



# Horticultural Fellowship Awards

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Interim Report Form

Project title: Working with the industry to develop the next generation of technical staff for the UK horticulture industry through a Summer Research Programme.

Project number: CP 87

Project leader: Dr Jim Monaghan

Report: Annual report, March 2015

Previous report: Annual report, May 2012  
Annual report, March 2013  
Annual report, March 2014

Fellowship staff: Josie Brough (Technical support); Dr Paul Hand (Associate); Prof Dave Pink (Associate); Dr Tom Pope (Associate)

Location of project: Harper Adams University

Industry Representative: N/A

Date project commenced: 8 July 2011 (back dated 1 April 2011)

Date project completed (or expected completion date): 31 March 2016

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The results and conclusions in this Annual Report are based on an investigation conducted over a one-year period. The 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 product 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.

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

Report authorised by:

[Name]

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## Progress Against Objectives and Annual Milestones

### Objectives

Objective	Original Completion Date	Actual Completion Date	Revised Completion Date
1. Recruit a minimum of 15 undergraduates from UK Higher Education Institutions to complete applied experiments in horticultural crop production and agronomy.	31/03/2016		
2. Deliver a minimum of 15 small-scale research projects for the industry.	31/03/2016		
3. Publicise the approach and outputs of the programme to the industry, Further Education and Higher Education Institutions.	31/03/2016		
4. Leverage additional funding for follow up projects.	31/03/2016		

### Summary of Progress

The fourth year of the Summer Research Programme (SRP) was successful. Four UK undergraduates were selected from Lancaster, Bristol, Cambridge and Oxford University. The students undertook four separate research projects at HAU linked Elsoms Seeds, Bulrush Ltd, BASF Agricultural Specialities Limited and G's, and also worked together on a number of on-going crop research experiments at HAU. Each student prepared and gave a presentation of their research to the representatives from HDC. The students also made a number of visits to businesses including strawberry, leafy salad, field vegetable, protected salad and ornamental producers.



More detailed reports of each of the four projects are appended to this report and a brief summary of each project is included here. The experiments are numbered sequentially throughout the fellowship and Experiments 11-14 are reported here.

***Experiment 11 – Effect of vine weevil on strawberry yields in first and second year crops (Liam Elliott – Cambridge University).***

Vine weevil (*Otiorhynchus sulcatus*) remains one of the most serious pests of soft fruit and ornamental crops (Moorhouse *et al.*, 1992). Damage is caused both by the adults, which feed on leaves, and larvae, which feed on plant roots, corms and tubers. As the larvae are root pests and the adult weevils are nocturnal an infestation may pass unnoticed for some time until adult leaf notching is noticed or plants show signs of wilting due to larval feeding damage, by which time they will have been damaged beyond recovery.

Growers are currently able to use Integrated Pest Management (IPM) compatible options to control vine weevil larvae, such as the entomopathogenic nematodes (EPNs) *Steinernema kraussei* (Nemasys L and Exhibitline sk), *Heterorhabditis bacteriophora* (Nemasys H, Nematop, Exhibitline h and Larvanem), a mix of *S. carpocapsae*, *S. feltiae* and either *H. bacteriophora* or *H. megidis* (SuperNemos) as well as the entomopathogenic fungus (EPF) *Metarhizium brunneum (anisopliae)* (Met52) (e.g. Bennison *et al.*, 2014). In contrast, growers are currently reliant on the use of broad spectrum insecticides such the pyrethroid lambda-cyhalothrin (Hallmark) for the control of vine weevil adults. Application of these insecticides against this pest is difficult, as they need to be applied at dusk, when the weevils become active. In addition, these insecticide applications have a negative impact on biocontrol agents used against other pests and naturally-occurring beneficials such as ground beetles that predate on vine weevil adults (Cross *et al.*, 2001).

Despite the importance of vine weevil to the soft fruit industry there is relatively little quantifiable information on the damage caused by this pest. In particular there is a lack of information on the effect of vine weevil on crop yield and quality in the absence of controls and where controls are applied. For strawberry crops it is currently estimated that even with available controls against both adult and larval stages of this pest, losses are approximately £14 million per annum (Wynn, 2010). However, such calculations are based on expert opinion rather than results from carefully designed experimental approaches.

**Liam studied the impact of substrate infested with vine weevil larvae on the yield and postharvest quality of fruit of table-top 60 day strawberry plants. He also studied the effect of two control strategies on overwintering vine weevil larvae. Liam concluded that:**

- **Vine weevil larvae are unlikely to affect the yield and quality of strawberry crops when plants are well established and become infested shortly before the harvest window.**
- **An EPN drench (*Nemasys L*) applied after the harvest window significantly reduced numbers of overwintering vine weevil larvae.**
- **Incorporation of an EPF (*Met52*) into the growing media did not significantly reduce numbers of overwintering vine weevil larvae.**

***Experiment 12 - The Effect of Variety and Irrigation on Splitting in Radishes. (Emma Micklewright – Oxford University).***

Radish (*Raphanus sativus*) is an economically important member of the mustard family, Brassicaceae. Hypocotyl splitting in radish is typically characterized by a radial longitudinal fracture which usually occurs pre-harvest, growth splits, or shortly (1-2 days) post-harvest, harvest splits, during storage. Splitting is a problem for growers as the amount of splitting can be as high as 30 % which exceeds supermarket tolerance to splitting which is usually 10 %. Splitting reduces the marketable yield as split radishes have to be removed by hand prior to packing which is time consuming and costly. Despite these problems, little is known about the environmental and physiological causes of splitting particularly in the smaller summer radishes which are predominantly grown in the UK.

There is evidence that timing of water availability during growth may affect splitting. Salter (1967) found dry conditions during mid-season carrot growth followed by rain prior to harvest resulted in an increased proportion of split carrots and significantly decreased marketable yield. Sorensen (1997) also found the timing of water stress had an effect on splitting in carrot, with carrots grown under fully irrigated conditions, or with an early drought stress, splitting more than carrots grown with a period of drought stress mid-growth when rapid radial expansion is occurring. Similar results have been found in tomato with cracking rates being at their highest when fruit growth is at a maximum (Dorais *et al.* 2004). Timing of water availability during growth may also affect splitting in summer radishes but this needs investigation.

**Emma grew three cultivars of radish under a dry or wet regime. She studied the effect of irrigation regime on growth and splitting at harvest. Emma concluded that:**

- **The dry watering regime reduced splitting in all 3 cultivars**
- **Dryer plants grew more slowly than the well watered plants**

- **Cultivar had no effect on splitting or other measures of plant growth**
- **Irrigation studies for one cultivar can be extrapolated to other cultivars without the requirement for additional experiments**

***Experiment 13 - Can drought stress change the flavour of Cos lettuce? (Jack Turner – Lancaster University)***

It is well known that roots in drying soil generate abscisic acid (ABA) and this is one of the pathways controlling stomatal aperture (Wilkinson and Davies, 2010). By manipulating soil water content in the rootzone, crop transpiration can be manipulated through ABA mediated stomatal closure following transient drought stress imposed during growth, known as deficit irrigation (DI) or through alternating portions of the root zone that dry down, termed partial rootzone drying (PRD). These techniques have been successfully implemented in tree fruit and vine crops with some commercial success in South America, Southern Europe and Australia and work through reducing leaf growth, redirecting resources to fruit growth, and/or increasing radiation interception by the fruit (Feres and Sorriano, 2007).

Techniques designed to increase ABA signalling by exposing crops to periods of drought stress are more difficult to implement with field vegetable crops such as lettuce that rely on an increase in leaf biomass for yield increases (Capra *et al.*, 2008). However, growing leafy plants using DI has been reported to change the biochemical and hence flavour profile of some herbs such as basil (Ekren *et al.*, 2012) and this project was developed in discussion with G's to assess whether the use of DI or PRD could influence the flavour of Cos lettuce.

**Jack grew Cos lettuce in pots with three irrigation regimes: well watered (Co), deficit irrigation (DI) and partial rootzone drying (PRD). The growth and yield of the plants was measured and the flavour of heads of the PRD and Co treatments was then assessed using a taste panel at HAU. Jack concluded that:**

- **Lettuce yield was reduced significantly by DI but not by PRD when compared to Co.**
- **The taste panel identified a trend that plants grown with PRD were sweeter than well watered plants but this response was not significant.**

**Experiment 14 - Stress priming kale – does it lead to more resilient plants?  
(Samantha Ball – Bristol University)**

There is a considerable background literature to support the hypothesis that preconditioning plants with a stress treatment provides a beneficial effect protecting from further stresses. Drought treatments of transplants of broccoli and other vegetables have been shown to improve future growth and stress resilience (e.g. Latimer, 1990). Bruce *et al.* (2007) describes priming, or hardening, as the initial exposure to abiotic or biotic stress to enhance resistance to the stresses later in the plants development. Such plants have a much stronger defensive response to later events of the stress event, forming what Bruce *et al.* (2007) describes as a stress memory.

Literature on this topic is mainly related to the priming of seeds but, in a recent HDC report, Mulholland (2013) showed that a brief treatment of cauliflower transplants/seedlings with salt resulted in increased resistance to pests and pathogens. An unexpected observation was that some salt treatments led to greater leaf and root growth compared to the controls and also improved subsequent yield and quality. The underlying basis of the growth benefit observed by Mulholland (2013) is not clear but has significant commercial potential.

Kale (*Brassica oleracea* v. *acephala*) was chosen as a model for this project as there is limited research done on this crop, as well as the fact that it has a fast turn over time in terms of growth rate.

