Horticultural Fellowship Awards

Final 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:	Final report, March 2016
Previous report:	Annual report, May 2012 Annual report, March 2013 Annual report, March 2014 Annual report, March 2015
Fellowship staff:	Carol-Ann Woolley and 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	31 March 2016

DISCLAIMER

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.

Dr Jim Monaghan

Fellow

Harper Adams university

Signature ....   Date ......10/03/2016.............................

Dr Andy Wilcox

Head of CES Department

Harper Adams University

Signature ....Date ....11/03/2016................................

CONTENTS

Progress against Objectives and Annual Milestones

Summary of Overall Progress Years 1-5

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	31/03/2016	
2. Deliver a minimum of 15 small-scale research projects for the industry.	31/03/2016	31/03/2016	
3. Publicise the approach and outputs of the programme to the industry, Further Education and Higher Education Institutions.	31/03/2016	31/03/2016	
4. Leverage additional funding for follow up projects.	31/03/2016	31/03/2016	

Objective 1. Recruit a minimum of 15 undergraduates from UK Higher Education Institutions to complete applied experiments in horticultural crop production and agronomy.

This objective was achieved. Eighteen students were recruited to the SRP from 12 universities. The 'home' universities and subsequent destinations following graduation of the students are shown in Table 1.1. The students were selected as having interest in further work or research within the fresh produce and horticulture sector but students with little prior experience of the sector were prioritised in the selection process.

Thirteen of the students are now working, or are intending to work with fresh produce and horticulture commercially or through research. Of these, 5 students have taken roles or have been accepted for roles at a graduate level in the industry e.g. MDS or company graduate scheme. Six of the students are pursuing a PhD in plant sciences with two students undertaking more applied work. Of the students working in the sector, all of them have stated that the SRP helped them when choosing a career in the sector.

> *"It has really made me consider going into the fresh produce sector - it is so much more different to conventional agriculture than I thought it would be, and to experience all that we did whilst at Harper and on the visits has made me very interested in it - I've even signed up to a Fresh Produce newsletter"* (Student from the 2015 crop).

Table 1.1. Post-graduation destinations of the Summer Research Programme students 2011-2015. Those students working in or intending to work in a role involved with fresh produce or horticultural crops are highlighted.

Year	Student	Undergraduate Studies	Destination following graduation
2011	Christina Bosworth	Newcastle University – BSc Agriculture	• Graduate Trainee MDS • Currently a Technical Graduate at Produce World
2011	Amy Davies	Newcastle University – BSc Agriculture	• Graduate Trainee MDS • Area Manager at British Sugar
2011	Isabel Webb	University of Cambridge – B.A. Natural Sciences (Plant Science)	• PhD in Molecular Microbiology at Oxford University/John Innes Centre on rhizobia-legume symbiosis.
2012	Rachel Carpenter	Bangor University – MEnvSci Environmental Science	• G's Graduate Scheme • NPD Concept Technologist at Bokomo Foods UK Ltd
2012	John Vaugh-Hirsch	Royal Holloway, University of London – BSc Biology	• PhD Student (funded by the BBSRC) investigating the cytokinin signally pathway in rice.

2012	Lara Boucher	Exeter University – BSc Biological Sciences	• Graduate Trainee MDS • Technical Manager at Riverford • Research Analyst at Good Growth Ltd
2013	William Johnson	Bangor University - MEnvSci Environmental Science	• Roehampton University – Environment and communications intern. **Aiming to get into Horticulture** (applied for post at Cambridge Botanic Gardens)
2013	Kathryn Hales	Exeter University – BSc Biological Sciences	• PhD Student at University of Warwick – Cavity spot in carrots.
2013	Jonathan Harvey	Lancaster University – BSc Environmental Biology	• Lancaster University – MSc International Innovation, Environmental Biology • Environmental Consultant at Stopford Energy and Environment
2013	Iain Place	University of Manchester – BSc Plant Sciences	• Integrated Pest Management (MSc) at Harper Adams University
2014	Samantha Ball	Bristol University – BSc Biology	• Post Grad Diploma in Agriculture at RAU. **Considering roles in Agronomy**.
2014	Liam Elliott	University of Cambridge – Natural Sciences (Plant Science)	• DPhil Student in Interdisciplinary Bioscience DTP at the University of Oxford

2014	Jack Turner	Lancaster University – BSc Environmental Biology	• PhD Student at Lancaster University – Water Use Efficiency in crops
2014	Emma Micklewright	University of Oxford – Biology/Biological Science	• Assistant Language Teacher at JET, in Japan. **Intends to pursue research on return.**
2015	Ryan Douglas	University of Edinburgh – BSc Biological Sciences (Plant Science)	• Applied to MDS and awaiting interview.
2015	Suzannah Franks	Newcastle University – BSc Agriculture	• Work experience at Marks and Spencer within the fresh produce sector following HAU placement • Graduate Trainee MDS (2017)
2015	Amy Newman	University of Bristol - MSc Biology	• PhD studentship at Warwick investigating circadian rhythms in plant-microbe interactions in the rhizosphere (Oct 2016)
2015	Nick Kuht	University of Leeds – BSc Biology	• Nothing organised yet.

Objective 2. Deliver a minimum of 15 small-scale research projects for the industry.

This objective was achieved. Eighteen small-scale research projects were completed and are listed below:

1. How best to inoculate lettuce with bacteria that are antagonists to E.coli?
2. Can we improve the shelf life of babyleaf spinach by stressing the plant with high EC water?
3. Does irrigation regime change the rooting pattern of leafy veg?
4. Does biochar addition improve growth of young containerised apple trees?
5. Does peat-free growing substrate significantly affect strawberry growth and yield?
6. Does irrigation regime affect rooting pattern of onion sets?
7. Does lettuce variety affect the performance of the generalist aphid pest *Myzus persicae*?
8. Can biochar products improve growth and/or quality in HONS?
9. Does radish hypocotyl water content affect susceptibility to post-harvest splitting?
10. Can a calcium foliar spray improve yield or post-harvest quality in strawberries?
11. Effect of vine weevil on strawberry yields in first and second year crops
12. The effect of variety and Irrigation on Splitting in Radishes
13. Can drought stress change the flavour of Cos lettuce?
14. Stress priming kale – does it lead to more resilient plants?
15. Stress responses of *Brassica oleracea*
16. A systems approach to disease resistance against necrotrophic fungal pathogens
17. Investigating the effects of relative water content on pinking in whole head lettuce (*Lactuca sativa*)
18. Assessing the effectiveness of control measures for vine weevil (*Otiorhynchus sulcatus*) damage of strawberry

The results of the work have been reported in the annual reports and all students presented their work to AHDB Horticulture representatives and businesses during their placement at HAU.

Objective 3. Publicise the approach and outputs of the programme to the industry, Further Education and Higher Education Institutions.

This objective was achieved.

1. A webpage and Facebook site was set up for the SRP and contains videos of each project.

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

https://www.facebook.com/HAUFreshProduce

2. The fellow was invited to present a student tutorial session at the Gatsby Plant Science Summer School, York, in 2014 and promoted jobs in the fresh produce and horticulture industry to first year plant science undergraduates as well as the SRP scheme.
3. The process of advertising the scheme led to direct contact with 30 universities and colleges. It is assumed that this interaction raised the profile of applied research opportunities within the area of fresh produce and horticulture.
4. Four news articles have been produced in AHDB publications which have raised the profile of the scheme within the industry.
    - HDC News. *University Challenge*. May 2015, pp 28-30.
    - HDC News. *Experts in the making*. September 2014, p13.
    - HDC News. *Tomorrow's World*. September 2013, pp14-15.
    - HDC News. *A taste of horticultural technology*. May 2013, pp16-18.

5. In addition, the SRP included visits to the following 19 businesses over 5 years covering a wide range of crops. A number of these businesses hosted repeat visits:

PDM (leafy salads), Lower Reule Farm (soft fruit), Elsoms Seeds (seeds), G's (field vegetables), Produce World (potatoes), Cornerways Nursery (tomatoes). FP Matthews (trees and HONS), Vitacress (field vegetables), VHB Herbs (herbs and tomatoes), Walberton and Binstead Nurseries (ornamentals), Eric Wall Nursery (tomatoes), Garden Organic – Ryton (organic production), Warwick Genebank (seed collections), H&H Duncalfe (soft fruit), Sutton Bridge Crop Research (postharvest research), MMUK (fruit and flowers), ADAS, Boxworth (crop research), Bakkavor Alresford Salads (watercress), Barfoots of Botley (sweetcorn and cucurbits).

Objective 4. Leverage additional funding for follow up projects.

This objective was achieved. A number of the research projects completed by the students produced outputs that contributed to on-going projects at HAU funded by BBSRC, Defra and AHDB Horticulture. Eight successfully funded research proposals have utilised research findings and/or method development completed by the summer students:

1. Additional funding was obtained from Elsoms Seeds for a summer student in 2012 who completed work on a phenotyping study in leafy salads
2. Syngenta funded PhD – Leafy vegetables: Resilient roots for rapid establishment under variable soil conditions. In collaboration with University of Nottingham.
3. BBSRC HAPI – A genetic approach to improving post-harvest quality. In collaboration with Reading and Warwick University, G's, Bakkavor and Rijkzwaan.
4. NERC – Developing a drought narrative resource in a multi-stakeholder decision-making utility for drought risk management. In collaboration with UWE and CEH.
5. Defra – Vegetable Genetic Improvement Network (VEGIN). In collaboration with Warwick University.
6. AHDB Horticulture – Inducing compact growth and improved shelf life in herbs by mimicking drought signals. Inducing compact growth and improved shelf life in herbs by mimicking drought signals (PE015).
7. AHDB Horticulture – Improving vine weevil control in hardy nursery stock (HNS 195). In collaboration with ADAS, University of Warwick and Natural Resources Institute.
8. AHDB Horticulture – Improving integrated pest management in strawberry (SF 156). In collaboration with EMR, Natural Resources Institute, CABI, ADAS, Keele University and Fera.

A proposal led by Dr Mark Else (NIAB-EMR) has been submitted this year to AHDB Horticulture in the area of precision and deficit irrigation for lettuce. This proposal incorporates findings from the SRP.

Summary of Progress Year 5

The final year of the Summer Research Programme (SRP) was undertaken by four UK undergraduates from Edinburgh, Bristol, Leeds and Newcastle University. The students undertook four separate research projects at HAU linked with G's, Tozers, Bulrush Ltd and BASF Agricultural Specialities Limited, 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 AHDB Horticulture. The students also made a number of visits to businesses including strawberry, leafy salad, field vegetable and protected salad producers.

More detailed descriptions 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 15-18 are reported here.

