Will elevated atmospheric carbon dioxide change how oak flowers?

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BACKGROUND

The timing of annual flowering in trees is crucial to ensure successful pollination and seed-set. This is not only vital to tree species but to the many species that depend on tree flowers and seeds as a food source. Evidence is emerging that tree species are responding to climate change by altering the time at which they flower[1], risking major disruption to ecosystems. The genetic control of flowering has been intensively studied in annual plants but not in trees, where the behaviour is more complex (**Fig. 1**). Determining if the same genes control flowering in trees will let us better predict their behaviour in future. **Elevated atmospheric CO_2 (eCO_2) also has a complex effect on flowering time[2], varying both within and between species**[3], but its effects have mostly been studied in annual plants[3].

Utilizing the BIFoR FACE experiment, **this project aims to identify the genes that control flowering in the tree species oak (*Quercus robur*) and test whether these genes are responsive to eCO_2.**

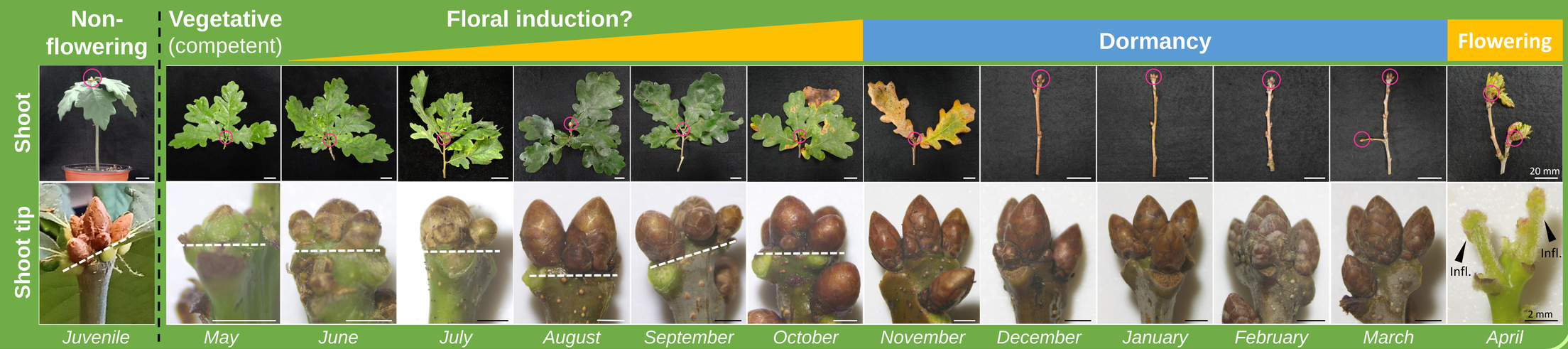

Fig. 1 Schematic of oak seasonal flowering.
'Adult' oak trees flower each year, with female flower spikes (inflorescences, infl.) emerging below the shoot apex during bud-burst (April). These are thought to be triggered by environmental signals received by the shoot growing tip (magenta circle) during the previous growing season (May – October). In contrast to this, 'juvenile' oak plants cannot flower even when grown under seasonal conditions that trigger flowering in 'adult' trees.

The genes that control flowering in oak are unknown, but could be the same as those that control flowering in annual plant genetic models[4].

METHOD

Fig. 2. On-site collection and preservation of oak shoot tissue.

To study gene expression, oak shoot tip samples were collected from adult trees in the BIFoR FACE arrays (x3 eCO_2, x3 ambient air) once per month across the growing season (May – October) for RNA extraction and RNA-seq analysis.

A major limitation to molecular analysis of trees is the need to snap-freeze tissue ASAP to preserve RNA in a forest environment. **We tested an alternative 'cut flowers' method** for collection (**Fig. 2**), whereby freshly-cut oak sprigs (**A**) were immediately wrapped in wet paper towel to maintain water flow to the shoot tip (**B**), and then sealed to reduce water loss (**C**). Samples were maintained at ambient temperature during collection. After collection, all samples were transported to UoB Biosciences where shoot tips were dissected out (**Fig. 1**, **white dotted lines**) and snap-frozen on liquid nitrogen for long-term preservation.

