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Investigating the utility of potato (*Solanum tuberosum* L.) canopy temperature and leaf greenness responses to water-restriction for the improvement of irrigation management

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ABSTRACT

Traits that rapidly respond to stress in important agricultural crops have the potential to provide growers with actionable feedback. Traits that respond to water-restriction could inform irrigation systems by identifying crop water status and requirements in real-time. This would be particularly useful for potato, which is extremely susceptible to drought. We conducted two pot experiments and one field experiment to evaluate the utility of two traits, canopy temperature and leaf greenness, for informing irrigation management in potatoes. We also evaluated the efficacy of Phenospex PlantEye F500 sensors for the remote sensing of leaf greenness. We found that canopy temperatures of the cvs. Maris Piper (Spring Pot Experiment, + 0.8 °C; Autumn Pot Experiment, + 5.3 °C) and Désirée (Autumn Pot Experiment, + 2.5 °C) increased with water-restriction and that the canopy temperatures of Maris Piper returned to its baseline within three days after the resumption of well-watered conditions. We also found that these responses varied between cultivars, with predictable outcomes based on reported and corroborated drought tolerance ratings. Leaf greenness was not affected by water restriction in the Spring pot experiment but had a significant interaction with sampling date and water restriction in the Autumn pot experiment. However, leaf greenness measurements from the Phenospex PlantEye F500 were significantly correlated with SPAD values, suggesting this tool might be useful in the screening for drought-tolerant cultivars in the future.

1. Introduction

Recent advances in plant phenotyping platforms have alleviated a significant bottleneck in our ability to understand useful traits in important agricultural crops (Furbank and Tester, 2011). The collection of phenotypic data has historically been destructive, expensive, and time-consuming, but now researchers are able to collect these data on agriculturally relevant scales (Furbank and Tester, 2011). A subset of phenotypic traits might therefore prove useful in providing growers with actionable feedback from their crops. For example, a trait that is found to reliably respond to drought stress could theoretically be used to inform and improve irrigation management, potentially reducing the substantial yield penalties (Jefferies and Mackerron, 1993) and irrigation costs associated with drought (Daccache et al., 2012). This is particularly relevant for potato, which is extremely susceptible to drought (Schafleitner et al., 2009), due to its high water-requirements (Knox et al., 1997) and shallow root system (van Loon, 1981).

The utility of such a trait should be defined by 1) the convenience and accuracy with which it can be measured on agriculturally relevant scales; 2) the rate, intensity, and reliability of its response to a relevant stress; 3) the practicability and cost/benefit ratio of an appropriate intervention; and 4) the rate, intensity, and reliability of its response to that intervention. With respect to convenience of measurement, advances in multi- and hyperspectral imaging, unmanned aerial vehicles, and image processing have made automatic remote sensing synonymous in the literature with “easy to measure” (Araus and Cairns, 2014). The suitability of a trait for remote sensing significantly narrows down those that might prove useful in increasing the efficiency of agricultural systems through a plant-feedback approach.

In the case of drought stress, previous research has highlighted canopy temperature (Chaudhuri and Kanemasu, 1985; Chaudhuri et al., 1986; Hatfield et al., 1987; Blum et al., 1989; Stark et al., 1991; Mahmud

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et al., 2016; Anderegg et al., 2021) and, more recently, leaf greenness (Ramírez et al., 2014; Rolando et al., 2015; Bai and Purcell, 2019; Li et al., 2019; Anderegg et al., 2021; Monteoliva et al., 2021) as effective traits with respect to the selection for drought tolerant cultivars. Canopy temperature indices have also been investigated as methods of measuring water stress (Rud et al., 2014) and for controlling irrigation systems in potato (Rinza et al., 2022). Within this research, both canopy temperature and leaf greenness have been measured with remote sensing technologies (Bai and Purcell, 2019; Rinza et al., 2019), satisfying the first criteria for screening candidate traits for a plant-feedback based agricultural system.

This research has also demonstrated the rate, intensity, and reliability of the responses of potato canopy temperature and leaf greenness to water-restriction, satisfying the second criteria. Water-restriction (high-frequency deficit irrigation to 50 % pot capacity) has been shown to increase canopy temperatures in five cultivars of potato, by an average of 2.3 °C ± 0.7 °C, at 1 pm (Mahmud et al., 2016). Similar results have since been found with the cv. Unica in the field, for which water-restriction was associated with an increase in canopy temperatures by ~4 °C between 3 and 4 pm (Rinza et al., 2019). Canopy temperatures are known to rise with increasing soil moisture deficits due to the reduced transpiration rates associated with stomatal closure (Fuchs, 1990). This process preserves plant water status under drought conditions but has downstream effects on carbon assimilation, increasing survivability at the cost of yield in agricultural species. Canopy temperature is therefore useful not only for estimating drought stress, but also for indicating periods of reduced yield accumulation.

Leaf greenness, as a proxy for chlorophyll content, has been proposed as an important trait for improving crop yields in the future, particularly under drought stress (Monteoliva et al., 2021). Increases in leaf greenness during periods of water-restriction are associated with reduced leaf growth in drought susceptible potato cultivars, suggesting that chlorophyll concentrations increase under water-restriction due to reductions in leaf area (Rolando et al., 2015). Severe water-restriction protocols in pots have been associated with an average increase in leaf greenness of ~10 SPAD units with the cv. Unica (Ramírez et al., 2014). Smaller, but still significant, increases in leaf greenness due to water-restriction were also observed in the field. In another pot experiment, increases in leaf greenness, of ~5 SPAD units, were observed within 10 days of water-restriction in the cvs. Sarnav, Unica, and Désirée (Rolando et al., 2015). These results have more recently been corroborated in six cultivars observed under short- and long-term water-restriction, although with less consistent differences in leaf greenness between the cultivars and treatments (Li et al., 2019).

However, very little research has investigated the practicability and cost/benefit ratios of basing irrigation management on these responses, or the rate, intensity, and reliability of the responses of these traits to irrigation. Before the viability of such systems can be evaluated, the responses of these traits must be understood to prevent systems being designed where the relevant intervention is not effective. Therefore, the effects of water-restriction and, uniquely, well-watered recovery periods on the canopy temperature and leaf greenness of potato, both in the glasshouse and in the field will be assessed. As droughts are predicted to become more frequent in many areas, we also aim to understand the effects of repeated water-restriction cycles on these traits. We hypothesised that water-restriction would be associated with increases in canopy temperature and leaf greenness, as seen in previous research, and that these responses would be stronger in less drought tolerant cultivars. We also hypothesised that these increases in canopy temperature and leaf greenness would be reversed with the resumption of well-watered conditions as transpiration and leaf expansion resume. Secondarily, we aimed to assess the utility of a remote sensing technology, Phenospex PlantEye F500 sensors, for measuring leaf greenness in potato and to quantify the relationship between these measurements and SPAD values.

2. Materials and methods

2.1. Plant material and growing conditions

Two pot experiments (spring and autumn) and one field experiment (summer) were carried out at the Crop and Environment Laboratory (N 51°26'13.0" W 0°56'31.0") at the University of Reading, UK. Both pot experiments were conducted in twelve bespoke plywood troughs (1140 ×300 x 412 mm, L x W x H; Fig. 1). Each trough was filled with 148 L of a 2:1 by volume mixture of John Innes No. 2 compost and sharp sand (Jubilee Building Supplies, Bracknell, UK). Each trough was fertilised with 576 g of Osmocote Pro (3–4 Mo). Ambient temperature and relative humidity during the summer field experiment were retrieved from the University of Reading Atmospheric Observatory (N 51°26'29.2" W 0°56'16.0") and were measured manually on each sample date in the glasshouse (Table 1).

For the pot experiments, the cv. Maris Piper was selected due to its popularity with UK growers, driven by its high yield and resistance to *Globodera rostochiensis* (Buckley, 2015). Melody and Désirée were selected for the spring and autumn pot experiments, respectively, for to their comparable maturity classes with Maris Piper and purportedly higher drought tolerance (Agriculture and Horticulture Development Board, 2023; Science and Advice for Scottish Agriculture, 2023). Pentland Javelin was selected for the summer field experiment to represent earlier maturing cultivars and inferred lower drought tolerance than Maris Piper (Hill et al., 2021). This was deemed necessary to cover a greater variety of the cultivated potato germplasm and to allow the detection of differences between the responses measured here in the relatively uncontrollable field environment.

2.2. Spring pot experiment

On 31st March 2022, eighteen pre-sprouted seed tubers of both *Solanum tuberosum* cvs. Maris Piper and Melody (treated with imazalil fungicide and provided by Branston Ltd., Lincoln, UK) were planted at a depth of 10 cm, with three tubers in each trough. All plants were grown under a glasshouse from planting to harvesting, 123 days after planting (DAP). Throughout the experiment, irrigation was controlled by a GP2 data logger and controller (Delta-T Devices, Cambridge, UK). Soil moisture was measured with four WET150 multi-parameter soil sensors (Delta-T Devices).

Each WET150 sensor was buried at a depth of 30 cm, at a 60° angle relative to the soil surface. One sensor for each combination of treatment and cultivar was buried in a sentinel trough. To impose the two treatment conditions, well-watered and water-restricted, each trough was connected to one of two irrigation loops. Each loop was independently controlled by the GP2, based on the soil moisture content measured by the WET150 sensors. Both loops could supply each trough with 12 L of water per hour through two drippers per plant.

Before the onset of water-restriction, the GP2 was programmed to check all the probes every hour for a soil moisture content (SMC) reading of < 36 % ($A_0 = 1.32$, $A_1 = 8.70$). If this condition was met by both probes within a treatment, dripper irrigation for each trough under that treatment was initiated automatically. The GP2 then rechecked each sensor every minute for an SMC of ≥ 36 %. Once this condition was met by both probes, irrigation would automatically stop. An SMC of 36 % was chosen as the irrigation threshold based on the WET150 readings at 80 % pot capacity (Turner, 2019), which was calculated gravimetrically.

These conditions were maintained until 8th June 2022 (69 DAP) when 50 % flower bud formation was reached, which coincides with tuber initiation (Li et al., 2019). On this date, the irrigation loop for the water-restricted troughs was manually turned off (Turner, 2019). The well-watered troughs remained under the same conditions as above. Irrigation for water-restricted troughs was reinitiated on 13th June and the plants were allowed to recover until 17th June (78 DAP). The second

Fig. 1. A photograph of the spring pot experiment on 22nd April 2022 (22 DAP) showing the twelve bespoke plywood troughs in which the experimental plants were grown. Each trough contained three plants and was irrigated through six 2 L $hour^{-1}$ drippers attached to one of two irrigation loops: well-watered or water-restricted. Each trough was palletised and could be disconnected from the irrigation loops to be moved into the adjacent glasshouse compartment to be scanned by two Phenospex PlantEye F500 multispectral 3D scanners (Phenospex, Heerlen, Netherlands).