Experiment 15 - Stress responses of* Brassica oleracea *(Ryan Douglas – Edinburgh University)

This project developed a protocol to allow large numbers of brassica seedlings to be analysed for their growth responses to three different abiotic stresses (cold, drought and waterlogging). This will allow the identification of brassica lines exhibiting increased stress tolerance that can be used in future crop breeding approaches.

There is some data from previous studies of abiotic stress responses in brassicas. In broccoli (*B. oleracea* var. Italica), chilling can increase bud stalk length and diameter, mature head diameter and weight (Kalisz *et al.,* 2014). Hadi *et al*., (2014) used polyethylene glycol (PEG) and mannitol treatments to examine the drought response of cauliflower (*B. oleracea* var. botrytis). Increasing concentrations of either treatment reduced seed germination, shoot and root length and biomass. In Chinese kale *(B. oleracea* var. alboglabra), both water deficit and waterlogging reduces leaf area, fresh and dry weight and leaf number, with drought leading to darker leaves and closed stomata (Issarakraisila *et al.*, 2007).

Ryan studied the response of young plants of three brassica lines (kale ‘nero di Toscana’, cabbage ‘greyhound’ and red cabbage ‘calibos’) to differing severities of cold, drought and waterlogging stress in order to determine a suitable stress level that will identify differential levels of response between the three lines.

Ryan concluded that:

- Red cabbage appeared to be more tolerant of salt stress compared to the other two lines, while for flood stress, kale appeared to be the most tolerant, followed by red cabbage.
- The results of the drought experiment are harder to interpret, however, the responses still differ between the lines.
- The range and severity of stresses tested here for salt (four days stress), drought (3 days stress) and flood (six days stress) can be used to screen further *B. oleracea* lines for tolerance to these abiotic factors and to identify lines with increased tolerance that will prove useful in downstream breeding programmes.

Experiment 16 – A systems approach to disease resistance against necrotrophic fungal pathogens (Amy Newman - University of Bristol)

This project was part of a larger project looking to identify novel alleles for increasing the resistance of lettuce to *Botrytis cinerea* to facilitate downstream breeding approaches.

B. cinerea causes substantial losses on field-grown and protected lettuce crops, an industry worth almost £200M/yr in the UK. Chemical control of *B. cinerea* is problematic due to spraying restrictions and the development of resistance to fungicides. Development of host resistance, meanwhile, may provide a more sustainable solution, but has been difficult for breeders to achieve.

Developing lettuce varieties with resistant to *B. cinerea* could present considerable economic benefits. For example, a 50% reduction in disease could provide significant savings both from reduced crop losses and lower spraying costs, while also providing environmental benefits.

Amy studied a Diversity Set of 96 lettuce accessions sourced from Warwick Crop Centre. These accessions were tested for *B. cinerea* resistance using a detached-leaf assay.

Amy showed that:

- Increasing whole plant age reduces *B. cinerea* lesion size, suggesting increased resistance of the host with age.
- This result is not found with leaves of different ages belonging to the same plant and so appears to occur at the whole plant level.
- The lines of the Lettuce Diversity Set show different levels of susceptibility to *B. cinerea*, indicating that this population may provide a useful genetic resource in breeding for resistance to this pathogen.

Experiment 17 - Investigating the effects of relative water content on pinking in whole head lettuce* (Lactuca sativa) *(Suzannah Franks - Newcastle University)

Pinking of lettuce tissue is serious concern to the fresh produce industry, causing extensive losses due to customer rejection of product with a poor appearance. Pinking is thought to represent a physiological response of the plant to wounding, whereby a series of enzymatic reactions lead to the production of pigments responsible for colour changes in tissues.

Pinking often occurs on lettuce ribs, a part of the plant most frequently exposed to damage during harvest and transport. There is evidence to suggest that the water content of lettuce affects the degree of pinking that will occur after harvest. This project seeks to clarify this relationship and determine whether this represents a result of the effects of water content on the tendency of the plant to undergo wounding.

Suzannah produced lettuce heads with differing relative water contents by taking harvested heads and either wilting them or rehydrating them and then impacting the lettuce ribs under controlled conditions.

Suzannah concluded that:

- Rib cracking following mechanical impact tends to be greater in lettuces that have a greater relative water content.
- Rib pinking is correlated to rib cracking.
- Actions that prevent mechanical damage to lettuce ribs will reduce rib pinking.

Experiment 18 - Assessing the effectiveness of control measures for vine weevil (Otiorhynchus sulcatus) *damage of strawberry* (Fragaria ananassa) *plants. (Nicholas Kuht - University of Leeds)*

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/year. However, such calculations are based on expert opinion rather than results from carefully designed experimental approaches.

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). In contrast, growers are currently reliant on the use of insecticides, such as the broad spectrum pyrethroid insecticide lambda-cyhalothrin (e.g. Hallmark) for the control of vine weevil adults in soft fruit crops. Application of these insecticides against this pest is difficult, as it is recommended that they are applied at dusk, when the weevils become active. In addition, applications of broad spectrum insecticides such as pyrethroids 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

Nick 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 (an epn drench or incorporation of an epf into the growing media) on overwintered vine weevil larvae. Nick concluded that:

- While Met52 did not affect plant height, fresh or dry weight, fruit production or fruit sugar content, it lead to significantly paler leaves and did not provide effective control of leaf notching or larval numbers but did appear to prevent root damage.
- The introduction of vine weevil eggs in the absence of a control measure, while leading to significantly increased numbers of both adult and larval weevils and leaf notching, did not affect plant height, fresh or dry weight, fruit production or fruit sugar content. A higher number of weevil eggs, larvae or adults may be required to see significant effects on these parameters and to be able to determine if the control measures can prevent such effects.

Visits by students

Six businesses hosted visits by the students: PDM (lettuce), Lower Reule Farm (strawberries), G's (field vegetables and mushrooms), Bakkavor Alresford Salads (watercress), Barfoots of Botley (sweetcorn and cucurbits), Eric Wall Nursery (tomatoes).

Milestones Year 5

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 AHDB Horticulture Research Manager.	31/05/2015	31/05/2015	
2. Commence experimental work.	31/05/2015	31/05/2015	
3. Complete mail shots and selected visits to other institutions.	31/05/2015	31/05/2015	
4. Recruit SRP students	06/04/2015	06/04/2015	
5. SRP students start	06/07/2015	06/07/2015	
6. SRP students finish	21/08/2015	21/08/2015	
7. Research reported to HDC	31/03/2016	31/03/2016	

Milestones not reached

N/A

Training undertaken

No training was undertaken by the Fellow in Year 5.

Expertise gained by Trainee

N/A

Other achievements in the last year not originally in the objectives

None

Grower Summary

Overall (Years 1-5)

Eighteen students were recruited to the Summer Research Programme (SRP) at HAU from 12 universities. The students were selected as having interest in further work or research within the fresh produce and horticulture sector but students with little prior experience of the sector were prioritised in the selection process. The students worked on 18 applied research projects that were each linked with commercial businesses ensuring that the work was of relevance to the industry. Each year the students visited a number of fresh produce and horticulture businesses during the SRP. Overall, 19 businesses were visited over 5 years covering a wide range of crops. A number of these businesses hosted repeat visits:

PDM (leafy salads), Lower Reule Farm (soft fruit), Elsoms Seeds (seeds), G's (field vegetables), Produce World (potatoes), Cornerways Nursery (tomatoes). FP Matthews (trees & HNS), Vitacress (field vegetables), VHB Herbs (herbs & tomatoes), Walberton & Binstead Nurseries (ornamentals), Eric Wall Nursery (tomatoes), Garden Organic (organic production), Warwick Genebank (seed collections), H&H Duncalfe (soft fruit), Sutton Bridge Crop Research (postharvest research), MMUK (fruit & flowers), ADAS, Boxworth (crop research), Bakkavor Alresford Salads (watercress), Barfoots of Botley (sweetcorn and cucurbits).

Thirteen of the students are now working, or are intending to work with fresh produce and horticulture commercially or through research. Of these, 5 students have taken roles or have been accepted for roles at a graduate level in the industry e.g. MDS or company graduate scheme. Six of the students are pursuing a PhD in plant sciences with two students undertaking more applied work. Of the students working in the sector, all of them have stated that the SRP helped them when choosing a career in the sector.

Headline

A Summer Research Programme (SRP) ran from 2011 to 2015 and was based at Harper Adams University (HAU) and led by Jim Monaghan. The SRP recruited 18 UK undergraduate students from 12 universities. These students carried out applied agronomy/crop production research projects within the Fresh Produce Research Centre and were supported by research staff associated with the centre.

Year 5

The final year of the Summer Research Programme (SRP) was undertaken by four UK undergraduates from Edinburgh, Bristol, Leeds and Newcastle University. The students undertook four separate research projects at HAU linked with G's, Tozers, Bulrush Ltd and BASF Agricultural Specialities Limited, 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 and protected salad 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 15-18 are reported here.

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.

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 containing 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 completed in 2015 are appended to this report:

Experiment 15 - Stress responses of *Brassica oleracea*

Experiment 16 – A systems approach to disease resistance against necrotrophic fungal pathogens

Experiment 17 - Investigating the effects of relative water content on pinking in whole head lettuce (*Lactuca sativa*)

Experiment 18 - Assessing the effectiveness of control measures for vine weevil (*Otiorhynchus sulcatus*) damage of strawberry

Experiment 15 - Stress responses of *Brassica oleracea*

Ryan Douglas - University of Edinburgh

15.1 Introduction

Because of a number of factors including climate change and the increasing global population and therefore demand for food, it is important to develop new crop varieties that are more tolerant of extreme conditions.

Brassicas represent a large group of crops, which for the species *Brassica oleracea* includes cabbage, cauliflower, broccoli, kale, sprouts and others. This project will aim to develop a protocol to allow large numbers of brassica seedlings to be analysed for their growth responses to three different abiotic stresses (cold, drought and waterlogging). This will then allow the identification of brassica lines exhibiting increased stress tolerance that can be used in future crop breeding projects.

There is some data from previous studies of abiotic stress responses in brassicas. In broccoli (*B. oleracea* var. Italica), chilling can increase bud stalk length and diameter, mature head diameter and weight (Kalisz *et al*., 2014). Hadi *et al*., (2014) used polyethylene glycol (PEG) and mannitol treatments to examine the drought response of cauliflower (*B. oleracea* var. botrytis). Increasing concentrations of either treatment reduced seed germination, shoot and root length and biomass. In Chinese kale (*B. oleracea* var. alboglabra), both water deficit and waterlogging reduces leaf area, fresh and dry weight and leaf number, with drought leading to darker leaves and closed stomata (Issarakraisila *et al*., 2007).

This project will examine the response of three brassica lines (kale 'nero di Toscana', cabbage 'greyhound' and red cabbage 'calibos') to differing severities of cold, drought and waterlogging stress in order to determine a suitable stress level that will identify differential levels of response between the three lines.