**Sam salt stressed kale plants at the seedling/module stage before transplanting into large tubs and growing on. The effect of drought and waterlogging at two timings on plant growth was measured. Sam concluded that:**

- **Salt pre-conditioning led to smaller kale plants after 5 weeks growth.**
- **Physiological response to seedling salt stress was observed for two weeks after transplanting.**
- **Overall, a pre-conditioning salt treatment is not beneficial to plants growing on in the range of environments studied.**

### **Visits by students**

Eight businesses hosted visits by the students: PDM (lettuce), Lower Reule Farm (strawberries), G's (field vegetables and mushrooms), Cornerways Nursery (tomatoes), H&H Duncalfe (soft fruit), Sutton Bridge Crop Research (postharvest research), MMUK (fruit and flower) and ADAS, Boxworth (crop research).

Informal feedback from the students was again very positive. One of the students has applied for a graduate agronomist's position in the field crop sector, two have applied for a PhD in the area of crop/plant science. The other one is unclear at this stage what they will do after graduation.

The fellow aims to keep contact with all the SRP students to track later career choices.

### **Milestones**

Annual Milestone	Original Completion Date	Actual Completion Date	Revised Completion Date
1. Select proposed project titles and outlines of work in agreement with Partner businesses and HDC Research Manager.	31/05/2014	31/05/2014	
2. Commence experimental work.	31/05/2014	31/05/2014	
3. Complete mail shots and selected visits to other institutions.	31/05/2014	31/05/2014	
4. Recruit SRP students	07/04/2014	07/04/2014	
5. SRP students start	07/07/2014	07/07/2014	
6. SRP students finish	30/08/2014	30/08/2014	
7. Research reported to HDC (end November)	31/03/2014	31/03/2014	

### **Milestones not being reached**

N/A

### **Do remaining milestones look realistic?**

Yes

### **Training undertaken**

No training was undertaken by the Fellow in Year 4.

### **Expertise gained by Trainee**

N/A

### **Other achievements in the last year not originally in the objectives**

The Gatsby Summer School for high achieving Plant Scientists targeted at first year UK undergraduates ([www.gatsbyplants.leeds.ac.uk](http://www.gatsbyplants.leeds.ac.uk)) has linked to this programme as an opportunity for applied research experience. The fellow was invited to attend the Gatsby Summer School and promote the SRP in 2014.

### **Changes to Project**

N/A

### **Are the current objectives still appropriate for the Fellowship?**

No changes proposed

## **Grower Summary**

The fourth year of the Summer Research Programme (SRP) was successful. Four UK undergraduates were selected from Lancaster, Bristol, Cambridge and Oxford University. The students undertook four separate research projects at HAU linked Elsoms Seeds, Bulrush Ltd, BASF Agricultural Specialities Limited and G's, and also worked together on a number of on-going crop research experiments at HAU. Each student prepared and gave a presentation of their research to the representatives from HDC. The students also made a number of visits to businesses including strawberry, leafy salad, field vegetable, protected salad and ornamental producers.

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## **Headline**

N/A

## **Background**

The recent Royal Society report and the Field and Vegetable Task Force report have both highlighted the shortage of applied technical expertise available to the UK horticulture industry. Reduction in government funding for applied horticulture research has led to a marked reduction in the pool of applied researchers available for employment in industry, research and advisory/agronomist roles. In addition the loss of many relevant crops focussed courses and modules from Universities have led to a marked shortage of opportunities for undergraduates to be exposed to, and trained in, applied research in horticulture crop production compared to 10-15 years ago. This limits the number of suitable candidates for technical roles in industry, research studentships, technical roles in universities or institutes, or agronomy and extension businesses.

We have launched a Summer Research Programme (SRP) based at Harper Adams University College (HAUC) and led by Jim Monaghan. The SRP will recruit three UK undergraduate students (and potentially seconded industry employees) each year. These students will then carry out applied agronomy/crop production research projects within the Fresh Produce Research Centre and be supported by other research staff associated with the centre.

## **Summary**

See appendices

## **Financial Benefits**

N/A

## **Action Points**

See appendices

## **Knowledge and Technology Transfer**

A webpage and facebook site has been set up for the SRP and contain videos of each project.

<http://www.harper-adams.ac.uk/initiatives/fresh-produce-research-centre/>

<https://www.facebook.com/HAUFreshProduce>

## **Glossary**

N/A

## **References**

See appendices

## **Appendices**

A detailed report of the four experiments are appended to this report:

Experiment 11 – Effect of vine weevil on strawberry yields in first and second year crops

Experiment 12 - The Effect of Variety and Irrigation on Splitting in Radishes

Experiment 13 - Can drought stress change the flavour of Cos lettuce?

Experiment 14 - Stress priming kale – does it lead to more resilient plants?





## Experiment 11 – Effect of vine weevil on strawberry yields in first and second year crops

Liam Elliott – Cambridge University

### 11.1. Background

Vine weevil (*Otiorhynchus sulcatus*) remains one of the most serious pests of soft fruit and ornamental crops (Moorhouse *et al.*, 1992). Damage is caused both by the adults, which feed on leaves, and larvae, which feed on plant roots, corms and tubers. As the larvae are root pests and the adult weevils are nocturnal an infestation may pass unnoticed for some time until adult leaf notching is noticed or plants show signs of wilting due to larval feeding damage, by which time they will have been damaged beyond recovery.

Growers are currently able to use Integrated Pest Management (IPM) compatible options to control vine weevil larvae, such as the entomopathogenic nematodes (EPNs) *Steinernema kraussei* (Nemasys L and Exhibitline sk), *Heterorhabditis bacteriophora* (Nemasys H, Nematop, Exhibitline h and Larvanem), a mix of *S. carpocapsae*, *S. feltiae* and either *H. bacteriophora* or *H. megidis* (SuperNemos) as well as the entomopathogenic fungus (EPF) *Metarhizium brunneum (anisopliae)* (Met52) (e.g. Bennison *et al.*, 2014). In contrast, growers are currently reliant on the use of broad spectrum insecticides such the pyrethroid lambda-cyhalothrin (Hallmark) for the control of vine weevil adults. Application of these insecticides against this pest is difficult, as they need to be applied at dusk, when the weevils become active. In addition, these insecticide applications have a negative impact on biocontrol agents used against other pests and naturally-occurring beneficials such as ground beetles that predate on vine weevil adults (Cross *et al.*, 2001).

Despite the importance of vine weevil to the soft fruit industry there is relatively little quantifiable information on the damage caused by this pest. In particular there is a lack of information on the effect of vine weevil on crop yield and quality in the absence of controls and where controls are applied. For strawberry crops it is currently estimated that even with available controls against both adult and larval stages of this pest, losses are approximately £14 million per annum (Wynn, 2010). However, such calculations are based on expert opinion rather than results from carefully designed experimental approaches.

This project was developed with Bulrush Ltd and BASF Agricultural Specialities Limited and asked two research questions:

- What is the effect of a vine weevil infestation on the yield and quality of a strawberry crop in years one and two after planting?

- To what extent does an EPN drench or incorporation of an EPF into the growing media protect the yield and quality of a strawberry crop from vine weevil damage?

## 11.2. Materials and methods

The experiment was carried out at Harper Adams University during the summer of 2014 (Year 1) and will be completed during the summer of 2015 (Year 2). The treatments studied are summarised in Table 11.1.

**Table 11.1** Summary of treatments

Treat No	No. of weevil eggs/plant	Product name	Active substance	Label recommended rate	Application method
1	0	-	-	-	-
2	15	-	-	-	-
3	15	Nemasys L	Steinernema kraussei	25,000 plant <sup>-1</sup>	Drench
4	0	Met52	Metarhizium brunneum	500 g m <sup>-3</sup>	Substrate incorporation
5	15	Met52	Metarhizium brunneum	500 g m <sup>-3</sup>	Substrate incorporation

### ***Experimental set up***

A 27 m x 10 m x 3 m polytunnel sited at CERC, Harper Adams University, supplied with mains power, potable and irrigation water was used for this experiment. The lower edges and ends of the tunnel was fitted with black netting to allow for ventilation but prevent entry of airborne pests and the outside perimeter of the tunnel was further protected by a 50 cm mesh electric fence.

4 x 24 m lengths of ridged profile aluminium container floor board width 22 cm x 3 cm deep was supported at intervals by plinths of 5 breeze blocks to a height of 53 cm arranged lengthwise down the tunnel at a spacing of 1.6 m apart. A 20 mm diameter irrigation line was attached with cable ties to one edge of each aluminium strip and the far end was

doubled over and secured with a cable tie. The other ends were connected to an in-line Dosatron DI-16 and feed stock tank and the irrigation and fertigation program was controlled by a Hunter ICC (Hunter Industries) irrigation controller. The controller was set to irrigate each line for 4 x 10 minute events each hour. During vegetative growth Solufeed strawberry starter feed (15:7:30) was used at a concentration of 1 kg per 10 l diluted to 1:200 during an irrigation event, this was then changed for Solufeed SF-C (7:12:35) at fruit formation and used at the same rate. Neither fertiliser included Ca (as this would lead to precipitation of phosphates out of the stock solution). The irrigation water was analysed before the start of the experiment and contained 51.4 mg l<sup>-1</sup> Ca.

Standard growing medium bags supplied by Bulrush Ltd were used. The growing medium was 80% peat and 20% wood fibre +/- Met52. Prior to laying the bags on the benching each bag was shaken to break up any compaction from storage. The bags were placed lengthwise on the bench and butted up to each other in pairs. Single-outlet drippers were attached to the main irrigation line and 2 drippers were placed in each bag with equal spacing.

Each bag was planted with 10 Elsanta 18-20 mm crowns (Hargreaves Plants Ltd, Spalding) in a double row formation on 28<sup>th</sup> May. After planting, the irrigation was set to constant for several days to thoroughly wet up the bags.

