DEFRA WGIN Sub-Contractor Interim Report – end of funding year 1

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1. Background and Objectives

1.1 Background

Carbon isotope discrimination ($\Delta^{13}C$) has been used in wheat to screen for genotypes with high transpiration efficiency (Rebetske et al., 2002) and this has led to the release of new wheat varieties (e.g. Rees and Drysdale) in Australia better suited to drier conditions during grain filling. More recently another isotope-based screen using ^{18}O enrichment has been shown in maize (Barbour, 2007) and wheat to correlate with yield potential and drought resistance (Cabrera-Bosquet et al. 2009). The $\Delta^{18}O$ signature of vegetative tissues is known to reflect variation in evaporative enrichment in leaves due to transpiration, and has been shown to be negatively correlated with transpiration rate (Barbour et al., 2000). Therefore, measurement of the $\Delta^{18}O$ signature might provide a powerful tool for plant breeders to track genotypic differences in drought resistance. In addition, the total leaf ash content of plant tissues has been suggested as a useful tool to predict yield performance under drought (Araus et al., 2002). The mechanism of mineral accumulation in plant tissues appears to be explained through the passive transport of minerals via xylem driven by transpiration. Thus, ash content measured in plant tissues may provide an indicator of transpirative gas-exchange activity and therefore of the total water transpired. This analysis, which is less expensive than analysis of $\Delta^{13}C$ or $\Delta^{18}0$ of plant tissues, could therefore have potential application with future prebreeding research activities as well as in breeders' trials. With a view to phenotyping large association genetics panels in future projects where costs may become prohibitive, it is worth testing relationships between ash content and genotypic differences in water use and grain yield in the present WGIN2 drought trials. If results are encouraging, this technique could then be applied more widely in future prebreeding research activities as well as in breeders' trials.

The $\Delta^{18}0$ value is expected to provide added value to the WGIN drought trials (in addition to the existing delta $\Delta\ ^{13}C$ value) because, although much attention has focused on improving WUE when breeding for drought adaptation, it seems that, except for very severe drought conditions, water use (WU, i.e. the total water absorbed and further transpired by the plant) is a more important adaptive trait than WUE (Slafer & Araus, 2007). This is related to the genotypic capacity to use available water and therefore to sustain transpiration under unfavourable conditions. The $\Delta^{18}0$ of vegetative tissues can be used as an indirect measure of transpiration and WU.

1.1 Objectives

In this sub-contract we explore the use of $\Delta^{18}0$ and $\Delta^{13}C$ of the flag leaf and total mineral ash content of flag-leaf in wheat as new tools for phenotyping wheat with respect to water inputs. We study a panel of 18 wheat genotypes which are being grown under irrigated and unirrigated conditions in the DEFRA WGIN Drought Tolerance sub-project (Activity 9) in experiments carried at University of Nottingham in 2009-10 and 2010-11. We compare genetic variability in $\Delta^{18}0$ (flag leaf), $\Delta^{13}C$ (flag leaf) and total mineral ash content (flag leaf) with that for $\Delta^{13}C$ (grain) which is being already being measured as a part of the core WGIN UoN funding and respective correlations with grain yield under drought.

2. Material and methods

2.1 Experimental design and plot management

Seventeen winter wheat cultivars and one spring wheat cultivar Paragon were used to identify physiological isotope screens indicative of water-use efficiency, water use and drought tolerance. The varieties were grown under irrigated and unirrigated conditions in a field experiment at Nottingham University in 2010-11 on a sandy loam soil type (Dunnington heath Series). The experimental design was a split plot block (randomised block) with three replicates. Irrigation treatments were randomised on main plots and cultivars on sub plots. Sub plot size was 12 x 1.65m.

Wheat Genotypes tested (18):

1. Avalon	10. M. Widgeon
2. Beaver	11. Oakley
3. Cadenza	12. Panorama
4. Capelle Desprez	13. Paragon
5. Cordiale	14. Rialto
6. Glasgow	15. Savannah
7. Hereward	16. Soissons
8. Hobbit	17. Xi19
9. Istabraq	18. Zebedee

Irrigation Treatment: In the irrigated treatment, a trickle irrigation system was used to maintain soil moisture deficit (SMD), calculated using the ADAS Irriguide model (Bailey & Spackman 1996), to <0·50 available water (AW) up to GS61+28 days and <0·75 AW thereafter. The AW capacity to 1·2m soil depth was 176 mm. No water was applied in the unirrigated treatment.