15.2 Materials and Methods

Plant growth

Three lines of *Brassica oleracea* (kale 'nero di Toscana', cabbage 'greyhound' and red cabbage 'calibos', 28 seeds of each) were grown in '345' module trays until the fourth true leaf was visible. Seedlings were watered as required. At the fourth leaf stage, five seedlings per line were placed into four separate module trays in a completely randomised arrangement. Each of the four trays was then used for the different stress treatments. In each case, one tray acted as a control and continued to be watered as required.

Stress Treatments

For the cold stress experiment, the module trays were placed at 3°C for 16 h overnight (5pm-9am = 16hrs) for one, two or three consecutive nights to provide three different stress regimes of increasing intensity. These trays were watered as the control tray during the day. For the salt stress experiment, the module trays were dipped for 10 s daily in a 57 x 38.5 x 5 cm plastic tray filled with 2 l of 30 g/l NaCl in water for one, four or seven consecutive days. For the drought stress experiment, watering was withheld for one, two or three consecutive days to provide the three stress regimes. For the flood stress experiment, the module trays were placed in a 57 x 38.5 x 5 cm plastic tray filled with water such that the water level was 10 mm below the top of the module tray and grown for one, three or six consecutive days.

For each stress experiment, after the end of the stress period, trays were watered as required as per the control until all stress regimes were complete. Seedlings were then potted on in 9 cm diameter pots equally filled with Levington M2 compost and placed in a split-plot randomised arrangement on top of module trays to allow drainage. The pots were then watered to saturation as required (usually 3 times per week), with the modules allowing the drainage of excess water. The seedlings were grown until the sixth true leaf was visible.

Measurements

At the emergence of the sixth true leaf, the overall height of each seedling was recorded. The colour and leaf area of the fourth true leaf was determined using a CR-300 Colorimeter (Konica Minolta, Nieuwegein, Netherlands) and leafarea meter (Eijkelkamp, Giesbeek, Netherlands) respectively. Leaf colour was determined using the L*, a*, b* colour spaces (Koukounaras *et al.*, 2009). The Hue angle (h°) was calculated as: $h° = 180 + \tan^{-1}(b^*/a^*)$ and Chroma (C*) as: $C^* = (a^{*2} + b^{*2})^{1/2}$.

The fresh and dry weight of each seedling was recorded (seedlings were dried in an oven for 48 h at 60°C). Data were analysed using Analysis of Variance (ANOVA) with Tukey’s multiple comparison test at p=0.05.

15.3 Results and Discussion

For the cold stress experiment, none of the measured parameters of the three *B. oleracea* lines were significantly affected by the stress. This could reflect the tolerances of the three chosen lines or a need to increase the duration and/or severity of the cold stress, perhaps by subjecting the plants to periods of below-zero temperatures (data not shown).

By comparison, in the salt stress experiment, the lines were adversely affected by the longer duration treatments. The height, leaf area, fresh and dry weight of both kale and white cabbage were significantly reduced by both four and seven day's salt stress. Red cabbage appeared to be more tolerant of salt stress with no significant effects of the four day stress. However, leaf area of this line was significantly reduced (by seven days stress). Figure 15.1 shows the effect of salt stress on fresh weight as an example.

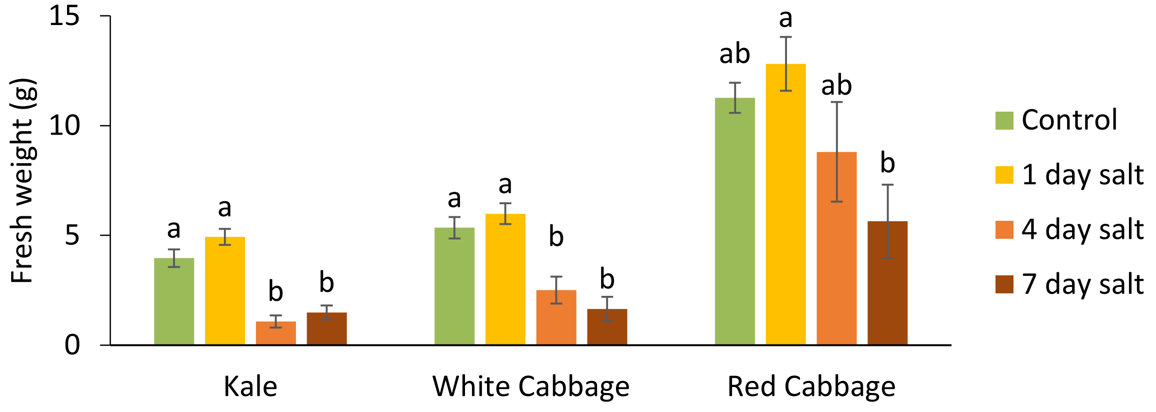


Figure 15.1. Effect of salt stress on the fresh weight of the three *B. oleracea* lines. Fresh weight of kale and white cabbage is significantly reduced by four or seven days stress. Red cabbage is less severely affected. Letters indicate significance groupings by Tukey's test (p=0.05).

A drought stress of two or three days significantly reduced the height of kale but not of the two cabbage lines (Figure 15.2). The effects of drought on other parameters were less clear. The leaf area and fresh weight of the two cabbages lines were unaffected, while the data suggested a negative effect of drought stress on these parameters in kale but the results were not significant. Conversely, the dry weight of the two cabbage lines was significantly reduced by the three day drought stress, while in kale, this reduction was not significant.

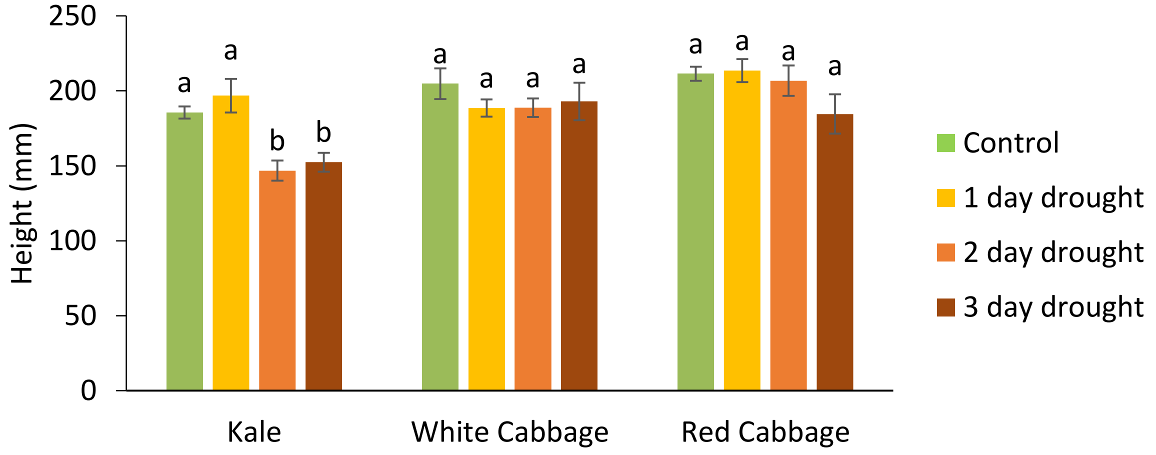


Figure 15.2. Effect of drought stress on the height of the three *B. oleracea* lines. Height of kale is significantly reduced by two or three days stress. White and red cabbage are unaffected. Letters indicate significance groupings by Tukey's test (p=0.05).

For the flood stress experiment, white cabbage appeared to be the most sensitive, with leaf area, fresh and dry weight being significantly reduced by three and six days of stress. In addition, the height of white cabbage was significantly reduced by six days of flood stress, while all three flood stresses reduced the lightness of leaves in this line. By contrast, in red cabbage, while fresh and dry weight were significantly reduced by six days of drought stress (no other stress durations were tested for this line due a lack of seedlings at the correct developmental stage), other parameters remained unaffected, suggesting a degree of flood tolerance compared to the white cabbage. Kale appeared to be more tolerant further still, with only leaf area significantly reduced by three and six days of stress. Figures 15.3 and 15.4 show the effect of flood stress on plant height and fresh weight, respectively.

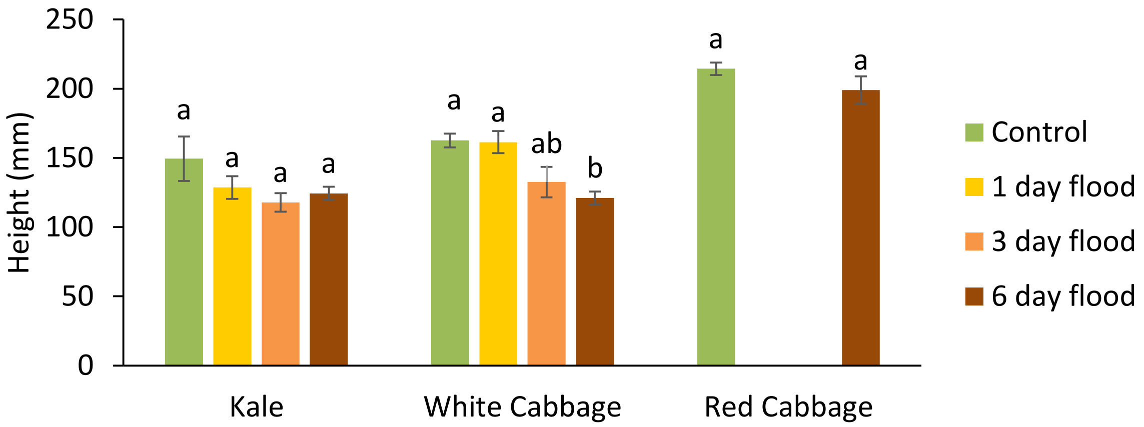


Figure 15.3. Effect of flood stress on the height of the three *B. oleracea* lines. Height of white cabbage is significantly reduced by six days of stress. Kale and red cabbage are unaffected. Letters indicate significance groupings by Tukey's test (p=0.05).