Plants were checked regularly for the presence of pests and diseases. Few pests or diseases were seen throughout the season but applications of Scala (applied at 2 l ha<sup>-1</sup>) were applied on 1<sup>st</sup> and 8<sup>th</sup> August to control grey mould while Nimrod (applied at 1.4 l ha<sup>-1</sup>) on 1<sup>st</sup> August and Systhane (applied at 0.23 l ha<sup>-1</sup>) on 8<sup>th</sup> August were applied to control powdery mildew. Majestik (applied at 25 ml ha<sup>-1</sup>) was applied on 1<sup>st</sup> and 8<sup>th</sup> August to control thrips towards the end of the season.

### ***Experimental design***

Each of the four raised benches was divided in half so that there were in total eight blocks. Each block had five experimental units consisting of two adjacent bags with a total of 20 plants. One experimental unit of each treatment was randomly allocated in each block.

### ***Infesting plants with vine weevil eggs***

Vine weevil eggs were supplied by S. J. Cockbill Vine Weevils. Vine weevil eggs were counted out onto a small piece of damp filter paper (2 cm x 2 cm) using a fine paint brush. Vine weevil eggs were then washed around the strawberry plant by first making a small hole in the compost next to the stem of the strawberry plant. Next a plastic wash bottle was used to carefully wash eggs into the hole, which was then covered with compost. This process

was repeated for each strawberry plant so that a total of 15 eggs were washed into the compost around each strawberry plant. Vine weevil eggs were washed onto the strawberry plants in batches between 14<sup>th</sup> and 18<sup>th</sup> July. Additional vine weevil eggs were maintained in the laboratory on moist filter paper in a Petri dish. These eggs were checked every 1-2 days recording the number of eggs that had hatched in order to estimate egg viability.

### ***Nematode drenches***

Nemasys L (*Steinernema kraussei*) was supplied by BASF Agricultural Specialities Limited. The nematodes were prepared following the label recommendations to aerate and dilute the solution of nematodes. Nematodes were applied on 19<sup>th</sup> August using a large syringe to deliver 25,000 nematodes per plant in 100 ml of water. Care was taken to agitate the nematode solution during this process to prevent the nematodes from settling out.

### ***Harvest and assessment***

Harvests took place bi-weekly (Monday and Thursday). All fully ripened fruit were harvested in one container then graded in Class I, as defined by International Standardisation of Fruit and Vegetables marketing standards OECD (Organisation for Economic Co-operation and Development) and Class II (waste, damaged and deformed fruit). The weight of each grade was recorded. At each harvest, three randomly selected Class I fruit were cut in half at the equator and the juice from each hemisphere was tested using a refractometer to measure total soluble solids (Brix).

### ***Vine weevil counts***

For each experimental unit, one of the two strawberry bags was destructively sampled between 1<sup>st</sup> and 2<sup>nd</sup> December in order to record the number of vine weevil larvae in each bag. Care was taken to remove the growing medium from the roots of strawberry plants to avoid missing any larvae. In addition an assessment of the root system of each plant was made.

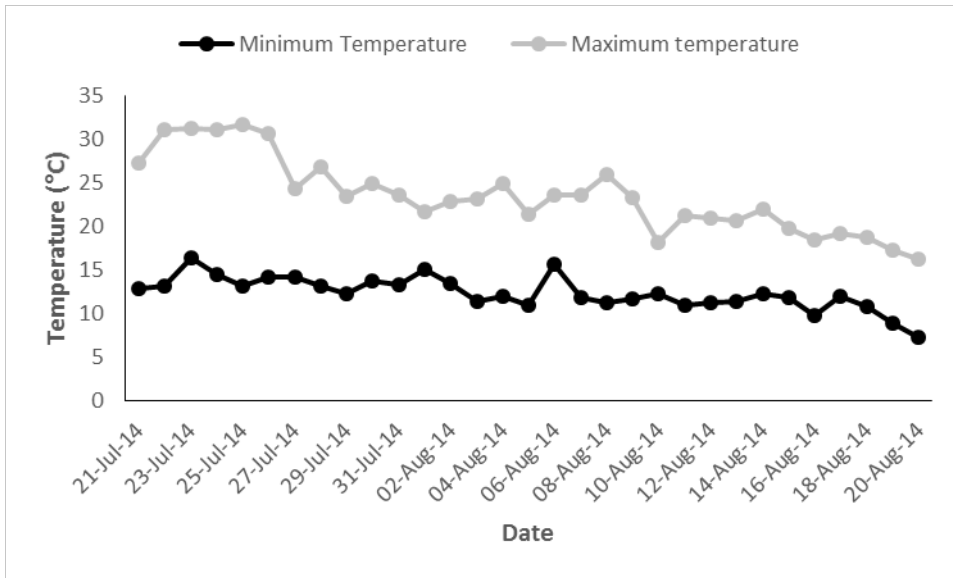
### ***Statistics***

All measurements were analysed by ANOVA using Genstat 16<sup>th</sup> Edition.

## **11.3. Results**

### ***Environmental conditions***

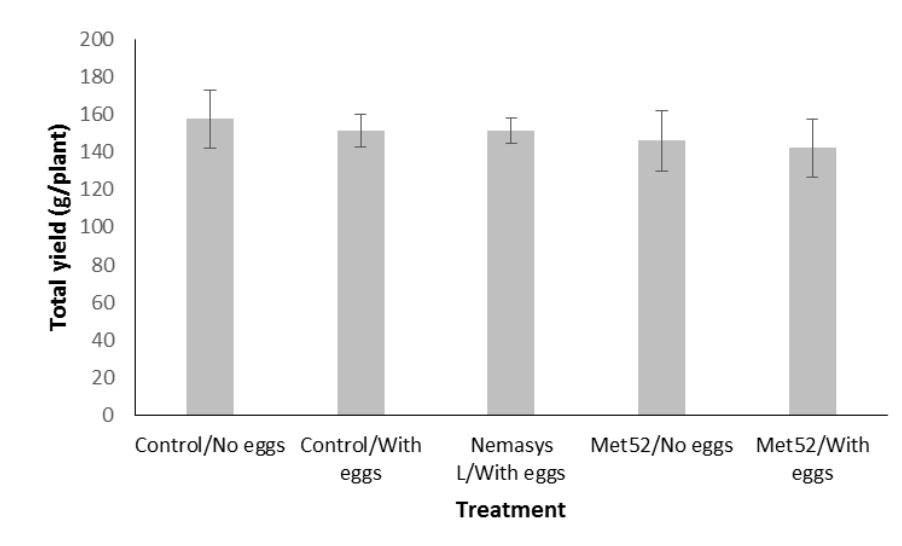
During fruit harvest the air temperature within the polytunnel ranged between 7.3°C and 31.7°C (Figure 11.1)



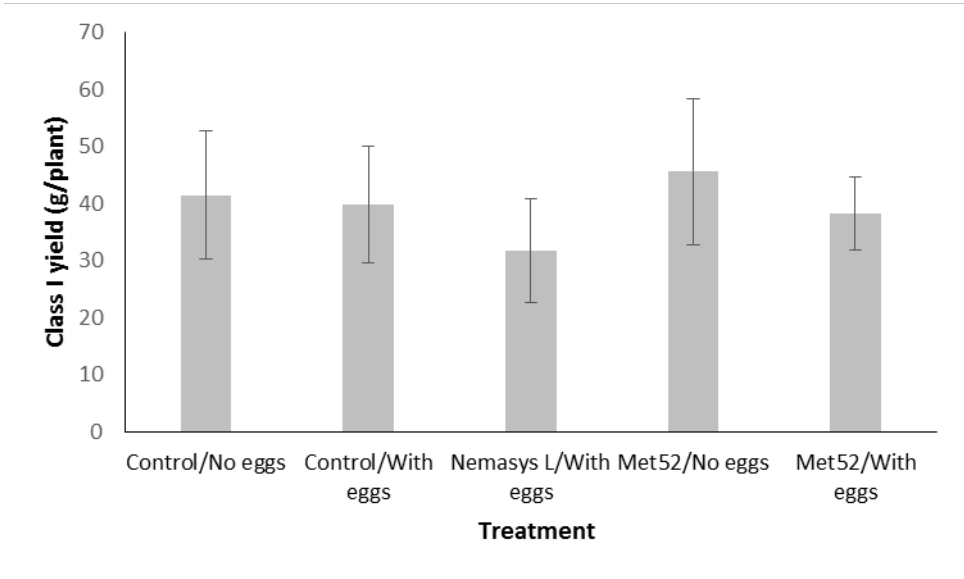
**Figure 11.1** Minimum and maximum temperatures during fruit harvest.

### Yield

The fruit was harvest over a four week period (21<sup>st</sup> July to 20<sup>th</sup> August). There was no difference ( $F = 0.33$ ,  $P = n.s.$ ) between treatments in total yield per plant (Figure 11.2). Similarly, there was no difference ( $F = 0.39$ ,  $P = n.s.$ ) between treatments in class 1 yield (Figure 11.3).



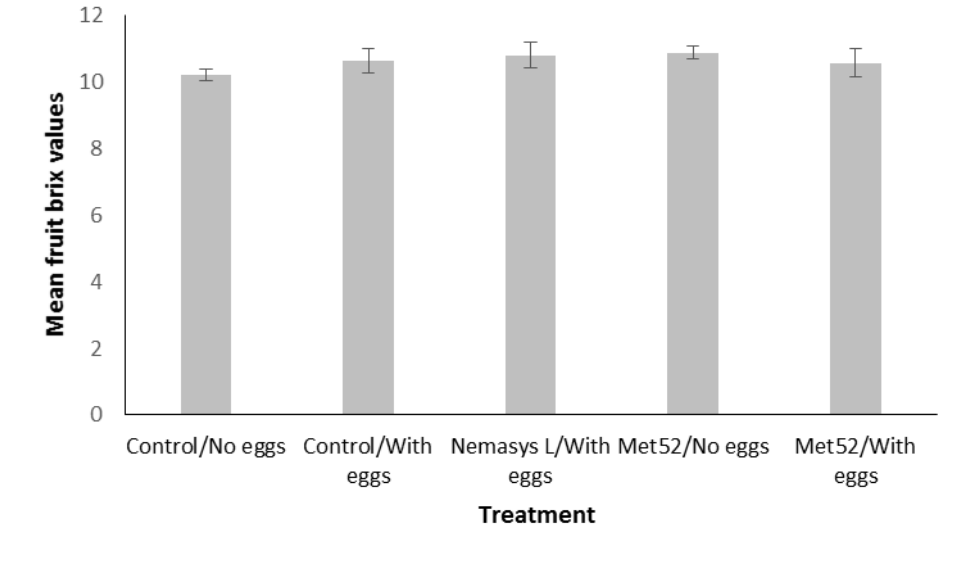
**Figure 11.2** Total strawberry yield (g plant<sup>-1</sup>) (mean ± S.E.,  $n = 8$ ).



