Project title:	Pre-adaptation of vegetable seedlings to increase their resistance to pest attack
Project number:	FV 402
Project leader:	Dr Barry Mulholland, ADAS
Report:	Final report, November 2013
Previous report	None
Key staff:	Chloe Whiteside, Angela Huckle and Barry Mulholland, ADAS Boxworth
Location of project:	Glasshouses and polytunnel, ADAS Boxworth Commercial grower, Lincs
Industry representative:	Andy Richardson, A & B Centre
Date project commenced:	1 April 2012
Date project completion due:	30 September 2013

The results and conclusions in this 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.

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GROWER SUMMARY

Headline

- By using a controlled stress technique, growers will be able to potentially reduce the use of chemical treatment in intensively raised plants before transplanting into the field.

Background

Two of the biggest issues facing propagators and growers are downy mildew and cabbage root fly. Control of downy mildew in young plants in propagation, particularly early spring sowings or those grown overwinter, is extremely difficult and recent approval losses mean fungicide options are limited. These problems impair optimum growth which results in a non-uniform crop, unmarketable produce and plant losses.

With typically 3-4 overlapping generations a year CRF is a perennial problem affecting all Brassica plantings from March to early October. Current CRF control on leaf and flowerhead Brassicas relies largely on insecticide module drenches at propagation of either spinosad (Tracer™) or more commonly chlorpyrifos (Dursban WG™). However, with the chlorpyrifos drench approval likely to be revoked in the near future (classed as a priority substance under the Water Framework Directive) alternative controls are therefore being actively sought to reduce the dependency on chemical control of pest and disease. The focus of the current project is to explore the potential of a non-chemical method to exploit the natural defence mechanisms of the plant and to explore whether this 'internal resistance' has a similar impact on CRF and downy mildew attack.

This project evaluates a brief period of stress that pre-adapts cauliflower plants to pest and disease attack; not all stress which plants experience is 'bad'; the internal 'natural' response of the stressed plant can be harnessed to repel pest and disease attack. The approaches adopted in the project are short periods of pre-adaptive stress with an application window of days, which is distinct from longer term 'hard raising' that can lead to poor quality 'woody' plant material. A key part of the production cycle is to confer resistance established in propagation to the main growth phase. To evaluate NaCl treated propagated material performance at maturity, pre-adapted module raised seedlings were grown on and monitored under commercial conditions in the field.

Expected deliverables

The expected deliverables for this project are:

(i) Project aim: To reduce the use of pesticides on Brassicas by increasing the host resistance of young plants.

(ii) Project objectives:

- To summarise current approaches to cabbage root fly (*Delia radicum)* and downy mildew (*Hyaloperonospora parasitica*) control;
- To explore the physiological and growth response of untreated and pre-adapted plants to pest challenge;
- To quantify the pest resistance of pre-adapted seedlings to selected inoculum levels of cabbage root fly and downy mildew.

Summary of the project and main conclusions

- Experimental data suggest that a moderate concentration of salinity (60 mM for Chassiron and 90-120 mM NaCl for Skywalker) applied in a solution to the roots, in the last week of the plug production cycle improved plant survival when attacked by CRF.
- Leaf expansion and root biomass was increased in cauliflower plants at 60 - 90mM NaCl compared with the zero salt control. The work highlighted a dual benefit of increased growth and pest resistance.
- Plants grown on to harvest that had been treated with salt, demonstrated a larger marketable curd compared with the control which had not been given additional salt. The size of the curd plus leaves was larger in plants that had not been salt treated. Salinity applied to seedlings may confer a favourable shift during crop maturation towards generative compared with vegetative growth.
- Whilst 'stressing' plants may appear at odds with the Horticulture business case of maximising productivity, improving our understanding and application of this research will ultimately lead to a reduction in the use of chemical application alone for pest control during plant propagation.

The data suggests that the cauliflower cultivars Chassiron and Skywalker can be pre-adapted by a brief period of NaCl solution feeding to the root-zone. Misting application to the foliage at the same dose provides less robust effects. Chassiron produced an increase in leaf expansion and a larger root system at 60 mM NaCl, whereas Skywalker exhibited enhanced growth at 90-120 mM NaCl compared with the zero NaCl control. Resistance to CRF damage was evident at 60 mM NaCl in Chassiron and 90-120 mM NaCl in Skywalker. In contrast NaCl had little impact on resisting infection by Downy mildew and in fact there

was weak evidence to suggest that at high levels of NaCl feeding concentrations (240 mM NaCl) then infection was increased. In summary:

- Skywalker and Chassiron show enhanced growth response to NaCl during propagation to a 5 day feeding window immediately prior to dispatch.
- Skywalker can beneficially utilise salt at higher concentrations (90-120mM NaCl) to promote growth compared with Chassiron (60 mM NaCl).
- The cultivar enhanced growth response to NaCl feeding coincides with a marked increase in the resistance or pre-adaptive potential of young plants to withstand CRF attack.
- The resistance of plants appears to be strongly linked with the ability to transport water from the root system and to drive cell expansion in the leaves.
- Transpiration is more markedly reduced in CRF attacked plants compared with photosynthesis alone.
- Growth responses under protected growth conditions appear to correspond with Chassiron and Skywalker performance in the field.
- Pre-adaptation appears to have promise for use with Brassica transplant crops and is a technique that has the potential to reduce chemical control dependency in production.
- Further field-scale trials that build upon this preliminary study will give the industry a clearer understanding of the benefits of pre-adaptive stress conditioning of Brassica transplants.

Table 1 below can be used to convert the units used in this report (mM) to g/l.

Table 1: Salinity conversions

NaCl Solution Concentration	EC Solution	EC Seawater

			~54dSm^{-1}
mM	**g/L**	**dS m^{-1}**	**Rel Strength**
60	3.5	5.5	0.1
120	7.0	11	0.2
240	14.0	22	0.4

Financial Benefits

The commercial objective of this project is to provide vegetable growers with guidelines on plant stress manipulation for control of CRF control in Brassicas species. This will allow growers to mitigate against the current level of chemical usage and reduce the costs of production and minimise the risk environmental pollution.

The potential benefits to the industry are:

- To understand and incorporate the practice of pre-adaptation treatments into plug raised Brassicas that confer pest resistance.
- Increased confidence in using the natural stress response functioning of plants to improve resilience to pest attack.
- To potentially reduce the dependence of chemical use to control pest attack.
- Increased profitability by decreasing the level of chemical control of CRF in Brassica plug plant production.
- To maintain productivity and minimise wastage whilst reducing chemical use.
- The current cost of Dursban (chlorpyrifos) is around £12/kg and Tracer £236/L. The rate of Dursban WG is 30g/5000 modules which = £0.36/5000 modules compared to Tracer which has a rate of 60ml/5000 modules which = £14.16/5000 modules. With chlorpyrifos likely to be revoked in the near future, this leaves growers with a treatment that is nearly 40 times more expensive for the control of CRF.
- If a suitable level of NaCl can be found, which will enhance the plants defence mechanisms, building resistance to cabbage root fly, then the total amount of Tracer can be reduced with direct replacement by pre-adaptation.
- In further experiments a combination of treatments could be evaluated to reduce direct chemical use. Either way the aspiration of the work is to produce the best quality plugs for transplant to the field at lowest plant raising cost and if residue free, lowest environmental impact.

Action points for growers

- NaCl fed to the root-zone as a controlled dose can enhance root growth and increase leaf expansion; pre-adaptation at known doses can also confer resistance to CRF.
- Pre-adaptation with NaCl at seedling stage can promote a shift from vegetative to generative growth and promote larger curd size at harvest.
- Very high salt levels can weaken growth by restricting water uptake and promote Downy mildew infection.
- Pre-adaption by using controlled stress offers the propagator an alternative technique to chemical treatment alone, which may prove cost effective and produce more robust and productive plants in the field.

Suggestions for further work

- Pre-adaptation with NaCl can be evaluated for an extended range of Brassica and other crop types.
- Combination doses of NaCl stress combined with Tracer could be evaluated to understand potential beneficial interactions on plant growth and resistance to CRF attack.
- Other stresses could be considered e.g. temperature combined with water / salt stress in combination or alone (see Dutch grower report).
- A greater emphasis on crop performance to maturity would give both propagators and growers more confidence to adopt pre-adaptation techniques for industry practice.
- Further work could be carried out to refine the pre-adaptation ‘window’ to evaluate the potential for non-chemical control of Downy mildew.
- At a fundamental level the work carried out could be extended to gene exploration and identification of specific mechanisms that trigger plant resistance to infection or attack.

SCIENCE SECTION

Introduction

Brassica plants are susceptible to attack by downy mildew (*Hyaloperonospora parasitica)* and cabbage root fly (*Delia radicum*). Both pests affect growth and may result in a non-uniform crop, unmarketable produce and plant death. Losses to cabbage root fly (CRF) can vary from 10% to near crop loss leading to losses up to the region of £145 million. Losses to downy mildew in untreated crops in 2009 caused a loss of 20,000 tonnes of Brassicas, a loss of £8.2 million (Wynn *et al*, 2010). Pesticide treatments are commonly applied during plug plant production or soon after transplanting to control these pests. There are concerns in the Brassica industry about the possible withdrawal of chlorpyrifos as a module drench for the control of CRF, as chlorpyrifos has been classed as a 'priority substance' under the Water Framework Directive. Alternative controls are therefore being actively sought and the focus of the current project is to explore the potential of a non-chemical method to exploit the natural defence mechanisms of the plant to provide resistance to downy mildew and CRF.

Environmental plant stress is often associated with increased susceptibility to pathogen attack and disease. Most research effort has and continues to focus on stress resistance in the context of long term tolerance to environmental stress such as drought, salinity, cold, nutrient shortage and water logging (Collier *et al.,* 2008). There is emerging evidence however, that relatively brief periods of controlled stress can lead to increased pathogen resistance in the leaves of tomato plants because of enhanced production of chemicals that dissuade pathogen attack (Achuo *et al.,* 2006).

The idea developed from commercial observations that seedlings which had been grown 'hard' with reduced water/fertiliser were much less susceptible to pest/disease attack. Growing plants 'hard' however takes longer and can result in poorer quality 'woody' plants which may establish slowly and unevenly. In this project a technique has been developed to stress young plants, and improve their robustness, using solutions of common salt (sodium chloride; NaCl). The science aspiration was that by applying salt solution to the plant for a short period of time just prior to planting out that it would pre-adapt the plant to pests and disease attack.

To use the plants 'in-built' stress response mechanisms to build natural resistance to pathogen attack has been little studied and is a candidate for a novel technique for control. HDC project FV 364 Novel approaches for the management of cabbage root fly considers the use of elicitors to trigger plant defences such as the increased release of glucosinolates. For this project, a number of abiotic stresses could be considered but a strong candidate for

pre-adaptation to further extreme events is the use of salinity, as it is relative straightforward to apply in solution to the plant root system in modules and can be removed by simply diluting through watering. There is experimental evidence in some crop species that plants which have suffered temporary environmental stress are more resistant to pest attack. Grower observations also suggest that plants which are subjected to a controlled period of stress are more resistant to pest attack compared with those grown 'soft'. The aim of this project is to explore the potential of using a controlled short duration salt-stress applied during production of Brassica plug plants for increasing natural host resistance to early attack by downy mildew and CRF. In this project we are advocating quantifying the effect of a brief period of stress that pre-adapts the plant, which is distinct from longer-term 'hard raising' that can lead to poor quality 'woody' plant material.

Pre-adaption of young plant material to salinity environmental stress is a novel technique which we envisage will confer resilience to pathogen attack during plug production and post-transplant into the field. Increased resistance in plant material may well reduce the need for chemical application to control pathogen attack and any reduction in chemical application will improve the profitability of production systems and minimise pesticide loss to the environment. The theory was tested on two commercial early season cauliflower varieties; Chassiron and Skywalker. Plants were fed a range of salt solutions at known concentrations, by hand watering direct to the roots for the final 5 days of propagation before planting out into larger pots and placing CRF eggs around the stem.

The challenge. The type of stress and the thresholds for triggering a beneficial response within the plant needs to be carefully selected, accurately quantified and the technique made relevant and clear for commercial practical application.

Materials and methods

Plant propagation, growing conditions and salinity treatment of Brassica plants

The project sourced 80-82 day cauliflower, cultivars Chassiron and Skywalker. The selected cultivars were seed sown into a peat (80%), green compost blend (20%) growing media using 345 cell trays by a commercial propagator. Plants were delivered to Boxworth for experimental work at cotyledon stage and grown on in insect-proof glasshouses at ADAS Boxworth until two to three leaves had emerged. Trays of plants were placed on capillary matting in glasshouses, watered to the mat surface and pot base by hand and grown at 12h

day 18°C/ night 15°C set point temperatures. These conditions were representative of air temperatures used under commercial growing conditions.

The trays of plants were treated with a range of salinity treatments for five days and then potted on approximately 7 days after the last application before being challenged with either CRF or downy mildew. Salinity treatments began when plants were at the two-to-three true leaf stage. Solutions of NaCl which ranged from 0 to 240 mM were prepared using technical grade NaCl and tap water and applied over the plants using a watering can and rose for once a day in the morning for five consecutive days. Approximately one litre of saline solution was applied per day until the compost was moist but not waterlogged. This replaced the standard pre-treatment hand watering regime.

Four experiments were conducted under glass for CRF (**Table 1** experiments 1, 2, 3 and 4). Also, four experiments were carried out for downy mildew under glass (**Table 1**, experiments 5, 6 and 8), and in a polytunnel **(Table 1**, experiment 7). A commercial field trial was also completed for pre-adapted cauliflower transplants with naturally occurring pest and disease challenges (**Table 1**, experiment 9). The polytunnel environment was selected for a single set of experiments to establish whether it promoted downy mildew compared with glasshouse conditions (**Table 1**, experiment 7). In experiments 4 and 8 saline solutions ranging from 0 to 120 mM were applied to the leaves using a spray mister for 15 days. This approach was implemented to evaluate whether similar plant responses could be arrived at by foliar application as distinct from feeding to the root-zone. The period for foliar application was extended so that the total amount of salt administered to the plants was similar for either approach. During misting normal watering with tap water continued to the pot base and capillary matting, so that plants were kept hydrated.

Table 1. Range of salinities applied to cauliflower seedlings during propagation - ADAS Boxworth, 2012-2013. G, P and F respectively denote glasshouse, polytunnel and field trial experiments.

Exp no and season	Date commenced	Date completed	Concentration (mM) of NaCl (five rates per experiment)*				
			1	2	3	4	5
Cabbage root fly							
1. Summer (G)	14.06.12	23.08.12	0	60	120	180	240
2. Autumn (G)	19.10.12	24.01.13	0	30	60	90	120
3. Summer (G)	30.05.13	06.08.13	0	30	60	90	120
4. Misting (G) (Autumn)	19.10.12	24.01.13	0	30	60	90	120
Downy mildew							
5. Summer (G)	14.06.12	23.08.12	0	60	120	180	240
6. Autumn (G)	19.10.12	25.01.13	0	30	60	90	120
7. Summer (P)	30.05.13	09.08.13	0	30	60	90	120
8. Misting (G) (Autumn)	19.10.12	25.01.13	0	30	60	90	120
Pest and disease							
9. Field trial (F)	24.05.12	18.10.12	0	30	60	90	120

***See equivalent salinities below.

NaCl Solution Concentration		EC Solution	EC Seawater ~54dSm^{-1}
mM	g/L	dS m^{-1}	Rel Strength
60	3.5	5.5	0.1
120	7.0	11	0.2
240	14.0	22	0.4

Approximately 48 hours after completion of NaCl application plants were then potted on. Once potted on plants were allowed at least 48 hours to recover from any 'transplant shock', before being inoculated. Full details of treatment and inoculation timings are given in the crop diaries (Appendix 1).

Measurements of leaf size were recorded daily during NaCl application and once per week for four weeks following the cessation of NaCl treatment, along with measurements of leaf gas exchange (photosynthesis and water vapour loss). Pest and disease incidence was recorded for the same time. The day before NaCl application commenced, a compost sample was taken for pH, % organic matter and nutrient analysis; temperature and humidity were recorded throughout the trial duration. Any phytotoxic effects seen during the trial were also recorded. The methodology has been split into root-zone application for CRF (experiments 1-3 (glass) and downy mildew (experiments 5 and 6 (glass) and 7 (polytunnel), foliar application for CRF under glass (experiment 4) and downy mildew (experiment 8) and field based assessment to maturity of pre-adapted cauliflower transplants (experiment 9).

Integration of pre-adaptation salinity treatments and challenge with P&D

Experiments 1 – 3: Pre-adaptation to challenge by Delia radicum (cabbage root fly)

Site and experiment design

Three fully replicated experiments were carried out at ADAS Boxworth in summer and autumn 2012, and spring/summer 2013, in insect-proof glasshouse compartments. Five replicate blocks of 10 treatment combinations (5 salinity treatments x 2 cabbage root fly treatments) were used on main plots, and the 2 cultivar treatments were arranged as sub-plots in a randomised split-plot design to give a total of 100 plots per trial. Data was analysed by ANOVA.

NaCl concentration treatments

The salinity range varied between experiments to establish a common response surface for the selected cauliflower cultivars to applied NaCl as a potential pre-adaptive treatment. To achieve this in Experiment 1 NaCl concentrations ranged from 0 to 240 mM, in Experiments 2 and 3 spanned 0 to 120 mM (**Tables 2** and **3**).

Table 2. Salinity and cabbage root fly treatments applied to cauliflowers, summer 2012 - ADAS Boxworth (Experiment 1)

Treatment – main plot	Treatment – sub plot	Salinity rate* (mM NaCl)	Salinity (g/L)	Cabbage root fly treatment (number of eggs)	Cultivar
1	1	0	0	0	Chassiron
	2	0	0	0	Skywalker
2	1	60	3.48	0	Chassiron
	2	60	3.48	0	Skywalker
3	1	120	6.96	0	Chassiron
	2	120	6.96	0	Skywalker
4	1	180	10.44	0	Chassiron
	2	180	10.44	0	Skywalker
5	1	240	13.92	0	Chassiron
	2	240	13.92	0	Skywalker
6	1	0	0	40**	Chassiron
	2	0	0	40**	Skywalker
7	1	60	3.48	40**	Chassiron
	2	60	3.48	40**	Skywalker
8	1	120	6.96	40**	Chassiron
	2	120	6.96	40**	Skywalker
9	1	180	10.44	40**	Chassiron
	2	180	10.44	40**	Skywalker
10	1	240	13.92	40**	Chassiron
	2	240	13.92	40**	Skywalker

* Applied daily for 5 days using a watering can and rose.

** Ten eggs were applied per week for 4 weeks or as many as were available from the culture (a minimum of 10 eggs/application).

Table 3. Salinity and cabbage root fly treatments applied to cauliflower seedlings, autumn 2012 and summer 2013 – ADAS Boxworth (Experiments 2 and 3)

Treatment – main plot	Treatment – sub plot	Salinity rate* (mM NaCl)	Salinity (g/L)	Cabbage root fly treatment (number of eggs)	Cultivar
1	1	0	0	0	Chassiron
	2	0	0	0	Skywalker
2	1	30	1.74	0	Chassiron
	2	30	1.74	0	Skywalker
3	1	60	3.48	0	Chassiron

	2	60	3.48	0	Skywalker
4	1	90	5.22	0	Chassiron
	2	90	5.22	0	Skywalker
5	1	120	6.96	0	Chassiron
	2	120	6.96	0	Skywalker
6	1	0	0	40**	Chassiron
	2	0	0	40**	Skywalker
7	1	30	1.74	40**	Chassiron
	2	30	1.74	40**	Skywalker
8	1	60	3.48	40**	Chassiron
	2	60	3.48	40**	Skywalker
9	1	90	5.22	40**	Chassiron
	2	90	5.22	40**	Skywalker
10	1	120	6.96	40**	Chassiron
	2	120	6.96	40**	Skywalker

* Applied daily for 5 days irrigated by hand.

** Ten eggs were applied per week for 4 weeks or as many as was available from the culture (a minimum of 4 eggs/application).

Inoculation methods and plant growth measurements

Plants were allowed to recover after the completion of NaCl treatment for at least 48 hours, and then transplanted into 1 L pots using the same growing media used for propagation. Each plot consisted of two pots (split-plot arrangement) with one plant in each plot. The pots were placed onto the capillary matting in a randomised block design. Three to four days post-transplanting, the 1st CRF treatments were applied and then all of the plants were covered with perforated clear bags to avoid cross-contamination between plots for the duration of the trial.

A laboratory culture of *D. radicum* was used to infest each potted cauliflower plant with 40 eggs over a four week period with up to 10 eggs being washed onto the compost around the stem base of the plant once per week for four weeks. On occasions when there were insufficient eggs available from the culture, the final inoculation of 10 eggs was omitted or the number of eggs at each inoculation was reduced. For each application an equivalent number of eggs were assessed *in vitro* to quantify a viability score for every batch used within an experiment. To do this eggs were placed onto a layer of damp filter paper in a Petri dish, and maintained in a CE room at 21°C, 16:8 L:D ensuring that the filter paper remained damp throughout. Eggs were monitored daily for hatching in order to score the viability of the eggs.

Plant measurement during salinity application

Every day for five days during the application of the salinity treatments leaf growth parameters were measured and recorded for selected plants. The leaf length and width on the youngest expanding leaf was recorded daily. Twenty leaves were measured per salinity treatment using a selection from across the module tray in a 'W' shape (10 from each cultivar keeping measurements from each cultivar separate) to give a total of 100 measurements. The selected leaves were tagged and the same leaf was measured each day. Treatments were not in randomised blocks at this stage because the salinity could be applied more evenly and accurately to a whole module tray. The module trays were arranged in treatment order in the centre of the glasshouse, to avoid any potentially confounding edge effects.

Plant growth measurements after the completion of NaCl application

Every week for at least four weeks after the application of the salinity treatments the leaf expansion was recorded for each plot. Leaf length and width was recorded once a week. The youngest expanding leaf was tagged on each plant and the same leaf was measured at each assessment date. Leaves were measured from one plant per cultivar per plot to give a total of 100 measurements.

Leaf gas exchange measurements

A Li-Cor LI-6400XT Portable Photosynthesis System machine (Li-Cor, USA) was used to measure net CO_2 leaf gas exchange for photosynthesis, transpiration rate for evaporative water loss, stomatal conductance, intercellular CO_2 concentration and instantaneous transpiration efficiency (ITE: µmol CO_2 / mmol H_2O). Measurements were taken for a period of 2 hours mid to late morning around the peak of diurnal photosynthesis, for example between 09:00 and 13:00, and if readings were stable, continued until 14:00. Each set of readings for each assessment was taken at the same time where possible and all under natural light conditions. Four sets of readings were made over a four week period.

To rationalise the number of readings and to gain sufficient data to allow interpretation of the treatment effects then leaves were selected from the control mid and highest concentrations for the NaCl treatments. So that data could be statistically analysed then one reading was taken from the youngest expanding leaf from each of the experimental plots across three blocks for uninoculated and inoculated treatments at 0, 120, 180 and 240 mM salinities for Experiment 1 and from uninoculated and inoculated treatments at 0, 60, 90 and 120 mM for Experiments 2 and 3. A measurement was taken from both cultivars in the plot, selecting leaves that were facing the sun, using the middle of the upper part of the leaf for the reading. When the data was analysed, there were no significant differences between the two cultivars

for the range of salinity treatments. Therefore, the data for the two cultivars were combined, in order to examine the effect of salinity treatment over time and increase the number of observations for treatment effects.

Final harvest destructive growth analysis

At four to five weeks after the first cabbage root fly applications were made, root damage and leaf area assessments were completed. Root damage was recorded as a percent of roots damaged and the whole roots system wash washed free of growing media, blotted dry and fresh root weight determined.

Experiments 5 – 7: Pre-adaptation to challenge by Hyaloperonospora parasitica (downy mildew)

Experiments were carried out on the same batch of source plants that were treated with NaCl, with the other half of the plants going as previously described for CRF treatment. The differences in measurement, transplantation and downy mildew inoculation are as set out below.

Treatments

In Experiment 5 NaCl concentrations ranged from 0 to 240 mM; in Experiments 6 and 7 NaCl concentration spanned 0 to 120 mM. (**Tables 4** and **5**).

Table 4. Salinity and downy mildew inoculation treatments applied to cauliflowers, summer 2012 - ADAS Boxworth

Treatment – main plot	Treatment – sub plot	Salinity rate* (mM NaCl)	Salinity (g/L)	Downy mildew treatment (spores/ml)	Cultivar
1	1	0	0	0	Chassiron
	2	0	0	0	Skywalker
2	1	60	3.48	0	Chassiron
	2	60	3.48	0	Skywalker
3	1	120	6.96	0	Chassiron
	2	120	6.96	0	Skywalker
4	1	180	10.44	0	Chassiron
	2	180	10.44	0	Skywalker
5	1	240	13.92	0	Chassiron
	2	240	13.92	0	Skywalker
6	1	0	0	1×10^4	Chassiron
	2	0	0	1×10^4	Skywalker

7	1	60	3.48	1 x 10^4	Chassiron
	2	60	3.48	1 x 10^4	Skywalker
8	1	120	6.96	1 x 10^4	Chassiron
	2	120	6.96	1 x 10^4	Skywalker
9	1	180	10.44	1 x 10^4	Chassiron
	2	180	10.44	1 x 10^4	Skywalker
10	1	240	13.92	1 x 10^4	Chassiron
	2	240	13.92	1 x 10^4	Skywalker

* Applied daily for 5 days using a watering can and rose.

Table 5. Salinity and downy mildew treatments applied to cauliflower seedlings, autumn 2012 and summer 2013 – ADAS Boxworth

Treatment – main plot	Treatment – sub plot	Salinity rate* (mM NaCl)	Salinity (g/L)	Downy mildew treatment (spores/ml)	Cultivar
1	1	0	0	0	Chassiron
	2	0	0	0	Skywalker
2	1	30	1.74	0	Chassiron
	2	30	1.74	0	Skywalker
3	1	60	3.48	0	Chassiron
	2	60	3.48	0	Skywalker
4	1	90	5.22	0	Chassiron
	2	90	5.22	0	Skywalker
5	1	120	6.96	0	Chassiron
	2	120	6.96	0	Skywalker
6	1	0	0	1 x 10^4	Chassiron
	2	0	0	1 x 10^4	Skywalker
7	1	30	1.74	1 x 10^4	Chassiron
	2	30	1.74	1 x 10^4	Skywalker
8	1	60	3.48	1 x 10^4	Chassiron
	2	60	3.48	1 x 10^4	Skywalker
9	1	90	5.22	1 x 10^4	Chassiron
	2	90	5.22	1 x 10^4	Skywalker
10	1	120	6.96	1 x 10^4	Chassiron
	2	120	6.96	1 x 10^4	Skywalker

* Applied daily for 5 days, irrigated by hand.

Inoculation methods, plant growth and gas exchange measurements

Plants were potted on as described for Experiments 1-3, but with 5 plants in a pot. At least 48 hours after transplanting, plants were spray inoculated with a spore suspension of downy

mildew prepared from active lesions maintained on a culture of cauliflower seedlings. All of the plants were then loosely covered with polythene to augment leaf wetness duration to promote the likelihood of infection. Assessments of plant growth and photosynthesis were carried out during and after salinity application as described in the previous section.

Disease assessments

Five days after inoculation, the plots were monitored daily for symptoms of downy mildew. A full assessment of incidence and severity of downy mildew symptoms was carried out at 7, 14, 21 and 28 days after inoculation.

Experiments 4 and 8: Foliar application of saline solutions.

