Project title:	Inducing compact growth and improved shelf life in herbs by mimicking drought signals
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The results and conclusions in this report are based on an investigation conducted over a nine month 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

High salinity treatments were found to be effective in reducing petiole length in flat leaf parsley and coriander. The sodium chloride treatments did not significantly reduce leaf area.

Background

Low light levels induce growth of longer, weaker stems and petioles in plants including culinary herbs (etiolation). Such plants show reduced shelf life and may fail to meet buyer specifications and customer expectations.

Under drought or high salinity conditions, plants respond by reducing growth via systems involving alkalinisation of xylem sap, hormone signalling via abscisic acid (ABA) and ethylene and other physical and physiological changes such as stomatal closure (Wilkinson and Davies, 2010).

Previous work on a wide range of species has indicated that supplying high pH artificial xylem sap or application of high pH solutions to the substrate can lead to reduced growth rates (Bacon *et al.,* 1998; Fung and Wong, 2001; Kaya *et al.,* 2002; Shi and Sheng, 2005; Aronsson *et al.,* 2009; Afshari *et al.,* 2011; Kang *et al.,* 2011; Kering and Kaps, 2011; Kettlewell *et al.,* 2012; Zhao *et al.,* 2013). This reduction in growth rate can lead to reduced plant height (Afshari *et al.,* 2011; Zhao *et al.,* 2013). High salinity has also been reported to reduce plant growth in a number of species (Shi and Sheng, 2005; Pitann *et al.,* 2009; Afshari *et al.,* 2011; Wang *et al.,* 2011; Babu *et al.,* 2012; Habibi and Amiri, 2013; Hajiaghaei-Kamrani *et al.,* 2013; Huckle and Mulholland, 2013; Hussain *et al.,* 2013). For example, when chervil seedlings were grown *in vitro* in sodium chloride solutions of up to 240 mM, the height of seedlings, along with hypocotyl and root length, was reduced by over 80% compared to control plants (Liopa-Tsakalidi and Barouchas, 2011).

Plant responses to drought and salinity appear to use an overlapping set of signals. For example, in broad bean (*Vicia faba*), apoplastic pH increased in response to treatment with potassium chloride or sodium chloride (Felle and Hanstein, 2002). In addition, both high salinity and drought conditions can result in an increase in ABA concentration in some species (Hartung *et al.,* 1988; Cramer, 1994; He and Cramer, 1996; Montero et al., 1998; Stoll *et al.,* 2000; Bahrun *et al.,* 2002; Christmann *et al.,* 2005; Babu *et al.,* 2012). Sunflower seedlings show reduced growth and leaf area at both higher salinity and pH (Shi and Sheng, 2005). When both stresses were combined together, the effect was much larger. This background led to this project where the effect of high pH and high salinity treatments on herb growth was investigated.

Summary

High pH and high salinity treatments were applied to four species of culinary herbs in an attempt to induce natural drought and salinity responses whilst still maintaining water provision.

The species investigated were: flat leaf parsley (*Petroselinum crispum* var. *neapolitanum*), coriander (*Coriandrum sativum*), basil (*Ocimum basilicum*) and mint (*Mentha sp.*). Two preliminary experiments (Experiments 1 and 2) were carried out to optimise the experimental design. These two experiments were performed with a minimum daytime temperature of 15°C and minimum night temperature of 5°C. The treatments in Experiment 1 were: foliar sprays of potassium bicarbonate ($KHCO_3$) from pH8 to 12, a 3 second soil drench of $KHCO_3$ at pH12 and both a foliar spray and 3 second soil drench of 0.24M sodium chloride (NaCl) with the water spray control being treatment 2. No significant effects on stem or petiole elongation or overall biomass differences were observed in these preliminary experiments. From the results, the experimental design was modified and was performed at minimum day and night temperatures of 18°C, with weekly treatments for up to four weeks before harvest.

In Experiment 3, $KHCO_3$ foliar sprays at pH8 and pH12, a $KHCO_3$ 3second soil drench at pH12 and a 0.24M NaCl 3 second soil drench were applied weekly. Again there was a water spray control. The optimisation of the experiment design appeared to be successful as several statistically significant results were observed in this experiment. The greatest effects were found with the 0.24M NaCl salt drench treatment. This treatment led to an average reduction in parsley 1st petiole length of 16% after 4 weeks and 11% for coriander after 2 weeks versus the water spray control. Parsley plants treated with the foliar spray of $KHCO_3$ at pH12 also showed reduced length of 1st petioles by 14% versus the water spray control after 4 weeks. The 0.24M NaCl drench, $KHCO_3$ pH12 spray and drench also led to a significant reduction in overall plant biomass for parsley only (an average reduction of 32%, 21% and 18% in wet weight, respectively, after 4 weeks versus the water spray control). Interestingly, the NaCl drench also showed an increase in the per cent dry weight of mint plants versus the water spray control by 15% after 4 weeks. The $KHCO_3$ treatments did not significantly affect coriander elongation or biomass in this experiment.

After Experiment 3 it was decided to proceed with flat leaf parsley and coriander as these species had shown the largest responses to the treatments and more reliable and consistent growth than mint and basil.

