatomie stomatique (taille, densité…) est susceptible d'impacter d'une part la conductance stomatique et donc Wi mais aussi d'autre part la dynamique de réponse aux changements environnementaux (Drake et al., 2013). Cette même dynamique de réponse stomatique peut à son tour influencer le gain de biomasse et la consommation hydrique des plantes sur le long terme et donc l'efficience de transpiration (TE ; Vialet-Chabrand et Lawson,2018; Papanastasiou et al., 2019).Si l'accumulation de biomasse est par définition une composante de l'efficience de transpiration, l'allocation du carbone à l'origine de cette biomasse est un élément fondamental à prendre en considération. En effet, comme mentionné plus tôt au sein de ce manuscrit les schémas d'allocation et d'investissement du carbone constituent un facteur essentiel impliqué dans l'écologie des espèces.

Dans le but d'adresser plus finement l'écologie des espèces ainsi que la différenciation de leurs niches écologiques respectives, il convient dès lors de caractériser ces schémas, la plasticité de ceux-ci sous des conditions de stress (notamment hydrique) et leurs possibles relations avec dynamique de réponse stomatique (Figure 7).

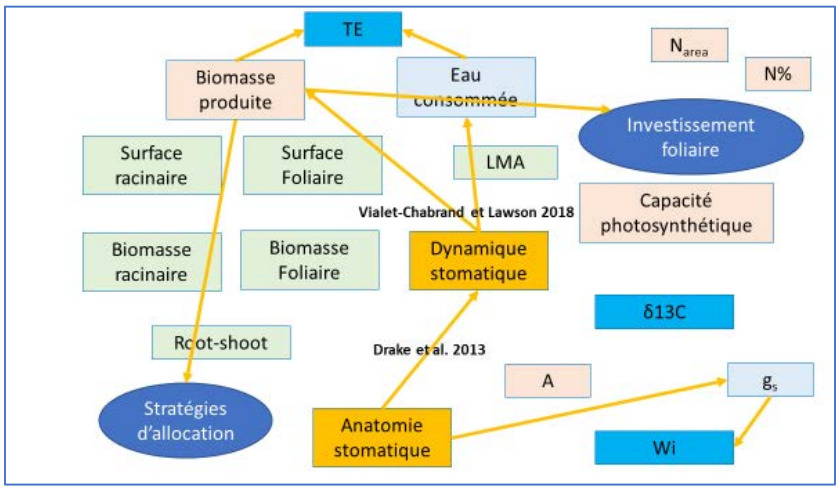

Figure 7 : liens théoriques entre anatomie stomatique (tailles, densité, longueur… des stomates) et paramètres descriptifs de la réponse dynamique à un changement environnemental (τ, λ, SL) ainsi que leurs possibles influences sur les échanges gazeux foliaires intégrés dans le temps (TE et ses composantes) et *in fine* les stratégies d'allocation et d'investissement du carbone réalisées par les plantes. Les flèches jaunes matérialisent les relations explorées dans le cadre de ces travaux

Considérations biochimiques des mouvements stomatiques

Les mouvements stomatiques sont la résultante de changements de pression de turgescence liés à l'ajustement osmotique en réponse aux flux potassiques (K+), chloriques (CL-) et d'anions organiques (malate) à travers la membrane plasmique et le tonoplaste (Blatt, 2000). La rapidité de réponse stomatique est donc intrinsèquement liée à l'activité de transport des solutés ainsi que la sensibilité de leurs voies de signalisation aux stimuli environnementaux (Lawson & Blatt, 2014 **;** Lawson & Vialet-chabrand, 2018). Le transport est déterminé par la densité ainsi que la capacité d'action des transporteurs disposés à la surface membranaire, elle-même liée au ratio de surface par volume des cellules et donc par extension à la taille des stomates (Franks et Farquhar, 2007). Cependant, il existe une large diversité interspécifique de l'activité de transport indépendante des traits anatomiques des stomates expliquant potentiellement le manque de corrélation entre rapidité de réponse et taille stomatique observé dans les récentes études traitant ce sujet **(**Lawson & Blatt, 2014**).** La manipulation des flux ioniques constitue une approche valable dans le but d'explorer la rapidité de réponse des stomates. Cependant l'osmorégulation ainsi que les voies de transduction des signaux au sein des cellules stomatiques sont des phénomènes complexes qu'il convient d'adresser à des fins d'optimisation des échanges gazeux foliaires et in fine de l'efficience d'utilisation de l'eau des plantes.

Objectifs

Il semblerait que la dynamique de réponse stomatique puisse jouer à long terme un rôle non négligeable sur l'efficience d'utilisation de l'eau des végétaux. L'accélération des mouvements stomatiques permet théoriquement d'améliorer l'assimilation de carbone et de limiter les pertes en eau inutiles par les plantes, optimisant ainsi leur efficience d'utilisation de l'eau (Papanatsiou et al., 2019). A ce jour de nombreuses études ont exploré la rapidité de réponse stomatique parmi une large gamme d'espèces associant la variabilité interspécifique observée à l'anatomie stomatique. Néanmoins, les informations dont nous disposons pour décrire la dynamique de réponse demeurent lacunaires. Par ailleurs il n'existe que peu d'études ayant étudié l'impact des conditions de croissance sur la dynamique de réponse stomatique. De récentes études ont suggéré que le statut hydrique des plantes (Qu et al., 2016 **;** Haworth et al., 2018) mais aussi la disponibilité en lumière (Matthews et al., 2018) au cours de leur développement influenceraient considérablement la réponse stomatique. L'asymétrie entre fermeture et ouverture stomatique constitueraient également une adaptation des écotypes végétaux à des environnements spécifiques notamment vis-à-vis de l'accès à la lumière et à l'eau (Ooba & Takahashi, 2009 ; Vico et al., 2011). Une asymétrie plus marquée due à une ouverture stomatique lente traduirait ainsi un comportement conservateur vis-à-vis de l'eau ; à l'inverse une ouverture rapide optimiserait l'assimilation carbonée. Ce caractère est donc susceptible de traduire le compromis qu'effectuent les plantes entre fixation du carbone et perte hydrique, afin de limiter le coût que requièrent les mouvements stomatiques en termes d'énergie et de changements osmotiques.

Les résultats ici mentionnés ont été obtenus suite à une étude sur le tabac réalisée au sein du laboratoire d'écologie et de physiologie végétale de l'Université des iles Baléares dans le cadre de la labelisation "Agreenium" par l'institut agronomique, vétérinaire et forestier de France (IAVFF). Outre l'aspect méthodologique, cette expérience a été réalisée dans le but de répondre à deux questions principales:

- Les conditions environnementales de croissance impactent-elles la dynamique de réponse stomatique suite à un changement d'intensité lumineuse chez Nicotiana tabacum?
- La morphologie stomatique impacte-t-elle cette même dynamique de réponse?

Article 1: *Gerardin et al. 2018: Shade and drought growth conditions strongly impact dynamic responses of stomata to variations in irradiance in Nicotiana tabacum : Environmental and Experimental Botany (publié:* https://doi.org/10.1016/j.envexpbot.2018.05.019*)*

Article 1: Introduction à la dynamique de réponse stomatique

Shade and drought growth conditions strongly impact dynamic responses of stomata to variations in irradiance in *Nicotiana tabacum*

Authors: Théo Gerardin[1,2], Cyril Douthe[2], Jaume Flexas[2], Oliver Brendel[1,3]

Adresses:

[1] Université de Lorraine, AgroParisTech, INRA, Silva, F-54000 Nancy, France

[2] Research Group on Plant Biology under Mediterranean Conditions, Universitat de les Illes Balears, Instituto de Investigaciones Agroambientales y de Economía del Agua (INAGEA), Carretera de Valldemossa Km 7.5, Palma de Mallorca, Illes Balears 07121, Spain

[3] Corresponding author postal address: Oliver BRENDEL, INRA, UMR Silva, F-542802 CHAMPENOUX, France

Tel : 0033.383394100

Emails:

theo.gerardin@inra.fr, cyrildouthe@me.com, jaume.flexas@uib.es, oliver.brendel@inra.fr

Keywords: drought, dynamic response, irradiance, shade, stomatal conductance

Published in Environmental and Experimental Botany 153 (2018) 188–197

https://doi.org/10.1016/j.envexpbot.2018.05.019

Received 9 February 2018; Received in revised form 24 May 2018; Accepted 24 May 2018

Abstract

Recent work has made progress in describing stomatal dynamics in terms of speed, amplitude of response, lag time and response time. However, little is known about the impact of growth conditions on the rapidity of stomatal movements, and their relationship with stomatal morphology within a species. We measured stomatal dynamics during opening and closing in response to changes in irradiance in tobacco plants (*Nicotiana tabacum*) grown under "Control", "Drought" and "Shade" treatments. Growth conditions strongly changed the rapidity of stomatal conductance response to irradiance. The "Drought" treatment considerably accelerated the response and "Shade" treatment slowed it down when compared to "Control". We confirmed for the "Control" treatment the known asymmetry of response, with closing faster than opening, but interestingly the asymmetry disappeared under both treatments. Only stomatal density and index were affected by the growing conditions, not stomatal size and form. Thus, the observed variation in stomatal closing and opening speed and related parameters (amplitude, lag time, response time) was not related to the size of the stomata, and only a marginal relationship between speed of the stomatal response and stomatal density was observed. These results suggest that physiological factors might be the main driver of variations in stomatal conductance dynamics within a species grown under different environmental conditions.

Highlights

Growth conditions (drought/shade) change dynamics of stomatal response to irradiance

Growth conditions (drought/shade) change asymmetry between opening and closing

Within species variation in stomatal speeds were not explained by stomatal size

Introduction

Stomatal morphology and their movements (opening and closing) are key components controlling exchange of water vapour and CO_2 between the leaf and the atmosphere, expressed as stomatal conductance (g_s). Stomatal conductance per leaf surface is mainly determined by stomatal density, size, pore area (aperture) and their distribution.

Since plants are subjected to a fluctuating environment through a diurnal cycle, with important variations of irradiance, vapour pressure deficit between the leaf and the atmosphere (VPD), temperature and soil water deficit, they need to balance gas exchange by adjusting stomatal conductance continuously during the day (Schulze and Hall, 1992; Pearcy et al., 2000). Usually stomata respond to low CO_2, low VPD and high irradiance by increasing their level of aperture, inducing an increase of g_s and vice versa (Outlaw, 2003; Haworth et al., 2018). Nevertheless, these environmental changes usually occur concomitantly, making g_s a complex resultant of various signals induced by their own signalling pathways and in a hierarchical manner (Lawson & Morison 2004; Lawson et al., 2010; Aasamaa & Sober, 2011; Haworth et al., 2018).