Table 1
Ambient mean temperatures (T) and relative humidities (RH) in the field between 12th May and 8th August 2022, and in the glasshouse between 6th June and 4th July 2022 and 23rd November and 17th December 2022 for the spring and autumn pot experiments, respectively. Ambient temperature and relative humidity during the summer field experiment were retrieved from the University of Reading Atmospheric Observatory (N 51°26'29.2" W 0°56'16.0") and were measured manually on each sample date in the glasshouse.

	Spring Pot Experiment		Autumn Pot Experiment		Summer Field Experiment	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
Mean	21.7	67	17.5	79	17.1	63
S.E.	0.8	1	1.5	2	0.4	1

drought period lasted from then until 24th June (85 DAP), after which all troughs were well-watered until harvest on 4th July (95 DAP).

2.3. Autumn pot experiment

On 12th September 2022, eighteen pre-sprouted seed tubers of both *Solanum tuberosum* cvs. Maris Piper and Désirée (treated with imazalil fungicide and provided by Branston Ltd., Lincoln, UK) were planted at a depth of 10 cm, with three tubers in each trough. Due to the observation of slightly abnormal plant development in the spring pot experiment (longer stems, which required staking), all plants were grown outside and uncovered from planting until 65 DAP, before being moved into the glasshouse. Before being covered, all plants were grown under rainfed conditions, which was sufficient to maintain a well-watered environment.

Once moved into the glasshouse, plants were grown under lights with a 16-hour photoperiod, to reduce variability in photosynthetically active radiation between this and the earlier experiments. Irrigation was controlled with the same method as above. In this experiment, all troughs were under the same well-watered conditions from planting until 11th October 2023 (29 DAP). On this date, the irrigation loop for the water-restricted troughs was manually turned off and water-restricted conditions were maintained for the remainder of the experiment. The treatment for the well-watered troughs remained the same. The drought period lasted until 17th December 2023 (96 DAP), when all the plants were harvested.

2.4. Summer field experiment

On 12th May 2022, 40 pre-sprouted seed tubers of both *Solanum tuberosum* cvs. Maris Piper and Pentland Javelin (treated with imazalil fungicide and provided by Branston Ltd., Lincoln, UK) were planted on the flat at a depth of 20 cm. The rows were manually ridged post-emergence to prevent lodging and greening. The seed tubers were planted in one plot split into four blocks. Each block contained four rows, spaced at 90 cm on centre. Each row contained five plants of a single cultivar, planted 38 cm apart. Within each block, the two cultivars alternated between the rows and one row of each cultivar was assigned to each treatment: well-watered and water-restricted.

To mitigate order effects, the cultivar and treatment assignment for each row varied between the blocks. To mitigate edge effects, guard plants of the cv. Arran Victory were planted surrounding each block. This cultivar was selected as it produces purple tubers, in contrast to the white tubers of Maris Piper and Pentland Javelin, which prevented the guard plant tubers from being attributed to experimental plants.

All plants were grown under a rain-out shelter and irrigated via lines of 2 L $hour^{-1}$ drippers, with one dripper per plant. These lines were supplied by one of two loops, which could be controlled independently to impose the two treatment conditions. Both were controlled manually to maintain well-watered conditions until 14th July (64 DAP). Irrigation for the water-restricted rows was then turned off until 18th July (68 DAP), after which it remained on until 3rd August (84 DAP). Water-restricted conditions were then maintained until the end of the experiment on 8th August (89 DAP).

2.5. Data collection

Between 6th June and 4th July (spring pot experiment), 23rd November and 17th December (autumn pot experiment), and 5th July and 8th August (summer pot experiment), average canopy temperature and SPAD values were regularly recorded for each plant. Canopy temperatures were measured with an AIR-801 infrared thermometer with a resolution of 0.1°C (ATP Instrumentation, Ashby-de-la-Zouch, UK) and SPAD values were measured with SPAD-502Plus (Konica-Minolta, Tokyo, Japan).

For each of these measurements three terminal leaflets were sampled per plant, each from distinct levels within the canopy. These measurements were averaged across each plant to give an accurate estimate of temperature and SPAD for the whole canopy (Víg et al., 2012). Canopy levels were defined as the third (Gervais et al., 2021), fifth, and seventh highest fully expanded leaves on the main stem of each plant. In the two pot experiments, these leaves were marked with cable ties around the petioles, so the same leaflets could be measured throughout the experiment.

As canopy temperature was particularly affected by ambient temperature fluctuations, all measurements were taken from the highest canopy level of each plant first, followed by the second level, and finally the third. All measurements were taken from 10:00–12:00 to minimise the variation caused by ambient changes throughout the day.

For the Spring experiment on 6th, 10th, 17th, and 23rd June 2022, each trough was scanned with two PlantEye F500 multispectral 3D scanners (Phenospex, Heerlen, Netherlands). PlantEye scanners have previously been used to measure "high-temperature-induced" (Lazarević et al., 2022) and drought-related (Hill et al., 2024) morphophysiological changes in potato. Integrated software (Phena; Phenospex) generated 3D point clouds of the plants, which were used by HortControl software (Phenospex) to calculate morphological parameters, including digital biomass, plant height, leaf area index, light penetration depth, leaf angle, average greenness, average NDVI, and average NPCI (Lazarević et al., 2021). Due to the high correlations between certain variables, only the previously stated variables were analysed.

2.6. Statistical analysis

All statistical analyses were conducted in RStudio (RStudio Team, 2020). Measurements for each plant within a trough (pot experiments) or row (field experiment) were grouped and averaged before analysis to prevent pseudo-replication. For each dependent variable, a linear model was formulated with treatment, cultivar, and sample date as interactive fixed effects and trough or row, depending on the experiment, as a random effect. Functions from the R package "easystats" (Lüdecke et al., 2022) were used to assess whether each model met these assumptions of ANOVA testing: homogeneity of variance, normality of residuals, and a lack of significant outliers. Homogeneity and normality were assessed both statistically and visually, as large sample sizes are often unable to be accurately assessed with statistics alone (Lumley et al., 2002; Lüdecke et al., 2022).

If any of these assumptions were not met, typically signified by a p-value > 0.05, the data were transformed, and the tests of normality and homogeneity of variance were reassessed. Once these assumptions were met, ANOVA testing was run on each model. ANOVA testing was selected as it allows for the comparison of effects across multiple independent variables, which included treatment, cultivar, and sample date here. Each experiment was analysed separately as comparing responses to water-restriction in different environments is beyond the scope of these experiments.

In the spring pot experiment, one group (well-watered, Melody, sampled on 20th June) had exceptionally low variance in canopy temperature. This resulted in the model violating the assumptions of ANOVA testing so two models were constructed, either including or excluding this group. Only the latter met the assumptions of ANOVA testing, but the results between the two ANOVAs were not significantly different, so results of the former are presented here.

All data presented are estimated marginal means. These were extracted from each model with the "emmeans" package in R (Lenth, 2023). These means ±CIs were plotted with the "ggplot2" package (Wickham, 2016). Any data that required transformation, as described above, were back transformed with the inverse function in R before being plotted with "ggplot2". Compact letters were calculated from the estimated marginal means and CIs with the "multcomp" package (Hothorn et al., 2008). Means not sharing any letter are significantly different by the Tukey-test at the 5 % significance level (Piepho, 2018).

3. Results

3.1. Spring pot experiment

3.1.1. Average canopy temperature

Water-restriction was associated with a slightly (+0.4 °C, 1.7 %) higher average canopy temperature across both cultivars and all sample dates in the spring pot experiment, but this difference was not statistically significant (Table 2). Although the overall difference in canopy temperature between the treatments was small, the overall temperature increase between the treatments for Maris Piper was higher (+0.8 °C, 3.5 % higher than well-watered). The overall canopy temperature of Melody was unaffected. There was a significant interaction effect between treatment and sample date on canopy temperatures ($p = 0.010$). Post hoc analysis showed that water-restriction was associated with significant increases in the canopy temperatures of Maris Piper relative to those of the well-watered control group on 10th (+3.9 °C, 16.8 %), 17th (+2.7 °C, 10.4 %), and 24th June (+1.8 °C, 9.4 %). All three of these dates occurred during the water-restricted periods. The canopy temperatures of Melody were not significantly affected by water-restriction on any sample dates (Fig. 2). There were few differences in canopy temperature between the cultivars overall (0.6 %), or under well-watered (1.2 %) or water-restricted (2.3 %) conditions. Sample date had a significant effect on canopy temperature ($p < 0.001$), due to fluctuations in ambient temperature throughout the experiment. There were no other significant or marginal interactions.

3.1.2. Average canopy SPAD

Water-restriction was associated with a very slightly (0.5 %) higher average canopy SPAD value across both cultivars and all sample dates,

Table 2
Main effects and interaction terms of three-way ANOVAs for average canopy temperatures (°C) and average canopy SPAD values of two potato cultivars (Maris Piper and Melody), grown in 148 L troughs inside a glasshouse, under either well-watered or water-restricted conditions. Canopy temperature and SPAD values were sampled between 6th June (67 DAP) and 4th July 2022 (95 DAP) with a handheld laser thermometer and a SPAD meter, respectively.

