Project title:	Improving quality and shelf-life of Romaine and Iceberg lettuce crops using precision, deficit and alternate wetting and drying irrigation techniques optimised for different soils
Project number:	FV 454
Project leader:	Dr Mark Else, NIAB EMR
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Key staff:	Mike Davies, June Taylor, NIAB EMR
Location of project:	NIAB EMR
Industry Representative:	Dave Edwards, Anglia Salads, Colchester, Essex
Date project commenced:	01 April 2017
Date project completed:	31 July 2018

DISCLAIMER

The results and conclusions in this report are based on an investigation conducted over a one-year period on potted lettuce plants. The controlled conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial irrigation recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headlines

1. Leaf responses of Romaine and Iceberg varieties to drying peat and silt soils were triggered at similar soil matric potentials, but at very different volumetric soil moisture contents
2. Thermal imaging of lettuce hearts detected a loss in transpirational cooling in response to very mild soil water deficits; this preceded significant reductions in stomatal conductance which were triggered at lower soil matric potentials
3. The Romaine varieties "Scala", *Actina", and Iceberg varieties "Challenge" and "Etude" have different sensitivities to drying soil therefore water management practice needs to be optimised for each variety
4. Post-harvest mid-rib pinking was not detected in any Romaine and Iceberg lettuce varieties grown in peat or silt soil under well-watered or mild soil drying treatments
5. Irrigation set points based on soil matric potentials have been identified for Romaine and Iceberg lettuce varieties grown in peat and silt soils

Background

UK lettuce growers strive to supply a consistently high quality product to achieve customer satisfaction but this can be challenging in changeable UK growing conditions. Anecdotal evidence suggests that lettuce crops grown overseas with reduced water inputs often have better leaf quality and a longer shelf-life than those grown under typical UK commercial conditions. Over-wet soils due to excessive rainfall or ineffective irrigation scheduling can promote postharvest mid-rib pinking and reduce shelf-life in some varieties *e.g*. Romaine and Iceberg. Although growers recognise the importance of optimising irrigation scheduling, matching crop demand for water with supply is challenging for many. New guidelines are needed to help UK growers to increase returns on investment through the efficient use of resources, and access to real-time field data is vital to avoid unplanned soil moisture deficits that have the potential to reduce head fresh weight and diameter, and lower leaf quality.

The removal of the Abstraction Licence exemption for trickle irrigators and the hot dry summer of 2018 have focussed attention on the availability and efficient use of water for irrigation in the production of leafy salads. UK's recent failure to meet the objectives set out in the Water Framework Directive around achieving "good quality status" of UK water bodies means that on-farm fertiliser use efficiencies must also be improved across the industry. At the same time, a greater consistency of supply of high quality fresh produce with an assured shelf-life must be achieved, alongside reductions in labour costs associated with crop management and harvesting.

There is evidence that using precision irrigation, alternate wetting and drying regimes and "beneficial stresses" such as deficit irrigation has the potential to improve leaf quality, and shelf-life in cut lettuce leaves, but head fresh weights are often reduced by more severe soil water deficits. A more rigorous scientific understanding of how the physiology of leafy salads is altered by mild and more severe soil drying is needed in order to identify new opportunities to use beneficial stresses in commercial production to improve leaf quality without reducing head fresh weight or diameter. In tandem, new uncomplicated grower-facing tools and approaches are needed to facilitate the integration of new innovative growing practices into existing commercial infrastructure.

In this work, we have imposed gradual soil drying to identify the most appropriate irrigation set points for Romaine and Iceberg lettuce varieties grown in two different soil types; this is the first step towards the longer-term aim of developing on-farm precision irrigation (PI) strategies that ensure marketable yields are maintained and leaf quality and resource use efficiency is optimised. Our approach is to derive irrigation set points based on soil matric potentials rather than using volumetric soil moisture contents or soil moisture deficits which are more commonly used in the industry. Soil matric potentials are not influenced by soil bulk density and so irrigation set points should be similar for specific varieties growing in a range of different soil types. Soil matric potentials can be converted to volumetric soil moisture contents by reference to a moisture release curve, which is a plot of the relationship between the water content, and the soil matric potential (see Science Section).

Summary

The aim of Phase I of this project was to develop scientifically-derived irrigation set points for use in precision irrigation, alternate wetting and drying regimes, and deficit irrigation to improve consistency of leaf quality and shelf-life potential of Romaine ("Actina" and "Scala") and Iceberg ("Challenge" and "Etude") lettuce without reducing head fresh weight. This was achieved by applying mild and gradual soil drying and measuring the first plant adaptive responses to declining soil water availability. The point of first wilting and widespread wilting was also noted.

Experimental details

Two varieties of Romaine lettuce ("Actina" and "Scala") and two varieties of Iceberg lettuce

Figure GS1. "Scala" and "Challenge" lettuce plants in 13 L pots of silt or peat soil in the GroDome at NIAB EMR (Experiment 1).

("Challenge" and "Etude") were used in three separate experiments. Each variety was grown in either a peat or a silt soil and two irrigation treatments (well-watered or drying down) were imposed. Lettuce blocks were supplied by Jepco Ltd and G's Fresh Ltd and planted in 13 L pots containing peat (G's, Cambs.) or silt (Jepco, Lincs.) soil. All plants were maintained in the GroDome (a controlled environment facility) at NIAB EMR (Figure GS1). Day and night temperatures were set to 18 °C and 12 °C, respectively; these values are the long-term historical averages for May-July at East Malling. Temperatures were adjusted to 22 °C and 14 °C for experiments in October to ensure plant establishment. Relative humidity was uncontrolled. There were 12 biological replicates for each variety, soil and treatment. A randomised block design that maximised statistical degrees of freedom was used and any statistically significant differences between treatments were identified.

Soil matric potential and volumetric moisture content sensors (Figure GS2) were buried at different depths within the pots. In addition, "spot measurements" of volumetric soil moisture content, soil temperature, and pore E.C. were made at three positions within the pot using a hand-held "WET" sensor and HH2 meter (Delta-T Devices Ltd, UK). Irrigation was scheduled to match demand with supply and applied *via* pressure compensated emitters and a four-way spider to help to ensure even distribution of water throughout the pot. Two weeks before plants reached market specifications, a drying down treatment was applied to half of the pots by reducing the daily irrigation volume to 80% of the volume of water lost by transpiration each day. In this way, gradual soil drying was imposed. Leaf physiological responses to drying soil including leaf chlorophyll content, leaf water relations, stomatal conductance, lettuce heart temperature and photosynthesis were measured three times a week and compared to those of well-watered plants to identify the degree of soil drying at which agronomically

Figure GS2. A) Sensors used to measure volumetric soil moisture content, soil temperature and pore E.C. (Delta-T Devices Ltd). B) Sensors used to compare soil matric potential and volumetric soil moisture content (METER Group, Inc. USA).

important traits were first affected. At harvest, measurements of whole lettuce fresh weight, lettuce head fresh and dry weights, the propensity for mid-rib pinking in cut leaves, and leaf antioxidant capacity at harvest were also made.

For brevity, results are reported here from experiments with Romaine “Actina” and Iceberg “Challenge” in peat and silt soils. Results from experiments with “Scala” and “Etude” in peat and silt soils are summarised in the Science Section.

Romaine “Actina” - changes in soil volumetric moisture content and matric potential

In the peat soil, the volumetric soil water content at pot / field capacity was around 40% and the corresponding soil matric potential was -6 kPa. Throughout the experiments, soil in the well-watered treatment was maintained around pot capacity, and in the drying down treatment, volumetric soil moisture content and soil matric potential fell to 27% and -300 kPa, respectively, by the end of the experiment.

In silt soil, the volumetric soil water content at pot (field) capacity was around 24% and the corresponding soil matric potential was –10 kPa. Throughout the experiments, soil in the well-watered treatment was maintained around pot capacity, and in the drying down treatment, volumetric soil moisture content and soil matric potential fell to 13% and -126 kPa, respectively, by the end of the experiment.

Romaine “Actina” - plant responses to soil drying

In drying peat soil, a small but significant increase in the temperature of lettuce hearts in plants under the drying down treatment was detected by thermal imaging. This reduction in transpirational cooling was first noted at a soil matric potential of -36 kPa (~VSMC of 36%). As the peat soil dried further, stomata began to close and a subsequent reduction in the rate of photosynthesis was detected (Figure GS3). Wilting was first detected at -160 kPa and was widespread at -280 kPa (Figure GS3). Leaf chlorophyll content was similar in plants under the two treatments. A significant difference in stomatal conductance between plants in well-watered

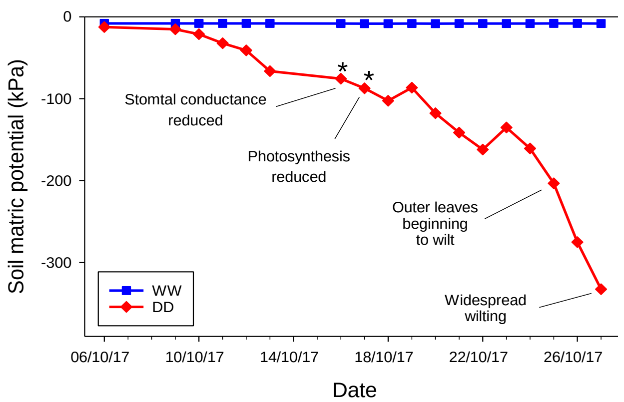

Figure GS3. The soil matric potentials at which statistically significant reductions in stomatal conductance and photosynthesis were detected in “Actina” lettuce growing in drying peat soil. The onset of mild and severe wilting is also shown.

Table 1. Volumetric soil moisture content (VSMC) and soil matric potential (soil ψ_m) at which statistically significant reductions in stomatal conductance (g_s) and photosynthesis (Pn) were first detected in "Actina" in response to gradual drying of peat and silt soil.

Soil	Pot Capacity		Significant reduction in g_s (p<0.05)		Significant reduction in Pn (p<0.05)	
	VSMC (%))	soil ψ_m (kPa)	VSMC (%))	Soil ψ_m (kPa)	VSMC (%)	Soil ψ_m (kPa)
Peat	40	-6	30	-78	27	-86
Silt	24	-10	14	-84	13	-94

peat soil and those in drying peat soil was first detected at -50 kPa (~VSMC of 30%), and photosynthesis was reduced significantly at -65 kPa (~VSMC of 27%). (Table 1).

In drying silt soil, a small reduction in transpirational cooling was first noted at a soil matric potential of -38 kPa, corresponding to a volumetric soil moisture content value of 18%. A significant difference in stomatal conductance was first detected at a soil matric potential of -84 kPa and a corresponding volumetric moisture content of *c.* 14%. Photosynthesis was reduced significantly at -94 kPa and a corresponding volumetric moisture content of 13% (Table 1). Plants began to wilt at -167 kPa and more widespread wilting was apparent at -230 kPa. Leaf chlorophyll content and water potential were similar in the well-watered and drying down treatments.

Romaine "Actina" - derivation of irrigation set points for use in alternate wetting and drying regimes

We have demonstrated conclusively that leaf adaptive physiological responses to drying soil occur at similar values of matric potential in the peat and silt soils, whereas the corresponding volumetric soil moisture contents were very different. This outcome was expected since matric potentials are not influenced by differences in soil bulk density, whereas volumetric water contents are. These results highlight the advantage of using matric potentials to schedule irrigation to crops in various soils with very different bulk densities, and moisture release curves linking soil matric potential with volumetric moisture content will be derived for different soils to help to inform growers' irrigation strategies.

In the pot experiments described above, "Actina" first perceived a moisture deficit stress at a soil matric potential between -78 and -84 kPa. Continued and sustained soil drying past this value would likely reduce final head weights and diameters, as well as leaf quality and so the recommended irrigation set point for further testing in field experiments is -65 kPa. This set point could be used to develop an Alternate Wetting and Drying treatment where the frequency and duration of irrigation is scheduled to return soil to field capacity once the average soil matric potential in the rooting zone reaches -65 kPa. This would correspond to an average volumetric soil moisture content in the rooting zone of 15 and 33% in silt and peat soils, respectively.

Iceberg "Challenge" - plant responses to soil drying

In drying peat soil, a significant difference in stomatal conductance was first detected at -44 kPa, and photosynthesis was also reduced significantly at -54 kPa (Table 2). The corresponding volumetric moisture content was 30%. Wilting was first detected at -180 kPa and was widespread at -320 kPa. Leaf chlorophyll content and water potentials were similar in the well-watered and drying down treatments.

In drying silt soil, a significant difference in stomatal conductance was first detected at a soil matric potential of -36 kPa and a corresponding volumetric moisture content of 16% (Table 2). Photosynthesis was reduced significantly at -65 kPa and a corresponding volumetric moisture content of below 14%. Plants began to wilt at -70 kPa and more widespread wilting was apparent at -155 kPa. Leaf chlorophyll content and water potentials were similar in plants under the well-watered and drying down treatments.

Table 2. Volumetric soil moisture content (VSMC) and soil matric potential (soil ψ_m) at which statistically significant reductions in stomatal conductance (g_s) and photosynthesis (Pn) were first detected in "Challenge" in response to gradual drying of peat and silt soil.

Soil	Pot Capacity		Significant reduction in g_s ($p<0.05$)		Significant reduction in Pn ($p<0.05$)	
	VSMC (%))	soil ψ_m (kPa)	VSMC (%))	Soil ψ_m (kPa)	VSMC (%)	Soil ψ_m (kPa)
Peat	45	-10	30	-54	30	-54
Silt	24	-10	16	-36	>14	-65

Iceberg "Challenge" - derivation of irrigation set points for use in alternate wetting and drying regimes

In the pot experiments described above, "Challenge" first perceived a moisture deficit stress at a soil matric potential between -36 and -54 kPa. Continued and sustained soil drying past this point would likely reduce final head weights and diameters, as well as leaf quality and so the recommended irrigation set point for further testing in field experiments is -25 kPa. This set point should be used in an alternate wetting and drying treatment where irrigation is scheduled to return soil to field capacity once the average soil matric potential in the rooting zone reaches -25 kPa, which is equivalent to a volumetric moisture content of 18 and 39% in silt and peat soils, respectively.

Mid-rib pinking was not observed in any variety in either well-watered or drying down treatments. The conditions that predispose leaves to mid-rib pinking are not yet known but over-irrigation or excessive rainfall are thought to be involved. Deficit irrigation has also been shown to increase the propensity for mid-rib pinking. In our work, over-watering was avoided by scheduling irrigation to match demand with supply and so the absence of any pinking was not unexpected.

Financial Benefits

The cost / benefit of adopting the irrigation strategies investigated in this project cannot be quantified at this early stage. The irrigation set points derived for potted Romaine and Iceberg varieties need to be tested in a controlled environment and validated in scientific field experiments where alternate wetting and drying strategies are deployed on commercial grower sites over the course of a full growing season, and are compared to commercial irrigation regimes.