**Figure 11.3.** Class 1 strawberry yield (g plant<sup>-1</sup>) (mean ± S.E.,  $n = 8$ ).

**Brix**

There was no difference ( $F = 0.87$ ,  $P = n.s.$ ) between treatments in mean fruit brix values recorded throughout the harvest window (Figure 11.4).

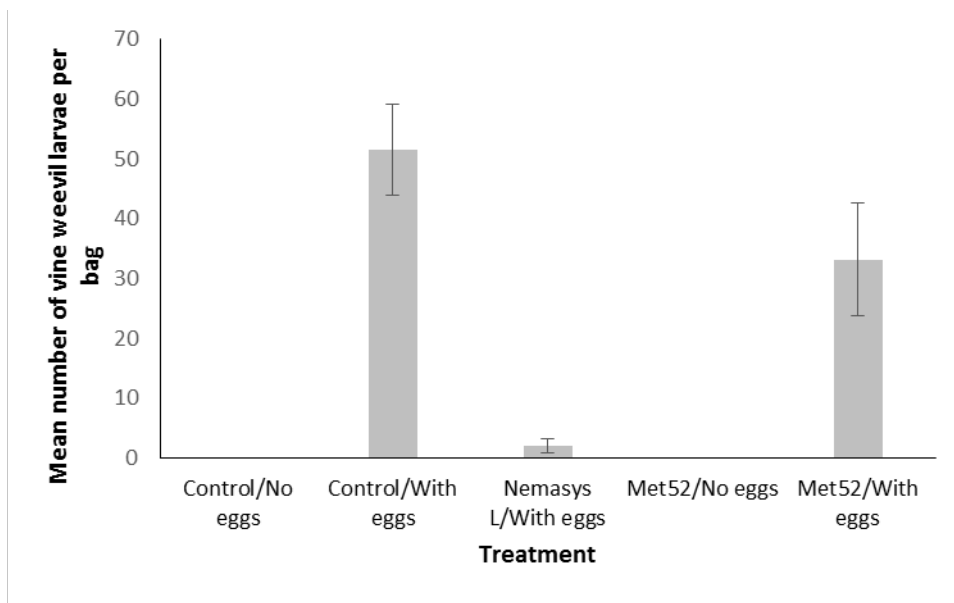


**Figure 11.4.** Mean fruit brix values (mean ± S.E.,  $n = 8$ ).

**Vine weevil counts**

Numbers of vine weevil larvae were assessed by destructively sampling one bag in each experimental unit during November (Figure 11.5). As expected no vine weevil larvae were

recorded in bags that had not been infested with vine weevil eggs. Analysis of numbers of vine weevil in bags that had been infested with 150 vine weevil eggs (15 eggs per plant) showed a clear difference ( $F = 14.14$ ,  $P < 0.001$ ) between treatments. Individual comparisons (LSD at 5%) between treatments shows that the EPN (*Nemasys L*) drench significantly reduced the number of vine weevil larvae in strawberry bags compared to the EPF (Met52) or the untreated control bags. Numbers of vine weevil larvae in EPF treated bags were similar to numbers in untreated control bags.



**Figure 11.5.** Mean numbers of vine weevil larvae in strawberry bags (mean  $\pm$  S.E.,  $n = 8$ ).

Plant health was not obviously affected by vine weevil larvae. All plants were recorded as having well developed root systems, however, anecdotally it was noted that plants in bags containing large numbers of vine weevil larvae were easier to separate, suggesting some reduction in root mass.

#### 11.4. Discussion

*What is the effect of a vine weevil infestation on the yield and quality of a strawberry crop in years one and two after planting?*

Data presented here indicate that the total yield and fruit quality, as measured by total yield, class 1 fruit and brix values, of established strawberry plants (cv. Elsanta) was not affected by vine weevil larvae in year one. This result reflects the limited opportunity for vine weevil larvae to cause significant damage during the harvest window. Plants were established for a



month and a half before being infested with vine weevil eggs between 14<sup>th</sup> and 18<sup>th</sup> July. Harvesting started on 21<sup>st</sup> July, just a week after the first eggs were applied. Although, the eggs washed around each plant were well developed and probably hatched after just a few days, the larvae developing from these eggs would have had little opportunity to damage the plants before the end of the harvest window.

Vine weevil overwinter either as larvae in earthen cells or as adults (Moorhouse *et al.*, 1992). Overwintering success of adult vine weevils appears to be temperature dependent. However, surviving adults may start egg laying as early as May and June (Blackshaw, 1996). Overwintering larvae are also sensitive to low temperatures but are to some extent protected from temperature extremes within the soil or growing media. While young larvae cause limited damage, feeding on fine roots, the older larvae emerging from the earthen cells in the spring feed voraciously on larger membranous roots before pupating (Moorhouse *et al.*, 1992). The effect of vine weevil damage in the spring on yield and quality of the strawberry crop will be investigated in year two of the experiment.

*To what extent does an EPN drench or incorporation of an EPF into the growing media protect the yield and quality of a strawberry crop from vine weevil damage?*

As already described total yield and fruit quality, as measured by class I fruit and brix values, of established strawberry plants was not affected by vine weevil larvae in year one. Therefore, an EPN drench (*Nemasys L*) applied after the harvest window on 19<sup>th</sup> August and incorporation of an EPF (Met52) into the growing media gave no measureable protection of yield and quality of the strawberry crop. However, the EPN drench did dramatically reduce numbers of overwintering vine weevil larvae. Although bags in which the EPF had been incorporated into the growing media had lower numbers of overwintering vine weevil larvae than untreated control bags the difference here was not statistically significant. Similar results for Met52 in a peat based growing media were reported by Bennison (2013).

## **11.5. Conclusions**

- Vine weevil larvae are unlikely to affect the yield and quality of strawberry crops when plants are well established and become infested shortly before the harvest window.
- An EPN drench (*Nemasys L*) applied after the harvest window significantly reduced numbers of overwintering vine weevil larvae.

- Incorporation of an EPF (Met52) into the growing media did not significantly reduce numbers of overwintering vine weevil larvae.

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## Experiment 12 - The Effect of Variety and Irrigation on Splitting in Radishes

Emma Micklewright – Oxford University

### 12.1. Background

Radish (*Raphanus sativus*) is an economically important member of the mustard family, Brassicaceae. Hypocotyl splitting in radish is typically characterized by a radial longitudinal fracture which usually occurs pre-harvest, growth splits, or shortly (1-2 days) post-harvest, harvest splits, during storage. Splitting is a problem for growers as the amount of splitting can be as high as 30 % which exceeds supermarket tolerance to splitting which is usually 10 %. Splitting reduces the marketable yield as split radishes have to be removed by hand prior to packing which is time consuming and costly. Despite these problems, little is known about the environmental and physiological causes of splitting particularly in the smaller summer radishes which are predominantly grown in the UK.

There is evidence that timing of water availability during growth may affect splitting. Salter (1967) found dry conditions during mid-season carrot growth followed by rain prior to harvest resulted in an increased proportion of split carrots and significantly decreased marketable yield. Sorensen (1997) also found the timing of water stress had an effect on splitting in carrot, with carrots grown under fully irrigated conditions, or with an early drought stress, splitting more than carrots grown with a period of drought stress mid-growth when rapid radial expansion is occurring. Similar results have been found in tomato with cracking rates being at their highest when fruit growth is at a maximum (Dorais *et al.* 2004). Timing of water availability during growth may also affect splitting in summer radishes but this needs investigation.

### 12.2. Materials and Methods

For the experiment 1.75 litre G18B half sized seed trays (Garland Products Ltd., Kingswinford, UK) were used to grow radish plants. The seed trays measured 230 mm in length, 170 mm in diameter and 60 mm in depth. All trays were filled level with the rim of the pot, to a weight of 1.5 kg, with John Innes No. 2 compost (Keith Singletons Horticultural products, Cumbria, UK). The compost in each pot was consolidated and levelled using a wooden pot tamper.

Three Radish (*Raphanus sativus*) cultivars: 'Rudi', 'Celesta' and 'Saxa 2'; were grown under wet and dry conditions similar to determine if the effects of water availability during growth were similar for different cultivars. The seeds were planted on 15<sup>th</sup> July 2014. The

seedlings were transplanted and treatments started on 21<sup>st</sup> July 2014. The experiment consisted of 10 pots plus 3 extra dry pots for each cultivar in a randomised block design. Pots were watered by bench capillary matting for 2 minutes three times a day giving a total of 17 mm day<sup>-1</sup>. Half the trays were placed on the bench for watering and half of the trays were placed in saucers to allow them to dry down. The trays which the plants were transplanted into were at the correct volumetric water content (VWC) for the treatments to begin. Transplanting was used to ensure even germination of seedlings and to allow treatments to begin immediately without the trays requiring a period of drying down.