To describe the stomatal behavior numerous steady-state models of g_s have been proposed (reviewed in Damour et al., 2010). However stomatal conductance variations induced by environmental changes are not instantaneous and show a temporal response that can be described by dynamic models (Vialet-Chabrand et al., 2017). Changes in stomatal aperture are performed via variations of water content of the guard cells, which are in turn produced by fluxes of potassium ions (K^+) in or out of the guard cell (Blatt, 2000; Shimazaki et al. 2007). Kirschbaum et al. (1988) proposed a temporal model where the dynamic response of g_s to irradiance was first initiated by a biochemical signal responding to the environmental change, followed by an osmotic adjustment inside the guard cells resulting in stomatal opening. The combination of these processes described the response of g_s as a sigmoidal curve. The dynamic change of g_s to atmospheric environmental variations takes from a few minutes to almost an hour, depending on species and irradiance variation (Vico et al., 2011; McAusland et al., 2016). Compared to stomatal dynamics in response to changes in irradiance, the variation of net CO_2 assimilation (A_n), if not limited by g_s, varies much faster, usually within a few seconds. A few studies highlighted the importance of response times of photosynthesis for carbon uptake (reviewed in Kaiser et al., 2018), however as these are generally an order of magnitude faster than changes in g_s, variation in the latter might be dominant in non-synchronicity situations.

Irradiance is the main environmental driver of photosynthesis, therefore the stomatal response to fluctuating light has been extensively studied (Kirschbaum et al., 1988; Shimazaki et al., 2007; Lawson et al., 2010 ; McAusland et al., 2016 ; Kardiman and Raebild, 2017 ; Matthews et al., 2018). Over time these fluctuations drive the temporal dynamics of carbon gain, water loss and by extension plants water use efficiency (Lawson and Blatt, 2014). Stable environmental conditions rarely occur in nature, therefore field measurements of g_s are unlikely to reach steady-states values (Lawson et al., 2010) resulting in decoupled A_n and g_s measures and

a non-representative Wi estimation (Lawson et al., 2010; McAusland et al., 2016, Vialet-Chabrand et al. 2017). A non-synchronicity in the temporal response between A_n and g_s can have repercussions on carbon fixation, the water lost by transpiration and long-term water use efficiency (McAusland et al., 2016). Due to the importance of dynamic stomatal regulation, modelling of g_s responses to environmental changes can improve the up-scaling of CO_2 and water vapour exchange from the leaf to the canopy level (Vialet-Chabrand et al., 2017). To parametrize such models, it is important to gain knowledge on the impact of growth conditions on the stomatal dynamics.

Morphological traits such as stomatal density and size regulate steady-state values of g_s (Franks and Farquhar, 2001) and set the theoretically achievable maximum stomatal conductance by the plant (Dow et al., 2014). A variation in stomatal morphology can lead to improved instantaneous and long-term water use efficiency, by impacting directly only g_s, but not A (Doheny et al., 2012; Franks et al., 2015). It has also been shown that an increase in g_s under high irradiance conditions was associated to an increase of stomatal density (Schlüter et al., 2003). Stomatal morphology and patterning is known to be influenced by both environmental growth conditions and plant hormones (Woodward, 1987; Hetherington and Woodward, 2003; Casson and Gray, 2008; Kardiman and Raebild, 2017). In tobacco leaves, Thomas et al. (2004) observed a 12.7-24.2% decrease of stomatal index (ratio between number of stomata to total number of epidermal cells) of developing leaves exposed to shading compared to a control treatment. Although growth conditions varying in atmospheric CO_2 concentration and irradiance have been shown to impact stomatal morphology, little is known about other environmental factors such as soil water stress. Jones (1977) showed for barley that a reduction of soil water availability could result in a decrease in stomatal index, but this is not always consistent as reported for groundnut (Clifford et al., 1995), where the stomatal index was not changed by water stress.

It has been hypothesized (Hetherington and Woodward, 2003; Drake et al. 2013, Raven, 2014; Kardiman and Raebild 2017) that stomatal traits such has density and size might be involved in the temporal response of g_s to an environmental change. These studies, based mainly on among species comparisons, suggested that a smaller stomatal size resulted in a faster stomatal response due to the higher surface-to-volume ratio and the lower subsequent solute transport required to drive stomatal movements (Lawson et al., 2014). Other recent evidence using a very large spectrum of species, including ferns, cycads, conifers, and angiosperms (Elliot-Kingston et al., 2016) had not found a relationship between stomatal size and closing speed, however the authors suggest a relationship with the atmospheric CO_2 levels during species diversification. Variations in stomatal size are often linked to variations in stomatal density by a negative correlation (Franks and Beerling, 2009; Doheny-Adam et al., 2012). Moreover, the product of size and density gives the overall stomatal exchange surface per leaf surface, suggesting that stomatal density could also act as driver of stomatal dynamics. Also, the relationship between stomatal response times and the shape of the guard cells (Hetherington and Woodwaard, 2003; Franks and Farquhar, 2007; McAusland et al., 2016) and their

patterning (Papanatsiou et al., 2016) have been studied. It has been shown that the stomatal shape (dumbbell or elliptical) might be a determinant driver of stomatal speed as dumbbell-shaped guard cells species tend to display faster responses to environmental fluctuations (Hetherington and Woodwaard, 2003; MacAusland et al., 2016). Moreover, the stomatal shape and patterning might confer different mechanical advantages via cell osmotic and turgor pressures influencing the rapidity of stomatal response (Franks and Farquhar, 2007). However, other parameters than stomatal morphology, such as variations in ion and water transport within guard cells, are likely to impact the speed of stomatal responses (Lawson and Blatt, 2014).

Growth conditions such as shade and soil water deficit have been shown to determine steady state stomatal conductance, however, only a few studies examined their influence on stomatal dynamics. In these studies, drier conditions have been related to faster stomatal response to irradiance (Vico et al., 2011; Lawson and Blatt., 2014; Qu et al., 2016a) suggesting an influence of plant water balance on the rapidity of stomatal response. In addition, Martins et al. (2016) demonstrated in conifers and ferns a major impact of leaf hydraulic status on g_s response time to vapour pressure deficit variations. However, less is known about the impact of different light environments during growth on the dynamics of stomatal responses to a change in irradiance. Matthew et al. (2018) have shown faster stomatal responses with an increased amplitude for Arabidopsis thaliana grown under fluctuating high light, compared to plants grown under shaded conditions. However, the acclimation of stomatal dynamics to the light environment might be species specific. Kardiman and Raebild (2017) showed that, although the dynamics of stomata of most tested species remained unaffected by their light environment, early successional species displayed faster stomatal responses when grown under shade conditions compared to the ones grown under full light, while late successional species displayed the opposite behaviour.

Differences in the speed of stomatal dynamics to a variation in irradiance have been shown among species and within individuals of the same species (Vico et al., 2011; McAusland et al., 2016). Stomatal speed has also been linked to plant functional types and environmental factors (Vico et al., 2011): graminoids tended to display shorter responses than forbs, woody gymnosperms or angiosperms and plants from dryer climates seemed to exhibit faster responses. Moreover, many species show an asymmetric response between stomatal opening and closing, ie, over 60% of the species reviewed by Ooba and Takahashi (2003) displayed a faster opening. Ooba and Takahashi (2003) argued that such an asymmetry could be related to the environmental growth conditions of the different species, where a light limited environment might favour a more rapid opening of stomata. However, Woods and Turner (1971) have suggested that a faster stomatal closure would reduce the temporal decoupling between A_n and g_s while a slower opening would reduce water loss without reducing A_n when g_s is not limiting, especially under well-watered conditions. Such a temporal asymmetry of stomatal movements might lead to a reduced transpiration and thus translate a conservative stomatal behaviour. Moreover, a slow opening might prevent situations of a continued g_s increase after A_n

has reached light saturation, which would result in an excessive water loss compared to carbon gains (Kirschbaum et al., 1988; Lawson et al., 2010; Vialet-Chanbrand et al., 2017). The literature therefore suggests that the asymmetry between opening and closing of stomata might have an important ecological impact, depending on the growing conditions.

Thus, our main objectives were to analyse:

the impact of three different growth conditions (control, shade and drought) on the paremeters conditionning the temporal response of g_s to step variations in irradiance;

the relationship between stomatal morphology and the dynamics of stomatal responses

The dynamics of the temporal stomatal response to a step variation in irradiance was characterised by using the Vialet-Chabrand et al (2013) model, which decomposes the sigmoidal response into a) the delay of stomatal response, b) the response time constant and c) the amplitude of stomatal movements. From the latter two parameters a maximal speed can be calculated. The stomatal response has been tested for opening and closing, which allowed also to estimate the asymmetry for the dynamic parameters.

Material and methods

Plant material and Experimental design

The experiment lasted for 8 weeks and was carried out on eighteen plants of *Nicotiana tabaccum* L. wild type (cv. Petite Havana SR1), grown at the University of the Balearic Island (UIB), Palma, Spain, 39°38'11.9"N 2°38'49.7"E in autumn 2015. Seeds were germinated in petri boxes with humidified filter paper and after one week transferred into 2L pots filled with 1/3 (V/V) of perlite and organic soil, respectively.

The two weeks old seedlings were then randomly divided into three growing condition groups until the end of the experiment 6 weeks later: "Control" (6 plants), "Shade" (7 plants) and "Drought" (5 plants). The "Control" treatment was characterized by ambient growth irradiance (400-450 µmol photons m^{-2} s^{-1}) and well-watered conditions. The "Shade" treatment was characterized by low irradiance (40 µmol photons m^{-2} s^{-1}) and the same well-watered conditions as in the "Control" treatment. The irradiance compensations point estimated from steady-state irradiance response curves (data not shown) were estimated at 30µmol m^{-2} s^{-1} for "Control" and 27 µmol m^{-2} s^{-1} for "Shade" treatments; 90% of maximum A was reached at about 1100µmol m^{-2} s^{-1} for "Control" and at about 990µmol m^{-2} s^{-1} for "Shade". Plants in "Control" and "Shade" treatments were watered every second day with Hoagland's solution, 50% dilution. The "Drought" treatment was characterized by the same ambient growth irradiance as the "Control" treatment, but the plants were submitted to a soil water deficit. The water deficit was controlled by weighing the pots at field capacity and watering to 50% of the weight. During the experiment the field capacity weight was retested on the plants of the "Control" treatment on a regular basis and the difference assumed to be the plant growth and added to the target weights of the "Drought" treatment. Before application of the treatments, the last emergent leaf was marked to ensure gas exchange measurement were done on leaves grown under treatment conditions. Other conditions in the growing chamber were 25ºC, air relative humidity 50-60% and a photoperiod of 12h/12h (8:00-20:00).

Measurement of leaf water potential

To determine the plant water status, midday leaf water potential (Ψ) was measured in fully expanded leaves with a Scholander pressure chamber (Soil moisture Equipment Corp., Santa Barbara, CA, USA). At the end of the experiment, seven leaves were measured for the control treatment from different plants and 4 leaves for each, the shade and the drought treatment.