Experiment 4 investigated the possibility of combining the high pH and high salinity treatments for an additive effect on plant elongation. The treatments used were a 0.24M NaCl foliar spray, $KHCO_3$ pH12 foliar spray either once or twice weekly, 0.24M NaCl soil drench either once or twice weekly and a combined 0.24M NaCl soil drench and $KHCO_3$ pH12 foliar spray. A water spray and water soil drench were used as controls. Treatments were applied for four weeks with additional lighting being provided.

For coriander, the NaCl drench once and twice weekly reduced petiole 2 length by 15% and 19% respectively (versus water drench once per week) and reduced petiole 3 length by 21% and 28%, respectively (versus water drench once per week). No significant effects on coriander elongation were noted when $KHCO_3$ sprays were applied alone. For parsley, NaCl drench twice weekly reduced petiole 2 and 3 length by 13% and 22%, respectively (versus water drench once per week). For coriander, the twice weekly NaCl drench led to 31% and 42% reductions in wet and dry weight, respectively (versus once weekly water drench). No significant effects on overall biomass were noted for parsley. The use of additional lighting may have reduced the effectiveness of the treatments in this experiment as two treatments per week were needed to obtain a significant reduction in petiole elongation. Also, some mild leaf tip scorching of coriander plants was noted, particularly for those treated twice weekly with NaCl, which could also result from the increased lighting and any resultant temperature increase. No additive effect from combining the high salinity and high pH treatments was detected as the high pH treatments appear largely ineffective in this experiment.

As the high salinity treatments appeared to be the most effective, Experiment 5 used variation in treatment frequency, duration and NaCl concentration to further investigate the effects of salt application on parsley and coriander. Soil drench treatments of either 0.24M or 0.12M NaCl supplied once or twice weekly for 5 second or 10 second durations were used. For parsley, no significant effects on petiole elongation were found. However, for coriander, the 0.24M NaCl 5s once weekly treatment significantly reduced the length of petiole 1 by 9%, the 0.12M NaCl 5s twice weekly treatment reduced the lengths of petioles 3 and 4 by 11% and 12%, respectively, while the 0.12M NaCl 10s twice weekly treatment significantly reduced the lengths of petioles 1 to 4 by 9%, 12%, 17% and 19%, respectively (in each case versus the corresponding water control).

For both parsley and coriander plants we could detect no significant difference in leaf area or colour of the third leaf in this experiment. However, leaf scorching and yellowing was noted on some coriander plants in this experiment, particularly when using the 0.12M NaCl 10s twice weekly treatment. The high light levels and day temperatures in this experiment, which was carried out in March and April could explain the reduced effectiveness of the treatments and increased severity of phytotoxicity, Interestingly, after a three day period of drying, the soil moisture content of salt-treated plants was significantly higher than for water control plants, suggesting a reduced water uptake rate by these plants, possibly involving reduced transpiration owing to stomatal closure in response to salt application. This effect should be considered when determining the relative water requirements of salt-treated and control plants.

Experiment 6 was designed to investigate the deterioration of salt- and water-treated plants during cold storage. The plants were treated as in experiment 5 and so some leaf damage (leaf scorching, yellowing) was noted for some salt-treated plants prior to storage. Plants were scored for leaf damage before and after 14 days storage at 4°C and the change in score calculated. In addition, plants were given a general appearance score based on colour and firmness after storage.

For parsley, significant differences were detected between the treatments in the ‘before’ and ‘general’ scores, with salt-treated plants appearing to have higher scores, indicating lower condition plants. For coriander, all four scores (‘before’, ‘after’, ‘change’ and ‘general’) showed significant differences between the treatments. Salt-treated plants appeared to have higher ‘before’, ‘after’ and ‘general’ scores than water-treated plants, indicating a worse plant condition. The ‘change’ score, indicating the difference in the condition of the plant from before to after storage, however, appeared to be lower for the salt-treated plants, suggesting a smaller change in condition during storage than for water-treated plants. These results may indicate that salt-treated plants deteriorate less quickly during storage than water-treated plants or that for salt-treated plants most deterioration had occurred prior to storage. It should be noted that the condition of coriander plants in this experiment was worse than noted in previous experiments, perhaps due to ambient light levels.

In the final experiment, Experiment 7, parsley plants were grown hydroponically in pots containing perlite partially submerged in an aerated commercially available nutrient solution. Treatments used were a once weekly foliar spray of $KHCO_3$ at pH 12, a once weekly exchange of the nutrient solution for 0.24M NaCl for 4 hours and a combination of both treatments. The NaCl treatment once again gave the largest reduction in elongation, decreasing the length of petioles 2 and 3 by 10% and 19%, respectively and reducing overall biomass by 39% versus the nutrient solution control. Again, the $KHCO_3$ pH 12 spray did not significantly affect elongation or biomass. Leaf area, colour and electrolyte leakage were unaffected by the treatments. Petiole firmness was also unaffected.