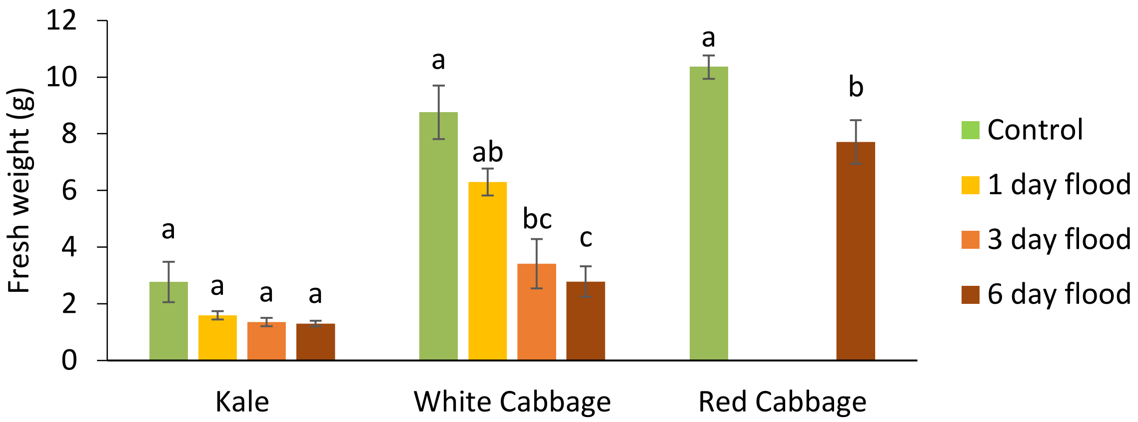


Figure 15.4. Effect of flood stress on the fresh weight of the three *B. oleracea* lines. Height of white and red cabbage is significantly reduced by six days of stress. Kale is unaffected. Letters indicate significance groupings by Tukey's test (p=0.05).

The reasoning behind these experiments is that some of the lines may be less affected by abiotic stress than others (such as smaller or larger reductions in growth rate after drought). Lines whose growth is altered less in response to stress may be better able to cope with climatic variation in the field.

15.4 Conclusion

- Red cabbage appeared to be more tolerant of salt stress compared to the other two lines, while for flood stress, kale appeared to be the most tolerant, followed by red cabbage.
- The results of the drought experiment are harder to interpret, however, the responses still differ between the lines.
- These differential responses to stress suggest that the range and severity of stresses tested here for salt (four days stress), drought (3 days stress) and flood (six days stress) can be used to screen further *B. oleracea* lines for tolerance to these abiotic factors and to identify lines with increased tolerance that will prove useful in downstream breeding programmes.

15.5 References:

Hadi. F., Ayaz, M., Ali, S., shafiq, M., Ullah, R., Jan, A.U. (2014). Comparative effect of polyethylene glycol and mannitol induced drought on growth (in vitro) of canola (*Brassica napus*), cauliflower (*Brassica oleracea*) and tomato (*Lycopersicum esculentum*) seedlings. *International Journal of Biosciences* 4: 34-41.

Issarakraisila, M., Qifu, M., Turner, R.W. (2007). Photosynthetic and growth responses of juvenile Chinese kale (*Brassica oleracea* var. *alboglabra*) and Caisin (*Brassica rapa* subsp. *parachinensis*) to waterlogging and water deficit. *Scientia Horticulturae* 111: 107-113.

Kalisz, A., Cebula, S., Kunicki, E., Gil, J., Sekara, A., Grabowska, A. (2014). Effect of chilling stress before transplanting on morphological parameters of broccoli heads. *Acta Scientiarum Polonorum-Hortorum Cultus* 13: 129-139.

Koukounaras, A., Siomos, A.S., Sfakiotakis, E. (2009). Impact of heat treatment on ethylene production and yellowing of modified atmosphere packaged rocket leaves. *Postharvest Biology and Technology,* 54, 172–176.

Experiment 16 - A Systems Approach to Disease Resistance Against Necrotrophic Fungal Pathogens

Amy Newman - University of Bristol

16.1 Introduction

This summer studentship forms part of a project with the overall objective of identifying sources of disease resistance in lettuce against *Botrytis cinerea,* an economically important fungal pathogen. The project aims to identify novel alleles for increasing the resistance of lettuce to *B. cinerea* to facilitate downstream breeding approaches. The project is run in association with A. L. Tozer.

B. cinerea causes substantial losses on field-grown and protected lettuce crops, an industry worth almost £200M/yr in the UK. Chemical control of *B. cinerea* is problematic due to spraying restrictions and the development of resistance to fungicides. Development of host resistance, meanwhile, may provide a more sustainable solution, but has been difficult for breeders to achieve.

Developing lettuce varieties with resistant to *B. cinerea* could present considerable economic benefits. For example, a 50% reduction in disease could provide significant savings both from reduced crop losses and lower spraying costs, while also providing environmental benefits.

The project makes use of a diverse set of 96 lettuce accessions sourced from Warwick Crop Centre. These accessions will be tested for *B. cinerea* resistance using a detached-leaf assay. This studentship aims to address four questions with regard to resistance in this diversity set:

1. Does plant age affect susceptibility to the pathogen?
2. Does resistance vary within individual plants (between leaves of different age)?
3. Does resistance vary between the accessions in the diversity set?
4. Can a whole-plant inoculation assay be developed and used to validate the detached leaf assay?

16.2 Materials and Methods

Plant growth

Lettuce *(Lactuca sativa)* of cultivars Iceberg and Saladin plus the ninety six accessions of the Warwick Crop Centre Lettuce Diversity Fixed Foundation Set were sown in 7 cm square pots containing Levington M2 compost and grown in a glasshouse at 15°C minimum day time and

5°C minimum night time temperature and watered as required. Supplemental lighting was provided to maintain a 16:8 day:night cycle and a minimum of 35,000 lux.

Detached leaf assay

B. cinerea spores were harvested in dH_2O and then diluted in a half strength potato dextrose broth (PDB) and 5% guar gum solution to create an inoculum. Leaves were placed in an 38 by 24 by 15 cm plastic box filled with 700 ml of 0.8% agar to a depth of approximately 10 mm. Single leaves were removed from plants at the age indicated and inoculated with 10 µl droplets of *B. cinerea* spore suspension (10^6 spores /ml). Each leaf was inoculated twice, once on either side of the midvein. The boxes were sealed with parafilm and incubated in a controlled environment chamber at a mean temperature of 22.7°C, 97.4% relative humidity and a 16:8 day:night cycle . Lesion size was recorded after 48 h and 72 h by photographing lesions followed by lesion size determination using ImageJ image analysis software. In experiments where only one leaf was removed per plant, the third leaf was used.

Whole plant inoculations

Plants were sprayed with a with *B. cinerea* spore suspension ($5x10^5$ spores /ml) using a commercial spray bottle. Each plant received twelve sprays, rotating the plant by 90 degrees after every third spray, delivering a final suspension volume of approximately 14.5 ml. Plants were then covered with a plastic bag and grown in a polytunnel. Plastic bags were removed after 48 hours and photos were taken. Plants were inspected for the presence of visible *B. cinerea* sporulation three and ten days post inoculation.

16.3 Results and Discussion

Effect of plant age on lesion development

On detached leaves of lettuce cv. Iceberg, the average *B. cinerea* lesion size 72 h after inoculation decreased as the age of the plant at inoculation increased (Figure 16.1.). This suggests perhaps an increased resistance to pathogen development with increasing plant age, which could result from host resistance mechanisms, or biochemical and/or morphological changes that occur during plant growth that reduce the ability of the pathogen to infection and proliferate.

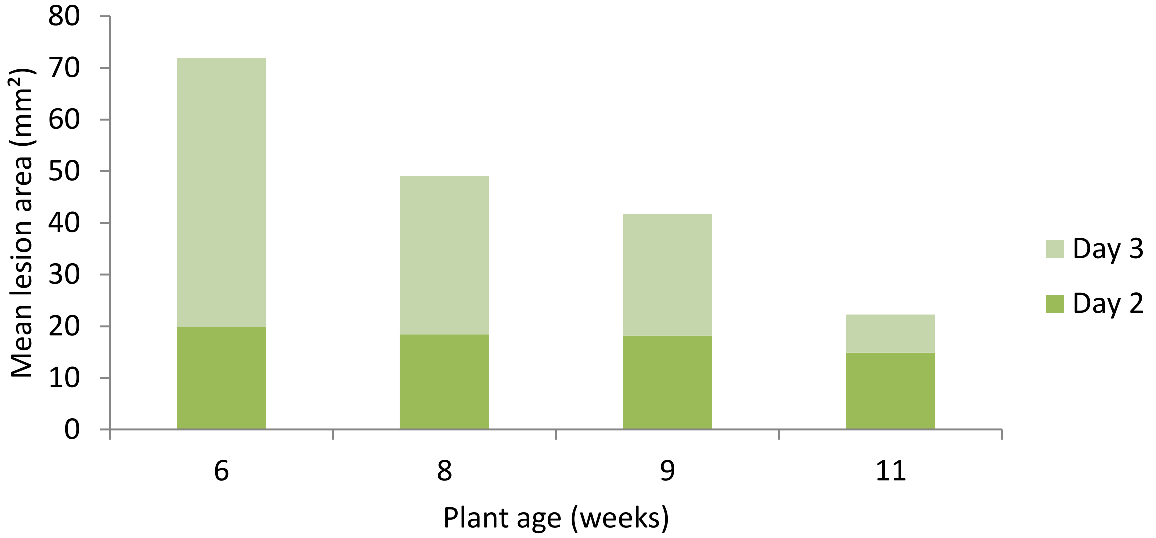


Figure 16.1. Effect of plant age on *B. cinerea* lesion development in lettuce (cv. Iceberg). Lesion area was recorded 48 and 72 h after inoculation.

Variation of resistance within plants

As whole plant age was shown to affect *B. cinerea* symptom development, so we wished to determine the effect of different leaf ages within the same plant on lesion size and so investigate the variation of resistance within plants. Six week old plants of cvs Iceberg and Saladin were used. Figures 16.2 A and B indicate that no correlation between leaf age and disease susceptibility was present. This indicates that the effects of age on symptom severity occur at the whole plant level and are not present between leaves of different ages belonging to the same plant.