Gas exchanges measurements

Gas exchange was measured using a portable photosynthesis system (LI-COR 6400; LI-COR, Lincoln, NE, USA) equipped with a 2cm² leaf chamber (Li-6400-40). Measured were: net CO_2 assimilation rate (A_n), stomatal conductance for water vapour (g_s) and leaf internal CO_2 concentration (C_i) (see Table 1 for units). All measurements were carried out between 10:00 and 19:00 h (Central European summer time). For each plant measured, gas exchange measurements were performed on the youngest, mature, fully expanded leaf, which

was grown under treatment conditions. This leaf has been measured three times at different days and different times during the day and a plant mean has been used in all statistics. Overall, the measurement of the stomatal response curves has taken 18 days. The environmental parameters inside the chamber were kept constant during the acclimation phase with [CO_2] entering the chamber of 400 µmol mol^{-1}, block temperature of 25°C, air flow of 300 µmol min^{-1} and a PPFD of 1500 µmol m^{-2} s^{-1} (red/blue irradiance 90/10%, respectively) until the leaf reached a steady-state of g_s (SS1; Fig. 1a). Then a measurement cycle consisted of two step changes in irradiance: first I) a single step change to low irradiance inducing stomatal closure and then II) a single step change back to the original high irradiance, inducing a stomatal reopening. For the low irradiance step, the PPFD was lowered from 1500 to 100 µmol.m^{-2} s^{-1} until the plant reached a new steady-state (SS2). After 10 minutes under this new steady-state, the PPFD was set back to its initial setting at 1500 µmol.m^{-2} $s^{-1}$and measurements were recorded until a new steady state was reached (SS3). The stomata were considered in steady-state when g_s did not vary more than ~0.005 mol m^{-2} s^{-1} during 10min. This resulted in a standard deviation over the 10 minutes of 0.0015 mol m-2 s-1. Data during the response curves were logged every 60sec. "Steady-state" data as mentioned through the manuscript were calculated for SS1 and SS2 as the mean of 5 points after stabilization of g_s, before each irradiance change (Fig. 1a).

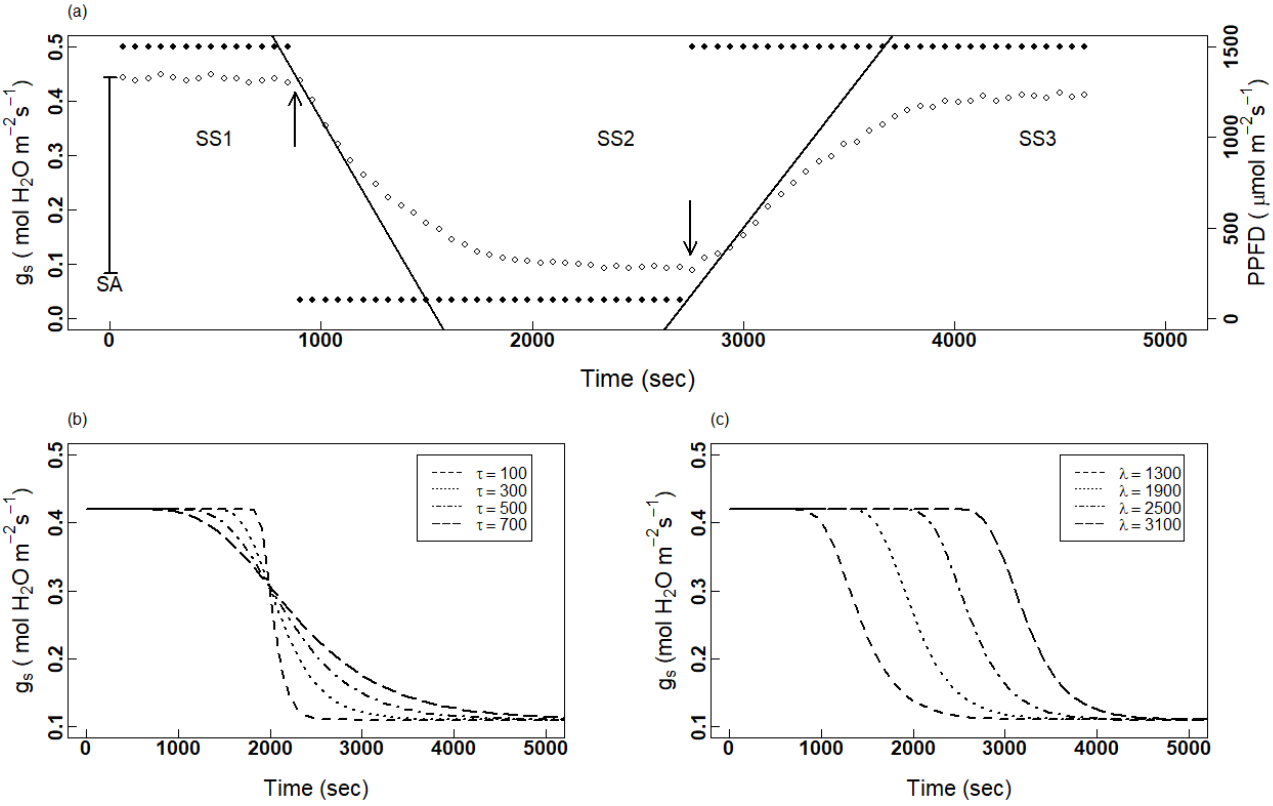

Figure 1: Illustration of the stomatal kinetics under irradiance changes. a: Example of a measured stomatal closure and opening (white dots) provoked by a change in irradiance (black dots). Black arrows represent the irradiance changes, the black lines are the maximal slopes (SL_{max}) of both opening and closing sequences and the amplitude of the stomatal response (SA). b: Simulation of the impact of increasing values by 200secs steps of □□ (response time) on the curvature, and c: Simulation of the impact of 600secs steps increasing values of □ (lag time) on the stomatal delay.

Stomata morphology

At the end of the experiment one leaf was sampled from five plants per treatment to determine the stomatal morphology of leaves on the abaxial and adaxial faces. Specifically, the following parameters were measured : the stomatal density (SD), epidermal cell density (CD), stomatal index (SI) defined as SD/(SD+CD), length of the stomatal guard cell complex (GCL), guard cells width (GCW), stomatal surface (SS) defined as an ellipse area: π*(GCL/2)*(GCW/2) and guard cell shape (GSH) defined as the ratio GCL/GCW. 1cm² portions of the leaves were collected and nail polish imprints of both leaf surfaces were taken using adhesive film and applied on microscope slides for analysis. Stomata and epidermis cells were counted in the obtained images using the ImageJ2 software (Schindelin et al. 2015). Six (500*370µm) images for both abaxial and adaxial surfaces of the leaf used for gaz exchange were taken per leaf (~200 stomata measured by treatment). For all stomatal morphology traits, mean values of adaxial and abaxial surfaces were used for ANOVA and correlations with gas exchange results, as gas exchanges is done for the whole leaf.

Model description

The stomatal responses of the irradiance curves were adjusted using a sigmoidal model based on Vialet-Chabrand et al. (2013). The sigmoidal model allows the estimation of parameters describing the temporal response of the stomata to an environmental change. The following equation was used:

$$gs = r0 + (G - r0) * exp\left(-exp\left(\frac{\lambda - t}{\tau}\right)\right)$$

where g_s is the fitted stomatal conductance, r0 is the starting value of the stomatal conductance (first steady-state obtained after the plant acclimation to the environmental conditions inside the Licor chamber, g_{min} or g_{max}), G the ending value of stomatal conductance (second steady-state reached after the full stomatal response to the irradiance change, g_{max} or g_{min}), λ is the lag time of the stomatal response (time needed to reach the inflection point of the curve from the moment of the irradiance change in each curve), and τ the response time. Compared to the sigmoidal equation used by Vialet-Chabrand et al. (2013), here, λ is mathematically independent from τ. From these parameters, the maximum slope (SL_{max}) as estimator of the speed of the stomatal response, can be calculated as:

$$SLmax = (1/\tau) * (G - r0)/exp$$

Where (G-r0) represents the amplitude of the stomatal response (SA). Increasing values of τ will affect the curvature of the stomatal response, the smaller a τ value is, the stronger the curvature and the higher SL_{max} will be, so the more rapidly g_s will increase/decrease.

This model was adjusted using the function “nlminb” of R (Team RC, 2015). To facilitate the adjustment of the sigmoidal model, five data points during the steady state before changing the irradiance were first included in the model adjustment. This affects only the lag time λ, which was then corrected by subtracting the added time period. The model adjustment is sensitive to the starting point and including five steady state

points made the starting steady state g_s more robust and decreases the dependency of the adjustment on measurement noise.

As SS1 and SS3 values for gas exchange variables were not significantly different (pairwise t-test p> 0.05) the mean amplitude between closing and opening in response to the step-change in irradiance were calculated for A_n and g_s as absolute and relative values (SA and RSA,respectively; Table 1) :

SA_{An} = ((*An* SS1 – *An* SS2) + (*An* SS3-*An* SS2)) /2

SA_{gs} = ((g_s SS1 – g_s SS2) + (g_s SS3-g_s SS2)) /2

RSA_{An} = (((*An* SS1 – *An* SS2) / *An* SS1) + ((*An* SS3-*An* SS2) / *An* SS3)) /2

RSA_{gs} = (((g_s SS1 – g_s SS2) / g_s SS1) + ((g_s SS3- g_s SS2) / g_s SS3)) /2

For the adjusted dynamic parameters, we also calculated the ratio between opening and closing, representing the asymmetry of the response. Further, the ratio between τ and λ was calculated, to characterise the relative impact of different treatments on both parameters.

Statistical analysis

Before statistical analysis, a mean was calculated for all repeated measurements on one leaf (~3). This allowed the estimation of leaf level parameters for gas exchange traits, which facilitated the comparison with leaf morphology traits. All subsequent statistics were based on mean-leaf values.

All statistical analyses were performed with R (Team RC, 2015). Treatment effects were analysed as a one factorial design analysis of variance (ANOVA). Significant differences were considered at $p < 0.05$. When the ANOVA showed a significant treatment effect, a Post-Hoc test using Tukey-HSD (package R, “agricolae”) was used to estimate significance of inter-group differences. Correlations were estimated using the Pearson method and p-values were adjusted for multiple comparisons using the “p.adjust” function with the “FDR” method.

Results

Steady-state gas exchange parameters under three different growing conditions

The different growing conditions significantly affected Ψ of tobacco plants, with the highest value for the "Shade" treatment (-0.37±0.06 Mpa), without being significantly different from the "Control" treatment (-0.51±0.09 Mpa, p>0.05 TukeyHSD), while the "Drought" treatment significantly decreased Ψ to –1.46±0.18 Mpa (p>0.05 TukeyHSD). A_n values measured under saturating irradiance (SS1, see Fig. 1) were significantly different among treatments, where the "Control" treatment showed the highest value and "Shade" the lowest (Table 1). g_s values differed among the treatments, showing the highest values in "Control" and the lowest in "Drought". These strong differences for A_n and g_s were associated with significantly different C_i among treatments, with the highest C_i in the "Shade" and the lowest under "Drought".