In summary, soil drenches of 0.24M NaCl 5s once weekly, 0.12M NaCl 5s twice weekly and 0.12M NaCl 10s twice weekly significantly reduced petiole elongation, particularly of coriander plants by 10-20% compared to control plants. No significant effects were found on leaf area or colour. The overall biomass of plants was reduced at times by the NaCl treatments but less consistently than elongation. Despite previously published data, we found no additive effects by combining high pH and high salinity treatments, likely due to the relative ineffectiveness of the high pH treatments. Further, in a hydroponics experiment, parsley leaf electrolyte leakage was unaffected by NaCl treatment, suggesting leaf quality was unchanged by the treatments. The firmness of petioles was similarly unaffected. However, given the reduction of petiole length, petioles could ultimately resist bending more than in untreated plants. NaCl treatment may also reduce plant quality deterioration during storage.

The effectiveness and phytotoxicity of the treatments appeared to depend on environmental conditions and treatment regime. Phytotoxicity was very low or absent in winter months but increased into spring. For example, increased phytotoxicity and reduced treatment efficacy was noted for Experiment 5 carried out in March-April compared to Experiment 3 performed in November-December. Given that the provision of artificial lighting in Experiment 4 also appeared to reduce treatment effectiveness, it is likely that ambient light levels and temperature are responsible for the differences seen. In addition, phytotoxicity also increased with higher frequency, higher duration NaCl treatments, particularly for the 0.12M NaCl 10s twice weekly treatment.

Financial Benefits

Provision of electrical lighting for large scale commercial glasshouses is very expensive. Using saline solutions to combat etiolation appears to represent a promising low cost alternative.

Action Points

- Despite the promise shown with these methods, there is insufficient evidence of benefits without the risk of phytotoxicity to make practical recommendations at this stage.

SCIENCE SECTION

Introduction

Under low light levels, such as occur during the winter months, the stem extension growth of plants is accelerated, producing taller, thinner stems of reduced strength leading to weaker plants with a lower leaf density (etiolation). For commercial fresh produce species such as herbs, such plants may not meet buyer specifications and may show reduced shelf life. Therefore, growers must reduce the stem extension growth rate of herbs under low light conditions. Provision of artificial lighting to large expanses of glasshouse is expensive. As such, low-cost methods to regulate plant growth at this time of year must be sought.

An alternative approach may be to exploit the drought stress response of plants in order to reduce plant elongation. Under drought conditions, a water deficit can occur as water is lost more quickly through the leaves and stems than it is taken up by the roots. To help cope with this deficit, plants reduce the growth rates of leaves and stems and increase or alter the growth of roots (Bacon *et al.,* 1998; Bahrun *et al.,* 2002; Wilkinson and Davies, 2010).

During drought conditions, the pH of plant xylem sap has been shown to increase in a number of species (Wilkinson and Davies, 1997; Bacon *et al.,* 1998; Wilkinson *et al.,* 1998; Stoll *et al.,* 2000; Bahrun *et al.,* 2002; Sobeih *et al.,* 2004; Jia and Davies, 2007; Aronsson, 2009) and this change can spread through plant tissues (Jia and Davies, 2007).

This suggests that it may be possible to reduce plant growth via the use of alkaline solutions intended to artificially increase the internal pH of the plant. Indeed, both the feeding of artificial xylem sap of increasing pH to detached seedlings or shoots and the application of alkaline solutions to the substrate of a wide range of plant species has been shown to provide a reduction in growth rate (Bacon *et al.,* 1998; Fung and Wong, 2001; Kaya *et al.,* 2002; Shi and Sheng, 2005; Aronsson *et al.,* 2009; Afshari *et al.,* 2011; Kang *et al.,* 2011; Kering and Kaps, 2011; Kettlewell *et al.,* 2012; Zhao *et al.,* 2013) and lead to reduced height of plants (Afshari *et al.,* 2011; Zhao *et al.,* 2013).

The application of foliar sprays may provide a convenient means to apply growth-retarding pH treatments to plants (Wilkinson and Davies, 2008; Kettlewell *et al.,* 2012). Sprays containing sodium bicarbonate have exhibited potential as a growth regulator for oil seed rape (*Brassica napus;* Aronsson *et al.,* 2009). Any pH treatment applied to herbs will be required to meet food safety standards. Food grade potassium bicarbonate has already been approved as a horticultural fungicide under the Health and Safety Executive Commodity Substance approval route (Health and Safety Executive, 2005). As such, its approval for use as a growth regulator could potentially be more rapidly obtained compared to other substances.

A number of studies have investigated the effect of bicarbonate application on the growth of plants and reductions in growth rate have been noted for a range of species (James *et al.,* 2002; Zribi and Gharsalli, 2002; Yang *et al.,* 2003; Bie *et al.,* 2004; Barhoumi *et al.,* 2007; Zhang and Mu 2009; Li *et al.,* 2010; Gao *et al.,* 2012;).

Salinity has also been reported to affect the growth of plants and could represent another means to control growth in low light conditions. Plant responses to salt stress include the regulation of growth. Slowing growth provides more cellular resources to combat the effects of the stress imposed and cell division and expansion could be affected directly (Zhu, 2001). *In vitro* growth of chervil seedlings in NaCl solutions of up to 240 mM showed reductions in seedling height, hypocotyl and root length by over 80% compared to water controls (Liopa-Tsakalidi and Barouchas, 2011).

