Measuring leaf elongation rate and transpiration in response to drought and elevation of atmospheric CO2 concentration

Victoria Acker, Jean-Louis Durand, Cédric Perrot, Eric Roy, Romain Barillot

Measuring leaf elongation and plant transpiration in response to drought and atmospheric CO_2 elevation

Victoria Acker[1], Jean-Louis Durand[1], Cédric Perrot[1], Eric Roy[1], Romain Barillot[1]

[1]INRAE, URP3F, 86600 Lusignan, France

1 Introduction

Grasses represent a large part of the flora found in terrestrial ecosystems and constitute the basis of many agrosystems. Their competitive ability and agronomic performance (*e.g.* biomass production for forage grasses or grain production for cereals) are largely determined by leaf growth. In grasses, leaf elongation occurs at the basis of the plant and depends on climatic conditions as well as water and nutrient availability (Maurice, 1997). Projected scenarios of climate change (IPCC, 2014) indicate that temperature and CO_2 concentrations will continue to increase by 2050 which have the potential to enhance plant growth (as long as temperatures do not reach extremes); but changes in seasonal precipitation and increased drought occurrences would reduce these benefits (Durand et al., 2013). Anticipating the effects of climate change on leaf growth therefore requires to integrate complex interactions between environmental factors and plant functioning.

Functional-structural plant modeling (FSPM) is a suitable tool for integrating the effects of climate change on resource acquisition by plants in interaction with their morphogenesis. Nevertheless, to our knowledge, there are no grass FSPM integrating both the trophic and water interactions on leaf growth. To that purpose, we aim at coupling two existing FSPMs: CN-Wheat (Barillot et al., 2016; Gauthier et al., 2020), which simulates leaf growth from environmental factors and metabolite concentrations at organ level; and a turgor-induced leaf growth model (Coussement et al., 2018) based on water relations at the scale of individual plant organs. Transpiration is a key variable in this coupling because it determines the water flow and resource allocation among plant organs and is highly affected by soil water availability and CO_2 concentration. However, limited information is available on transpiration dynamics of individual plants exposed to contrasting environmental conditions in terms of CO_2 level and water availability.

Therefore, we conducted an experiment to study the effects of the interaction between water deficit and CO_2 on leaf growth and transpiration of three grass species. In order to monitor the transpiration flow of individual plants, we developed a system of connected load cells. The objective of this experiment was to provide data for model implementation and evaluation.

2 Material and methods

Experimental protocol

The protocol consists of growing plants of winter bread wheat (*Triticum aestivum* L.); perennial ryegrass (*Lolium perenne* L.); and tall fescue (*Festuca arundinacea*), in growth chambers under two CO_2 levels: 200 and 800 ppm. A total of 960 seedlings were transplanted in 0.7L pots filled with vermiculite and coarse gravels. An aluminum foil was placed on the surface of the pot around the stem to prevent from water loss by evaporation. From transplantation to stage leaf 5 growing, plants were irrigated daily with a complete nutrient solution. At stage leaf 5, irrigation was stopped on half of the plants to induce a severe water stress until leaf elongation cessation. At different stages of the drought treatment (start, mid-drought and cessation of growth), ten plants per treatment were sampled to

measure the length of the stem, specific leaf area, water potential of mature leaves and growth zones, osmotic potential of growth zones, biomass of roots, leaves and stems, carbon and nitrogen content, and the discrimination of carbon ^{13}C.