The previous crops was oats. The experiment was sown in the 2nd week in October 2010. Nitrogen and P, K fertilizers were applied to ensure that nutrients were not limiting. Herbicides. fungicides and pesticides were applied as required to minimise effects of weeds, diseases and pests. PGRs (Chlormequat) was applied at GS31 to reduced the risk of lodging.

2.2.. Experimental measurements

Flag leaf samples at GS61 and grain dry matter samples at harvest were assessed for carbon 12/13 isotope discrimination (grain samples through core funding). Flag leaf samples at GS61 were also assessed for oxygen 16/18 isotope enrichment and for mineral ash content.

Date of GS61 was measured by recording Zadoks' stages for each sub-plot every 3-4 days through the flowering window. Plant height was recorded in all 108 sub-plots from GS75 to harvest. Growth analysis samples were taken from a 0.64 m^2 area and 10% sub-sample of plant material processed and dry weighted. Flag leaves from the 10% sub-sample were used for the isotope analysis. A sub-plot area of at least 5 m^2

was machine-harvested at harvest and grain weighed and grain yield expressed at 15% mc.

Carbon 12/13 isotope (Δ^{13}C) and Oxygen isotope (Δ^{18}O) discrimination analysis
From each-sub-plot dried flag leaves and grain were milled separately for use in carbon12/13 and oxygen 16/18 isotope analysis. The samples were dried at 48 hours at 80 °C then ground to a fine powder using a cyclotec 1093 sample machine. The milled samples were then submitted to the isoptope laboratory at The James Hutton Institute and analysed through an online system composed of an elemental analyser, a TripleTrap and a mass spectrometer to determine carbon isotope composition.

Mineral ash analysis
From each plot, milled flag leaf samples were submitted to the chemical analysis laboratory for mineral ash determination by combustion analysis.

Crop water uptake
Soil water uptake was measured directly for eight cultivars (Cordiale, Hobbit, Glasgow, M.Widgeon, Xi19, Rialto, Savannah, Soissons) in the unirrigated plots by gravimetric analysis of four soil cores per plot to 120 cm. Soil cores were taken on 21 April and 10 August and the water uptake calculated as the difference between these two estimates of soil water content plus rainfall in the intervening period.

3. Results for WGIN Drought Tolerance trial 2009-10

From the core WGIN DEFRA funding grain Δ 13C results showed a highly significant linear relationship with grain yield amongst the eighteen cultivars under both irrigated (R^2 = 0.72. P< 0.001) and unirrigated (R^2 = 0.30. P< 0.001) conditions (Fig.1). The corresponding relationship between the grain yield and flag-leaf Δ ^{13}C was not statistically significantly under irrigation, and was only weakly significant under irrigated conditions (R^2 = 0.18, P< 0.10; Fig. 2). Fitting a linear relationship across both the irrigated and unirrigated treatment combinations, there was a significant linear regression (R^2 = 0.35, P< 0.001). For both grain and flag-leaf Δ ^{13}C results indicated that WUE was negatively associated with grain yield (i.e. suggesting water use was positively correlated with grain yield). The linear relationship between grain yield and flag-leaf Δ ^{18}O was not statistically significant amongst the 18 genotypes under either irrigated or unrrigated conditions; however, fitting a linear relationship across irrigated and unirrigated treatment combinations there was a significant relationship (R^2 = 0.32, *P*< 0.001) (Fig. 3). Nevertheless, the statistical significance of the relationship between grain yield and either of flag-leaf Δ ^{13}C or Δ ^{18}O was much lower than that for the relationship between grain yield and grain Δ ^{13}C**.**

Grain yield was not significantly associated with mineral ash content of the flag leaf either under irrigated or unirrigated conditions amongst the 18 genotypes, or combining data across the two irrigation treatments (Fig. 4).