Growth reduction in high salinity conditions has also been found for species including *Brassica* seedlings (Huckle and Mulholland, 2013), maize (*Zea mays*; Pitann *et al.,* 2009) sunflower (*Helianthus anuum*; Shi and Sheng, 2005) and tomato (Afshari *et al.,* 2011; Babu *et al.,* 2012; Hajiaghaei-Kamrani *et al.,* 2013; Wang *et al.,* 2011). In addition, an *in vitro* study of wheat seedlings showed that increased concentration of NaCl solution reduced seed germination and seedling growth (Hussain *et al.,* 2013), while *in vitro* salt concentrations up to 200 mM led to a reduced growth rate, leaf chlorophyll content, total protein, rate of photosynthesis, stomatal conductance and mineral uptake in citrus root stocks (Habibi and Amiri, 2013). These effects could be utilised by careful treatment of herbs with mild, sub-phytotoxic, salt stress to provide shorter plants.

Project Aims

The aim of the pilot study is to explore the potential for mimicking the natural drought signals, which move from root to shoot, to give more –compact growth and improved shelf life in a arrange of herb species without the detrimental effects of water stress.

Project objectives

1. To screen four pot-grown herb species (coriander, flat parley, mint, basil) for response of leaf, petiole and stem internode growth to alkaline pH buffer application and to saline application.
2. For the most-responsive herb species, to explore responses of leaf, petiole and stem internode growth to varying pH and treatment frequency and saline concentration and treatment frequency.
3. For the most-responsive herb species, to evaluate effects on potential shelf life.

Materials and Methods

Plant Growth

Seedlings of flat leaf parsley (*Petroselinum crispum* var. *neapolitanum*), coriander (*Coriandrum sativum*), basil (*Ocimum basilicum*) and mint (*Mentha sp.*) were obtained from Lincolnshire Herbs (Bourne, UK) at around 5 days post germination in 7 cm square plastic pots filled with substrate. Plants were thinned to an equal number of plants per pot and grown in temperature-controlled glasshouse facilities at HAU at either minimum 15°C day time, 5°C night time temperature (experiments 0 and 1) or at a minimum day and night temperature of 18°C (experiment 2 onwards) and watered from below using a bench top irrigation system. No additional lighting was used, except for experiments 0 and 1 and 3, where supplemental lighting was supplied for 12h per day). For experiments 0 and 1, 5 plants were used per pot. From experiment 2 onwards, 6 plants were used per pot. In experiment 2, coriander plants were grown from seed due to lack of availability of seedlings. In all cases, 1 plant per pot was used for mint.

For hydroponics experiments, seeds of flat leaf parsley were obtained from Lincolnshire Herbs and grown in 7 cm square plastic pots filled with perlite. The holes in the pot bases were covered and new ones made at the bases of the pot sides. 12 pots were submerged in a 25 cm by 35 cm black plastic box containing water mixed with Ionic HydroGrow (Growth Technology, UK) hydroponic nutrient solution at a concentration according to the manufacturer's instructions. A small air pump (200 l/h Aquarium Internal Filter 200IF, All Pond Solutions, UK) was used for water aeration. Four boxes were used per treatment. Seeds were germinated in the dark then thinned to two plants per pot. The first treatment was applied 7 days after germination and weekly thereafter. Nutrient solution in all boxes was replaced with a fresh batch 28 days after the first treatment.

Experimental design

Designs were generated in Genstat (16th Edition, VSN International Ltd, UK). For Experiment 1, 5 pots were used per species per treatment. Pots were arranged in a blocked design as shown in table 1 (B=basil, C=coriander, M=mint, P=parsley).

Table 1. Pot arrangement for Experiment 1.

Block 1				2				3				4				5			
B7	M4	P7	C9	M5	B4	B8	M6	B5	P2	P5	M9	C5	M3	C1	C7	P9	M6	C5	B9
C3	P2	B3	P8	P7	C7	C9	M4	C7	M8	M4	B2	B7	B4	M2	P1	P4	B2	C7	P1
P4	B9	C5	M1	C4	C5	M1	P2	P6	C1	B6	C8	B1	C2	P4	B6	M7	M5	B1	M2
C2	B8	M6	M7	M3	P1	B6	B5	M2	C5	B3	P1	C4	P5	M7	P3	C2	P7	B4	P5
M9	C6	P9	C4	B2	P6	P4	C1	M1	B9	C9	M5	M8	C9	B5	M5	P6	M1	P3	B7
M8	C7	M2	B2	C6	B9	C3	B7	P9	B4	M7	P3	M9	P7	B3	M4	B5	C4	P2	C8
B5	P3	C8	B6	P8	M8	M7	M9	M3	C3	C2	P4	C3	B8	P8	C8	C1	B3	M9	M4
P6	C1	B4	P5	B1	M2	P5	C8	B8	P7	B1	C6	P6	M1	C6	B2	M3	C9	B6	C3
B1	M5	P1	M3	P9	B3	C2	P3	C4	M6	P8	B7	P2	M6	B9	P9	B8	P8	M8	C6

For Experiment 2, 10 pots were used per species per treatment in a blocked design shown in table 2 (each species was arranged separately).

Table 2. Pot arrangement for Experiment 2.