Action Points

1. Consider using changes in soil matric potential to inform irrigation decisions for crops in different soils rather than changes in soil volumetric moisture content
2. Review commercial irrigation strategies and identify where improvements could be made to optimise soil water availability in the rooting zone
3. Recognise that varieties have differential sensitivity to soil drying and schedule irrigation accordingly to avoid limiting photosynthesis and leaf quality
4. Improve irrigation scheduling to avoid unplanned soil water deficits and optimise leaf transpirational cooling in hot weather
5. Avoid over-irrigation, especially after rainfall, which exacerbates post-harvest pinking

SCIENCE SECTION

Introduction

Optimising leaf quality and shelf-life under changeable UK growing conditions is challenging for many leafy salad growers. Irrigation scheduling in lettuce production relies largely on experience and there has been little research into identifying the ranges of soil water availability that optimise marketable yields, leaf quality and shelf-life in the different soils in which lettuce is grown. To avoid yield penalties resulting from unplanned soil water deficits, growers often apply sufficient irrigation to maintain soils at or near to "field capacity". Results from EMR's ERDF-funded WATERR project, in which Irrigation Business Reviews with 10 UK lettuce growers were completed, showed that the average volume of water applied per ha in 2011-2013 was 784 m^3, with a range of 281 – 2,142 m^3/ha. Average yields of 20 t/ha (range 7-34) were achieved and the average water productivity (WP) value was 79 m^3/t marketable yield (range 16-94). A lower WP value indicates a more efficient use of irrigation water. This range is typical of many horticultural sectors and although the WP value is influenced by many factors (soil type, cropping system, weather *etc*.), the range indicates that some UK lettuce growers are already delivering 'better practice' whilst others are not so water conscious.

More efficient water, fertiliser, energy and pesticide use in the production of higher yields of better quality crops with improved shelf-life and nutritional content is vital to the future success of agri-businesses. Under new regulations introduced by Defra at the end of 2017, the abstraction licence exemption which previously applied to trickle irrigators was removed. Until then, no licence had been required, and with no limit on the volume of water they could abstract, most trickle irrigators were able to expand production each year in line with the growth in demand for UK leafy salads. However, under the New Authorisations regulations, all trickle irrigators must apply for a licence by the end of 2019, and the volume of water they will be allowed to abstract from 1 January 2018 onwards was restricted to historical peak volumes. If growers wish to expand production and increase their abstraction volumes, they will have to apply for a new licence which the Environment Agency have advised will be more restrictive than those available under the New Authorisations.

Furthermore, all growers will be under increasing pressure to reduce the effects of intensive horticulture on groundwater quality to ensure that the target of 100% of England's water bodies classified as of "good status" is met by the revised deadline of 2027. The previous target was 2016 by which time only 27% of water bodies were of "good status". To comply with these legislative demands, growers must be able to demonstrate an efficient use of irrigation water and applied fertilisers. New guidelines are needed to improve the economic and environmental sustainability of UK leafy salad production, and to help growers to comply with current and future legislation.

These legislative pressures mean that on-farm water and fertiliser use efficiencies must be improved across the industry. At the same time, a greater consistency of supply of high quality fresh produce with an assured shelf-life must be achieved, alongside reductions in labour costs associated with crop management and harvesting. To achieve these aims, new, uncomplicated grower-facing tools and approaches that can be integrated into existing commercial infrastructure are needed. A scientifically-derived blueprint to improve on-farm water and fertiliser use efficiencies is needed that will enable growers to demonstrate compliance with new legislation introduced to safeguard the environment and reduce the impacts of intensive horticulture on ground water quality.

The effects of imprecise irrigation scheduling and/or excessive rainfall on leaf quality in Romaine and Iceberg lettuce varieties are well-known (Luna *et al*., 2013). Increased irrigation led to a greater biomass production in Iceberg lettuce, with heads containing a greater proportion of water, but after 20 days in cold storage, pinking was significantly greater in treatments where higher volumes of irrigation were applied (FV 413, Vickers *et al*., 2015 but see Hilton *et al*., 2009). Because differences in response to excess and limited soil water availability occur with distinct lettuce types (Luna *et al*., 2013), the form of water management needs to be optimised for each type and, perhaps, each variety.

In this work, we have imposed gradual soil drying to identify the most appropriate irrigation set points for Romaine and Iceberg lettuce varieties grown in two different soil types; this is the first step towards the longer-term aim of developing on-farm precision irrigation (PI) strategies that ensure marketable yields are maintained and leaf quality and resource use efficiency is optimised. Our approach is to derive irrigation set points based on soil matric potentials (soil ψ_m), rather than using volumetric soil moisture contents (VSMC) or soil moisture deficits (SMD) which are more commonly used in the industry. Soil matric potentials are not influenced by soil bulk density and so irrigation set points should be similar for specific varieties growing in a range of different soil types. Although the opportunities, constraints and challenges of implementing PI on commercial sites have been discussed by Monaghan et al. (2013), our results should help to inform on-farm irrigation scheduling for lettuce crops growing in different soils. Further work is needed in which these irrigation set points are tested and validated in scientific field experiments using alternate wetting and drying (AWD) strategies on commercial grower sites over the course of a full growing season, and are compared to commercial irrigation regimes. The results from this project will also inform further research work on testing the potential to use Regulated Deficit Irrigation (RDI) and to improve aspects of leaf quality, and will help to avoid some of the pitfalls encountered in previous work (*e.g*. Monaghan *et al.* 2016) where Deficit Irrigation (DI) was applied rather arbitrarily.

Our industry partners include Jepco Marketing Ltd and G's Fresh Vegetables Ltd, each of whom provided plant modules, soils and agronomy advice. A peat and a silt soil were used in GroDome (a controlled environment facility) experiments at NIAB EMR to derive and test irrigation set points for potted Romaine and Iceberg varieties. In addition, moisture release curves linking soil ψ_m with VSMC for the peat and silt soils, a sandy loam (Betts, Kent) and a silty clay (G's, south coast) will be generated to help to inform irrigation strategies for lettuce production on these soils.

Materials and methods

Plant material and controlled environment conditions

Two varieties of Romaine ("Scala" and "Actina") and two varieties of Iceberg ("Challenge" and "Etude") lettuce were used in separate experiments; the variety used was governed by the availability of plant material. Lettuce blocks were supplied by Jepco Ltd and G's Fresh Ltd and planted in 13 L containers containing silt soil provided by Jepco, Lincs., or peat soil provided by G's, Cambs., from commercial field sites.

For Experiment 1, "Scala" and "Challenge" lettuce plants were delivered to NIAB EMR on 17 May 2017 and planted in 13 L pots containing either peat or silt soil (Figure 1). Plants were grown in a compartment of the GroDome at NIAB EMR. Day and night temperatures were set to 18 °C and 12 °C, respectively; these values are the long-term historical averages for May-July at East Malling. Relative humidity was uncontrolled.

Figure 1. "Scala" and "Challenge" lettuce blocks were delivered to NIAB EMR and planted into 13 L pots of peat or silt soil. In Experiment 1, there were two dripper stakes per pot. Photo taken on 19 May 2017.

For Experiment 2, "Actina" and "Etude" lettuce plants were delivered to NIAB EMR on 3 October 2017 and planted in 13 L pots containing either peat or silt soil in the GroDome. To increase the speed of plant establishment, day time temperature was set to 22 °C and 14 °C at night, during the soil drying phase of the experiment the compartment was set at 18 °C day time temperature and 12 ºC at night; relative humidity was uncontrolled.

For Experiment 3, “Challenge” lettuce plants were delivered to NIAB EMR on 4 May 2018 and planted in 13 L pots containing silt soil in the GroDome. Day and night temperatures were set to 18 °C and 12 °C; relative humidity was uncontrolled.

Experimental design

Experiments were conducted simultaneously, one for each variety, to identify the soil ψ_m at which leaf physiological adaptive responses were first triggered. Discussions with Dr Phil Brian, NIAB EMR’s statistician, prior to the beginning of the project ensured that our experimental designs, our replication and our recording protocols were statistically sound. Our research was carried out with sufficient statistical rigour to be able to determine whether effects of small differences in water availability on crop quality were statistically significant. Forty-eight plants of each variety were used in each experiment. Twenty-four were planted in peat soil and 24 in silt soil. Each set of 24 plants was arranged in two rows in a randomised block design with 12 blocks (Figure 2) and two irrigation treatments were imposed: i) well-watered (WW) in which the average soil ψ_m throughout the rooting zone was maintained at *c.* -10 kPa ii) Drying Down (DD) in which daily irrigation volumes were gradually reduced so that the decreasing soil ψ_m triggered a range of leaf physiological responses that could be measured.

Figure 2. “Scala” and “Challenge” lettuce plants in 13 L pots of silt or peat soil in the GroDome at NIAB EMR (Experiment 1). Photo taken on 14 June 2017.

Fertiliser applications

Soil analyses provided by Jepco and G’s were used by David Norman of Fresh Produce Consultancy Ltd to calculate nitrate, phosphate and potassium rates. Fertilisers were added to the pots of peat and silt and mixed thoroughly into the soil prior to planting. Commencing from 7 days after planting, weekly sprays of foliar nutrients and fungicides were applied as recommended.

Soil water measurements and environmental monitoring

Volumetric soil moisture content, pore electrical conductivity (E.C.) and soil temperature at different depths within the rooting zone were measured continuously using WET-2, SM300 and SM150T sensors (Figure 3) connected to GP2 Advanced Datalogger and Controller with telemetry (Delta-T Devices Ltd, Cambridge, UK). Soil matric potentials were measured using Decagon MPS2 sensors connected to Decagon EM50G dataloggers (Decagon Devices Ltd). Data were downloaded daily and averaged within treatments. Photosynthetically active radiation, air temperature, and relative humidity were measured in the GroDome compartment and daily vapour pressure deficits (VPDs) were calculated.

Figure 3. Sensors used to measure volumetric soil moisture content, soil temperature and pore E.C. Photo taken on 15 June 2017.

To facilitate transfer of the results to commercial growers who typically use volumetric measures of soil moisture content to inform their irrigation strategies, corresponding values of soil ψ_m and VSMC were obtained throughout the drying down phase using METER MPS-2 and 10HS sensors (Figure 4) so that preliminary calibration curves for the silt and peat soils could be generated *in situ*. More detailed moisture release curves for peat, silt, a silty loam and a sandy loam will be generated during August to October 2018 using a pressure plate system recently purchased by NIAB EMR.

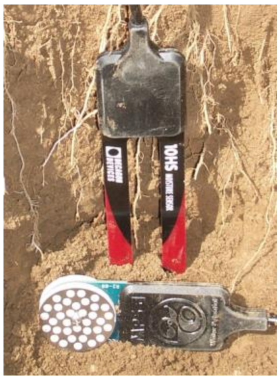
Figure 4. Sensors used to compare soil matric potential and volumetric measure volumetric soil moisture content. Photo taken on 15 June 2014.

Imposition of the Well-watered and Drying Down irrigation treatments

Eight irrigation lines were installed to ensure that irrigation could be delivered to each variety and treatment individually. The irrigation lines were connected to two Galcon DC-4S units (City Irrigation Ltd); each unit had four solenoid valves that could be operated independently.

Experiment 1: Irrigation was delivered to each pot via two pressure-compensated 2 L h^{-1} emitters with individual dripper stakes. During establishment, all pots were irrigated to maintain the average soil ψ_m at -10 kPa (field/pot capacity). Once established, all pots received 100% of their daily evaporative demand for water. Changes in fresh weight of four

WW plants-and pots, in each set of 24 plants, were measured over 24 h to calculate crop co-efficients. The crop co-efficient was used in conjunction with an estimate of potential evapotranspiration (ET_p) over 24 h to estimate plant water use. An SKTS500 Evaposensor (Syke instruments UK) was used to calculate daily ET_p values. The DD treatment was first imposed on 10 June 2017. Plants received only 50% of the WW treatment irrigation volume.

Experiments 2 and 3: The approach to irrigation scheduling was modified to try to prevent the limited rooting in silt soils that was seen in Experiment 1 (see Figure 11). Irrigation was delivered to each pot *via* four pressure-compensated 2 L h^{-1} emitters with individual dripper stakes; this ensured a more even distribution of water in each quarter of the pot. To encourage roots to grow down into the pot during establishment, irrigated was applied once the soil ψ_m in the uppermost 10 cm of soil reached -30 kPa. Once established, irrigation was supplied as for Experiment 1. In Experiments 2 and 3, the DD treatment was first imposed on 3 October 2017, and 12 May 2018, respectively.

Plant physiological measurements

Stomatal conductance, photosynthesis and leaf chlorophyll index

Stomatal conductance (g_s) and photosynthesis (Pn) of fully expanded leaves were measured using a portable infra-red gas analyser (LI-6400XT, LiCor Biosciences). A SPAD meter was used to estimate leaf chlorophyll content. All measurements were made between 11:00 and 13:00 each day as gradual soil drying was imposed. Once significant differences in Pn were detected in DD-treated plants, the physiological measurements were terminated. Eight plants of each variety and soil type were used to quantify leaf quality parameters at harvest and after shelf-life, while irrigation to the other four DD pots was withheld to determine the points at which wilting first occurred and more widespread wilting occurred.

Leaf water potential, osmotic potential and turgor

To determine the leaf water potential and osmotic potential of the leaves during the first stages of soil drying, three leaf discs were excised with a cork borer (Figure 5). Six plants per soil, variety and irrigation treatment were sampled at each time point. Discs were taken from the mid-rib of a mature leaf to minimise effects on transpirational losses. Each set of three discs was placed in sealed containers

Figure 5. Leaf discs were excised from using a cork borer for measurement of leaf water relations. Photo taken on 16 June 2017.

containing moist filter paper. The discs were blotted dry before sealing in the thermocouple chamber of a Wescor Water Potential System (Wescor Inc., UT, USA). Previously, the thermocouples were placed in a covered polystyrene box to equilibrate for 1 h before taking water potential measurements. Leaf discs were then wrapped in aluminium foil and frozen in liquid nitrogen before storage at -20 ºC until needed for measurements of osmotic potentials. Discs were allowed to defrost, were blotted dry and sealed into the chambers and measurements were taken as described previously. Leaf turgor was calculated as the difference between osmotic potential and leaf water potential.

Thermal imaging

Thermal images were taken daily with a ThermaCam P25 (Flir UK) during the initial soil drying phases. Images were taken from above for all plants (Figure 6). Maximum, minimum and average temperature of the whole lettuce, the heart of the lettuce and a flat leaf for each image was recorded.

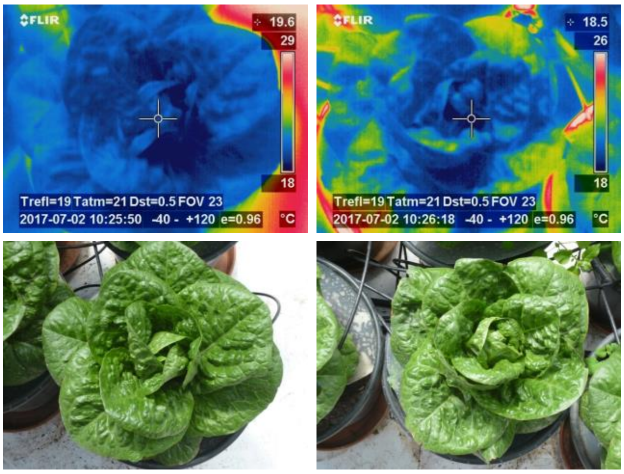

Figure 6. The potential of using thermal imaging to detect very early changes in leaf temperature as a result of soil drying-induced partial stomatal closure was tested. Photo taken on 2 July 2017.

Harvest and shelf life

Eight WW- and eight DD-treated plants of each variety grown in peat and silt soils were cut at the base. Fresh weights of the whole lettuce and the lettuce heart were recorded. Half of the harvested plants were placed in cold storage for 10 days at 4ºC, and on removal from storage, the lettuces were assessed for “pinking” on the 10 cm visible length of mid-ribs from the base. The remaining harvested lettuces were cut in half, one half was fresh weighed and placed in a drying oven at 80˚C until constant weight to determine dry matter content. The remaining lettuce half was stored at -80˚C until analysis to determine total anti-oxidant capacity by the Trolox equivalent anti-oxidant capacity (TEAC) assay.