Decreasing irradiance from high to low intensity (SS1 to SS2, see Fig. 1), A_n and g_s decreased drastically as expected (Table 1), but not C_i. A_n and g_s were lower in "Drought" than in "Control" and "Shade". For C_i, no significant differences were detected among the three steady-states, within each treatment. Interestingly, when measurement-irradiance was increased back to high intensity (SS3), plants recovered in each case to the same values as for SS1, for each parameter and whatever the growing conditions.

Amplitude of response under changing measuring irradiance

For both A_n and g_s, their respective variations were similar during closing and opening as there was no significant difference between the two high-irradiance steady state measurements (SS1 and SS3; pairwise t-test p > 0.05) indicating a full reopening, therefore the analysis of the absolute and relative amplitude of the responses (SA and RSA (%), respectively) was based on the mean values of closing and opening sequences (presented in Table 1). The absolute amplitude of the A response (SA_{An}) was highest for "Control" and lowest for "Shade, where the absolute amplitudes for gs (SA_{gs}) were highest for "Control", but lowest for "Drought". Although the SA were significantly different among treatments for both A and g_s, the relative amplitudes (RSA) were only significantly lower for the "Shade" treatment for both traits.

Table 1: Means per treatment (± standard error of the mean) of net CO_2 assimilation (A_n), stomatal conductance (g_s), internal CO_2 concentration (C_i) at each steady-state reached during the measurement cycle (SS1-SS2-SS3, see Figure 1a for details). The amplitude of variation of the parameters between each steady state (SA and RSA, respectively in absolute values and percentage). For C_i, amplitude of variations was not shown as no differences between the steady states were observed. Different letters show the significant differences among treatments using a Tukey-HSD test. "*" represents the significant differences between two steady states from a paired t-test at $p<0.05$. There was no significant difference between SS1 and SS3, which was therefore not indicated in the table.

Treatments		SS1		SS2		SS3	SA	RSA (%)
Control	A_n	19.9 ± 0.4^{a} (15)	*	4,7 ± 0.4^{a} (16)	*	20.3 ± 0.4^{a} (16)	15.5 ± 0.2^{a} (15)	77.0 ± 1.6^{a} (15)
Drought	(µmol m^{-2} s^{-1})	11.1 ± 0.5^{b} (18)	*	2.8 ± 0.2^{b} (17)	*	12.1 ± 0.7^{b} (17)	8.9 ± 0.7^{b} (17)	75.2 ± 2.9^{a} (17)
Shade		8.5 ± 0.5^{c} (18)	*	3.7 ± 0.2^{ab} (18)	*	7.9 ± 0.7^{c} (18)	4.6 ± 0.6^{c} (18)	54.2 ± 2.8^{b} (18)
Control	g_s	0.41 ± 0.03^{a} (15)	*	0.11 ± 0.01^{a} (16)	*	0.38 ± 0.03^{a} (16)	0.28 ± 0.02^{a} (15)	72.6 ± 1.6^{a} (15)
Drought	(mol m^{-2} s^{-1})	0.08 ± 0.00^{c} (18)	*	0.02 ± 0.01^{b} (17)	*	0.09 ± 0.01^{c} (17)	0.06 ± 0.01^{c} (17)	71.9 ± 0.9^{a} (17)
Shade		0.22 ± 0.01^{b} (18)	*	0.1 ± 0.01^{a} (18)	*	0.2 ± 0.01^{b} (18)	0.11 ± 0.01^{b} (18)	54.2 ± 2.4^{b} (18)
Control	Ci	292.0 ± 5.5^{b} (15)	ns	312.5 ± 9^{a} (16)	ns	286.4 ± 5.9^{a} (16)	-	-
Drought	(µmol mol^{-1})	160.3 ± 11.9^{c} (18)	ns	193.5 ± 11^{b} (17)	ns	159.7 ± 15.9^{b} (17)	-	-
Shade		320.3 ± 4.4^{a} (18)	ns	323.5 ± 4.1^{a} (18)	ns	316.0± 7.7^{a} (18)	-	-

Kinetics parameter of stomatal response under irradiance changes

During the closing sequence (from SS1 to SS2), the response time (τ) ranged from ~90 s for the faster responses to ~600 s for the slowest (Table 2). Each treatment was significantly different from each other, with "Shade" (slowest) < "Control" < "Drought" (fastest). Interestingly, we only observed significantly different τ values between opening and closing sequence for the "Control" treatment, with an asymmetrical response of ~2 times slower opening, whereas "Drought" and "Shade" did not show significantly asymmetric responses.

Table 2: Kinetic parameters for opening and closing sequence, with τ the time constant (sec), λ the delay of stomatal response to reach the inflection point (sec) and SL_{max} the maximal slope of the response. Different letters show the significant differences between treatments from an ANOVA model including treatment effects followed by a post-hoc Tukey test. (. * show the significant differences between closing and opening from a paired t-test (P-values < 0.05), so a significant difference indicates an asymmetric response.

Treatment		Closing SS1-SS2		Opening SS2-SS3	Ratio Closing/Opening
Control	τ	378 ± 38^{a} (15)	*	695 ± 118^{a} (16)	0.59 ± 0.04^{b} (15)
Drought	(sec)	87 ± 4^{c} (18)	ns	101 ± 18^{b} (17)	1.09 ± 0.16^{a} (17)
Shade		577 ± 38^{b} (18)	ns	637 ± 52^{a} (18)	0.92 ± 0.06^{a} (18)
Control	Λ	348 ± 15^{a} (15)	*	693 ± 41^{a} (16)	0.51 ± 0.01^{a} (15)
Drought	(sec)	151 ± 9^{c} (18)	*	430± 81^{b} (17)	0.39 ± 0.03^{b} (17)
Shade		278 ± 16^{b} (18)	*	495 ± 21^{b} (18)	0.57 ± 0.02^{a} (18)
Control		1.08 ± 0.08^{b} (15)	ns	0.99 ± 0.13^{a} (16)	-
Drought	τ/λ	0.58 ± 0.04^{c} (18)	*	0.25 ± 0.05^{b} (17)	-
Shade		2.09 ± 0.1^{a} (18)	*	1.3 ± 0.12^{a} (18)	-
Control	SI_{max}	-3.13 ± 0.4^{b} (15)	*	1.79 ± 0.16^{b} (16)	-1.84 ± 0.11^{b} (15)
Drought	(mol m^{-2} s^{-2} x 10^{5})	-3.16 ± 0.3^{b} (18)	*	3.72 ± 0.59^{a} (17)	-0.97 ± 0.11^{a} (17)
Shade		-0.96 ± 0.1^{a} (18)	*	0.75 ± 0.06^{b} (18)	-1.33 ± 0.06^{a} (18)

The lag time of the response (λ) showed a different pattern compared to τ. During the closing sequence, "Drought" showed again the shortest delay (fastest response, lowest λ values), then "Shade" treatment, then "Control" (longest delay, highest λ values). In all treatments, λ was significantly higher for the opening than for the closing sequence (Table 2), with the strongest λ asymmetry for the "Drought" treatment while the ratios were similar between "Shade" and "Control" treatments.

For the "Control" treatments, λ and τ showed very similar values (ratio τ/λ ~1) in both closing and opening sequences, for "Drought" and "Shade" treatments a significant deviation from unity was observed but in opposite directions: the "Drought" treatment induced a shift to a longer λ, whereas the "Shade" treatment induced a shift to higher τ.

SL_{max} for closing was significantly slower in the "Shade" treatment, whereas for opening it was significantly faster for the drought treatment. This was due to the strong asymmetry observed for the "Control" treatment, which was much smaller in the "Drought" and "Shade" treatments.

In Figure 2, the mean of the estimated parameters for each treatment was applied to the sigmoidal model to visualize the differences in the responses, using a normalized g_s scale (setting g_{min} to 0 and g_{max} to 1; Fig. 2a and Fig. 2b), as well as the measured g_s values (Fig. 2c and 2d). Plant from the "Drought" treatment reach the new steady state after the step change in irradiance significantly more rapidly compared to the other two treatments. As the normalized graphs do not depend on the amplitude, they illustrate the difference between the "Control" and "Drought" treatments in terms of τ and λ : for a similar overall response time, the "Shade" treatment shows a shorter time lag of the response.

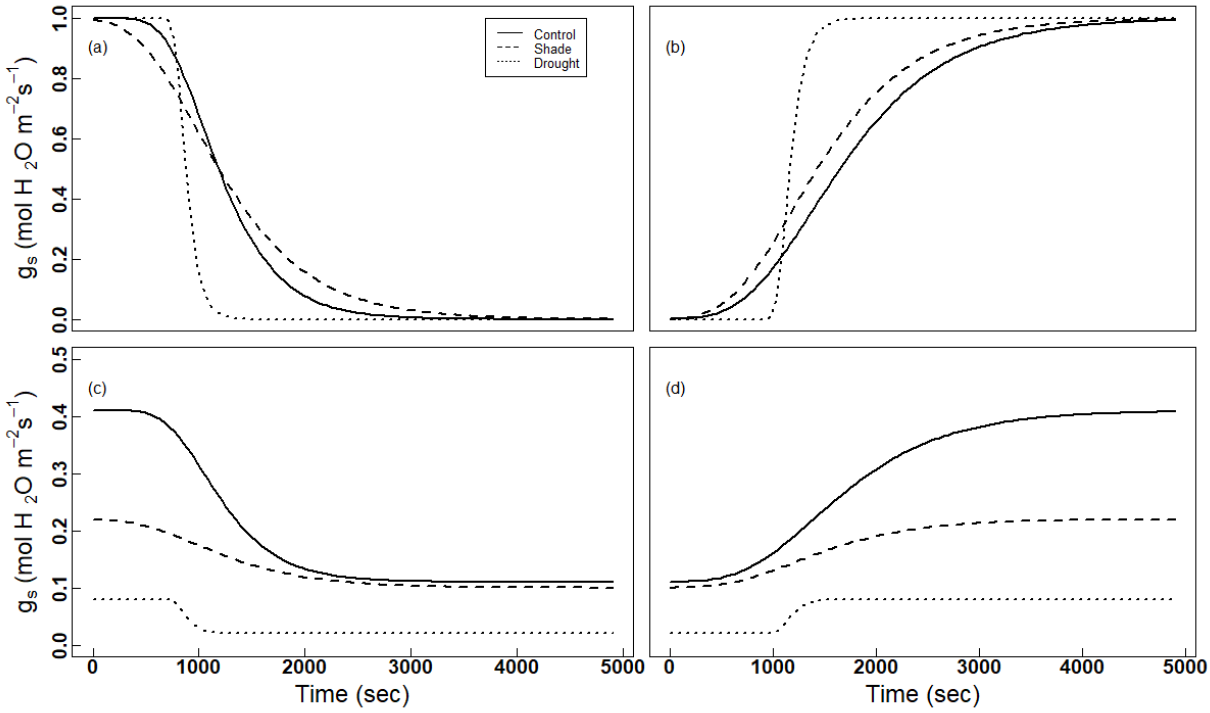

Figure 2: Fitted stomatal dynamics induced by changes in light intensity in closing (a, c) and opening (b, d) sequences. Plain lines are for "Control", simple dashed are for "Shade" and dotted lines are for "Drought" treatment. Figures (a) and (b) show normalized conductance responses, whereas the absolute values of g_s are shown in Figures (c) and (d). Each curve was estimated by using the mean values of the dynamic parameters from Table 2 and the sigmoidal model.