		Average Canopy Temperature			Average Canopy SPAD		
Effect	DenDF	F	p	Sig.[1]	F	p	Sig.[1]
Treatment (T)	8	0.94	0.361	ns	0.35	0.568	ns
Cultivar (C)	8	0.11	0.747	ns	1.04	0.339	ns
Sample Date (SD)	118	123.61	0.000	***	36.47	0.000	***
T x C	8	0.98	0.351	ns	0.53	0.487	ns
T x SD	118	2.18	0.010	*	0.49	0.943	ns
C x SD	118	0.69	0.785	ns	2.16	0.011	*
T x C x SD	118	0.92	0.548	ns	0.51	0.930	ns

1Significant p-values are indicated at the following levels: ns, not significant; $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

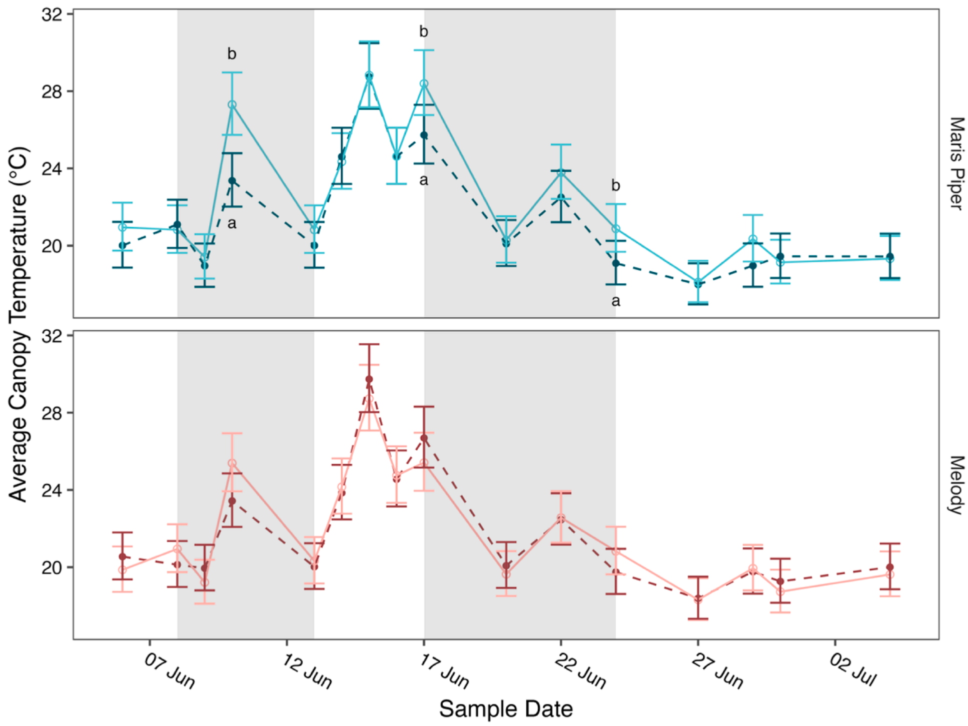

Fig. 2. Mean canopy temperatures of potato (cvs. Maris Piper and Melody) over time, grown in 148 L troughs under either well-watered (dashed line) or water-restricted (solid line) conditions. Plants were grown in the glasshouse between 31st March and 4th July 2022 (95 DAP). Canopy temperatures were measured between 6th June and 4th July 2022. Water-restricted conditions were imposed between 8th June and 13th June and again between 17th June and 24th June (shaded areas). Outside of these dates, water-restricted plants were well-watered and allowed to recover from drought stress. Means represent canopy temperatures averaged across three canopy levels: top, middle, and bottom, from three plants per trough (n = 3) ± CI. Means with different letters within each facet and sample date were significantly different by Tukey's test ($p < 0.05$). Letters denoting non-significant differences were removed for readability.

but this difference was not statistically significant (Table 2). SPAD values increased with water-restriction in Maris Piper (+0.6 SPAD units, 1.6 %), but not in Melody. Unlike with canopy temperature, there was not a significant interaction effect between treatment and sample date on canopy SPAD. Whilst the main and interaction effects of treatment on canopy SPAD were not significant, canopy SPAD values for water-restricted Maris Piper did noticeably increase (3.4 %) after the first period of water-restriction. The difference between the treatments decreased over time but the relationship was maintained for the duration of the experiment. Post hoc analysis showed that these differences were not significant on any of the sample dates (Fig. 3).

There was a small non-significant difference in canopy SPAD between the cultivars (1.0 %), but there was a significant interaction effect between cultivar and sample date on canopy SPAD values ($p = 0.011$). Post-hoc analysis demonstrated that Maris Piper had slightly, but significantly, higher canopy SPAD values than Melody on 17th June (4.5 %) and 4th July (3.9 %).

3.1.3. *Phenospex PlantEye F500s*

None of the variables measured by the Phenospex PlantEye F500s were significantly affected by water-restriction across the whole experiment (Supplementary Table 2). There was a marginally insignificant effect of treatment on NDVI ($p = 0.051$). Post hoc analysis revealed this to be a result of a significant (5.9 %) increase in the NDVI of well-watered Maris Piper, relative to the water-restricted Maris Piper, on the last sample date. Melody remained unaffected by treatment on all sample dates (Supplementary Figure 1). The overall effect of treatment on the greenness index was insignificant ($p = 0.226$). However, there was a significant difference in the greenness of Maris Piper between the treatments on the last sample date. Water-restriction was associated with a large (12.0 %) increase in the greenness of Maris Piper on this date. There was also a moderate to strong, significant correlation between average greenness as measured by the PlantEye sensors and the average canopy SPAD values recorded on the same sample dates: 6th, 10th, and 17th June 2022 (r (34) = 0.59, $p < 0.001$). When the two cultivars were analysed separately, the correlations for both cultivars remained significant but was stronger in Melody than Maris Piper (Maris Piper, r (17) = 0.57, p = 0.013; Melody, r (17) = 0.84, $p < 0.001$; Fig. 4).

There was a significant interaction effect between treatment and sample date on light penetration depth ($p = 0.025$). Post hoc analysis showed that this was a result of the significant (39.8 %) increase in light penetration depth associated with water-restriction in Melody on the last sample date (Supplementary Figure 1). Post hoc analysis showed that there were no other significant differences between treatments for any of the other digitally measured variables (Supplementary Figures 1 & 2). In contrast with treatment, there were significant differences between the cultivars in half of the digitally measured variables. Digital biomass, height, leaf area index, and NPCI were all significantly affected by cultivar (p = 0.009, 0.017, 0.010, and 0.011, respectively). There was also a marginally non-significant effect of cultivar on the greenness index ($p = 0.060$).

3.1.4. *Fresh tuber yield*

Water-restriction was associated with a significant (48.7 %) reduction in fresh tuber yield per trough across both cultivars ($p < 0.001$) compared to yields from well-watered plants (Table 3). There was a significant (21.9 %) difference in the mean fresh tuber yields between Melody (2.4 kg) and Maris Piper (1.9 kg) under well-watered conditions. However, water-restriction was associated with similar yield decreases in Melody (48.9 %) and Maris Piper (48.4 %), accounting for the lack of an interaction effect (Fig. 7).

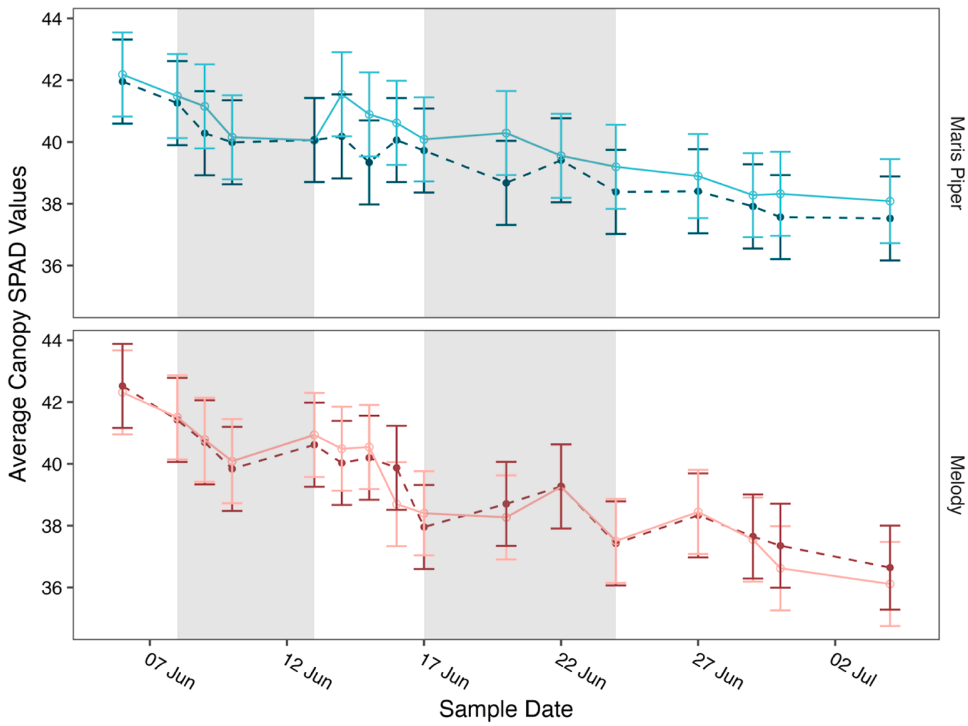

Fig. 3. Mean canopy SPAD values of potato (cvs. Maris Piper and Melody) over time, grown in 148 L troughs under either well-watered (dashed line) or water-restricted (solid line) conditions. Plants were grown in the glasshouse between 31st March and 4th July 2022 (95 DAP). Canopy SPAD values were measured between 6th June and 4th July 2022. Water-restricted conditions were imposed between 8th June and 13th June and again between 17th June and 24th June (shaded areas). Outside of these dates, water-restricted plants were well-watered and allowed to recover from drought stress. Means represent canopy SPAD values averaged across three canopy levels: top, middle, and bottom, from three plants per trough (n = 3) ± CIs. Means with different letters within each facet and sample date were significantly different by Tukey's test (p < 0.05). Letters denoting non-significant differences were removed for readability.

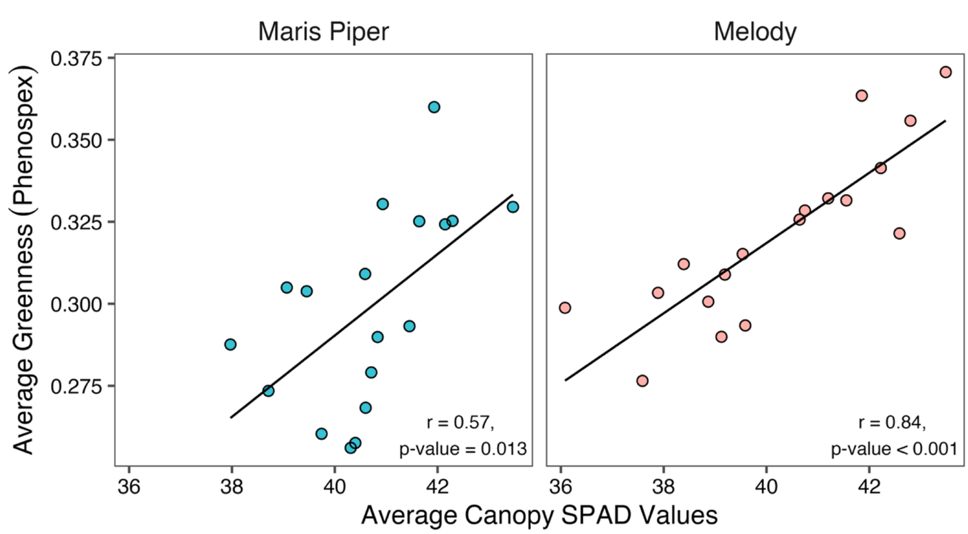

Fig. 4. Correlation between digital average greenness and average canopy SPAD values for two cultivars of potato, Maris Piper and Melody, grown in 148 L troughs under either well-watered or water-restricted conditions (Maris Piper, r (17) = 0.57, p = 0.013; Melody, r (17) = 0.84, $p < 0.001$; Combined, r (34) = 0.59, $p < 0.001$). Plants were grown in the glasshouse between 31st March and 4th July 2022 (95 DAP). Digital average greenness and average canopy SPAD values were measured on 6th, 10th, and 17th June 2022. Points represent digital average greenness from three plants per trough (n = 3) and canopy SPAD values averaged across three canopy levels: top, middle, and bottom, from three plants per trough (n = 3). Digital average greenness was measured by HortControl (Phenospex, Heerlen, Netherlands).

