Science and Innovation Awards for Young People in Agriculture, Fisheries and Forestry

2021 round - 12-month and final report

As part of your 2021 Science Award grant you are required to provide milestone reporting at the 6-month and 12-month marks, which will be provided to ABARES and your Award sponsor. This is the 12-month and final report template. Please complete by providing detailed and clear information on your project and project outcomes. It must capture the full results of your project and be explained in depth. Challenges or unexpected obstacles, and how these were managed, can also be reported. Photos, charts, graphs and other documentation can be included. Refer to your grant agreement and milestones when completing this report.

As noted in your grant agreement, this final report should also include

1. a financial acquittal -a statement accompanied by a reconciliation of the expenditure against budget, signed and certified but not audited. It is to be on the grantee's letterhead and in their preferred format;
2. a signed statutory declaration as to whether the Project was carried out in accordance with the objectives, milestones and key performance indicators. The signatory can be the chairperson, managing director, chief executive, or equivalent officer of the Grantee.

We understand that these two items may take longer to coordinate and are agreeable to receiving the 12-month report in the first instance, with the financial acquittal and signed statutory declaration to follow within one month.

The due date of your 12-month progress report will be advised by the Community Grants Hub/ Department of Agriculture, Water and the Environment

Name: Demi Sargent
Award Sponsor: CRDC

1. **Description of your project: provide a short paragraph describing your project, including any variations.**

My project, "The Gatekeeper to Photosynthesis: Mesophyll Conductance and Abiotic Stress in Cotton" endeavoured to build on the limited understanding of mesophyll conductance – the process of CO_2 diffusion through the leaf to reach the site of photosynthesis – and how is responds in different cotton (*Gossypium*) species to heat and drought stress. Initially, we also aimed to use microscopic analysis to also understand whether anatomical characteristics and variation played a role in variation as a result of abiotic stress and inter-species differences.

However, due to delays as a result of the Covid-19 pandemic restrictions in Sydney and lowest-on-record radiation levels during the La Nina, these samples were taken but were not able to be analysed in time for this report due to significantly longer processing and imaging timeframes and having this task outsourced. Therefore, the milestones were changed to remove protein and RNAseq analysis, and instead add a more time-efficient experiment to investigate the temperature response of a broader range of *Gossypium* species.

2. Project milestones completed: Describe the milestones and outcomes completed in this stage. If any milestones were not undertaken or varied from your grant agreement, please provide an explanation. Refer to your grant agreement for the agreed list of milestones.

Deliverables/activity	**Status**
Heat- and drought-stress experiments: - Expose three species (n=6) to combinations of heat and drought simulations - Measure response of mesophyll conductance combined with gas-exchange analysis to determine changes in CO_2 assimilation and water use. Take leaf samples for microscopy, proteomics and RNAseq	Mesophyll conductance was measured in two species, *Gossypium hirsutum* cv. Sicot 71 and *Gossypium bickii*, in response to prolonged elevated temperature (+6°C) and soil water deficit (60 – 70%). Samples for microscopy, proteomics and RNAseq were taken and stored at -80°C.
- Microscopy: Anatomical variation in chloroplast distribution and cell wall thickness to assess the pathlength of CO_2 diffusion. Outsource sectioning and imaging to CAM at ANU. - Confocal/light microscopy; SEM; TEM. - Three *Gossypium* (cotton) species (*G. hirsutum* cv. Sicot 71 and two wild species – *G. australe* and *G. raimondii*). ~10 samples per method (30 samples each species)	Samples have been fixed and embedded in resin blocks. Sectioning and imaging has begun with the assistance of CAM at ANU. Completing this microscopic analysis has been planned for future work.
Proteomics/RNAseq analysis: - Cellular fractionation for enrichment of chloroplast and plasma membranes. This will be used to extract membrane proteins from three species (n=5); purify membrane proteins using GC-MS; outsource proteomic analysis - Analyse RNAseq data for variation in membrane protein expression between three species	Due to the delayed start of the project due to campus closures associated with the Covid-19 pandemic and lowest-on-record light levels hindering the growth of summer crops such as cotton, this experiment was replaced with a more time efficient experiment to investigate the temperature response of mesophyll conductance in five species from diverse geographic origins: *Gossypium hirsutum* cv. Sicot 71, *G. arboreum*, *G. harknessii*, *G. bickii* and *G. sturtianum*. Completing the proteomics and RNAseq analysis has been planned for future work.