Stomatal morphology in response of different growing treatments

No significant differences were found between abaxial and adaxial faces for the considered stomatal traits. Overall, the stomatal ratio abaxial/adaxial for the measured tobacco plants was 2.46±0.4 while the epidermal cell ratio was lower at 1.39±0.1.

Significantly lower SD and CD as well as SI values were observed for the "Shade" treatment (Table 3), whereas no significant differences among treatments were observed for GCW, GSH and SS,

Table 3: Stomatal morphology means for treatments. GCL: guard cell length (µm), GCW: pore width (µm), SS: stomatal surface (µm²), GSH: stomatal shape (GCL/GCW), SD: stomatal density (mm^{-2}), CD: epidermis cell density (mm^{-2}), SI: stomatal index (SD/SD+CD). Different letters show the significant differences between the treatments (Tukey-HSD).

Treatment		Value
Control	GCL	33.1 ± 1.2^{a} (5)
Drought	(µm)	35.1 ± 1.7^{a} (5)
Shade		34.0 ± 0.5^{a} (5)
Control	GCW	26.7 ± 0.8^{a} (5)
Drought	(µm)	26.4 ± 0.8^{a} (5)
Shade		24.1 ± 0.3^{a} (5)
Control	SS	695 ± 55.2^{a} (5)
Drought	(µm²)	731 ± 73^{a} (5)
Shade		666 ± 22^{a} (5)
Control	GSH	1.24 ± 0.02^{b} (5)
Drought		1.33 ± 0.03^{ab} (5)
Shade		1.41 ± 0.02^{a} (5)
Control	SD	126 ± 7^{a} (5)
Drought	(mm^{-2})	131 ± 12^{a} (5)
Shade		58 ± 8^{b} (5)
Control	CD	478 ± 15^{a} (5)
Drought	(mm^{-2})	544 ± 73^{a} (5)
Shade		318 ± 25^{b} (5)
Control	SI	0.2 ± 0.01^{a} (5)
Drought		0.19 ± 0.01^{a} (5)
Shade		0.15 ± 0.01^{b} (5)

Table 4 Correlation table of dynamic parameters and the stomatal morphology across treatments. The upper-right triangle displays the number of observations and the p-values (with "***" for p<0.001; "**" for p<0.01 and "*" for p<0.05, corrected for multiple comparisons using FDR), while the lower-left displays the r-values (Pearson test). With the dynamic parameters: τ, λ, SL_{max} (where closing slopes are negative), their closing/opening ratio, the absolute amplitude of stomatal conductance response (SA), and the stomatal parameters: GCW (guard cells width), GCL (guard cells length), SS (stomatal size), SD (stomatal density), and SI (the stomatal index). *n*=15-18, bold r-values are highly significant (***).

	τ_{cl}	τ_{op}	λ_{cl}	λ_{op}	$SI_{max\ cl}$	$SI_{max\ op}$	τ_{ratio}	λ_{ratio}	$SL_{max\ ratio}$	SA_{cl}	SA_{op}	GCW	GCL	SS	SD	SI
τ_{cl}		***18	**18		***18	***18		**18							*15	*15
τ_{op}	0,83		***18	***18	* 18	***18	**18	*18	***18	*18						
λ_{cl}	0,66	0,87		***18		*18	**18	*18	***18	**18	*18					
λ_{op}	0,46	0,75	0,9			*18	**18		***18	**18	**18					
$SI_{max\ cl}$	0,78	0,48				***18		*18							*15	**15
$SI_{max\ op}$	-0,85	-0,76	-0,58	-0,47	-0,78		*18	.	*18							
τ_{ratio}		-0,62	-0,67	-0,69		0,55			***18		**18					
λ_{ratio}	0,68	0,51	0,48		0,48	-0,44										*15
$SL_{max\ ratio}$		-0,71	-0,79	-0,84		0,54	0,83			**18	**18					
SA_{cl}		0,59	0,84	0,85					-0,77		***18					
SA_{op}			0,77	*0,82*			-0,69		0,69	0,96						
GCW													**15	***15		*15
GCL												0,68		***15		
SS												0,9	0,93			
SD	-0,63				-0,64											**15
SI	-0,52				-0,69			-0,54				0,64		0,45	*0,72*	

Cross-correlations between stomatal kinetics and morphology

The dynamic parameters τ, λ SL_{max} and SA displayed significant and high correlations between their opening and closing values (Table 4). τ, λ and SL_{max} also correlated with each other, especially within the same irradiance change sequences, except for λ vs. SL_{max} where the correlations were lower or not significant during opening and closing respectively. However, within the same sequence, SA did only correlate with λ and not with τ or SL_{max}.

There were no correlations between τ, λ, SL_{max}, SA and the stomatal size parameters (GCW, GCL, SS). For closure, τ and SL_{max} showed small negative correlations with SD and SI (Fig. 3), associating more stomata with faster responses. The τ relationships were more clearly driven by the treatments differences than the SL_{max} relationships (Fig. 3). Whereas λ did not correlate with stomatal size or density parameters, its asymmetry (λ ratio) correlated negatively with SI, expressing a tendency for the opening delay to be longer with more guard cells per total cells.

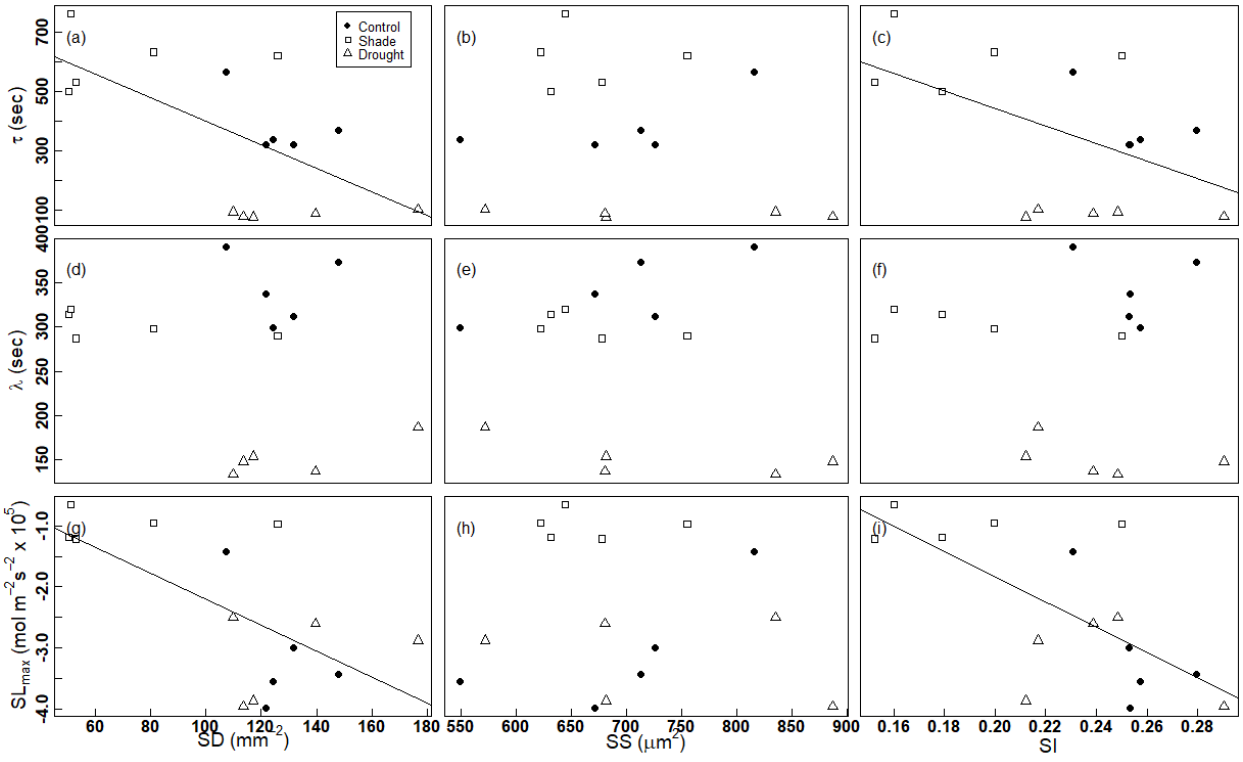

Figure 3: Cross correlations between dynamic parameters of the closing sequences (τ – time constant, λ – lag time and SL_{max} – maximum slope) and stomatal parameters (SS -stomatal size, SD -stomatal density and SI -the stomatal index). Black dots for "Control", white squares for "Shade" and white triangles for "Drought" treatment.

Discussion

Treatments impact on steady states values

Under the high irradiance conditions of SS1 and SS3, both treatments reduced gs and A compared to control, where "Drought" had a stronger impact on gs and "Shade" a stronger impact on A. The latter is probably due to a reduced photosynthetic capacity for the plants of the "Shade" treatment (confirmed by unpublished data). Stomatal closure under drought is a well studied response (Turner, 1974; Tardieu and Davies, 1992; Giorio et al., 1999), whereas the stomatal closure under shade might be due to a C_i mediated signal to optimize the leaf internal CO_2 concentration (Mott, 1988).

Impact of the treatments on the dynamic response to irradiance

Kirschbaum et al. (1988) proposed a dynamic model in which the response to irradiance was hypothesized to be composed of three functional steps: first, a biochemical signal that responds directly to irradiance, then the subsequent variation of osmotic potential causing finally the movement of water, in/out the guard cells, inducing the actual stomatal movement. From our model we extracted two parameters (τ and λ) both expressed as time constants, where λ (as a lag time estimate) could be related to the time needed for the first biochemical signal induction, such as the phototropin I and II or zeaxanthin (Demming-Adams et al., 1989; Christie, 2007). Further, τ, describing the steepness of the sigmoidal shape (Fig. 1b), could be related to the response time of the stomatal movement itself, which might be related to the ion and water fluxes operating during stomatal movements (Blatt, 2000).