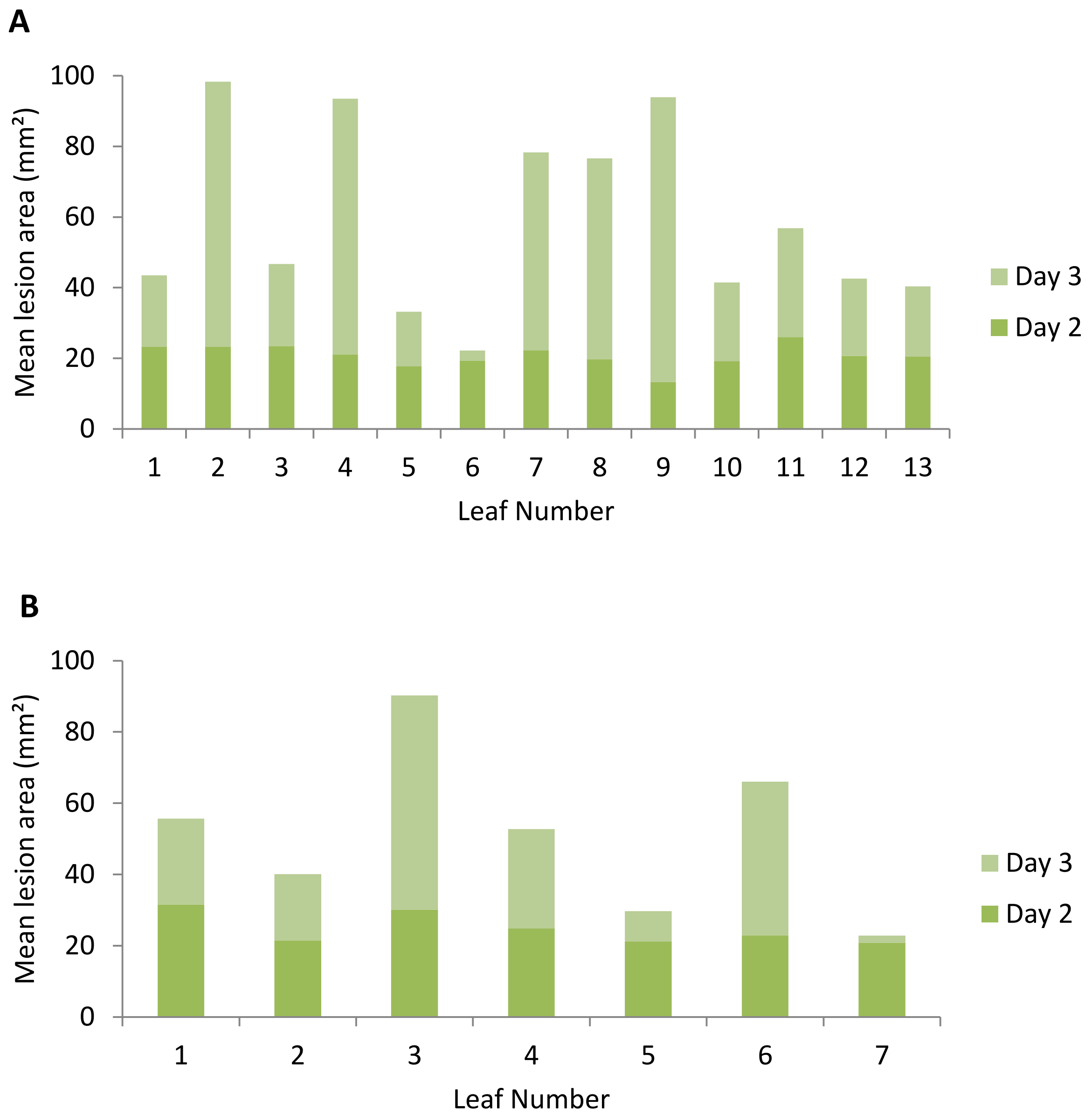


Figure 16.2. Effect of leaf age on *B. cinerea* lesion development in lettuce (A. cv. Iceberg, B. cv. Saladin). Lesion area was recorded 48 and 72 h after inoculation.

Variation in resistance between accessions of the diversity set

Inoculation of the ninety six accessions of the Warwick Crop Centre Lettuce Diversity Fixed Foundation Set showed that a large amount of variation in *B. cinerea* susceptibility was present within the set (Figure 16.3). This suggests that the diversity set may provide a good source of data and genetic material for further investigation of *B. cinerea* resistance. Lines with small or no lesions can be taken forward for mapping and transcriptome analysis to determine the gene(s) responsible.

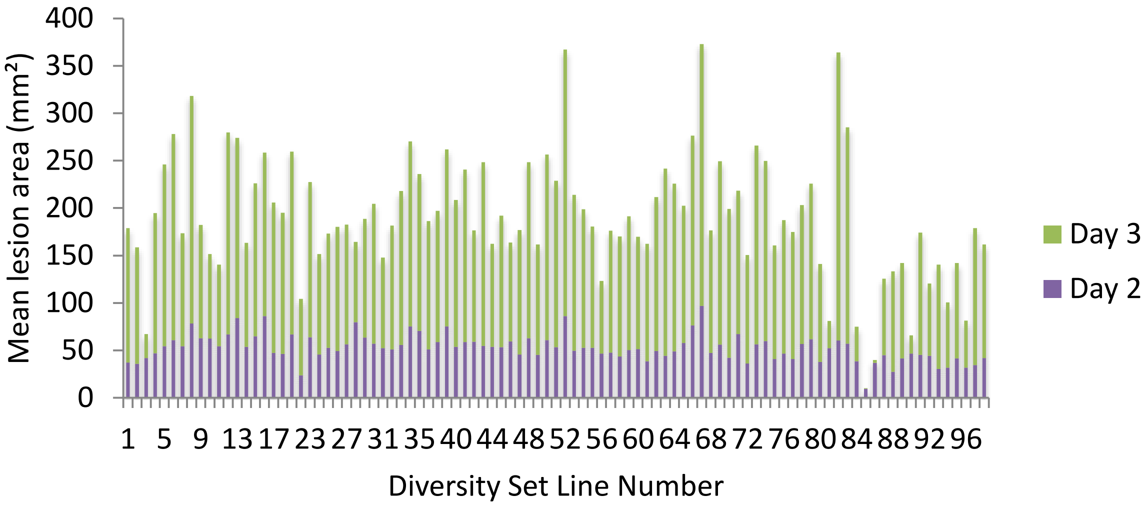


Figure 16.3. *B. cinerea* lesion development on the ninety six accession of the lettuce diversity set. Lesion area was recorded 48 and 72 h after inoculation.

Whole plant inoculation

Ten days after inoculation, 17 of 31 lettuces inoculated as whole plants showed signs of *B. cinerea* sporulation, 16 of which were cv. Iceberg. This indicates the ability to obtain symptoms using the whole plant inoculation method, particularly with cv. Iceberg, but further investigation may be required to ensure that results obtained with this approach match those from the detached leaf assay.

16.4 Conclusion

- Increasing whole plant age reduces *B. cinerea* lesion size, suggesting increased resistance of the host with age.
- This result is not found with leaves of different ages belonging to the same plant and so appears to occur at the whole plant level.
- The lines of the *L. sativa* diversity set show different levels of susceptibility to *B. cinerea*, indicating that this population may provide a useful genetic resource in breeding for resistance to this pathogen.

Experiment 17 - Investigating the effects of relative water content on pinking in whole head lettuce (*Lactuca sativa*)

Suzannah Franks - Newcastle University

17.1 Introduction

Leafy salads often suffer from discolouration on the butt and leaf ribs within a few days after harvest, limiting their shelf life. Enzymatic and non-enzymatic oxidative processes cause 'browning' and 'pinking' which results in the emergence of coloured pigments (brown and pink/red respectively) are produced via the phenylpropanoid (PPO) pathway (Toivonen and Brummell 2008). Pinking continues to present substantial problems for producers with both UK and imported crops. Poor product on the shelf reduces sales and leads to more complaints and consumer dissatisfaction.

Studies also report that increased irrigation can decrease storability with higher subsequent pinking expression postharvest (Wurr *et al*., 2003; Monaghan *et al*., 2007; Luna *et al*., 2012). There is no work studying the effect of rainfall but it can be assumed that the response to heavy rain would be similar. Higher water contents in lettuce heads could affect tissue turgor pressure and cell expansion. Changes in turgor pressure could result in the lettuce rib being more susceptible to rupture, resulting in the induction of PPO activity. Increased irrigation could impact on growth, with rapid growth in lettuce contributing to the occurrence of tipburn. However, the level of irrigation/rainfall that would lead to increased pinking has not been reported.

This project tests the following hypotheses:

1. Relative water content (RWC) of lettuce ribs can be modified by post-harvest treatments.

2. Ribs with high RWC are more prone to damage and pinking.

17.2 Materials and Methods

The first three weeks of the placement were spent optimising the experimental system used (data not presented). Whole head iceberg lettuce (*Lactuca sativa*, obtained from PDM Produce, Great Chatwell, Shropshire, UK) was subjected to four different treatments on the day of harvest. Prior to treatment, outer leaves were removed and the butt trimmed and the whole head fresh weight recorded. In the first treatment, the whole head was placed in a plastic grip seal bag (53 x 38 cm) containing 250 g silica crystals. In the second, a small net was pulled taught around a tray filled with fresh tap water. The lettuce butt was placed on the netting so that the butt and base of the lettuce were submerged in the water. This was then put in a plastic grip seal bag (53 x 38 cm). The third was wrapped using an industry packaging bag (a plastic bag with a few holes in 30 x 27 cm) and the fourth simply unwrapped. Lettuce of all four treatments were stored at ambient temperature in the laboratory. Five heads were used per treatment.

The following day, the whole head fresh weight was recorded again and a rib sample (3.5 x 7 cm template) removed and its weight recorded (FW). Afterwards, these strips of rib were fully submerged in distilled water and stored in the cold store at 5°C for a minimum of 4 hours. The ribs were then gently dried using paper towels and weighed again to give the turgid weight (TW). Dry weight (DW) was calculated after 48 h of drying in an oven at 60°C. Relative water content (RWC) was calculated as: RWC=(FW-DW)/(TW-DW). The lettuces were scored for rib pinking and rib cracking (based on a specification provided from G's, using a 1 to 4 scale system with 4 being the worst-affected) prior to being placed in turn on a platform with a rib positioned at the top and a 6 x 7 x 6 cm plastic pot containing 150 g sand was dropped onto the rib from a height of 1 m. The whole heads were then securely wrapped using industry bags and put in the cold store at 5°C. Six days later, rib damage and pinking were rescored and the change in score for each head calculated. The experiment was repeated three times. Data were analysed using Analysis of Variance (ANOVA) with Tukey's multiple comparison test at p=0.05.

17.3 Results and Discussion

Relative water content (RWC) of lettuce ribs

The 'wrapped' and 'wet' treatments showed a significantly higher RWC than the 'unwrapped' and 'dry' treatments, indicating that the treatments were effective in altering the water status of the ribs (Figure 17.1) with a range of RWC from 76% to 88%. The highest RWC was achieved through either hydrating the tissue or wrapping the harvested head in plastic suggesting that the rib was turgid on delivery to HAU as no additional hydration was achieved. The use of a desiccant did not increase dehydration of the tissue compared to an unwrapped head.