Total anti-oxidant capacity

Hydrophilic anti-oxidants were extracted in 10 mL of [80:20] [methanol: water]. To determine the anti-oxidant capacity of leaf samples, the TEAC method was used. A solution of 7 mM 2,2’–Azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) in ultrapure water was converted to its mono-cationic form (ABTS+) by the addition of 2.45 mM (final concentration) potassium persulphate ($K_2S_2O_8$). A 30 µL aliquot of solvent extract was pipetted into a 3 mL cuvette containing diluted ABTS+ solution. Measurements of absorbance were then made with a Pharmacia ultraspec III spectrophotometer at a wavelength of 734 nm. The absorbance of the standards of 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) in concentration range of 0 to 2 mM were measured and the % inhibition of the ABTS+ was calculated and a linear graph with an r^2 value of 0.999 was plotted. The regression equation was used to determine the amount of anti-oxidant capacity in the samples relative to the reactivity of Trolox.

Statistical analyses

Statistical analyses were carried out using Genstat 14 Edition (VSN International Ltd). To determine whether differences between irrigation treatments were statistically significant, analysis of variance (ANOVA) tests were carried out and least significant difference (LSD) values for p<0.05 were calculated.

Results

Experiment 1 - Romaine “Scala” in peat soil

Soil volumetric moisture content and soil matric potential

At the beginning of the experiment, the VSMC in both the WW and DD treatments was c. 40%, with a corresponding soil ψ_m of -6 kPa (pot/field capacity) (Figure 7A&B). On 10 June 2017, water was withheld from plants in the DD treatment and the average VSMC and soil ψ_m throughout the rooting zone began to decline. The soil in the WW treatment was maintained at or near to pot capacity throughout the experiment (VSMC values from 36 - 40%, and soil ψ_m values from -6 to -27 kPa) (Figure 7A&B). To ensure that soil dried gradually in the DD treatment, 80% of the evaporative losses over the previous 24 h was applied to the DD pots from 14 June 2017, so that VSMC and soil ψ_m continued to decline on successive days (Figure 7A&B). The VSMC in the DD treatment fell to 27% and the soil ψ_m to -300 kPa on 23 June 2017 when measurements of physiological responses ended.

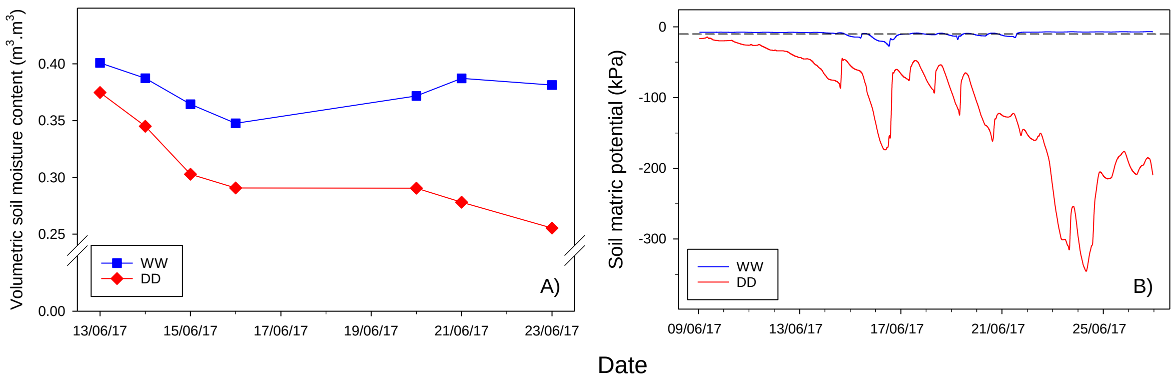

Figure 7. Changes in A) average volumetric soil moisture content and B) average soil matric potential during the phase of gradual drying of peat soil in the rooting zone of “Scala” lettuce.

Plant physiological responses to drying peat soil

Leaf physiological responses including *gs*, Pn, leaf water potential, osmotic potential and leaf temperature were measured in DD plants subjected to decreasing soil moisture availability / content and compared to the plants grown in soil at or close to pot capacity (WW). Thermal images taken of the heart detected a small and transient but statistically significant increase in temperature in plants in the DD treatment on 13 June 2018 (Figure 8) when the soil ψ_m reached -36 kPa. On subsequent measurement days, heart and leaf temperatures were similar in both irrigation treatments. The small but significant rise in leaf temperature was detected 24 h before the first statistically significant reduction in *gs* was noted in the DD-treated plants (Figure 9A) when the soil ψ_m reached -50 kPa. Continued soil drying caused further reductions in *gs* when measured on subsequent days, except on 16 June 2017 when soil ψ_m increased temporarily following an irrigation event (see Figure 7B) that was applied to prevent the soil from drying too quickly. Photosynthesis was reduced significantly on 20 June (Figure 9B) at a soil ψ_m of -145 kPa.

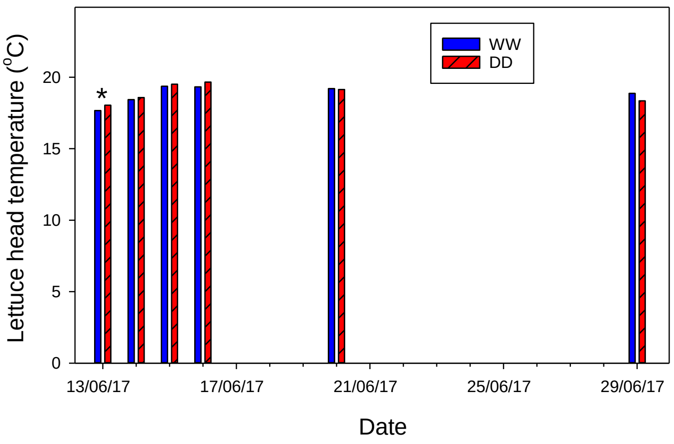

Figure 8. The effects of drying peat soil on "Scala" lettuce heart temperatures detected using thermal imaging. Data are mean values of 12 replicate plants, asterisks indicate statistically significant differences between WW and DD values (p<0.05).

Throughout the early stages of gradual soil drying, leaf water potentials, osmotic potentials and turgor pressures were similar in both treatments (data not shown). Visible signs of wilting in the outer leaves first occurred at -270 kPa and more widespread wilting was noted at -320

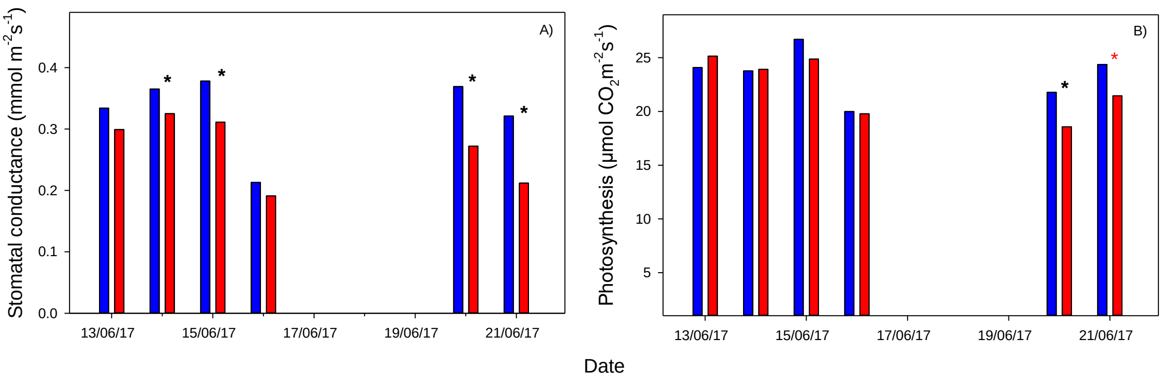

Figure 9. Soil drying induced changes in A) stomatal conductance and B) photosynthesis of Romaine "Scala" lettuce in peat soil. Data are mean values of 12 replicate plants, black asterisks indicate statistically significant differences between WW and DD values (p<0.05). Red asterisks indicate differences between WW and DD values are approaching significance.

kPa (data not shown). The soil ψ_m at which statistically significant differences in leaf physiology between WW- and DD-treated "Scala" were first detected in peat soil are plotted in Figure 10. The reduction in stomatal conductance in response to drying soil indicate that "Scala" plants first perceived a mild soil drying stress when the peat soil ψ_m reached -76 kPa (Figure 10). On the previous day at the time of leaf physiological measurements, the average soil ψ_m within the rooting zone was -47 kPa. Assuming similar light intensities and VPDs to those in our experiments, we would expect that an average soil ψ_m throughout the rooting zone above -47 kPa would not trigger leaf adaptive response to declining soil water availability. In future work, we would use an irrigation set point of -47 kPa to test the potential of using AWD irrigation regimes to improve water use efficiency and leaf quality attributes of "Scala" growing in peat soil, The corresponding VSMC for the peat soil used in the experiment was 37%.

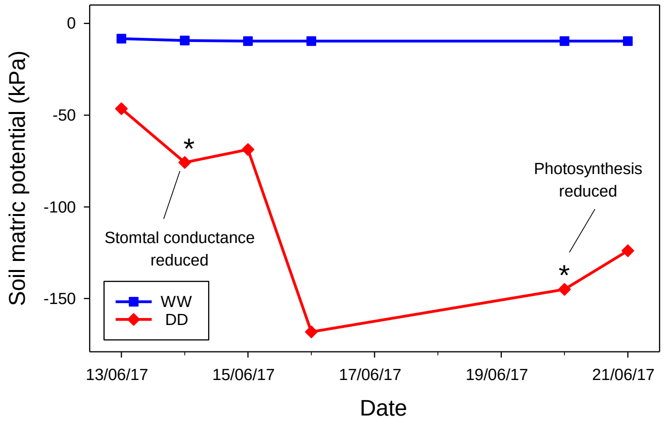

Figure 10. The soil matric potentials at which statistically significant reductions in stomatal conductance and photosynthesis were detected in "Scala" lettuce growing in drying peat soil.

Experiment 1 – "Scala" leaf quality at harvest

At maturity, there was no significant difference in the fresh weight of the whole lettuce, lettuce head, or in the leaf % dry weight between plants in the WW or DD treatments (Table 1). There was no significant difference in leaf total antioxidant capacity between the two treatments. There was very little evidence of mid-rib pinking in the cut lettuce leaves from either treatment that were placed into shelf life (data not shown).

Experiment 1 - Romaine "Scala" in silt soil

Soil volumetric moisture content and soil matric potential

Table 1. Effects of irrigation treatments on "Scala" lettuce quality attributes at harvest in response to gradual drying of peat soil.

Soil	Whole lettuce F.W. (g)		Head F.W. (g)		Leaf dry matter (%)		TEAC (µM g^{-1} F.W.)	
	WW	DD	WW	DD	WW	DD	WW	DD
Peat	709	676	320	292	5.6	5.6	16.2	16.6
Prob (f)	ns		ns		ns		ns	

ns – not significant; TEAC – Trolox Equivalent Antioxidant Capacity

At the beginning of the experiment, the VSMC in both the WW and DD treatments was c. 23% and a soil ψ_m of -14 kPa (pot/field capacity) (data not shown). On 10 June 2017, irrigation water was withheld from the plants in the DD treatment and the average pot VSMC and soil

ψ_m began to decline gradually, whilst plants in the WW treatment were maintained at or near pot capacity throughout the experiment.

Plant physiological responses to drying silt soil

Significant reductions in *gs* and Pn, and the first signs of wilting appeared in DD-treated plants at a surprisingly high average soil ψ_m of -28 kPa (data not shown). On further investigation, it was found that the root systems of these plants were restricted to the top 15 cm of soil (Figure 11) whilst the moisture sensors were at 15 and 30 cm depths. Consequently, the results from this experiment were deemed unreliable and the protocol was modified accordingly. In Experiment 2, matric potential sensors were placed at three depths in the soil profile (the uppermost sensor being 10 cm from the soil surface), and following establishment, water was withheld temporarily from all the pots to force the roots down into the soil profile (see Figure 19).

Figure 11. Limited root growth of “Scala” in silt soil at the end of Experiment 1. Photo taken on 4 July 2018.

<u>Experiment 1 - Iceberg “Challenge” in peat soil</u>

Soil volumetric moisture content and soil matric potential

Before the drying down treatment was imposed, the VSMC in both the WW and the soon-to- be DD treatments was *c*. 38% with a soil ψ_m of -10 kPa (pot/field capacity) (Figure 12). Soil in the WW treatment was maintained at or near pot capacity throughout the experiment. On 4 July 2017, water was withheld from plants in the DD treatment and the average VSMC and soil ψ_m began to decline. Gradual soil drying was imposed successfully with the VSMC in the DD treatment declining to 24% and a soil ψ_m of -100 kPa on 12 July 2018 when measurements of physiological responses were terminated.

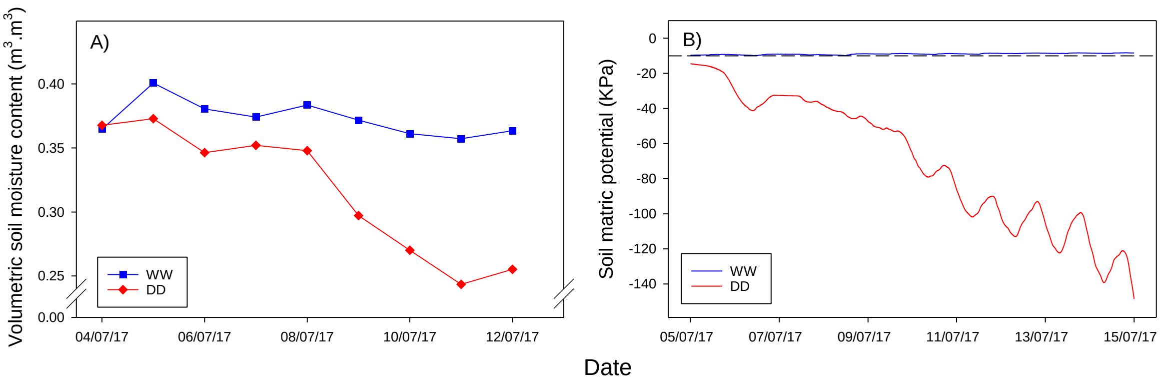

Figure 12. Changes in A) average volumetric soil moisture content and B) average soil matric potential during the phase of gradual drying of peat soil in the rooting zone of “Challenge” lettuce.

Plant physiological responses to drying peat soil

Thermal imaging did not detect any significant lettuce heart temperature differences between the two treatments during the early stages of soil drying (data not shown). Stomatal conductance and Pn was significantly reduced in the DD-treated plants on 9 July 2018 at a soil ψ_m of -44 kPa (Figure 13) when compared to WW plants. On subsequent measurement dates, values of *g*s and Pn were consistently lower in DD-treated plants although the differences to WW plants were not always statistically significant due to varying evaporative demands.