3. Results: What results do you have from your project? Have the original aims and objectives of your project been achieved? Were there any factors that caused difficulties or setbacks in achieving your goals? Be detailed in documenting the project outcomes.

We developed an accurate pipeline for gravimetrically applying drought by monitoring and controlling pot weight to obtain a certain level of soil water deficit (SWD, %; Fig. 1) in order to produce a leaf water potential indicative of drought stress (~<-1.5 MPa for cotton; Fig. 2A).

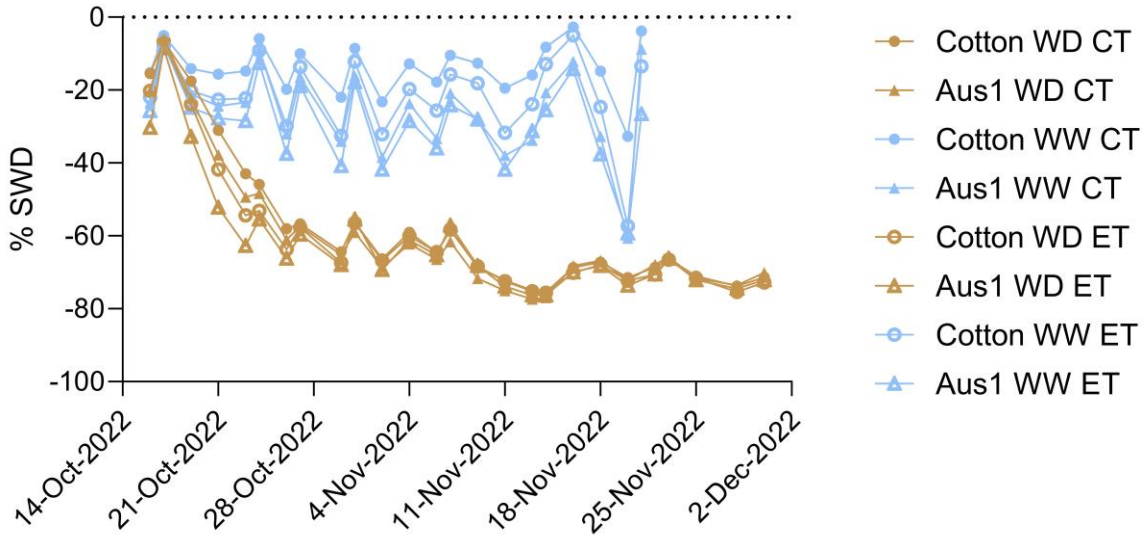

Fig. 1. Percentage Soil Water Deficit (% SWD) of potted *G. hirsutum* cv. Sicot 71 (Cotton) and *G. bickii* (Aus 1) plants throughout the elevated temperature and drought experiment. Treatments included control temperature (CT; triangles), elevated temperature (ET; circles), well-watered (WW; blue), and water-deficit (WD; brown).