Site and trial design

These two experiments were carried out at ADAS Boxworth in autumn 2012 in a glasshouse. In Exp 4, five replicate blocks of 10 treatment combinations of 5 salinity treatments x 2 cabbage root fly treatments were used on main plots, and the 2 cultivar treatments were arranged as sub-plots in a randomised split-plot design to give a total of 100 plots per trial. In Exp 8 the same design was used but the inoculum was downy mildew. Data was analysed by ANOVA.

Treatments

Treatments were as in Table 4 and 5 for the main autumn 2012 trials, using a range of salinities from 0 -120 mM, applied as a foliar spray over a longer period, of 15 days. This was done so that the plants were exposed to the same quantity of NaCl compared with root-zone treated plants.

NaCl treatments began when plants were in module trays at the one true leaf stage. Saline solutions were applied daily using a hand held mister for 15 days. For the first five days, 25 ml of the required treatment was applied as a fine mist to the leaves of the plants of each tray. For the next five days (days 6 – 10) 50 ml of the required treatment was applied as a fine mist to the leaves of the plants of each tray. For the final five days (days 11 – 15) 75 ml of the required treatment was applied as a fine mist to the leaves of the plants of each tray. Each module tray represented one treatment. Saline solutions were adjusted for the three time periods so that the same concentration of salt was applied each day although the volume of water differed. This was to account for increasing leaf expansion as the plants grew. During foliar treatment application, plants were irrigated to the pot base to avoid confounding water deficits in the root zone.

Inoculation and assessments

Inoculation with cabbage root fly and downy mildew, assessment of infection and plant growth analysis was as described previously.

Experiment 9 – Field trial with natural pest and disease challenge

A field experiment was carried out using seedlings subjected to pre-adaptive salinity stress before being transplanted in the field during a commercial planting operation.

Site and trial design

The trial comprised ten treatments; five salinity treatments and two 80-82 day-old commercial cultivars, Chassiron and Skywalker (**Table 6**). Each treatment was replicated four times, making a total of 40 plots arranged in a fully randomised design. Each plot consisted of 60 plants, planted at standard commercial spacing of 45 cm down the row in three rows of 20 plants using commercial equipment supplied and operated by the grower.

Seedlings were sown by a commercial propagator and delivered to ADAS, Boxworth in module trays at cotyledon-one true leaf stage. Seedling trays were placed on capillary matting and watered from below. NaCl treatments were applied by hand to the root-zone to tray plants at ADAS Boxworth for 5 consecutive days prior to planting at the site. The trial was planted at a commercial grower site, Lincolnshire within a commercial crop of cauliflower. The trial site was approximately 130 m long by nine rows wide. There were 10 m long guard plots positioned at either end of the trial. The trial was planted using a nine row planter with three supervisors monitoring the correct planting of each of the plots (Figure 1). Plants were arranged in modular trays to correspond to the trial plan prior to planting out. Each plot consisted of three rows of 20 plants. The host grower applied an herbicide to the trial area but no plant protection products were applied to plants in the experiment in order to allow natural pest and disease attack.

Table 6. Salinity treatments used in the field trial, Lincs – 2012 (Experiment 9)

Treatment	Salinity rate (mM NaCl)	Salinity (g/L)	Cultivar
1	0	0	Skywalker
2	30	1.74	Skywalker
3	60	3.48	Skywalker
4	90	5.22	Skywalker
5	120	6.96	Skywalker
6	0	0	Chassiron
7	30	1.74	Chassiron
8	60	3.48	Chassiron
9	90	5.22	Chassiron
10	120	6.96	Chassiron

Figure 1. Pre-adapted plants being transplanted at a commercial site in Lincolnshire in June 2012.

Assessments

Plants were assessed on 26 July and 29 August 2012 for vigour and occurrence of pests and diseases. At harvest, ten cauliflowers per plot were cut and trimmed as advised by the grower. The cauliflowers were assessed for vigour, pest and disease damage and blemishes on enclosing leaves. Five roots systems were excavated and removed from each plot, cleaned and weighed together, to provide an integrated average root weight per treatment plot. Each root system was assessed individually for damage and given a root damage index score. Data was analysed by ANOVA.

Results and discussion

Experiments 1 – 3: Pre-adaptation to challenge by cabbage root fly

Experiment 1

Leaf expansion during salinity application

NaCl concentrations of 240 mM reduced leaf length and width in Chassiron (**Figure 2** and **3**). Conversely a concentration of 60 mM, although leaf expansion was reduced compared with the control the effect was not statistically significant.

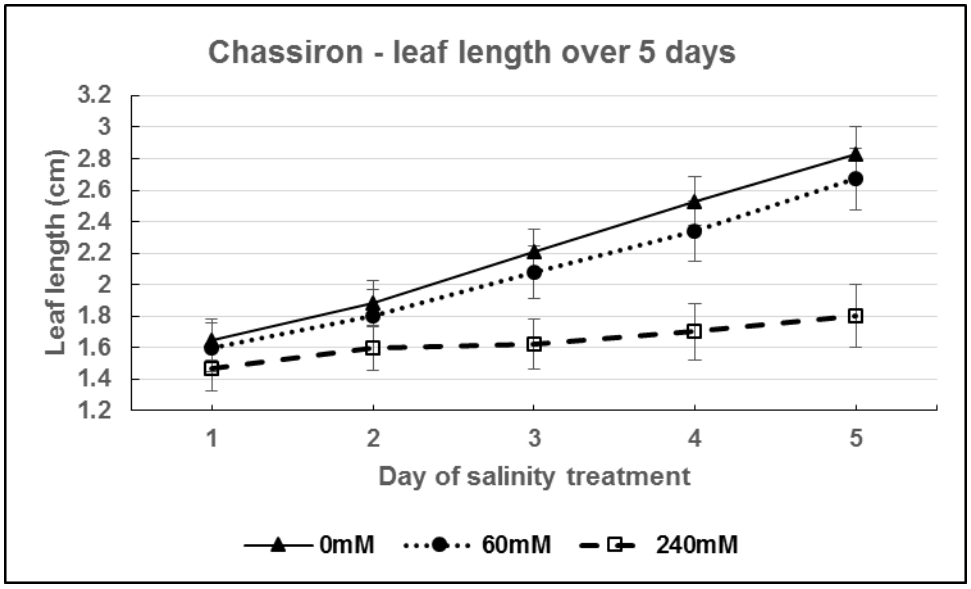

Figure 2: Leaf length during 5 days of salinity treatment, Exp 1, 2012. Vertical bars are ± standard errors, with 48 degrees of freedom (d.f).

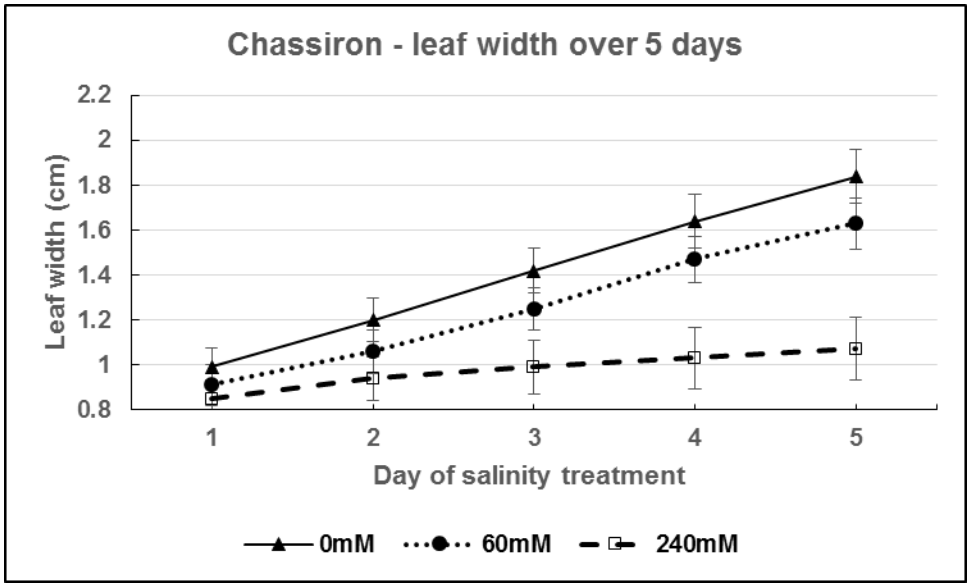

Figure 3: Leaf width during 5 days of salinity treatment, Exp 1, 2012. Vertical bars are ± standard errors, with 48 d.f.

Whilst Skywalker concurred with Chassiron and exhibited a reduced leaf length and width (**Figures 4** and **5**), leaf growth parameters were clearly enhanced under NaCl applied at a concentration of 60 mM (**Figures 4** and **5**), which remained constant over the 5 day treatment period.

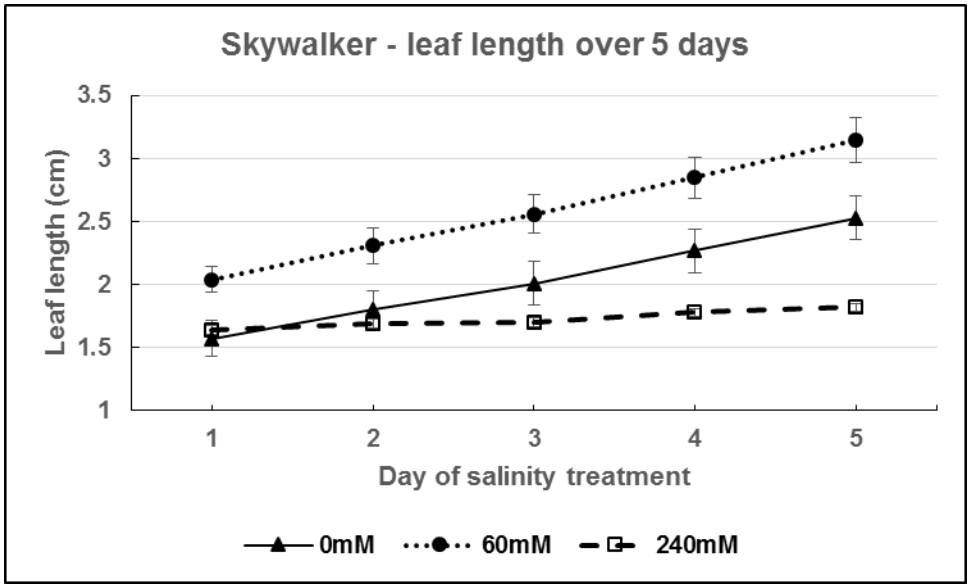

Figure 4: Leaf length during 5 days of salinity treatment, Exp 1, 2012. Vertical bars are ± standard errors, with 48 d.f.

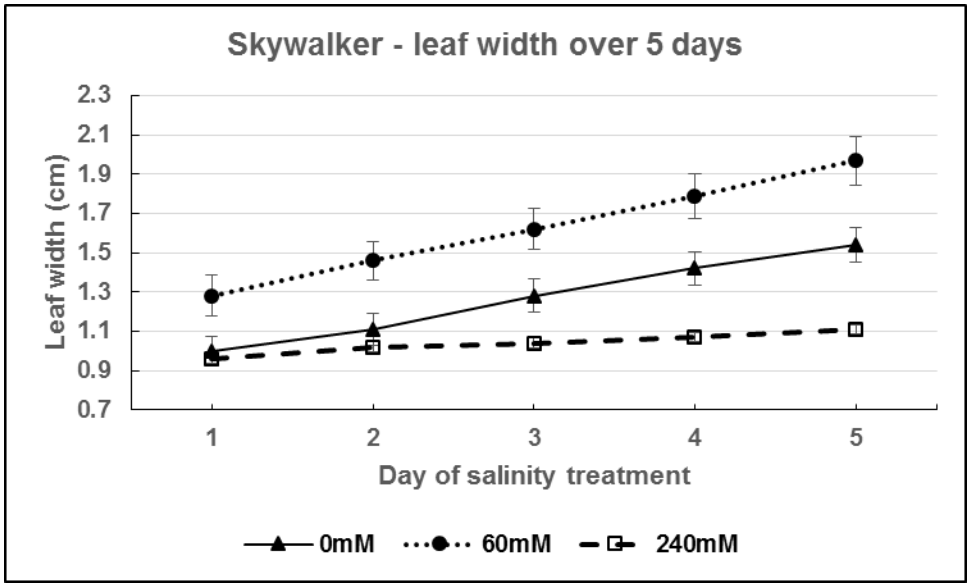

Figure 5: Leaf width during 5 days of salinity treatment, Exp 1, 2012. Vertical bars are ± standard errors, with 48 d.f.

Leaf area performance from the completion of NaCl treatment

From the completion of NaCl application and transplanting into larger pots, Chassiron exhibited reduced leaf areas in the 240 mM treatment (**Figure 6**); plants treated with 60 mM NaCl performed similarly to the control. Skywalker leaf area was similar across all treatments and appeared to be considerably more able to incorporate and metabolise enhanced NaCl supply to the expanding leaf tissues compared with Chassiron (**Figure 7**).

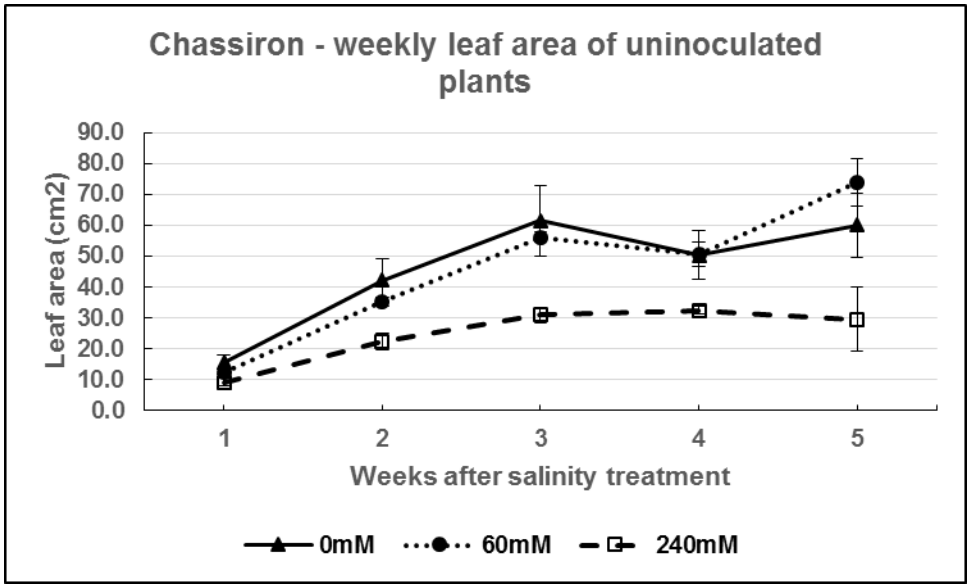

Figure 6: Chassiron leaf area in weeks following salinity treatment, Exp 1, 2012. Vertical bars are ± standard errors, with 48 d.f.

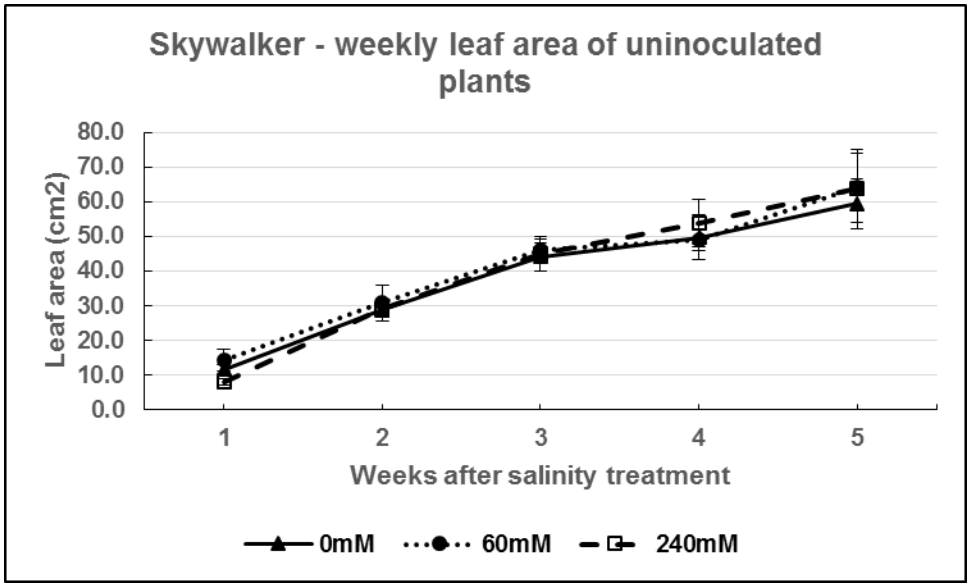

Figure 7: Skywalker leaf area in weeks following salinity treatment, Exp 1, 2012. Vertical bars are ± standard errors, with 48 d.f.

Effect of salinity treatments on leaf gas exchange

Photosynthesis was lower in the highest salinity rate, with and without CRF inoculum, however, this was not statistically significant (P=0.202; **Table 7**). With CRF present, photosynthesis appeared to be reduced and was noticeably additive at the highest NaCl treatment 240 mM NaCl (**Table 7**).

Table 7. Effect of salinity treatments on Brassica photosynthetic net CO_2 exchange (µmol CO_2 m^{-2} s^{-1}) - Summer 2012 – Exp 1.

Treatment	Salt concentration (mM)	With or without CRF eggs	CO_2 exchange at weeks after salinity application: 3 (25 Jul 12)	5 (9 Aug 12)	6 (16 Aug 12)
1	0	-	10.94	2.45	4.42
3	120	-	10.73	3.13	4.82
5	240	-	9.93	3.26	4.44
6	0	+	10.05	3.02	2.23
9	180	+	10.25	2.10	3.32
10	240	+	9.75	0.45	2.10

s.e.d – 1.602, with 72 d.f.

Stomatal conductance was significantly reduced at the highest salinity treatment with CRF inoculum (P<0.001; **Table 8**). The untreated plants and the 240 mM treated plants, with CRF inoculum (T6 and T10), had a much lower stomatal conductance towards the end of the experiment,; the presence of CRF had a significant impact on stomatal conductance and the effect appeared to be additive to the NaCl treatment particularly at 240 mM in weeks 3-5 (P<0.001; **Table 8**).

Table 8. Effect of salinity treatments on Brassica leaf stomatal conductance (mol H_2O m^{-2} s^{-1}) – Summer 2012 – Exp 1.

Treatment	Salt concentration (mM)	With or without CRF eggs	Stomatal conductance at weeks after salinity application:		
			3 (25 Jul 12)	5 (9 Aug 12)	6 (16 Aug 12)
1	0	-	0.49	0.95	0.55
3	120	-	0.51	0.79	0.75
5	240	-	0.44	0.77	0.44
6	0	+	0.49	0.37	0.12
9	180	+	0.40	0.53	0.20
10	240	+	0.38	0.12	0.14

s.e.d – 0.090, with 72 d.f.

Plants inoculated with CRF had a significantly lower transpiration rate than uninoculated plants (P<0.001; **Table 9**). By 2 August 2012, T10 was the lowest, showing both CRF and high salinity treatment were impacting on photosynthesis. Two weeks later, T6 and T10 were also very low, but T9 was higher.

Table 9. Effect of salinity treatments on Brassica leaf instantaneous transpiration rate (ITE µmol CO_2 / mmol H_2O) – Summer 2012 – Exp 1.

Treatment	Salt concentration (mM)	With or without CRF eggs	ITE at weeks after salinity application:		
			3 (25 Jul 12)	5 (9 Aug 12)	6 (16 Aug 12)
1	0	-	6.02	5.48	4.92
3	120	-	6.25	5.15	5.62
5	240	-	5.67	4.95	4.49
6	0	+	5.96	3.42	1.86
9	180	+	5.33	3.92	2.55
10	240	+	5.07	1.65	1.84

s.e.d – 0.572, with 72 d.f.

Number of collapsed plants at end of experiment

Figure 8 shows that a greater number of plants collapsed at the higher salinity levels of 180 mM and 240 mM, than in the untreated, so too much salt causes too much stress on the plants, and they are unable to recover from it, therefore they are unable to resist attack by CRF. There was 100% difference in the number of collapsed Chassiron and collapsed Skywalker at both 0 mM and 180 mM. At 60 mM, there were no collapsed plants for either cultivar, showing that lower levels of salt are beneficial to the plant, enabling the plants to become stronger and fight off pest attack (P<0.05).

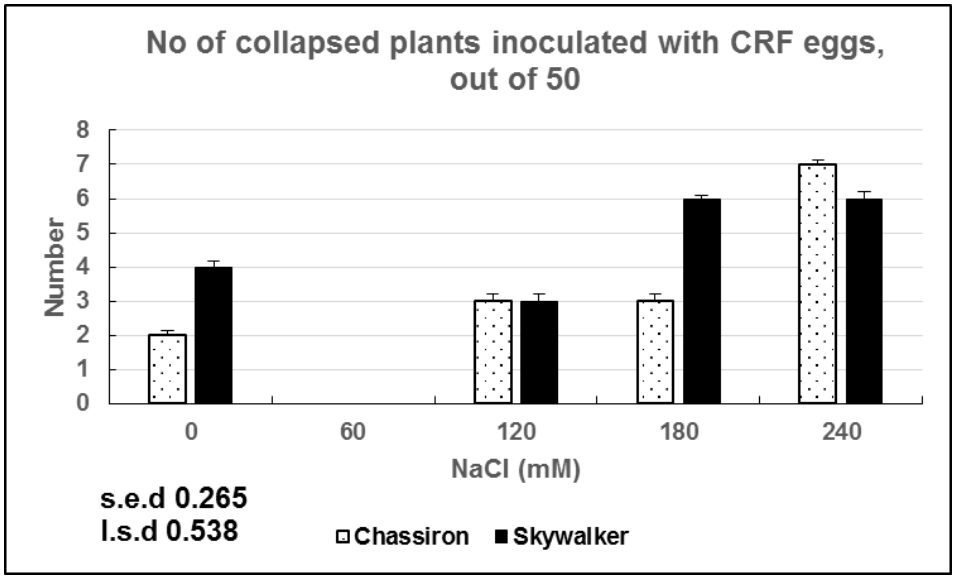

Figure 8: Number of collapsed plants for each salinity treatment due to inoculation with CRF eggs, Exp 1, 2012. Vertical bars are ± standard errors, with 48 d.f.

Root weight at end of experiment

Figure 9 shows the average root weight of both cultivars for each salinity treatment, without the addition of CRF eggs. Skywalker is more tolerant of higher salinity levels, as root weight increases up to 120 mM. Both cultivars suffer at 240 mM, where root weight greatly decreases, however Skywalker is still slightly better. For Chassiron, root weight peaks at 60 mM. This shows that some salt is beneficial to the plant in producing a stronger, heavier root.

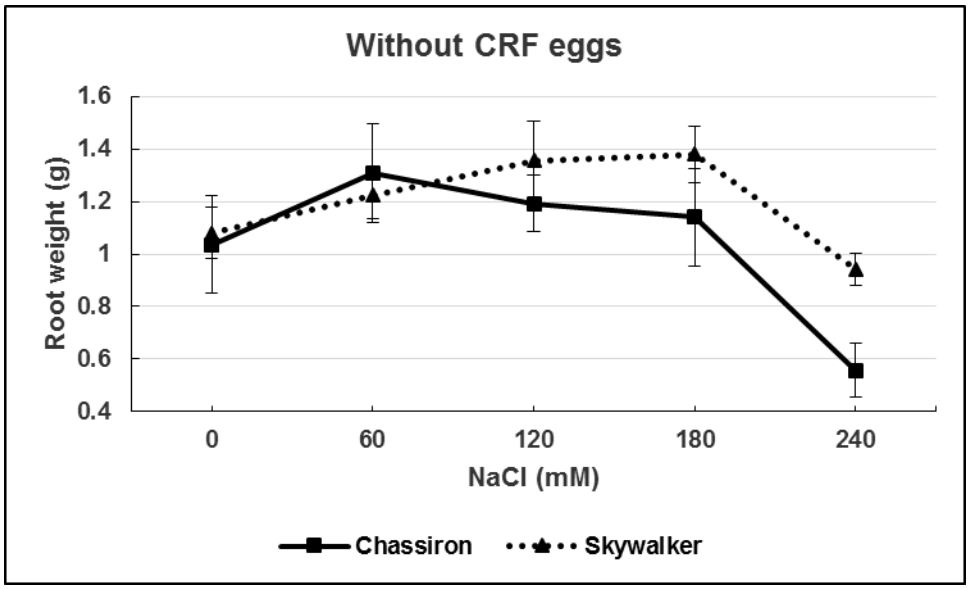

Figure 9: Weight of roots for both cultivars without CRF inoculation, Exp 1, 2012. Vertical bars are ± standard errors, with 48 d.f.

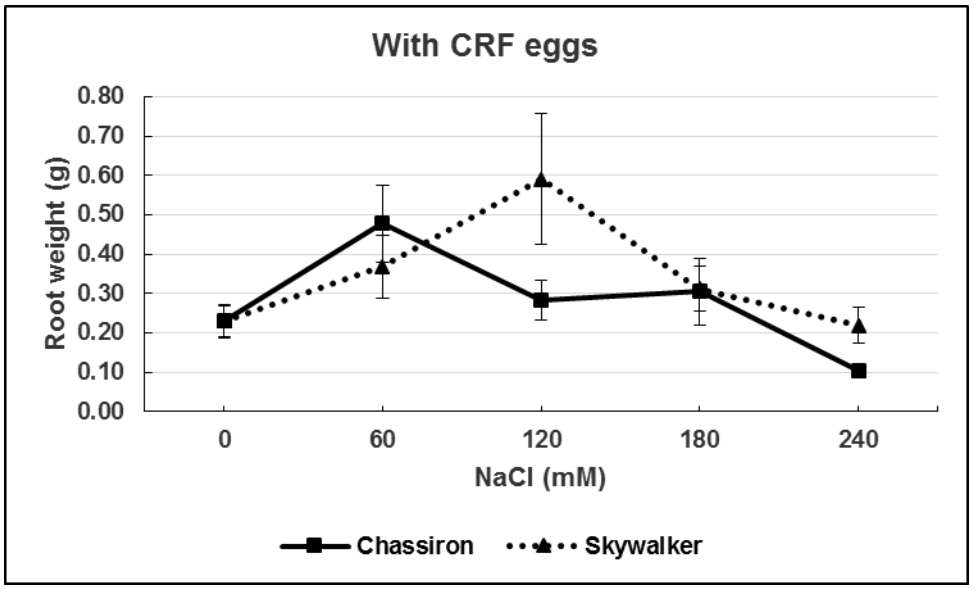

Figure 10: Weight of roots for both cultivars with CRF inoculation, Exp 1, 2012. Vertical bars are ± standard errors, with 48 d.f.

Figure 10 shows the average root weight of both cultivars, which are approximately half the root mass compared with no CRF (cf. Figure 9) for each salinity treatment, with the addition of CRF eggs. Skywalker is more tolerant, and the average root weight is highest at 120 mM, whereas it peaks at 60 mM NaCl for Chassiron ($P<0.01$; **Figure 10**). There is no difference between the root weight of an untreated Skywalker plant and one treated with 240 mM of NaCl. For Chassiron, plants treated with 240 mM plus CRF eggs weighed less than the untreated, showing that too much salt has a detrimental effect and doesn't provide any protection against CRF attack. Both cultivars show some resistance to CRF attack when they are treated with 60 mM of NaCl.