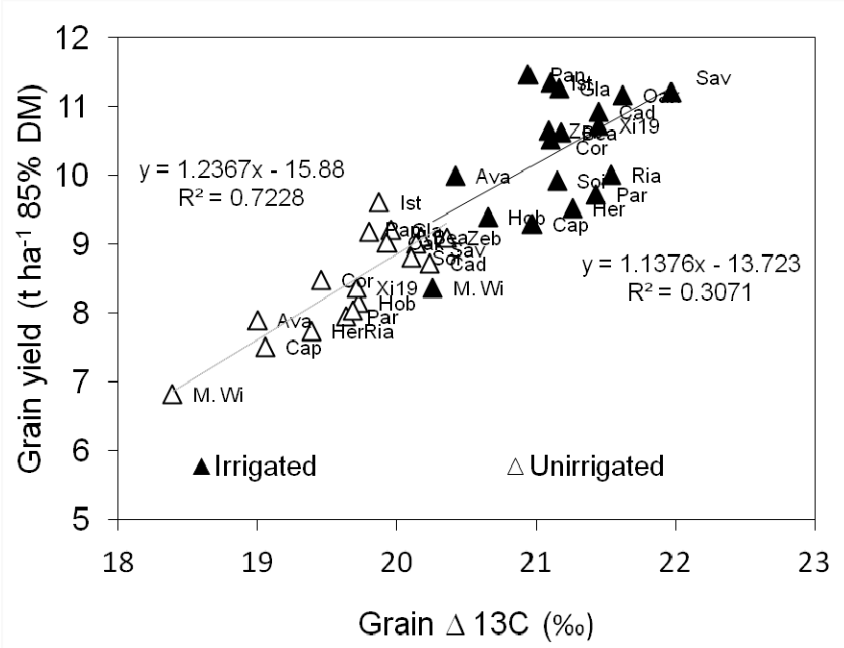

Fig. 1 Linear regression of grain yield on flag leaf Δ ^{13}C discrimination amongst 18 wheat genotypes under irrigated and unirrigated conditions at Sutton Bonington 2009-10.

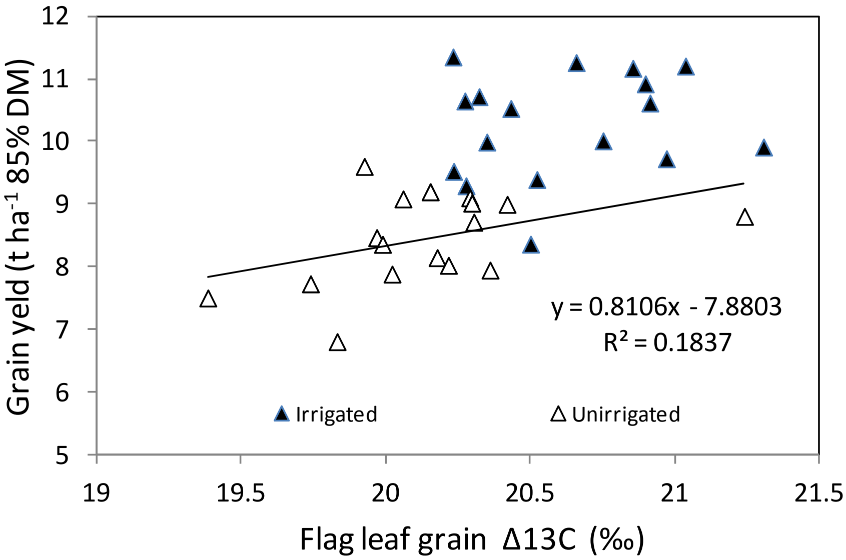

Fig. 2 Linear regression of grain yield on flag leaf Δ ^{13}C amongst 18 wheat genotypes under irrigated and unirrigated conditions at Sutton Bonington 2009-10.