Leaf water potential in WW- and DD-treated plants was similar throughout most of the experiment but a statistically significant increase was noted in DD-treated plants on 12 June 2018 when soil ψ_m was -98 kPa (Figure 14), presumably as a consequence of soil drying-

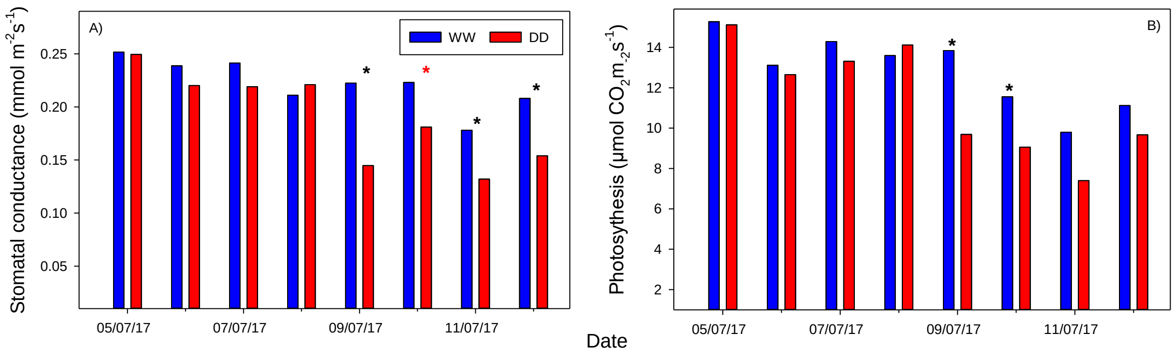

Figure 13. Soil drying induced changes in A) stomatal conductance and B) photosynthesis of Iceberg "Challenge" lettuce in peat soil. Data are mean values of 12 replicate plants, black asterisks indicate statistically significant differences between WW and DD values (p<0.05). Red asterisks indicate differences between WW and DD values are approaching significance.

induced stomatal closure which served to improve leaf water balance. Although subsequent measurements of leaf osmotic potential were made, the calculated turgor pressures were sometimes negative which implied that some of the measurements of osmotic potential were inaccurate. Consequently, data for leaf osmotic potential and turgor potential were considered to be unreliable and have been omitted. As the degree of soil drying progressed, visible signs of wilting in the outer leaves was first noted at a soil ψ_m -163 kPa with more widespread wilting at -288 kPa.

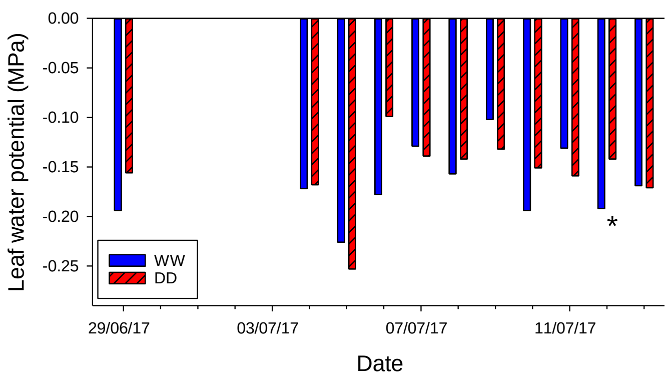

Figure 14. Soil drying induced changes in leaf water potential measured in leaf discs excised from Iceberg "Challenge" lettuce in peat soil. Data are mean values of 6 replicate plants, the asterisk indicates a statistically significant difference between WW and DD values (p<0.05).

The soil matric potentials at which statistically significant differences in leaf physiology between WW- and DD-treated “Challenge” were first detected in peat soil are plotted in Figure 15. These leaf adaptive responses to limiting soil water availability indicate that “Challenge” plants first perceived a mild soil drying stress when the peat soil ψ_m reached -44 kPa (Figure 15). On the previous day at the time of leaf physiological measurements, the average soil ψ_m within the rooting zone was -35 kPa. In future work to test the potential of using alternate wetting and drying (AWD) irrigation regimes to improve water use efficiency and leaf quality attributes of “Challenge” growing in peat soil, an irrigation set point of -35 kPa should be used (see below). The corresponding VSMC for the peat soil used in the experiment was 35%.

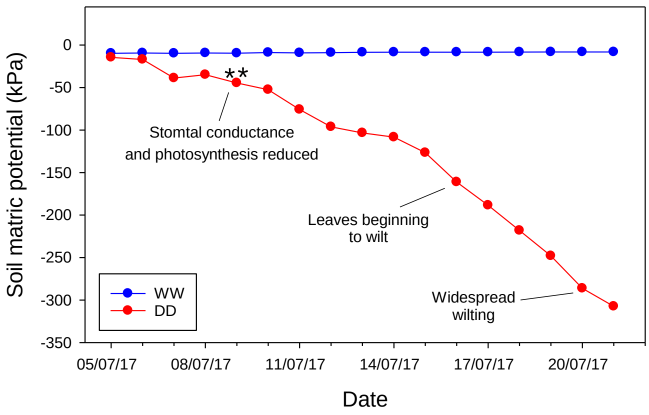

Figure 15. The soil matric potentials at which statistically significant reductions in stomatal conductance and photosynthesis were first detected in “Challenge” lettuce growing in drying peat soil. The onset of mild and severe wilting is also shown.

<u>Experiment 1 - Iceberg “Challenge” in silt soil</u>

Soil volumetric moisture content and soil matric potential

At pot capacity, the VSMC in both the WW and the DD treatments was *c*. 23%, with a corresponding soil ψ_m of -10 kPa (Figure 16). In the WW treatment, the soil was maintained at or near pot capacity throughout the experiment. On 4 July 2017, irrigation water was withheld from plants in the DD treatment so that gradual soil drying was imposed. On 12 July 2018 when the leaf physiological measurements were terminated, VSMC had decreased to 14% and the soil ψ_m to -70 kPa (Figure 16).

Plant physiological responses to drying silt soil

Thermal imaging did not detect any significant lettuce heart temperature differences between the two treatments during the early stages of soil drying (data not shown). Stomatal

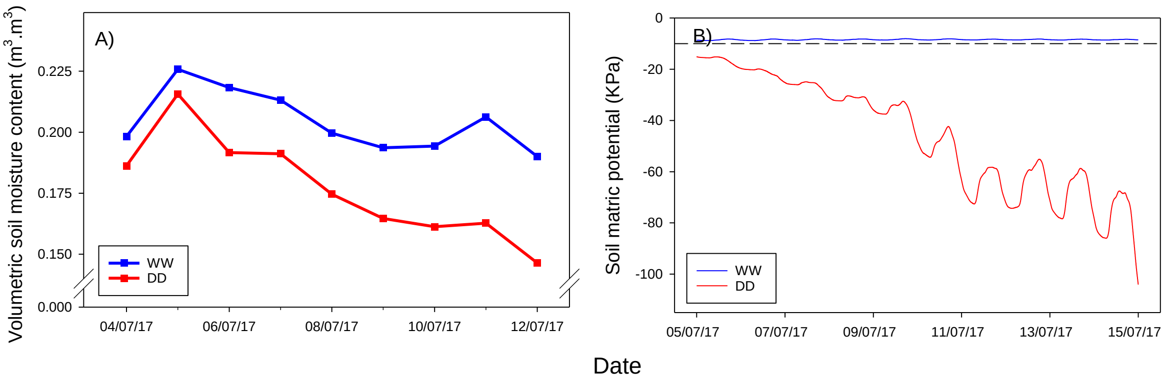

Figure 16. Changes in A) average volumetric soil moisture content and B) average soil matric potential during the phase of gradual drying of silt soil in the rooting zone of “Challenge” lettuce.

conductance was significantly reduced in DD-treated plants on 9 July 2018 at a soil ψ_m of -36 kPa (Figure 17), although *gs* was lowered in DD-treated plants from 15 July 2017, and differences from WW values approached statistical significance on 7 and 8 July 2017. The lowered *gs* values in DD-treated plants persisted until 13 July 2017 when the evaporative demand was lowered due to cloud cover and a corresponding fall in light intensity (data not shown). Photosynthesis was significantly reduced in DD-treated plants on 13 July 2018, at a

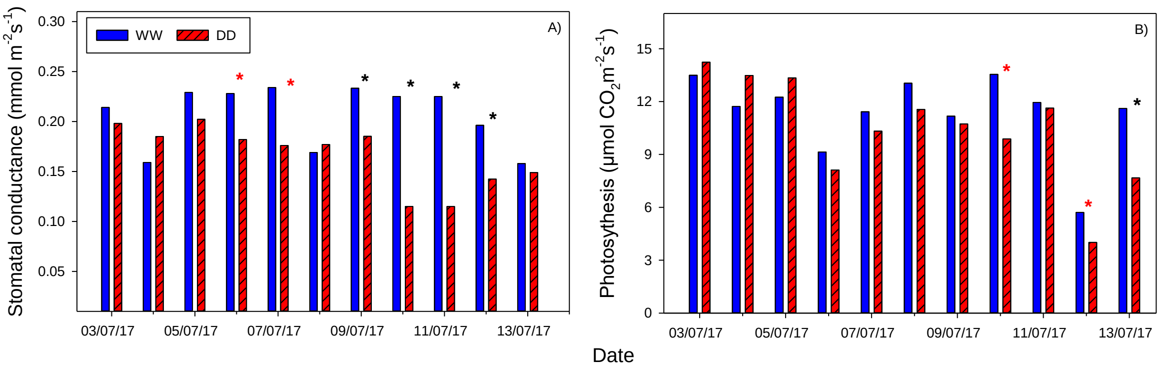

Figure 17. Soil drying induced changes in A) stomatal conductance and B) photosynthesis of Iceberg "Challenge" lettuce in silt soil. Data are mean values of 12 replicate plants, black asterisks indicate statistically significant differences between WW and DD values (*p*<0.05). Red asterisks indicate differences between WW and DD values are approaching significance.

soil ψ_m of - 65 kPa, although treatment differences approached significance on 10 and 12 July 2013 (Figure 17).

Leaf water potentials in WW and DD-treated plants were similar throughout the experiment (Figure 18); the only significant effect of soil drying was to lower the leaf water potential on 11 July 2017 when the soil ψ_m was -60 kPa. Again, data for leaf osmotic potential and turgor potential were considered to be unreliable and have been omitted. Visible signs of wilting occurred at -70 kPa with more widespread wilting at -155 kPa.

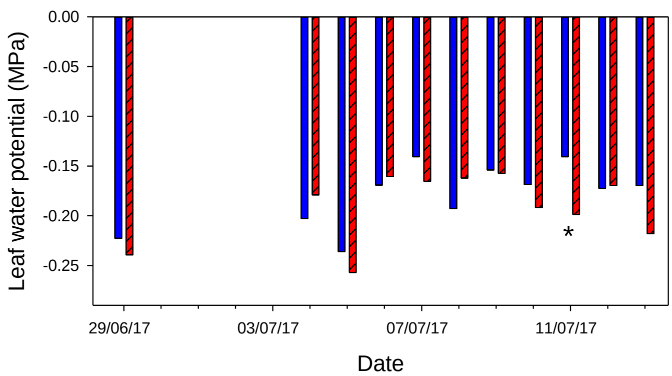

Figure 18. Soil drying induced changes in leaf water potential measured in leaf discs excised from Iceberg "Challenge" lettuce in silt soil. Data are mean values of 6 replicate plants, asterisks indicate statistically significant differences between WW and DD values (*p*<0.05).

Challenge" plants first perceived a mild soil drying stress when the silt soil ψ_m reached -36 kPa (Figure 19). On the previous day at the time of leaf physiological measurements, the average soil ψ_m within the rooting zone was -25 kPa. Stomatal limitation of Pn occurred at a soil ψ_m of -65 kPa. In future work to test the potential of AWD irrigation regimes to improve water use efficiency and leaf quality attributes of "Challenge" growing in silt soil, an irrigation set point of -25 kPa should be used (see below). The corresponding VSMC for the silt soil used in the experiment was 18%.

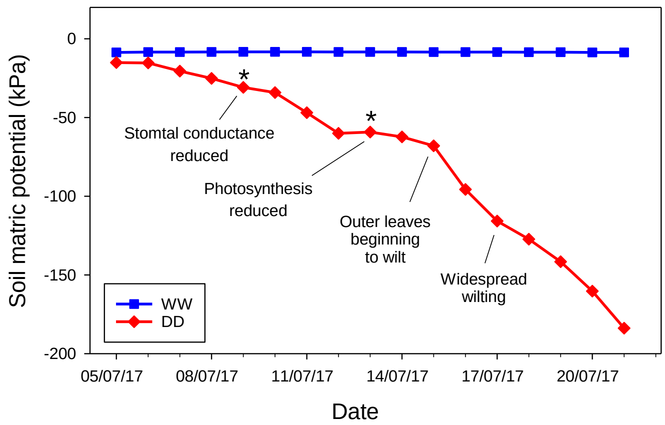

Figure 19. The soil matric potentials at which statistically significant reductions in stomatal conductance and photosynthesis were detected in "Challenge" lettuce growing in drying silt soil. The onset of mild and severe wilting is also shown.

<u>Experiment 1 - Measurements made at harvest</u>

For "Challenge" lettuce grown in peat soil, there was no significant difference in the fresh weight of the whole lettuce, lettuce head, or in the leaf % dry weight between plants in the WW treatment and those subjected to the DD treatment (Table 2). There was no significant difference in leaf total antioxidant capacity between the two treatments (Table 2). There was very little evidence of mid-rib pinking in the cut lettuce leaves from either treatment that were placed into shelf life (data not shown).

For "Challenge" lettuce grown in silt soil, whole lettuce fresh weight at harvest was significantly reduced in plants under the DD treatment (Table 2). The reduction in head fresh weight in DD-treated plants was close to statistical significance, as was the associated increase in % leaf dry matter content (Table 2), compared to WW values. There was no

Table 2. Effects of irrigation treatments on "Challenge" lettuce quality attributes at harvest in response to gradual drying of peat and silt soils. Values are means of measurements taken on six replicate plants, with the Lsds at 5%.

Soil	Whole lettuce F.W. (g)		Head F.W. (g)		Leaf dry matter (%)		TEAC (µM g^{-1} F.W.)	
	WW	DD	WW	DD	WW	DD	WW	DD
Peat	858	843	583	567	5.0	5.3	11.8	14.0
Prob (f)	ns		ns		ns		ns	
Silt	597	499*	443	312	4.6	7.5	11.2	15.2
Prob (f)	<0.001		0.09		0.08		ns	
Lsd (5%)	55		117		3.5			

ns – not significant
* denotes a significant statistical difference (p<0.05)

significant difference in leaf total antioxidant capacity between the two treatments (Table 2). There was very little evidence of mid-rib pinking in the cut lettuce leaves from either treatment that were placed into shelf life (data not shown).

In these experiments, the analysis of leaf quality attributes at harvest was made on plants growing in increasingly dry soil, to the point where the declining soil ψ_m significantly affected photosynthesis in DD-treated plants. The aim of the experiment was to determine "how dry is too dry", and was not to determine the effect of soil drying on leaf quality attributes since the soil ψ_m was deliberately allowed to fall beyond the value which would be used in regulated deficit irrigation (RDI) treatments. The value in these results is that they provide an indication of how leaf quality attributes could be manipulated by mild soil drying, and will inform further work on developing PI and RDI regimes that optimise head fresh weight and leaf quality attributes.

Experiment 2 – Rooting depth in peat and silt soils

To encourage roots to grow down into the pot during establishment, the irrigation delivery system, the approach to irrigation scheduling and the positioning of soil matric potential sensors was modified in Experiment 2. Rather than maintaining soil around field capacity, irrigation was applied once the soil ψ_m in the uppermost 10 cm of soil reached -30 kPa. This approach was effective, and rooting in the peat and silt soils was similar after the establishment phase (Figure 20). At maturity, water was extracted from the entire soil volume as evidenced by daily falls in soil ψ_m at each of the three sensor positions (10, 20 and 30 cm) within the pots.