Block	1	2	3	4	5
	3	**5**	**2**	**1**	**2**
	2	**4**	**3**	**3**	**5**
	6	**6**	**4**	**2**	**1**
	5	**2**	**1**	**6**	**3**
	4	**3**	**5**	**4**	**4**
	1	**1**	**6**	**5**	**6**
	6	**6**	**3**	**1**	**6**
	5	**1**	**5**	**5**	**5**
	4	**5**	**6**	**3**	**2**
	1	**4**	**1**	**4**	**1**
	3	**2**	**2**	**6**	**3**
	2	**3**	**4**	**2**	**4**

For Experiment 3, 10 pots were used for each species per time point per treatment. The pots were arranged in a blocked split plot design as shown in table 3 (each species was arranged separately).

Table 3. Pot arrangement for Experiment 3.

Block	**Treatments**									
1	4	4	2	2	1	1	3	3	5	5
2	1	1	3	3	5	5	2	2	4	4
3	5	5	4	4	2	2	3	3	1	1
4	3	3	2	2	5	5	1	1	4	4
5	2	2	5	5	4	4	1	1	3	3
6	3	3	4	4	5	5	2	2	1	1
7	3	3	1	1	4	4	2	2	5	5
8	5	5	1	1	3	3	2	2	4	4
9	1	1	3	3	4	4	5	5	2	2
10	4	4	2	2	5	5	1	1	3	3

For Experiments 4-7, a Latin Square design was used with the number of replicates indicated below. Each species was arranged separately.

Table 4. 8x8 Latin Square design for pot arrangement for Experiment 4. Plants were placed on two trays (shown in grey).

1				2			
5	**1**	**8**	**3**	**6**	**4**	**7**	**2**
1	**5**	**4**	**7**	**2**	**8**	**3**	**6**
3	**7**	**2**	**5**	**4**	**6**	**1**	**8**
6	**2**	**7**	**4**	**5**	**3**	**8**	**1**
4	**8**	**1**	**6**	**3**	**5**	**2**	**7**
2	**6**	**3**	**8**	**1**	**7**	**4**	**5**
7	**3**	**6**	**1**	**8**	**2**	**5**	**4**
8	**4**	**5**	**2**	**7**	**1**	**6**	**3**

Table 5. 6x6 Latin Square design for pot arrangement for Experiments 5 and 6. Pots were again placed on two trays.

1			2		
2	**5**	**3**	**6**	**1**	**4**
4	**1**	**5**	**2**	**3**	**6**
1	**4**	**2**	**5**	**6**	**3**
6	**3**	**1**	**4**	**5**	**2**
3	**6**	**4**	**1**	**2**	**5**
5	**2**	**6**	**3**	**4**	**1**

Table 6. 4x4 Latin Square design for box arrangement for the hydroponics experiment (Experiment 7). Each box contained 12 pots of parsley.

4	**1**	**2**	**3**
2	**3**	**4**	**1**
1	**4**	**3**	**2**
3	**2**	**1**	**4**

Treatments

Treatments used for each experiment are shown in Table 7.

Table 7a-c. Treatments used in the experiments (Expt = experiment, Trt No. = treatment number, KOH = potassium hydroxide).

a. Treatments for Experiments 1 to 4.

Expt	Trt No.	Treatment	Frequency
1	**1**	Water foliar spray (control)	Once
	2	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 8	Once
	3	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 9	Once
	4	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 10	Once
	5	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 11	Once
	6	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 12	Once
	7	Foliar spray of 0.24M NaCl	Once
	8	Soil drench of 0.02M KHCO3 adjusted using KOH to pH 12	Once
	9	Soil drench of 0.24M NaCl	Once
2	**1**	Water foliar spray (control)	Once per week
	2	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 8	Once per week
	3	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 10	Once per week
	4	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 12	Once per week
	5	Soil drench of 0.02M KHCO3 adjusted using KOH to pH 12	Once per week
	6	Soil drench of 0.24M NaCl	Once per week
3	**1**	Water foliar spray (control)	Once per week
	2	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 8	Once per week
	3	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 12	Once per week
	4	Soil drench of 0.02M KHCO3 adjusted using KOH to pH 12	Once per week
	5	Soil drench of 0.24M NaCl	Once per week
4	**1**	Water foliar spray (control)	Once per week
	2	Water control soil drench	Once per week
	3	Soil drench of 0.24M NaCl	Once per week
	4	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 12	Once per week
	5	Soil drench of 0.24M NaCl plus foliar spray 0.02M KHCO3, pH12	Once per week
	6	Foliar spray 0.24M NaCl	Once per week
	7	Soil drench of 0.24M NaCl	Twice per week
	8	Foliar spray 0.02M KHCO3, adjusted using KOH to pH 12	Twice per week

b. Treatments for Experiments 5 and 6.

Expt.	Trt No.	Treatment	Conc.	Duration	Frequency
5/6	**1**	Water soil drench		5s	Once weekly
	2	NaCl soil drench	0.24M	5s	Once weekly
	3	Water soil drench		5s	Twice weekly
	4	NaCl soil drench	0.12M	5s	Twice weekly
	5	Water soil drench		10s	Twice weekly
	6	NaCl soil drench	0.12M	10s	Twice weekly

c. Treatments for Experiment 7 – hydroponics.