Similarly to the steady state parameters, the dynamic response to irradiance has been significantly changed by both treatments and resulted in contrasting stomatal behaviours in terms of opening and closing. For the "Control" treatment, the range values of τ, λ and SL_{max} were comparable to a study on multiple species (including *Nicotiana tabaccum*) using a similar dynamic model and irradiance variations (McAusland et al., 2016). This study also showed a strong relationship between τ and SL_{max} across species. The different treatments used in our study allowed a more detailed analysis of the overall coordination between lag and response times. The "Drought" treatment decreased lag (λ) and response (τ) times for opening as well as closing, with a stronger impact on closing for λ, but a stronger impact on opening for τ. No impact of drought was visible for SL_{max} due to a simultaneous decrease in amplitude. It has been suggested that plants from drier climates or experiencing a drought stress showed similar faster responses (Vico et al., 2011; Lawson and Blatt, 2014). To the best of our knowledge, only a few other studies have investigated experimental drought impact on the dynamic of stomatal response (Qu et al., 2016; Haworth et al., 2018). Both studies observed faster responses associated to drought during stomatal closing, however, Haworth et al. (2018) found no impact of drought on the opening sequence, which differs from our results. Therefore, literature results as well

as our study seem to suggest an increase in stomatal speed under drought, however a conclusion on a differential impact between opening and closing will still need more experimental evidence. However, the coordinated response of the two time constants towards more rapid stomatal responses (reduced τ and λ values) during drought suggest a tighter coupling between A_n and g_s. This might reduce the loss of water, both at the instantaneous and long-term scale (McAusland et al. 2016) and thereby improve water use efficiency.

To our knowledge, only few studies have estimated stomatal dynamics on experimental shade or low irradiance growth conditions (Kardiman and Raebild, 2017; Matthews et al., 2018). Our results on τ and SL_{max} for closing tended to be in agreement with the previous studies in which shade grown plants displayed slower stomatal responses to irradiance, however for opening no differences to "Control" was shown. Whereas for the lag time (λ) an acclimation in the opposite direction to a faster response, similarly to the "Drought" treatment, was shown. These results suggest that response (τ) and lag times (λ) not only acclimated independently to the prevailing environmental treatments, but also that opening and closing mechanisms were not affected similarly by environmental conditions. Such differences between opening and closing response times (τ) might be partly due to the differential ion flux pathways involved in solute uptake and loss involved in stomatal opening and closing, respectively (Blatt, 2000; Shimazaki et al., 2007; Lawson and Blatt 2014). Moreover, Haworth et al (2018) have suggested that the free-ABA content might also have a large influence of the speed of stomatal movements. The acclimations observed for lag time might be more dependent on signalling pathways for irradiance signals (Blatt, 2000), but also via leaf internal CO_2 concentration, modified by the irradiance impact on photosynthesis (Hiyama et al. 2017).

There was a strong asymmetry towards slower stomatal opening in the "Control" treatment for SL_{max} as well as τ and λ. After Woods and Turner (1971) an asymmetry in this direction might be an adaptation to reduce water loss as a fast-stomatal closing allows a tighter coupling between A_n and g_s thus reducing excessive loss of water (Tinoco-Ojanguren and Pearcy, 1992; Ooba and Takahashi, 2003). Whereas a slower opening might avoid overshooting situations where stomata continue to open after an increase in irradiance, even when photosynthesis is actually saturated (MacAusland et al., 2016). However, no asymmetry was found for the "Drought" treatment concerning SLmax and τ, suggesting a stronger impact of drought on the physiological mechanisms affecting the opening speed. Whereas for the λ the asymmetry was even significantly stronger under drought, suggesting that the speed of the biochemical signalling of the irradiance change was more increased for closing, even if also the opening lag time was more rapid compared to control.

Ooba and Takahashi (2003) suggested that light limited environments would favour a more rapid increase in g_s as a faster stomatal opening allows a better gaz exchanges coupling and should theoretically increase the overall CO_2 uptake. The experimental "Shade" treatment used here did increase the response time for closing relatively more than for opening, however this was not seen for the closing speed, which was actually reduced more for closing. By dissecting the speed into several parameters as τ, the response time (independent of amplitude), the amplitude itself and λ, the lag time, we were able to show that these parameters were affected differently, both "Drought" and "Shade" treatments equilibrated the response times between opening and closing, whereas the asymmetry of the lag time was significantly accentuated by the "Drought". This could suggest that irradiance response signalling pathways as well as physiological mechanisms relating to stomatal movements might be different between opening and closing and acclimate differently to environmental constraints. However, to substantiate such a hypothesis, more detailed studies are necessary on the molecular level.

Acclimation of stomatal morphology to drought and shade

Plants are known to adjust stomatal density, index and size during leaf development to the prevailing environmental conditions (Rawson and Craven, 1975; Carins Murphy et al., 2012; Kalve et al., 2014; McAusland et al., 2016). The decrease of stomatal density (SD) with increasing atmospheric CO_2 concentration is well documented (Woodward, 1987; Pal et al., 2005; Franks and Beerling, 2009); however, there is no clear consensus about the impact of drought on stomatal morphology. Theodorou et al. (2013) observed antagonistic responses of SD among genotypes of grapevine cultivars submitted to drought. Similar contrasting results have been reported in tree (Laajimi et al., 2011) or grass species (Xu and Zhou, 2008). Concerning shade growth conditions, most of the literature suggests a decrease of SD (Brodribb and Jordan, 2011; Aasamaa and Aphalo, 2016; Kardiman and Raebild, 2017; Matthews et al., 2018), linked to a lower stomatal index (SI) (Ashton and Berlyn, 1994, Sun et al., 2003; Aasamaa and Aphalo, 2016). In this study, tobacco plants displayed no acclimation to water deficit in terms of stomatal size, density or index, despite the high intensity of the water stress during the whole growing period, and thus the development of the measured leaves. However, the plants grown under shade displayed a significantly reduced SD, linked to a decrease in SI but no change of the stomatal size, which corroborates the literature results on woody and herbaceous species other than tobacco (Gay and Hurd, 1975; Aasama and Aphalo 2016; Carins-Murphy et al., 2016; Kardiman and Raebild, 2017). The lack of such a correlation in our study suggests that the decrease in stomatal density under "Shade" was due to an impact on stomatal pattering during leaf development.

Relationship between dynamic parameters and stomatal morphology

Most of the studies on the relationship between stomatal morphology and dynamics report a faster stomatal response associated with smaller stomata or a higher stomatal density (Hetherington and Woodward 2003; Franks and Farquhar, 2007; Drake et al., 2013; Raven, 2014, Xiong et al., 2017). Nevertheless, other recent studies did not detect any significant correlation between stomatal density, size and the rapidity of response (Haworth et al., 2015; Aasamaa and Aphalo, 2016; Elliot-Kingston et al., 2016). Most of these studies focused on the inter-specific diversity while only one explored within species variation induced by an experimental shade (Aasama and Aphalo, 2016). In all of these studies the rapidity of response was expressed as speed that is g_s variation over time. In our study we were able to decompose the speed of stomatal response (SL_{max}) into several parameters (τ and SA_{gs}) and put in evidence that the similar SL_{max} found in "Control" and "Drought" treatments resulted from significantly different response times (τ) and amplitude of stomatal response and therefore very different dynamic responses under these two treatments (Table 2). McAusland et al., (2016) have used a similar model and have found across species with elliptical shaped stomata that pore length correlated with speed (SL_{max}, for opening only), probably related to the amplitude of the response (strong correlation with steady state g_s), but not with the response time (τ). In our study, within one species but across treatments, we did not find any correlation between dynamic parameters and guard cell length, width or surface, similarly to Aasama and Aphalo (2016). This result might be due to the low variability of stomatal sizes within species, despite the range of environmental conditions. However, there were significant correlations between stomatal density or index and closing related parameters such as SL_{max} and □□ For the latter this was clearly a co-variation related to the differences among treatments, however for SL_{max} this was less clear (Fig. 3) and might therefore suggest an effect of the number of stomata on stomatal closing, with more stomata resulting in faster dynamics. Such a more rapid stomatal response with higher stomatal density had already been suggested for opening sequences by Vialet-Chabrand et al (2016) using simulations. Finally, the observation of strong differences in stomatal dynamics without (and not related to) a strong variation in stomatal size leads us to the conclusion that within-species, acclimation of stomatal lag and response times involve other mechanisms than stomatal morphology. Such mechanisms could include physiological de-/activation of ion transport in the stomatal guard cells, or a genetic control on the expression of ion transport channels.

Conclusion

In this study we highlighted the strong impact of "Drought" and "Shade" treatments on the dynamic response of stomata to variations in irradiance in tobacco plants. The rapidity of response was affected

by both treatments but in different ways, where “Drought” reduced both the delay and response times (both faster), “Shade” treatment also reduced the delay but slowed the response time, thus significantly changing the shape and thus the dynamic of the response. Moreover, we show different stomatal dynamics between opening and closing sequences among treatments, suggesting the existence of different signalling pathways and/or mechanisms involved in the asymmetrical response to irradiance in tobacco plants. However, these tests were only performed with one very large step change in irradiance. An important perspective would be to confirm the coherence of these results with step changes of different amplitudes and different starting irradiances.

The impact of the “Shade” treatment on the stomatal dynamics could be an indication that when introducing such dynamics into canopy scale models, a different parameterization between sun and shade leaves might have to be taken into account. To gain a more mechanistic insight into the acclimation of stomatal dynamics to the growth environment, a more molecular approach would be necessary to observe short-term variations of guard-cell gene expression, ion channel functioning or abundance.

Declaration of interest

All authors disclose any financial or personal conflict of interest.

Author contributions

TG, CD, OB designed the experiment, CD and JF provided study material and environment, TG and CD conducted the experiment, TG, CD, OB did the data analysis, and TG, CD, OB, JF wrote the manuscript and were involved in the interpretation and critical discussion of the results, OB and JF obtained funding.

Acknowledgement

TG acknowledges the PhD grant from the H20ak ANR-14-CE02-0013 project. The research stay of TG was supported by a grant overseen by the French National Research Agency (ANR) as part of the "Investissements d'Avenir" program (ANR-11-LABX-0002-01, Lab of Excellence ARBRE) and financial support of INRA / DARESE. JF and CD acknowledge funding from the MINECO project CTM2014-53902-C2-1-P.

Bibliography

Aasamaa, K., Sõber A., 2011. Responses of stomatal conductance to simultaneous changes in two environmental factors. Tree Physiol. 31: 855–864

Aasamaa, K., Aphalo, PJ., 2016. The acclimation of Tilia cordata stomatal opening in response to light, and stomatal anatomy to vegetational shade and its components. Tree Physiol. 37, 209-219

Ashton, P.M.S., Berlyn, G.P., 1994. A comparison of leaf physiology and anatomy of Quercus (section Erythrobalanus-Fagaceae) species in different light environments. Am J Bot. 81, 589–597

Blatt, M.R., 2000. Cellular signaling and volume control in stomatal movements in plants. Annu Rev Cell Dev Biol. 16, 221-241

Brodribb, T.J., Jordan, G.J., 2011. Water supply and demand remain balanced during leaf acclimation of Nothofagus cunninhamii trees. New phytologist. 192, 437-448

Carins Murphy, M.R, Jordan, G.J., Brodribb, T.J., 2016. Cell expansion not cell differentiation predominantly co-ordinates veins and stomata within and among herbs and woody angiosperms grown under sun and shade. Annals of Botany. 118, 1127-1138

Carins Murphy, M.R., Jordan, G.J., Brodribb, T.J., 2012. Differential leaf expansion can enable hydraulic acclimation to sun and shade. Plant, Cell & Environment. 35, 1407–1418.