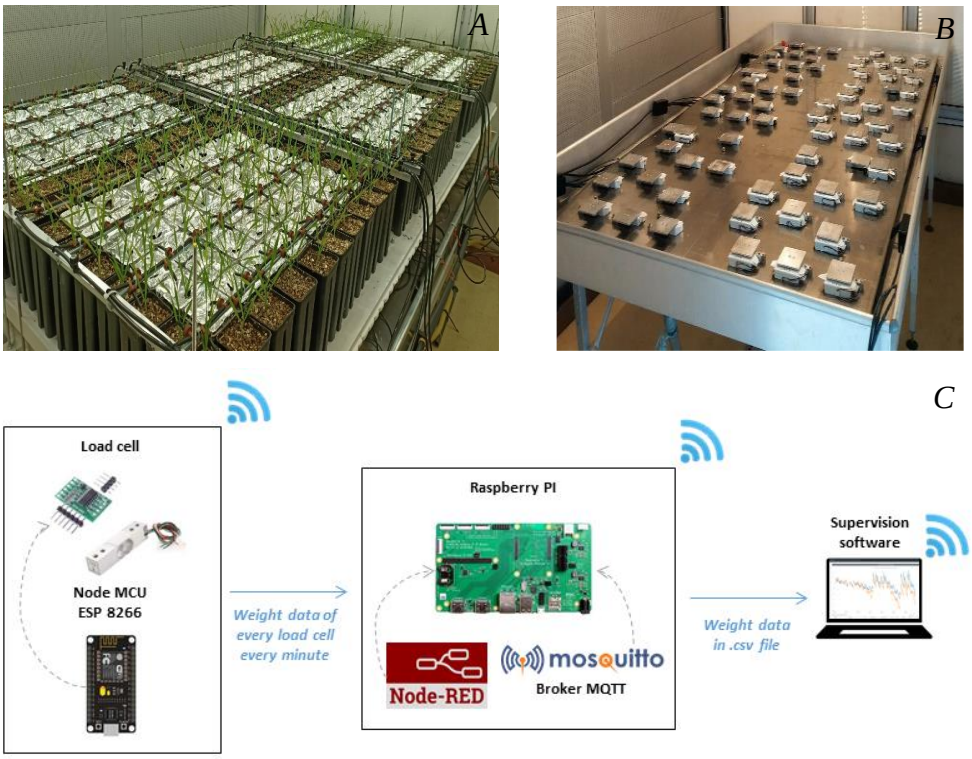

Figure 1. A) Experimental setup of one of the two growth chambers. B) System of connected load cells to measure the transpiration rate of individual plants C) Explanatory diagram of the connection between load cells and the supervision software. Each load cell (2 kg capacity) was linked to a Node MCU card and connected to a WiFi network. The software Node-RED and the Broker MQTT Mosquitto were installed on a Raspberry PI connected to the same WiFi network than the load cells.

LER measurement

Leaf length was measured daily on the first tiller of ten plant per treatment with a ruler. We calculated Leaf Elongation Rate (LER) per tiller by cumulating the elongation of all visible leaves of the first tiller.

Transpiration measurement

Plant transpiration rate was estimated gravimetrically by using a system of load cells placed under the 120 plants used for LER determination (fig. 1). We hypothesized that weight variations were only due to plant transpiration. Indeed, on the one hand the evaporation of the substrate was limited by the aluminum foil placed on the surface of the pots; and on the other hand, the transpiration flow being calculated with a short time step (1 min), the variation in plant weight due to growth was neglectable. To overcome the vibration disturbances related to the functioning of the growth chambers, the load cells were fixed on an 8 mm thick aluminum plate. An insulator was placed on the top of each load cell to limit temperature variations. To acquire plant weight every minute, a Node-RED program was installed on a Raspberry PI and interrogated a NodeMCU ESP8266 card installed on each load cell through a Broker MQTT Mosquitto. Weight values were concatenated, timestamped and saved in a csv file that was sent to a supervision software.

3 Results

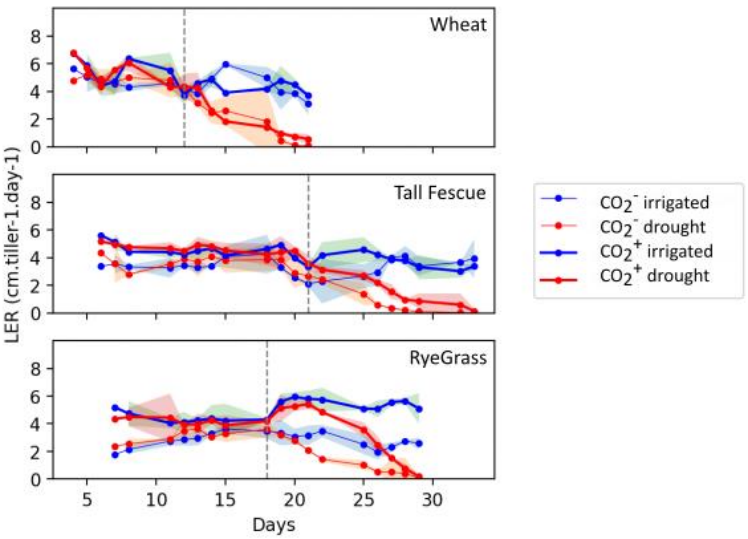

Figure 2. Leaf elongation rate (LER) in cm per day and per tiller. The vertical dotted line represents the beginning of the drought for the plants in red. The plants in blue are irrigated throughout the experiment. Low CO_2^- is represented by the thinner curve and high CO_2^+ by the thicker curve.

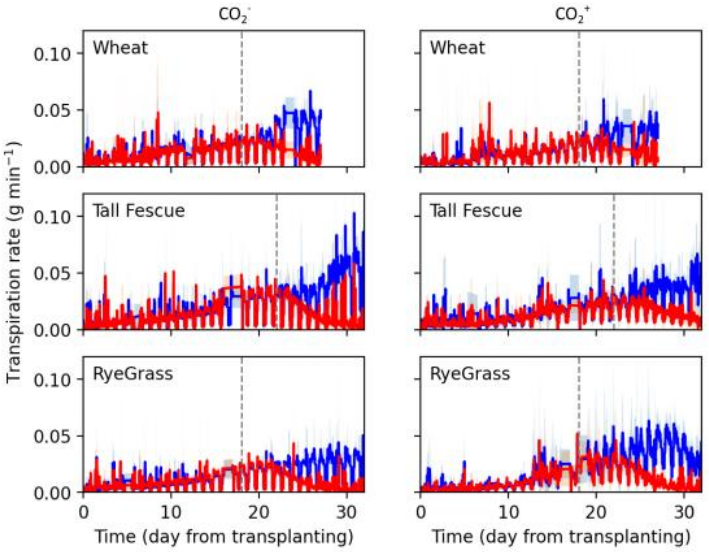

Figure 3. Instantaneous transpiration rate in gram per minute for each day since the plant pots were placed in the chamber. The vertical dotted line represents the beginning of drought for the plants in red. The plants in blue are irrigated throughout the experiment. Low CO_2^- is on the left and high CO_2^+ on the right. The horizontal line at day 10 is a lack of data following a power cut.