3.2. Autumn pot experiment

3.2.1. Average canopy temperature

Water-restriction was associated with a significantly (+3.9 °C, 9.5 % compared to well-watered plants) higher average canopy temperature across both cultivars and all sample dates in the autumn pot experiment. This difference was found to be statistically significant (p = 0.001; Table 4). There was also a significant interaction effect between treatment and sample date ($p < 0.001$). Post hoc analysis showed that water-restriction was associated with significantly higher canopy temperatures in Maris Piper on all but the first two sample dates (+4.2 °C, 10.9 %; +6.7 °C, +16.9 %; +7.1 °C, 20.1 %; +5.2 °C, 13.6 %; +5.4 °C, 14.4 %;

Table 3
Main effects and interactions terms of a two-way ANOVA for the fresh tuber yield of two potato cultivars (Maris Piper and Melody), grown in 148 L troughs, inside a glasshouse, under either well-watered or water-restricted conditions. Plants were harvested on 4th July 2022 (95 DAP).

Effect	DenDF	F	p	Sig.[1]
Treatment (T)	8	121.24	0.000	***
Cultivar (C)	8	13.71	0.006	**
T x C	8	1.59	0.243	ns

[1]Significant *p*-values are indicated at the following levels: ns, not significant; $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

Table 4
Main effects and interactions terms of three-way ANOVAs for average canopy temperatures (°C) and average canopy SPAD values of two potato cultivars (Maris Piper and Désirée), grown in 148 L troughs, inside a glasshouse, under either well-watered or water-restricted conditions. Canopy temperature and SPAD values were sampled between 23rd November and 17th December 2022 with a handheld laser thermometer and a SPAD meter, respectively.

		Canopy Temperature			Canopy SPAD		
Effect	DenDF	F	p	Sig.[1]	F	p	Sig.[1]
Treatment (T)	8	28.95	0.001	***	3.90	0.084	ns
Cultivar (C)	8	12.74	0.007	**	2.76	0.136	ns
Sample Date (SD)	55	5.13	0.000	***	265.46	0.000	***
T x C	8	4.06	0.079	.	0.02	0.904	ns
T x SD	55	4.48	0.001	***	3.16	0.007	**
C x SD	55	2.93	0.011	*	1.90	0.087	ns
T x C x SD	55	0.81	0.584		1.31	0.262	ns

[1]Significant *p*-values are indicated at the following levels: ns, not significant; $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

+8.0 °C, 20.6 %). Similar results were found with Désirée, where water-restriction was associated with significantly higher temperatures on three (+3.7 °C, 8.8 %; +4.3 °C, 9.9 %; +5.5 °C, 13.0 %) of the eight sample dates (Fig. 5). The overall increase in canopy temperature across the experiment was higher in Maris Piper (+5.3 °C, 13.6 %) than in Désirée (+2.5 °C, 5.8 %).

There was a small (2.5 °C, 5.8 %) significant ($p = 0.007$) difference in the average canopy temperatures of the two cultivars across both treatments and all sample dates, with Désirée observed to be slightly warmer than Maris Piper. There was a difference in canopy temperature between the cultivars under the well-watered treatment (9.8 %), but not the water-restricted (2.5 %) conditions, with Désirée being warmer than Maris Piper under both. There was also a significant interaction effect between cultivar and sample date on canopy temperature ($p = 0.011$). Post hoc analysis showed that the average canopy temperature of Désirée was significantly warmer than that of Maris Piper on all but the first sample dates.

3.2.2. *Average canopy SPAD*

Water-restriction was associated with a small (6.9 %) non-significant ($p = 0.084$) increase in average canopy SPAD values across both cultivars and all sample dates (Table 4). However, there was a significant interaction between treatment and sample date ($p = 0.007$). Post hoc analysis demonstrated that water-restriction was associated with significant increases in canopy SPAD on 15th December 2022 (11.9 %) for Maris Piper and on 7th (11.6 %) and 15th December (16.2 %) for Désirée (Fig. 5).

There was a small (4.0 %), non-significant difference in average canopy SPAD values between the two cultivars across both treatments and all sample dates, with that of Maris Piper being slightly higher than that of Désirée.

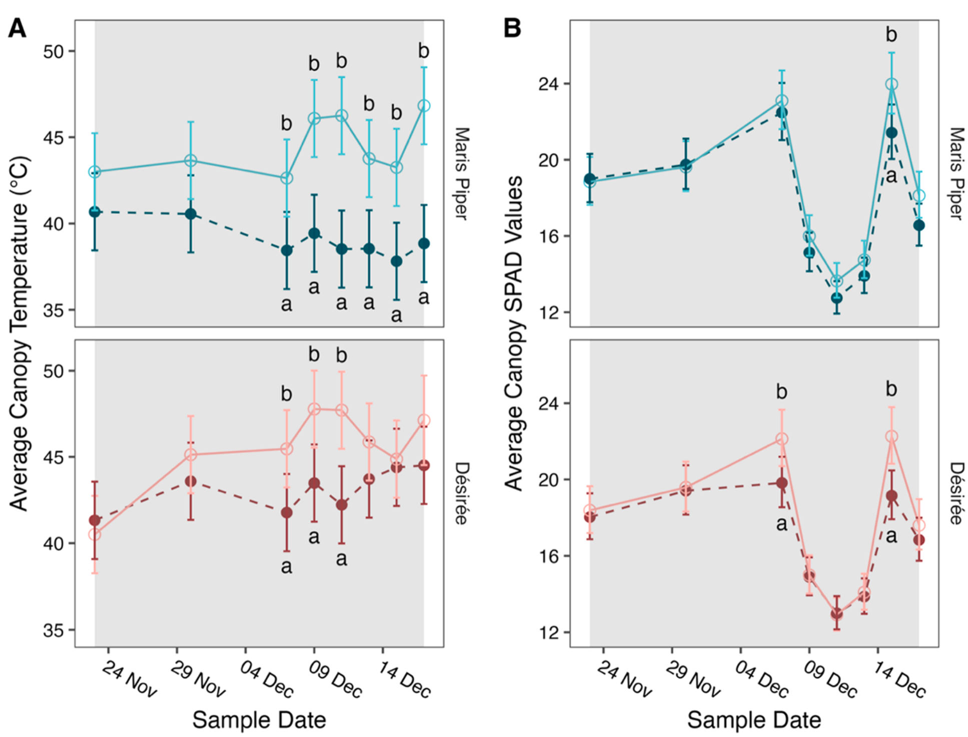

Fig. 5. Mean canopy temperatures (A) and SPAD values (B) of potato (cvs. Maris Piper and Désirée) over time, grown in 148 L troughs under either well-watered (dashed line) or water-restricted (solid line) conditions. Plants were grown in the glasshouse between 12th September and 17th December 2022 (95 DAP). Canopy temperatures and SPAD values were measured during a single water-restriction period, imposed between 23rd November and 17th December 2022 (shaded area). Means represent canopy temperatures and SPAD values averaged across three canopy levels: top, middle, and bottom, from three plants per trough (n = 3) ± CIs. Means with different letters within each facet and sample date were significantly different by Tukey's test (p <0.05). Letters denoting non-significant differences were removed for readability.

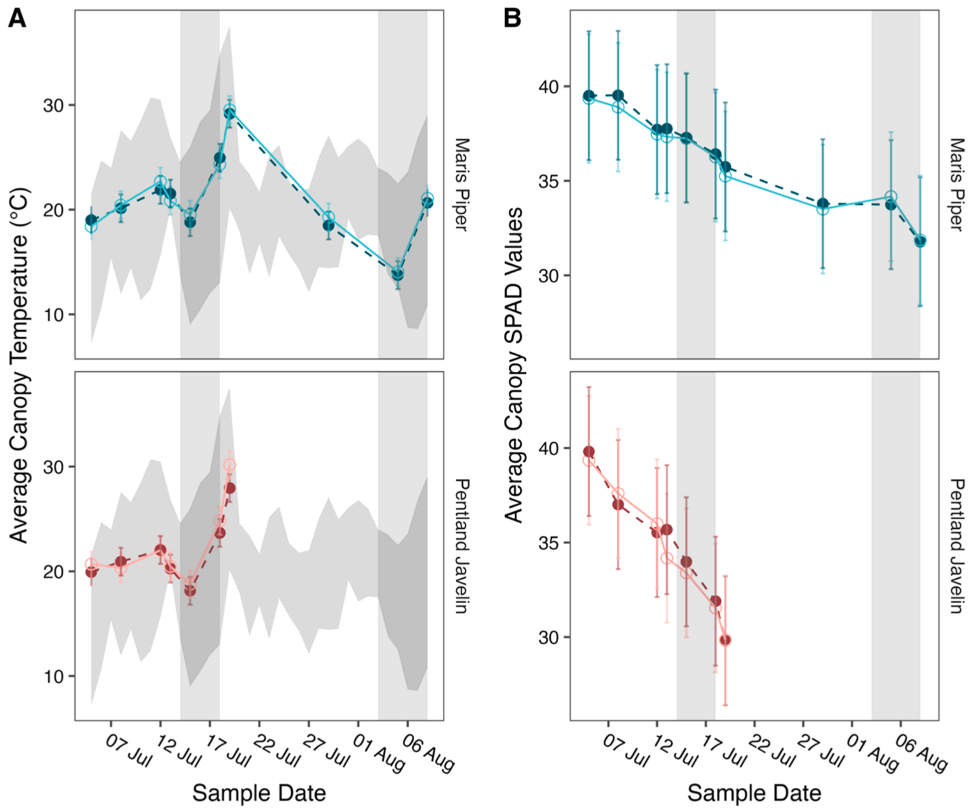

Fig. 6. Mean canopy temperatures (A) and SPAD values (B) of potato (cvs. Maris Piper and Pentland Javelin) over time, grown under a rain-out shelter in the field between 12th May and 8th August 2022 (89 DAP). All plants were grown under well-watered conditions, with irrigation supplied by dripper lines, until 14th July (64 DAP). Subsequently, each row of plants was subjected to either well-watered conditions for the duration of the experiment (dashed lines) or two water-restricted periods (vertical grey bars) between 14th July (64 DAP) and 18th July (68 DAP) and between 3rd August (84 DAP) and 8th August (89 DAP) (solid lines). Between these periods, the water-restricted plants were well-watered and allowed to recover from drought stress. After these periods of water-restriction, all plants were harvested, and fresh tuber yields for each row were measured. Canopy temperature and SPAD values were sampled between 5th July (55 DAP) and 8th August 2022 (89 DAP). Daily ambient temperature ranges are shown by the grey ribbon. Means represent canopy temperatures and SPAD values averaged across three canopy levels: top, middle, and bottom, from five plants per row (n = 4) ±CIs. Means with different letters within each facet and sample date were significantly different by Tukey's test (p <0.05). Letters denoting non-significant differences were removed for readability.