Figure 20. Root growth of "Actina" in silt soil and peat soil during the establishment phase in Experiment 2. Photo taken on 25 August 2017.

Experiment 2 - Romaine "Actina" in peat soil

Soil volumetric moisture content and soil matric potential

Before the DD treatment was imposed, average VSMC in all pots was c. 45% with a soil ψ_m of -10 kPa (pot/field capacity) (Figure 21). Soil in the WW treatment was maintained at pot/field capacity throughout. Irrigation water was withheld from plants in the DD treatment on 3 October 2017 and the average VSMC and soil ψ_m fell gradually to 26% and -120 kPa respectively, by 20 October 2018 when physiological measurements were terminated.

Plant physiological responses to drying peat soil

Thermal images of the heart detected a small but significant increase in temperature in DD-treated plants on 9 October 2018 at a soil ψ_m of -33 kPa (Figure 21). Again, this preceded

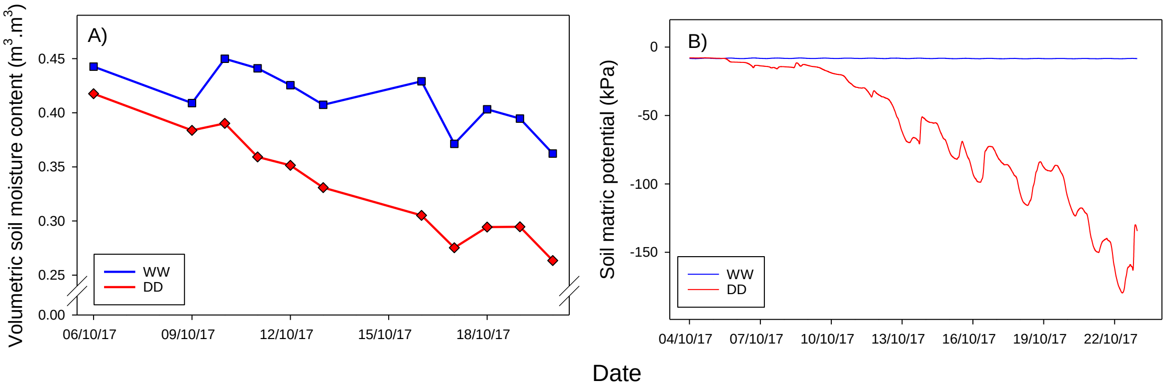

Figure 21. Changes in A) average volumetric soil moisture content and B) average soil matric potential during the phase of gradual drying of peat soil in the rooting zone of "Actina" lettuce.

any detectable change in stomatal conductance. Throughout the remainder of the experiment, lettuce leaf temperature was higher in DD-treated plants and, in most cases, the differences to WW values were statistically significant (Figure 22). Stomatal conductance was significantly reduced in the DD treatment, compared to WW values, on 16 October 2018 at a soil ψ_m of -78 kPa (Figure 23A) and despite differences in absolute values caused by changing evaporative demand, a soil-drying induced reduction in *gs* persisted throughout the experiment. Soil-drying induced reductions in Pn was first detected on 17 October 2018 at a soil ψ_m -86 kPa (Figure 23B), and again on 20 October 2017.

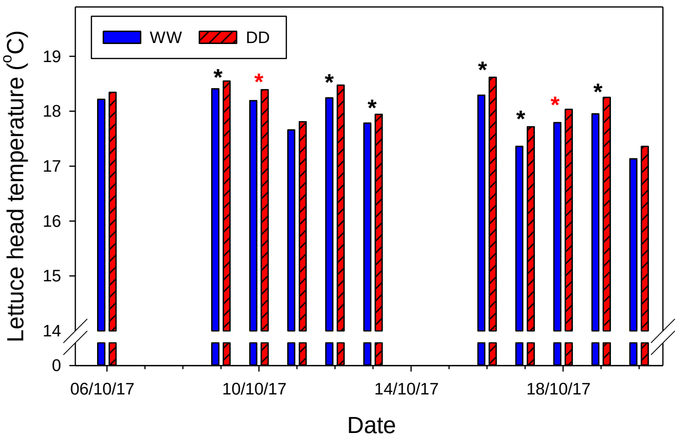

Figure 22. The effects of drying peat soil on "Actina" lettuce leaf temperatures detected using thermal imaging. Data are mean values of 12 replicate plants, asterisks indicate statistically significant differences between WW and DD values (p<0.05). Red asterisks indicate differences are approaching significance.

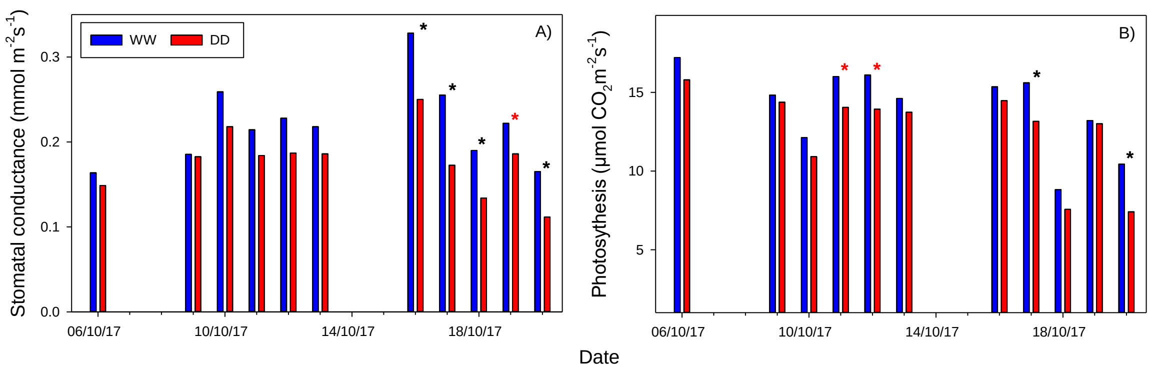

Figure 23. Soil drying induced changes in A) stomatal conductance and B) photosynthesis of Romaine "Actina" lettuce in peat soil. Data are mean values of 12 replicate plants, black asterisks indicate statistically significant differences between WW and DD values (p<0.05). Red asterisks indicate differences are approaching significance.

Leaf water potential values in DD-treated plants were significantly reduced compared to WW values on 3 October 2017 (Figure 24), and again on 14 and 18 October 2017 as soil drying continued. Progressive stomatal closure in DD-treated plants acted to conserve leaf water balance and so leaf water potentials in DD-treated plants were higher (*i.e.* less negative) by the end of the experiment (Figure 24). Leaf osmotic potential and turgor potential data were considered to be unreliable and so have been omitted. Plants began to show visible signs of wilting at -160 kPa and more sustained wilting at -_280 kPa.

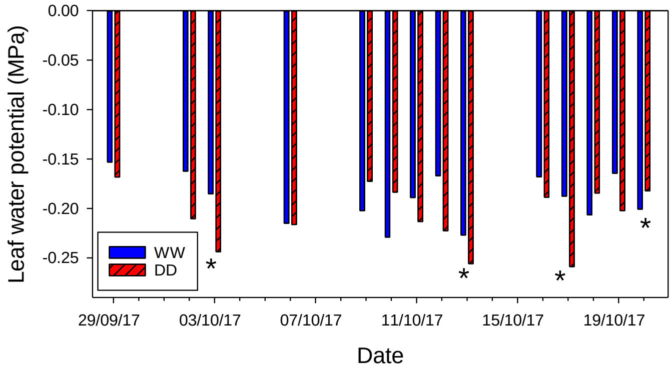

Figure 24. Soil drying induced changes in leaf water potential measured in leaf discs excised from Iceberg "Actina" lettuce in peat soil. Data are mean values of 6 replicate plants, asterisks indicate statistically significant differences between WW and DD values (p<0.05).

"Actina" plants first perceived a mild soil drying stress when the peat soil ψ_m reached -78 kPa (Figure 25). On the previous day at the time of leaf physiological measurements, the average soil ψ_m within the rooting zone was 67- kPa. In future work to test the potential of using alternate wetting and drying (AWD) irrigation regimes to improve water use efficiency and leaf quality attributes of "Actina" growing in peat soil, irrigation set points of -65 kPa should be used (see below). The corresponding VSMC for the peat soil used in the experiment was 33%.

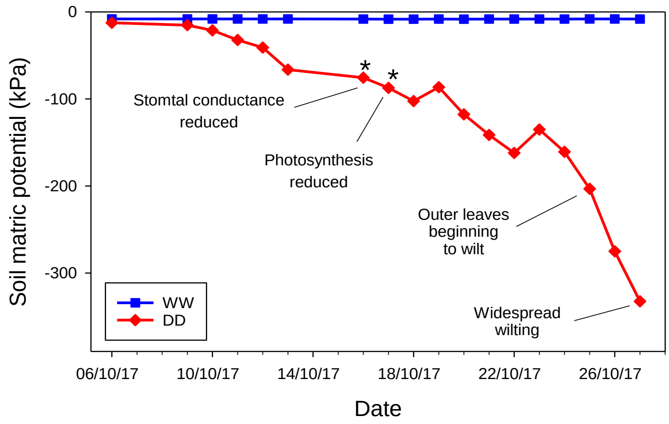

Figure 25. Soil matric potentials at which statistically significant reductions in stomatal conductance and photosynthesis were detected in "Actina" lettuce growing in drying peat soil. The onset of mild and severe wilting is also shown.

<u>Experiment 2 - Romaine "Actinia" in silt soil</u>

Soil volumetric moisture content and soil matric potential

Before the DD treatment was imposed, average VSMC in all pots was c. 24% with a soil ψ_m of -10 kPa (pot / field capacity) (Figure 26). Soil in the WW treatment was maintained at pot/field capacity throughout the experiment. Irrigation water was withheld from plants in the DD treatment on 3 October 2017 and the average VSMC and soil ψ_m fell gradually to 13% and -126 kPa respectively, by 20 October 2018 when physiological measurements were

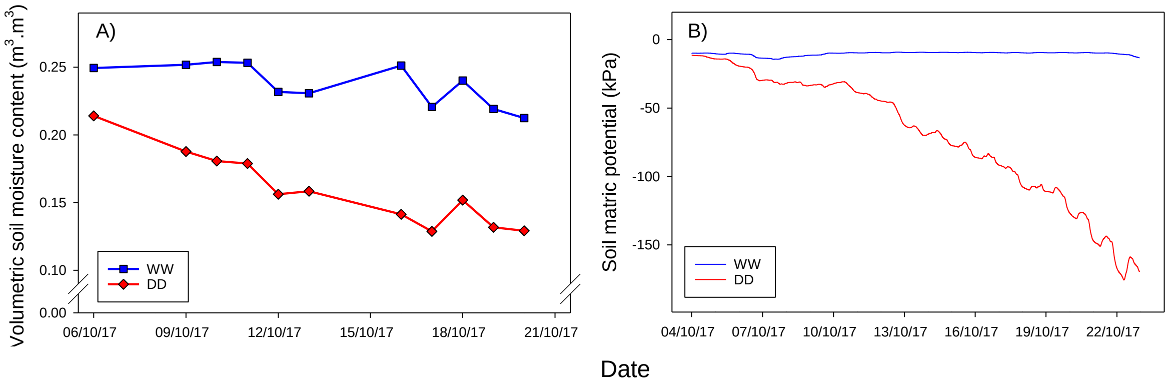

Figure 26. Changes in A) average volumetric soil moisture content and B) average soil matric potential during the phase of gradual drying of silt soil in the rooting zone of "Actina" lettuce.

terminated.

Plant physiological responses to drying silt soil

Thermal images of the leaves detected a small but significant increase in temperature in DD-treated plants on 10 October 2018 at a soil ψ_m of -38 kPa (Figure 27). This effect was noted 6 days before soil-drying induced reductions in *gs* were detected. Lettuce leaf temperature was also higher in DD-treated plants on several other occasions during the experiment, and the temperature response was generally consistent with the stomatal response to soil drying (Figure 28A), as expected since the temperature increase is a direct result of the loss of transpirational cooling caused by the onset of stomatal closure. Stomatal conductance was significantly reduced in

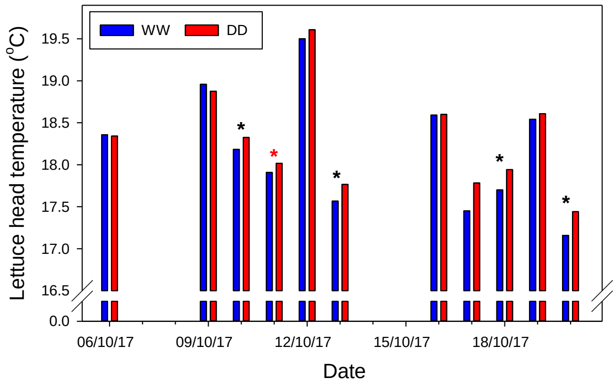

Figure 27. The effects of drying silt soil on "Actina" lettuce leaf temperatures detected using thermal imaging. Data are mean values of 12 replicate plants, asterisks indicate statistically significant differences between WW and DD values (p<0.05). Red asterisks indicate differences are approaching significance.

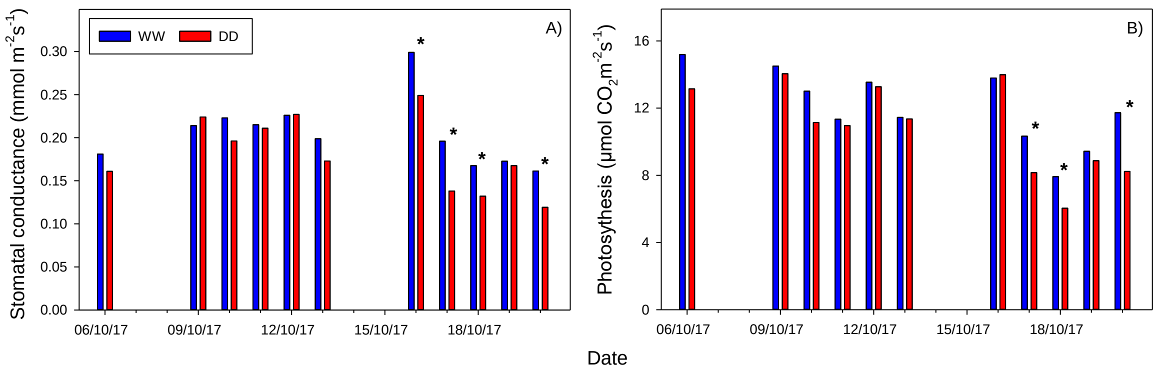

Figure 28. Soil drying induced changes in A) stomatal conductance and B) photosynthesis of Romaine "Actina" lettuce in silt soil. Data are mean values of 12 replicate plants, black asterisks indicate statistically significant differences between WW and DD values (p<0.05). Red asterisks indicate differences between WW and DD values are approaching significance.

the DD treatment, compared to WW values, on 16 October 2018 at a soil ψ_m of -84 kPa (Figure 28A), and *gs* values remained lower for the rest of the experiment. Photosynthesis was significantly reduced in the DD treatment a day later at -94 kPa (Figure 28B) and again on 18 and 20 October 2017.

Leaf water potential was significantly reduced in DD-treated plants on 3 and 6 October 2017, compared to WW values (Figure 29), and values were then similar until the end of the experiment irrespective of irrigation treatments. Measurements of leaf osmotic potential and turgor potential were considered to be unreliable and so are not presented. Plants began to show visible signs of wilting at -167 kPa and more sustained wilting at -230 kPa.