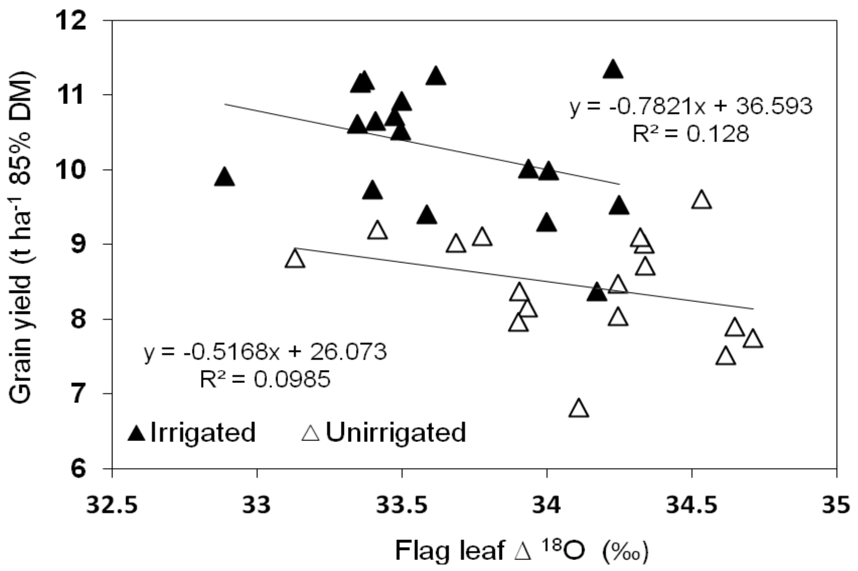

Fig. 3 Linear regression of grain yield on flag leaf Δ ^{18}O amongst 18 wheat genotypes under irrigated and unirrigated conditions at Sutton Bonington 2009-10.

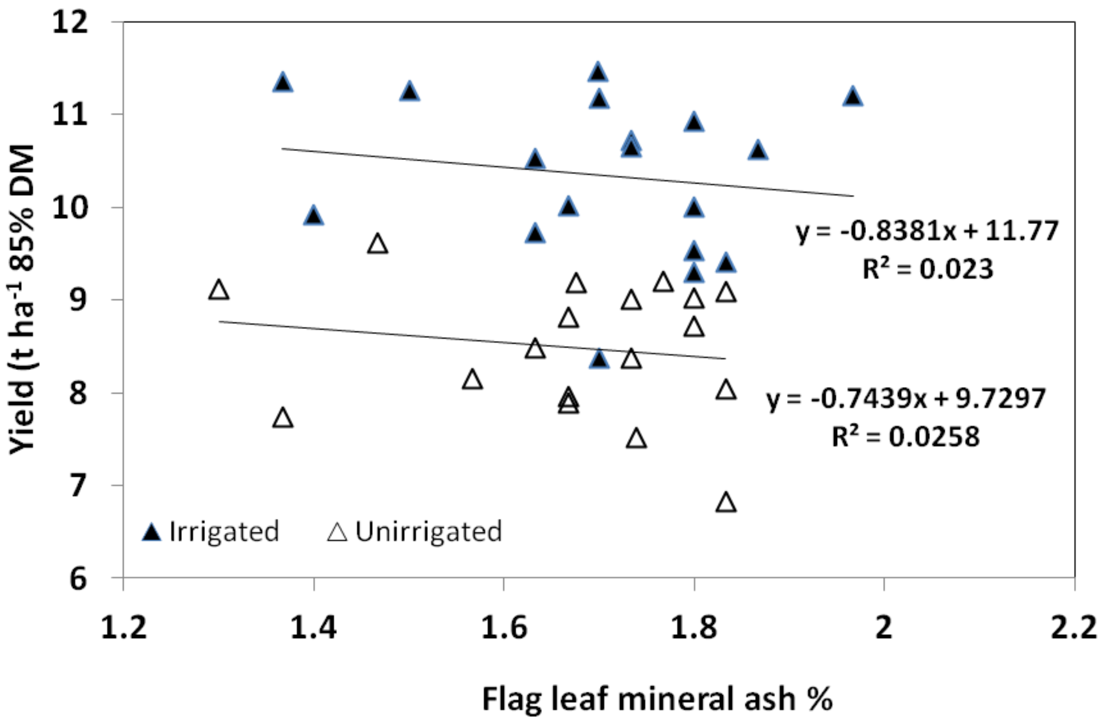

Fig. 4. Linear regression of grain yield on total mineral ash content of flag leaf (%) amongst 18 wheat genotypes under irrigated and unirrigated conditions at Sutton Bonington 2009-10.