3.2.3. *Fresh tuber yield*

Results for fresh tuber yield in the autumn pot experiment were consistent with those for the spring pot experiment. Water-restriction was associated with a significant (70.9 %) reduction in fresh tuber yield per trough across both cultivars ($p < 0.001$; Table 5). Unlike in the previous experiment, there was a smaller (and non-significant;17.3 %) difference in well-watered fresh tuber yield between Maris Piper (1.8 kg) and Désirée (2.1 kg). Interestingly, there was only a small (5.8 %) difference in the fresh tuber yield of Maris Piper between the pot experiments, despite the latter being conducted after the northern hemisphere summer. Water-restriction had a similar effect on both cultivars, being associated with a significant (69.1 % and 72.6 %, respectively) decreases in fresh tuber yield in Maris Piper and Désirée (Fig. 7).

Table 5
Main effects and interactions terms of a two-way ANOVA for the fresh tuber yield of two potato cultivars (Maris Piper and Désirée), grown in 148 L troughs, inside a glasshouse, under either well-watered or water-restricted conditions. Plants were harvested on 17th December (96 DAP).

Effect	DenDF	F	p	Sig.[1]
Treatment (T)	8	129.16	0.000	***
Cultivar (C)	8	1.11	0.323	ns
T x C	8	0.29	0.604	ns

[1]Significant *p*-values are indicated at the following levels: ns, not significant; $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

3.3. *Summer field experiment*

3.3.1. *Average canopy temperature*

Water-restriction was not associated with a significant increase in average canopy temperature in the field across both cultivars and all sample dates (Table 6). There were no significant interaction effects between temperature and either of the other two grouping factors.

3.3.2. *Average canopy SPAD*

Water-restriction had no effect on average canopy SPAD values in the field across both cultivars and all sample dates (Table 6). There was a significant interaction effect between cultivar and sample date on canopy SPAD in the field but, which was due to the 100 % mortality of Pentland Javelin. Post hoc analysis showed no differences in canopy SPAD between the treatments on any sample dates (Fig. 6).

3.3.3. *Fresh tuber yield*

Water-restriction was associated with a significant (29.1 %) decrease in fresh tuber yield per row in the summer field experiment ($p < 0.001$; Table 7). Similar results were found within each cultivar. Water-restriction was associated with a larger decrease in the fresh tuber yield of Pentland Javelin (41.3 %) than in Maris Piper (24.1 %), although both yield reductions were significant (Fig. 7). Across the two water treatments, there was a significant (90.1 %) difference in fresh tuber yield between the two cultivars ($p < 0.001$).

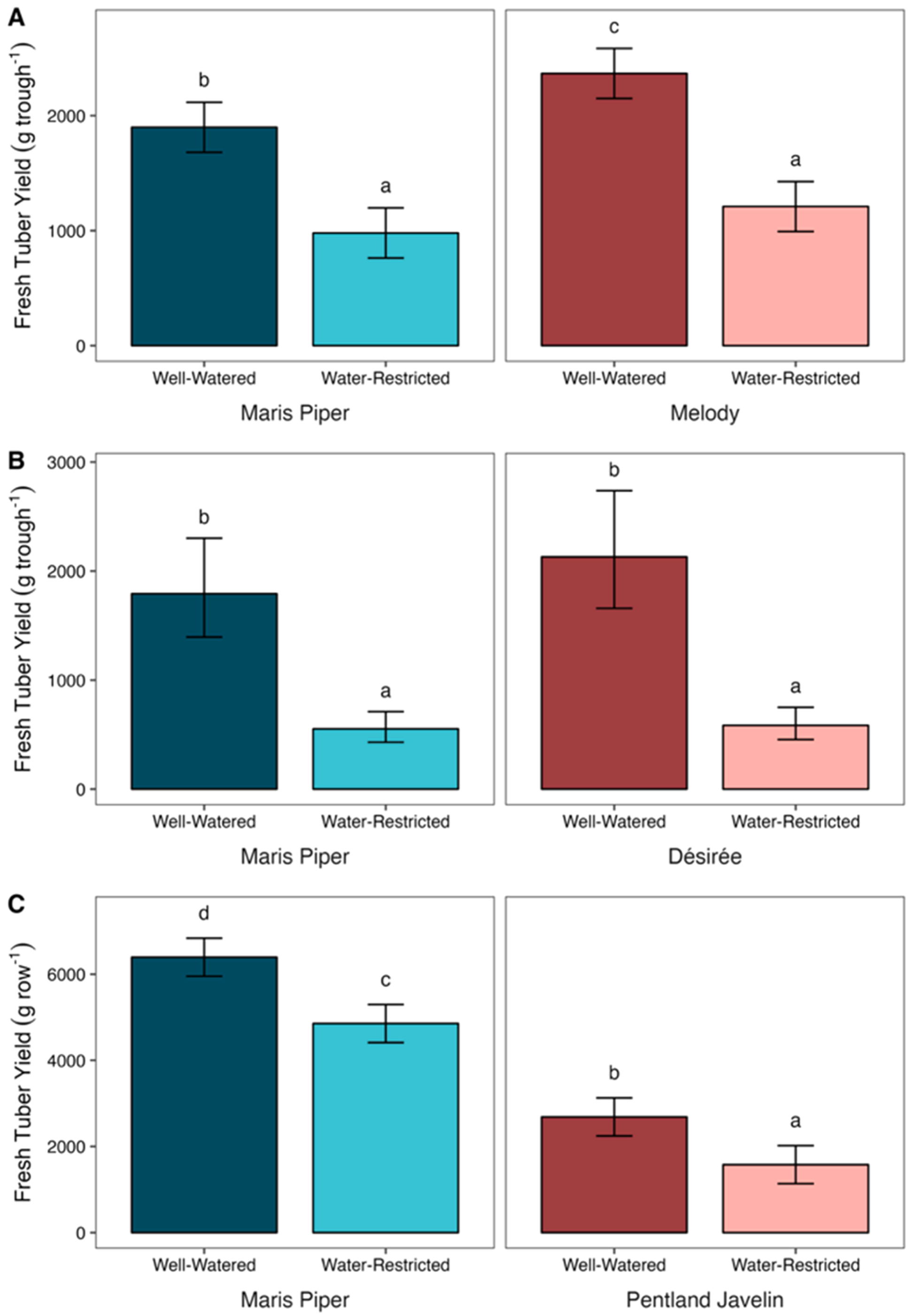

Fig. 7. Mean fresh tuber yields of potato (cvs. Maris Piper, Melody, Désirée, and Pentland Javelin), grown in either (A & B) 148 L troughs, inside a glasshouse, or (C) in the field, inside a rain-out shelter, both under either well-watered (dark bars) or water-restricted (light bars) conditions. Plants were grown in the glasshouse between (A) 31st March and 4th July 2022 (95 DAP) or (B) 12th September and 17th December 2022 (96 DAP). Plants were grown in the field (C) between 12th May and 8th August 2022 (89 DAP). Tubers were harvested on (A) 4th July (95 DAP), (B) 17th December (96 DAP), and (C) 8th August 2022 (89 DAP). Means represent FTY averaged across (A & B) three plants per trough or (C) five plants per row (A & B, n = 3; C, n = 4) ±95 % CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$).

4. Discussion

4.1. *Canopy temperatures increased under water-restriction and returned to baseline after irrigation resumed*

In both pot experiments, water-restriction was associated with an increase in the average canopy temperature of Maris Piper. In the spring, the overall increase in canopy temperature of Maris Piper due to water-restriction was relatively small: 0.8 °C. However, on individual sample dates during both water-restriction periods, significant increases in canopy temperatures of 3.9 °C, 2.7 °C, and 1.8 °C were observed (Fig. 2). These differences returned to baseline by the first sample date after both water-restriction periods. Similar results were found in the autumn pot experiment, where the canopy temperature of Maris Piper increased significantly due to water-restriction by 4.2 °C, 6.7 °C, 7.1 °C, 5.2 °C, 5.4 °C, and 8.0 °C after the first two sample dates (Fig. 5). Canopy

Table 6
Main effects and interactions terms of three-way ANOVAs for average canopy temperatures (°C) and average canopy SPAD values of two potato cultivars (Maris Piper and Pentland Javelin), grown in the field, inside a rain-out shelter, under either well-watered or water-restricted conditions. Canopy temperature and SPAD values were sampled between 5th July (55 DAP) and 8th August 2022 (89 DAP) with a handheld laser thermometer and a SPAD meter, respectively.

		Canopy Temperature			Canopy SPAD		
Effect	DenDF	F	p	Sig.[1]	F	p	Sig.[1]
Treatment (T)	12	1.24	0.286	ns	0.02	0.898	ns
Cultivar (C)	13	0.04	0.853	ns	3.73	0.077	ns
Sample Date (SD)	90	145.48	0.000	***	50.71	0.000	***
T x C	13	0.55	0.471	ns	0.00	0.989	ns
T x SD	90	0.65	0.754	ns	0.22	0.990	ns
C x SD	90	2.04	0.068	ns	7.81	0.000	***
T x C x SD	90	1.03	0.411	ns	0.33	0.918	ns

[1]Significant *p*-values are indicated at the following levels: ns, not significant; $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

Table 7
Main effects and interactions terms of a two-way ANOVA for the fresh tuber yield of two potato cultivars (Maris Piper and Pentland Javelin), grown in the field, inside a rain-out shelter, under either well-watered or water-restricted conditions. Plants were harvested on 8th August (89 DAP).

Effect	DenDF	F	p	Sig.[1]
Treatment (T)	8	42.49	0.000	***
Cultivar (C)	8	296.00	0.000	***
T x C	8	1.12	0.310	ns

[1]Significant *p*-values are indicated at the following levels: ns, not significant; $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

temperature responses to water-restriction in the other cultivars, Melody and Désirée, were less consistent. There were no significant differences in the canopy temperatures of Melody on any sample dates during the spring pot experiment (Fig. 2). Significant increases in canopy temperature due to water-restriction were only observed on three sample dates for Désirée in the autumn pot experiment, although the temperature increases on these sample dates were comparable to those of Maris Piper (Fig. 5). In the summer field experiment, canopy temperatures were not consistently affected by water-restriction across or within the sample dates for either cultivar (Fig. 6).