Casson, S., Gray, J.E., 2008. Influence of environmental factors on stomatal development. New Phytologist. 178, 9-23

Christie, J.M., 2007. Phototropin blue-light receptors. Annu Rev Plant Biol. 58, 21-45

Clifford, S.C., Black, C.R., Robert, J.A., Stronach, I.M., Singleton-Jones, P.R., Mohamed, A.D., Azam-Ali, S.N., 1995. The effect of elevated atmospheric CO_2 and drought on stomatal frequency in groundnut (Arachis hypogaea (L.)). J Exp Bot. 46, 847-852

Demming-Adams, B., Winter, K., Kruger, A., Czygan, F.C., 1989. Light Response of CO2 Assimilation, Dissipation of Excess Excitation Energy, and Zeaxanthin Content of Sun and Shade Leaves. Plant Physiol. 90, 881-886

Doheny-Adams, T., Hunt, L., Franks, P.J., Beerling, D.J., Gray, J.E., 2012. Genetic manipulation of stomatal density influences stomatal size, plant growth and tolerance to restricted water supply across a growth carbon dioxide gradient. Philos Trans R Soc Lond B Biol Sci. 367, 547–555

Dow, G.J., Berry, J.A., Bergmann, D.C., 2014.The physiological importance of developmental mechanisms that enforce proper stomatal spacing in Arabidopsis thaliana. New Phytol. 201, 1205–1217

Drake, P.L., Froend, R.H., Franks, PJ., 2013. Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. J Exp Bot. 64, 495–505

Engineer, C., Hashimoto-Sugimoto, M., Negi, J., Israelsson-Nordstrom, M., Azoulay-Shemer, T., Rappel, W.J., Iba, K., Schroeder, J., 2016. CO2 sensing and CO2 regulation of stomatal conductance: advances and open questions. Trends Plant Sci. 21, 16-30

Elliot-Kingston, C., Haworth, M., Yearsley, J.M., Batke, SP., Lawson, T., McElwain, J.C., 2016. Does Size Matter? Atmospheric CO2 May Be A Stronger Driver of Stomatal Closing Rate Than Stomatal Size in Taxa That Diversified under Low CO2. Front Plant Sci. 7, 1253

Franks, P.J., Beerling, D.J., 2009. Maximum leaf conductance driven by CO2 effects on stomatal size and density over geologic time. Proc Natl Acad Sci USA. 106, 10343–10347

Franks, P.J., Doheny- Adams, T., Britton- Harper, Z.J. and Gray, J.E., 2015. Increasing water- use efficiency directly through genetic manipulation of stomatal density. New Phytologist, 207, 188-195

Franks, P.J, Farquhar, G.D., 2007. The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiol. 143, 78–87

Gay, A.P., Hurd, R.G., 1975. The influence of light on stomatal density in the tomato. New Phytol. **75**, 37-46

Giorio, P., Sorrentino, G., d'Andria, R., 1999. Stomatal behavior, leaf water status and photosynthetic response in field-grown olive trees under water deficit. Env Exp Bot. 42, 95-104

Haworth, M., Killi, D., Materassi, A., Raschi, A., 2015. Coordination of stomatal physiological behaviour and morphology with carbon dioxide determines stomatal control. American Journal of Botany. 102, 677-688

Haworth, M., Marino, G., Cosentino, S. L., Brunetti, C., De Carlo, A., Avola, G., . & Centritto, M., 2018. Increased free abscisic acid during drought enhances stomatal sensitivity and modifies stomatal behaviour in fast growing giant reed (Arundo donax L.). Environmental and Experimental Botany. 147, 116-124.

Hetherington, A.M., Woodward, F.I., 2003. The role of stomata in sensing and driving environmental change. Nature 424, 901–908

Hiyama, A., Takemiya, A., Munemasa, S., Okuma, E., Sugiyama, N., Tada, Y,Murata, Y., Shimazaki, K.I., 2017. Blue light and CO_2 signals converge to regulate light-induced stomatal opening Nat. Commun. 8, 1284

Jones, H.G., 1977. Transpiration in barley lines with differing stomatal frequencies. J Exp Bot 28, 162–168

Kardiman, R., Ræbild, A., 2017. Relationship between stomatal density, size and speed of opening in Sumatran rainforest species, *Tree Physiology*, https://doi.org/10.1093/treephys/tpx149

Kaiser, E., Morales, A. and Harbinson, J., 2018. Fluctuating light takes crop photosynthesis on a rollercoaster ride. Plant physiology, 176, 977-989.

Kalve, S., Fotschki, J., Beeckman, T., Vissenberg, K., Beemster, G.T.S., 2014. Three-dimensional patterns of cell division and expansion throughout the development of Arabidopsis thaliana leaves. Journal of Experimental Botany 65, 6385–6397.

Kirschbaum, M.U.F., Gross, L.J., Pearcy, R.W., 1988. Observed and modelled stomatal responses to dynamic light environments in the shade plant Alocasia macrorrhiza. Plant Cell Environ. 11, 111–121

Laajimi, N.O., Boussadia, O.,Skhiri, F.H., Teixeira da Silva, J.A., Rezgui, S., Hellali, R., 2011. Anatomical adaptations in vegetative structures of Apricot tree (Prunus armeniaca L.) cv. 'Amor El Euch' grown under water stress. Fruit, Vegetable and Cereal Sci. Biotech. 5, 46-51

Lawson, T., Blatt, M.R., 2014. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. Plant Physiol. 164, 1556–1570

Lawson, T., Morison, J.I.L., 2004. Stomatal function and physiology. In AR Hemsley, I Poole, eds, The Evolution of Plant Physiology; from whole plants to ecosystem. Elsevier, Amsterdam, pp. 217–242

Lawson, T., von Caemmerer, S., Baroli, I., 2010. Photosynthesis and stomatal behaviour. In UE Lüttge, W Beyschlag, B Büdel, D Francis, eds, Progress in Botany. Springer, Berlin, pp. 265–304

Martins, S.C., McAdam, S.A., Deans, R.M., DaMatta, F.M. and Brodribb, T.J., 2016. Stomatal dynamics are limited by leaf hydraulics in ferns and conifers: results from simultaneous measurements of liquid and vapour fluxes in leaves. Plant, cell & environment. 39, 694-705

Matthews, J.S., Vialet-Chabrand, S.R. and Lawson, T., 2018. Acclimation to fluctuating light impacts the rapidity and diurnal rhythm of stomatal conductance. Plant physiology. 176, 1939-1951

McAusland, L., Vialet-Chabrand, S., Davey, P., Baker, N.R., Brendel, O., Lawson, T., 2016. Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. New Phytol. 211, 1209–1220

Merilo, E., Laanemets, K., Hu, H., Xue, S., Jakobson, L., Tulva, I., Gonzalez-Guzman, M., Rodriguez, P.L., Schroeder, J.I., Broschè, M., Kollist, H., 2013. PYR/RCAR Receptors Contribute to Ozone-, Reduced Air Humidity-, Darkness-, and CO_2-Induced Stomatal Regulation. Plant Physiol. 162, 1652-1668

Mott, K.A., 1988. Do Stomata Respond to CO2 Concentrations Other Than Intercellular. Plant Physiol. 86, 200–203

Ooba, M., Takahashi, H., 2003. Effect of asymmetric stomatal response on gas-exchange dynamics. Ecol Modell. 164, 65–82

Outlaw, W.H., 2003. Integration of cellular and physiological functions of guard cells. CRC Crit Rev Plant Sci. 22, 503–529

Pal, M., Rao, L.S., Jain, V., Srivastava, A.C., Pandey, R., Raj, A., Singh, K.P., 2005. Effects of elevated CO_2 and nitrogen on wheat growth and photosynthesis. *Biolo. Plantarum*. 49**,** 467-470

Pearcy, R.W., Schulze, E.D., Zimmermann, R., 2000. Measurement of transpiration and leaf conductance. Plant Physiological Ecology, pp. 137-160

Qu, M., Hamdani, S. and Bunce, J.A., 2016. The physiology and genetics of stomatal adjustment under fluctuating and stressed environments. Applied Photosynthesis-New Progress

Qu, M., Hamdani, S., Li, W., Wang, S., Tang, J., Chen, Z., Song, Q., Li, M., Zhao, H., Chang, T., 2016a Rapid stomatal response to fluctuating light: an under-explored mechanism to improve drought tolerance in rice. Funct. Plant Biol. 43, 727–738

Raven, J.A., 2014. Speedy small stomata? J Exp Bot. 65, 1415–1424

Rawson, H., Craven, C., 1975. Stomatal development during leaf expansion in to- bacco and sunflower. Australian Journal of Botany. 23, 253–261

Schindelin, J.; Rueden, C. T., Hiner, M. C., Eliceiri K.W., 2015 The ImageJ ecosystem: An open platform for biomedical image analysisis. , Mol Rep Dev. 82,518-529

Schlüter, U., Muschak, M., Berger, D., Altmann, T., 2003. Photosynthetic performance of an Arabidopsis mutant ith elevated stomatal density (sdd1-1) under different light regimes. J Exp Bot. 54, 867-874

Schulze, E.D., Hall, A.E., 1982. Stomatal responses, water loss and CO2 assimilation rates of plants in contrasting environments. Physiological Plant Ecology II, pp. 181-230

Shimazaki, K.I., Doi, M., Assmann, S., Kinoshita, T., 2007. Light Regulation of Stomatal Movement. Annu Rev Plant Biol. 58, 219-247

Sun, B, Dilcher D.L., Beerling D.J., Zhang C., Yan D., Kowalski E. 2003. Variation in Ginkgo biloba L. leaf characters across a climatic gradient in China. Proc Natl Acad Sci USA. 100,7141–7146

Tardieu, F., Davies, W.J., 1992. Stomatal response to abscisic acid is a function of current plant water status. Plant Physiol 98, 540-545

Team, R. C., 2015. R Foundation for Statistical Computing; Vienna, Austria: 2014. R: A language and environment for statistical computing, 2013

Theodorou, N., Koundouras, S., Zioziou, E., & Nikolaou, N. (2013). Responses of leaf stomatal density and anatomy to water deficit in four winegrape cultivars (Vitis vinifera L.). AMPELOS 2013 - 3rd international symposium on trends in world vitiviniculture development conference; 30-31 May 2013 Santorini island, Greece

Thomas, P.W., Woodward, F.I., Quick, W.P., 2004. Systemic irradiance signaling in tobacco. New Phytol. 161, 193-198

Tinoco-Ojanguren, C, Pearcy, R.W., 1992. Dynamic stomatal behavior and its role in carbon gain during lightflecks of a gap phase and an understory Piper species acclimated to high and low light. Oecologia. 92, 222–228

Turner, N.C., 1974. Stomatal behavior and water status of maize, sorghum, and tobacco under field conditions II. At low soil water potential. Plant Physiol. 53, 360-365

Tuteja, N., 2007. Abscisic Acid and Abiotic Stress Signaling. Plant Signaling & Behavior. 2, 135-138