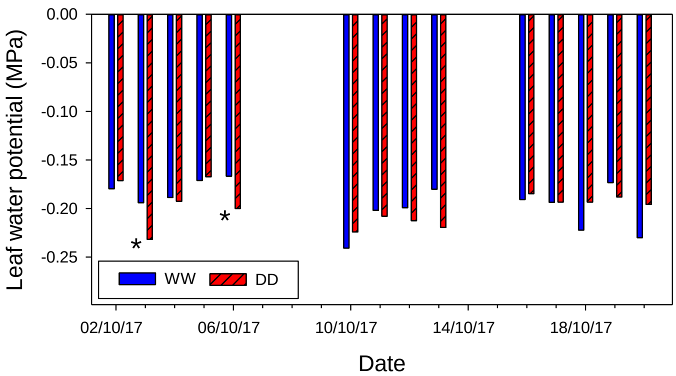

Figure 29. Soil drying induced changes in leaf water potential measured in leaf discs excised from Romaine “Actina” lettuce in silt soil. Data are mean values of 6 replicate plants, asterisks indicate statistically significant differences between WW and DD values (p<0.05).

“Actina” plants first perceived a mild soil drying stress when the silt soil ψ_m reached -84 kPa (Figure 30). On the previous measurement day at the time of leaf physiological measurements, the average soil ψ_m within the rooting zone was -65 kPa. In future work to test the potential of using AWD irrigation regimes to improve water use efficiency and leaf quality attributes of “Actina” growing in silt soil, irrigation set points of -65 kPa should be used (see below). The corresponding VSMC for the silt soil used in the experiment was 16%.

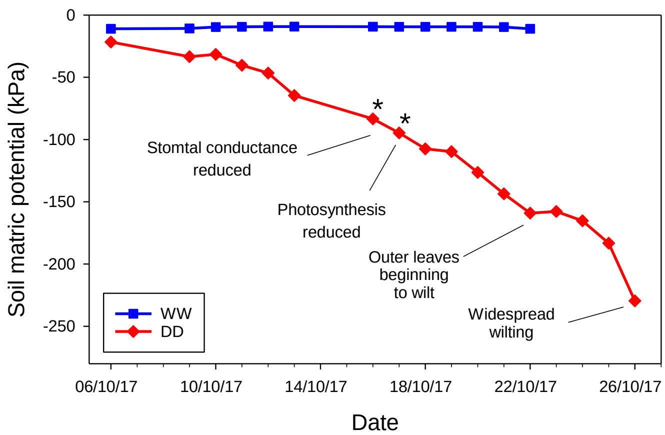

Figure 30. The soil matric potentials at which statistically significant reductions in stomatal conductance and photosynthesis were detected in “Actina” lettuce growing in drying silt soil. The onset of mild and severe wilting is also shown.

<u>Experiment 2 - Measurements made at harvest</u>

For “Actina” grown in peat soil, there was a significant decrease in the fresh weight of the whole lettuce and the lettuce head in DD-treated plants compared to their WW counterparts

(Table 3). The associated increase in the leaf % dry weight between plants in the WW treatment and those subjected to the DD treatment was also close to statistical significance (Table 3).

Table 3. Effects of irrigation treatments on "Actina" lettuce quality attributes at harvest in response to gradual drying of peat and silt soils.

Soil	Whole lettuce F.W (g)		Head F.W. (g)		Leaf dry matter (%)		TEAC (µM g^{-1} F.W.)	
	WW	DD	WW	DD	WW	DD	WW	DD
Peat	510	376*	338	259*	6.1	7.4*	7.1	7.3
Prob (f)	0.005		0.008		0.056		ns	
Lsd (5%)	79.5		56.4		1.37			
Silt	496	343*	320	230*	6.0	4.9	7.4	8.1
Prob (f)	<0.001		0.001		0.064		ns	
Lsd (5%)	55.7		39.7		1.22			

ns – not significant
* denotes a significant statistical difference (p<0.05)

There was no significant difference in leaf total antioxidant capacity between the two treatments (Table 3), and there was very little evidence of mid-rib pinking in the cut lettuce leaves from either treatment (data not shown).

For "Actina" lettuce grown in silt soil, whole lettuce fresh weight at harvest was significantly reduced in plants under the DD treatment (Table 3). The reduction in head fresh weight in DD-treated plants was also significant, and the effects of soil drying on % leaf dry matter content were almost significant (Table 3), compared to WW values. There was no significant difference in leaf total antioxidant capacity between the two treatments (Table 3). There was very little evidence of mid-rib pinking in the cut lettuce leaves from either treatment that were placed into shelf life (data not shown).

Experiment 2 - Iceberg "Etude" in peat soil

Soil volumetric moisture content and soil matric potential

Before the DD treatment was imposed, VSMC and soil ψ_m at pot/field capacity in the peat soil was *c*. 44% and -10 kPa, respectively (Figure 31A&B). Soil in the WW-treated plants

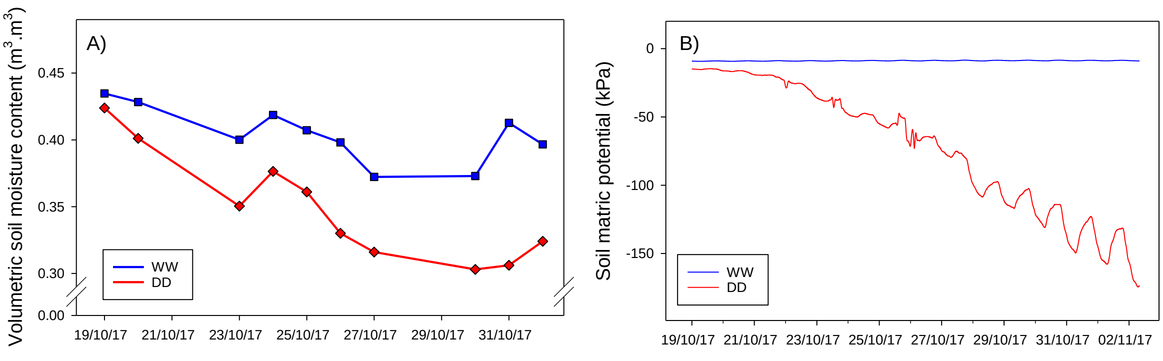

Figure 31. Changes in A) average volumetric soil moisture content and B) average soil matric potential during the phase of gradual drying of peat soil in the rooting zone of "Etude" lettuce.

was maintained at these values throughout the experiment. Irrigation water was withheld from the plants in the DD treatment on 18 October 2017, and the average pot VSMC and soil ψ_m began to decline gradually. By the end of physiological measurements on 1 November 2017, VSMC in the DD treatment had fallen to 30 %, and a corresponding soil ψ_m of -136 kPa (Figure 31A&B).

Plant physiological responses to drying peat soil

Thermal images of the lettuce hearts showed a small but significant increase in temperature for those in the DD treatment on 31 October 2017, corresponding to a soil ψ_m of -125 kPa (Figure 32). Stomatal conductance was significantly reduced in the DD treatment on 23 October 2017 when compared to those plants in the WW treatment (Figure 33A), at an average soil ψ_m of -36 kPa Photosynthesis was significantly reduced in DD-treated plants on 30 October 2017 at a soil ψ_m of -116 kPa (Figure 33B), although the decrease noted on 25 October was close to significance.

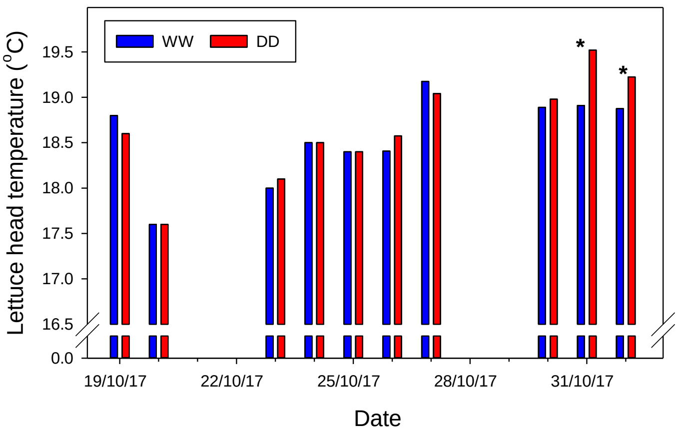

Figure 32. The effects of drying peat soil on "Etude" lettuce heart temperatures detected using thermal imaging. Data are mean values of 12 replicate plants, asterisks indicate statistically significant differences between WW and DD values (p<0.05).

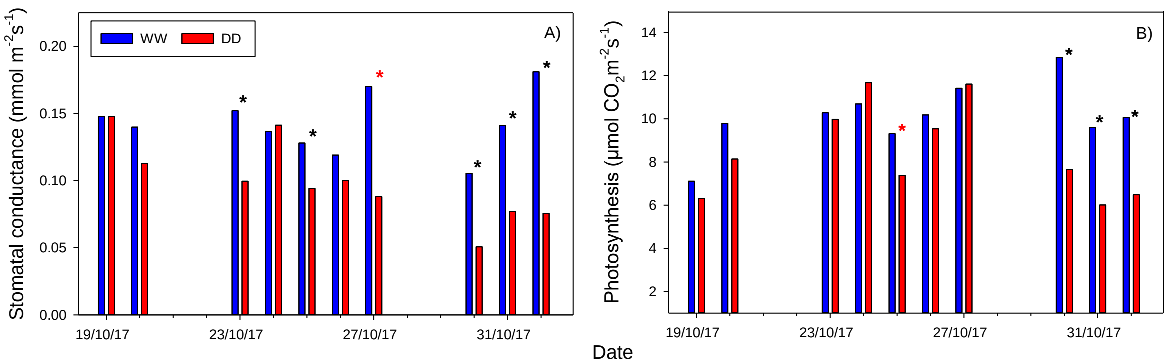

Figure 33. Soil drying induced changes in A) stomatal conductance and B) photosynthesis of Iceberg "Etude" lettuce in peat soil. Data are mean values of 12 replicate plants, black asterisks indicate statistically significant differences between WW and DD values (p<0.05). Red asterisks indicate differences between WW and DD values are approaching significance.

Leaf water potential was lowered significantly in DD-treated plants on 23 October 2017 but then was significantly higher than WW values the next day (Figure 34), perhaps as a consequence of the onset of stomatal closure on 23 October 2017. Values were then similar between treatments for the remainder of the experiment. Data on osmotic potential and turgor pressure were deemed to be unreliable and have been omitted. Plants began to show visible signs of wilting at -75 kPa and more sustained wilting at -180 kPa.

"Etude" plants first perceived a mild soil drying stress when the peat soil ψ_m reached -36 kPa (Figure 35). On the previous day at the time of measurements, the average soil ψ_m within the rooting zone was -25 kPa. In future work to test the potential of using AWD regimes to improve water use efficiency and leaf quality attributes of "Etude" growing in peat soil, irrigation set points of -25 kPa should be used (see below). The corresponding VSMC for the peat soil was 40%.

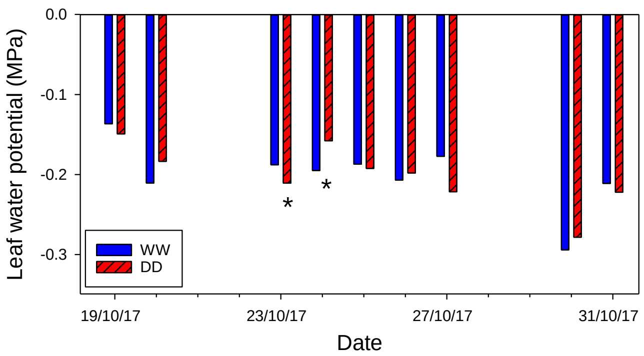

Figure 34. Soil drying induced changes in leaf water potential measured in leaf discs excised from "Etude" lettuce in peat soil. Data are mean values of 6 replicate plants, asterisks indicate statistically significant differences between WW and DD values (*p*<0.05).

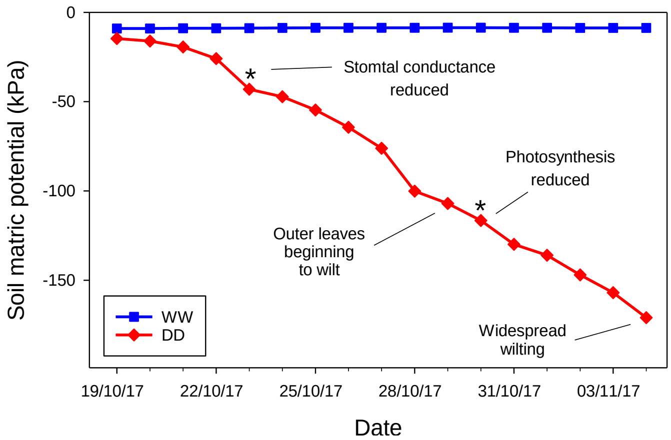

Figure 35. Soil matric potentials at which statistically significant reductions in stomatal conductance and photosynthesis were detected in "Etude" lettuce growing in drying peat soil. The onset of mild and severe wilting is also shown.

<u>Experiment 2 - Iceberg "Etude" in silt soil</u>

After establishing well, some plants began to develop chlorosis and failed to grow away. The cause was not identified although improper nutrition was ruled out. Since the number of remaining healthy plants was fewer than needed to carry out an experiment with 12 biological replications for each treatment, the decision was taken to terminate the experiment and repeat the work in Spring 2018 using "Challenge".

<u>Experiment 2 - Measurements made at harvest</u>

For "Etude" grown in peat soil, there were no significant treatment differences in any of the quality parameters measured (Table 4) and there was very little evidence of mid-rib pinking in the cut lettuce leaves from either treatment (data not shown).

Table 4. Effects of irrigation treatments on "Etude" lettuce quality attributes at harvest in response to gradual drying of peat soil.

Soil	Whole lettuce F.W. (g)		Head F.W. (g)		Leaf dry matter (%)		TEAC (μM g^{-1} F.W.)	
	WW	DD	WW	DD	WW	DD	WW	DD
Peat	579	498	456	399	4.6	4.6	8.6	7.8
Prob (f)	ns		ns		ns		ns	

ns – not significant

Experiment 3 - Iceberg “Challenge” in silt soil

Soil volumetric moisture content and soil matric potential

Pot / field capacity was *c.* 23% with a soil ψ_m of -10 kPa (Figure 36A&B). On 12 May 2018, water was withheld from plants in the DD treatment and the average pot VSMC and soil ψ_m gradually declined. By 18 May 2018, VSMC in the DD treatment had fallen to 16 % and soil ψ_m was -70 kPa (Figure 36A&B).

Plant physiological responses to drying silt soil

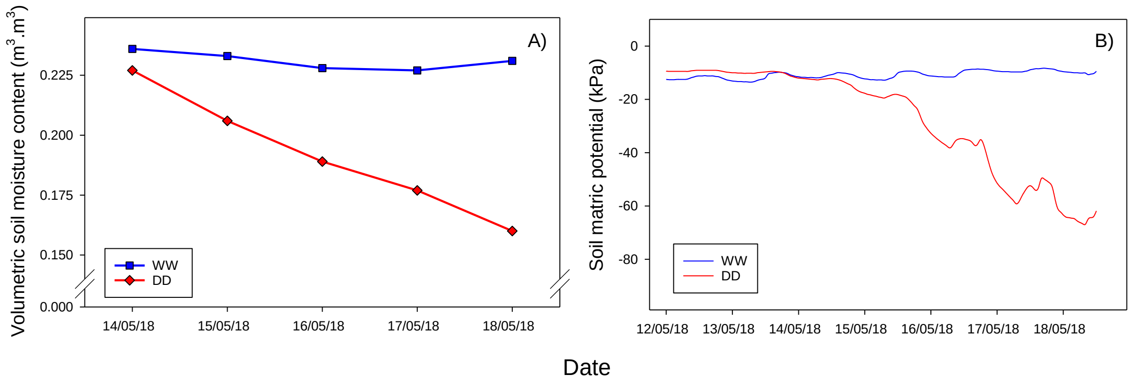

Figure 36. Changes in A) average volumetric soil moisture content and B) average soil matric potential during the phase of gradual drying of silt soil in the rooting zone of “Challenge” lettuce.