Figure 11 shows plants that were representative of treatments 0 mM, 60 mM and 240 mM, with CRF inoculum. It is clear that water alone (0 mM), is not enough to provide resistance to CRF. 240 mM is too much for the plant and it is unable to recover from the stress caused by the high salt content. However, plants treated with 60 mM appear able to resist CRF attack, and the plant remains strong and healthy.

Figure 11: L-R; 0mM + CRF; 60 mM + CRF; 240 mM + CRF, Exp 1, 2012.

Experiment 2

Leaf expansion during salinity application

In Experiment 2, Chassiron grew steadily during the 5 days of salinity treatment, particularly at 60 mM (**Figure 12**). NaCl concentrations of 120 mM also stimulated plant growth in Chassiron. The untreated plants were smaller to begin with at the start of the experiment, but grew quickly and were the same length as plants treated with 120 mM at day 4.

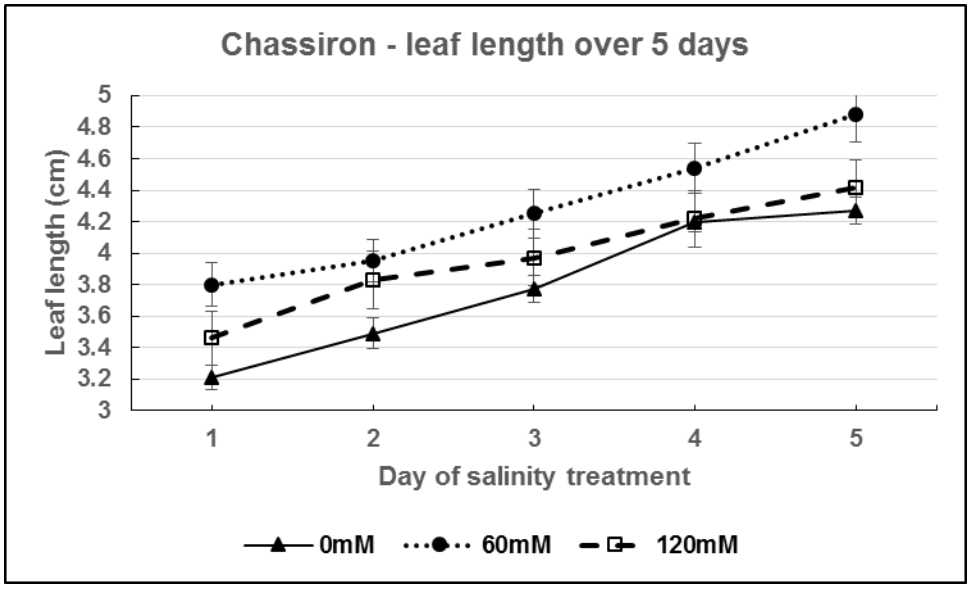

Figure 12: Leaf length during 5 days of salinity treatment, Exp 2, 2012. Vertical bars are ± standard errors, with 48 d.f.

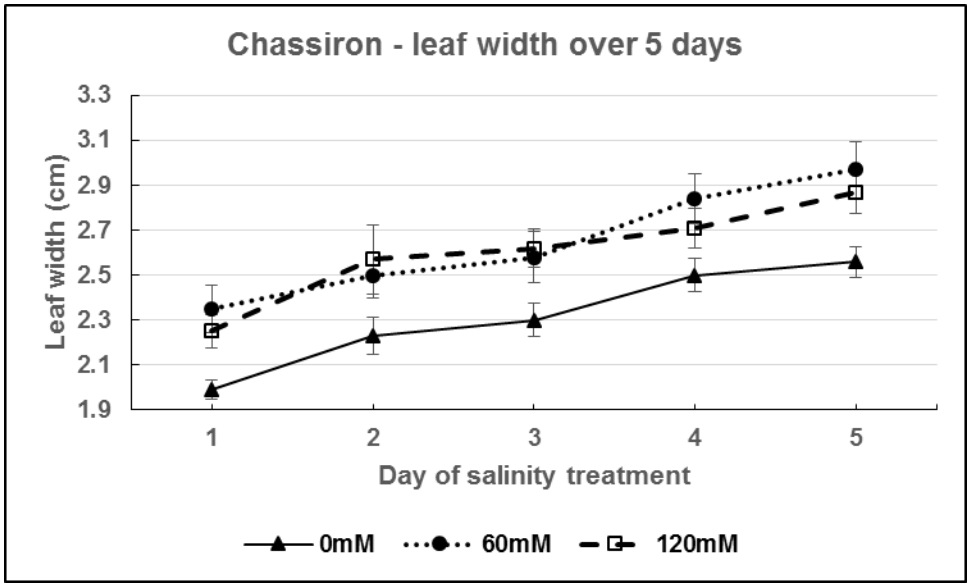

Figure 13: Leaf width during 5 days of salinity treatment, Exp 2, 2012. Vertical bars are ± standard errors, with 48 d.f.

Whilst Skywalker concurred with Chassiron and exhibited an increased leaf length and width (**Figures 14** and **15**), leaf growth parameters were clearly enhanced in the untreated plants, although these plants were larger at the start of treatment. NaCl applied at a concentration of 60 mM and 120 mM (**Figures 14** and **15**), promoted growth at the same rate over the 5 day treatment period.

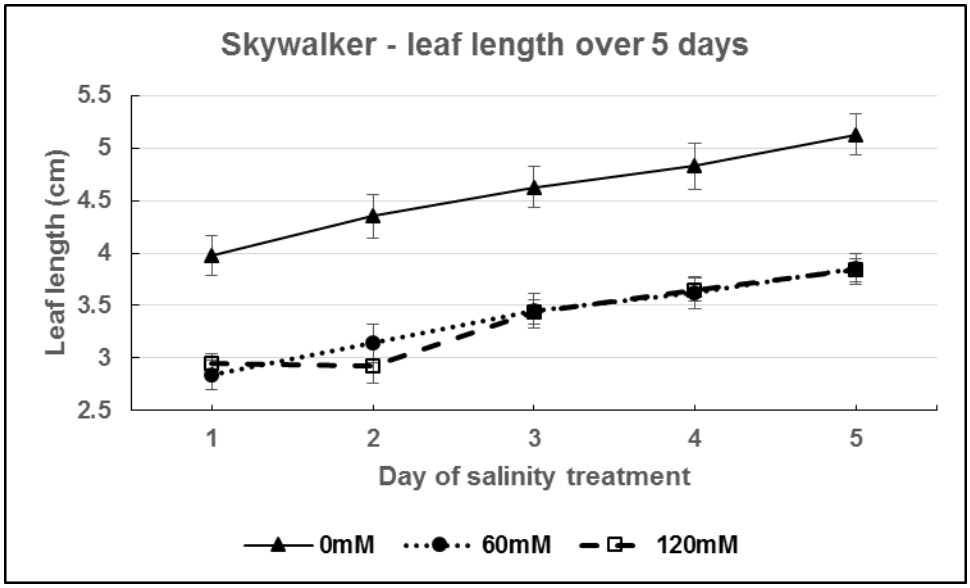

Figure 14: Leaf length during 5 days of salinity treatment, Exp 2, 2012. Vertical bars are ± standard errors, with 48 d.f.

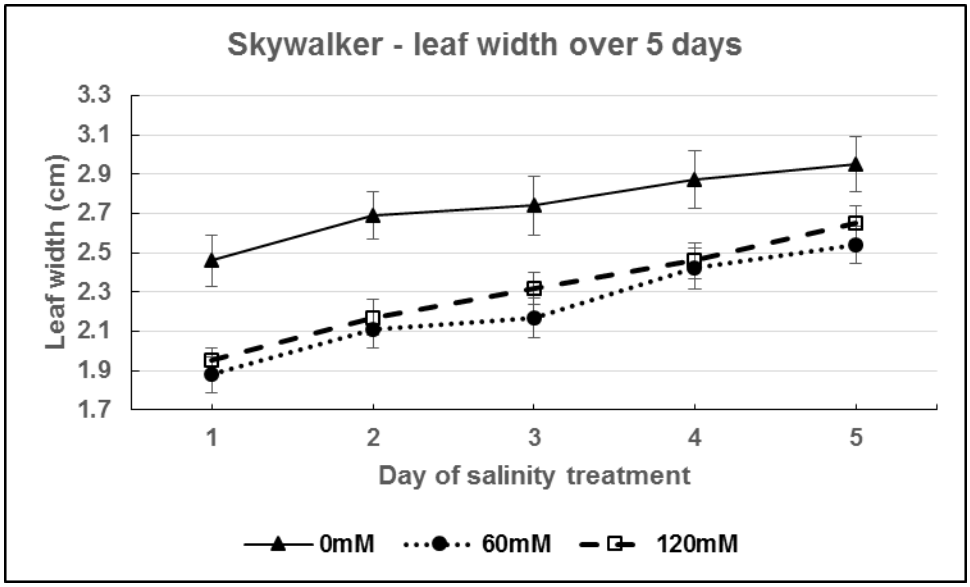

Figure 15: Leaf width during 5 days of salinity treatment, Exp 2, 2012. Vertical bars are ± standard errors, with 48 d.f.

Leaf area performance from the completion of NaCl treatment

From the completion of NaCl application and transplanting into larger pots, Chassiron exhibited increased leaf areas in all treatments (**Figure 16**); plants treated with 60 mM and 120 mM NaCl performed similarly to the control. Skywalker leaf area was similar across all treatments and appeared to be considerably more tolerant of higher salinity rates, as leaf area was greatest at 120 mM (**Figure 17**). 6 weeks after treatment both cultivars were the same size for both the untreated and the 120 mM treated plants, without CRF inoculum.

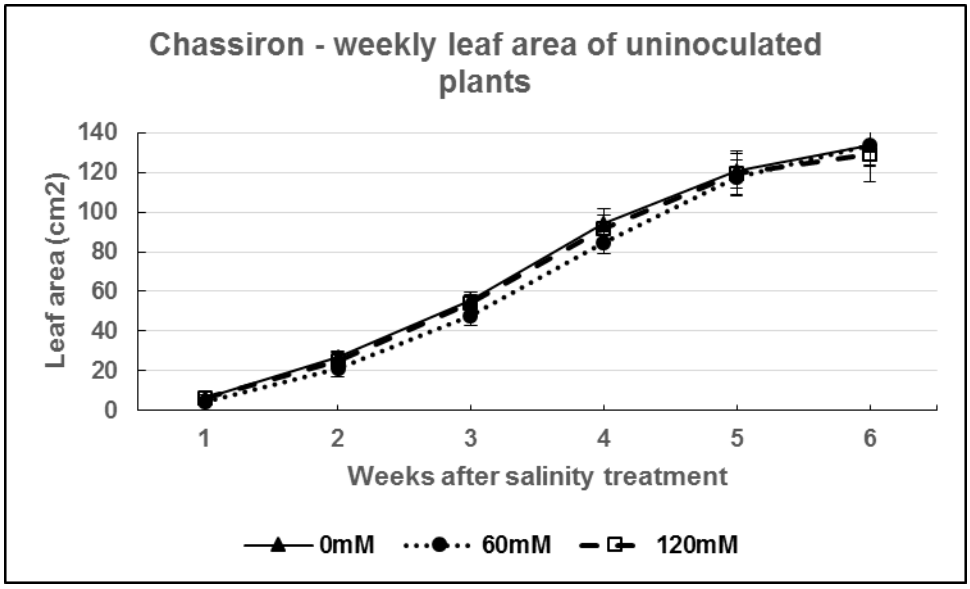

Figure 16: Chassiron leaf area in weeks following salinity treatment, Exp 2, 2012. Vertical bars are ± standard errors, with 48 d.f.

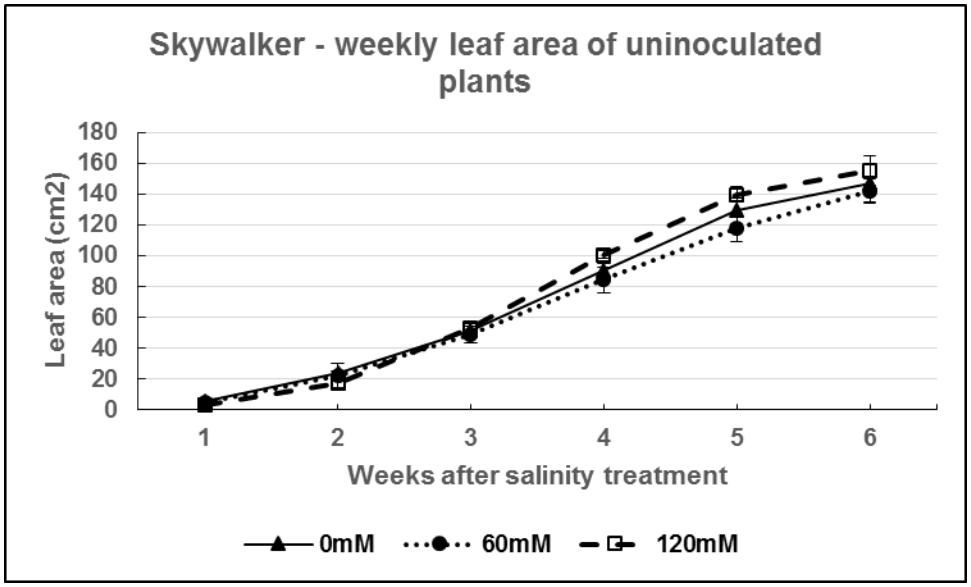

Figure 17: Skywalker leaf area in weeks following salinity treatment, Exp 2, 2012. Vertical bars are ± standard errors, with 48 d.f.

Effect of salinity treatments on leaf gas exchange

Photosynthesis was in general higher in the uninoculated plants, and low versus high NaCl treatments, although not significant (**Table 10**). Photosynthesis was generally lowest under high NaCl concentrations, particularly in uninoculated plants. A much flatter response surface was observed in inoculated plants, where CRF was impacting more on the root system of non NaCl treated plants (**Table 10**).

Table 10. Effect of salinity treatments on Brassica photosynthetic net CO_2 exchange (µmol CO_2 m^{-2} s^{-1}) – Autumn 2012 – Exp 2.

Treatment	Salt concentration (mM)	With or without CRF eggs	CO_2 exchange at weeks after salinity application:					
			2 (22 Nov 12)	3 (29 Nov 12)	4 (6 Dec 12)	5 (13 Dec 12)	6 (19 Dec 12)	7 (3 Jan 13)
1	0	-	3.17	2.98	4.86	3.70	2.66	2.75
3	60	-	2.88	3.14	4.84	3.58	2.86	2.90
5	120	-	2.81	3.14	4.72	3.58	2.51	2.52
6	0	+	2.92	2.95	4.88	3.60	2.84	2.54
9	90	+	2.50	3.08	4.66	3.58	2.54	2.20
10	120	+	3.31	2.90	4.81	3.59	2.89	2.33

s.e.d – 0.335, with 120 d.f.

Stomatal conductance was highest in the untreated plants with CRF inoculum until 19 December, when conductance then decreased, and was highest in plants treated with 60 mM, without CRF inoculum (**Table 11**). Untreated and treated plants inoculated with CRF had a much lower stomatal conductance at the end of the experiment; the presence of CRF

had a significant impact on stomatal conductance and the effect appeared to be additive to the NaCl treatment particularly at 90 mM and 120 mM in weeks 5-7 (P<0.01; **Table 11**).

Table 11. Effect of salinity treatments on Brassica leaf stomatal conductance (mol H_2O m^{-2} s^{-1}) – Autumn 2012 – Exp 2.

Treatment	Salt concentration (mM)	With or without CRF eggs	Stomatal conductance at weeks after salinity application:					
			2 (22 Nov 12)	3 (29 Nov 12)	4 (6 Dec 12)	5 (13 Dec 12)	6 (19 Dec 12)	7 (3 Jan 13)
1	0	-	0.33	0.32	0.32	0.19	0.27	0.46
3	60	-	0.34	0.33	0.38	0.23	0.35	0.55
5	120	-	0.33	0.35	0.37	0.20	0.30	0.38
6	0	+	0.38	0.38	0.42	0.28	0.24	0.31
9	90	+	0.36	0.37	0.38	0.22	0.32	0.23
10	120	+	0.36	0.30	0.36	0.24	0.25	0.31

s.e.d – 0.054, with 120 d.f.

Plants inoculated with CRF had a significantly lower transpiration rate than uninoculated plants (P<0.001; **Table 12**). Transpiration rate was higher in plants treated with 60 mM without CRF inoculum. This shows that CRF inoculum is having an effect on the plants, and reducing the rate of transpiration, and this was statistically significant (P<0.01).

Table 12. Effect of salinity treatments on Brassica leaf instantaneous transpiration rate (ITE µmol CO_2 / mmol H_2O) – Autumn 2012 – Exp 2.

Treatment	Salt concentration (mM)	With or without CRF eggs	ITE at weeks after salinity application:					
			2 (22 Nov 12)	3 (29 Nov 12)	4 (6 Dec 12)	5 (13 Dec 12)	6 (19 Dec 12)	7 (3 Jan 13)
1	0	-	2.29	2.13	1.95	1.72	2.43	2.83
3	60	-	2.35	2.15	2.13	1.94	2.92	3.15
5	120	-	2.35	2.19	2.11	1.76	2.68	2.50
6	0	+	2.39	2.34	2.21	2.20	2.26	1.90
9	90	+	2.42	2.30	2.13	1.87	2.76	1.83
10	120	+	2.45	2.05	2.02	1.90	2.35	1.95

s.e.d – 0.251, with 120 d.f.

Number of collapsed plants at end of experiment

In Exp 2, Chassiron appeared to perform better than Skywalker, with less collapsed plants (**Figure 18**). There were more collapsed plants in the untreated for Skywalker, and no collapsed plants for either cultivars at 90 mM (P<0.01; **Figure 18**). There were a greater number of collapsed plants for Skywalker at 120 mM compared with Chassiron at 120 mM.

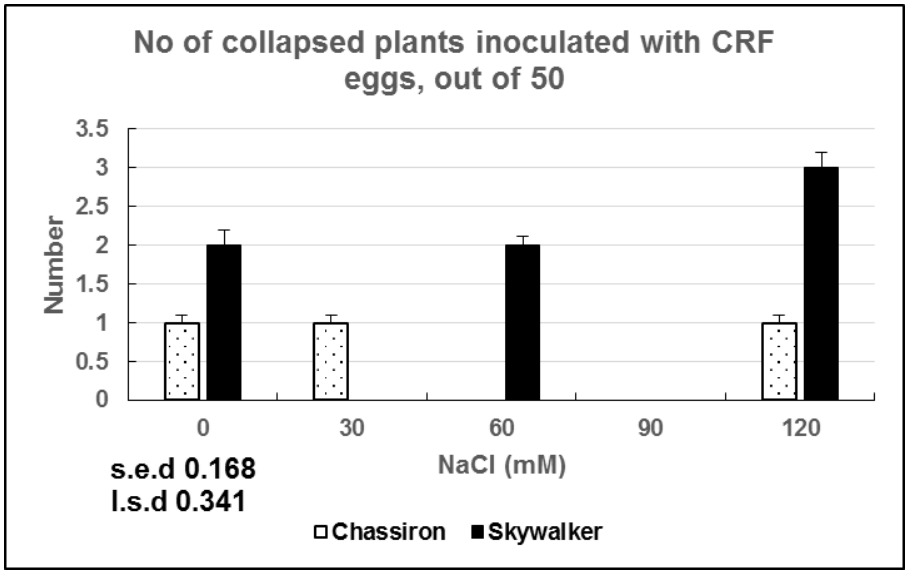

Figure 18: Number of collapsed plants for each salinity treatment due to inoculation with CRF eggs, Exp 2, 2012. Vertical bars are ± standard errors, with 48 d.f.

Root weight at end of experiment

Figure 19 shows the average root weight of both cultivars for each salinity treatment, without CRF inoculation. Root weight varied, and for Skywalker was lower at 60 mM than at 0 mM or 120 mM. With Chassiron, root weighed increased and reached a peak at 60 mM, where it then fell by approximately 2 g at 90 mM.

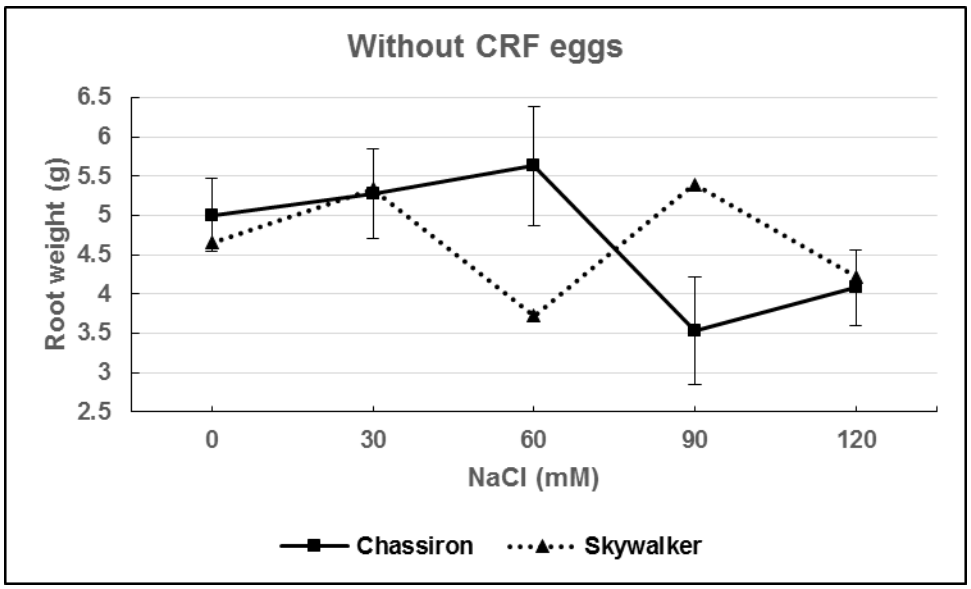

Figure 19: Weight of roots for both cultivars without CRF inoculation, Exp 2, 2012. Vertical bars are ± standard errors, with 48 d.f.

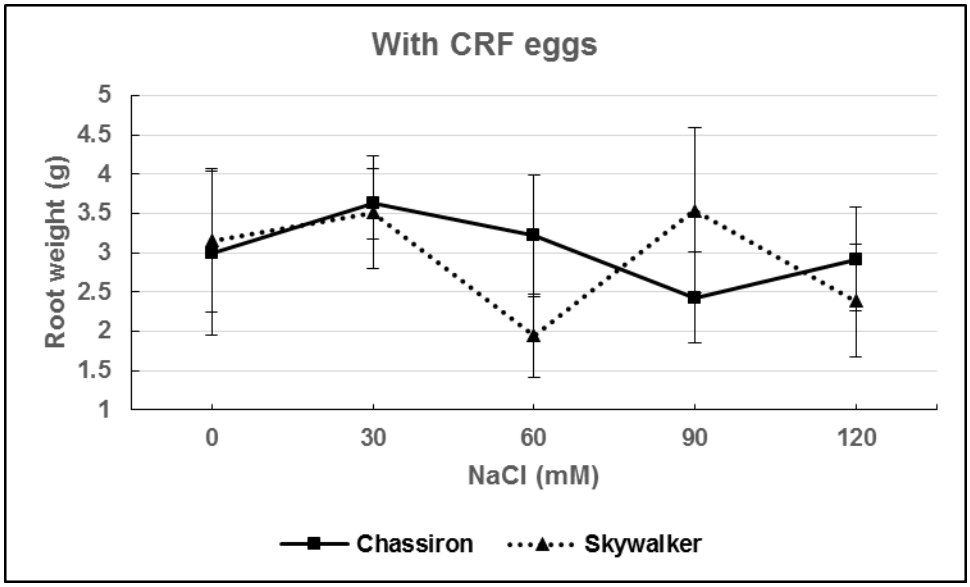

Figure 20: Weight of roots for both cultivars with CRF inoculation, Exp 2, 2012. Vertical bars are ± standard errors, with 48 d.f.

Figure 20 shows the average root weight of both cultivars for each salinity treatment, with CRF inoculation. Both cultivars show an increase in weight at 30 mM. Chassiron decreases after this, but does increase again at 120 mM. Skywalker shows an increased root biomass at 90 mM as well, but decreases at 120 mM.

Figure 21 shows plants representative of treatments at 0 mM, 60 mM and 120 mM, with CRF inoculum. It can be seen that untreated plants and those treated with 120 mM respond in the same way and are unable to resist CRF attack. However, those treated with 60 mM are able to provide some protection.

Figure 21: L-R; 0 mM + CRF; 60 mM + CRF; 120 mM + CRF, Exp 2, 2012.

Experiment 3

Leaf expansion during salinity application

In Experiment 3, Chassiron were a lot smaller than Skywalker at the start of the salinity treatments, as this was how they had arrived from the nursery. Chassiron were at 1 true leaf stage, whereas Skywalker were at 2-3 leaf stage at the start of treatment. With Chassiron, the plants increased in size at all salinity levels (**Figure 22** and **23)**. Plants treated with 60 mM appeared to perform better, and grew quickly after day 3. Plants treated with 120 mM still increased in size, but growth was slower.

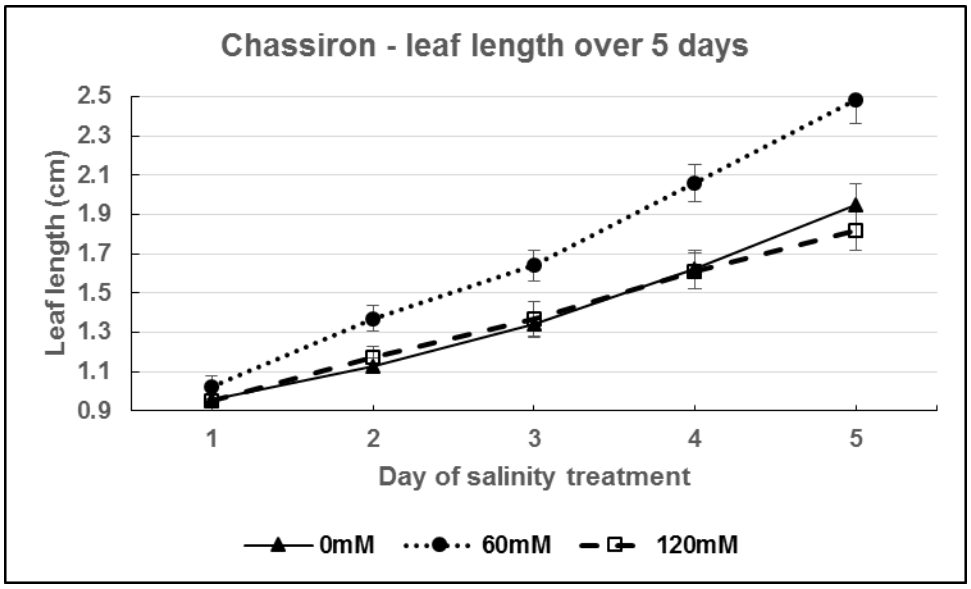

Figure 22: Leaf length during 5 days of salinity treatment, Exp 3, 2013. Vertical bars are ± standard errors, with 48 d.f.