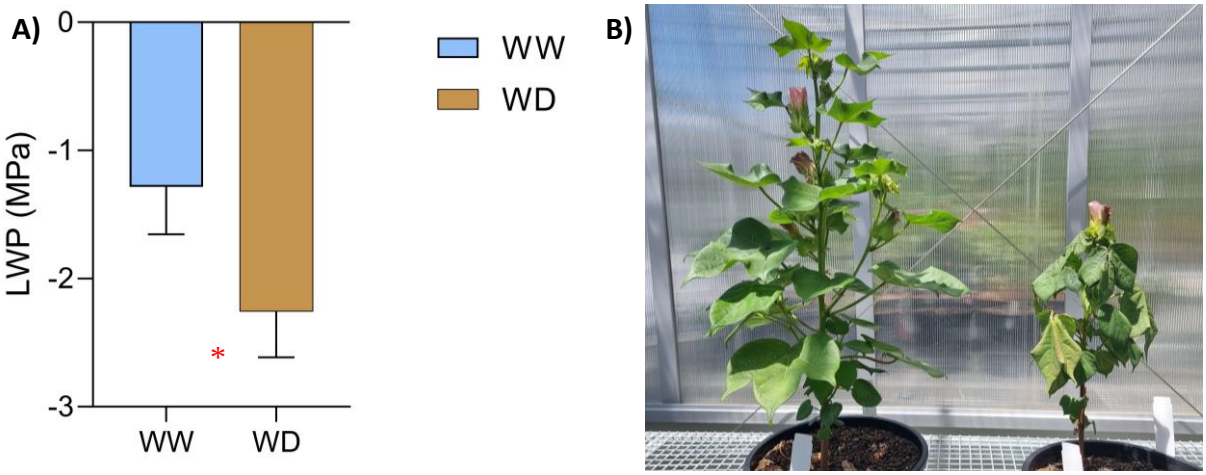

Fig. 2. A) Leaf Water Potential (LWP; MPa) of well-watered (WW; blue) and water-deficit (WD; brown) treated plants (*F pr.* = 0.03). B) well-watered (left) and water-deficit (right) treated *G. hirsutum* cv. Sicot 71.

Overall, a 60-70% soil water deficit was applied for 6-7 weeks (17/10/2022 – 30/11/2022; Fig. 1). The degree of soil water deficit was increased as plants showed signs of acclimation (reduced wilting or improved stomatal conductance/gas exchange function).

Substantial changes to biomass in water-deficit treated plants across both species were observed. Biomass data including leaf number, leaf area and dry weight of stems, leaves, reproductive material and stems have been collected and will be analysed and presented at the Australian Cotton Research Conference in September 2023.

Mesophyll conductance was negatively affected by soil water deficit (*F pr.* = 0.008), where plants subjected to water-deficit experienced a 27% reduction in mesophyll conductance compared to well-watered plants. Elevated temperature (38°C) increased mesophyll conductance by 30% compared to plants under control temperature (32°C). (*F pr.* <0.001) and species.

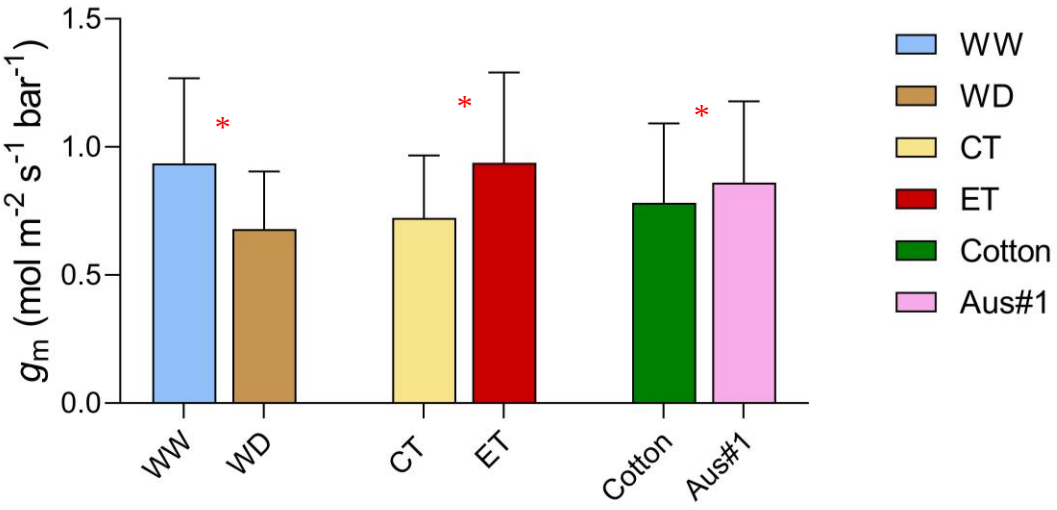

Fig. 3. The effects of water, temperature and species on mesophyll conductance (g_m, mol m^{-2} s^{-1} bar^{-1}). Well-watered (WW), water-deficit (WD), control temperature (CT); elevated temperature (ET)

In *G. hirsutum* cv. Sicot 71, the rate of net photosynthetic CO_2 assimilation, decreased in response to soil water deficit and the combination of soil water deficit and elevated temperature compared to well-watered plants (Fig. 4A). Noteworthy differences in net photosynthetic CO_2 assimilation between *G. hirsutum* cv. Sicot 71 and *G. bickii* were a greater CO_2 assimilation rate in *G. bickii* under soil water deficit (orange asterisk) and the combination of soil water deficit and elevated temperature (red asterisk; Fig. 4A). This could indicate superior abiotic stress tolerance of this native Australian species, supporting further investigation into this species for heat and drought tolerance traits for use in cotton cultivar development programs.