Vico, G., Manzoni, S., Palmroth, S., Katul, G., 2011. Effects of stomatal delays on the economics of leaf gas exchange under intermittent light regimes. New Phytol. 192, 640–652

Uhl, S., Dreyer, E., Brendel, O., 2013. Performance of a new dynamic model for predicting diurnal time courses of stomatal conductance at the leaf level. Plant, Cell & Environment 36, 1529-1546

Vialet-Chabrand, S., Matthews, J.S.A., Brendel, O., Blatt, M.R., Wang, Y., Hills, A., Griffiths, H., Rogers, S. and Lawson, T., 2016. Modelling water use efficiency in a dynamic environment: an example using Arabidopsis thaliana. Plant Science. 251, 65-74

Vialet-Chabrand, S.R.M., Matthews, J.S.A., McAusland, L., Blatt, M.R., Griffiths, H., Lawson, T., 2017. Temporal Dynamics of Stomatal Behavior: Modeling and Implications for Photosynthesis and Water Use. Plant Physiol. 174, 603-613

Woods, D.B., Turner, N.C., 1971. Stomatal response to changing light by four tree species of varying shade tolerance. New Phytol. 70, 77–84

Woodward, F.I., 1987. Stomatal numbers are sensitive to increases in CO_2 from preindustrial levels. Nature. 327, 617–618

Xiong, D., Douthe, C., Flexas, J., 2017. Differential coordination of stomatal conductance, mesophyll conductance and leaf hyraulic conductance in response to changing light across species. doi: 10.1111/pce.13111

Xu, Z., Zhou, G., 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. J. Exp. Bot. 59, 3317-3325

Synthèse du chapitre méthodologique

Les données présentées ci-dessus démontrent l'impact des conditions de croissance sur la dynamique de réponse stomatique. Lorsque les plantes grandissent sous des conditions environnementales différentes, la dynamique de réponse stomatique à un changement de lumière s'en trouve fortement altérée. Il est par ailleurs notable que ces altérations sont spécifiques aux facteurs environnementaux limitants propres à chaque groupe de traitement. Ainsi, une exposition à la sécheresse n'a pas les mêmes conséquences qu'une croissance sous ombrage sur la dynamique de réponse stomatique. Au cours de cette expérimentation, des plants de tabacs (*Nicotiana tabacum)* ont grandi pendant 21 jours en étant exposés à un stress hydrique ou un faible accès à la lumière. Une campagne de mesure visant à caractériser la dynamique de fermeture puis d'ouverture stomatique ainsi que l'asymétrie de ces deux séquences fut alors menée. Au-delà des mesures d'échanges gazeux foliaires, l'utilisation d'un modèle dynamique nous a permis de décrire la réponse stomatique par trois paramètres distincts (SL_{max}, lambda et tau), tau étant une constante de temps dessinant l'allure de la courbe, lambda étant le délai de réponse de la conductance stomatique (le temps entre le changement de lumière et l'atteinte du point d'inflexion de la sigmoïde). La pente max (SL_{max}) est calculée en intégrant l'amplitude du changement de conductance d'un état stable (steady-state) à l'autre et de la constante k, SL_{max} décrit une vitesse de réponse maximale suite au changement du paramètre environnemental.

Impact de la morphologie stomatique sur la dynamique de réponse à la lumière

Dans cet article, nous avons également testé la possible relation entre morphologie stomatique et vitesse de réponse. Il faut préalablement noter que le traitement de stress hydrique n'a pas eu d'impact sur la morphologie stomatique en comparaison du groupe témoin. En revanche, les plants de tabac s'étant développés sous ombrage affichent une densité stomatique (SD) ainsi qu'un index stomatique (SI) significativement plus faible que les autres traitements sans pour autant diverger des deux autres groupes en termes de taille des stomates (SS). Si dans le cas d'une croissance à faible lumière un impact de l'anatomie stomatique sur la rapidité des mouvements stomatiques à la lumière n'est pas à exclure, l'absence de différence d'anatomie entre les groupes témoins et sous stress hydrique suggère que la diversité des réponses temporelles de g_s observées n'est pas liée à la morphologie chez les plants de tabac.

Perspectives au regard de nos travaux sur les chênes

Outre les considérations théoriques de l'impact de la dynamique de réponse des stomates aux stimuli environnementaux, nous avons mis en évidence par ces travaux que les conditions de croissance pouvaient considérablement impacter la dynamique de réponse à la lumière chez les plantes indépendamment des caractéristiques anatomiques des stomates. Il n'existe que peu d'études traitant la dynamique de réponse temporelle chez le chêne. Dans un premier temps, Roussel et al., (2009) observèrent des comportements stomatiques particulièrement divergents au cours de cinétiques journalières monitorant les échanges gazeux foliaires (A, g_s , Wi) chez deux génotypes sélectionnés pour leurs efficiences d'utilisation de l'eau extrêmes (hautes et faibles valeurs de discrimination isotopique : $\Delta^{13}C$: figure 8). En outre, les plants sélectionnés pour leur faible efficience affichaient des valeurs de conductance stomatique bien plus élevées que le groupe de forte efficience, caractérisé par une ouverture stomatique relativement plus rapide et de fortes amplitudes en début de matinée, résultant en des valeurs de Wi élevées tout le long de la journée. Ces travaux préliminaires insufflèrent donc l'idée qu'il pourrait exister des comportements stomatiques impactant l'assimilation et l'eau consommée au cours d'un cycle diurne et par extension à l'efficience d'utilisation de l'eau intégrée sur le long terme.

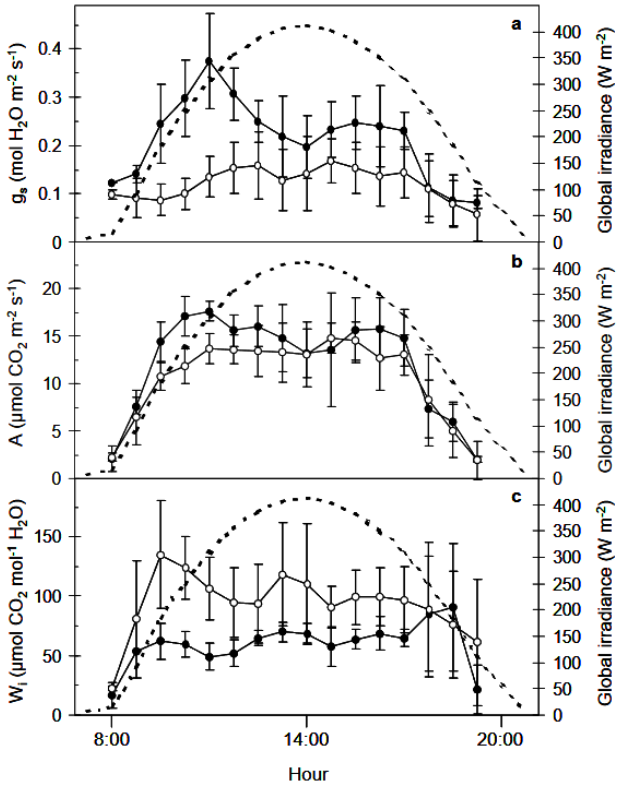

Figure 8 : Exemple de cinétique journalière de la conductance stomatique (a), de l'assimilation de CO_2 (b) et de l'efficience d'utilisation de l'eau intrinsèque qui en résulte (Wi) : d'après la thèse de Magali Roussel.

Par la suite, au cours de ses travaux de thèse, Silvère Vialet-Chabrand (Fig 9) mit en évidence une large diversité interspécifique de réponses stomatiques à la lumière au cours de cinétiques journalières et de changement instantanés d'irradiance chez cinq espèces de chênes faisant état d'une importante diversité au niveau de l'anatomie stomatique et de l'efficience intrinsèque d'utilisation de l'eau (Vialet-chabrand et al., 2013b).

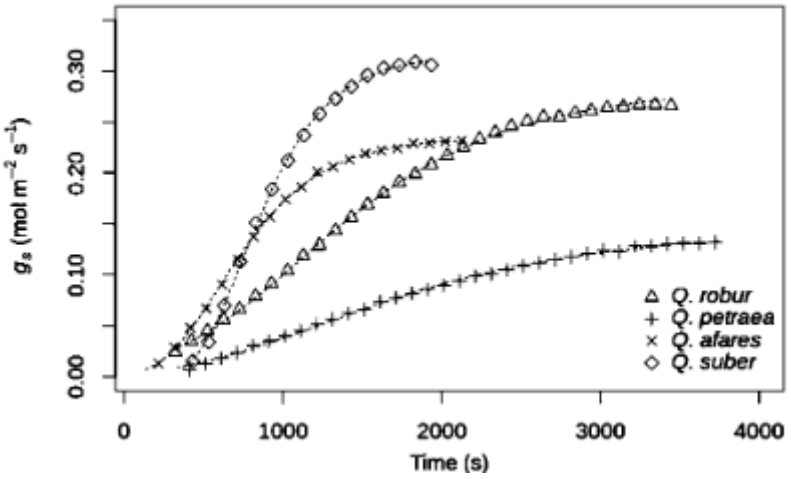

Figure 9 : Diversité de la réponse stomatique à une augmentation de la lumière de 0 à 800 µmol $m^{-2}s^{-1}$ chez le genre Quercus (D'après Vialet-Chabrand Thèse)

Par l'usage de la modélisation dynamique des échanges gazeux foliaires, il mit en relief l'importance de l'intégration en continu des variations microclimatiques afin de décrire les variations de g_s chez le chêne. Explorant la réponse temporelle de g_s à un changement brutal des conditions environnementales, Vialet-chabrand (2013c) identifia également une réponse différentielle à la lumière bleue entre les chênes sessiles et pédonculés. En effet l'exposition à différentes qualités du spectre lumineux entrainait une diminution plus forte de l'efficience d'utilisation de l'eau intrinsèque chez *Q. robur*. En complément de ces différences interspécifiques de perception du spectre lumineux, il observa une diversité de perception du niveau de CO_2 dans la feuille, les chênes afares et liège présentant une réponse différentielle à celle de *Q. robur* et *Q. petraea*. Cependant ces travaux ne furent réalisés que sur un nombre restreint de plants et ne sont donc probablement pas représentatifs des espèces.

Cet ensemble d'éléments laisse supposer que la dynamique de réponse stomatique pourrait jouer un rôle majeur dans les échanges gazeux qu'entretiennent *Q. robur* et *Q. petraea* avec leur environnement, particulièrement lorsque ceux-ci sont intégrés sur le long terme (Efficience de transpiration). Les disparités de réponses entre ces deux espèces pourraient également être associées à la différentiation de leurs niches écologiques respectives. Ce faisant, dans un effort

d’identification et de caractérisation des traits sous-jacents impliqués dans la diversité et la plasticité de l’efficience d’utilisation de l’eau chez ces deux espèces, la modélisation et la description de la réponse temporelle de g_s à des stimuli environnementaux (lumière, CO_2 , VPD) constitueront des axes majeurs de réflexion au sein des chapitres suivants de ce manuscrit.