Stomatal conductance was reduced significantly in the DD treatment on 17 May at a soil ψ_m of -53 kPa (Figure 37A). Photosynthesis was significantly reduced in DD-treated plants on 18 May 2018 at a soil ψ_m of -65 kPa (Figure 37B).

Experiment 3 - Measurements made at harvest

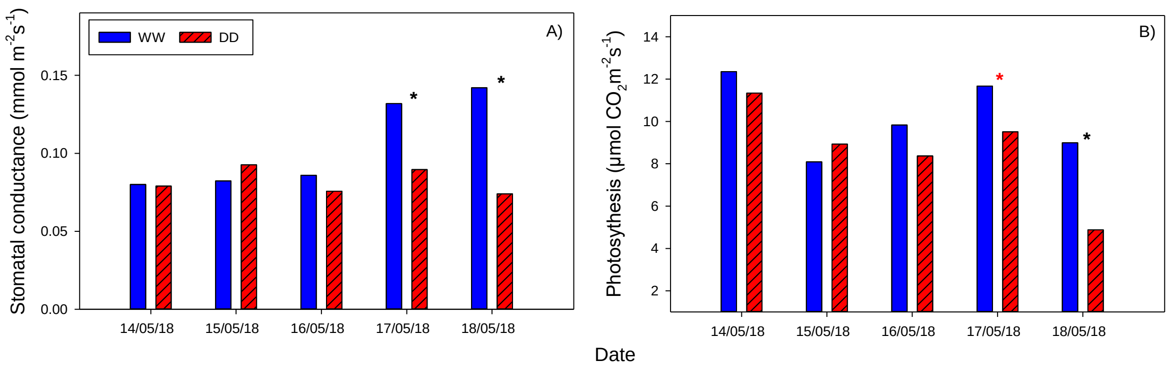

Figure 37. Soil drying induced changes in A) stomatal conductance and B) photosynthesis of Iceberg “Challenge” lettuce in silt soil. Data are mean values of 12 replicate plants, black asterisks indicate statistically significant differences between WW and DD values (p<0.05). Red asterisks indicate differences between WW and DD values are approaching significance.

For “Challenge” lettuce grown in silt soil, whole lettuce fresh weight, head fresh weight and % leaf dry matter content were similar at harvest, irrespective of irrigation treatment (data not shown). There was no significant difference in leaf total antioxidant capacity between the two treatments, and no evidence of mid-rib pinking in the cut lettuce leaves from either treatment that were placed into shelf life (data not shown).

Discussion

Delivery of project objectives and milestones

The aim of the original 5-year project submission was to improve leaf quality and shelf-life potential in commercial Romaine and Iceberg lettuce crops using PI, DI and AWD irrigation techniques optimised for different soils. The FV Panel agreed to fund the first phase of this research but at a reduced cost. The following revised project objectives were agreed:

(i) Project objective(s):

- To derive and test irrigation set points based on soil matric potentials for Romaine and Iceberg lettuce in a peat and a silt soil
- To communicate and demonstrate the results to the industry

These objectives have been met in full.

In addition, the following milestones were agreed:

#	Milestone Summary	Start	Finish
1.1	Soil matric potentials that trigger plant responses to drying soils determined in potted "Scala" and "Challenge" lettuce	April 2017	Sept 2017
1.2	Effects of water deficit stress on "Scala" and "Challenge" lettuce leaf quality in different soils determined	Jun 2017	Dec 2017
1.3	Lower irrigation set points identified for testing in potted "Scala" and "Challenge" lettuce	Jan 2018	Feb 2018

These milestones have been delivered in full. A 4-month project extension until 31 July 2018 was agreed with the AHDB in order to be able to repeat the experiment with Iceberg lettuce in silt soil.

Optimising lettuce leaf quality

The aim of Phase 1 of this research was to derive irrigation set points based on soil matric potentials for Romaine and Iceberg lettuce varieties in a peat and a silt soil. The longer term aim of the research programme is to develop guidelines and approaches to enable leafy salad growers to supply a consistently high quality product to achieve customer satisfaction whist using resources responsibly in a challenging and changing climate and in accord with current legislative demands.

Many studies have shown that lettuce leaf quality can be affected by both abiotic and biotic factors, but our focus is to understand the effects of over- and under-irrigation so that practical solutions can be tested and developed for irrigated field-grown crops. These factors were also investigated in the HortLINK project (HL0196 - Developing an intelligent overhead irrigation system for high quality horticultural field crops) and the results are discussed in recent papers from the teams at Cranfield University, Harper Adams University and Lancaster University – (see References).

To inform future work on developing PI and AWD strategies for a range of high value horticultural crops, the approach developed at NIAB EMR is to investigate and understand plant or tree physiological responses to progressive but mild soil drying, and to use this information to develop low risk irrigation strategies for commercial production systems that optimise water and fertiliser use efficiencies, crop productivity and fresh produce quality. The initial work is often carried out in controlled environments using potted plants but with all relevant commercial agronomy inputs to try to ensure that the plants are grown to a similar specification as commercial crops. The next phase is to implement and adjust the irrigation set points in commercial production systems so that grower-facing solutions and technologies are delivered that improve production efficiency. A two-way exchange of knowledge exchange through engagement with the industry, the provision of high quality grower training and support, and collaboration with leading agri-businesses at an early stage are essential components of this process.

In Phase 1 of this work, we have imposed progressive but mild soil drying to identify when potted Romaine and Iceberg lettuce varieties first perceive a soil water deficit. This was achieved by measuring some of the leaf physiological responses to drying soils and identifying the soil ψ_m at which changes in leaf physiology diverged significantly from control values measured in well-watered plants.

<u>Plant responses to drying soils</u>

Plants respond to mild soil water deficits by initiating and maintaining a range of leaf adaptive responses that are designed to limit foliar water loss until a more favourable water supply is restored. The regulation of these adaptive responses is achieved by a combination of root-sourced hydraulic and chemical signals that are transported in the xylem to shoot tissues where they trigger various leaf morphological and physiological responses. In many plants, there is a hierarchy of responses that are triggered by increasingly dry soils. A decrease in midday xylem stem water potential is often the first detectable response to drying soil, and this is often followed by slowed leaf extension, and partial stomatal closure which serve to limit both the transpirational leaf area and the rate of water loss through stomatal pores on the leaves. Complete and prolonged stomatal closure will limit CO_2 diffusion into the sub-stomatal cavity which then reduces the rate of photosynthesis; this is known as a stomatal limitation to photosynthesis. Further soil drying will result in metabolic limitations to photosynthesis, osmotic adjustment and a myriad of other changes in leaf metabolism until eventually leaf turgor is lost and progressive wilting occurs.

The traditional use of the Scholander pressure chamber to measure midday stem water potential and leaf water potential is only practicable in leaves with a stem or petiole and it is difficult to measure leaf extension accurately in lettuce, and so in this project we focussed on soil-drying induced changes in stomatal conductance as the first reliable and consistent response to declining soil ψ_m. We also used the onset of a lowered photosynthetic rate to identify the soil ψ_m at which leaf quality attributes would be affected in the longer term.

One limitation of the methodology used in this project is that, in order to impose mild soil drying gradually so that the onset of leaf adaptive responses could be readily identified, irrigation water has to be added at certain points in the drying cycle to prevent the soil ψ_m from falling too quickly. On these occasions, it was sometimes the case that the adaptive responses observed on the previous day were temporarily lost as the plants responded to the increased availability of soil water. An example of this effect can be seen in the temporary increase in *gs* in DD-treated plants in Figure 30 on 24 October 2017. A similar stomatal response to irrigation of drying soil was reported for “Chancellor” by Knox *et al*. (2014). In our work for the soft fruit sector, we have overcome this problem by using automated irrigation scheduling using soil moisture sensors and a proportional–integral–derivative (PID) controller in a control loop feedback to maintain VSMC within +/- 0.5% of a predetermined set point. Due to a combination of budget restrictions and a greater soil volume (and therefore a greater number of sensors needed), we were not able to use the PID approach in the current project. In future work, we would prefer to use the PID approach to impose the required soil water deficits more accurately and precisely. A further complication is the effect of variable VPDs on plant response to soil water deficits. On a cool cloudy day, plants will be under less stress at a given value of soil Ψ_m than on a sunny day with a high evaporative demand. This can also lead to the apparent recovery of the previously stressed plants but this is only temporary and more sustained leaf responses are seen as the soil continues to dry.

Opportunities to improve consistency of leaf quality

As already mentioned, the analysis of leaf quality attributes at harvest was made on plants growing in increasingly dry soil, to the point where the declining soil ψ_m triggered stomatal inhibition of photosynthesis in DD-treated plants. Our aim was to determine “how dry is too dry”, and was not to determine the effect of deficit irrigation on leaf quality attributes since the soil ψ_m was deliberately allowed to fall beyond the value which would be used in AWD or DI treatments. Further work is needed to investigate the potential of using AWD or mild DI treatments to improve aspects of leaf quality (see below), but the treatments need to be applied judiciously. In our previous work with the Romaine variety “Little Gem” we showed that the leaf fresh weight to dry weight ratio was significantly lowered under DI treatment, without a corresponding loss of the fresh weight of harvestable yield (Else *et al.,* unpublished). In this work, we have identified the soil matric potentials that should be used in AWD treatments to investigate the potential to improve resource use efficiency and consistency of leaf quality in Romaine and Iceberg varieties.

In our work, mid-rib pinking was not observed in any variety in either well-watered or drying down treatments. The conditions that predispose leaves to mid-rib pinking are not yet known but over-irrigation or excessive rainfall are thought to be involved (FV413, Vickers *et al.,* 2015 but see Hilton *et al*., 2009). Deficit irrigation has also been shown to increase the propensity for mid-rib pinking (Monaghan *et a*l., 2016). In our work, over-watering was avoided by scheduling irrigation to match demand with supply and so the absence of any pinking was not unexpected.

Irrigation set points for use in future AWD experiments

Using the approach outlined above, we have identified the soil ψ_m values that trigger leaf adaptive responses in potted Romaine and Iceberg lettuce varieties growing in a peat and a silt soil. We also identified the equivalent VSMCs and have shown that whilst the irrigation set points based on soil ψ_m are very similar in both peat and silt soils for a particular variety, the corresponding VSMC values are very different. We intend to test these irrigation set points in further experiments in which AWD is used throughout crop development (see below). In addition to the set points identified in Table 6, we would also use irrigation set points that we would expect to result in stomatal limitation of photosynthesis to identify “how dry is too dry” under changeable VPDs. The first stage of this work would be carried out with potted plants in the GroDome at NIAB EMR to test whether repeated AWD from plant establishment to harvest affects head fresh weight and diameter, and alters leaf quality attributes. The second stage would be to test these AWD regimes on commercial grower sites in different soils under a range of VPDs to determine whether the AWD set points need to be adjusted during a typical growing season. We would also use weather probability forecasting and remote monitoring of plant stress to inform our irrigation decision making.

Deficit Irrigation and Regulated Deficit Irrigation

Deficit irrigation is the application of irrigation water below crop requirements throughout the entire growth stage. Regulated Deficit Irrigation (RDI) is the imposition of DI at a specific stage or stages of crop development. In some crops, improved production efficiency and fresh produce quality can be gained using RDI techniques. However, both DI and RDI must be applied judiciously to avoid yield penalties and lowered leaf quality (see Monaghan *et al*., 2016). For example, severe DI in which only 50% of water lost by transpiration was replaced resulted in yellowing and lowered quality of processing spinach leaves (Leskovar & Piccinni, 2005). These authors also reported that a 75% DI treatment improved water use efficiency without reducing yields, but they did not investigate effects on post-harvest leaf quality. Similarly, Luna *et al.* (2012) reported that the quality and shelf-life of fresh-cut Iceberg lettuce was better preserved in plants receiving DI during the growing period. These authors also reported higher overall visual quality and fewer off-odours after 13 days of storage in fresh-cut Romaine lettuce grown under DI (Martinez-Sanchez *et al.,* 2012).

Monaghan *et a*l. (2016) applied a series of DI treatments to Iceberg lettuce in which both the timing and duration of DI were adjusted; they concluded that DI could be used to provide smaller but higher quality heads which are less likely to be rejected, due largely to a reduction in post-harvest rib pinking. However, the choice of DI treatments in those experiments was rather arbitrary. The degree of soil moisture deficits imposed were uncontrolled and were dependent on the weather conditions at the time of application, rather than being based on a detailed understanding of the variety's physiological and biochemical responses to decreasing soil ψ_m (increasing soil moisture deficits) at different growth stage and in different soils, as was done here. Preliminary work using partial rootzone drying (PRD - a different DI technique) to improve leaf quality at HAU was inconclusive (Jim Monaghan, pers. comm.).

To inform further research on DI strategies for leafy salads, work to establish a critical value of soil moisture content for the lettuce cv. "Chancellor" grown in 4 L pots of John Innes No. 2 compost was carried out at Lancaster University as part of HL0196. Partial stomatal closure was triggered at a VSMC of *c.* 12%, with the corresponding soil ψ_m estimated to be -500 kPa (Knox *et al*., 2014). However, the yield response to irrigation was highly linear between the 60% and 100% of Evapotranspiration (ET) treatments, with even a small decrease in irrigation volume (to just 90% of ET) resulting in a significant decrease in yield. Consequently, yield reduction was first observed at a VSMC of 16% (equivalent to *c.* -100 kPa), well before the "critical soil moisture value" of 12% was reached. However, in that work, sustained DI treatments were applied throughout plant growth and development, and so the degree of stress encountered by the plants would have been far greater than in our intended AWD treatments where the soil will be returned to field capacity between mild soil drying events.

The soil ψ_m at which a significant reduction in *gs* was observed in the HL0196 work, -500 kPa, was very low compared to the values reported here for the peat and silt soils (-36 to -84 kPa). Widespread wilting would be expected at a soil ψ_m of -500 kPa. The reasons for this disparity are not known, but our work on stomatal responses to gradual soil drying in a range of different horticultural crops is consistent with that reported here. In the Lancaster work, VSMC at field capacity was c. 27% and so it is surprising that partial stomatal closure was not triggered before the 12% value reported. This work was carried out in 4 L pots but it is not clear where in the pots the VSMC was measured, or whether several measurements were made to give an average VSMC throughout the rooting zone.

Thermal and hyperspectral imaging to detect the onset of water deficit stress

Our work shows that it might be possible to use thermal imaging to identify the early physiological responses to mild soil drying and to inform irrigation scheduling to commercial lettuce crops. This is contrary to results reported in the HL0196 project. In that work, partial stomatal closure was detected at VSMC values below 15%, and so by the time rises in leaf temperature were noted, head fresh weight had already been reduced. Knox *et al*. (2014) concluded that it was not possible to identify a relationship between plant "growth" [*sic*] measures and the soil moisture deficit. In our experiments in a controlled environment, a significant rise in leaf temperature was sometimes detected before a significant reduction in stomatal conductance, but in other cases, there was no detectable rise in leaf temperature. Further work is needed to investigate the potential of using thermal imaging to detect the very early leaf responses to soil water deficits under controlled conditions, and if the approach is shown to be reliable, to then determine whether it would be feasible to deploy the thermal imaging under field conditions.