### ***Treatments***

Two treatments were studied: wet and dry. For the first treatment the compost in trays was maintained at high water content close to pot capacity using capillary irrigation, this was the wet treatment. The dry treatment was maintained at low water content by hand watering to a low water content which was above permanent wilting point.

### ***Harvest***

Treatments were harvested when more than 50 % of plants were 25 mm in diameter or greater. This was to ensure there were no effects on splitting due to size. The diameter 25 mm was chosen because this is the median commercial hypocotyl diameter. There were differences in rate of growth between cultivars and treatments therefore they were harvested on different days. 'Celesta' and 'Saxa 2' which were grown under wet conditions were harvested first on day 27, followed by 'Rudi' grown under wet conditions on day 29. The three dry treatments were harvested last, 'Saxa 2' was harvested on day 34 then 'Rudi' and 'Celesta' were both harvested on day 36.

### ***Statistics***

All measurements were analysed by ANOVA using Genstat 16<sup>th</sup> Edition.

## **12.3. Results**

The mean temperature of the glasshouse during the experiment was 24°C with a range of 42°C to 11°C. The mean relative humidity was 70 % ranging between 100 % and 29 %.

### ***Substrate water content***

The 'Rudi' wet treatment had an average VWC of 65.0 % with a maximum of 70.5 % and a minimum of 58.6 %. The 'Rudi' dry treatment had an average VWC of 17.2 %, a maximum of 23.3 % and a minimum of 7.8 %. The 'Saxa 2' wet treatment had an average VWC of 64.9 % with a maximum of 69.5 % and a minimum of 55.2 %. The 'Saxa 2' dry treatment had an average VWC of 16.0 %, a maximum of 23.0 % and a minimum of 8.5 %. The 'Celesta' wet treatment had an average VWC of 64.6 % with a maximum of 69.9 % and a

minimum of 58.5 %. The 'Celesta' dry treatment had an average VWC of 16.3 %, a maximum of 22.9 % and a minimum of 7.9 % (Table 12.1).

**Table 12.1** Mean volumetric water content (VWC) of the trays from the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta') during the experiment

Cultivar	Treatment	Mean VWC (%)	Max VWC (%)	Min VWC (%)
Rudi	Wet	65.0	70.5	58.6
Saxa 2	Wet	64.9	69.5	55.2
Celesta	Wet	64.6	69.9	58.5
Rudi	Dry	17.2	23.3	7.8
Saxa 2	Dry	16.0	23.0	8.5
Celesta	Dry	16.3	22.9	7.9

### **Splitting**

Substrate water content during growth had a significant effect on the number of radishes which split during growth ( $P < 0.001$ ). Splits were significantly higher for radishes grown under wet conditions. Cultivar had no effect on splitting ( $P = 0.746$ ) (Table 12.2).

**Table 12.2** Mean number of split radishes per tray at harvest for the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

Treatment	Rudi	Saxa 2	Celesta	Mean	P	L.S.D.
Wet	37.94	35.56	33.33	35.61	<0.001	5.59
Dry	1.00	6.56	10.78	6.11		
Mean	19.47	21.06	22.60	20.86		
P	0.746				0.118	
L.S.D.	6.85					9.68

### **Hypocotyl**

Radishes were harvested when the treatment reached a commercial hypocotyl harvest size rather than on a specific day. The radishes from cultivar 'Rudi' that were grown under wet conditions were harvested five days before those grown under dry condition. For the cultivars 'Saxa 2' and 'Celesta', the period between the harvest of the radishes grown under wet conditions and those grown under dry conditions was seven days.

Hypocotyl fresh weight was significantly ( $P < 0.001$ ) affected by irrigation treatment with the radishes which received more water having a greater weight. This result was consistent for

all cultivars. There was no effect of cultivar on hypocotyl fresh weight ( $P=0.189$ ) (Table 12.3).

**Table 12.3** Hypocotyl fresh weight (g) for the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

<i>Treatment</i>	<i>Rudi</i>	<i>Saxa 2</i>	<i>Celesta</i>	<i>Mean</i>	<i>P</i>	<i>L.S.D.</i>
Wet	100.9	119.7	86.6	12.4	<0.001	14.3
Dry	66.8	72.1	75.3	71.4		
Mean	83.9	95.9	80.9	86.9		
P	0.189				0.111	
L.S.D.	17.9				24.31	

Hypocotyl water content was significantly ( $P<0.001$ ) affected by irrigation treatment with the radishes which received more water having a greater water content at harvest. This result was consistent for all cultivars. There was no effect of cultivar on hypocotyl water content ( $P=0.594$ ) (Table 12.4).

**Table 12.4** Hypocotyl water content (%) for the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

<i>Treatment</i>	<i>Rudi</i>	<i>Saxa 2</i>	<i>Celesta</i>	<i>Mean</i>	<i>P</i>	<i>L.S.D.</i>
Wet	89.86	90.57	89.80	90.08	<0.001	1.02
Dry	86.84	87.40	87.68	87.31		
Mean	88.35	88.98	88.74	88.69		
P	0.594				0.660	
L.S.D.	1.25				1.76	

### Leaves

Number of leaves was not affected by irrigation treatment or cultivar (data not shown).

Leaf area was significantly ( $P<0.001$ ) affected by irrigation treatment with the radishes which received more water having a greater leaf area at harvest. This result was consistent for all cultivars. There was no effect of cultivar on leaf area at harvest ( $P=0.982$ ) (Table 12.5).

**Table 12.5** Leaf area (cm<sup>2</sup>) for the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

<i>Treatment</i>	<i>Rudi</i>	<i>Saxa 2</i>	<i>Celesta</i>	<i>Mean</i>	<i>P</i>	<i>L.S.D.</i>
Wet	199.2	211.1	204.4	204.9	<0.001	28.06
Dry	147.0	136.6	148.0	143.9		
Mean	173.1	173.9	176.2	174.4		
P	0.982				0.788	
L.S.D.	34.37					48.60

Leaf fresh weight was significantly ( $P < 0.001$ ) affected by irrigation treatment with the radishes which received more water having a greater leaf fresh weight at harvest. This result was consistent for all cultivars. There was no effect of cultivar on leaf fresh weight at harvest ( $P = 0.396$ ) (Table 12.6).

**Table 12.6** Leaf fresh weight (g) for the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

<i>Treatment</i>	<i>Rudi</i>	<i>Saxa 2</i>	<i>Celesta</i>	<i>Mean</i>	<i>P</i>	<i>L.S.D.</i>
Wet	88.7	93.2	97.0	93.0	<0.001	4.30
Dry	65.5	66.3	64.0	65.2		
Mean	77.1	79.7	80.5	79.1		
P	0.396				0.181	
L.S.D.	5.26					7.44

## 12.4. Discussion

### *The effect of irrigation on rate of growth*

Slower growth was observed for the radishes grown under dryer conditions. The cultivar 'Rudi' had a five day difference in harvest time for the two irrigation treatments but 'Celesta' and 'Saxa 2' were effected to a greater extent by the treatments as they had a seven day difference in harvest time. This finding is supported by previous research in which drought conditions were found to reduce or stop cellular division and cellular expansion in radishes (Joyce *et al.* 1983). Leaf growth was also reduced in the radishes grown under dry conditions. At harvest when the radishes grown under dry conditions had been grown for an additional five days the leaf area, number and fresh weight were all significantly less than

the results for the radishes grown under wet conditions. Smaller leaves would have resulted in a reduced photosynthetic area and may explain in part the reduced growth rate of the radish hypocotyls. As leaves are removed from the majority of radishes prior to sale in the UK, it is not thought leaf size would be of great importance to the consumer.

#### *The effect of irrigation on splitting*

The amount of splitting observed at harvest was lower in radishes grown under dry conditions despite the radishes being grown for an additional five days allowing a greater amount of time of splitting to occur. The reduction in splitting may have been due to a reduction in pressure within the hypocotyl. The radishes grown under dry conditions had lower water content at harvest ( $P < 0.001$ ) suggesting they may have had a lower turgor pressure and the cells were under less pressure making them less susceptible to splitting. However, as turgor pressure was not determined it is impossible to determine if this theory is correct. Differences in splitting susceptibility may have also been due as a result of difference in growth rate a slower growth rate may have resulted in less stress within the hypocotyl, however this would not explain the difference in postharvest splitting susceptibility. Difference in splitting during growth and in postharvest splitting susceptibility could be due to differences in cellular composition. Joyce *et al.* (1983) suggested lignin synthesis may be reduced to a lesser extent by water deficit than cell division and expansion resulting in a build-up of cell wall material. Changes in the structure and strength of cell walls may affect splitting susceptibility both during growth and postharvest as splits have been shown to propagate through cells rupturing the cell walls.

#### *Do cultivars differ significantly in their responses?*

No significant effect of cultivar was found for any of the variables measured. This knowledge is of use to growers because it suggests results from irrigation studies for one cultivar can be extrapolated to other cultivars without the requirement for additional experiments.

### **12.5. Conclusion**

- The dry watering regime reduced splitting in all 3 cultivars
- Drier plants grew more slowly than the well watered plants
- Cultivar had no effect on splitting or other measures of plant growth
- This suggests that irrigation studies for one cultivar can be extrapolated to other cultivars without the requirement for additional experiments

### **12.6. References**



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## Experiment 13 - Can drought stress change the flavour of Cos lettuce?

Jack Turner – Lancaster University

### 13.1. Background

It is well known that roots in drying soil generate abscisic acid (ABA) and this is one of the pathways controlling stomatal aperture (Wilkinson and Davies, 2010). By manipulating soil water content in the rootzone, crop transpiration can be manipulated through ABA mediated stomatal closure following transient drought stress imposed during growth, known as deficit irrigation (DI) or through alternating portions of the root zone that dry down, termed partial rootzone drying (PRD). These techniques have been successfully implemented in tree fruit and vine crops with some commercial success in South America, Southern Europe and Australia and work through reducing leaf growth, redirecting resources to fruit growth, and/or increasing radiation interception by the fruit (Feres and Sorriano, 2007).

