

**HORIZON
2020**

A combination of approaches from the molecular to the integrative plant level to understand the bases of stomatal responses to CO₂ and their variability

Reporting

Project Information

STOCOVAR

Grant agreement ID: 740222

[Project website](#) 

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Project closed

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€ 183 454,80

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UNIVERSITY OF BRISTOL

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Periodic Reporting for period 1 - STOCOVAR (A combination of approaches from the molecular to the integrative plant level to understand the bases of stomatal responses to CO₂ and their variability)

Reporting period: 2018-09-01 to 2020-08-31

Summary of the context and overall objectives of the project



Understanding plant responses to rising atmospheric CO₂ is of major interest given the necessity to select and develop new crop cultivars that will show resilience to climate change while maintaining yields. CO₂ influences gas-exchange by controlling the aperture of the stomata, microscopic pores found on aerial surfaces of most plants. Their opening and closure controls CO₂ uptake for photosynthesis and water vapour loss by transpiration. Photosynthesis and transpiration co-vary with the aperture and density of stomatal pores, depending on plant species, varieties and environmental conditions. In addition, photosynthesis and transpiration rates are codetermined by the leaf area, which is involved in light capture and is subjected to evaporation. Thus, plant productivity is positively coupled with water losses through stomatal characteristics and shoot development. This coupling has prompted plant scientists to define water-use efficiency (WUE) as the amount of biomass produced per unit of water used through transpiration. Plants respond to elevated CO₂ levels by reducing stomatal aperture, and recent advances have shed light on the intracellular machinery responsible for this response. However, little is understood about the variability in stomatal responses to CO₂, both among and within species. This project first aims at deciphering the main factors responsible for variations in stomatal responses to CO₂ and their impacts on whole plant performance (growth and WUE). It specifically addresses (i) the CO₂ response mechanisms that take place at the molecular level and its interplay with stress hormones that lead to stomatal closure when the CO₂ level increases, and (ii) the molecular mechanisms mediating the repression of stomatal development when plants are growing under elevated CO₂ concentrations. Another crucial question is how plants respond to CO₂ when in combination with other environmental constraints. Soil water availability is considered the primary factor limiting growth and productivity and thus, there is a clear and obvious need to study its interactive effects with elevated CO₂ on a range of plant water relations. Although CO₂ is often predicted to stimulate the yield of crops, counteracting the negative impacts of drought on food production, the positive or negative outcomes of these combinations might actually depend on the intensity and timing of drought stress relative to the development of the crop. Here, we also aim at revealing whether and how the drought- and CO₂- signalling cascades overlap and interact depending on the timing of drought to ultimately alter the trade-offs between carbon gain and water loss by leaves.

Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far



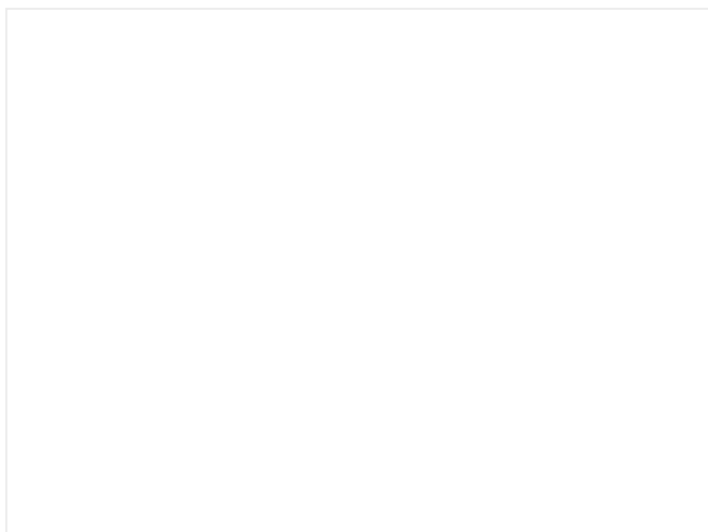
We have been investigating these questions from the cellular to the level of the whole plant by using the model plant *Arabidopsis thaliana*. We have used mutants in which particular genes are disrupted, to investigate their stomatal behaviour and thus understand the role played by these specific genes in the traits of interest. We gathered a unique collection of 50 mutants affected in stomatal development or function. We set up original phenotyping tools to allow a tight control of the CO₂ scenarios experienced by the plants and by setting up a platform made of connected balances for continuous measurement of plants transpiration dynamics. This was coupled with RGB imaging, with automated image analysis pipelines to measure plant growth, and with thermal imaging to track changes in plants

temperature, a proxy for stomatal conductance. Building on these biological resources and phenotyping facilities, we examined the role of the signalling pathway of a stress hormone (abscisic acid, ABA) in the plant response to elevated CO₂, depending on the timing of a drought event during its development cycle. We used mutants deficient in ABA biosynthesis or in the receptors for the hormone. Plants were subjected to different scenarios of drought while growing in elevated CO₂ and we measured their growth, water use, stomatal opening and photosynthesis to understand the role of these genes in maintaining stomatal function upon these complex combinations of environmental factors, and how this impacts on whole plant performance. We further addressed how the elevated CO₂ signal is sensed by the plants on the long term, by investigating the role of a carbonic anhydrase newly identified by the group. In order to extend our study to many mutants and many combinations of CO₂ x drought scenarios, we used the PHENOPSIS high-throughput phenotyping platform (Montpellier, EPPN2020 funding). We further extended our study to the natural variability within a major European crop, grapevine, by screening large populations for stomatal responses to CO₂, and linking their variability to allelic variations. In collaboration with INRAE (secondment and funding The Company of Biologists), we deployed an experiment in which grapevine leaves from 279 varieties were measured for their transpiration in response to elevated CO₂, together with leaf morphological structures. Genome wide analysis studies have allowed the identification of genomic regions controlling the variations in the traits of interest.