Flag-leaf Δ ^{18}O is not strongly influenced by photosynthesis rate, so measurement of grain Δ ^{13}C and flag-leaf Δ ^{18}O allows stomatal and photosynthesis effects on Δ ^{13}C to be teased apart. Fig. 5 shows that under drought there is a tendency for a negative linear assocation (R^2 = 0.13, P = 0.16) between grain Δ ^{13}C and flag-leaf Δ ^{18}O, i.e. higher water-use effciency is associated with lower water use (hence yield). Although this relationhsip was not statisitically signficant under either irrigated or unirrigated conditions, it was for values averaged across irrigation treatments; there was negative linear relationship between grain Δ ^{13}C and flag-leaf Δ ^{18}O amongst the 18 cultivars (R^2 = 0.22, P = 0.06). Also there was a negative linear relationship between grain Δ ^{13}C and flag-leaf Δ ^{18}O when fitting a linear relationship across irrigated and unirrigated treatment combinations (R^2 = 0.32, P< 0.001). Therefore, present results suggested that there was a trade-off between water-use effciency and water use. However, there were departures from this overall relationship for indidvual cultivars. Thus, under drought, M. Widgen, Glasgow and Soissons maintained high WUE relative to their water use (as indcated by points below the regression line in Fig. 5), and the opposite was the case for Hereward and Istabraq. Hereward and Istabraq also showed a similar response under irrigated conditions, i.e. exhibited a low WUE relative to their water use in comparison with other cultvars.

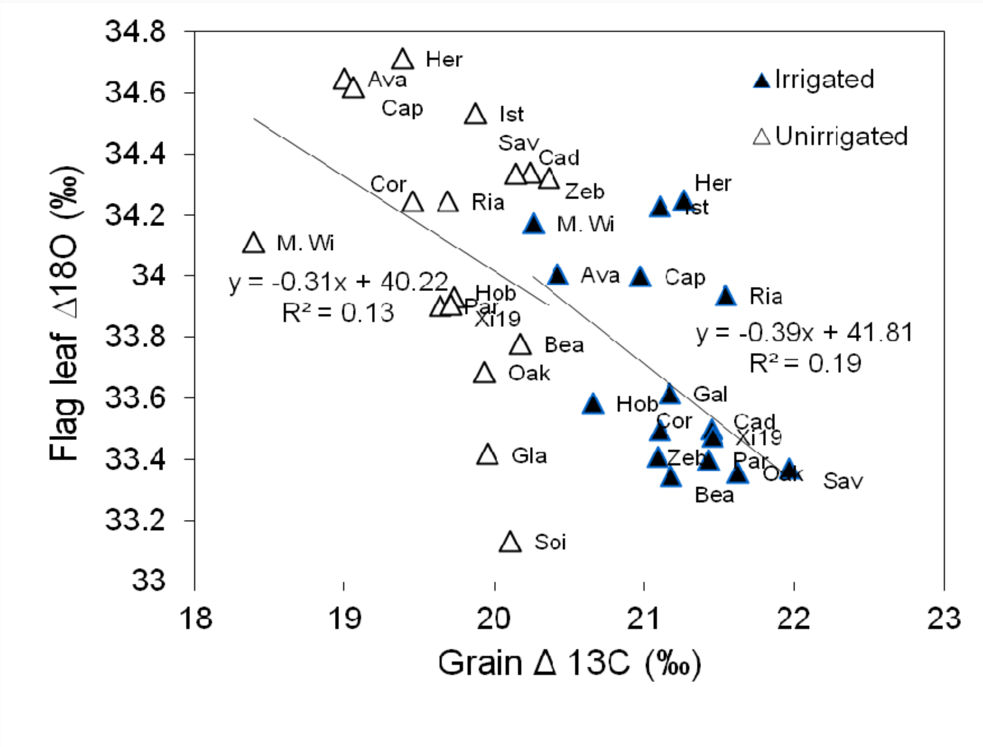

Fig. 5 Linear regression of flag-leaf Δ ^{18}O on grain Δ ^{13}C amongst 18 wheat genotypes under irrigated and unirrigated conditions at Sutton Bonington 2009-10.

There was a negative linear relationship between flag-leaf Δ ^{18}O and crop water uptake from 21 April to harvest measured by gravimteric analysis of soil cores (Fig. 6, P< 0.05).