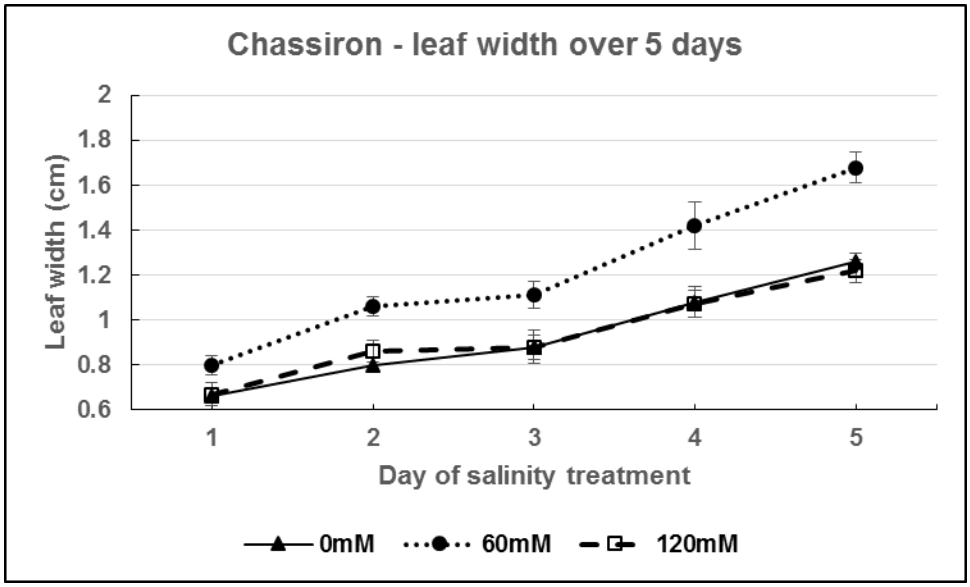

Figure 23: Leaf width during 5 days of salinity treatment, Exp 3, 2013. Vertical bars are ± standard errors, with 48 d.f.

When salinity treatments started on Skywalker, they were already at 2-3 leaf stage, and therefore the rate of growth over the next 5 days was not quite as rapid as it was for Chassiron. Leaf length was reduced at 120 mM NaCl (**Figure 24**), whereas leaf width was similar at 60 mM and 120 mM NaCl (**Figure 25**).

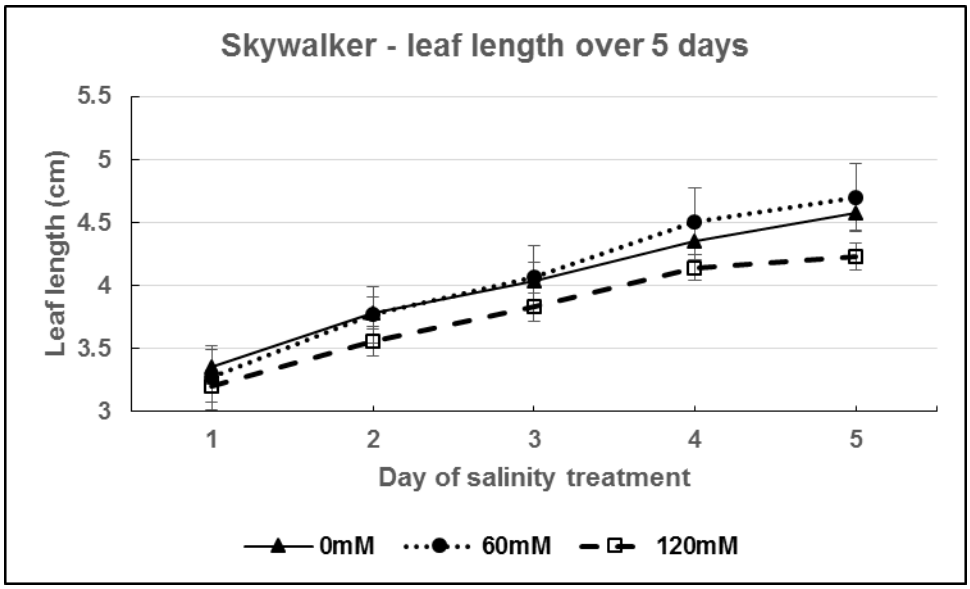

Figure 24: Leaf length during 5 days of salinity treatment, Exp 3, 2013. Vertical bars are ± standard errors, with 48 d.f.

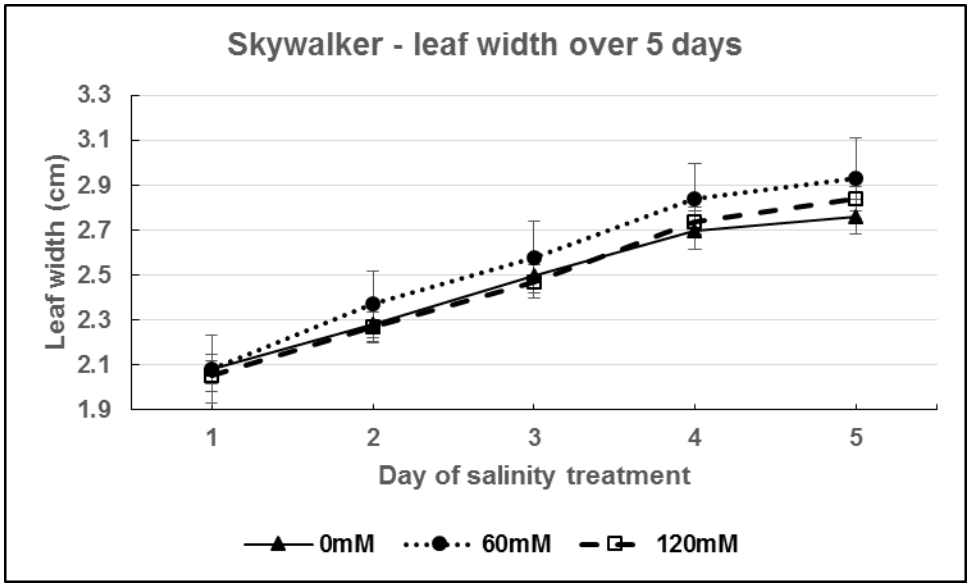

Figure 25: Leaf width during 5 days of salinity treatment, Exp 3, 2013. Vertical bars are ± standard errors, with 48 d.f.

Leaf area performance from the completion of NaCl treatment

In the weeks following salinity treatment, the plants continued to grow, although the rate was slower (**Figure 26** and **27**). Untreated Chassiron grew better than the treated plants, although Skywalker performed well at 60 mM, with a larger starting leaf area. Both cultivars grew until week 3. After that leaf area decreased. This coincided with a period of relatively high temperatures (up to 33.5°C), and the plants began to visibly suffer (wilting).

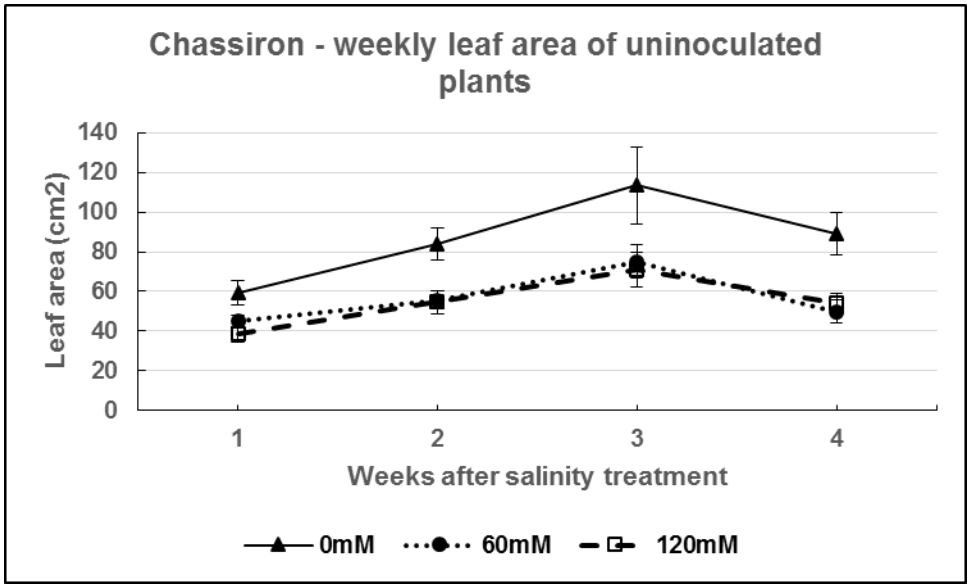

Figure 26: Chassiron leaf area in weeks following salinity treatment, Exp 3, 2013. Vertical bars are ± standard errors with 48 d.f.

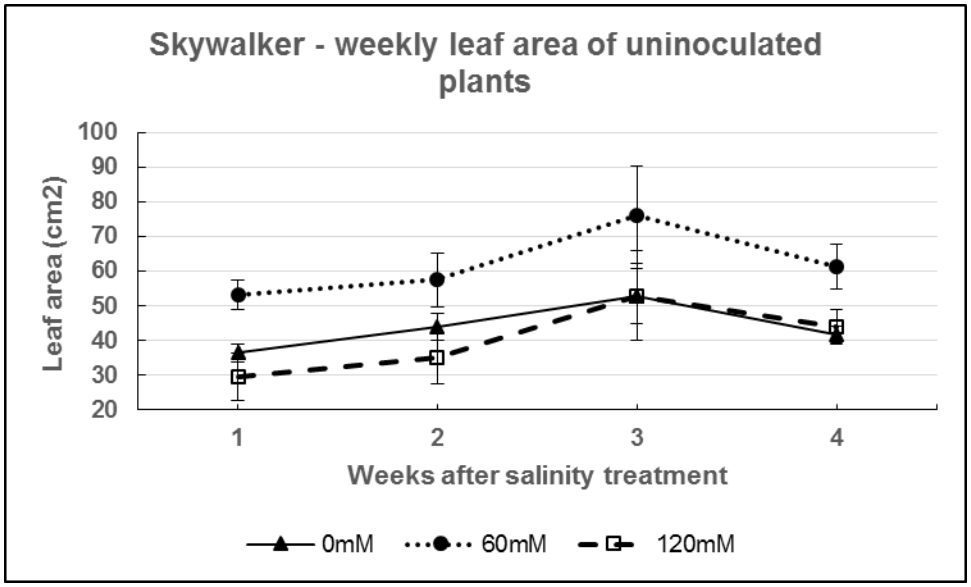

Figure 27: Skywalker leaf area in weeks following salinity treatment, Exp 3, 2013. Vertical bars are ± standard errors, with 48 d.f.

Effect of salinity treatments on leaf gas exchange

Table 13 shows that in the first few weeks following treatment, untreated plants with CRF inoculum and plants treated at 60 mM without CRF inoculum had the highest levels of CO_2 exchange. In week 4, levels had decreased in all treatments, and by week 5 CO_2 exchange was similar for all treatments, with and without CRF inoculum. However, this was not statistically significant (P=0.755).

Table 13. Effect of salinity treatments on Brassica photosynthetic net CO_2 exchange (µmol CO_2 m^{-2} s^{-1}) – Summer 2013 – Exp 3.

Treatment	Salt concentration (mM)	With or without CRF eggs	CO_2 exchange at weeks after salinity application:		
			3 (4 Jul 13)	4 (11 Jul 13)	5 (17 Jul 13)
1	0	-	9.16	5.99	9.97
3	60	-	12.74	4.68	9.46
5	120	-	11.97	8.07	9.99
6	0	+	13.50	6.93	9.88
9	90	+	11.66	5.71	9.83
10	120	+	11.14	6.31	9.00

s.e.d – 1.858, with 46 d.f.

In week 3, stomatal conductance was highest in the untreated plants with CRF inoculum (**Table 14**). It was lowest in plants treated with 90 mM, with CRF inoculum. By week 5, plants treated with 90 mM and with CRF inoculum had the highest stomatal conductance, and plants treated with 120 mM, without CRF inoculum, were the lowest. This was not statistically significant (P=0.483).

Table 14. Effect of salinity treatments on Brassica leaf stomatal conductance (mol H_2O m^{-2} s^{-1}) – Summer 2013 – Exp 3.

Treatment	Salt concentration (mM)	With or without CRF eggs	Stomatal conductance at weeks after salinity application:		
			3 (4 Jul 13)	4 (11 Jul 13)	5 (17 Jul 13)
1	0	-	0.69	0.50	0.65
3	60	-	0.62	0.67	0.69
5	120	-	0.67	0.52	0.46
6	0	+	0.72	0.58	0.62
9	90	+	0.58	0.49	0.73
10	120	+	0.62	0.67	0.60

s.e.d – 0.120, with 46 d.f.

In week 3, untreated plants had the highest transpiration rate of 6.16 (**Table 15**). By week 5, plants treated at 60 mM and 90 mM were performing better, with plants treated at 120 mM having the lowest transpiration rate. This was not statistically significant (P=0.520).

Table 15. Effect of salinity treatments on Brassica leaf instantaneous transpiration rate (ITE µmol CO_2 / mmol H_2O) – Summer 2013 – Exp 3.

Treatment	Salt concentration (mM)	With or without CRF eggs	ITE at weeks after salinity application:		
			3 (4 Jul 13)	4 (11 Jul 13)	5 (17 Jul 13)
1	0	-	6.16	3.35	4.99
3	60	-	5.87	4.17	5.03
5	120	-	5.53	3.69	4.22
6	0	+	6.13	4.07	4.84
9	90	+	5.70	3.37	5.17
10	120	+	5.78	4.05	4.54

s.e.d – 0.466, with 46 d.f.

Number of collapsed plants at end of experiment

In Exp 3 there were less collapsed plants (**Figure 28**). There were no collapsed Skywalker plants at 0 mM, 30 mM and 60 mM, showing that Skywalker are more tolerant to salt and can use it to help fight pest attack by CRF. There were also no collapsed Chassiron at 60 mM, which suggests this is a good salt level to help strengthen plants and make them more resistant to CRF attack. Plants treated with 120 mM had the highest number of collapsed plants, for both cultivars, which shows that this salinity rate is too high and the plants remain stressed and are unable to fully recover from it (not significant).

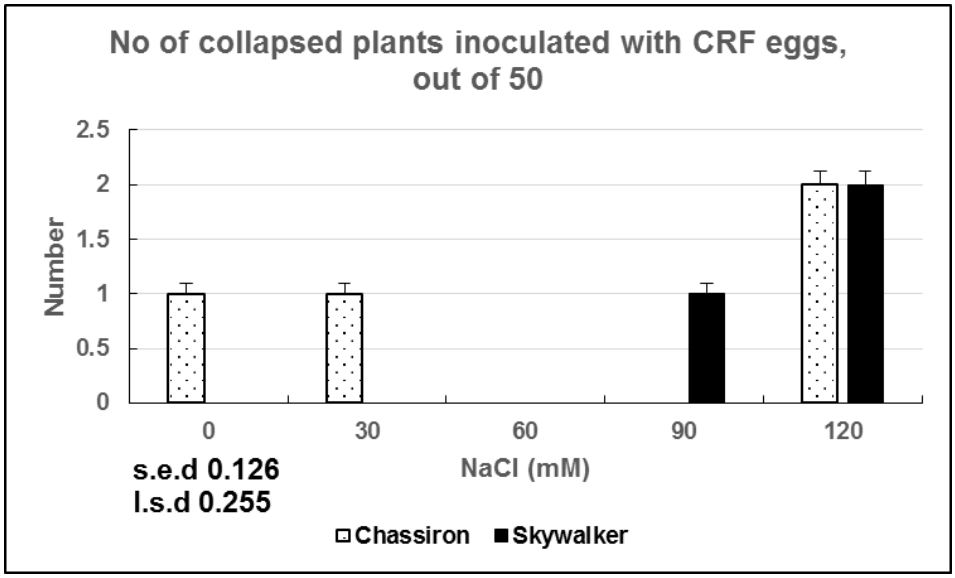

Figure 28: Number of collapsed plants for each salinity treatment due to inoculation with CRF eggs, Exp 3, 2013. Vertical bars are ± standard errors, with 48 d.f.

Root weight at end of experiment

Figure 29 shows the average root weight of plants at each salinity rate, without CRF inoculum. The untreated root weight for both cultivars is very similar. However, Chassiron produces a stronger root at 30 mM, whereas Skywalker is stronger at 60 mM and 90 mM. After 30 mM the root weight of Chassiron decreases, and Skywalker decreases at 120 mM. This shows that Skywalker is more tolerant of salinity levels up to 90 mM, but cannot cope with salinity levels over this.

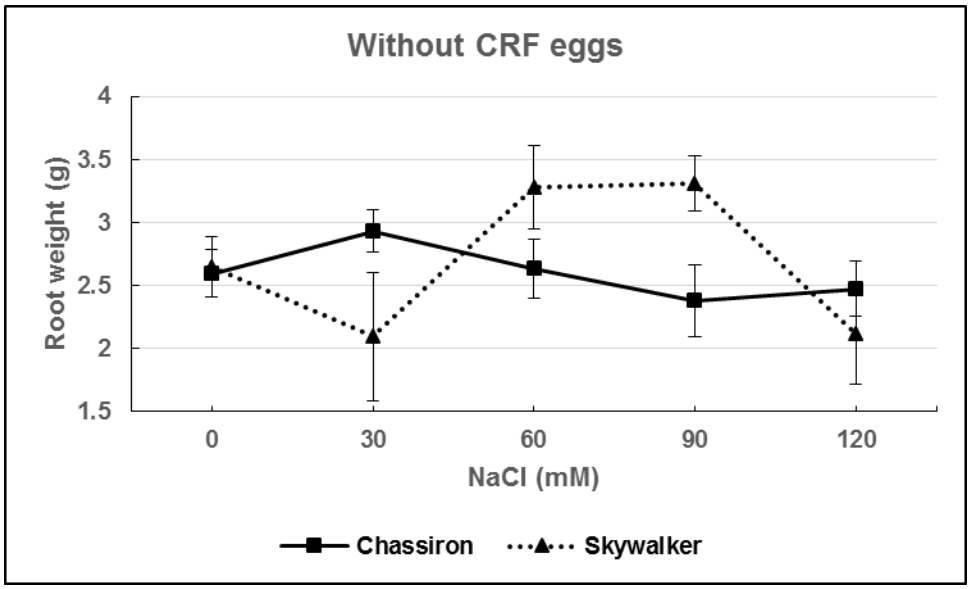

Figure 29: Weight of roots for both cultivars without CRF inoculation, Exp 3, 2013. Vertical bars are ± standard errors, with 48 d.f.

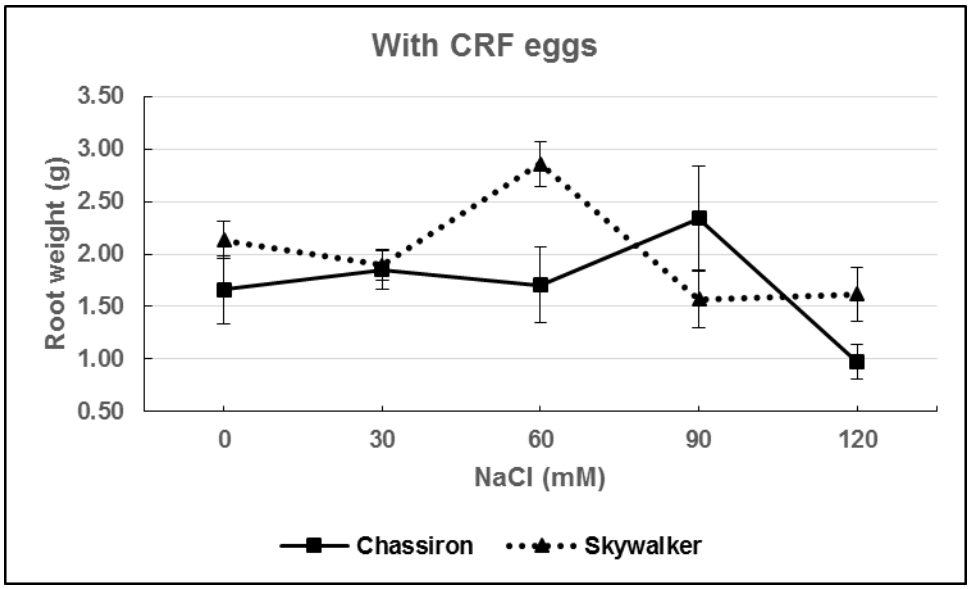

Figure 30: Weight of roots for both cultivars with CRF inoculation, Exp 3, 2013. Vertical bars are ± standard errors, with 48 d.f.

Figure 30 shows the average root weight of plants at each salinity rate, with CRF inoculum. Chassiron produces a stronger root at 90 mM, Skywalker at 60 mM. This shows that some salt is beneficial to the plant in helping it to resist CRF attack.

Figure 31 shows plants representative of each treatment at 0 mM, 60 mM and 120 mM, with CRF inoculum. As seen in Exp 1 and 2, untreated plants and those treated with the highest salinity level are unable to resist attack by CRF, and consequently collapse. However, plants treated with 60 mM show some resistance and the plant remains healthy.

Figure 31: L-R; 0 mM + CRF; 60 mM + CRF; 120 mM + CRF, Exp 3, 2013.

Experiments 5 – 7: Pre-adaptation to challenge by Hyaloperonospora parasitica (downy mildew)

Experiment 5

Leaf expansion during salinity application

Salinity treatments were applied daily for 5 days, to the whole 345 tray. Each tray represented one treatment. After the 5 days, the plants were given time to recover, and were then potted on into 1 L pots, for both the CRF and downy mildew trials. Therefore data on leaf expansion during salinity treatment is the same for the CRF experiments and the downy mildew experiments.

Leaf area performance from the completion of NaCl treatment

In the weeks following salinity treatment, the plants grew steadily, at a similar rate to the CRF trial (Exp 1). However, plants were smaller overall as there were 5 plants to a pot, rather than 1 to a pot in the CRF experiment. Chassiron grew at 240 mM until week 4, and then leaf area began to decrease as the plants started to visibly struggle (wilt) (**Figure 32**). After week 3, Skywalker plants treated with 240 mM stopped growing and remained the same size (**Figure 33**). Plants treated with 60 mM NaCl grew just as well as the untreated for both cultivars, and actually promoted growth in Chassiron from week 4 onwards.

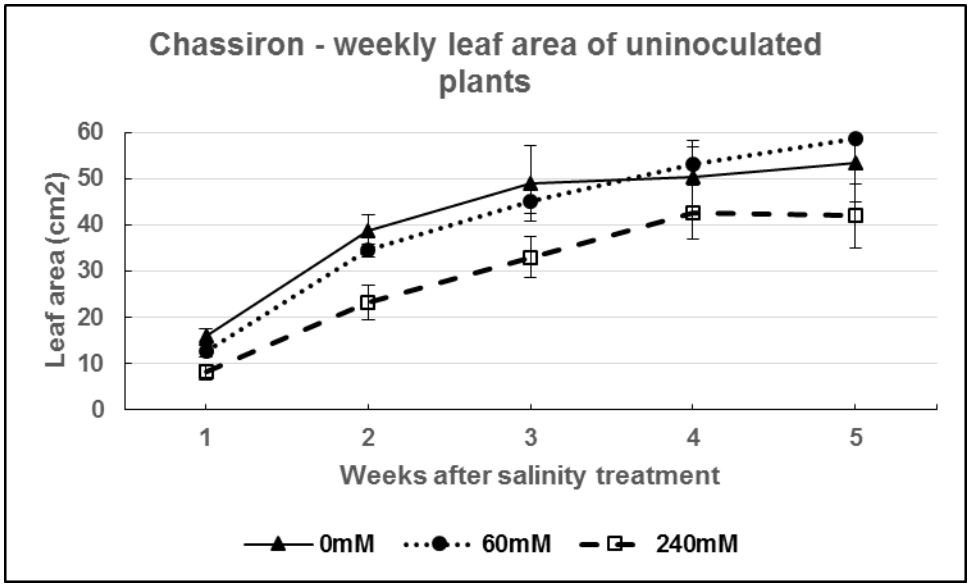

Figure 32: Chassiron leaf area in weeks following salinity treatment, Exp 5, 2012. Vertical bars are ± standard errors, with 48 d.f.

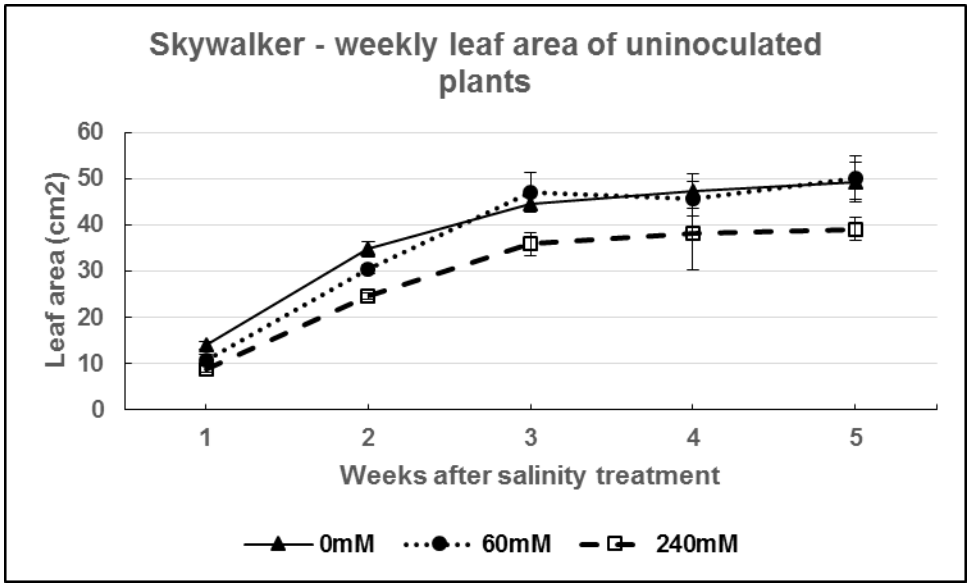

Figure 33: Skywalker leaf area in weeks following salinity treatment, Exp 5, 2012. Vertical bars are ± standard errors, with 48 d.f.

Incidence of downy mildew

Figure 34 shows that uninoculated plants still became infected, albeit on a small scale. Plants that were inoculated with downy mildew also only showed a small level of infection. However, it can be seen that plants treated with a salinity of 240 mM showed the highest levels of infection for Chassiron, indicating that the plants were already under high stress and therefore were more susceptible to infection by downy mildew (P= 0.071).

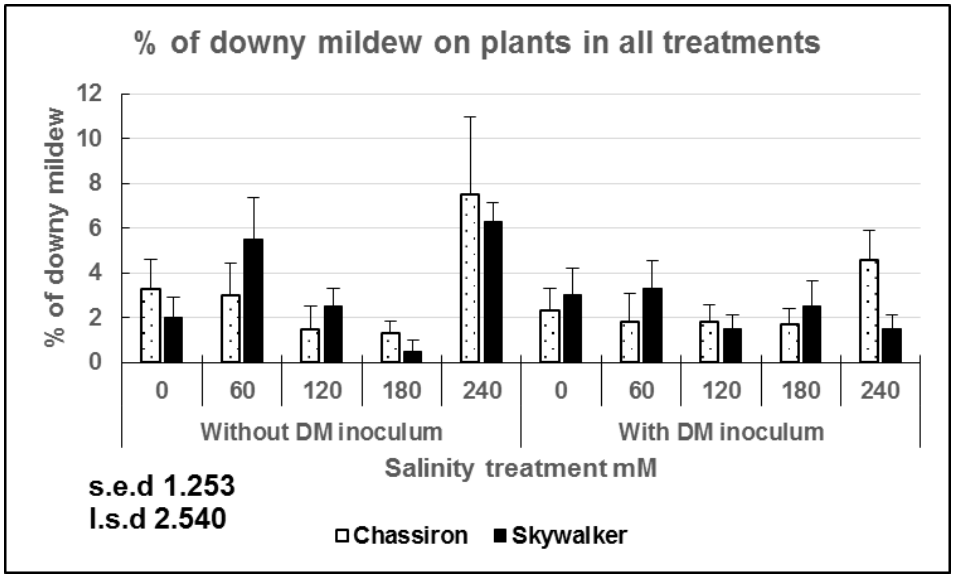

Figure 34: Percentage of leaf area infected with downy mildew, Exp 5, 2012. Vertical bars are ± standard errors, with 48 d.f.