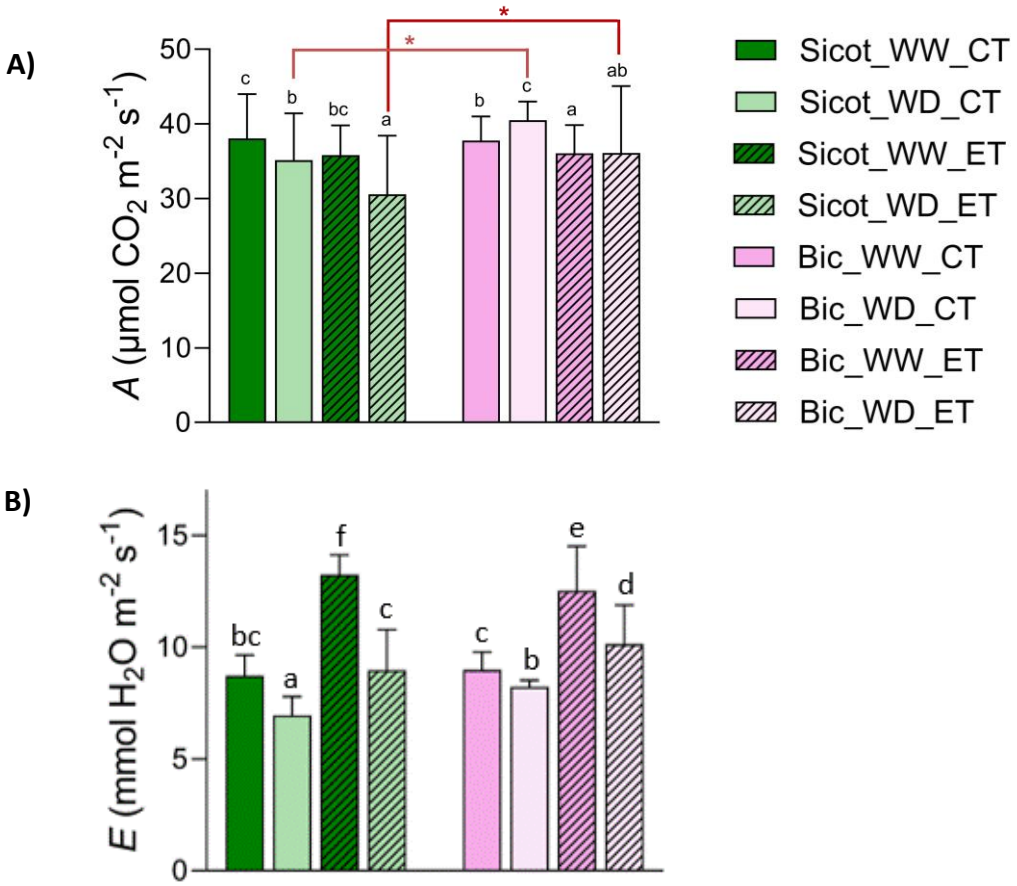

Fig. 4. The response of A) net CO_2 assimilation rate (*A*) and B) transpiration rate (*E*) to water deficit (pale bars) and elevated temperature (striped bars) in *G. hirsutum* cv. Sicot 71 (green bars) and *G. bickii* (pink bars). Letters denote statistical differences (*F pr.* <0.05) between groups.

Transpiration (evaporative loss of water from the leaf's surface) increased in both species in response to elevated temperature under both well-watered and water-deficit conditions (Fig. 4B). This is a common response to high temperature stress, as evaporative cooling allows plants to avoid heat stress by maintaining physiologically "comfortable" leaf temperatures. The degree of increase in transpiration was lower in water-deficit plants, likely due to the reduced water available to transpire. Notably, *G. bickii* was capable of greater rates of transpiration under water deficit conditions compared to *G. hirsutum* cv. Sicot 71 (Fig. 4B). This would indicate that *G. bickii* was not behaving conservatively under water deficit conditions in favour of maintaining greater photosynthetic rates by maintaining higher stomatal (leaf surface pores) openness.