It was hypothesised that the canopy temperatures of Melody and Désirée would respond less strongly to water-restriction than that of Maris Piper, due to the latter's relative drought susceptibility. According to the AHDB potato variety database, the drought tolerance ratings of Maris Piper, Melody, and Désirée are 3, 5, and 7 out of 9, respectively (Agriculture and Horticulture Development Board, 2023). The European Cultivated Potato Database (ECPD) concurs, classifying the drought tolerance of Maris Piper as "low to medium" and Désirée as "high to very high" (Science and Advice for Scottish Agriculture, 2023). The ECPD has no Information on the drought tolerance of Melody. However, the extent to which the canopy temperatures of Melody were unaffected by water-restriction was not expected. Also unexpectedly, the reduction in the effect size of water-restriction on Désirée towards the end of the autumn pot experiment was not a result of decreasing canopy temperatures under water-restriction. Rather, the canopy temperatures of Désirée under well-watered conditions increased after 11th December. This was likely due to early senescence, which has previously been reported in Désirée under long photoperiods and high temperatures (Demagante and Vander Zaag, 1988), comparable to the conditions in this experiment.

The design of the spring pot experiment accounted for the small overall effect of water-restriction on the canopy temperatures of Maris Piper. This study aimed to evaluate the utility of traits including canopy temperature for a plant-feedback irrigation system. Therefore, it was necessary to include well-watered periods in the water-restricted treatment to investigate whether these traits would provide useful evidence of both stress and recovery. In this experiment, canopy temperature was shown to respond to both water-restriction and the subsequent well-watered conditions (Fig. 2). After a significant temperature increase due to water-restriction on the last sample date of the second water-restricted period, the canopy temperature of Maris Piper returned to that of the well-watered control group within three days. However, the ambient temperature on the last sample date of the first water-restriction period was relatively cool, reducing the need for canopy cooling by transpiration, and thus was not driving a difference in canopy temperature between the treatments. Therefore, the rate with which canopy temperature is restored post-drought remains unclear, and likely depends on ambient temperature, irrigation rate, and cultivar.

To understand the size of the effect of water-restriction on canopy temperatures in potato, it was also important to investigate the effects of a single, terminal period of water-restriction on this crop. Water-restriction was associated with a much larger, 3.9 °C, overall increase in canopy temperatures in this experiment, compared to the spring pot experiment. As was predicted due to the relative drought tolerances of the cultivars used, the canopy temperatures of Maris Piper were more affected by water-restriction than that of Désirée, with overall average increases of 5.3 °C and 2.5 °C, respectively. The difference in effect size between the two pot experiments presented here highlights the confounding effects that non-standardised growing conditions and drought protocols can have on potato morphophysiology (Hill et al., 2021).

Intermittent drought stress is more analogous to conditions in the field (Turner, 2019), especially for potato, which is typically irrigated intermittently with booms or rain guns (Daccache et al., 2012). However, the results presented here demonstrate the difficulty with detecting meaningful effect sizes in canopy temperature due to intermittent water-restriction. This is compounded by the high variance in canopy temperatures related to fluctuations in ambient temperature, both between and within sample dates. Post hoc analysis can be targeted at individual sample dates to mitigate the effects of the former issue. The latter could be addressed in future research with the use of imaging technologies that can phenotype multiple plants concurrently. Previous research has used infrared cameras to sample the canopy temperature of multiple potato plants in parallel and allowed for the detection of a significant difference between treatments of <1 °C (Rinza et al., 2019). Significant *p*-values are perhaps over relied upon (Greenland et al., 2016) but, if statistical models are to be used to control crop irrigation systems, then some method of detecting meaningful deviations from well-watered canopy temperatures must be defined.

The results presented here from the pot experiments are consistent with previous research that showed reduced canopy temperature depressions (CPD) in potato due to high-frequency deficit irrigation (Mahmud et al., 2016). CPD was defined as the difference between ambient air temperature and average canopy or leaf temperatures and is thus a measure of the cooling effect of transpiration. This effect was consistent across all five cultivars investigated but varied in magnitude throughout the day and between the cultivars. For example, the differences in CPD between the treatments were smallest at 8 am, when the ambient temperatures were cool, and greatest at 1 pm, when they peaked. The more drought tolerant cultivars, CIP 393371.58 and CIP 396244.12, were also found to have smaller differences in CDP between the treatments, which is consistent with our findings. Similar results have also been found by another study on the cv. Unica, with the greatest differences in canopy temperature between treatments occurring at 3 – 4 pm (Rinza et al., 2019). Other studies have investigated the utility of screening canopy temperatures to detect drought tolerant potato cultivars (Stark et al., 1991; Ninanya et al., 2021) or to control potato irrigation systems with temperature-based crop water-stress indices (Rinza et al., 2022). However, we are not aware of any studies that investigated both the effects of water stress and subsequent recovery on potato canopy temperatures.

Understanding the effects of water-restriction on potato canopy temperatures is further complicated by moving from the glasshouse to the field. The results from our summer field experiment were not consistent with those found in the pot experiments, or with previous research (Mahmud et al., 2016; Rinza et al., 2019). Canopy temperatures were not reliably affected by water-restriction across or within the sample dates. It's likely that the less homogenous conditions of the field experiment were partly responsible for this. Variations in ambient temperatures, relative humidity, and soil water holding capacity are greater in the field compared to the relatively controlled conditions of the glasshouse, and therefore increase the canopy temperature variance within each treatment. In this experiment, extremely high ambient temperatures for the region may also have confounded the effects of water-restriction on potato canopy temperatures.

The first water-restriction period during the summer field experiment coincided with an "unprecedented extreme heatwave", where ambient temperatures exceeded 40 °C for the first time on record in the UK (Met Office National Climate Information Centre, 2022). This could account for the lack of temperature differences between the treatment groups, as it's likely that all the plants experienced significant heat stress, which would confound the effects of drought stress (Hill et al., 2021). It's also possible that the irrigation system was unable to provide sufficient volumes of water to prevent drought stress in the well-watered plants, due to the extreme requirements for evapotranspiration caused by the heatwave. High ambient temperatures were certainly responsible for the 100 % fatality rate observed in Pentland Javelin after 19th July 2022 (Fig. 6). Early maturing cultivars, including Pentland Javelin, are known to be less robust to heat and drought stress due to their smaller root systems and the reduced capacity to recover associated with greater determinacy (Hill et al., 2021).

The issue of greater environmental variability in the field could be overcome with larger scale, remote sampling of canopy temperature. This approach has previously been successfully implemented in cotton, where sixteen infrared thermocouples were used to detect elevated canopy temperatures in cotton (Peters and Evett, 2008; O'Shaughnessy and Evett, 2010). These thermocouples were attached to a centre pivot irrigation boom to remotely collect canopy temperatures across large plots of field-grown cotton. Water use efficiency was significantly improved compared to manual irrigation with this method (O'Shaughnessy and Evett, 2010). However, centre pivot irrigation is not used in potato, at least in the UK, and it's unlikely remote sensing of canopy temperature could be used to inform irrigation management with sprinkler irrigation. Drip irrigation would be more suitable but would require complex infrastructure to differentially control irrigation within the field. Field-wide drip irrigation management would be more feasible but may only be commercially viable in areas with limiting water availability. This may soon become the case in the UK, where water availability is predicted to limit potato production in 50 % of years by 2050 (Daccache et al., 2011).

4.2. Water-restriction was associated with small increases in SPAD which were maintained for the duration of the spring pot experiment

In these experiments, overall average canopy SPAD values (leaf greenness) had similar relationships with water-restriction as canopy temperature. In both the pot experiments, water-restriction was associated with a small overall increase in leaf greenness. As with temperature, this difference was larger across the single water-restriction period of the autumn pot experiment (+6.9 %) than across the intermittent water-restriction periods of the spring pot experiment (+0.5 %). However, neither of these differences were found to be significant. In the spring pot experiment, there was a noticeable increase in the leaf greenness of Maris Piper immediately after the first water-restriction period (Fig. 3). This relative increase, compared to the well-watered Maris Piper, was maintained until the end of the experiment. Post hoc testing showed that this difference was not significant on any sample dates (Fig. 3). While the overall difference in leaf greenness in the autumn pot experiment was also not significant ($p = 0.084$). There were also three dates on which leaf greenness was significantly higher with water-restriction than without: one for Maris Piper and two for Désirée (Fig. 5). There was also a large U-shaped dip in leaf greenness for both cultivars in the autumn pot experiment between 7th and 15th December 2022 (Fig. 5). As with temperature, water-restriction had no effect on leaf greenness in the summer field experiment (Table 6).

To our knowledge, the experiments presented here are the first to assess the utility of leaf greenness as a trait to inform irrigation management through its response to cycles of well-watered and water-restricted conditions. This is certainly the case for potato but may also be true for all agricultural crops. Earlier research in potato has demonstrated that, in cultivars where leaf greenness increases under drought stress, it typically remains elevated for 20–50 days (Rolando et al., 2015; Li et al., 2019). However, these experiments were designed to assess the utility of leaf greenness as a marker of drought tolerance in large panel breeding programmes (Rolando et al., 2015; Li et al., 2019), and therefore were only interested in the initial response. While the latter did include a "cyclical" water-restriction treatment, the well-watered recovery periods were not differentiated from the water-restriction periods in the final analysis, as this was beyond the scope of the experiment. However, our initial findings suggest that leaf greenness does not return to baseline after well-watered conditions are restored to previously water-restricted plants. Therefore, we cannot recommend leaf greenness as a useful trait for irrigation management in potato.

Previous research found much stronger evidence of a positive effect of water-restriction on leaf greenness than that observed here. In a study on the potato cv. Unica, a "stay-green" effect was observed under the most severe water-restriction treatments in both the glasshouse and the field (Ramírez et al., 2014). The size of this stay-green effect, defined as the maintenance of SPAD values over time, appeared to be positively correlated with the severity of water-restriction. The differences in leaf greenness between treatments over time were found to be significant on all sample dates in the experiment, but it's unclear whether each of the less severe treatments were significantly different from the control. In a subsequent study on three potato cultivars, significant short-term increases in leaf greenness were consistently observed under water-restricted conditions (Rolando et al., 2015) similar to the moderate water-restriction used previously (Ramírez et al., 2014). In this experiment, greater short-term increases in leaf greenness due to water-restriction were associated with reductions in tuber yield, suggesting maintenance of leaf greenness under water-restriction is associated with drought tolerance in potato.

In a more recent leaf greenness study of six potato cultivars with similar maturities but varying drought tolerances, four cultivars demonstrated higher leaf greenness under both short- and long-term water-restriction compared to well-watered treatment conditions (Li et al., 2019). One cultivar (Favorita) showed smaller increases in leaf greenness under both water-restricted treatments towards the end of the experiment. In another cultivar (Atlantic), leaf greenness decreased under both water-restricted treatments relative to control conditions. The authors concluded that leaf greenness increases were consistently and negatively associated with drought tolerance under both water-restricted conditions. This conclusion was reached despite the observations of Atlantic, which maintained leaf greenness under water-restricted conditions but produced low yields under all conditions. The authors suggested that the effects of water-restriction on very drought susceptible cultivars may be inconsistent with less susceptible cultivars, or that the growing conditions confounded these results.