The experiment was replicated in Autumn 2012 (Exp 6) and Summer 2013 (Exp 7) to try and get more detailed results.

Only low levels of downy mildew were seen within all three of these experiments despite gaining a sufficient concentration of spores for inoculation. This was most likely due to the glasshouse conditions being too dry for infection. The final experiment was run in a polytunnel as conditions should have been cooler, but summer 2013 proved to be hot and dry. The trials were run alongside the cabbage root fly trials as the transplants were obtained at the same time, and the peaks in the cabbage root fly culture determined the timing of the trials.

No significant differences between treatments were seen in any of the experiments.

Experiments 4 and 8: Foliar mist application of saline solutions

Experiment 4 – cabbage root fly

Leaf expansion during salinity application

In the first 5 days of treatment, both cultivars grew at the same rate for all treatments (**Figure 35** and **36**). After day 5, Chassiron appeared to grow slightly better at 120 mM (**Figure 35**). However, growth then slowed from day 10 to day 15, but the untreated and 60 mM treated plants continued to grow at a steady rate, resulting in all treatments ending on day 15 with a similar leaf area.

Skywalker also remained constant across all treatments from day 1 to day 5 (**Figure 36**). From day 5 – 15, plants treated with 60 mM grew bigger and had a greater leaf area on day 15. Growth was hindered in plants treated with 120 mM, with a smaller leaf area than the untreated by day 15.

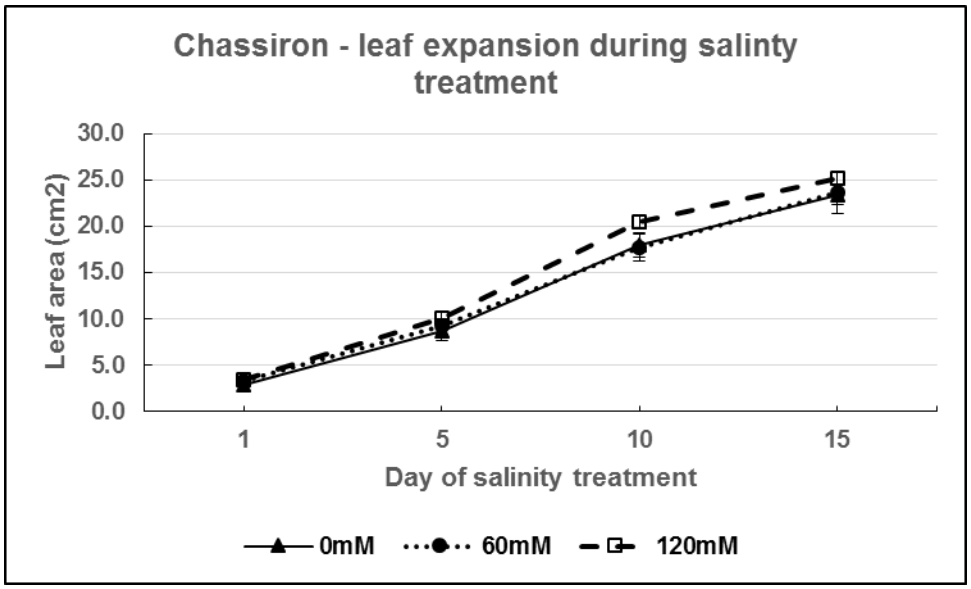

Figure 35: Chassiron leaf area during salinity treatment, Exp 4, 2012. Vertical bars are ± standard errors, with 48 d.f.

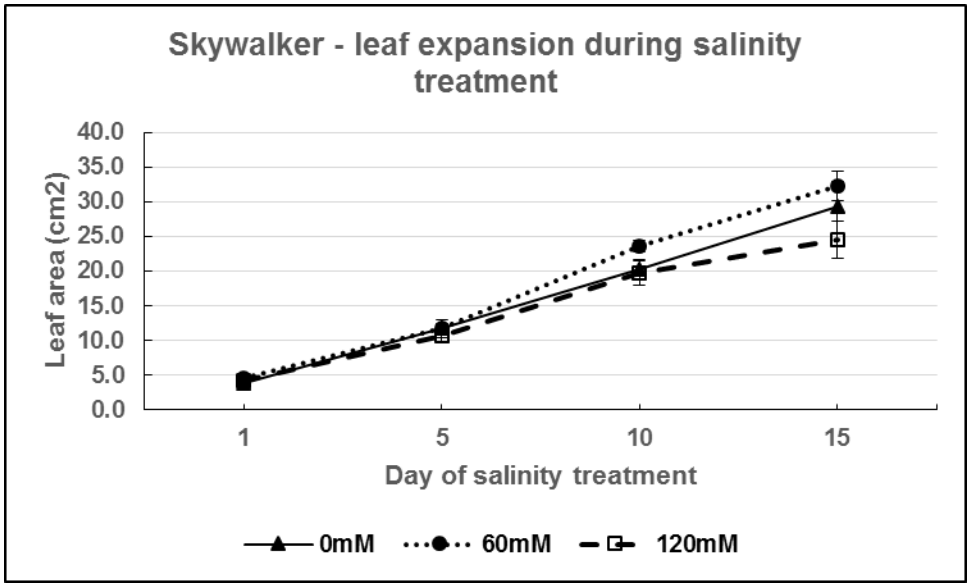

Figure 36: Skywalker leaf area during salinity treatment, Exp 4, 2012. Vertical bars are ± standard errors, with 48 d.f.

Leaf area performance from the completion of NaCl treatment

Both cultivars grew well in the first 3 weeks following salinity treatment by misting. After week 3 the rate of growth began to level out. **Figure 37** shows that Chassiron grew better when it was untreated, and those treated with 60 mM and 120 mM grew in a similar way. **Figure 38** shows that Skywalker actually grew better at 120 mM after week 3, but then growth ceased after week 4. Untreated plants and plants treated with 60 mM grew in a similar way.

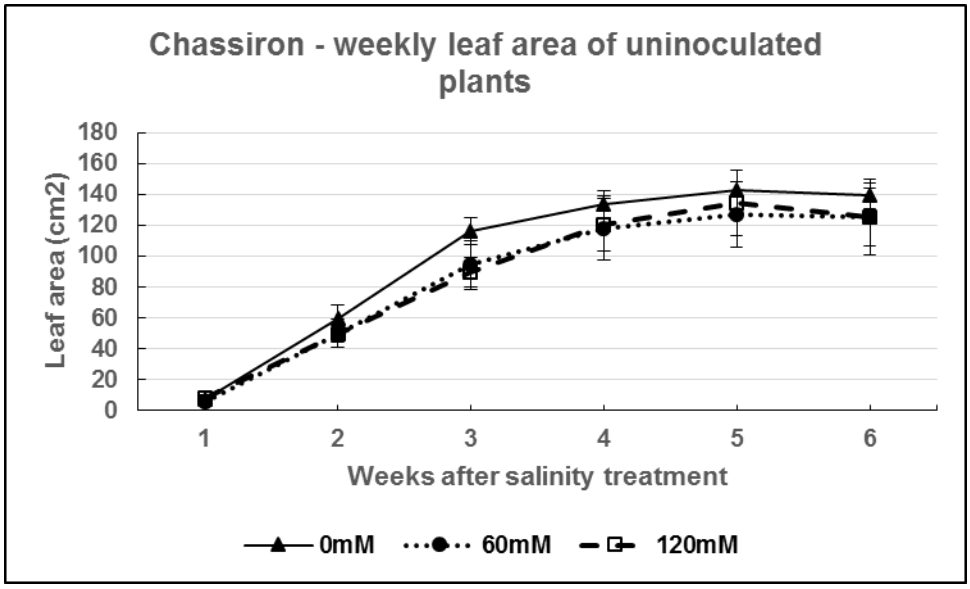

Figure 37: Chassiron leaf area in weeks following salinity treatment, Exp 4, 2012. Vertical bars are ± standard errors, with 48 d.f.

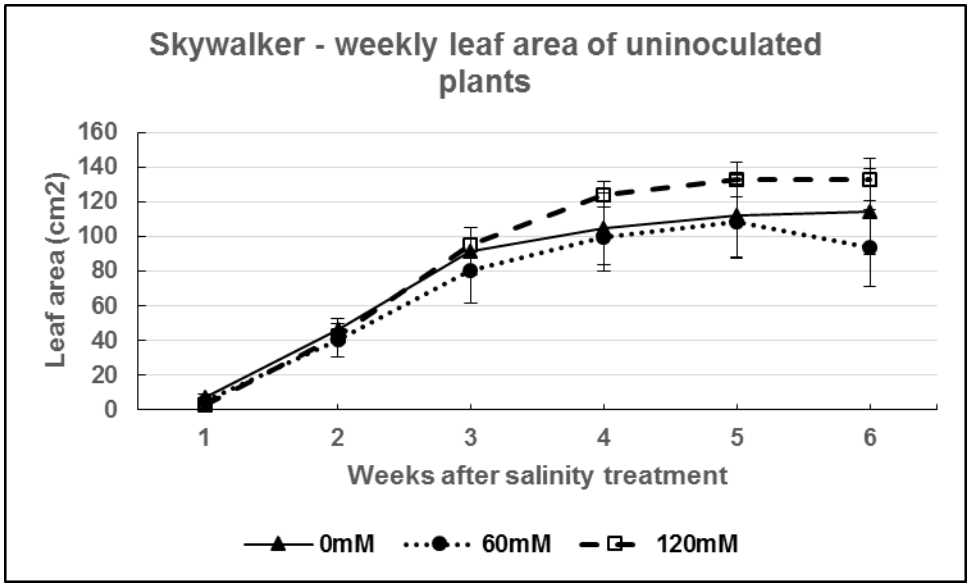

Figure 38: Skywalker leaf area in weeks following salinity treatment, Exp 4, 2012. Vertical bars are ± standard errors, with 48 d.f.

Number of collapsed plants at end of experiment

At the end of the experiment, only 2 Chassiron plants had collapsed, one treated with 60 mM of NaCl and uninoculated with CRF and one treated with 120 mM of NaCl and inoculated with CRF.

Root weight at end of experiment

Figure 39 shows the average root weight of plants at each salinity level, without CRF inoculum. Untreated Chassiron had a much heavier, stronger root than Skywalker. None of the salinity treatments increased root weight for Chassiron, whereas Skywalker had a heavier root weight at 30 mM, 60 mM and 90 mM compared with the untreated. 30 mM helped Skywalker grow a stronger root, but 120 mM affected both cultivars and root weight decreased.

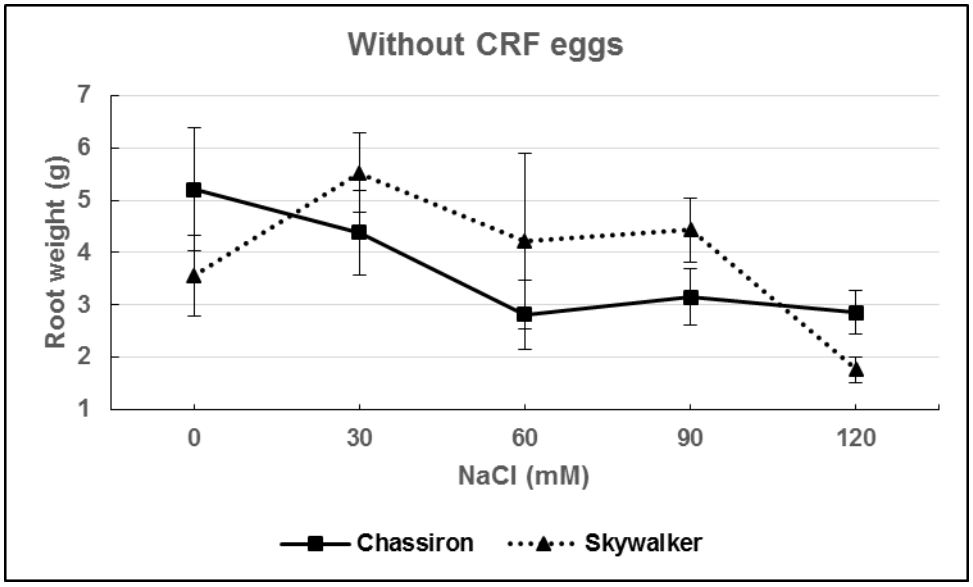

Figure 39: Weight of roots for both cultivars without CRF inoculation, Exp 4, 2012. Vertical bars are ± standard errors, with 48 d.f.

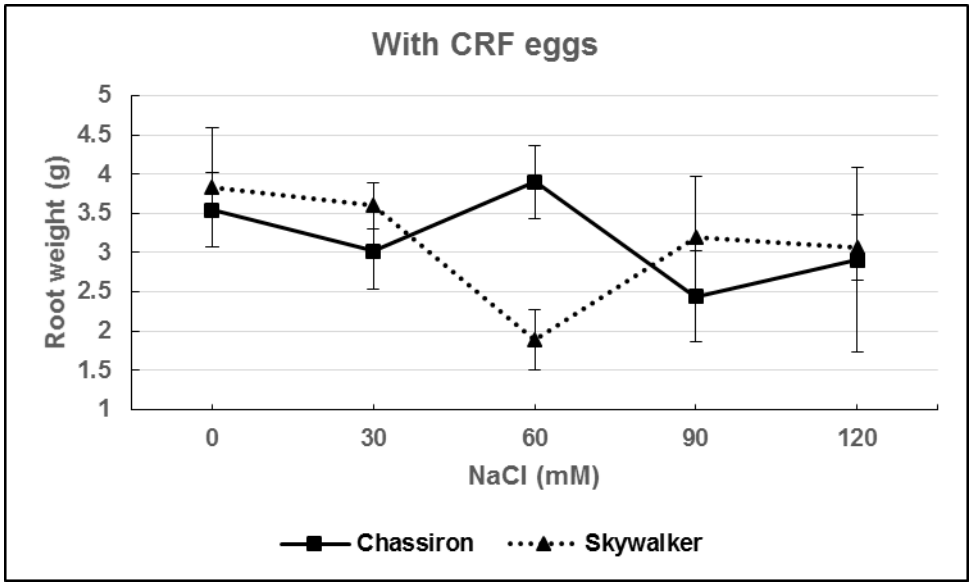

Figure 40: Weight of roots for both cultivars with CRF inoculation, Exp 4, 2012. Vertical bars are ± standard errors, with 48 d.f.

Figure 40 shows the average root weight of plants at each salinity level, with CRF inoculum. Skywalker had a better root weight in the untreated than in any other salinity level, although 90 mM and 120 mM provided a stronger root than 60 mM. Chassiron had a better, stronger root at 60 mM, suggesting that this salinity rate helped provide resistance to CRF.

Experiment 8 – downy mildew

Leaf expansion during salinity application

Salinity treatments were applied daily for 15 days, by misting over 50 plants per treatment. After the 15 days, the plants were given time to recover, and were then potted on into 1 L pots, for both the CRF and downy mildew trials. Therefore data on leaf expansion during salinity treatment is the same for Experiments 4 and 8.

Leaf area performance from the completion of NaCl treatment

Both cultivars continued to grow in the weeks following treatment, although Skywalker were slightly bigger (**Figure 41** and **42**). With Chassiron, plants treated with 60 mM and 120 mM grew in a similar way, and were larger than the untreated, suggesting that the addition of NaCl helped to promote growth. For Skywalker, the untreated were bigger than the treated plants, but those treated with 60 mM and 120 mM again grew in a similar way.

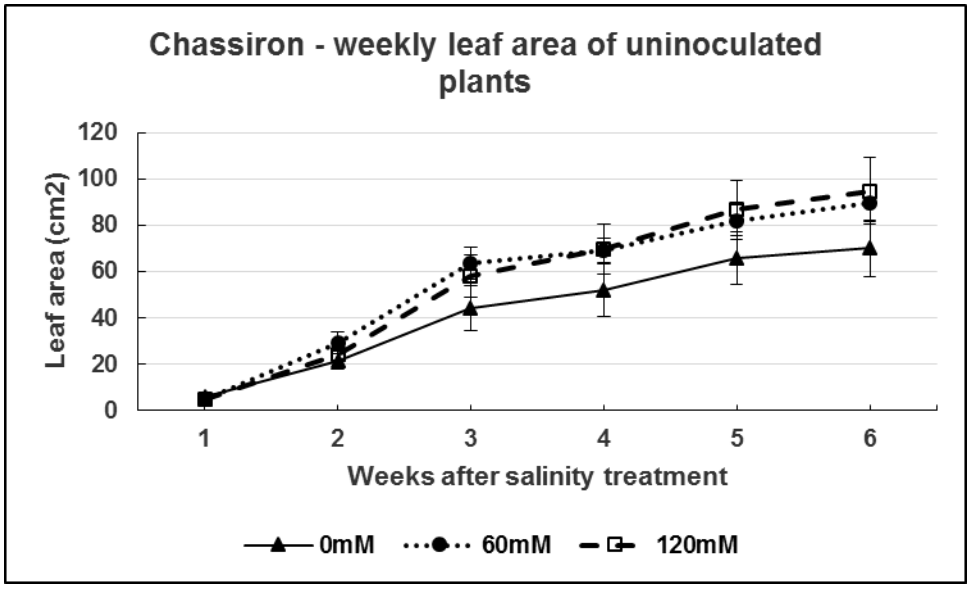

Figure 41: Chassiron leaf area in weeks following salinity treatment, Exp 8, 2012. Vertical bars are ± standard errors, with 48 d.f.

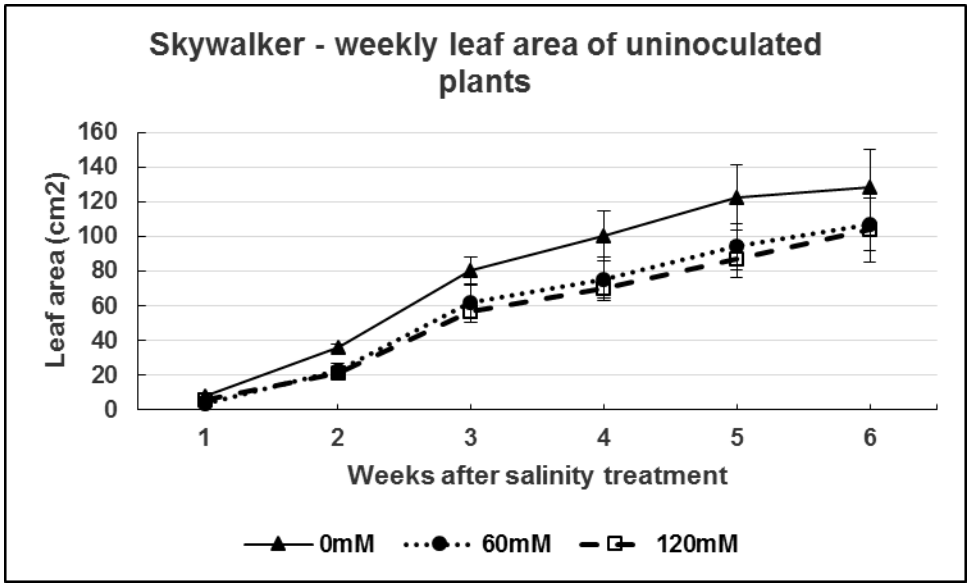

Figure 42: Skywalker leaf area in weeks following salinity treatment, Exp 8, 2012. Vertical bars are ± standard errors, with 48 d.f.

Incidence of downy mildew

The plants were inoculated twice; first with a spore suspension of $1x10^4$, then again at $5x10^4$ when downy mildew failed to develop. Unfortunately glasshouse conditions were too cold and dry as the experiment was carried out in late autumn, and no downy mildew developed during the experiment.

Experiment 9: Field trial with natural pest and disease challenge

Table 16: Effect of salinity treatments applied during propagation on growth, quality and yield of cauliflower in the field – Lincs, 2012.

Salinity rate (mM)	% healthy plants/plot 26 July 12	Plant vigour 26 July 12	% healthy plants/plot 29 Aug 12	Plant vigour 29 Aug 12	% of caulis at harvest GS	Weight of 10 caulis with leaves (kg)	Weight of 10 trimmed caulis (kg)	% of caulis discoloured	% of caulis with ricing	Weight of 5 roots (kg)	RDI from CRF
Chassiron											
0	88.8	4.5	88.8	4.75	20.5	18.25	10.38	2.50	15.0	0.59	22.2
30	97.5	5.0	97.5	5.0	28.8	19.05	10.35	2.50	15.0	0.64	16.8
60	92.5	5.0	91.2	4.5	25.0	17.78	10.45	0.00	15.0	0.59	21.5
90	95.0	5.0	93.8	5.0	13.8	17.23	10.58	10.00	20.0	0.52	22.0
120	78.8	4.0	77.5	4.5	32.0	18.40	9.18	2.50	17.5	0.60	30.0
Skywalker											
0	90.0	5.5	88.8	4.75	31.2	22.75	10.15	3.75	3.8	0.63	13.3
30	93.8	5.25	93.8	4.75	25.0	23.40	11.75	0.00	0.0	0.66	17.3
60	93.8	4.75	93.8	5.0	26.2	21.28	11.00	5.00	0.0	0.61	24.3
90	96.2	5.0	95.0	5.25	26.2	23.00	12.12	1.25	0.0	0.64	18.8
120	90.0	5.0	90.0	5.0	25.0	21.50	11.03	3.75	2.5	0.66	21.2
L.S.D (27 d.f)	11.55	0.77	11.44	0.75	17.80	2.36	1.61	7.49	11.11	119.9	16.40

Plant vigour for Chassiron on 29 August was improved in plants which had been treated with 30 mM and 90 mM at seedling stage ($P<0.05$; **Table 16**). For Skywalker, plant vigour was highest at 60 mM and 120mM, which suggests that Skywalker responds positively to increased NaCl feeding during plant establishment in modules. For Chassiron, the weight of 10 trimmed cauliflowers was lower for plants treated with 120 mM. For Skywalker, untreated trimmed cauliflowers weighed the least. Root weight was lower for Chassiron than for Skywalker. However, for both cultivars, root weight was highest at 30 mM. Skywalker also had the same root weight at 120 mM. Chassiron suffered more root damage by CRF than Skywalker, with the most damage in plants treated at 120 mM. The lowest damage was in plants treated with 30 mM. For Skywalker, the most CRF damage was in the 60 mM treated plants, and the least damage was in the untreated. On 29 August, the amount of healthy plants per plot was greatest for the 30 mM treated Chassiron, and the 90 mM treated Skywalker.

Discussion

Leaf expansion, both length and width proved to be a sensitive indicator of salt stress conditions in the root-zone. This concurs with previous studies in tomato where salinity was found to have instantaneous effects on the rate of leaf growth (Mulholland *et al.,* 2003). The size and function of the root system is a critical factor in producing robust transplant material for the field. Root weight appeared to correlate strongly with the ability of plants to withstand CRF attack, when plants were grown on under controlled glasshouse conditions in pots. Root weight enhanced under moderate levels of NaCl feeding to the root-zone at 60 mM for Chassiron and between 90-120 mM NaCl for Skywalker. Both leaf expansion and root weight, demonstrated differences in growth response to NaCl, with Skywalker being able to metabolism more NaCl to promote growth and enhance CRF resistance compared with Chassiron. The data nevertheless suggest that feeding with salt can have a beneficial effect on growth; this growth promotion has been linked with enhanced production of abscisic acid in roots and leaf tissues, stomatal closure and improved leaf water relations (Mulholland *et al.*, 2003). In the current project gas exchange measurements suggested a greater impact on stomatal conductance and transpiration compared with carbon assimilation or photosynthesis at the leaf level.

Summary of plant development and function

- The addition of some NaCl to cauliflower plants at propagation stage, in module trays, is beneficial in promoting growth later on in the development of the plant.
- Leaf expansion is a sensitive indicator of plant response to NaCl, with plants treated with lower concentrations, (30 – 90 mM) producing bigger leaves than those untreated.
- 60 mM NaCl appears to be the acceptable level of salinity in promoting growth in both cultivars, however Skywalker showed improved growth rates up to 90 mM.
- Higher salinities of 120 – 240 mM proved to be too much for plants to handle. Growth was slower and stopped altogether 3-5 weeks after treatment.
- Photosynthesis and stomatal conductance was greatly reduced in the highest salinity rates.
- The rate of transpiration was lowest in plants treated with high salinity levels, and plants inoculated with CRF. Plants treated at 60 mM, without CRF inoculum, showed the highest levels of transpiration.

- In field conditions, Chassiron was more healthy and vigorous at 30 and 90 mM, whereas Skywalker was more vigorous at 120 mM, showing that Skywalker is more tolerant of higher salinities.
- Root weight was highest for both cultivars at 30 mM, although Skywalker had a heavier root.
- The weight of trimmed cauliflowers was highest for both cultivars at 90 mM, but lowest at 120 mM for Chassiron and 0 mM and 120 mM for Skywalker.
- Plants treated with salt by foliar misting, rather than by root drenching, were able to tolerate the higher salinities more, with both cultivars continuing to grow in the weeks following treatment. In fact, Skywalker plants in the cabbage root fly experiment grew bigger at 120 mM, compared to 0 mM and 60 mM.

Summary of plant resistance to Cabbage Root Fly

- Low levels of salinity (30 and 60 mM) helped to increase root biomass in plants uninoculated with CRF. Higher levels, particularly 240 mM, significantly reduced root biomass.
- In plants inoculated with CRF, roots were generally larger at 30 mM, suggesting there is some resistance here to CRF attack.
- Some salt helped plants to resist attack by CRF, reducing the number of collapsed plants. In each experiment, the highest number of collapsed plants was at the highest salinity rates of 120 – 240 mM, for both cultivars. A treatment of 60 mM NaCl seemed to be most beneficial to the plant, with no collapsed plants for either cultivar at this rate, in 2 out of 3 experiments.
- Plants treated with the highest salinity rate, and inoculated with CRF, showed a large decrease in stomatal conductance, compared to plants treated with lower salinities.
- Plants treated by foliar misting, rather than by root drenching, showed a larger root biomass at 30 mM for Skywalker, without CRF inoculum. 120 mM greatly reduced root biomass for both cultivars.
- Chassiron plants treated with 60 mM by misting developed a stronger root biomass.
- There were less collapsed plants in the experiment where plants were treated by foliar misting, compared to root drenching.

Summary of plant resistance to Downy mildew

- Plants treated with 60 – 180 mM NaCl showed low levels of infection by downy mildew.
- Plants treated at the highest salinity rate of 240 mM had the highest level of infection, showing that too much salt had a negative effect on the plant, reducing their defence system and making it easier for fungal pathogens to attack the plant.

General discussion

As agricultural production intensifies with the aspiration of 'sustainable intensification', horticulture systems (high input and relatively short growing season) are susceptible to salinity build in the rooting zone. The findings from the current study concur with previous findings where high concentrations of NaCl significantly decreased shoot and root dry weights, but for selected cultivars growth performance was actually improved under specific saline conditions. This improvement or reduction in growth may have been strongly linked to leaf chlorophyll contents and water relations of different cauliflower varieties (Batool *et al.,* 2013).

An offshoot of this project in being able to understand the mechanism of 'stress tolerance' may be to understand the key attributes or 'traits' of salt tolerant crops. At a global scale the area of cultivated land continues to decrease due to an increase in the salinity content of the soil (Shahbaz *et al.,* 2012). Whilst research has primarily focused on salt tolerance in staple crops, less work has been done on vegetable crops. All vegetable crops are affected by salinity in some way, and salinity affects every aspect of plant development, including morphology, physiological function and yield. Therefore, work is timely in terms of crop system productivity with the additional and hitherto largely unrecognised ability of controlled NaCl stress to enhance the plants natural defence system.