Expt.	Trt No.	Treatment	Frequency
7 -Hydroponics	**1**	Nutrient solution control	Once weekly
	2	Nutrient solution plus 0.24M NaCl	Once weekly
	3	$KHCO_3$ pH12 spray	Once weekly
	4	0.24M NaCl + $KHCO_3$ pH12 spray	Once weekly

For Experiment 1, plants were treated once at 12 days post germination. For subsequent experiments, treatments were applied for four weeks (two weeks in Experiment 2 for parsley, coriander and mint). Foliar sprays were applied using the HAU pot sprayer at a density of 200 l/Ha (two passes were made over the pots). Soil drenches were applied by dipping pots into a tray containing 1 l of the treatment solution for 3 s (5 s for Experiment 4, as indicated for Experiments 5 and 6). For the hydroponics experiment, nutrient solution was removed and stored and replaced with salt solution for four hours, after which the pots and box were rinsed in clean water and replaced together with the original nutrient solution.

Assessment of Treatments

Measurements of stem, petiole or internode length were made using digital callipers (Draper Expert 46610 0-150 mm Dual-Reading Digital Vernier Calliper, Draper Tools, UK). For overall biomass measurements of wet and dry weight, the plants in each pot were cut at soil level and weighed together before and after drying for at least 3 days at 80°C. In Experiment 1, measurements of length were made at 2, 6 and 9 days after treatment. For Experiment 2, measurements were made at 2, 6 and 9 days after the first treatment (for coriander and parsley, continuing to 13, 17 and 20 days for basil), and at 3, 7 and 10 days after the first treatment (for mint). In Experiment 3, assessment was made 2 and 4 weeks after the first treatment (2 weeks only for coriander). Wet weight was recorded after 4 weeks. For subsequent experiments, measurements were made beginning one week after the final (4^{th}) treatment.

Leaf colour was determined using a Minolta CR-300 Colorimeter (Minolta, Japan) using the L*, a*, b* colourspaces, as according to Koukounaras *et al.* (2009). The hue angle (H°) was calculated as $180+\tan^{-1}(b^*/a^*)$ and Chroma (C*) as $(a^{*2} + b^{*2})^{1/2}$. For the hydroponics experiment the second leaf was used for colour determination. Leaf area was recorded using a LI-COR LI-3000A portable area meter (LI-COR, Nebraska, USA). For Experiment 5, the third leaf was used for colour and area determination.

Electrolyte leakage was determined according to the method of Wagstaff *et al.* (2007). For the hydroponics experiment, 0.75g of third leaves was collected per box, placed in a 50 ml tube and 40 ml deionised water added. The samples were incubated for 3 hrs at 18°C. Electrical conductivity was determined using a Jenway 4510 Conductivity Meter, after which the samples were frozen at -20°C for 3 days and the measurement repeated. Electrical conductivity prior to freezing was calculated as a percentage of that after freezing to indicate per cent of maximal electrolyte leakage.

Petiole strength was assessed using a Stable Micro Systems TA HD plus Texture Analyser with an AACC Cake-Pan1_P36R program (determination of firmness of panetone and sponge cake using the AACC (74-09) standard method (originally developed for bread firmness)). A 25 mm Perspex cylinder and 5 kg load cell were used. For the hydroponics plants, 12 5^{th} petioles per treatment were cut to 80 mm long and centred on the cutting surface of the analyser for firmness determination.

For analysis of leaf area and petiole strength in the hydroponics experiment, 3 pots from each replicate were grouped into a single box per treatment and analysed 2 weeks after the final treatment date.

Soil moisture content was determined for Experiment 5 using an ML2 ThetaProbe Soil Moisture Sensor (Delta-T Devices, UK) calibrated for organic substrate. Plants were treated and watered as normal then left to dry for 3 days to determine residual soil moisture.

For assessment of storage performance, plants were not thinned out and were treated as per Experiment 5. Pots were placed closely packed in a cold room at 4°C under low light conditions for 14 days, one week after the 4^{th} and final treatment. Plants used for cold storage were scored for yellowing and leaf scorch before and after storage using the scale below (Table 8). A general score of appearance was also made after storage according to Table 9.

Table 8. Scoring system for analyse of plant storage performance.

Score	
1	Good, green appearance
2	Hint of yellowing
3	Slight yellowing or scorching
4	Slight yellowing and scorching or medium yellowing
5	Moderate yellowing or scorching
6	Yellowing and scorching, one bad one mild
7	Moderate yellowing and scorching

Table 9. General scoring system of plant appearance after storage.

Score	
1	Very good colour, no wilting
2	Slight loss of colour
3	Moderate wilting, colour loss
4	Severely wilted

Data were analysed using a generalised ANOVA in Genstat (16^{th} Edition, VSN International Ltd, UK). A Latin Square Design was specified as required. Data were checked for normality using residuals plots. Tukey's multiple comparison test was used to determine significant differences between treatment means (p=0.05). The column number in the pot arrangement was added as a covariate for Experiment 3 and petiole diameter added as a covariate in analysis of petiole strength. The storage performance scores data was analysed using Friedman's Nonparametric ANOVA. Figures are shown indicating the mean value for each treatment with error bars of +/- one standard error of the mean.