In the spring pot experiment, a small but non-significant increase in the leaf greenness of Maris Piper was observed after the first water-restriction period and a stay-green effect was observed in the water-restricted group for the duration of the experiment. Melody exhibited greater maintenance of leaf greenness under water-restricted conditions, remaining consistent with the well-watered group. Melody is known to be more drought tolerant than Maris Piper (Agriculture and Horticulture Development Board, 2023) and this was reflected in the fresh tuber yields of Maris Piper and Melody under water-restriction observed here (Fig. 7). Therefore, these findings provide only tentative support to the hypothesis of this research and evidence from previous work, and the lack of statistical significance observed here must be noted.

In the autumn experiment, the larger increase in leaf greenness due to water-restriction was observed in the Désirée. This cultivar is purported to be more drought tolerant than Maris Piper (Agriculture and Horticulture Development Board, 2023; Science and Advice for Scottish Agriculture, 2023), and was shown to produce higher fresh tuber yields under water-restriction (Fig. 7). This result is inconsistent with our hypothesis and contradicts the evidence from previous research, including the spring pot experiment. However, there was a large confounding effect on the leaf greenness measurements in this experiment, the cause of which is unclear. U-shaped dips in SPAD values of a similar magnitude have been observed before, but these were not consistent between treatment groups (Li et al., 2019). It's probable that some systemic physiological effect, e.g., pot binding (Sinclair et al., 2017), or environmental change was therefore responsible for the inconsistent leaf greenness results found here.

Exactly why water-restriction was associated with such inconsistent effects on leaf greenness is unclear. The allocation of nitrogen (N) to chlorophylls is strongly affected by N supplementation conditions (Makino and Osmond, 1991), but this was considered in the experimental design. Slow-release fertiliser and conservative irrigation protocols were used to minimise the confounding effects of leeching on N availability. N availability could have been greater under well-watered conditions due to the faster dissolution of the fertiliser, but SPAD values were generally higher under water-restricted conditions. Therefore, differences in N availability are unlikely to have contributed to the inconsistent effects of water-restriction on leaf greenness observed here.

Previous research has shown that the severity and duration of water-restriction has a strong effect on SPAD values in potato (Ramírez et al., 2014). In pots, only the most severe water-restricted conditions, 30 % of transpired water replaced daily by drip irrigation or partial rootzone drying, were associated with a significant increase in SPAD values. The two less severe water-restricted conditions, 60 and 45 %, did not cause significantly different SPAD values from controls. Similar results were also found in the field, with the addition of the largest effects occurring on the last sample date. It's therefore possible that our water-restricted conditions were not severe enough to observe significant differences in SPAD values. The confounding effects of ambient light on SPAD values are discussed below.

4.3. Phenospex PlantEye F500 measurements of greenness were strongly correlated with canopy SPAD values

None of the variables measured by the Phenospex PlantEye F500s in the spring pot experiment were found to be significantly affected by water-restriction. There were significant increases in NDVI and average greenness in Maris Piper (Supplementary Figure 2) and in light penetration depth in Melody (Supplementary Figure 1) on the final sample dates for the PlantEye. For Maris Piper, the increases in NDVI and greenness occurred in the well-watered plants and not in the water-restricted plants, although greenness was trending up for both treatments. It's unclear why this occurred, but it did coincide with an increase in the average canopy SPAD values of well-watered Maris Piper two days earlier (Fig. 3). Anomalous fluctuations in canopy SPAD values were observed in both pot experiments and have been observed in previous research in pots (Li et al., 2019). Our data from previous experiments (Hill et al., 2024) has shown that small pots can have important and significant confounding effects on potato, which are likely associated with inadvertent drought stress caused by the insufficient water-holding capacity of small substrate volumes. However, this was considered in the design of the troughs and the irrigation protocol used in these experiments, which should have been sufficient to prevent water-availability related pot binding (Sinclair et al., 2017; Turner, 2019).

Phenospex report that data from their PlantEye F500 sensors are unaffected by ambient light conditions (PlantEye, F500 - Multispectral 3D laser scanner for plant phenotyping, 2018), although this has not been independently verified. However, there was a moderate to strong correlation between average greenness as measured by the PlantEye F500s and average canopy SPAD values (Maris Piper, $r\,(17) = 0.57$, $p = 0.013$; Melody, $r\,(17) = 0.84$, $p < 0.001$; Combined, $r\,(34) = 0.59$, $p < 0.001$; Fig. 4). SPAD values have previously been shown to decrease under greater ambient light intensities in tobacco (Nauš et al., 2010), soybean, and rice (Xiong et al., 2015), due to intracellular light-dependent chloroplast movement. Therefore, it's likely that variation in ambient light conditions were associated with the fluctuations in average greenness and canopy SPAD observed here and in previous research (Li et al., 2019), rather than a systemic error.

5. Conclusions

These experiments demonstrate for the first time that potato canopy temperatures rapidly return to baseline with the resumption of well-watered conditions. Taken with the support that these experiments provide to previous research, showing that water-restriction is associated with increases in canopy temperatures of potato (Mahmud et al., 2016; Rinza et al., 2019), we have shown that direct measurements of canopy temperatures have potential for informing irrigation systems in potato. We found that this response is cultivar-dependent, as the canopy temperatures of the more drought tolerant cvs., Melody and Désirée, were less affected by water-restriction than those of Maris Piper. Further research should therefore include a range of potato cultivars with contrasting maturities and drought tolerance ratings. Extremely high temperatures during the field experiment also dramatically confounded the effects of water-restriction. Thus, more research is needed to assess the utility of canopy temperature for plant-feedback irrigation systems in the field. Our results for leaf greenness in Maris Piper and Désirée provide weak support for previous research (Ramírez et al., 2014; Rolando et al., 2015; Li et al., 2019), showing much smaller increases in average canopy SPAD values than those observed before. Uniquely in potato, we have shown that the resumption of well-watered conditions did not return the leaf greenness of Maris Piper to baseline. Therefore, this research suggests that leaf greenness is a more useful trait for selecting drought-tolerant cultivars than for a plant-feedback irrigation system. The moderate to strong correlation observed between the Phenospex PlantEye F500s and the SPAD-502Plus measurements of leaf greenness suggest the former may be useful in this screening process.

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CRediT authorship contribution statement

Dominic Hill: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Antreas Koryzis:** Investigation. **David Nelson:** Writing – review & editing, Supervision, Resources. **John Hammond:** Writing – review & editing, Supervision. **Luke Bell:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

David Nelson reports a relationship with Branston Ltd. that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is publically available at ⟨https://doi.org/10.5281/zenodo.10805257⟩.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2024.109063.

References

Agriculture and Horticulture Development Board (2023) Potato Varieties Database. Available at: ⟨https://potatoes.agricrops.org/varieties⟩ (Accessed: 3 January 2024).

Anderegg, J., Aasen, H., Perich, G., Roth, L., Walter, A., Hund, A., 2021. Temporal trends in canopy temperature and greenness are potential indicators of late-season drought avoidance and functional stay-green in wheat (Available at:). Field Crops Res. 274 (108311), 108311. https://doi.org/10.1016/j.fcr.2021.108311.

Araus, J.L., Cairns, J.E., 2014. Field high-throughput phenotyping: the new crop breeding frontier (Available at:). Trends Plant Sci. 19 (1), 52–61. https://doi.org/10.1016/j.tplants.2013.09.008.

Bai, H., Purcell, L.C., 2019. Evaluation of soybean greenness from ground and aerial platforms and the association with leaf nitrogen concentration in response to drought (Available at:). Crop Sci. 59 (6), 2763–2773. https://doi.org/10.2135/cropsci2019.03.0159.

Blum, A., Shpiler, L., Golan, G., Mayer, J., 1989. Yield stability and canopy temperature of wheat genotypes under drought-stress (Available at:). Field Crops Res. 22 (4), 289–296. https://doi.org/10.1016/0378-4290(89)90028-2.

Buckley, D., 2015. The potential of resistant cultivars to control the white potato cyst nematode Globodera pallida (Available at:). Asp. Appl. Biol. 130, 17–22. https://doi.org/10.5555/20163320953.

Chaudhuri, U.N., Deaton, M.L., Kanemasu, E.T., Wall, G.W., Marcarian, V., Dobrenz, A. K., 1986. A procedure to select drought-tolerant sorghum and millet genotypes using canopy temperature and vapor pressure deficit[1]' (Available at:). Agron. J. 78 (3), 490–494. https://doi.org/10.2134/agronj1986.00021962007800030020x.

Chaudhuri, U.N., Kanemasu, E.T., 1985. Growth and water use of sorghum (*Sorghum bicolor* (L.) moench) and pearl millet (*Pennisetum americanum* (L.) leeke) (Available at:). Field Crops Res. 10, 113–124. https://doi.org/10.1016/0378-4290(85)90019-X.

Daccache, A., Keay, C., Jones, R.J.A., Weatherhead, E.K., Stalham, M.A., Knox, J.W., 2012. Climate change and land suitability for potato production in England and Wales: impacts and adaptation (Available at:). J. Agric. Sci. 150 (2), 161–177. https://doi.org/10.1017/S0021859611000839.

Daccache, A., Weatherhead, E.K., Stalham, M.A., Knox, J.W., 2011. Impacts of climate change on irrigated potato production in a humid climate (Available at:). Agric. For. Meteorol. 151 (12), 1641–1653. https://doi.org/10.1016/j.agrformet.2011.06.018.

Demagante, A.L., Vander Zaag, P., 1988. The response of potato (*Solanum* spp.) to photoperiod and light intensity under high temperatures (Available at:). Potato Res. 31 (1), 73–83. https://doi.org/10.1007/BF02360023.

Fuchs, M., 1990. Infrared measurement of canopy temperature and detection of plant water stress (Available at:). Theor. Appl. Climatol. 42 (4), 253–261. https://doi.org/10.1007/BF00865986.

Furbank, R.T., Tester, M., 2011. 'Phenomics–technologies to relieve the phenotyping bottleneck (Available at:). Trends Plant Sci. 16 (12), 635–644. https://doi.org/10.1016/j.tplants.2011.09.005.

Gervais, T., Creelman, A., Li, X.-Q., Bizimungu, B., De Koeyer, D., Dahal, K., 2021. Potato response to drought stress: physiological and growth basis (Available at:). Front. Plant Sci. 12, 698060. https://doi.org/10.3389/fpls.2021.698060.

Greenland, S., Senn, S.J., Rothman, K.J., Carlin, J.B., Poole, C., Goodman, S.N., Altman, D.G., 2016. Statistical tests, P values, confidence intervals, and power: a guide to misinterpretations (Available at:). Eur. J. Epidemiol. 31 (4), 337–350. https://doi.org/10.1007/s10654-016-0149-3.