Studies on the combined effects of salt-stress and *esca* (young vine decline) disease in grapevines showed that nutrients in the roots and shoots was reduced at higher salt concentrations, and the presence of fungi reduced nutrients even more, showing that the addition of salt combined with disease aggravated effects (Oliveira *et al.,* 2011). However, the range of salinities used was rather broad; more specific concentration may have had a different effect. The data from the current study and the literature suggest that the control of plant fungal infection is more problematic compared with CRF. It may be that more precise timings and a more precise quantification of the pre-adaptation treatment is required to enable to correct change in plant metabolism to dissuade fungal infection.

Conclusions

The data suggests that the cauliflower cultivars Chassiron and Skywalker can be pre-adapted by a brief period of NaCl solution feeding to the root-zone. Misting application to the foliage at the same dose provides less robust effects. Chassiron produced an increase in leaf expansion and a larger root system at 60 mM NaCl, whereas Skywalker exhibited enhanced growth at 90-120 mM NaCl compared with the zero NaCl control. Resistance to CRF damage was evident at 60 mM NaCl in Chassiron and 90-120 mM NaCl in Skywalker. In contrast NaCl had little impact on resisting infection by Downy mildew and in fact there was weak evidence to suggest that at high levels of NaCl feeding concentrations (240 mM NaCl) then infection was increased. In summary:

- Skywalker and Chassiron show enhanced growth response to NaCl during propagation to a 5 day feeding window immediately prior to dispatch.
- Skywalker can beneficially utilize salt at higher concentrations (90-120mM NaCl) to promote growth compared with Chassiron (60 mM NaCl).
- The cultivar enhanced growth response to NaCl feeding coincides with an marked increase in the resistance or pre-adaptive potential of young plants to withstand CRF attack.
- The resistance of plants appears to be strongly linked with the ability to transport water from the root system and to drive cell expansion in the leaves.
- Transpiration is more markedly reduced by CRF attached compared with photosynthesis alone.
- Growth responses under protected growth conditions appear to correspond with Chassiron and Skywalker performance in the field.
- Pre-adaptation appears to have promise for use with Brassica transplant crops and is a technique that will reduce chemical control dependency in production.
- Further field scale trials that build upon this preliminary study will give the industry a clearer understanding of the benefits of pre-adaptive stress conditioning of transplants.

Suggestions for further work

- Pre-adaptation with NaCl can be evaluated for an extended range of Brassica and other crop types.
- Combination doses of NaCl stress combined with Tracer could be evaluated to understand potential beneficial interactions on plant growth and resistance to CRF attack.

- Other stresses could be considered e.g. temperature combined with water / salt stress in combination or alone (see Dutch grower report).
- A greater emphasis on crop performance to maturity would give both propagators and growers more confidence to adopt pre-adaptation techniques for industry practice.
- Further work could be carried out to refine the pre-adaptation 'window' to evaluate the potential for non-chemical control of Downy mildew.
- At a fundamental level the work carried out could be extended to gene exploration and identification of specific mechanisms that trigger plant resistance to infection or attack.

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Technology transfer

Presentations

Presentation to Plant Propagator AGM, Technical Seminar, Stoneleigh, Wed 2 Oct 2013.

Article

Brassica Research Newsletter 'Taking cabbage root fly with a pinch of salt' 13 September, 2013.

HDC News 'Pre-adaptation of Brassicas to pest attack. March 2013.

Overseas travel (Barry Mulholland) and discussion of pre-adaptation stress techniques with Syngenta and Dutch grower. Report summary:

Report to UK Plant propagators - Visit to Gitzels Enkhuizen NE Holland

10 January 2013

Participants

William Gitzels – Nursery owner

Syngenta – Guido de Wit, Key account manager, young plant raisers, North Europe

Dr Barry Mulholland – Head of Horticulture ADAS

Rationale of visit

To explore the concept of pre-adaptation in Brassica seedling production. The current HDC project FV402 Brassica pre-adaptation with salinity was used as an example of plant hardening and development of internal plant resistance; to be used in combination with existing or future chemical controls.

Gitzels Nursery

Situated in Enkhuizen the main R&D and production / sourcing of seed in Holland. All heated and vented glass production area. For 2013 120 million plug plants are being produced, and are only for Dutch growers. Tomatoes are propagated for supply into a commercial breeding programme and cut flowers are also produced (chrysanthemums) for supply mainly into Eastern Europe.

Discussion on plant management and pre-adaptation

There is much debate amongst Dutch propagators and growers concerning how hard to grow plants. There is anecdotal evidence that growing plants too hard reduced crop yield potential. It is a difficult balancing act to achieve as transplant success in the field requires a degree of plant hardening. Recent events have presented growers with the task of scheduling plants under relatively warm and low light conditions.

Gitzels approach to growth control and P&D attack in Brassica propagation

Holding plants and producing plants of the right quality is achieved by temperature control and during the recent dull warm spell by reducing the frequency of irrigation (gaps of up 1 week or longer depending upon the weather). Close attention is paid to the differential between day/ night temperatures to remove rapid cooling and condensation of water on plant surfaces. The precise details reside with William Gitzels, but the technique of differential manipulation was explained and works very well for downy mildew control.

Withholding irrigation is a form of pre-adaptation control and OK to carry out under cool conditions, when the evaporative demands of the plant are low, during the early part of the year. Plugs are irrigated with nutrient solution to achieve a growing media plug EC of 3.0-3.5 dSm^{-1} (this will rise to 5.0 dSm^{-1} as the plug dries out, because of evaporative demand). Irrigation was overhead with the pot base being air pruned on raised benches. CRF and aphids are controlled with the use of dummy pills at the seed sowing stage. The propagation process does not use salinity as a form of CRF or DM control. There is however scope for

further thought and discussion on the range of pre-adaptive controls and how these link with existing chemical treatments and final yield potential when grown on in the field.

The Brassica tray module operation is not fully automated, Gitzels nursery in comparison with neighbouring producers, have found that their manual operation was faster and more efficient for loading and dispatch.

Way forward and information exchange

On a national level there appears to be little competitive advantage to be gained by information exchange between Dutch and UK based growers as Brassicas are produced for regional supply only. William Gitzels was keen to travel to the UK and exchange experience and information on Brassica propagation with a small group of similar producers. If there is an appetite for this interaction then it should happen soon, end of February 2013 or early 2014 was suggested to maintain momentum. Syngenta can act to coordinate a small focus group and draw together relevant propagators and ADAS would be happy to be involved with a science translation role.

Appendix 1 – Crop diaries

Exp 1 - Summer 2012

14/06/2012 Plants collected from Delflands (5 trays of Chassiron and 5 trays of Skywalker). Plants at cotyledon stage-looking healthy. Placed in glasshouse 2. Data logger placed in glasshouse.

30/06/2012 Salinity day 1. Photos taken and vigour score given. Tagged 10 young leaves per tray, to give 20 measurements for each treatment, 10 from each variety, so 100 measurements in total. Measured leaf length and width. Plants at growth stage 3 leaves. pH and EC measured for each treatment. EC is measured in mS. Unfortunately, because the salinity rates have been increased for the pot trial, the EC meter cannot give a reading for treatment 5, (240mM NaCI), as it is too high. Meter will only read up to 20mS. 1L of treatment applied to the module tray, with 1L of fresh water applied to the untreated trays.

01/07/2012 Salinity day 2. Photos taken and vigour score given. Skywalker treatment 5 looks the most affected, with some plants wilting. Leaf length and width measurements taken. pH and EC measured and 1L of treatment applied to the relevant module trays with a watering can and rose.

02/07/2012 Salinity day 3. Photos taken and vigour scores given. Skywalker treatment 5 still the most affected. Leaf length and width measured. pH and EC measured and 1L applied to each tray.

03/07/2012 Salinity day 4. Photos taken and vigour scores given. The leaves on treatment 5 Chassiron and treatment 4 Skywalker are greener than the other trays. Skywalker treatment 5 is wilting. Leaf length and width measurements taken. pH and EC measured and 1L of treatment applied to the relevant tray.

04/07/2012 Salinity day 5. Photos taken and vigour scores given. Skywalker treatment 5 had perked upa bit and was not quite as wilted but still looked the worst tray. T5 Chassiron and T4 Skywalker still had greener leaves. Phytotoxicity score given. Most treatments showed some yellowing to the leaves. T5 Skywalker had a few crinkled and dying young leaves. Leaf length and width

measurements taken. pH and EC measured and 1L of treatment applied to the relevant tray.

17/07/2012 Trays potted into trial plan.

18/07/2012 Li-Cor assessment week 1. Machine would not settle properly, so not as many readings were taken. Leaf length and width measured on every plant. Youngest fully expanded leaf was measured and the leaf was tagged so that the same leaf can be measured again.

20/07/2012 Cabbage root fly eggs applied to relevant plots. 10 eggs were washed around base of the plant. Both cultivars were treated, plants 1 and 2, so all 4 pots per plot treated. Total of 1000 eggs on the trial. 10 eggs also placed on damp filter paper and kept in bug lab for viability test. 90% viability.

25/07/2012 Li-Cor assessment week 2. Sunny day although 2/3 of the trial was in shade when measurements were taken. Machine working well so measurements taken on youngest fully expanded leaf for treatments T1, T3, T5, T6, T9 and T10 across blocks 1,2 and 3. Leaf length and width measurements taken on tagged leaves. Vigour scores given although there was no real difference yet in plots.

27/07/2012 Cabbage root fly eggs applied to relevant plots, 10 eggs per pot. All pots then covered with clear perforated bread bags and secured with elastic band. 95% viability.

02/08/2012 Li-Cor assessment week 3. Measurements taken on youngest expanding leaf for treatments T1, T3, T5, T6, T9 and T10 across blocks 1, 2 and 3. Leaf length and width measurements taken on tagged leaves. Vigour scores given. CRF treated plots are beginning to struggle. Few plants had downy mildew on them, plots noted.

03/08/2012 CRF eggs not added this week as culture is struggling. Will let culture recover and will add eggs next week and the week after.

06/08/2012 Assessment to count the number of collapsed plants in the trial. T6, 9 and 10 affected. Photos taken.

09/08/2012 Li-Cor assessment week 4. Cloudy morning. Measurements taken on youngest expanding leaf for treatments T1, T3, T5, T6, T9 and T10 across blocks 1, 2 and 3. Leaf length and width measurements taken on tagged

leaves. Vigour scores given, definite differences between plots now.

10/08/2012 CRF eggs added to T6-10 (week 3). 10 eggs washed onto soil around plant stem. Bags put back over pots. 20 eggs put on damp filter paper in bug lab for viability test. 90% viability.

13/08/2012 2nd assessment to count number of collapsed plants. More have collapsed now with T8 being affected as well now. Photos taken.

16/08/2012 Li-Cor assessment week 5. Cloudy again. Measurements taken on youngest expanding leaf for treatments 1, 3, 5, 6, 9 and 10 across blocks 1, 2 and 3. Some leaves are practically dead on T6, 9 and 10. Leaf length and width measurements taken. This was bit difficult for some plants as the leaves were curling, dying and going crispy. Vigour scores given.

17/08/2012 4th application of CRF eggs is omitted as not enough eggs have been produced by the culture. Also, some of the plants that have been inoculated with CRF are almost dead soputting on more eggs will not have any effect. So the inoculated plants have had a total of 30 eggs.

21-23/8/12 Destructive assessment. 25 fully expanded leaves removed from each treatment across all blocks for both varieties, so 500 leaves in total. This was a bit tricky for T5 and 10 as the plants were really struggling and dying so there wasn't 25 leaves in total. Leaves were scanned through a leaf area machine borrowed from Rosemaund. Each plant was removed from its pot and the root cut off. They were washed, left to air dry overnight and then weighed. Root damage index scores were given. Compost from the plants was quickly checked for CRF pupae and larvae, which was collected and will go back into the culture. Plants disposed of in skip and glasshouse cleaned. Temperature logger downloaded.

Exp 2 - Autumn 2012

19/10/2012 Plants collected from Delflands, 5 trays each of Chassiron and Skywalker, and placed in GH2 on capillary matting. Plants at cotyledon stage, looking healthy. They will be watered daily for the next week or so.

23/10/2012 2 temperature loggers placed in GH. 1st leaf emerging.

25/10/2012 50 plants removed from each tray to be used in the mini GH trial C. Plants for mini trial moved to GH3, along with one of the temperature loggers.

31/10/2012 Another temperature logger placed in GH2.

05/11/2012 Day 1 of salinity treatments. Plants will have no extra watering this week. 10L of each treatment made up and stored in water containers in the GH. Vigour scores given for each set of plants. All plants currently look the same. Leaf length and width measurements taken on 10 plants per tray. The youngest fully emerged leaf (leaf 2) was measured and tagged so the same leaves will be measured all week. pH and EC measurements taken for each treatment, and 1L of treatment applied to the relevant tray.

06/11/2012 Day 2 of salinity treatments. Vigour scores given for each set of plants. Leaf length and width measurements taken on tagged leaves. 1L of treatment applied to each tray.

07/11/2012 Day 3 of salinity treatments. Vigour scores given. Leaf length and width measurements taken. 1L of treatment applied to each tray.

08/11/2012 Day 4 of salinity treatments. Vigour scores given and leaf length and width measurements taken. 1L of treatment applied to each tray.

09/11/2012 Day 5 of salinity treatments. Vigour and phytotoxicity scores given. No significant phytotoxicity seen other than some slight leaf curling. Leaf length and width measurements taken. Plants will now be given a few days to recover and will be lightly watered overhead.

13/11/2012 Plants potted up into organic compost supplied by Delflands nursery. 1 plant per pot. 2 pots per plot. Trial set up in GH2 on capillary matting, which will be watered, and plants will be watered lightly overhead if needed.

15/11/2012 Compost sample taken.

16/11/2012 10 Chassiron and 10 Skywalker taken from the untreated trays and placed in another 345 tray. Dursban mixed up to a solution of 1.5g in 0.25L of water. Each set of leaves was misted with 4ml of water. Each plug was then treated with 1ml of dursban, using a syringe. The leaves were then misted with 10ml of water after treating. Each plant was potted up individually into 1L pots using Delflands compost. Another randomisation and trial plan was produced, so 5 plants of each cultivar will have dursban and no CRF eggs, and the other 5 plants for each cultivar will have dursban and CRF eggs added. The 20 pots were placed in GH2 along with the main CRF trial.

21/11/2012 Lights turned off and heating turned down as CRF eggs cannot be added this week as there is not enough in the culture. Therefore we need to slow the growth of the plants down for a week.

22/11/2012 Leaf lengthxwidth measurements taken on youngest expanding leaves. Leaves were tagged so the same leaves can be measured each week. Vigour and phytotoxicity scores given. No real phytotoxicity seen. Li-Cor measurements taken for both cultivars for T1, 3, 5, 6, 9 and 10 across blocks 1, 2 and 3. Photosynthesis readings were low as it was cloudy. Leaf measurements and vigour scores also taken on Dursban plants and leaves tagged.

27/11/2012 NRM results received.

29/11/2012 Leaf lengthxwidth and vigour assessment completed. One of the plants that was measured last week had died, (P32 Skywalker), so the 2nd plant will be measured from now on. Li-Cor assessment on T1, 3, 5, 6, 9 and 10 in blocks 1-3 for both cultivars. Photosynthesis readings were low again, fairly cloudy day.

30/11/2012 1st application of CRF eggs. Only 4 eggs could be washed onto each plant, and block 5 didn't have any eggs as there was not enough available from the culture. Eggs that were collected on the 19.11.12, 26.11.12, 28.11.12 and 30.11.12 were used. Eggs were divided evenly, so each plant would have had an egg from each collection date. 10 eggs from each date were also used for viability. 4 eggs per plant were also used for the dursban pots. Lights and heating turned back on.

06/12/2012 Leaf lengthxwidth and vigour assessment completed. Li-Cor assessment completed on usual plots.

07/12/2012 2nd application of CRF eggs delayed due to low number of eggs in the culture. It has been decided that eggs will be applied once a fortnight in order to get enough eggs. Li-Cor and leaf measurements will still take place weekly. Bags will be left off of pots as it takes 7-8 weeks for an egg to turn into a fly, and as the plants get bigger there is a risk of the plants getting damaged.

13/12/2012 Leaf lengthxwidth and vigour assessment completed. Li-Cor assessment completed on usual plots.

14/12/2012 2nd CRF app. 7 eggs washed onto each plant. (3 eggs from either 10.12.12 or 12.12.12, 1 egg from 6.12.12 and 3 eggs from either 3.12.12 or 4.12.12). Block 5 was omitted again. Eggs for viability set up in the bug lab.

19/12/2012 Leaf lengthxwidth and vigour assessment completed. Li-Cor assessment completed on usual plots. No sign of any damage from CRF yet.

03/01/2013 Leaf lengthxwidth assessment completed. Li-Cor assessment completed on usual plots. There are now differences between some of the plots, with some of the T6, 8 and 10 plants wilting and collapsing. The lower leaves are unlikely to expand much more, so leaf measurements will now finish. None of the dursban treated plants have been affected.

04/01/2013 3rd CRF app. 7 eggs washed onto each plant. (3 eggs from 27.12.12, 3 eggs from 2.1.13 and 1 egg from 4.1.13). Block 5 omitted. Eggs for viability set up in the bug lab.

08/01/2013 Temperature logger removed and downloaded, as logger need calibrating.

11/01/2013 Temperature logger returned to glasshouse.

21/01/2013 Started destructive assessment. The number of collapsed plants was recorded, and 25 leaves per treatment/per variety, were collected, bagged and stored in CS01. 500 leaves in total. Leaves were also collected from the dursban treated plants, 25 leaves/treatment, so 100 leaves in total. Fully expanded leaves were taken. Each plant was dug up, and the root was removed. Roots were bagged individually and stored in CS01. All plants were

disposed of in the site skip. GH2 was cleared and swept, and temperature logger was downloaded.

22/01/2013 Roots removed from CS01 and washed to remove all compost. Roots wrapped in paper towel to let them dry quicker. All roots from the main trial were weighed and given an RDI score. Roots kept in the lab for photos to be taken.

23/01/2013 Dursban treated roots weighed and given an RDI score.

24/01/2013 Leaves removed from CS01 and measured using a leaf area machine borrowed from Rosemaund. The machine was calibrated before use, using pieces of paper that added up to 200cm2, and the machine was adjusted until the correct reading was shown. The machine was also calibrated throughout the day, once an hour. Main trial and dursban plants both measured.

Exp 3 - Summer 2013

24/05/2013 GH 2 disinfected.

30/05/2013 Plants collected from Delflands, 5 trays of Chassiron and 5 trays of Skywalker. Plants at cotyledon stage, although Skywalker are taller,

Chassiron have only just emerged. Trays placed on individual pieces of capillary matting in GH2, and will be watered from below, unless the compost gets very dry, then they will be watered overhead. 2 temperature loggers, (DL202 and DL206) set up and placed in GH. Bio-controls for aphids introduced. 500L of organic modular compost also collected from Delflands and stored in the potting shed, which will be used for potting up the plants.

04/06/2013 Plants checked. There is some downy mildew present on the cotyledons but they seem to be growing through it. Chassiron still at cotyledon, Skywalker at 1 leaf.

10/06/2013 Salinity day 1. Chassiron 1-2 leaves, Skywalker 2-3 leaves. DM still present on cotyledons but seems to be drying up and leaves are clean. Each tray given a little bit of water overhead as the compost was looking quite dry on some trays. 2nd leaf tagged on 10 plants per tray, so 100 leaves in total. Salinity treatments mixed up, 10L of each treatment, which will be stored in the GH. Leaf length and width measured. Vigour scores given. Untreated given a score of 5, and then the others will score either above or below that. pH and EC of each treatment measured using a pH meter which was calibrated using buffer solutions of pH4 and pH7. 1L of each treatment applied to the relevant tray. Photos taken.

11/06/2013 Salinity day 2. Leaf length and width measured on tagged leaves. Vigour scores given and photos taken. pH and EC of each treatment measured. 1L of treatment applied to the tray.

12/06/2013 Salinity day 3. Leaf length and width measured on tagged leaves. Vigour scores given and photos taken. pH and EC of each treatment measured. 1L of treatment applied to the tray.

13/06/2013 Salinity day 4. Leaf length and width measured on tagged leaves. Vigour scores given and photos taken. pH and EC of each treatment measured. 1L of treatment applied to the tray.

14/06/2013 Salinity day 5. Leaf length and width measured on tagged leaves. Vigour scores given and photos taken. Phytotoxicity scores given. All plants treated with salt showed very slight leaf crinkling and treatments 4 and 5 for both cultivars were shorter. pH and EC of each treatment measured. 1L of treatment applied to the tray.

17/06/2013 Plants potted up according to trial plan, 1 plant per pot. 2nd leaf tagged on plant in pot 1, which will be measured each week. Pots placed in GH2 on capillary matting, which will be watered automatically. Plants can also be watered overhead if necessary. Compost sample taken and stored in CS/01. Chassiron 2-3 leaves, Skywalker 3-4 leaves.

21/06/2013 Phytotoxicity scores given.

27/06/2013 Length and width of one leaf per plant per cultivar per plot measured. Vigour scores given to all plots. Li-Cor measurements taken on six treatments on three blocks (2, 3, 4).

28/06/2013 1st CRF application. 10 eggs washed onto each plant from T6-10. Viability set up in the lab.

04/07/2013 Leaf length and width measured on tagged leaves. Vigour scores given. Li-Cor measurements taken for both cultivars from T1, 3, 5, 6, 9 and 10, from blocks 1, 2 and 3.

05/07/2013 2nd CRF application. 10 eggs per plant using eggs collected from 2/7/13. Viability set up in the lab.

11/07/2013 Leaf length and width measured on tagged leaves. Vigour scores given. Li-Cor measurements taken for both cultivars from T1, 3, 5, 6, 9 and 10, from blocks 1, 2 and 3. Readings were lower as it was cloudy.

12/07/2013 3rd CRF application. 6 eggs washed onto each plant for T6-10, using eggs collected from 8.7.13 (3) and 11.7.13 (3). Viability set up in the lab.

16/07/2013 Collapsed plant assessment. Only a few plants are wilting due to CRF damage, mostly in T10. Majority of T6 are looking healthy. Some pots have become quite damp from the automatic watering, could be a factor? So this has been turned down to once a day and plants will be watered overhead if needed.

17/07/2013 Leaf length & width measured on tagged leaves. If the leaves had died or fallen off, no measurement was taken. Vigour scores given. Li-Cor measurements taken for both cultivars from T1, 3, 5, 6, 9 and 10 in blocks 1, 2 and 3.

19/07/2013 CRF application for this week delayed until next week, due to lack of eggs in the culture.

22/07/2013 Collapsed plant assessment. No change from last week, & one of the plants that had collapsed last week had recovered this week.

26/07/2013 Due to lack of eggs in the culture. CRF applications will now stop.

29/07/2013 Collapsed plant assessment. No change from last week.

02/08/2013 Final plant assessment, no more collapsed plants.

04/08/2013 Started destructive assessment. All plants removed from pots and root chopped off at soil level. Roots put in labelled bags and stored in CS/01. Plants, pots and compost disposed of in site skip.

05/08/2013 Roots removed from CS/01 and washed to remove all compost. Roots wrapped in paper towel and left in lab to dry overnight. Temperature logger downloaded.

06/08/2013 Roots weighed and RDI scores given. Not so much root damage seen, with some plants treated with CRF eggs scoring 0-10 RDI.

Exp 4 - Autumn 2012

19/10/2012 Plants collected from Delflands, 5 trays each of Chassiron and Skywalker, and placed in GH2 on capillary matting. Plants at cotyledon stage, looking healthy. They will be watered daily for the next week or so.

23/10/2012 2 temperature loggers placed in GH. 1st leaf emerging.

25/10/2012 50 plants removed from each tray and transplanted into separate 345 trays. 5 trays in total as the same treatments for each separate cultivar will fit in the same tray, so 1 tray per treatment. The 5 trays were moved to GH3 and placed on separate pieces of capillary matting. 1 temp logger moved to GH3. The plants will not be watered overhead, only onto the matting.

29/10/2012 Majority of plants have recovered well over the weekend. T1 Chassiron has not recovered as well as the others, but will keep these as T1 so we know it was a transplanting stress not a treatment stress. 5 healthy plants from each treatment numbered, to be used for leaf measurements. Leaf length and width measurements taken on the 1st leaf. Photos taken of each treatment and vigour scores given. Capillary matting watered. 1L stock solution made up for each treatment. pH meter calibrated using pH 4 and pH7 buffer solutions, and pH and EC measured for each treatment. 25ml of each treatment applied using a hand-held mister.

30/10/2012 2nd spray of dilution rate 1 applied. Capillary matting watered 1hour before.

31/10/2012 3rd spray of dilution rate 1 applied.

01/11/2012 4th spray of dilution rate 1 applied.

02/11/2012 5th spray of dilution rate 1 applied. Leaf length and width measurements taken on leaves 1 and 2. Vigour and phytotoxicity scores given. Some plants of both cultivars in treatments 4 and 5 are beginning to curl slightly on the leaf edges.

03/11/2012 1st spray of dilution rate 2 (50ml) applied.

04/11/2012 2nd spray of dilution rate 2 applied.

05/11/2012 3rd spray of dilution rate 2 applied.

06/11/2012 4th spray of dilution rate 2 applied.

07/11/2012 5th spray of dilution rate 2 applied. Leaf length and width measurements taken on all fully emerged leaves for 5 plants/cv/treatment. Some plants now have 3 leaves. Vigour and phytotoxicity scores given. No phytotoxic effects other than very slight leaf curling.

08/11/2012 1st spray of dilution rate 3 (75ml) applied.

09/11/2012 2nd spray of dilution rate 3 applied.

10/11/2012 3rd spray of dilution rate 3 applied.

11/11/2012 4th spray of dilution rate 3 applied.

12/11/2012 5th spray of dilution rate 3 applied. Final salinity day. Leaf length and width measurements taken on tagged plants. All emerged leaves measured. Some plants now have 4 leaves. Vigour scores given. Plants generally looking good. Phytotoxicity scores given. From T3 up there was some slight curling on the edge of a few leaves. In T5, both varieties showed some crispiness to the edges of some leaves and a few leaves were stunted and slightly deformed. Photos taken. There was quite a bit of dried salt on some of the leaves for T3-5. Plants will now be left to recover for a couple of days, with just watering onto the capillary matting, and then they will be potted up at the end of the week.

15/11/2012 Plants potted up into 1L pots using organic compost supplied by Delflands nursery. 1 plant per pot, 1 pot per plot. Trial set up on capillary matting on bench 1 in the main glasshouse. Plants will be lightly watered overhead.

15/11/2012 Compost sample taken.

21/11/2012 Vigour and phytotoxicity scores given. A couple of plants are struggling, this may be because their roots were quite small when they were potted on. The youngest expanding leaf for each plant was measured, lengthxwidth. These leaves were tagged so that they can be measured each week.

22/11/2012 CRF application delayed 1 week as there was not enough eggs available from the culture.

27/11/2012 NRM results received.

28/11/2012 Leaf lengthxwidth and vigour assessment completed. Caulis 4-8 leaves.