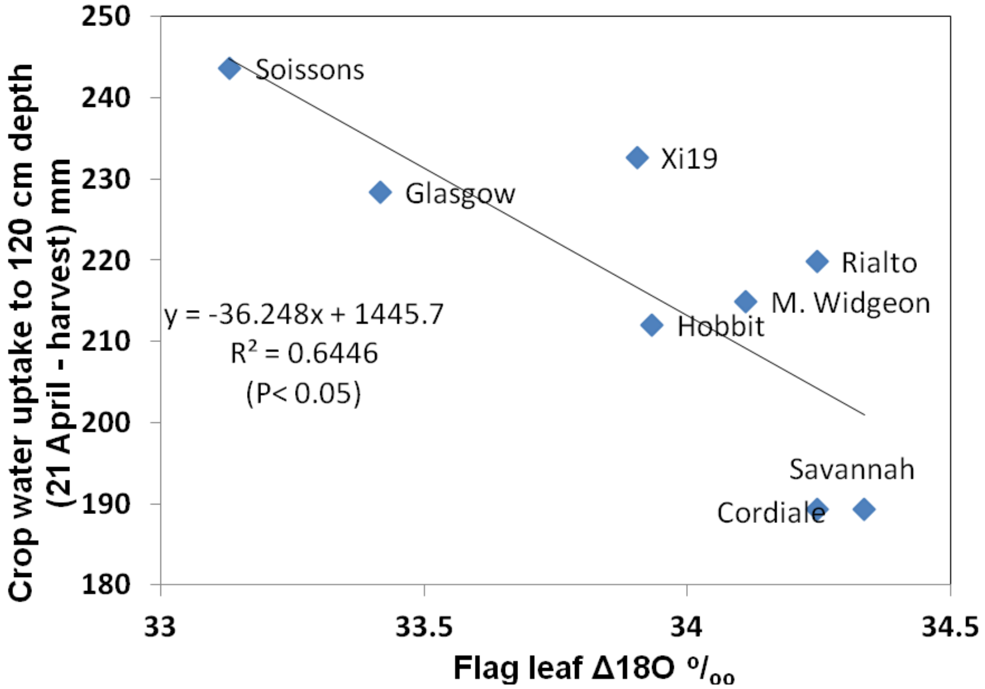

Fig. 6. Linear regression of crop water use on flag-leaf Δ ^{18}O amongst 8 wheat genotypes under unirrigated conditions at Sutton Bonington 2009-10.

4. Preliminary conclusions

In WGIN, this trait analysis directly relates to the “Targeted Traits” section of “Resource Development”, specifically “improvement of water use efficiency and drought tolerance traits”. The potential value of Δ^{18}0 and mineral ash content as new phenotypic screens in wheat for adaptation and yield potential under varying levels of water availability has been evaluated in one year. Initial results indicated the simpler mineral ash content screen may not be a useful technique for indicating drought performance under UK conditions. By contrast, the presented results indicate there was a negative linear relationship between flag-leaf Δ ^{18}O and crop water use and this may have scope for use as a screen for crop water use. The relationship between Δ ^{18}O and crop water use is being quantifed again in 2011 to test further the potential utility of this trait as an indirect measurement of water use. The flag-leaf Δ ^{13}C and flag-leaf Δ ^{18}O were correlated with yield but not as strongly as grain flag-leaf Δ ^{13}C. Although these screens may not be applicable as direct screens of grain yield under drought, the combination of flag-leaf Δ ^{18}O and grain Δ ^{13}C is potentially useful in that it provides a mechanism to identify those cultivars combining high WUE with high water use, and hence new germplasm for incorporation into breeding programmes for drought tolerance. These novel phenotypic screens are being repeated in 2010-11, to test for consistency of these positve departures from the overall negative relationships between WUE and WU. The consistency of the genotypic rankings for the physiological screens across years will also be quantified. Generally the isotope screens seemed to be operating in a constitutive manner in 2010, with genotype rankings generally similar with and without drought and linear relationships between isotope ratios and WU/WUE also observed under irrigated conditons, although R^2 values were higher under drought. This is encouraging since it, for example, indicates grain $\Delta\ ^{13}C$ may be selected in non-drought seasons and that high expression of grain $\Delta\ ^{13}C$ is not detrimental in the absence of drought.

The flag-leaf $\Delta\ ^{13}C$, flag-leaf $\Delta\ ^{18}O$ and flag-leaf mineral ash content screens have not previously been been assessed on winter wheat on UK germplasm.

The team who carried out this work at University of Nottingham included Jayalath De Silva (field sampling and preparation of samples for isotope analysis and data analyis) and John Alcock and Matt Tovey (management of experimental plots).

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