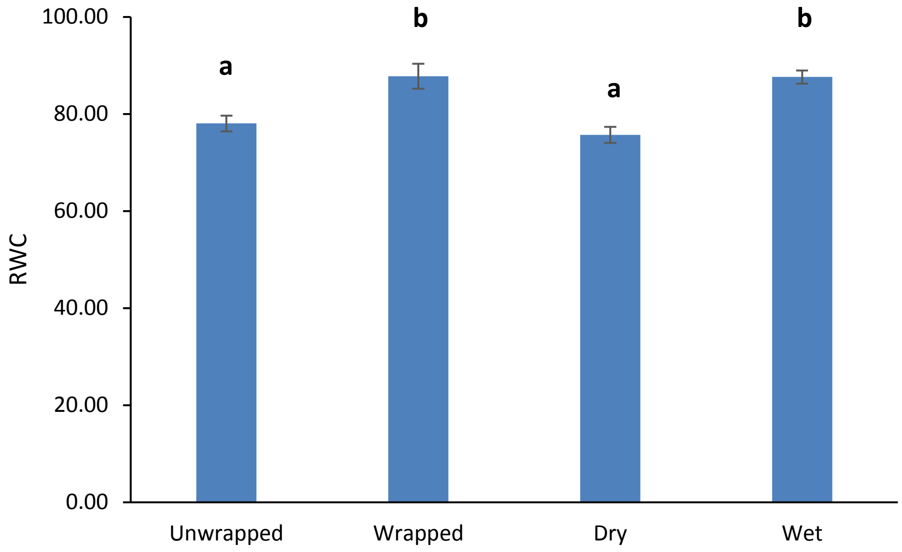


Figure 17.1. Effect of the treatments on relative water content (RWC) of whole head Iceberg lettuce. Letters indicate significance groupings by Tukey's test (p=0.05).

Rib cracking and pinking of whole lettuce heads

While rib cracking score appeared to be lowest for the 'dry' treatment, intermediate for the 'unwrapped' and 'wrapped' treatments, and highest for the 'wet' treatment, there was no significant difference detected in the rib cracking score between lettuce heads undergoing the different treatments (Figure 17.2, blue bars). However, the 'wet' treatment led to a significantly higher rib pinking score compared to the 'dry' and 'unwrapped' treatments (Figure 17.2, red bars). This suggests that the extent of rib cracking is unaffected by RWC within the range achieved by these treatments, while higher RWC may be associated with higher pinking scores.

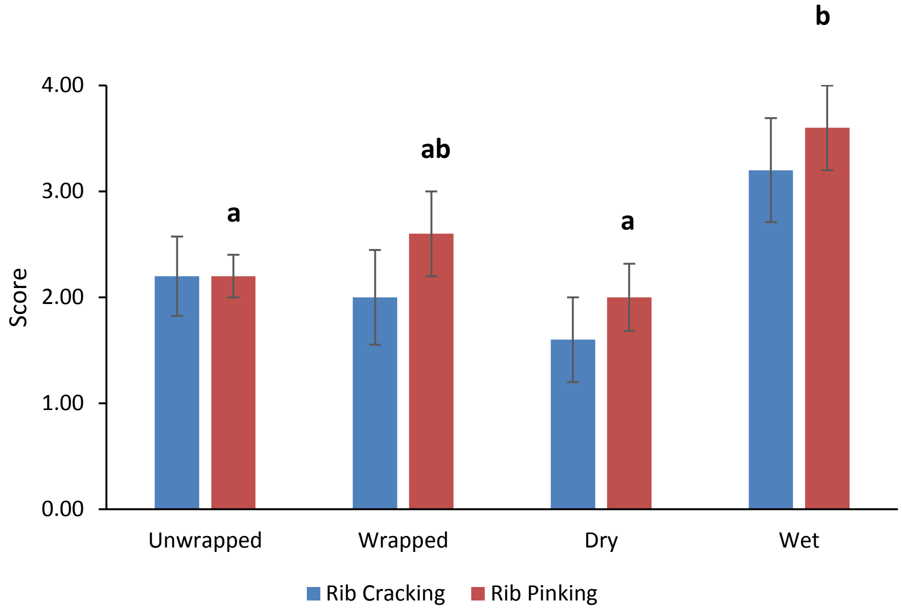


Figure 17.2. Effect of the treatments on rib cracking and pinking of whole head Iceberg lettuce. Letters indicate significance groupings by Tukey's test (*p*=0.05, rib cracking and pinking were analysed separately).

The pattern of response of both rib cracking and rib pinking differed compared to that observed for RWC. The greatest level of both rib cracking and pinking was observed in the hydrated heads and the least for both measures was observed in the desiccated heads. One explanation for this response is that the RWC was calculated from the outer most leaf rib, and the rib cracking and pinking was assessed from the next rib. The RWC of the outer leaf may not be representative of the second rib and further work is needed to explain the more marked response to the 'wet' treatment.

17.4 Conclusion

- Rib cracking following mechanical impact tends to be greater in lettuces that have a greater relative water content.
- Rib pinking is correlated to rib cracking.
- Actions that prevent mechanical damage to lettuce ribs will reduce rib pinking.

17.5 References:

Luna, M. C., Tudela, J. A., Martínez-Sánchez, A., Allende, A. and Gil, M. I (2012). Long-term deficit and excess of irrigation influences quality and browning related enzymes and phenolic metabolism of fresh-cut iceberg lettuce (*Lactuca sativa* L.) Postharvest Biology and Technology 73: 37-45

Monaghan, J., Hilton, H., Akehurst, J., Fellows, J., Wood, M., Simmonds, L. and Rahn, C. (2007). Improved efficiency of nutrient and water use for high quality field vegetable production using dynamic fertigation. Hortlink Final Report for project HL0165

Toivonen, P. M. A. and Brummell, D. A. (2008). Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. Postharvest Biology and Technology 48 (1): 1-14

Wurr, D., Parr, A., Feuerhelm, S., Kennedy, S., Pennings, H., Oost, E., Cornai, I., Harriman, M., Sawday, J. and Tucker, A. (2003) Improving the quality and shelf-life of cut salad products. Department for Environment, Food and Rural Affairs final report for project number HLO142

Experiment 18 - Assessing the effectiveness of control measures for vine weevil (*Otiorhynchus sulcatus*) damage of strawberry (*Fragaria ananassa*) plants.

Nicholas Kuht - University of Leeds

18.1 Introduction

Vine weevil (*Otiorhynchus sulcatus*) remains one of the most serious pests of soft fruit and ornamental crops (Moorhouse *et al*., 1992; Bennison, 2014). 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 insecticides, such as the broad spectrum pyrethroid insecticide lambda-cyhalothrin (e.g. Hallmark) for the control of vine weevil adults in soft fruit crops. Application of these insecticides against this pest is difficult, as it is recommended that they are applied at dusk, when the weevils become active. In addition, applications of broad spectrum insecticides such as pyrethroids 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/year (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?

Results from year one of this project are presented in the 2015 Annual report for AHDB Horticulture project CP 087 (Monaghan, 2015).

18.2 Materials and Methods

Plant Growth

Strawberry (*Fragaria ananassa*, cv. Elsanta) plants were grown in standard growing medium bags supplied by Bulrush Ltd. The growing medium was 80% peat and 20% wood fibre +/- Met52. Each bag had ten planting holes and a 18-20 mm crown (Hargreaves Plants Ltd, Spalding) was planted in into each hole on 28th May 2014. Grow bags were arranged lengthways on benches approximately 53 cm above ground level in an open-ended, close-sided polytunnel at the Crop and Environment Research Centre at Harper Adams University. 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/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 Ca.

Treatments

Vine weevil (*Otiorhynchus sulcatus*) eggs were added to selected bags by first counting eggs out onto a small piece of damp filter paper (2 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 to be infested. Vine weevil eggs were washed onto the strawberry plants in batches between 14th and 18th July 2014. Standard grow bags were supplied +/- Met52 (Bulrush Ltd, Londonderry). Met52 is based on the entomopathogenic fungus *Metarhizium brunneum* (*anisopliae*) and was suppled at a rate of 0.5 kg/m^3 pre-mixed in the bags. Drenches of Nemasys L (BASF Agricultural Specialities Limited, Littlehampton) containing the entomopathogenic nematode *Steinernema kraussei*, were applied on 19th August 2014 using a large syringe to deliver 25,000 nematodes/plant in 100 ml of water. Eight growbags were set up for each of five different treatments: 1. No weevil eggs, no control measure (no W + no con), 2. Weevil eggs, no control measure (W + no con), 3. Weevil eggs, Nemasys L (W + Nem), 4. No weevil eggs, Met52 (no W + Met52) and 5. Weevil eggs, Met52 (W + Met52).

Measurements

After treatment, plants were grown and fruit harvested in 2014 (see CP 087 Annual Report 2014). At the end of the season grow bags were placed on the ground in an uncovered polytunnel to overwinter. In April 2015 grow bags were placed back onto the benches in wooden frames (approx. 0.8 x 1.6 m) with a horticultural fleece base, to allow drainage, and the inside of the wooden frames was coated with Ecotak (Oecos, Kimpton) to ensure the prevention of weevil movement between boxes. The irrigation was re-connected by threading drippers through the horticultural fleece to ensure that the wooden frame remained weevil proof. Fruit was harvested twice a week between 3rd June 2015 and 9th July 2015. The degree of leaf notching caused by weevil herbivory was scored for 10 leaves per plant (1: no notching, 2: moderate notching, 3: severe notching). The height of each plants was measured using a ruler from the top of the growbag to the top of the tallest leaf for each planting hole. Five mature leaves were removed per growbag and the colour determined using a CR-300 Colorimeter (Konica Minolta, Nieuwegein, Netherlands), taking care to avoid the main leaf vein. Leaf colour was determined using the L*, a*, b* colour spaces (Koukounaras *et al.*, 2009). The Hue angle (h°) was calculated as: $h° = 180 + \tan^{-1}(b^*/a^*)$ and Chroma (C*) as: $C^* = (a^{*2} + b^{*2})^{1/2}$. Above ground fresh and dry weight of strawberry plants was recorded for each growbag at the end of the experiment. An assessment of the numbers of weevil larvae in each grow-bag and root damage scored. Each bag was cut open lengthways using a knife, plants were then separated from each other by pulling two adjacent plants apart. A detailed search was then completed for vine weevil larvae feeding within the root mass of each plant.

During the fruiting season in 2015 (3rd June to 9th July), all fully ripened fruit were harvested twice a week into 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).

18.3 Results and Discussion

Leaf Notching

Leaf notching scores were lowest for the two treatments in which weevil eggs were noted added (Treatments 1 and 4, Figure 18.1). The addition of weevil eggs significantly increased the extent of leaf notching observed on the plants (Treatment 2, Figure 18.1). Nemasys L, but not Met52, (Treatments 3 and 5, respectively) was able to significantly reduce leaf notching caused by adult developing from introduced weevil eggs to a level comparable to treatments where eggs had not been added. This suggests that Nemasys L was more effective than Met52 in preventing the successful development of weevil eggs into adults. The fact that a small amount of notching was recorded in treatments in which weevil eggs had not been added suggested that despite the use of Ecotak, a small number of weevils were able to infest these plots.