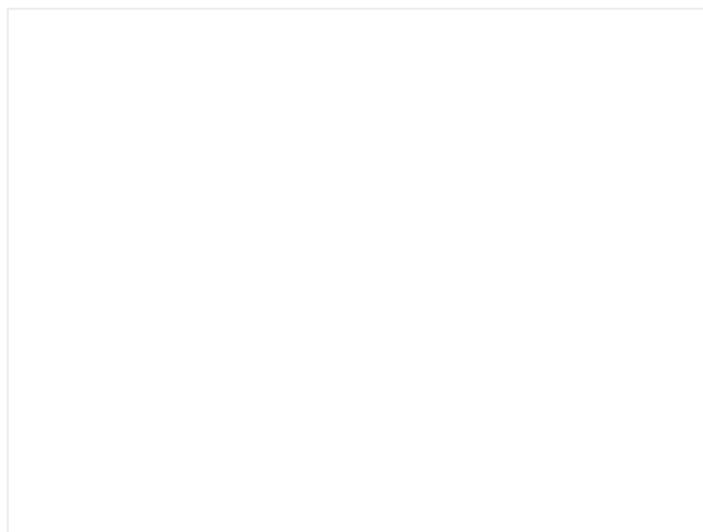
Progress beyond the state of the art and expected potential impact (including the socio-economic impact and the wider societal implications of the project so far)

This project has gathered massive and original datasets, both in Arabidopsis and grapevine. Despite the postponing of experiments due to the Covid-19 crisis, that led in turn, to postponing data analysis, all the experimental plans have finally been achieved. On the one hand, the original experiments based on thousands of Arabidopsis plants allowed us to study how plants respond to elevated CO₂ depending on the timing of a drought event during their growth cycle. This angle has rarely been explored despite its major relevance for agricultural production. By using mutants affected for different genes involved in the control of stomatal development, or stomatal closure, we found a wide range of responses (transpiration, growth, biomass allocation to vegetative or reproductive organs) between mutants, depending on the scenario. This finding showed that the relative importance of the signalling pathways studied depends on the timing of drought in the cycle. The ongoing analyses will allow us to evaluate which genes play a major role in growth maintenance and water saving in the face of these combinations of environmental factors. On the other hand, the work focused on grapevine is, to the best of our knowledge, the first to study responses to elevated CO₂ across the genetic diversity of this species. GWAS analyses have already led to the identification of genomic regions specifically controlling stomatal closure in response to high CO₂. Overall, the work carried out will contribute to understanding the effects of increased atmospheric concentrations of CO₂ on plants in order to have a better understanding of the effects of global environmental change on crop production. The outputs will be not only new fundamental knowledge, but also will pave the way to plant breeding by guiding the choice of new varieties that maintain production while saving water in the face of climate change. This project has already had impacts, with articles published and in preparation, and on Dr Coupel-

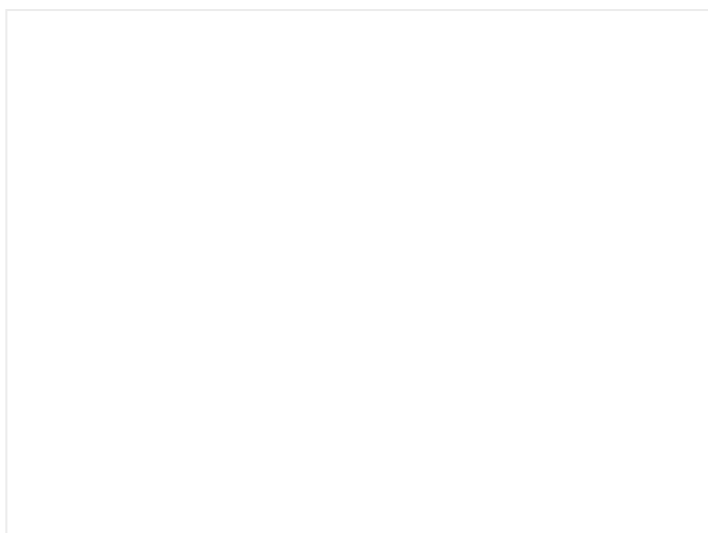
Ledru's career, who has been awarded an INRAE fellowship and is preparing an application to an ERC funding. There is no doubt that the STOCOVAR project has initiated a strong collaboration between the University of Bristol and the INRAE that will be pursued in the next years.



leaf imprint for measurements of stomatal density



thermal imaging of arabidopsis plants to assess rosette temperature



Mutants of *Arabidopsis thaliana* in the PHENOPSIS platform

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Permalink: <https://cordis.europa.eu/project/id/740222/reporting>

European Union, 2025