Results

Experiment 1 - Preliminary

Plant elongation

For coriander and basil no significant difference was noted when the change in stem or petiole/internode length from 2 to 9 days after treatment was compared, despite some differences in length between treatments at individual time points, suggesting perhaps an initial shorter length in the control plants (Figures 1 and 2). No significant effects on the elongation of parsley and mint plants were noted in this experiment (data not shown).

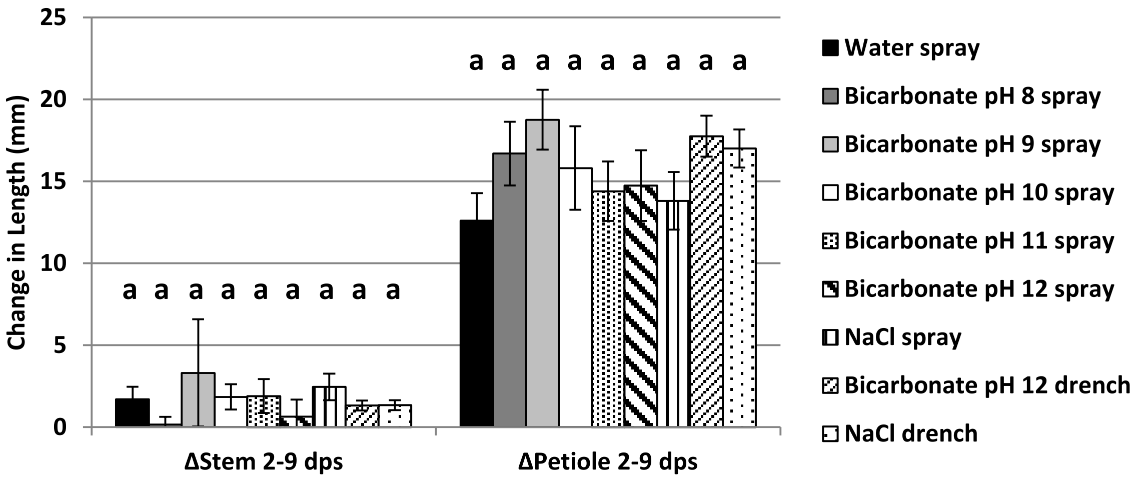

Figure 1. Effect of high pH and salinity treatments on coriander stem and 1st petiole length in Experiment 1. dps = days after first treatment. Error bars indicate +/- one standard error of the mean.

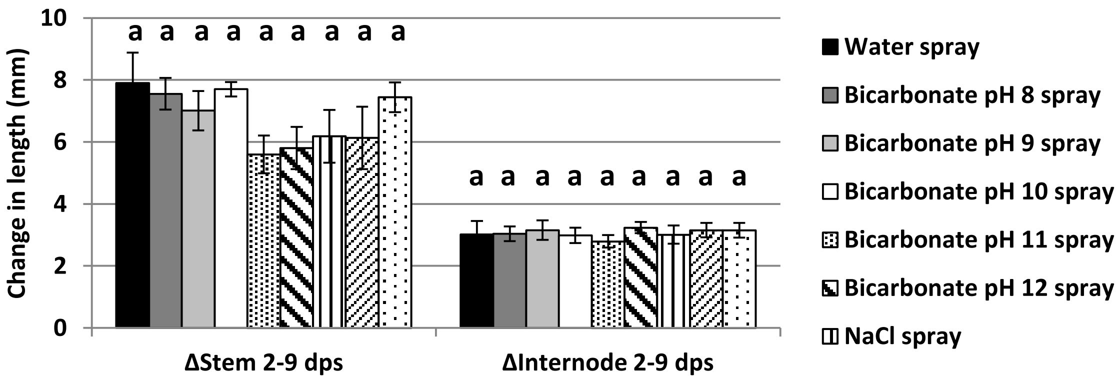

Figure 2. Effect of high pH and salinity treatments on basil stem and 1st internode length in Experiment 1. dps = days after first treatment. Error bars indicate +/- one standard error of the mean.

Experiment 2 - Preliminary

Plant elongation

As in the previous experiment, for parsley, mint and basil, no significant difference was noted when the change in stem or petiole/internode length from 2 to 9 days (or 2-20 days for basil) after the first treatment was compared, despite some differences in length between treatments at individual time points, suggesting perhaps an initial shorter length in the control plants (Figures 3 to 5). No significant effects on the elongation of coriander plants were noted in this experiment (data not shown).