As a control, shoot tips were also dissected from glasshouse-grown oak saplings and immediately snap-frozen.

We are extremely grateful to Gael Denny, Kris Hart and Rob Keyzor Tree Surgeons for their help with facilitating monthly tissue harvesting from the BIFoR FACE arrays.

RESULTS

RNA was extracted from shoot tips using a modified QIAGEN Rneasy protocol and tested for quality prior to mRNA-seq analysis by Novogene (Cambridge, UK).

RNA extracted from forest-collected adult shoot tips was found to be of similar quality to that extracted from juvenile shoot tips snap-frozen immediately after collection, with no evidence of degradation (**Fig. 3**). For all samples RNA Integrity (RIN) values were ≥7.0, sufficient for mRNA-seq.

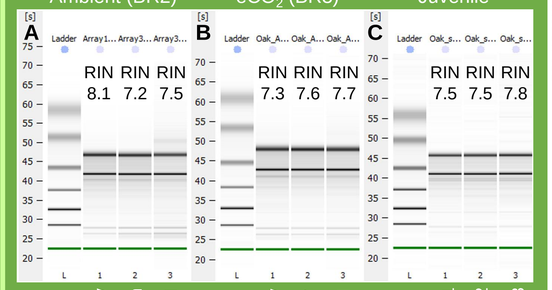

Fig 3. Agilent Bioanalyzer RNA 6000 nano electrophoresis analysis of RNA from adult ambient (**A**), adult eCO_2 (**B**) and juvenile (**C**) oak shoot tips.

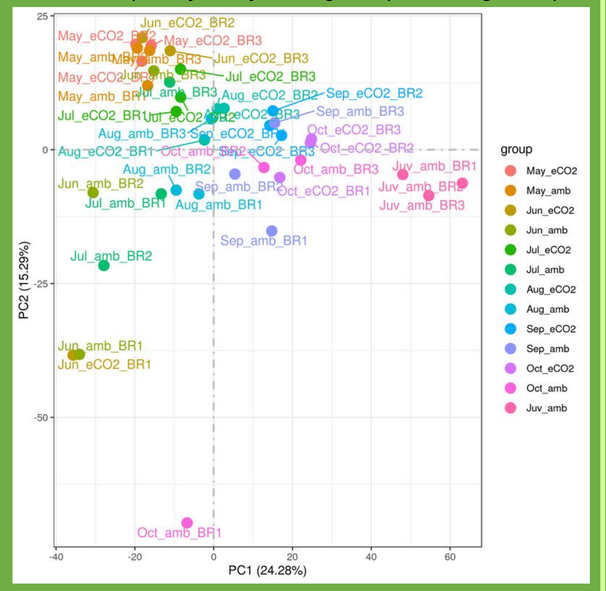

Fig 4. Principal Component Analysis of global gene expression by sample.

Principal Component Analysis (PCA) of global gene expression between samples (**Fig. 4**) found that, in most cases, biological replicates within each month+CO_2 treatment combination cluster more closely together than with samples from other months. Adult samples clustered separately from juvenile. Within adult samples there is consistent separation of variation between months (horizontal axis) and, within most months, separation between eCO_2 and ambient (vertical axis). **This suggests consistent gene expression changes relating to time and eCO_2 are present in this dataset.**

CONCLUSIONS (SO FAR…)

- Preserving collected oak shoot tissue on-site using the 'cut flowers' method apparently prevents RNA degradation in the shoot tip at ambient temperature.
- Need to test for any artefactual effects on gene expression within the shoot tip caused by this method, but PCA analysis suggests that the mRNA-seq data generated contains genuine signals of seasonal and eCO_2 response.
- If successful, this low-tech collection method could be applied to other field studies involving tree material.

FUTURE WORK

- Differential Gene Expression (DGE) analysis of mRNA-seq data is needed to identify candidate seasonal regulators of oak flowering and eCO_2-responsive genes.
- Candidate expression changes will be validated via qPCR analysis across three separate growing seasons.
- Corresponding leaf samples harvested alongside shoot tips will be sequenced to identify candidate flowering-promoting signals originating in the canopy.

REFERENCES

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