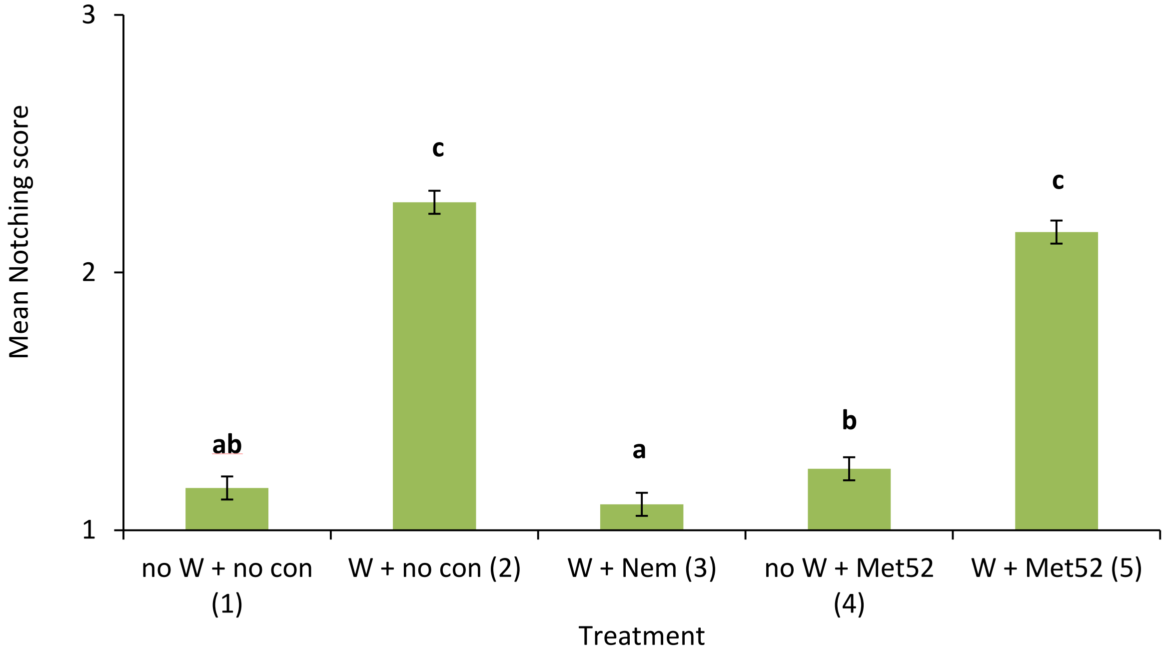


Figure 18.1. Effect of treatment on notching score of strawberry leaves. Letters indicate significance groupings by Tukey's test (p=0.05).

Plant height and mass

There was no significant difference in plant height between any of the five treatments (Figure 18.2), indicating that neither weevil egg addition alone, nor the two vine weevil control measures significantly affected plant size. In addition, no significant differences between the treatments were found for plant fresh or dry weight (data not shown).

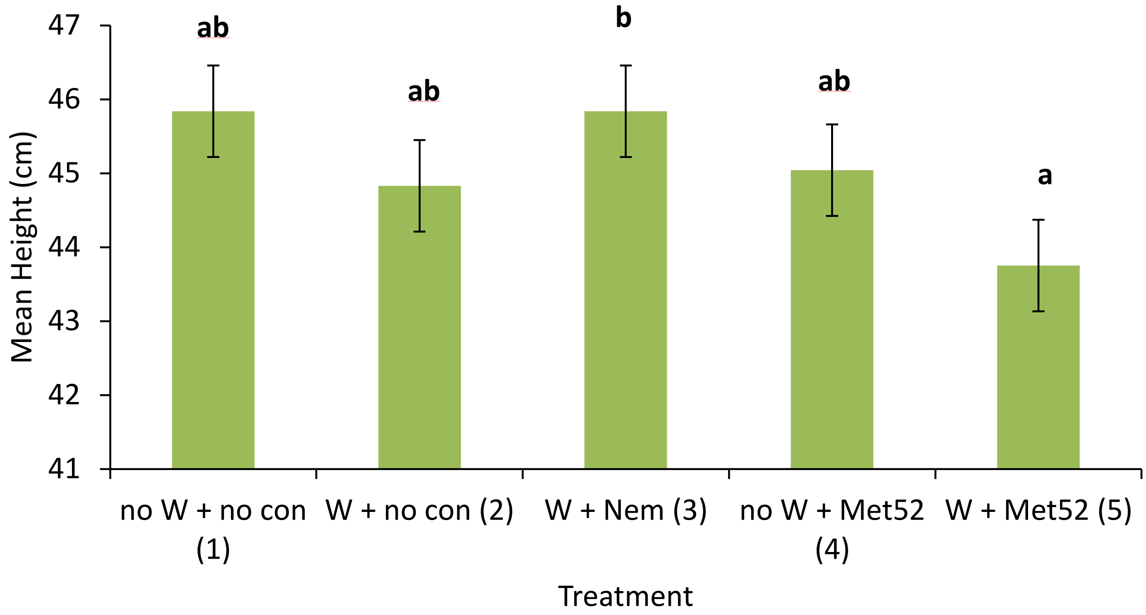


Figure 18.2. Effect of treatment on height of strawberry plants. Letters indicate significance groupings by Tukey's test (*p*=0.05).

Leaf Colour

Both treatments containing Met52 caused the development of significantly paler leaves than treatments lacking Met52 (Figure 18.3). The addition of Nemasys L, however, did not significantly alter leaf lightness compared to the control (Treatment 1). This indicates that while Nemasys L had no effect, Met52 significantly altered the colour of strawberry plant leaves, which could reflect a lower chlorophyll content and reduced photosynthetic capacity.

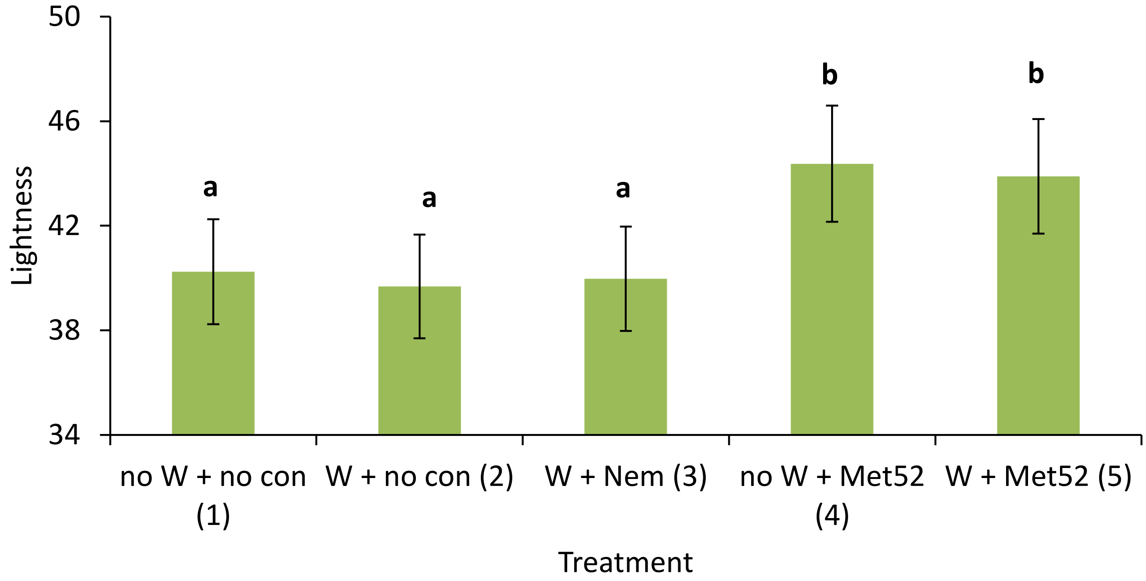


Figure 18.3. Effect of treatment on leaf lightness of strawberry plants. Letters indicate significance groupings by Tukey's test (p=0.05).

Fruit sugar content

There was no significant difference in fruit sugar content between any of the five treatments (Figure 18.4), indicating that neither the introduction of weevil eggs alone, nor the control measures significantly affected fruit sweetness.

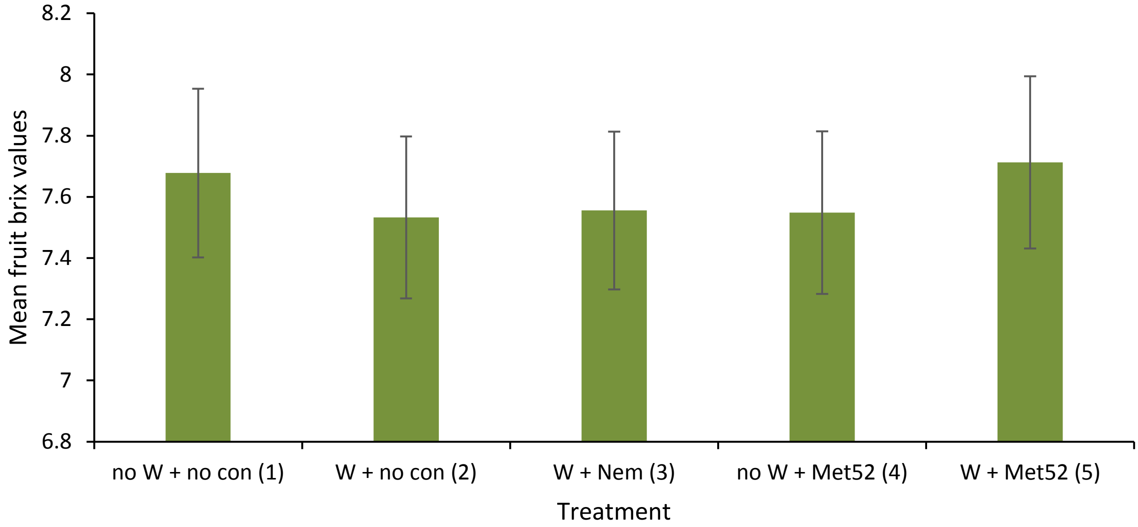
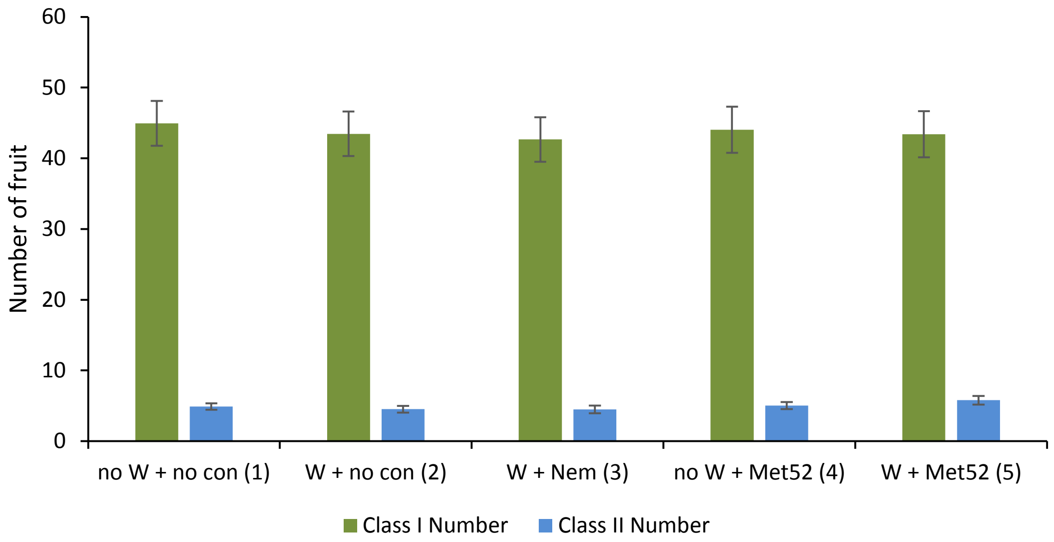


Figure 18.4. Effect of treatment on mean fruit brix values.