Hatfield, J.L., Quisenberry, J.E., Dilbeck, R.E., 1987. Use of canopy temperatures of identify water conservation in cotton germplasm (Available at:). Crop Sci. 27 (2), 269–273. https://doi.org/10.2135/cropsci1987.0011183x002700020030x.

Hill, D., Conte, L., Nelson, D., Hammond, J., Bell, L., 2024. Investigating the water availability hypothesis of pot binding: small pots and infrequent irrigation confound the effects of drought stress in potato (*Solanum tuberosum* L.) (Available at:). Front. Plant Sci. 15, 1399250. https://doi.org/10.3389/fpls.2024.1399250.

Hill, D., Nelson, D., Hammond, J., Bell, L., 2021. Morphophysiology of Potato (*Solanum tuberosum*) in Response to Drought Stress: paving the way forward (Available at:). Front. Plant Sci. 11, 597554. https://doi.org/10.3389/fpls.2020.597554.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 346–363.

Jefferies, R.A., Mackerron, D.K.L., 1993. Responses of potato genotypes to drought. II. Leaf area index, growth and yield (Available at:). Ann. Appl. Biol. 122 (1), 105–112. https://doi.org/10.1111/j.1744-7348.1993.tb04018.x.

Knox, J.W., Weatherhead, E.K., Bradley, R.I., 1997. Mapping the total volumetric irrigation water requirements in England and Wales (Available at:). Agric. Water Manag. 33 (1), 1–18. https://doi.org/10.1016/S0378-3774(96)01285-1.

Lazarević, B., Carović-Stanko, K., Safner, T., Poljak, M., 2022. Study of high-temperature-induced morphological and physiological changes in potato using nondestructive plant phenotyping (Available at:). Plants 11 (24). https://doi.org/10.3390/plants11243534.

Lazarević, B., Šatović, Z., Nimac, A., Vidak, M., Gunjača, J., Politeo, O., Carović-Stanko, K., 2021. Application of phenotyping methods in detection of drought and salinity stress in basil (*Ocimum basilicum* L.) (Available at:). Front. Plant Sci. 12, 629441. https://doi.org/10.3389/fpls.2021.629441.

Lenth, R.V. (2023) 'emmeans: Estimated Marginal Means, aka Least-Squares Means'. Available at: ⟨https://CRAN.R-project.org/package=emmeans⟩.

Li, X., Ramírez, D.A., Qin, J., Dormatey, R., Bi, Z., Sun, C., Wang, H., Bai, J., 2019. Water restriction scenarios and their effects on traits in potato with different degrees of drought tolerance (Available at:). Sci. Hortic. 256, 108525. https://doi.org/10.1016/j.scienta.2019.05.052.

van Loon, C.D., 1981. The effect of water stress on potato growth, development, and yield (Available at:). Am. Potato J. 58 (1), 51–69. https://doi.org/10.1007/BF02855380.

Lüdecke, D., Ben-Shachar, M.S., Patil, I., Wiernik, B.M., Bacher, E., Thériault, R. and Makowski, D. (2022) easystats: Framework for Easy Statistical Modeling, Visualization, and Reporting. Available at: ⟨https://easystats.github.io/easystats/⟩.

Lumley, T., Diehr, P., Emerson, S., Chen, L., 2002. The importance of the normality assumption in large public health data sets (Available at:). Annu. Rev. Public Health 23, 151–169. https://doi.org/10.1146/annurev.publhealth.23.100901.140546.

Mahmud, A.-A., Hossain, M.M., Karim, M.A., Mian, M.A.K., Zakaria, M., Kadian, M.S., 2016. Plant water relations and canopy temperature depression for assessing water stress tolerance of potato (Available at:). Indian J. Plant Physiol. 21 (1), 56–63. https://doi.org/10.1007/s40502-015-0202-3.

Makino, A., Osmond, B., 1991. Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat (Available at:). Plant Physiol. 96 (2), 355–362. https://doi.org/10.1104/pp.96.2.355.

Met Office National Climate Information Centre (2022) Unprecedented extreme heatwave, July 2022. Available at: ⟨https://www.metoffice.gov.uk/binaries/content/assets/metofficegovuk/pdf/weather/learn-about/uk-past-events/interesting/2022/2022_03_july_heatwave_v1.pdf⟩ (Accessed: 1 September 2024).

Monteoliva, M.I., Guzzo, M.C. and Posada, G.A. (2021) 'Breeding for drought tolerance by monitoring chlorophyll content', Gene technology [Preprint]. Available at: ⟨https://repositorio.inta.gob.ar/handle/20.500.12123/9833⟩ (Accessed: 8 January 2024).

Nauš, J., Prokopová, J., Rebíček, J., Spundová, M., 2010. SPAD chlorophyll meter reading can be pronouncedly affected by chloroplast movement (Available at:). Photosynth. Res. 105 (3), 265–271. https://doi.org/10.1007/s11120-010-9587-z.

Ninanya, J., Ramírez, D.A., Rinza, J., Silva-Díaz, C., Cervantes, M., García, J., Quiroz, R., 2021. Canopy temperature as a key physiological trait to improve yield prediction under water restrictions in potato (Available at:). Agronomy 11 (7), 1436. https://doi.org/10.3390/agronomy11071436.

O'Shaughnessy, S.A., Evett, S.R., 2010. Canopy temperature based system effectively schedules and controls center pivot irrigation of cotton (Available at:). Agric. Water Manag. 97 (9), 1310–1316. https://doi.org/10.1016/j.agwat.2010.03.012.

Peters, R.T., Evett, S.R., 2008. Automation of a center pivot using the temperature-time-threshold method of irrigation scheduling (Available at:). J. Irrig. Drain. Eng. 134 (3), 286–291. https://doi.org/10.1061/(asce)0733-9437(2008)134:3(286).

Piepho, H.-P., 2018. Letters in mean comparisons: what they do and don't mean (Available at:). Agron. J. 110 (2), 431–434. https://doi.org/10.2134/agronj2017.10.0580.

PlantEye F500 - Multispectral 3D laser scanner for plant phenotyping (2018) PHENOSPEX. Available at: ⟨https://phenospex.com/products/plant-phenotyping/planteye-f500-multispectral-3d-laser-scanner/⟩ (Accessed: 15 January 2024).

Ramírez, D.A., Yactayo, W., Gutiérrez, R., Mares, V., De Mendiburu, F., Posadas, A., Quiroz, R., 2014. Chlorophyll concentration in leaves is an indicator of potato tuber yield in water-shortage conditions (Available at:). Sci. Hortic. 168, 202–209. https://doi.org/10.1016/j.scienta.2014.01.036.

Rinza, J., Ramírez, D.A., García, J., de Mendiburu, F., Yactayo, W., Barreda, C., Velasquez, T., Mejía, A., Quiroz, R., 2019. Infrared radiometry as a tool for early water deficit detection: insights into its use for establishing irrigation calendars for potatoes under humid conditions (Available at:). Potato Res. 62 (2), 109–122. https://doi.org/10.1007/s11540-018-9400-5.

Rinza, J., Ramírez, D.A., Ninanya, J., de Mendiburu, F., García, J., Quiroz, R., 2022. Water saving using thermal imagery-based thresholds for timing irrigation in potatoes under drip and furrow irrigation systems (Available at:). Agronomy 12 (12), 2921. https://doi.org/10.3390/agronomy12122921.

Rolando, J.L., Ramírez, D.A., Yactayo, W., Monneveux, P., Quiroz, R., 2015. Leaf greenness as a drought tolerance related trait in potato (*Solanum tuberosum* L.) (Available at:). Environ. Exp. Bot. 110, 27–35. https://doi.org/10.1016/j.envexpbot.2014.09.006.

RStudio Team (2020) 'RStudio: Integrated Development Environment for R'. Boston, MA: RStudio, PBC. Available at: ⟨http://www.rstudio.com/⟩.

Rud, R., Cohen, Y., Alchanatis, V., Levi, A., Brikman, R., Shenderey, C., Heuer, B., Markovitch, T., Dar, Z., Rosen, C., Mulla, D., Nigon, T., 2014. Crop water stress index derived from multi-year ground and aerial thermal images as an indicator of potato water status (Available at:). Precis. Agric. 15 (3), 273–289. https://doi.org/10.1007/s11119-014-9351-z.

Schafleitner, R., Gutierrez, R. and Legay, S. (2009) 'Drought stress tolerance traits of potato', in Tropical roots and tubers in a changing climate: a convenient opportunity for the world. Fifteenth Triennial Symposium of the International Society for Tropical Root Crops. International Society for Tropical Root Crops - Peru Branch, pp. 1–5. Available at: ⟨https://www.researchgate.net/profile/Sylvain-Legay-3/publication/268204380_Drought_stress_tolerance_traits_of_potato/links/550ab9810cf290bdc10ffdb5/Drought-stress-tolerance-traits-of-potato.pdf⟩.

Science and Advice for Scottish Agriculture (2023) European Cultivated Potato Database, Varieties. Available at: ⟨https://live_euro.sasa.gov.uk/varieties⟩ (Accessed: 3 January 2024).

Sinclair, T.R., Manandhar, A., Shekoofa, A., Rosas-Anderson, P., Bagherzadi, L., Schoppach, R., Sadok, W., Rufty, T.W., 2017. Pot binding as a variable confounding plant phenotype: theoretical derivation and experimental observations (Available at:). Planta 245 (4), 729–735. https://doi.org/10.1007/s00425-016-2641-0.

Stark, J.C., Pavek, J.J., McCann, I.R., 1991. Using canopy temperature measurements to evaluate drought tolerance of potato genotypes (Available at:). J. Am. Soc. Hortic. Sci. 116 (3). https://doi.org/10.21273/JASHS.116.3.412.

Turner, N.C., 2019. Imposing and maintaining soil water deficits in drought studies in pots (Available at:). Plant Soil 439 (1), 45–55. https://doi.org/10.1007/s11104-018-3893-1.

Víg, R., Huzsvai, L., Dobos, A., Nagy, J., 2012. Systematic Measurement Methods for the Determination of the SPAD Values of Maize (*Zea mays* L.) Canopy and Potato (*Solanum tuberosum* L.) (Available at:). Commun. Soil Sci. Plant Anal. 43 (12), 1684–1693. https://doi.org/10.1080/00103624.2012.681740.

Wickham, H. (2016) 'ggplot2: Elegant Graphics for Data Analysis'. Springer-Verlag New York. Available at: ⟨https://ggplot2.tidyverse.org⟩.

Xiong, D., Chen, J., Yu, T., Gao, W., Ling, X., Li, Y., Peng, S., Huang, J., 2015. SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics (Available at:). Sci. Rep. 5, 13389. https://doi.org/10.1038/srep13389.