Techniques designed to increase ABA signalling by exposing crops to periods of drought stress are more difficult to implement with field vegetable crops such as lettuce that rely on an increase in leaf biomass for yield increases (Capra *et al.*, 2008). However, growing leafy plants using DI has been reported to change the biochemical and hence flavour profile of some herbs such as basil (Ekren *et al.*, 2012) and this project was developed in discussion with G's to assess whether the use of DI or PRD could influence the flavour of Cos lettuce.

This project was developed with G's and asked two research questions:

- *Do deficit irrigation techniques reduce plant growth?*
- *Does partial rootzone drying influence the flavour of Cos leaves?*

### 13.2. Materials and method

Split pots were prepared using the following method: 1.4 litre square rose pots (Neva 2, AGK, NL) were prepared by removing a 4 cm wide x 5 cm deep rectangle from the top of one sides. Two prepared pots were positioned so the cut recesses matched up and the pots were joined and sealed along the cut edges with strong tape. The pots were filled with John Innes No. 2 substrate which was tamped down level to the rim the pot. A small hole in the compost in the centre of each paired pot above the recess was made using two fingers and a three to four week old cos lettuce transplant cv Hunter supplied by PDM was planted into the central recess and firmed in, each pot of the pair was placed in a plant saucer to contain water runoff. Sixty prepared paired pots were arranged in a fully randomised design of 6

rows of 10 pots on the right hand bench of CERC glasshouse No 4 at HAU. The bench was prepared with a polystyrene base topped with capillary matting. The pots were well watered for 8 days to establish the transplants. On day 9 watering regimes were applied to 20 pots per treatment by adding the calculated water volume gradually to the substrate surface of each pot.

### ***Irrigation treatments***

Soil moisture readings using a soil moisture meter probe DTHH2 Theta probe (Delta T Devices Cambridge) were taken daily, where possible. Water volumes lost from each pot were calculated and the required measured volume was added slowly to the surface of the soil on Monday, Wednesday and Friday each week. The following treatments were imposed:

1. Co - control (both pots irrigated to pot capacity)
2. DI - deficit irrigation (both pots irrigated to 75% pot capacity when they reached a critical deficit of 400 ml per pot)
3. PRD – partial rootzone drying (1 pot treated as Co and one pot as DI and pots switched when the DI pot reached a critical deficit of 400 ml per pot)

Each week the number of visible leaves were counted and chlorophyll measurements were done on the youngest fully expanded leaf using a Minolta SPAD 502 meter. Stomatal conductance readings were taken using a Delta T porometer AP4 (Delta T Devices Cambridge) every third day throughout the experiment. Weekly leaf counts were also recorded and leaf area of the 5<sup>th</sup> and 7<sup>th</sup> leaves were also estimated.

### ***Harvest assessments***

Plants were harvested earlier than planned after 33 days due to the presence of mildew. Each plant was cut off just above soil level then the fresh weight was recorded. The heads were then placed individually in bread bags and placed in the drying ovens at 60°C until completely dry then the dry weights were recorded. The pots and roots from five plants from each treatment were replaced in the glasshouse to dry out before the roots were separated from the compost and the root mass weighed.

### ***Taste panel***

During the harvest, five of the best lettuce heads from Co and PRD treatments were selected for the taste test. These samples were prepared in the RFA labs. Leaves were separated from the heads and pooled together for each treatment. A directional paired comparison taste test to assess sweetness and bitterness on the prepared samples was

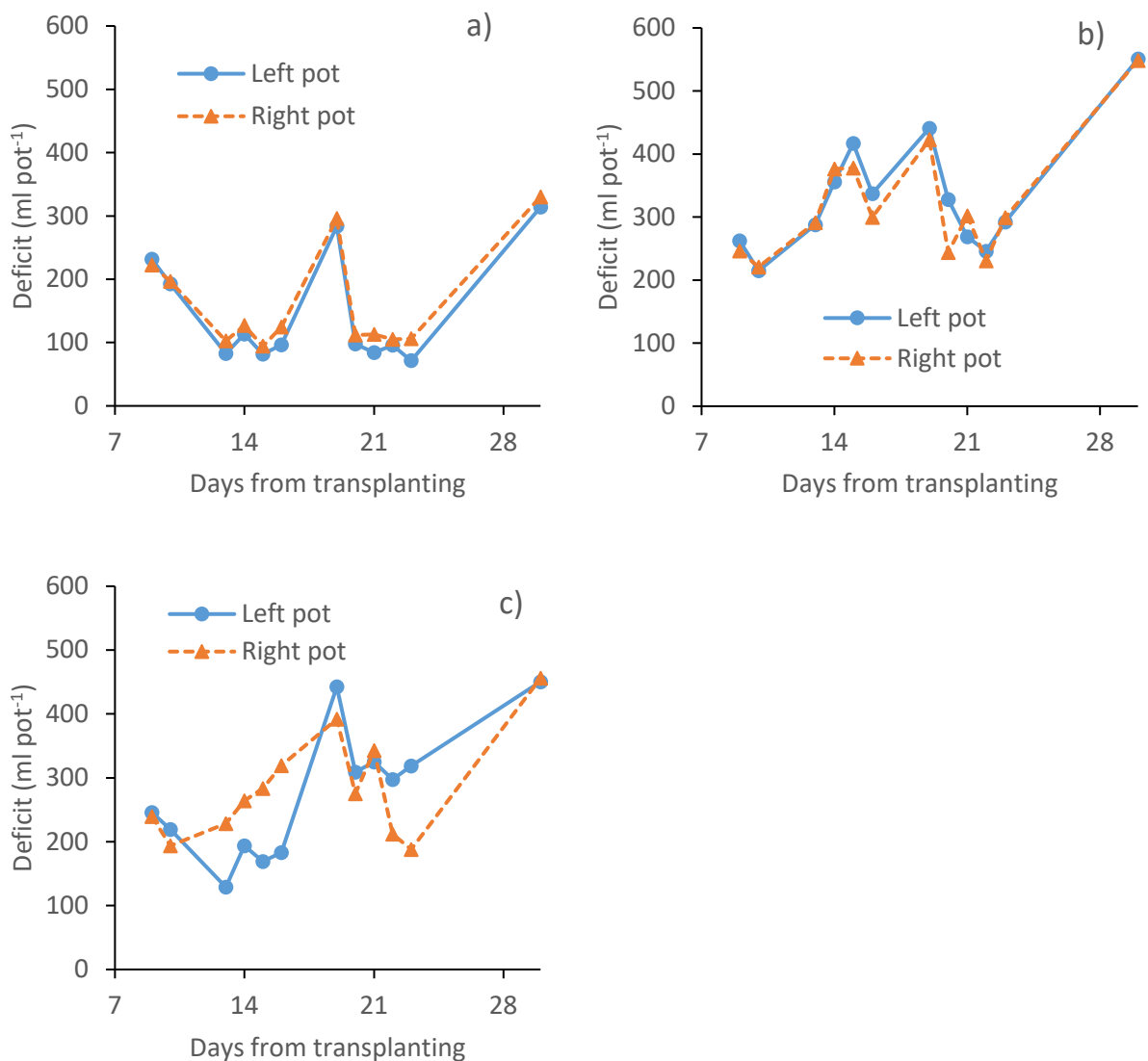
done by a panel of 21 untrained volunteers in the sensory awareness lab under controlled conditions.

### ***Statistics***

All measurements were analysed by ANOVA using Genstat 16<sup>th</sup> Edition.

### **13.3. Results**

The treatments generated different patterns of deficit in the pots (Figure 13.1a-c). The left and right pots in the Co treatment had similar deficits. Deficits developed over each weekend but were returned to pot capacity and showed that plants were using approximately 200 ml per day (i.e. 100 ml each for the left and right pots, respectively) (Figure 13.1a). The DI treatment showed no difference between the left and right pots but had a greater deficit (i.e. was dryer) than the control (Figure 13.1b). The PRD treatment shows a different pattern of deficit for the left and right pots with the right pot dryer from day 13-19 and the left pot dryer from day 22 to harvest (Figure 13.1c).



**Figure 13.1.** Average water deficit (ml) from pot capacity for left and right paired pots for a) control, b) deficit irrigation and c) partial rootzone drying treatments.

### **Plant growth**

The fresh weight of the harvested heads differed significantly between Co and DI treatments (Table 13.1). The Co treatment producing significantly heavier heads (236.0g) than DI (205.8g); PRD produced heads of an intermediate fresh weight (221.6g) that were not significantly different from either of the other treatments (Table 13.1). The dry weight of the heads followed a similar pattern (data not presented).

The number of leaves produced during the experiment was reduced by the irrigation treatments compared to the Co. The reduction was significant for DI with an average of 2.4 fewer leaves produced per plant compared to control plants which had an average of 9

leaves per plant (Table 13.1). The PRD plants had an average of 0.5 fewer leaves than the control plants but this was not significant. The area of the fully expanded leaf 7 showed no significant differences between treatments (Table 13.1). Stomatal conductance was reduced in DI plants from Day 12-19 but only significantly less than Co, on Day 19 (Table 13.2).

**Table 13.1** Average fresh weight of the lettuce heads at the end of the experiment. Different letters indicate that values are significantly different ( $P < 0.05$ ).

Treatment	Fresh weight (g plant <sup>-1</sup> )	Change in leaf number from day 6-21 (leaves plant <sup>-1</sup> )	Estimated area of leaf 7 (cm <sup>2</sup> )
Co	236.0 b	9.0 b	299 a
DI	205.8 a	7.6 a	268 a
PRD	221.6 ab	8.5 ab	263 a
Mean	221.1	8.4	277
SE	10.2	0.4	14.8

**Table 13.1** Stomatal conductance from day 6 to 30. Different letters indicate that values are significantly different ( $P < 0.05$ ).