On average, LER of irrigated plants was about 5 cm day^{-1} for wheat, 4 cm day^{-1} for tall fescue and 5 cm day^{-1} for ryegrass. Unlike other species, LER of ryegrass plants strongly increased in response to elevated CO_2, from 4 cm day^{-1} at CO_2^- to 6 cm day^{-1} at CO_2^+. The LER dropped for all treatments after stopping irrigation. LER was null after 10 days for wheat whatever the CO_2 treatment. The drop in LER was faster at CO_2^- for tall fescue and ryegrass (LER = 0 after 6 and 8 days, respectively) than at CO_2^+ (LER = 0 after 12 and 11 days, respectively).

The daily transpiration rate of irrigated plants throughout the experiment increased with time (fig. 3). This was due to the development of plants and the increase in their foliar surface (data not shown). The cumulated transpiration at the end of the experiment for the irrigated plants in CO_2^- was 1000, 800, 500 g for wheat, tall fescue and rye grass, respectively. Elevated CO_2, decreased plant transpiration in wheat and tall fescue (900 and 550 g, respectively). The lower transpiration rate found at CO_2^+ was expected as elevated CO_2 induces stomatal closure, which limits water loss while maintaining high photosynthetic activity and plant growth. On the contrary, the transpiration of ryegrass increased with CO_2 (820 g), which also strongly enhanced leaf growth compared to the other species: at the end of the experiment, total biomass was three times higher at CO_2^+ for ryegrass, while it doubled for tall fescue and was multiplied by 1.5 times for wheat (data not shown).
Under conditions of water deficit, the rate of transpiration decreases progressively at both levels of CO_2 and stopped after a few days, as the LER.

4 Discussion

The innovative automatic weighing device presented in this study allowed us to measure the transpiration of individual plants and thus observe their water status under different environmental

conditions. It promotes understanding of the functioning of the plant at local scales (i.e. the organ) which explains what is happening on a larger scale (i.e. plant). Working on FSPMs, this analysis is needed and it seems promising to anticipate the response of leaf growth to the future environmental conditions and identify adaptation traits of plants. The results highlighted intraspecific variations in leaf growth response, especially for perennial plants (tall fescue and ryegrass), which gives us perspectives to work on the genericness of our model to different grass species

We studied the leaf growth response of three grass species to water deficit and increase of CO_2 concentration. The LER and transpiration flow showed that CO_2 boosts the plant development while limiting water loss by closing stomata. Plants respond differently to drought. In water deficit conditions, elevated CO_2 maintained growth of tall fescue and ryegrass for a longer period than at low CO_2 but finally we observed the growth cessation in any case. Growth of wheat stopped at the same time at both CO_2 level. Interspecific differences occurred for the plant development. All species presented greater plant development at elevated CO_2. But the ryegrass had the fastest LER. It resulted in a higher transpiration rate and in a higher water consumption than at low CO_2. For this species, the ability of CO_2 to mitigate the effects of water deficit seems to be limited.

This study provides quantitative information to be used for implementing the trophic and water dynamics interactions in a FSPM. Results on water and osmotic potentials, C and N contents and plant surface and biomass are currently being analyzed and will be used to validate the model's ability to simulate leaf growth in a wide range of environments.

5 References

Barillot, R., Chambon, C., Andrieu, B. (2016). CNWheat, a functional–structural model of carbon and nitrogen metabolism in wheat culms after anthesis. I. Model description. *Annals of Botany* 118, 997-1013.

Coussement, J. R., De Swaef, T., Lootens, P., Roldán-Ruiz, I., Steppe, K. (2018). Introducing turgor-driven growth dynamics into functional–structural plant models. *Annals of botany* 121, 849-861.

Durand, J. L., Lorgeou, J., Picon-Cochard, C., Volaire, F. (2013). Ecophysiologie de la réponse et de l'adaptation des plantes fourragères et prairiales au changement climatique. *Fourrages, Association Française pour la Production Fourragère*, 111-118.

Gauthier, M., Barillot, R., Schneider, A., Chambon, C., Fournier, C., Pradal, C., Robert, C., Andrieu, B. (2020). A functional structural model of grass development based on metabolic regulation and coordination rules. *Journal of Experimental Botany* 71, 5454-5468.

IPCC. (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K. and Meyer, L.A. (eds.)].

Maurice, I. (1997). Mise en place du volume et dépôt de composés carbonés et azotés au cours de la croissance de la feuille de fétuque élevée. Sciences Agronomiques. Université de Lorraine.