Fruit production

There was no significant difference between the five treatments in the number or mass of Class I and II fruit produced (Figure 18.5 A and B.). This indicates that neither the introduction of weevil eggs alone, nor the control measures significantly affected fruit production by the plants.

A



B

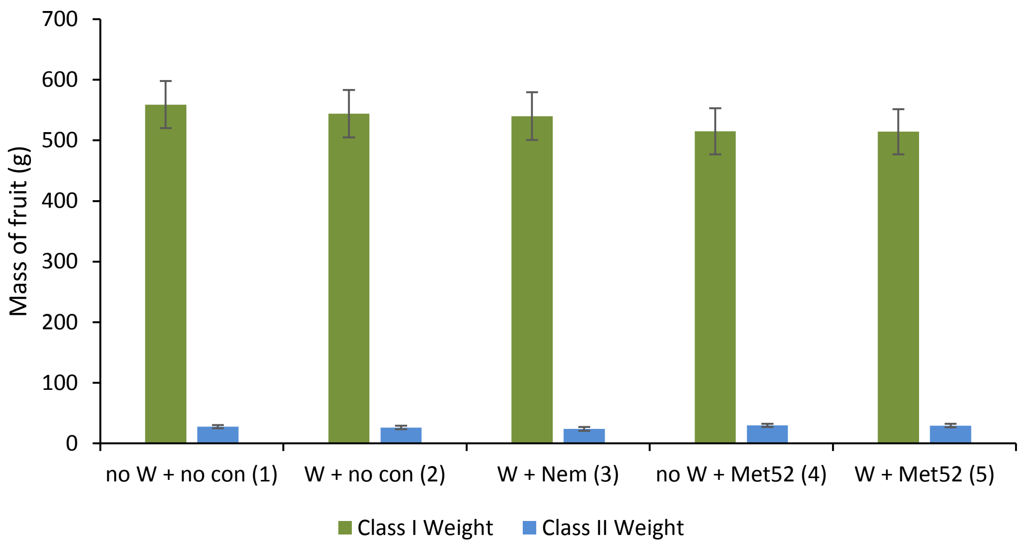


Figure 18.5. A. Effect of the treatments on fruit number per growbag. **B.** Effect of the treatments on fruit mass per growbag.

Presence of adult vine weevils and larvae

It appears that the introduction of vine weevil eggs in the absence of a control measure, while leading to significantly increased leaf notching did not affect plant height, fresh or dry weight, fruit production or fruit sugar content. Despite this the data indicate that the addition of vine weevil eggs in the absence of a control measure (Treatment 2) resulted in significantly more vine weevil adults being found in these plots compared to when eggs were not added (Treatment 1). For both Nemasys L and Met52, the number of adult weevils recovered was at an intermediate level between Treatments 1 and 2 (lower for Nemasys L), suggesting some degree of reduction of weevil numbers by both control measures, however, the numbers of adult weevils recovered was not significantly different from either of the treatments lacking control measure (Treatments 1 and 2). As adult weevils are responsible for leaf damage, this data is somewhat consistent with that of the leaf notching, at least for Nemasys L.

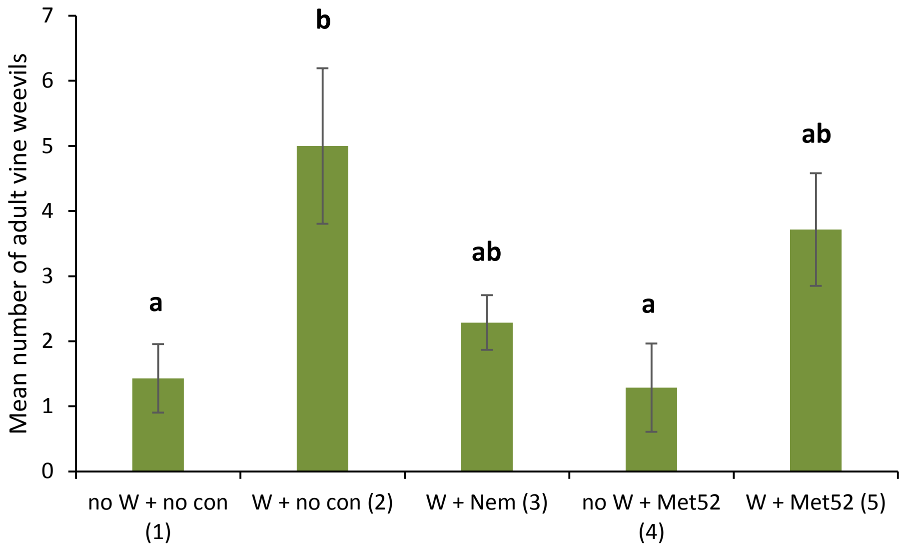


Figure 18.6. Effect of the treatments on the mean number of adult vine weevils recovered at the end of the growing season. Letters indicate significance groupings by Tukey's test (*p*=0.05).

For vine weevil larvae, the addition of vine weevil eggs in the absence of a control measure (Treatment 2) resulted in a significant increase in the number of vine weevil larvae compared to when eggs were not added (Treatment 1, Figure 18.7). The addition of Nemasys L, but not Met52, was able to maintain larval numbers comparable to the uninoculated Treatment 1, indicating Nemasys L, but not Met52, is an effective control measure against vine weevil larvae in this study. When roots were examined for damage, the addition of vine weevil eggs in the absence of a control measure (Treatment 2) caused a significant increase in the root damage score compared to when eggs were not added (Treatment 1, Figure 18.8). Both control measures showed root health scores that were not significantly different to the uninoculated Treatment 1, suggesting that they may both be effective in preventing root damage.

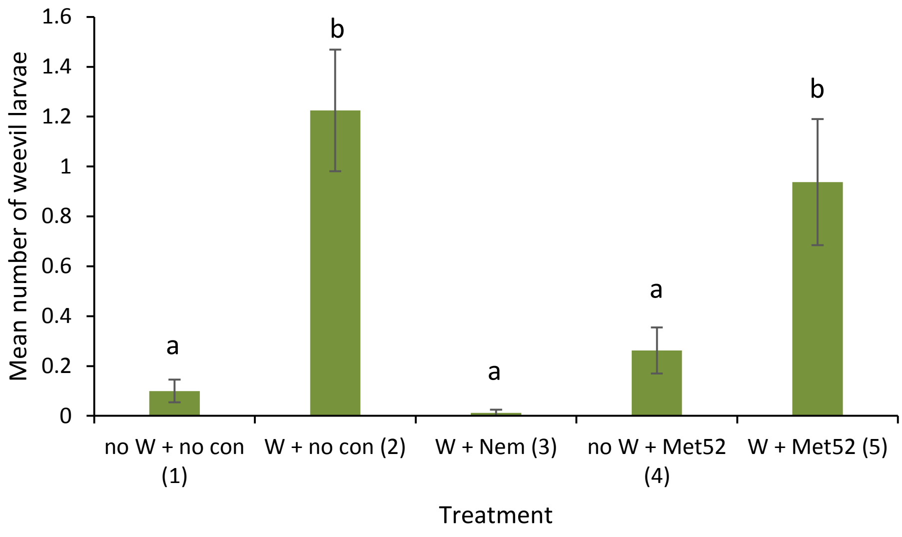


Figure 18.7. Effect of the treatments on mean number of vine weevil larvae recovered at the end of the growing season. Letters indicate significance groupings by Tukey's test (*p*=0.05).

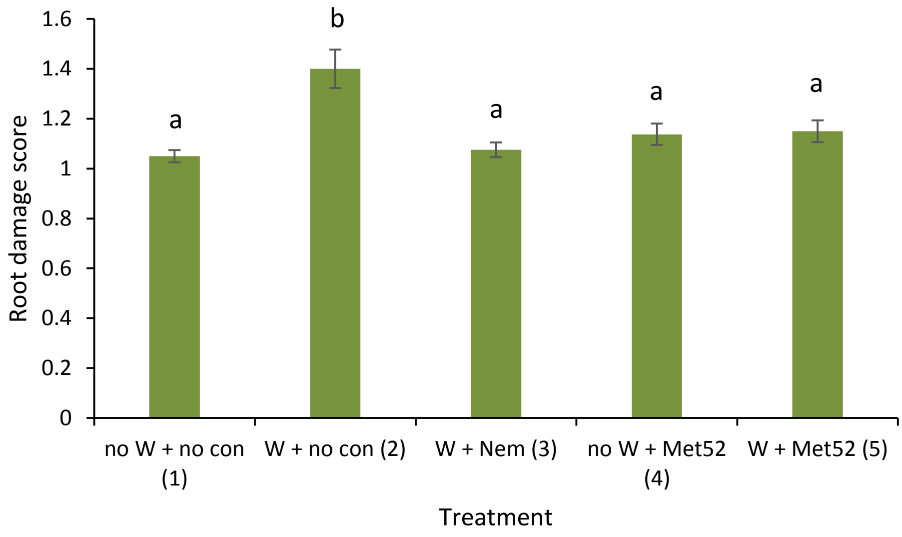


Figure 18.8. Effect of the treatments on strawberry root damage at the end of the growing season. Letters indicate significance groupings by Tukey's test (*p*=0.05).

18.4 Conclusion

- Nemasys L, but not Met52, appeared to provide effective control of leaf notching and root damage by vine weevil and significantly reduced numbers of vine weevil larvae in the substrate, while not affecting plant height, fresh or dry weight, leaf colour, fruit production or fruit sugar content.

- While Met52 did not affect plant height, fresh or dry weight, fruit production or fruit sugar content, it lead to significantly paler leaves and did not provide effective control of leaf notching or larval numbers but did appear to prevent root damage.
- The introduction of vine weevil eggs in the absence of a control measure, while leading to significantly increased numbers of both adult and larval weevils and leaf notching, did not affect plant height, fresh or dry weight, fruit production or fruit sugar content. A higher number of weevil eggs, larvae or adults may be required to see significant effects on these parameters and to be able to determine if the control measures can prevent such effects.

18.5 References

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