Treatment	Stomatal conductance (mmol m <sup>-2</sup> s <sup>-1</sup> )							
	Day 6	Day 12	Day 14	Day 16	Day 19	Day 21	Day 23	Day 30
Co	186 a	660 a	522 a	529 a	504 b	278 a	218 a	187 a
DI	215 a	597 a	421 a	404 a	320 a	272 a	230 a	162 a
PRD	196 a	569 a	518 a	513 a	345 ab	297 a	248 a	174 a
Mean	199	609	487	482	390	283	232	174
SE	24.1	45.1	45.5	56.2	75.3	22.2	26.1	23.1

### ***Taste panel***

The tasters were unable to identify significantly the different leaves correctly when presented as one leaf different and two leaves the same in a choice of three leaves. Only 3/21 tasters identified the correct leaf i.e. the taste differences were not clear. However, when the panel was given a directed choice of four responses 10/21 chose the response that the PRD samples were sweeter than Co. This was not significant as 15 correct choices were required. Fewer tasters (3/21) identified the PRD samples as being more bitter.

### **13.4. Discussion**

#### *Did treatments give different patterns of drought stress?*

Compared to the well watered controls both DI and PRD treatments generated different deficits in the pots. The DI treatment generated larger deficits in both pots than the drying pot in the PRD pair, suggesting that the well watered paired pot in the PRD treatment was supplying a disproportionate amount of water to the growing plant i.e. the DI paired pot was not being depleted of water as rapidly.

#### *Did treatments affect plant growth?*

The stomatal conductance values showed that the stomata were observed to be responding to drought stress on only one day. It may be that either the drought stress was limited or that stomata only respond at severe stress. The DI plants were significantly affected by the reduced water availability. Compared to the Co plants they had significantly reduced Fresh Weight, and fewer leaves at the end of the experiment. Interestingly, the PRD plants were similar to the Co plants in both fresh weight, and leaf number as although both values were smaller they were not significantly different.

#### *Did PRD influence the flavour of Cos leaves?*

The taste panel was unable to distinguish between PRD and Co leaves for the overall taste profile. However, when given a directed choice half the tasters identified that the Cos grown with PRD was sweeter. This result is not significant but suggests that this may be an area of further work. The taste panel was untrained and the number of tasters was relatively small for a study of this nature and it may be that subtle differences could be detected more clearly in a larger panel of trained tasters.

### **13.5. Conclusion**

- Lettuce yield was reduced significantly but DI but not by PRD
- The taste panel identified a trend that plants grown with PRD were sweeter than well watered plants but this response was not significant.

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## **Experiment 14 - Stress priming kale – does it lead to more resilient plants?**

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### **14.1. Background**

There is a considerable background literature to support the hypothesis that preconditioning plants with a stress treatment provides a beneficial effect protecting from further stresses. Drought treatments of transplants of broccoli and other vegetables have been shown to improve future growth and stress resilience (e.g. Latimer, 1990). Bruce *et al.* (2007) describes priming, or hardening, as the initial exposure to abiotic or biotic stress to enhance resistance to the stresses later in the plants development. Such plants have a much stronger defensive response to later events of the stress event, forming what Bruce *et al.* (2007) describes as a stress memory.

Literature on this topic is mainly related to the priming of seeds but, in a recent HDC report, Mulholland (2013) showed that a brief treatment of cauliflower transplants/seedlings with salt resulted in increased resistance to pests and pathogens. An unexpected observation was that some salt treatments led to greater leaf and root growth compared to the controls and also improved subsequent yield and quality. The underlying basis of the growth benefit observed by Mulholland (2013) is not clear but has significant commercial potential.

Kale (*Brassica oleracea* v. *acephala*) was chosen as a model for this project as there is limited research done on this crop, as well as the fact that it has a fast turn over time in terms of growth rate.

This project was developed with help from Elsoms Seeds and asked the question:

- *Does salt stress in the module stage confer drought stress resilience in later growth*

### **14.2. Materials and Methods**

#### ***Seedling production***

Elsoms Seeds provided *Brassica oleracea* black kale E33706 seed for this experiment. Separate 30cm x 30cm cut down units of 345 modules were filled with John Innes No. 2 substrate. Single seeds were sown into each module. The module units were placed on capillary matting squares in trays. The seedlings were grown on in the glasshouse for 2-3 weeks until they reached 2 -3 true leaf stage with standard watering.

### ***Polytunnel layout***

Forty 30 litre black pots (H33 cm Ø40 cm) were filled with a power harrowed, de-stoned field soil on top of a base layer of gravel. The pots were arranged in a randomised block of 10 pots in 4 rows on a geotex membrane in a polytunnel protected with insect proof netting along the sides and ends. Watermark sensors (IRR Meter Company Inc.) were fitted into 8 tubs to a depth of 5cm below the soil surface to monitor the soil moisture between the different treatments. Previous work had established the field capacity of the experimental soil as 23% volumetric moisture content.

### ***Salt stress treatment***

A 100mM NaCl (5.844g NaCl l<sup>-1</sup>) solution was prepared to prime the kale seedlings, with deionised water used for the control priming. Each unit was immersed into 1 litre of 100mM NaCl for 5 minutes every day for 4 days and grown on for 3 days with watering on capillary matting with dH<sub>2</sub>O, before transplanting.

### ***Transplanting***

Two primed and two controlled seedlings per tub were transplanted on day 9 into the prepared tubs at an equal spacing of 12 cm. After the plants had established for one week the following watering treatments were imposed over a six week period

### ***Treatments:***

The following treatments were studied:

- Control – aim to maintain ~20% moisture content in the soil
- Drought – not watered
- Waterlog 1 – watered to >5% above field capacity (28% moisture content in soil from day 22 – 33 after salt stress treatment)
- Waterlog 2 – watered to >5% above field capacity (28% moisture content in soil from day 36 – 45 after salt stress treatment)

### ***Measurements***

Soil moisture readings to calculate the water volumes to add to the treatments were taken daily using Watermark Sensors and a Field Scout TDR100 (Spectrum Technologies Inc.) fitted with a 12cm probe. Water volumes were calculated for each tub and the measured volume was added slowly to the surface of the soil on Monday, Wednesday and Friday each week.

Stomatal conductance was measured twice weekly on Tuesday and Friday using an AP4 Porometer (Delta-T Devices) and daily SPAD recording were made using Minolta SPAD-502 chlorophyll meter. The number of visible leaves were also counted weekly.

At the end of the trial, 47 days after starting the experiment, the kale was harvested and fresh weights were recorded. The plants were separated for measurement of leaf number and size. The kale was then bagged and oven-dried at 60°C for dry weights.

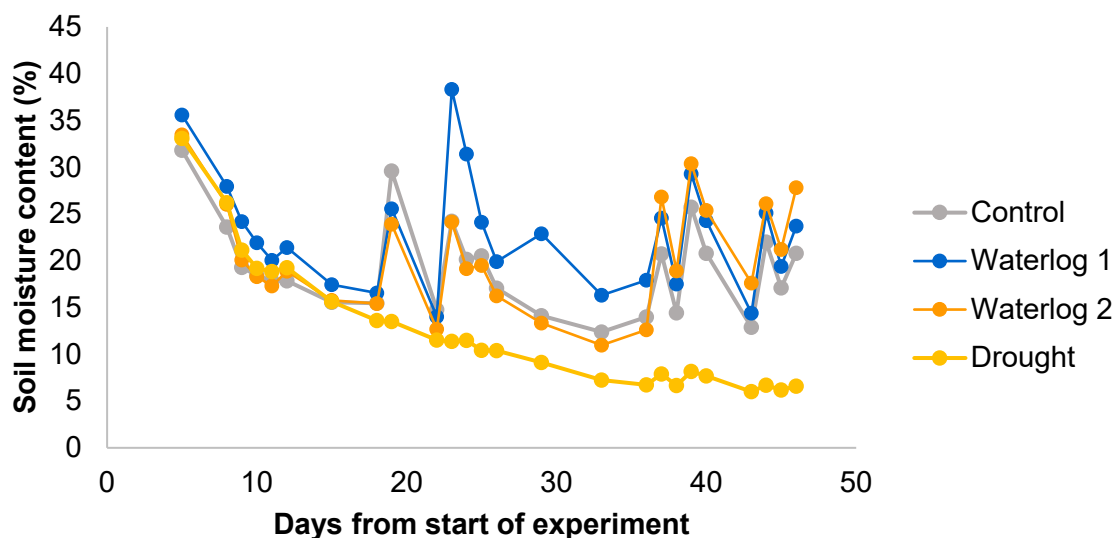
### Statistics

All measurements were analysed by ANOVA using Genstat 16<sup>th</sup> Edition.