The temperature response of mesophyll conductance in *G. gossypioides* from Central Oaxaca was statistically different from the other four *Gossypium* species studied here (Fig. 5). This species consistently had the lowest mesophyll conductance across all temperatures, although it did increase substantially with temperature. Of particular note is the lack of temperature sensitivity of mesophyll conductance in *G. arboreum* and the strong positive response of mesophyll conductance with temperature in *G. bickii* and *G. sturtianum*. It is notable that the climate of origin of *G. bickii* and *G. sturtianum* – both originate from areas of Australia including Central Australia – is hot (mean summer daily maximum temperature >35°C) and arid to low mean annual precipitation. In contrast, *G. gossypioides* is from Central Oaxaca, which is a warm (mean summer daily maximum temperature ~33°C) and average precipitation climate of origin.

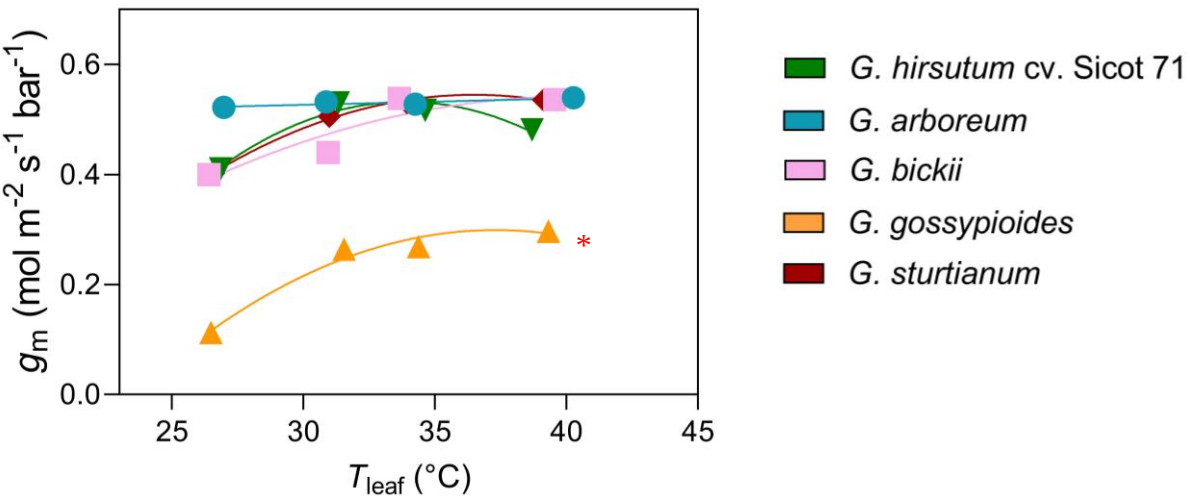

Fig. 5. The response of mesophyll conductance (g_m) to temperature in five diverse species: *G. hirsutum* cv. Sicot 71, *G. arboreum*, *G. bickii*, *G. gossypioides* and *G. sturtianum*.

The start of the project was delayed by 6 months due to campus closures associated with the Covid-19 pandemic. An additional delay of several months occurred due to the La Nina resulting in the lowest-on-record light levels which significantly hindered the growth of summer crops such as cotton. We overcame the significant delay by replacing the time-consuming RNAseq and proteomics analyses with a temperature response experiment. Cotton requires maximal radiation in order to grow, thus we overcame the issue of extremely low light by using the funds allocated for the RNAseq and proteomics analyses to purchase LED lights to hang in the glasshouse.

4. **Benefits to industry: Describe how your industry will benefit from your project results. Have there been any steps already taken with industry, or plans to engage stakeholders? Are you working with your Award sponsor to reach industry stakeholders?**

The industry will benefit from our drought pipeline which is more accurate and allowed for even fine changes in gas exchange to be distinguished. Our approach of exploring wild *Gossypium* species to screen for superior abiotic stress responses is novel. Additionally, this has unveiled *G. bickii* as a potential candidate species for sourcing novel heat and drought resilience traits from for cotton cultivar development programs. Further, this research has improved our understanding of the poorly-understood mechanism of mesophyll conductance, and importantly, how it is impacted by high temperatures and drought stress. Through the experiment we replaced the RNAseq and proteomics analyses with, we have gained crucial insight into how the temperature sensitivity of mesophyll conductance varies between species. This new knowledge is important for forecasting changes to crop productivity associated with climate change and identifying avenues to improve crop yield potential through targeting mesophyll conductance as the gatekeeper to photosynthesis.