Although not a part of this project, we have also been investigating the use of hyperspectral imaging to detect spectral changes that coincide with the onset of stress perception in plants exposed to progressive soil drying. These data are currently being analysed and the results will underpin future research proposals on using imaging to detect responses to abiotic and biotic stresses, and to develop non-destructive tools to estimate leaf quality attributes in leafy salad crops.

New opportunities to improve production efficiency and leaf quality

The opportunities, constraints and challenges of implementing PI on commercial sites have been discussed by Monaghan *et al. (*2013), but new approaches in which models predicting changes in crop coefficients and rooting depth over the growing season are informed by digital estimates of ground cover offer exciting opportunities to scale-up PI approaches for lettuce and other leafy salad crops (Escarabajal-Henarejos *et al*., 2015a, b).

Rather than applying DI *per se* which has been shown to reduce marketable yields even when mild DI is applied, (90% of ET but see discussion above), the AWD approach suggested here may deliver more consistent leaf quality without a loss in marketable fresh weight or head diameter by avoiding over and under-irrigation.

<u>Relating soil volumetric moisture content to soil matric potential</u>

To be able to use the new information generated in this project to inform irrigation scheduling strategies for lettuce crops growing in different soil types, growers will need either to deploy sensors across the rooting zone to measure soil matric potentials directly, or be able to convert measurements of VSMC to values of soil matric potential. There are a variety of sensors that growers can deploy to measure the soil matric potential within the rooting zone of their crops (see below). To ensure that the project results would have relevance to growers of lettuce in all the major soils, an agreed output from this project was to derive moisture release curves for peat, silt, silty loam and sandy loam soils (see below).

Soil matric potential sensors

There are a variety of sensors that growers can deploy to measure the soil ψ_m within the rooting zone of their crops. The most commonly used sensor for soil ψ_m is the water-filled hydraulic tensiometer. When working correctly, tensiometers give a direct measurement of matric potential. A widely perceived limitation in their use is the view that the water-filled tensiometer can only be used to measure matric potentials as low as –90 kPa due to cavitation of water at lower pressures. Although careful saturation of commercially available tensiometers can allow matric potentials as low as –200 kPa to be measured (Whalley *et al*., 2009), their use in the field is often problematic and it may not be obvious to the grower if a tensiometer is not working reliably.

A significant effort has been made to develop sensors which can reliably measure matric potentials which are much lower than –90 kPa. The most common design is the porous matrix sensor. Here a porous material is allowed to equilibrate with the soil water, then the water content of the porous matrix is measured and converted to a matric potential using a calibration curve. An example of a gypsum block sensor is the ‘Water Mark®’ sensor which is currently widely used in irrigation control in some countries. Although the accuracy of these sensors is not sufficient for most scientific purposes (Whalley *et al*., 2013), when coupled to the G-Dot system (Figure 38), this sensor deployed at different depths through the rooting zone provides simple visual information of the likely soil water availability throughout the rooting zone.

Figure 38. The 'Water Mark®' sensor and G-Dot display system.

Decagon Inc. (now METER Group, Inc. USA) developed a commercial porous matrix sensor which uses dielectric measurement to monitor the water content of a ceramic material (Figure 39). This is a relatively low cost sensor with an accuracy of ± 10% (±2 kPa) from -9 to -100 kPa and lower accuracy past -100 kPa. Although the sensor accuracy is again not ideal, highly accurate matric potential data are not needed to schedule irrigation effectively to field crops where other sources of variability will have a more significant effect on outcomes. These sensors can be deployed at multiple depths and connected to dataloggers with telemetry so that data can be viewed remotely several times a day if necessary. This system was used in this research project.

Figure 39. The Decagon MPS6 soil matric potential sensor.

In addition to providing information on changes in soil ψ_m throughout the rooting zone, these sensors can also help growers to decide when to resume irrigation following rainfall events. Avoiding irrigating soils already close to field capacity will help to reduce the propensity for mid-rib pinking (Luna *et al*., 2013; FV413, Vickers *et al*., 2015).

Soil moisture release curves

Soil matric potentials can be converted to VSMC by reference to a moisture release curve, which is a plot of the relationship between the water content, and the soil ψ_m. The s soil ψ_m is the sum of four components: matric potential, osmotic potential, gravitational potential, and pressure potential, but for most agricultural and horticultural situations, soil ψ_m has the most significance.

The general features of a moisture release curve can be seen in Figure 40, in which the volumetric soil moisture content is plotted against the soil ψ_m. At matric potentials close to zero, a soil is close to saturation, and water is held in the soil primarily by capillary forces. As the water content decreases, binding of the water becomes stronger, and at small potentials (more negative, approaching wilting point) water is strongly bound in the smallest of pores, at contact points between soil grains and as films bound by adsorptive forces around particles.

Sandy soils will involve mainly capillary binding, and will therefore release most of the water at higher potentials, while clayey soils, with adhesive and osmotic binding, will release water at lower (more negative) potentials. At any given potential, peaty soils will usually display much higher moisture contents than clayey or silty soils, which would be expected to hold more water than sandy soils. The water holding capacity of any soil is due to the porosity and the nature of the bonding in the soil.

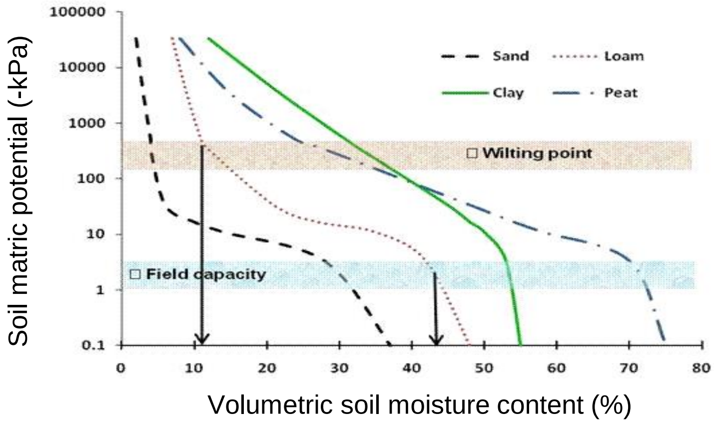

Figure 40. Soil moisture release curves for different soils. For a loam soil, field capacity is around 44% and -5 kPa; plant wilting point for the same soil is 11% and 550 kPa. See the NRCCA Soil and Water Management Study Guide.

The traditional and most accurate way of constructing a moisture release curve is to use a pressure plate apparatus. The apparatus consists of strongly built metal chambers containing one or more ceramic plates, on to which uniform soil samples are placed. Having attained maximum moisture retention (field capacity) the samples within the cells are then subjected to controlled positive air pressures and water is gradually removed. By careful control of pressure, the various equilibrium conditions of pressure and soil water tension or suction are obtained and a moisture release curve can be constructed. NIAB EMR has recently purchased a pressure plate system and this is currently being used to generate moisture release curves for the four soils. In the meantime, preliminary moisture release curves over a limited but horticulturally important range have been generated for the soils used in this project (Figure 41), using *in situ* measurements of both soil water content and soil matric potential in pots of peat and silt soils.

It should be noted that the bulk density of soils used in this pot experiment will be different to those in the rooting zone in the fields from where the soil was taken and so the moisture release curves derived here would be different to those constructed from soil cores taken from fields.

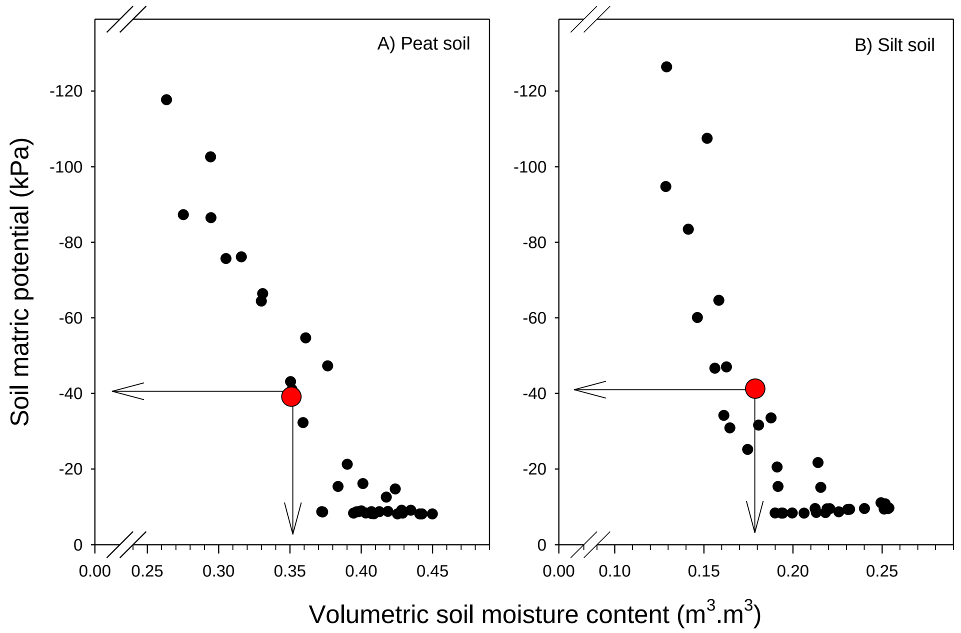

Figure 41. Preliminary soil moisture release curves for A) the peat soil and B) the silt soil used in this project. Note the different scales on the x axes. At -40 kPa, the corresponding VSMC in peat soil is 0.35 $m^3.m^3$, or 35%; in silt soil it is 0.18 $m^3.m^3$, or 18%.

Future work

The next phase will be to develop Alternate Wetting and Drying irrigation strategies in a controlled environment and then test these in commercial field trials on successive crops throughout the growing season so that the validity of the approach can be tested under different transpirational demands. We will evaluate any effects on head fresh weight and diameter, and aspects of leaf quality over the growing season. There is also great potential to use hyperspectral imaging to develop non-destructive predictive models for leaf quality attributes, and to test the feasibility of using thermal imaging to detect the very early plant responses to mild soil water deficits.

Conclusions

We have demonstrated that leaf adaptive physiological responses to drying soil occur at similar values of matric potential in the silt and peat soils, whereas the corresponding volumetric soil moisture contents were very different (Table 6). This outcome was expected since matric potentials are not influenced by differences in soil bulk density, whereas

Table 6. The soil matric potentials and volumetric soil moisture contents at which leaf adaptive responses in DD-treated Romaine ("Actina", "Scala") and Iceberg ("Challenge", "Etude") varieties grown in peat and silt soils diverged significantly from WW values, and the suggested irrigation set points for to be tested in alternate wetting and drying treatments.

Lettuce variety	First adaptive response				Irrigation set point			
	Soil ψ_m (kPa)		VSMC (%)		Soil ψ_m (kPa)		VSMC (%)	
	Peat	Silt	Peat	Silt	Peat	Silt	Peat	Silt
"Actina"	-78	-84	30	14	-65	-65	33	16
"Scala"	-76	*	34	*	-47	*	37	*
"Challenge[1]"	-54	-36	30	16	-35	-25	35	18
"Challenge[2]"	*	-54	*	18	*	-34	*	19
"Etude"	-36	*	35	*	-25	*	40	*

* Value not determined
[1]Experiemnt 1 – July 2017
[2]Experiemnt 3 – May 2018

volumetric water contents are. These results highlight the advantage of using matric potentials to schedule irrigation to crops in various soils, rather than volumetric soil moisture contents which are influenced by the different bulk densities in the two soils.

Continued and sustained soil drying past the values at which leaf adaptive responses were first detected would likely reduce final head weights and diameters, as well as leaf quality. Plant adaptive responses to drying soil will also be affected by the prevailing evaporative demand and so we have taken the soil ψ_m values recorded on the previous measurement days to those where significant reductions in *gs* were noted to use as irrigation set points for further testing in field experiments. These values could be used in an AWD treatment where irrigation is scheduled to return soil to field capacity once the irrigation set point is reached. This alternate wetting and drying would be carried out at different times of the growing season so that the validity of the approach could be tested under different transpirational demands. Funding for this research, for translational work, for grower support and training and demonstration of "best" and "better" practice will be sought after further consultation with the AHDB FV Panel, the BLSA and the wider industry.

Knowledge and Technology Transfer

1. Project Kick-off Meeting, NIAB EMR, 5 April 2017. Present were Mark Else (NIAB EMR), Mike Davies (NIAB EMR), Emma Garfield (G's) (by Facetime), Dave Edwards (Jepco), Industry representative, Kim Parker (AHDB).
2. Quarter 1 Review Meeting, NIAB EMR, 3 July 2018. Present were Mark Else (NIAB EMR), Mike Davies (NIAB EMR), Emma Garfield (G's) (by Facetime), Dave Edwards (Jepco), Industry representative, Kim Parker (AHDB).
3. Quarter 2 Review Meeting, AHDB AHDB Stoneleigh Park, 5 December 2017. Present

were Mark Else (NIAB EMR), Mike Davies (NIAB EMR), Rob Parker (G's) (by Conference call, Dave Edwards (Jepco), Industry representative, Jim Monaghan (Harper Adams University), Kim Parker (AHDB), Grace Choto (AHDB).

4. The project leader submitted a presentation for the AHDB FV Lettuce Workshop on 1 March but the event was postponed due to bad weather. The Project Leader was unable to attend the rescheduled event held on 20 March 2018 due to prior work commitments.
5. A summary of the project aims, objectives and progress was presented at 'Solutions for the Future', an event hosted by The Produce Quality Centre (PQC) at East Malling, Kent on 12 June 2018. The PQC is a collaboration between the Natural Resources Institute (NRI) and NIAB EMR.
6. The project results and conclusions will be presented at the AHDB Leafy Salads R&D Meeting to be held on 2 October 2018.
7. A Factsheet summarising the results from FV 454 is currently being prepared for the AHDB and will be available in 2019.
8. An article is being prepared for AHDB News.

Glossary

Available water capacity - the difference in the water content at field capacity and wilting point.

Field capacity - the water content of a soil or substrate after it has been fully saturated and allowed to drain freely.

Matric potential - A measure of the capillary forces holding water in the growing medium and therefore the force required to remove it.

Potential evapotranspiration - the rate at which a crop would lose water under prevailing environmental conditions if water supply was non-limiting. It includes evaporation from the plants (transpiration) and from the soil or growing medium.

Tensiometer – A device for measuring the water tension in s soil or substrate. Tensiometers measure matric potential rather than volumetric water content, and it is measured in kiloPascals (kPa). Matric potentials become more negative as the growing medium dries.

Transpirational cooling – Cooling of plant organs or tissues caused by the evaporation of water from aerial parts, such as leaves, stems and flowers.

Volumetric substrate moisture content - the water content of the soil or substrate expressed as a fraction or percentage of the total volume occupied by water. Its optimum value depends on the type of soil and is influenced by the bulk density of the soil.

Wilting point - the water content of soil or substrate when a plant can no longer draw water from it. At this point, the capillary forces holding the water in the growing medium just exceed the capillary pull, "suction" or substrate water tension capable of being exerted by the plant.

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Notes