30/11/2012 1st application of CRF eggs. Only 4 eggs could be washed onto each plant, and block 5 didn't have any eggs as there was not enough available from the culture. Eggs that were collected on the 19.11.12, 26.11.12, 28.11.12 and 30.11.12 were used. Eggs were divided evenly, so each plant would have had

an egg from each collection date. 10 eggs from each date were also used for viability.

05/12/2012 Leaf lengthxwidth and vigour assessment completed. A few of the tagged leaves had broken so the next youngest leaf was tagged instead and these will be measured from now on. Caulis 5-8 leaves.

07/12/2012 2nd application of CRF eggs delayed due to low number of eggs in the culture. It has been decided that eggs will be applied once a fortnight in order to get enough eggs. Leaf measurements will still take place weekly. Bags will be left off of pots as it takes 7-8 weeks for an egg to turn into a fly, and as the plants get bigger there is a risk of the plants getting damaged.

12/12/2012 Leaf lengthxwidth and vigour assessment completed.

14/12/2012 2nd CRF app. 7 eggs washed onto each plant. Block 5 was omitted again. Eggs for viability were set up in the bug lab.

18/12/2012 Leaf lengthxwidth and vigour assessment completed.

02/01/2013 Leaf lengthxwidth and vigour assessment completed. Some CRF plants are showing signs of wilting. However, most of block 1 is wilting, as well as some of the plants along the edge of the bench, where they have got a bit dry.

04/01/2013 3rd CRF app. 7 eggs washed onto each plant. (3 eggs from 27.12.12, 3 eggs from 2.1.13 and 1 egg from 4.1.13). Block 5 omitted. Eggs for viability set up in the bug lab.

08/01/2013 Temperature logger removed and downloaded, as logger need calibrating.

11/01/2013 Temperature logger returned to glasshouse.

21/01/2013 Started destructive assessment. The number of collapsed plants was recorded, and 20 leaves per treatment/per variety, were collected, bagged and stored in CS01. 400 leaves in total. Fully expanded leaves were taken. Each plant was dug up, and the root was removed. Roots were bagged individually and stored in CS01. All plants were disposed of in the site skip.

22/01/2013 Roots removed from CS01 and washed to remove all compost. Roots wrapped in paper towel and left to dry.

23/01/2013 Roots weighed and RDI scores given. Roots kept in the lab for photos to be taken. Temperature logger downloaded.

24/01/2013 Leaves removed from CS01 and measured using a leaf area machine borrowed from Rosemaund. The machine was calibrated before use, using pieces of paper that added up to 200cm2, and the machine was adjusted until the correct reading was shown. The machine was also calibrated throughout the day, once an hour.

Exp 5 - Summer 2012

14/06/2012 Plants collected from Delflands (5 trays of Chassiron and 5 trays of Skywalker). Plants at cotyledon stage-looking healthy. Placed in glasshouse 2. Data logger placed in glasshouse.

29/06/2012 Salt weighed out for each treatment and solutions made up ready to start treatments tomorrow.

30/06/2012 Salinity day 1. Photos taken and vigour score given. Tagged 10 young leaves per tray, to give 20 measurements for each treatment, 10 from each variety, so 100 measurements in total. Measured leaf length and width. Plants at growth stage 3 leaves. pH and EC measured for each treatment. EC is measured in mS. Unfortunately, because the salinity rates have been increased for the pot trial, the EC meter cannot give a reading for treatment 5,

(240mM NaCI), as it is too high. Meter will only read up to 20mS. 1L of treatment applied to the module tray, with 1L of fresh water applied to the untreated trays.

01/07/2012 Salinity day 2. Photos taken and vigour score given. Skywalker treatment 5 looks the most affected, with some plants wilting. Leaf length and width measurements taken. pH and EC measured and 1L of treatment applied to the relevant module trays with a watering can and rose.

02/07/2012 Salinity day 3. Photos taken and vigour scores given. Skywalker treatment 5 still the most affected. Leaf length and width measured. pH and EC measured and 1L applied to each tray.

03/07/2012 Salinity day 4. Photos taken and vigour scores given. The leaves on treatment 5 Chassiron and treatment 4 Skywalker are greener than the other trays. Skywalker treatment 5 is wilting. Leaf length and width measurements taken. pH and EC measured and 1L of treatment applied to the relevant tray.

04/07/2012 Salinity day 5. Photos taken and vigour scores given. Skywalker treatment 5 had perked upa bit and was not quite as wilted but still looked the worst tray. T5 Chassiron and T4 Skywalker still had greener leaves. A few aphids were spotted on Chassiron T5 only. Phytotoxicity score given. Most treatments showed some yellowing to the leaves. T5 Skywalker had a few crinkled and dying young leaves. Leaf length and width measurements taken. pH and EC measured and 1L of treatment applied to the relevant tray.

04/07/2012 Trays of caulis with downy mildew collected, which will be kept for inoculum.

16/07/2012 Trays potted into trial plan.

18/07/2012 Li-Cor assessment week 1. Machine would not settle properly, possibly due to cloud cover and people in the glasshouse, so not as many readings were taken. Leaf length and width measured on every plant. Youngest fully expanded leaf was measured and the leaf was tagged so that the same leaf can be measured again.

19/07/2012 Trial supposed to be inoculated with downy mildew today but the culture is dying and there are not enough spores. Checked with study director and it will

be ok to inoculate next week. Few leaves with small amount of DM placed in damp chamber to encourage spores to grow.

23/07/2012 Checked leaves in damp chamber and spores have grown, checked them under microscope. Did preliminary scrape and there should be enough spores for inoculating tomorrow.

24/07/2012 24 caulis at 2-3 leaf stage with DM collected from Delflands which will be used to keep DM culture going. Some of them potted up into 1L pots. Placed on capillary matting with perforated plastic over the top, along with netting for shade, and kept outside for the mean time as the polytunnels are so hot and don't want the DM to die. 5 1/2 trays of caulis also sown.

24/07/2012 Trial inoculated with DM inoculum at a spore concentration of $5x10^4$. Plants inoculated using a hand held mister with 300ml of inoculum. Pots staggered in a zig-zag pattern to prevent inoculum getting on uninoculated pots. Trial covered with insect netting rather than polythene as it is very hot and don't want to kill plants.

25/07/2012 Li-Cor assessment week 2. Sunny day although 2/3 of the trial was in shade when measurements were taken. Machine working well so measurements taken on youngest fully expanded leaf for treatments T1, T3, T5, T6, T9 and T10 across blocks 1,2 and 3. Leaf length and width measurements taken on tagged leaves. Vigour scores given although it was difficult to give an accurate score as the plants were wilting a bit as it was very hot and the plants had not been watered that morning due to Li-Cor assess, so scores of 4 given. Once measurements taken, plants were watered onto soil, not leaves, and recovered with netting.

26/07/2012 Trial sprayed with Chess to try and clear aphids.

27/07/2012 Trial uncovered.

31/07/2012 Downy mildew assessment 7 days after inoculation. Not much to be seen yet, although there is some DM on a few plants, mostly Chassiron. T5 and 10 are most affected. Aphids still present although numbers are decreasing. Photos taken.

02/08/2012 Li-Cor assessment week 3. Cloudy day so machine would not always settle. Measurements taken on youngest fully expanded leaf for treatments T1, T3,

T5, T6, T9 and T10 across blocks 1, 2 and 3. Leaf length and width measurements taken on tagged leaves. Vigour scores given, DM is now starting to develop more.

07/08/2012 Downy mildew assessment 14 days after inoculation. More DM has developed although it is still fairly minor, and only affecting 1-2 leaves/plant at most. Still some aphids. GS 7-9 leaves.

09/08/2012 Li-Cor assessment week 4. Cloudy. Measurements taken on youngest fully expanded leaf for treatments 1, 3, 5, 6, 9 and 10 across blocks 1, 2 and 3. Leaf length and width measurements taken on tagged leaves. Vigour scores given. Plants looking healthy.

13/08/2012 Downy mildew assessment 21 days after inoculation. Presence of downy mildew has not greatly increased and there are a few untreated plants that have 1 or 2 infected leaves.

16/08/2012 Li-Cor assessment week 5. Cloudy day. Measurements taken on youngest fully expanded leaf for treatments 1, 3, 5, 6, 9 and 10 across blocks 1, 2 and 3. Leaf length and width measurements taken on tagged leaves. Vigour scores given.

22-23/8/12 Destructive assessment. 28 day DM assessment completed. Not much mildew present so scores given for percentage of leaf area affected in whole pot. There is also DM present in untreated plots. 25 fully expanded leaves collected from across all blocks for each treatment for both varieties, so 500 leaves in total. Leaves scanned through a leaf area machine borrowed from Rosemaund. Some leaves with DM also collected to keep culture going. Plants disposed of in skip and glasshouse cleaned.

Exp 6 - Autumn 2012

19/10/2012 Plants collected from Delflands, 5 trays each of Chassiron and Skywalker, and placed in GH2 on capillary matting. Plants at cotyledon stage, looking healthy. They will be watered daily for the next week or so.

23/10/2012 2 temperature loggers placed in GH. 1st leaf emerging.

25/10/2012 50 plants removed from each tray to be used in the mini GH trial C. Plants for mini trial moved to GH3, along with one of the temperature loggers.

31/10/2012 Another temperature logger placed in GH2.

05/11/2012 Day 1 of salinity treatments. Plants will have no extra watering this week. 10L of each treatment made up and stored in water containers in the GH. Vigour scores given for each set of plants. All plants currently look the same. Leaf length and width measurements taken on 10 plants per tray. The youngest fully emerged leaf (leaf 2) was measured and tagged so the same leaves will

be measured all week. pH and EC measurements taken for each treatment, and 1L of treatment applied to the relevant tray.

06/11/2012 Day 2 of salinity treatments. Vigour scores given for each set of plants. Leaf length and width measurements taken on tagged leaves. 1L of treatment applied to each tray.

07/11/2012 Day 3 of salinity treatments. Vigour scores given. Leaf length and width measurements taken. 1L of treatment applied to each tray.

08/11/2012 Day 4 of salinity treatments. Vigour scores given and leaf length and width measurements taken. 1L of treatment applied to each tray.

09/11/2012 Day 5 of salinity treatments. Vigour and phytotoxicity scores given. No significant phytotoxicity seen other than some slight leaf curling. Leaf length and width measurements taken. Plants will now be given a few days to recover and will be lightly watered overhead.

14/11/2012 Plants potted up into 1L pots using organic compost supplied by Delflands nursery. 5 plants per pot, 2 pots per plot. Trial set up in GH3 on capillary matting, which will be watered and plants will also be lightly watered overhead until they are inoculated.

15/11/2012 Compost sample taken.

16/11/2012 One 345 tray of caulis infected with DM collected from Delflands to go with the DM culture.

19/11/2012 DM inoculum made up using plants collected on 16.11.12 as well as plants from the culture. 600ml of inoculum was made at a concentration of $1x10^4$. Relevant plots were sprayed with inoculum using a hand-held mister, to the point of run-off. Uninoculated plots were shielded with a board to prevent inoculum spraying onto them. The trial was then covered with polythene which will stay on for 3 days.

22/11/2012 Trial uncovered. Leaf lengthxwidth measurements taken on youngest expanding leaf for 1 plant per plot. Leaf tagged so the same leaves can be measured each week. Vigour and phytotoxicity scores given. Only phytotoxicity was some slight leaf curling. Li-Cor measurements taken on youngest fully expanded leaf for both cultivars for T1, 3, 5, 6, 9 and 10. Measurements taken on blocks 1, 2 and 3 only. Photosynthesis readings quite low as it was cloudy.

26/11/2012 7-day downy mildew assessment. No DM present so an assessment sheet hasn't been filled in as each plot would score 0. Plants looking healthy. GS 4-6 leaves. Trial is now being watered with automatic watering system onto capillary matting.

27/11/2012 NRM results received.

29/11/2012 Leaf lengthxwidth and vigour assessment completed. No sign of DM. Li-Cor assessments done on T1, 3, 5, 6, 9 and 10 in blocks 1-3 for both cultivars. Photosynthesis readings were low again, fairly cloudy day.

03/12/2012 14-day downy mildew assessment. There was still no sign of any DM, so plants were re-inoculated. 700ml of inoculum was made up with a concentration of $5x10^4$. Plants were inoculated using a hand-held mister, and uninoculated plants were sheilded with a board to prevent contamination. The whole trial was then covered with polythene which will stay on for 3 days. GS 5-8 leaves.

06/12/2012 Trial uncovered. Leaf lengthxwidth and vigour assessment completed. Li-Cor assessments carried out on usual plots. There was a powercut just as Li-Cor measurements started to be taken. Lights went off and temperature dropped quite a bit.

10/12/2012 7-day downy mildew assessment. No DM present yet, so an assessment sheet hasn't been filled in yet. GS 6-9 leaves.

13/12/2012 Leaf lengthxwidth and vigour assessment completed. Li-Cor assessments carried out on usual plots.

17/12/2012 14-day downy mildew assessment. There is no sign of any DM on any of the plots. Plants are too big and vigorous to be re-inoculated, so will continue with measurements as normal, and keep monitoring to see if any DM develops later on.

19/12/2012 Leaf lengthxwidth and vigour assessment completed. Li-Cor assessments carried out on usual plots.

02/01/2013 28-day downy mildew assessment. No DM has developed. This could be because when the trial was inoculated and covered, there was not enough humidity and so the DM just didn’t take. Plants will be checked one last time when the destructive assessment is done.

03/01/2013 Leaf lengthxwidth and vigour assessment completed. Li-Cor assessments carried out on usual plots. The lower leaves are unlikely to extend anymore, so leaf measurements will finish now.

08/01/2013 Temperature logger removed and downloaded, as logger needs calibrating.

11/01/2013 Temperature logger returned to glasshouse.

22/01/2013 Final check on plants. There was no downy mildew present on the plants. Leaves were collected from the trial, 25 leaves/treatment/variety, so 500 leaves in total. They were bagged and stored in CS01. Plants disposed of in the site skip, and GH cleared. Temperature logger downloaded.

25/01/2013 Leaves removed from CS01 and measured using a leaf area machine borrowed from Rosemaund. The machine was calibrated before use, using pieces of paper that added up to 200cm2, and the machine was adjusted until the correct reading was shown. The machine was also calibrated throughout the day, once an hour.

Exp 7 - Summer 2013

24/05/2013 GH 2 disinfected.

30/05/2013 Plants collected from Delflands, 5 trays of Chassiron and 5 trays of Skywalker. Plants at cotyledon stage, although Skywalker are taller, Chassiron have only just emerged. Trays placed on individual pieces of capillary matting in GH2, and will be watered from below, unless the compost gets very dry, then they will be watered overhead. 2 temperature loggers, (DL202 and DL206) set up and placed in GH. Bio-controls for aphids introduced. 500L of organic modular compost also collected from Delflands and stored in the potting shed, which will be used for potting up the plants.

04/06/2013 Plants checked. There is some downy mildew present on the cotyledons but they seem to be growing through it. Chassiron still at cotyledon, Skywalker at 1 leaf.

10/06/2013 Salinity day 1. Chassiron 1-2 leaves, Skywalker 2-3 leaves. DM still present on cotyledons but seems to be drying up and leaves are clean. Each tray

given a little bit of water overhead as the compost was looking quite dry on some trays. 2nd leaf tagged on 10 plants per tray, so 100 leaves in total. Salinity treatments mixed up, 10L of each treatment, which will be stored in the GH. Leaf length and width measured. Vigour scores given. Untreated given a score of 5, and then the others will score either above or below that. pH and EC of each treatment measured using a pH meter which was calibrated using buffer solutions of pH4 and pH7. 1L of each treatment applied to the relevant tray. Photos taken.

11/06/2013 Salinity day 2. Leaf length and width measured on tagged leaves. Vigour scores given and photos taken. pH and EC of each treatment measured. 1L of treatment applied to the tray.

12/06/2013 Salinity day 3. Leaf length and width measured on tagged leaves. Vigour scores given and photos taken. pH and EC of each treatment measured. 1L of treatment applied to the tray.

13/06/2013 Salinity day 4. Leaf length and width measured on tagged leaves. Vigour scores given and photos taken. pH and EC of each treatment measured. 1L of treatment applied to the tray.

14/06/2013 Salinity day 5. Leaf length and width measured on tagged leaves. Vigour scores given and photos taken. Phytotoxicity scores given. All plants treated with salt showed very slight leaf crinkling and treatments 4 and 5 for both cultivars were shorter. pH and EC of each treatment measured. 1L of treatment applied to the tray.

17/06/2013 Plants potted up according to trial plan, 5 plants per pot. 2nd leaf tagged on 1 plant in pot 1, which will be measured each week. Pots placed in poly 3, raised up on crates lined with plastic & capillary matting. Plants can be watered overhead until they are inoculated, then they are to be watered onto matting only. Compost sample taken and placed in CS/01. Chassiron 2-3 leaves, Skywalker 3-4 leaves.

21/06/2013 Phytotoxicity scores given.

24/06/2013 Treatments six to ten inoculate with downy mildew. Quite a lot of downy mildew present on plants in culture. Spore concentration of solution 6 x 10,000 spores per ml. Plants watered beforehand. All plots covered with perforated polythene.

27/06/2013 Polythene removed first thing in the morning to give leaves time to dry before Li-Cor measurements taken. Li-Cor measurements taken on six treatments on three blocks (2,3,4). Leaf length and width measured and vigour scores given. Treatments five and ten: plants are small, discoloured and a little wilted. Skywalker more severely affected than Chassiron. Photos taken of full trial and of stunted plants.

01/07/2013 Seven day assessment for downy mildew, no disease present on any of the plots.

04/07/2013 Leaf length and width measured on tagged leaves. Vigour scores given.

05/07/2013 Li-Cor measurements taken from both cultivars for T1, 3, 5, 6, 9 and 10 on blocks 1, 2 and 3.

08/07/2013 14 day DM assessment. Small amount of DM seen in some plots on both cultivars. Some dead plants, mainly Skywalker.

11/07/2013 Leaf length and width measured on tagged leaves. Vigour scores given. Li-Cor measurements taken from both cultivars for T1, 3, 5, 6, 9 and 10 on blocks 1, 2 and 3.

16/07/2013 21 day DM assessment. Not much difference between this week and last week. Some of the lower leaves are beginning to fall off, which means some plants that had DM last week don't have it now as the leaf has come off. Skywalker in T5 and 10 seem to have suffered quite a bit, with plants either stunted or dying.

17/07/2013 Leaf length & width measured on tagged leaves. There were a couple of plots where the lower leaves had died and fallen off, so no measurement was taken. Vigour scores given. Not much change from last week. Plants could do with a feed next week. Li-Cor measurements taken for both cultivars for T1, 3, 5, 6, 9 and 10 on blocks 1, 2 and 3.

22/07/2013 28 day disease assessment. No different from last week, except a few more plants had died. Although assessments have now finished, the plants will be left for at least another week & monitored to see if any more disease develops.

09/08/2013 Trial has been monitored since 22.7.13, and no more downy mildew has appeared, so trial will now finish. All plants cleared from polytunnel and disposed of in site skip. Temperature logger downloaded.

Exp 8 - Autumn 2012

19/10/2012 Plants collected from Delflands, 5 trays each of Chassiron and Skywalker, and placed in GH2 on capillary matting. Plants at cotyledon stage, looking healthy. They will be watered daily for the next week or so.

23/10/2012 2 temperature loggers placed in GH. 1st leaf emerging.

25/10/2012 50 plants removed from each tray and transplanted into separate 345 trays. 5 trays in total as the same treatments for each separate cultivar will fit in the same tray, so 1 tray per treatment. The 5 trays were moved to GH3 and placed on separate pieces of capillary matting. 1 temp logger moved to GH3. The plants will not be watered overhead, only onto the matting.

29/10/2012 Majority of plants have recovered well over the weekend. T1 Chassiron has not recovered as well as the others, but will keep these as T1 so we know it was a transplanting stress not a treatment stress. 5 healthy plants from each treatment numbered, to be used for leaf measurements. Leaf length and width measurements taken on the 1st leaf. Photos taken of each treatment and vigour scores given. Capillary matting watered. 1L stock solution made up for each treatment. pH meter calibrated using pH 4 and pH7 buffer solutions, and pH and EC measured for each treatment. 25ml of each treatment applied using a hand-held mister.

30/10/2012 2nd spray of dilution rate 1 applied. Capillary matting watered 1hour before.

31/10/2012 3rd spray of dilution rate 1 applied.

01/11/2012 4th spray of dilution rate 1 applied.

02/11/2012 5th spray of dilution rate 1 applied. Leaf length and width measurements taken on leaves 1 and 2. Vigour and phytotoxicity scores given. Some plants of both cultivars in treatments 4 and 5 are beginning to curl slightly on the leaf edges.

03/11/2012 1st spray of dilution rate 2 (50ml) applied.

04/11/2012 2nd spray of dilution rate 2 applied.

05/11/2012 3rd spray of dilution rate 2 applied.

06/11/2012 4th spray of dilution rate 2 applied.

07/11/2012 5th spray of dilution rate 2 applied. Leaf length and width measurements taken on all fully emerged leaves for 5 plants/cv/treatment. Some plants now have 3 leaves. Vigour and phytotoxicity scores given. No phytotoxic effects other than very slight leaf curling.

08/11/2012 1st spray of dilution rate 3 (75ml) applied.

09/11/2012 2nd spray of dilution rate 3 applied.

10/11/2012 3rd spray of dilution rate 3 applied.

11/11/2012 4th spray of dilution rate 3 applied.

12/11/2012 5th spray of dilution rate 3 applied. Final salinity day. Leaf length and width measurements taken on tagged plants. All emerged leaves measured. Some plants now have 4 leaves. Vigour scores given. Plants generally looking good. Phytotoxicity scores given. From T3 up there was some slight curling on the edge of a few leaves. In T5, both varieties showed some crispiness to the edges of some leaves and a few leaves were stunted and slightly deformed. Photos taken. There was quite a bit of dried salt on some of the leaves for T3-5. Plants will now be left to recover for a couple of days, with just watering onto the capillary matting, and then they will be potted up at the end of the week.

15/11/2012 Plants potted up into 1L pots using compost supplied by Delflands nursery. 4 plants per pot, 1 pot per plot. Some pots could only have 3 plants as there were a few plants across the treatments which had either died or their roots broke off, and there were no spare plants. Trial set up on capillary matting on

bench 1 in the main glasshouse. Plants will be lightly watered overhead until they are inoculated.

15/11/2012 Compost sample taken.

16/11/2012 One 345 tray of caulis infected with DM collected from Delflands to go with the DM culture.

19/11/2012 Some plants have not recovered very well, so a quick assessment was done of each pot, counting the total number of plants in each pot, the number that were healthy and the number that were struggling. None of the plants have actually died so may still recover.

19/11/2012 DM inoculum made up using plants collected on 16.11.12 as well as plants from the culture. 600ml of inoculum was made at a concentration of $1x10^4$. Relevant plots were sprayed with inoculum using a hand-held mister, to the point of run-off. Uninoculated plots were sheilded with a board to prevent inoculum spraying onto them. The trial was then covered with polythene which will stay on for 3 days.

22/11/2012 Trial uncovered. Leaf lengthxwidth measurements taken on youngest expanding leaves for each cultivar in each plot. Leaves tagged so that the same leaf can be measured each week. Vigour and phytotoxicity scores given.

26/11/2012 7-day downy mildew assessment. No DM present so an assessment sheet wasn't filled in as each plot would score 0. Plants have now recovered from transplanting. GS 4-6 leaves.

27/11/2012 NRM results received.

28/11/2012 Leaf lengthxwidth and vigour assessment completed. Still no signs of DM.

03/12/2012 14-day downy mildew assessment. There was still no sign of any DM, so plants were re-inoculated. 700ml of inoculum was made up with a concentration of $5x10^4$. Plants were inoculated using a hand-held mister, and uninoculated plants were sheilded with a board to prevent contamination. The whole trial was then covered with polythene which will stay on for 3 days. GS 5-8 leaves.

06/12/2012 Trial uncovered. Leaf lengthxwidth and vigour assessment completed.

10/12/2012 7-day downy mildew assessment. No DM present yet, so an assessment sheet hasn't been filled in. GS 6-8 leaves.

12/12/2012 Leaf lengthxwidth and vigour assessment completed. Still no signs of DM.

17/12/2012 14-day downy mildew assessment. There is no sign of any DM on any of the plots. Plants are too big and vigorous to be re-inoculated, so will continue with measurements as normal, and keep monitoring to see if any DM develops later on.

18/12/2012 Leaf lengthxwidth and vigour assessment completed. Still no signs of DM.

02/01/2013 28-day downy mildew assessment. No DM has developed. This may be because humidity wasn't high enough when the trial was inoculated and covered. Leaf lengthxwidth and vigour assessment completed.

08/01/2013 Temperature logger removed and downloaded, as logger needed calibrating.

11/01/2013 Temperature logger returned to glasshouse.

23/01/2013 Final check on plants. There was some downy mildew on a few plants, but seeing as there was downy mildew on untreated plots as well, it was probably caused by humidity in the glasshouse and therefore wasn't recorded. Leaves were collected from the trial, 20 leaves/treatment/variety, so 400 leaves in total. They were bagged and stored in CS01. Plants were disposed of in the site skip. Temperature logger downloaded.

25/01/2013 Leaves removed from CS01 and measured using a leaf area machine borrowed from Rosemaund. The machine was calibrated before use, using pieces of paper that added up to 200cm2, and the machine was adjusted until the correct reading was shown. The machine was also calibrated throughout the day, once an hour.

Exp 9 - Summer 2012

24/05/2012 Plants collected from Delflands, 5 trays of Chassiron and 5 of Skywalker, and placed in glasshouse 3. Temperature logger placed in glasshouse.

06/06/2012 Trays set out in treatment order onto capillary matting. 5 youngest leaves tagged on each tray to give 50 leaves in total. Leaf length and width measured. Vigour score given and photos taken. 5L of salinity treatments made up. pH meter calibrated and pH of treatments recorded. 1L of treatment applied to relevant tray.

07/06/2012 2nd salinity. pH meter calibrated and pH of treatments measured. Leaf length and width measured. Vigour scores given. 1L applied to each tray. Photos taken.

08/06/2012 3rd salinity. pH meter calibrated and pH of treatments measured. Leaf length and width measured. Vigour scores given. 1L applied to each tray. Photos taken. Treatments made up for the weekend.

09/06/2012 4th salinity. pH meter calibrated and pH of treatments measured. Leaf length and width measured. Vigour scores given. 1L applied to each tray. Photos taken.

10/06/2012 5th salinity. pH meter calibrated and pH of treatments measured. Leaf length and width measured. Vigour scores given. 1L applied to each tray. Photos taken.

11/06/2012 Phytotoxicity score given for each treatment. Plants are looking ok, slightly yellow on some leaves. Plants re-arranged into trays so that they are in randomised order ready for planting in the field.

20/06/2012 Trays moved to hard standing and covered with insect netting.

25/06/2012 Plants fed with Vitax 214 mixed to a solution of 10g/1L. 10L made to feed 9 trays.

27/06/2012 Trial set up at site in Lincolnshire, with grower assistance. First 2 rows were planted first, (so block 4 and 1 containing 3 rows of plants, and block 4 and 2 containing 3 rows of plants) going up the field, and then the 3rd row was planted on the way back down the field, (so block 3 and 4 containing 3 rows

of plants). This means the 3rd block is a bit out of line with the other 2 blocks. Planting was mostly successful although a few plants were missed and a couple may have been placed into the wrong plot. Checked the distribution of the plants after planting to make sure the rows were even and there was the right number of plants. Plots labelled and flags placed in each corner of the trial. Temperature logger placed in field and 2 bird scarers. Photos taken and soil sample taken.