With the support of our research manager from CRDC, we have been working on engaging CRDC again in addition to Cotton Seed Distributors (CSD) to invest in future research into mesophyll conductance to determine whether it is a trait of value for the cotton industry. We hold biannual steering committee meetings with CRDC and CSD, during which I have been updating them of the progress of this research and proposing future directions of this work including characterising the anatomical and biochemical components of mesophyll conductance and identifying additional species with superior mesophyll conductance.

5. **Budget expenditure: How has your grant funding been expended? (Refer to the budget from your grant agreement and your six-month report). Include details of any unspent funds. A financial acquittal is required at the conclusion of your project. Refer to page one of this report for advice.**

The entire allocation of $20,000 was spent. See financial acquittal attached below:

6. **Dissemination: Describe the communication activities you have undertaken (eg speaking at external events and seminars, media coverage, contributions to newsletters, journals or published articles etc). How effective were these activities in raising awareness of your project?**

All results presented in this report were presented via oral presentation and poster presentation at the international Gordon Research Conference and Seminar "CO2 Assimilation in Plants from Genome to Biome" in Lucca, Italy in May 2023. This was a valuable opportunity to speak with the world's leaders in photosynthesis, including world-renowned mesophyll conductance expert Associate Professor Jaume Flexas and photosynthesis experts Professor Carl Bernacchi, Professor Tom Sharkey and Assistant Processor Berley Walker. The in-depth discussions I had throughout this conference gave me additional avenues to investigate such as looking into the influence of cell wall composition on mesophyll conductance, and more refined measurements to consider for analysing leaf and canopy temperature. This event helped establish my visibility as a researcher of mesophyll conductance and photosynthesis amongst the highest calibre researchers in the field of photosynthesis.

7. **Personal benefits: What benefits has this Science Award grant provided you? (eg assisted in career or skills development? presentation skills and opportunities? to continue working in your chosen field of research?)**

The Science and Innovation Award has given me an opportunity to focus on an area of research that I otherwise would not have had during my postdoctoral fellowship, that will likely result in a high impact publication. This has given me the opportunity to explore more fundamental scientific questions, in contrast to my very applied postdoctoral research. This prestigious award has also improved my visibility as a researcher, which I believe will strengthen my CV and ability to obtain future funding and research opportunities.

8. **Future work: Have you identified any future work opportunities to build on your project outcomes? What will you do in your career over the next 12 months?**

We plan to complete the microscopic analyses from preserved samples as future work to complete this research for publication in a Q1 journal, which should be fairly simple with the immense amount of data we have already collected from this grant. Over the next 12 months I will finalise my postdoctoral research which includes a variety of molecular biology experiments and crop scale modelling to further develop our pipeline for enhancing photosynthesis in cotton through synthetic biology. During this time, I aim to publish four papers from my PhD which was conferred July 2021.

7. Contact with your Award sponsor: Have you maintained contact with your Award sponsor during your project? Do you intend to continue this contact in the future?

Contact has been maintained with our award sponsor throughout the project, especially during challenges such as the Covid-19 pandemic restrictions in Sydney and while troubleshooting the low radiation levels resulting from the La Nina. We intend to maintain our

connection with CRDC, both for my current postdoctoral research fellowship, and for future research if possible.

8. Declaration and signature

I certify that the information presented in this report is an accurate statement of my project for the 2021 Science and Innovation Awards for Young People in Agriculture, Fisheries and Forestry.

If not included with this final report, I confirm that a financial acquittal and a signed statutory declaration will be provided within one month of this final report submission.

Signature:	
Date:	31/07/2023

Return the completed report to the Community Grants Hub.

For enquiries contact Maree Finnegan, Science Awards Manager, ABARES, GPO Box 858,

Canberra, ACT, 2601; Ph: 02 6272 2260 / 0417 689 567 / scienceawards@awe.gov.au

Thank you, and we hope that you have found your involvement in the 2021 Science and Innovation Awards to be a valuable and worthwhile experience. We wish you success in the next stages of your career.