## 14.3. Results

### *Soil moisture content*

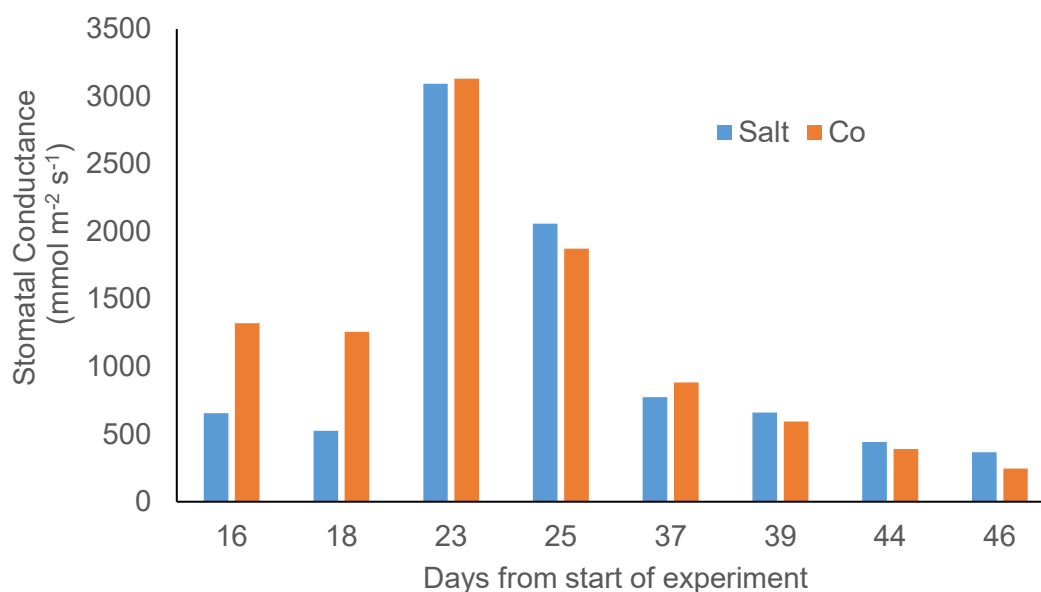
Following an initial heavy irrigation prior to planting the moisture content in the pots declined towards the estimated FC of 23% by day 10 (Figure 14.1). The drought treatment received no further water and moisture content declined to approximately 5% by the end of the trial. The control treatment varied throughout the trial as a consequence of more variable irrigation by the student than was hoped for. The waterlogging treatments showed a markedly higher moisture content than control for waterlog 1 but less so for waterlog 2.



**Figure 14.1.** Soil moisture content over the duration of the trial

## **Stomatal conductance**

Following transplanting the control (well watered) plants that had been exposed either to salt stress or not in the seedling stage showed a difference in stomatal conductance at day 16 and 18 after the seedlings were treated (Figure 14.2). The plants that had received the salt pre-stress had a lower conductance suggesting that the stomata were more restricted. By day 23 there was no difference between the seedling treatments and this relationship continued to the end of the experiment.



**Figure 14.2.** Stomatal conductance of plants exposed to salt stress at the seedling stage before transplanting into control treatment pots.

## **Plant growth**

### *Effect of pre-stress treatments*

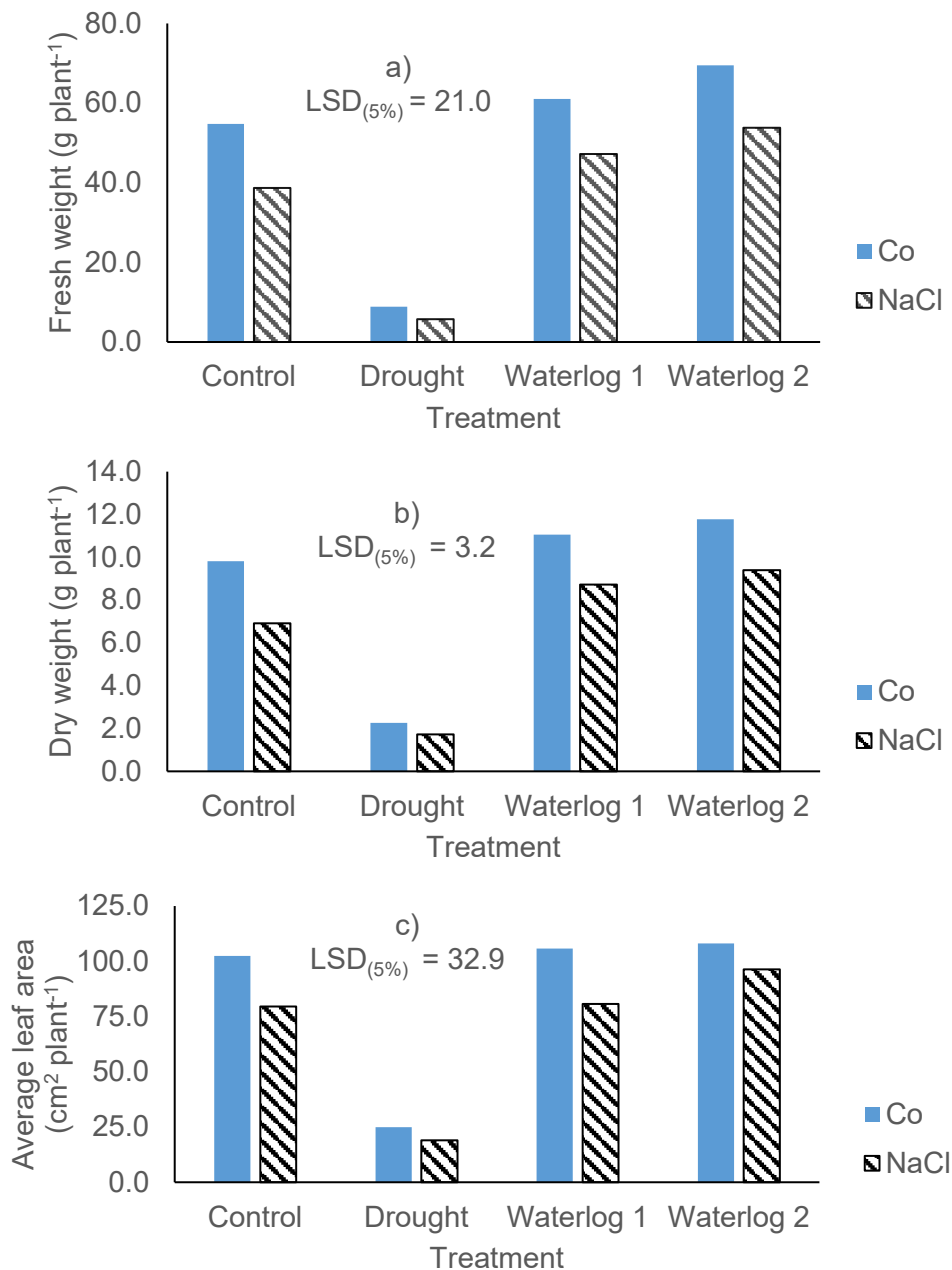
Overall, pre-stress treatment had a significant effect on the fresh weight, dry weight and leaf area of the plants by the end of the experiment (Table 14.1). In all three variates the salt pre-stress treatment led to significantly smaller values, being approximately 25% smaller for each.

**Table 14.1.** Main effect of pre-stress treatment on plant growth variates.

Pre-stress treatment	Fresh wt (g plant <sup>-1</sup> )	Dry wt (g plant <sup>-1</sup> )	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )
Co	48.5	8.73	85.3
Salt	36.4	6.69	68.9
<i>Mean</i>	42.5	7.71	77.1
<i>SE</i>	10.5	1.61	16.5
<i>P value</i>	0.02	0.01	0.05

#### *Effect of growing treatments*

The control, well watered, treatments had significantly greater fresh weight, dry weight and average leaf area compared to the drought treatments (Figure 14.3 a-c). Both waterlogging treatments had no significant effect compared to control. By the end of the experiment the growth differences between the pre-stress treatments within each growing treatment were not significant but the trend was consistent as discussed previously and no interactions were observed.



**Figure 14.3.** Graphs showing a) Fresh weight, b) Dry weight and c) Average leaf area of the plants after 47 days. LSD<sub>(5%)</sub> are included for each figure.

#### 14.4. Discussion

Most stress tolerance studies to date have involved prolonged stress treatments during growth. The brief (1-2 week) period of stress investigated here is more comparable to conditions UK crops experience in the field.

*Does salt stress in the module stage affect plant growth?*

Salt stress of seedlings during the module phase affected plant growth significantly leading to smaller leaves and less biomass by the end of the trial. This was in contrast to the results with cauliflower reported by Mulholland (2013) where salt stress led to larger plants. In this experiment the limited time did not allow optimisation of the treatments although preliminary work (not reported) indicated that a root dip of 100mM NaCl for 5 minutes daily was sufficient stress the seedlings without leading to leaf yellowing. This experiment shows that the level of salinity was too high for the seedlings. Further work is needed to optimise salinity treatments for seedlings to establish whether a lower level of stress can confer benefits to kale plants.

#### *Does salt stress affect responses to drought or waterlogging in the transplanted crop?*

The response to drought and waterlogging was consistent for both pre-stress treatments. Drought led to a marked reduction in plant growth as would be expected. However, the drought was extreme with water withheld from transplanting. The waterlogging treatments did not show any effect on kale growth and soil moisture readings suggest that moisture content was not close to saturation with soil moisture levels higher at the start of the experiment than most days following i.e. the soil was not waterlogged as hoped. This could be due to a number of factors. The soil was recently disturbed through filling the tubs and was not compacted and would be relatively free draining. The student irrigated the pots three times a week and there is a suggestion that the levels of water applied were inconsistent through human error.

An interesting observation from the experiment was that stomatal closure in response to salt stress was observed until day 23 suggesting that the physiological response to the seedling treatments persisted for at least 14 days.

#### **14.5. Conclusion**

- Salt pre-conditioning led to smaller kale plants after 5 weeks growth.
- Physiological response to seedling salt stress was observed for two weeks after transplanting.
- Overall, a pre-conditioning salt treatment is not beneficial to plants growing on in the range of environments studied.

#### **14.6. References**

Bruce, T. J. A., Matthes, M. C., Napier, J. A. and Pickett, J. A. (2007). Stressful “memories” of plants: Evidence and possible mechanisms. *Plant Science*, 173 (6), pp. 603-608.

Latimer, J. G. (1990). Drought or mechanical stress affects broccoli transplant growth and establishment but not yield. *HortScience*, 25(10), 1233-1235.

Mulholland, B. (2013) Preadaptation of vegetable seedlings to increase their resistance to pest attack. *Final report on HDC project FV402, 99pp.*