29/06/2012 Soil sample sent to NRM for analysis.

26/07/2012 Visit to trial site. There is obvious scorch on the leaves from herbicide spray, although the rest of the field is not affected as much. Yellowing around the edge of the leaves. Majority of plants had suffered from bird damage but not severe. We assessed 20 plants in the middle of the plot by giving an overall vigour score and counting how many plants were missing or damaged. Few samples of damaged plants were brought back to BX for inspection. Some of the plots at the bottom of block 4 may have been waterlogged during the heavy rain as the plants were a lot smaller. There didn't appear to be any obvious differences between the plots.

29/08/2012 Visit to trial site. Same assessment as before, giving plots a vigour score and counting number of healthy plants out of middle 20. Plants a lot bigger, 12-15 leaves. Plants that were alive at last visit are still healthy, apart from 1 plant in plot 3. Some downy mildew present on lower leaves in virtually every plot. Leaves appear slightly twisted and warped, more so in some plots. Scorch seen at previous visit has now gone. No evidence of pests or disease.

09/10/2012 Trial harvested. Each plot was assessed for % leaf area affected by ringspot across the plot. Plots were also assessed for % of caulis that were at harvestable growth stage. Some caulis had gone too far and were beginning to go off and show ricing. 10 caulis that were as close to harvest GS as possible were removed from each plot, cutting the stem at soil level. The 10 roots were removed and bagged separately for RDI assessing. The 10 caulis were weighed together, with the leaves, and then the leaves were removed. Caulis were bagged up by plot and returned to BX. Temp logger was removed and all canes and flags removed.

10/10/2012 Temp logger downloaded. Caulis were weighed again to get a trimmed weight of 10 caulis. Numbers of caulis in each plot with discolouration and/or ricing was recorded. No bracketing seen. Roots stored in cold store.

17/10/2012 Cauliflower roots washed and air-dried. Weighed as a bulk sample, so 5 roots to a plot. Each root given an RDI score. Results very variable, ranging from 0-10 to 40-60 in the same plot. Roots returned to cold store (CS/01). 2 pupae and 2 larvae found.

09/01/2013 Roots disposed of in site skip.

Appendix 2 – Egg viability

Exp 1 - Summer 2012

Date of application	% of viable eggs
20 July 2012	90%
27 July 2012	95%
10 August 2012	90%

Exp 2 - Autumn 2012

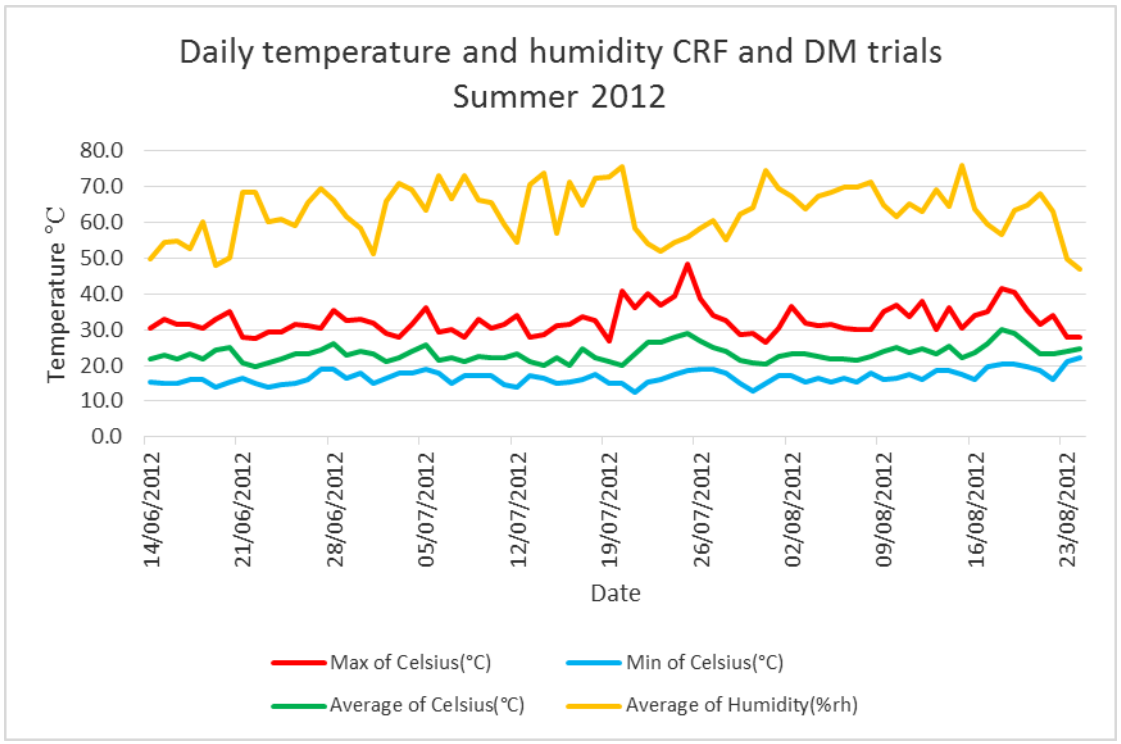
Date of application	% of viable eggs
30 November 2012	80%
14 December 2012	95%
4 January 2013	95%

Exp 3 - Summer 2013

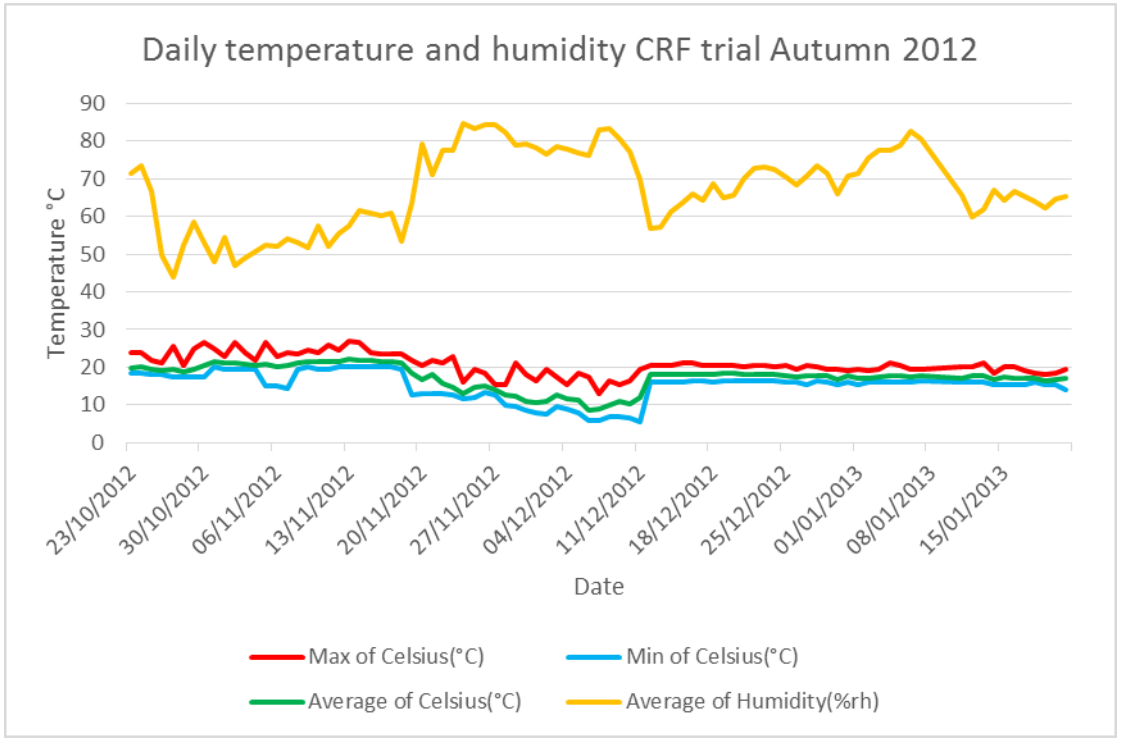
Date of application	% of viable eggs
28 June 2013	100%
5 July 2013	80%
12 July 2013	95%

Appendix 3 –Logger temperature data

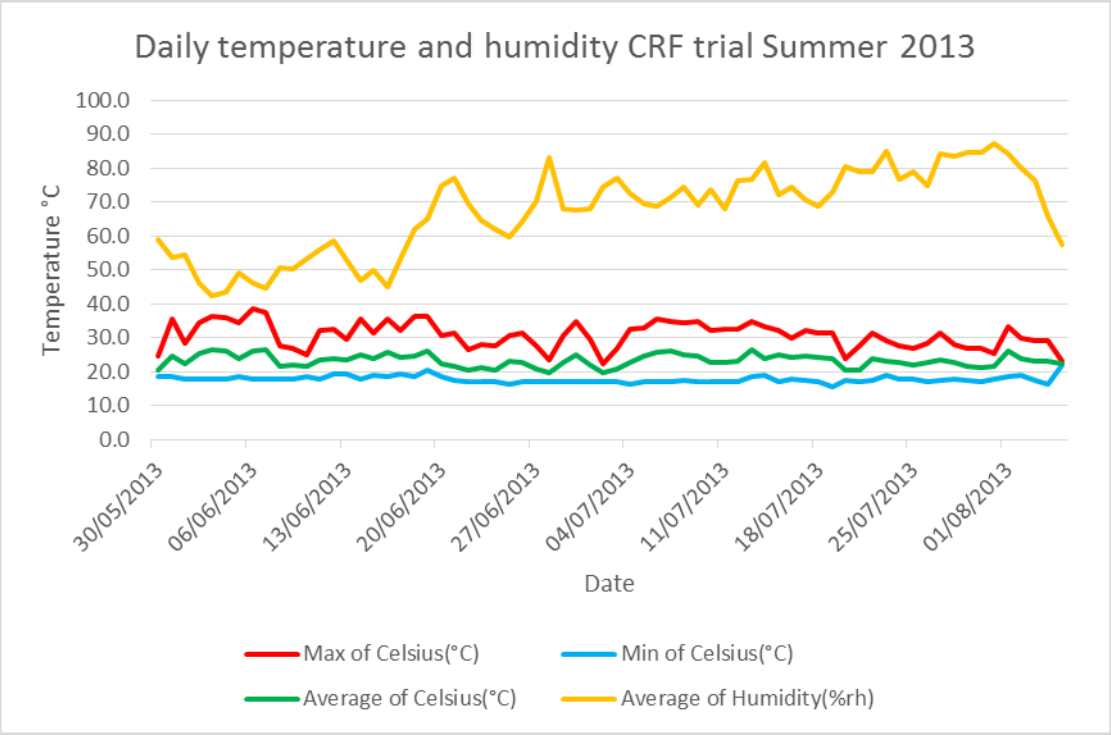
Exp 1 and 5 – Summer 2012



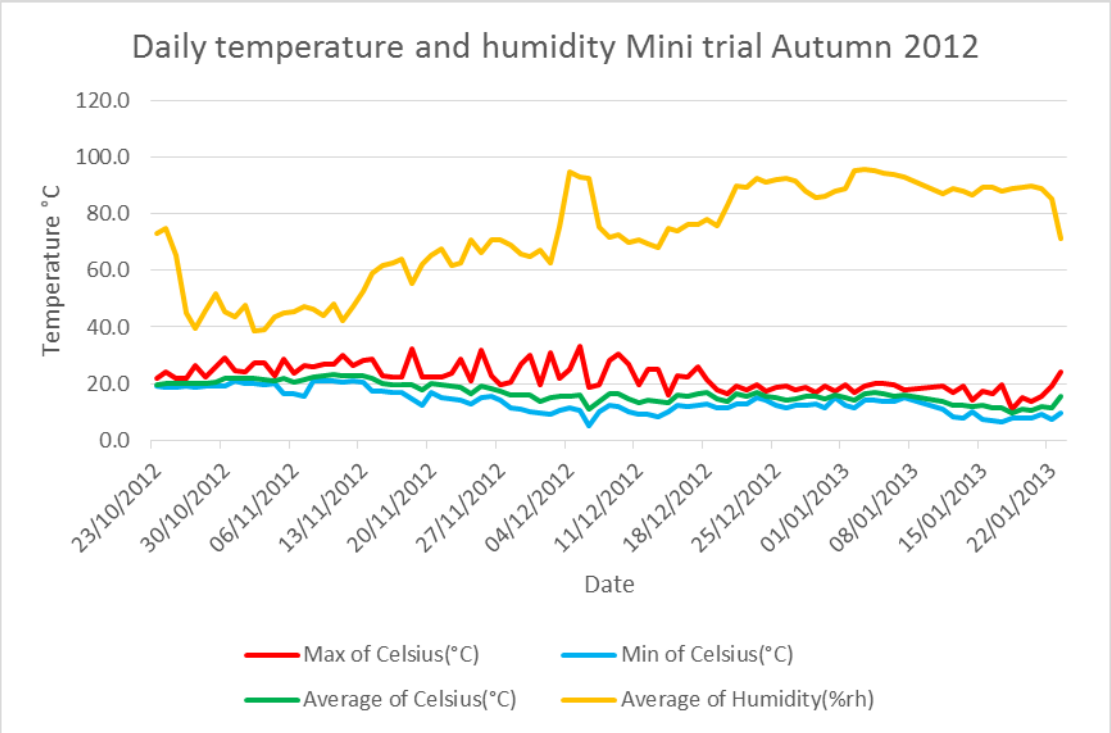
Exp 2 – Autumn 2012



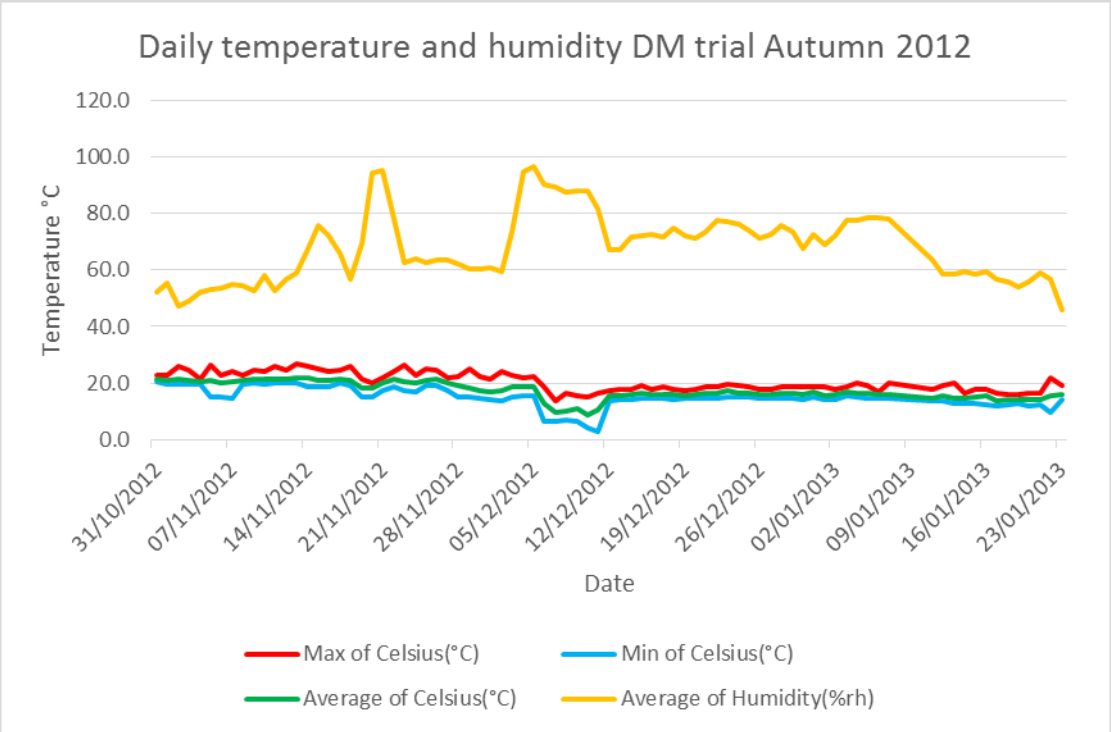
Exp 3 – Summer 2013



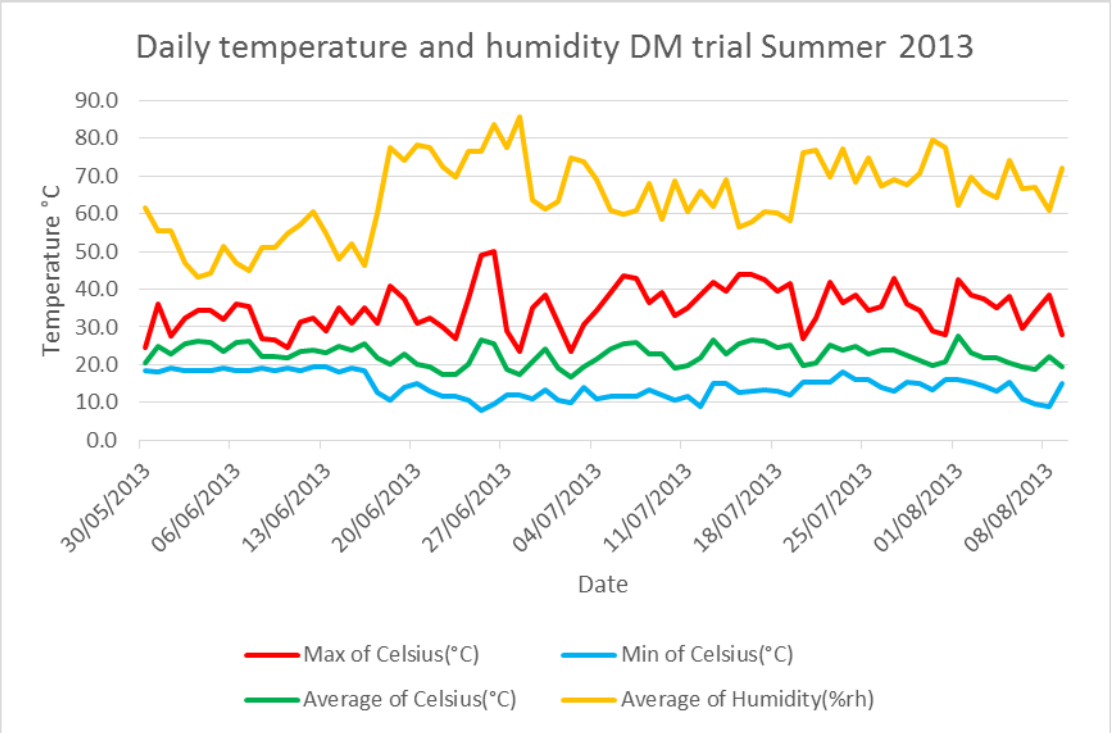
Exp 4 and 8 – Autumn 2012



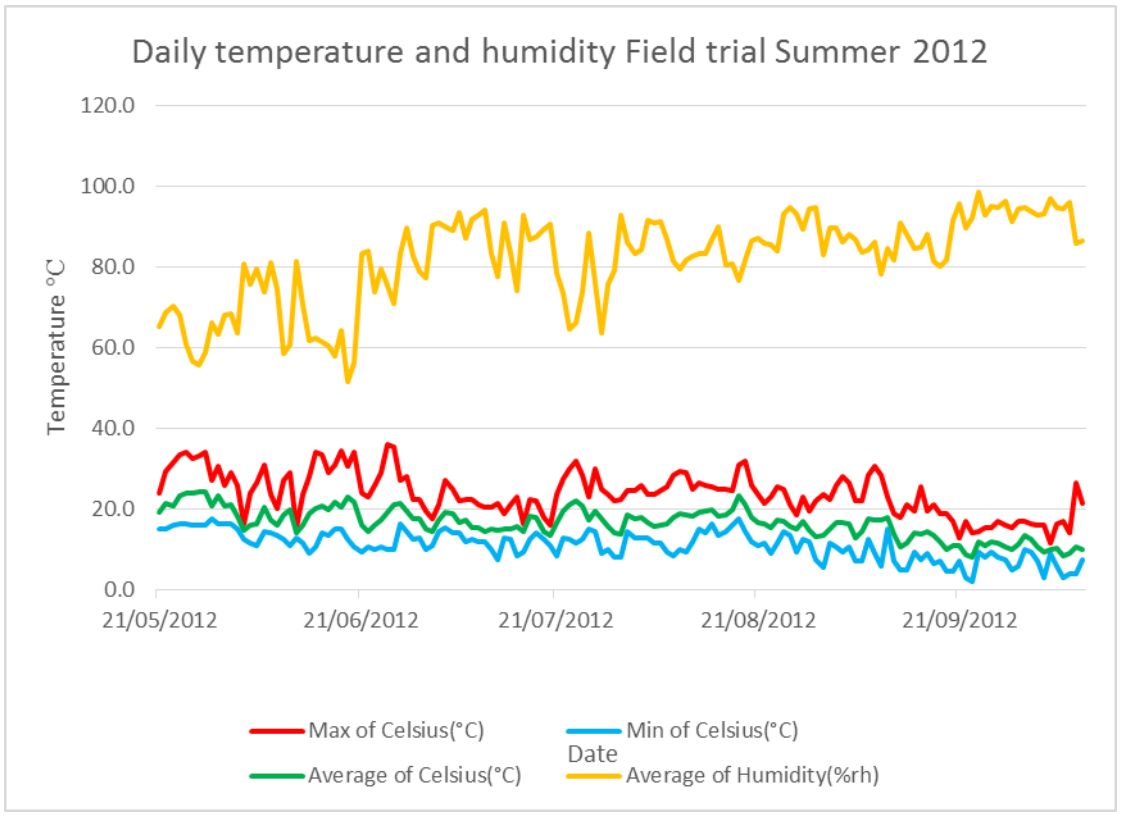
Exp 6 – Autumn 2012



Exp 7 – Summer 2013



Exp 9 – Summer 2012



Appendix 4 – Li-Cor measurements

Experiment 1

Table 1. Effect of salinity treatments on vapor pressure deficit (VpdL kPa) – Summer 2012 – Exp 1

Treatment	Salt concentration (mM)	With or without CRF eggs	VpdL at weeks after salinity application: 3 (25 Jul 12)	5 (9 Aug 12)	6 (16 Aug 12)
1	0	-	1.42	0.78	1.11
3	120	-	1.42	0.84	0.96
5	240	-	1.57	0.86	1.18
6	0	+	1.46	1.21	1.86
9	180	+	1.55	1.12	1.69
10	240	+	1.69	1.64	1.84

s.e.d – 0.133 with 72 d.f

Table 2. Effect of salinity treatments on Intercellular CO_2 / Ambient CO_2 (Ci_Ca) – Summer 2012 – Exp 1.

Treatment	Salt concentration (mM)	With or without CRF eggs	Ci_Ca at weeks after salinity application: 3 (25 Jul 12)	5 (9 Aug 12)	6 (16 Aug 12)
1	0	-	0.87	0.97	0.94
3	120	-	0.87	0.97	0.95
5	240	-	0.86	0.96	0.93
6	0	+	0.87	0.94	0.91
9	180	+	0.84	0.95	0.87
10	240	+	0.81	1.02	0.97

s.e.d – 4.457 with 72 d.f

Table 3.Effect of salinity treatments on Water Use Efficiency (WUEi) – Summer 2012 – Exp 1.

Treatment	Salt concentration (mM)	With or without CRF eggs	WUEi at weeks after salinity application: 3 (25 Jul 12)	5 (9 Aug 12)	6 (16 Aug 12)
1	0	-	0.18	0.05	0.09
3	120	-	0.17	0.06	0.09
5	240	-	0.18	0.07	0.10
6	0	+	0.17	0.09	0.09
9	180	+	0.20	0.06	0.15
10	240	+	0.21	-0.05	0.01

s.e.d – 0.072 with 72 d.f

Experiment 2

Table 4. Effect of salinity treatments on vapor pressure deficit (VpdL kPa) – Autumn 2012 – Exp 2.

Treatment	Salt concentration (mM)	With or without CRF eggs	VpdL at weeks after salinity application: 2 (22 Nov 12)	3 (29 Nov 12)	4 (6 Dec 12)	5 (13 Dec 12)	6 (19 Dec 12)	7 (3 Jan 13)
1	0	-	0.78	0.74	0.68	0.97	1.01	0.73
3	60	-	0.77	0.72	0.65	0.91	0.93	0.68
5	120	-	0.79	0.70	0.65	0.96	0.99	0.78
6	0	+	0.74	0.70	0.61	0.86	1.04	0.95
9	90	+	0.75	0.69	0.65	0.94	0.97	0.97
10	120	+	0.76	0.75	0.67	0.93	1.05	0.93

s.e.d – 0.058 with 120 d.f

Table 5. Effect of salinity treatments on Intercellular CO_2 / Ambient CO_2 - Ci_Ca – Autumn 2012 – Exp 2.

Treatment	Salt concentration (mM)	With or without CRF eggs	Ci_Ca weeks after salinity application: 2 (22 Nov 12)	3 (29 Nov 12)	4 (6 Dec 12)	5 (13 Dec 12)	6 (19 Dec 12)	7 (3 Jan 13)
1	0	-	0.94	0.95	0.92	0.90	0.94	0.96
3	60	-	0.95	0.95	0.93	0.91	0.95	0.96
5	120	-	0.95	0.95	0.93	0.90	0.95	0.96
6	0	+	0.95	0.95	0.94	0.93	0.93	0.90
9	90	+	0.96	0.95	0.93	0.90	0.95	0.93
10	120	+	0.95	0.95	0.92	0.90	0.93	0.92

s.e.d – 0.014 with 120 d.f

Table 6. Effect of salinity treatments on Water Use Efficiency WUEi – Autumn 2012 – Exp 2.

Treatment	Salt concentration (mM)	With or without CRF eggs	WUEi at weeks after salinity application: 2 (22 Nov 12)	3 (29 Nov 12)	4 (6 Dec 12)	5 (13 Dec 12)	6 (19 Dec 12)	7 (3 Jan 13)
1	0	-	0.14	0.14	0.25	0.22	0.11	0.10
3	60	-	0.12	0.15	0.23	0.19	0.10	0.09
5	120	-	0.12	0.14	0.22	0.22	0.09	0.10
6	0	+	0.13	0.13	0.22	0.17	0.13	0.20
9	90	+	0.10	0.14	0.22	0.21	0.09	0.13
10	120	+	0.14	0.14	0.25	0.21	0.13	0.15

s.e.d – 0.024 with 120 d.f

Experiment 3

Table 7. Effect of salinity treatments on vapor pressure deficit (VpdL kPa) – Summer 2013 – Exp 3.

Treatment	Salt concentration (mM)	With or without CRF eggs	VpdL at weeks after salinity application: 3 (4 Jul 13)	4 (11 Jul 13)	5 (17 Jul 13)
1	0	-	1.11	1.04	0.95
3	60	-	1.16	0.79	0.91
5	120	-	1.16	0.97	1.11
6	0	+	1.06	0.87	1.02
9	90	+	1.14	1.01	0.90
10	120	+	1.12	0.86	1.01

s.e.d – 0.117 with 46 d.f

Table 8. Effect of salinity treatments on Intercellular CO_2 / Ambient CO_2 (Ci_Ca) – Summer 2013 – Exp 3.

Treatment	Salt concentration (mM)	With or without CRF eggs	Ci_Ca at weeks after salinity application: 3 (4 Jul 13)	4 (11 Jul 13)	5 (17 Jul 13)
1	0	-	0.94	0.86	0.91
3	60	-	0.87	0.95	0.92
5	120	-	0.86	0.90	0.87
6	0	+	0.88	0.93	0.89
9	90	+	0.89	0.87	0.92
10	120	+	0.89	0.93	0.90

s.e.d – 0.036 with 46 d.f