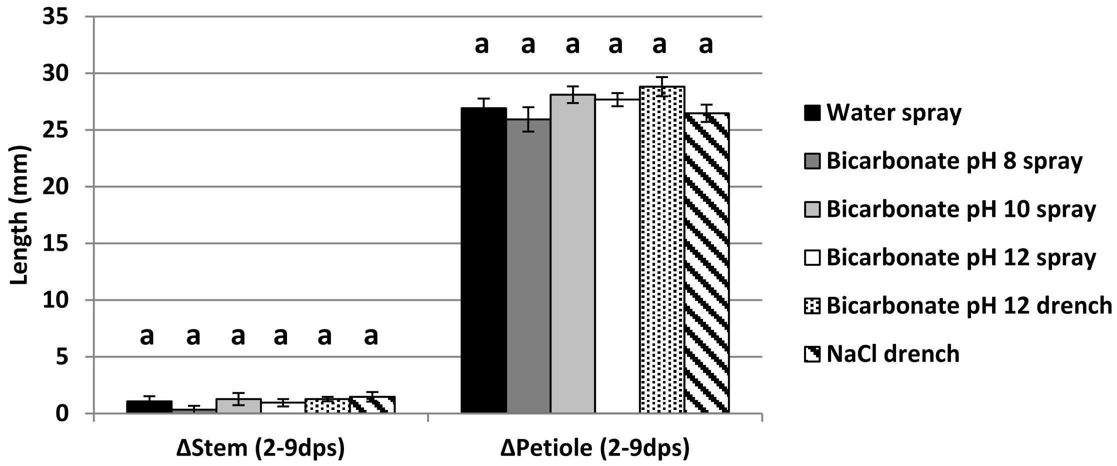

Figure 3. Effect of high pH and salinity treatments on flat leaf parsley stem and 1st petiole length in Experiment 2. dps = days after first treatment. Error bars indicate +/- one standard error of the mean.

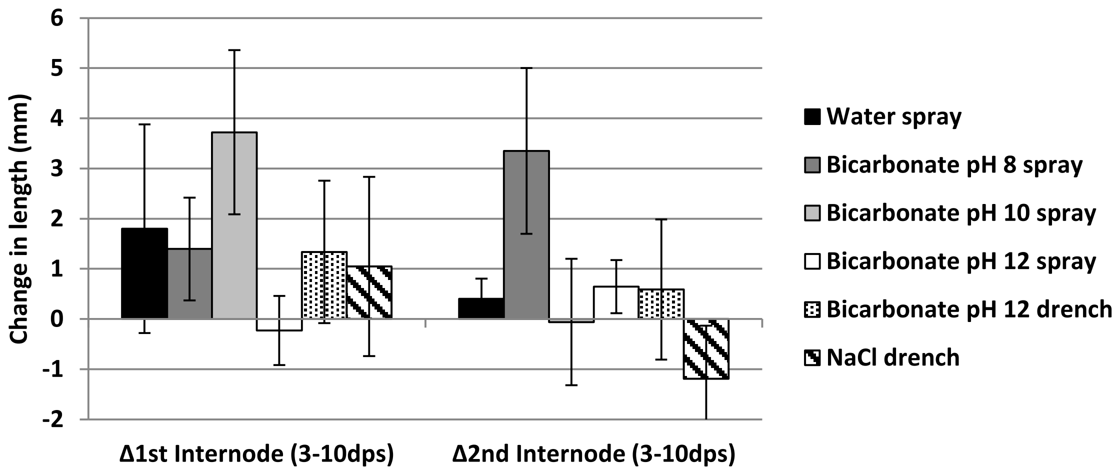

Figure 4. Effect of high pH and salinity treatments on mint 1st and 2nd internode length in Experiment 2. dps = days after first treatment. Error bars indicate +/- one standard error of the mean.

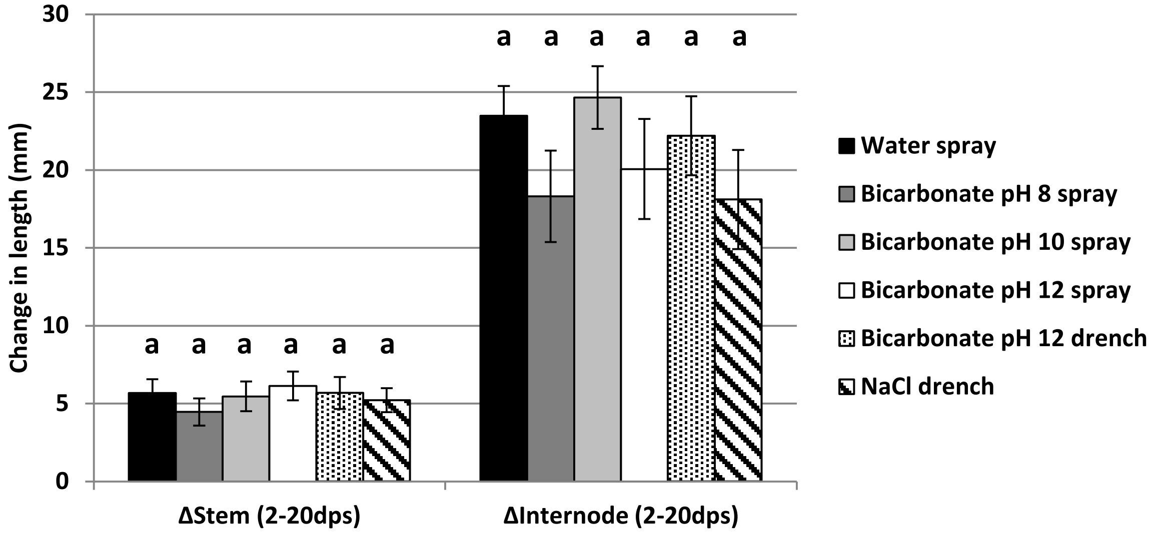

Figure 5. Effect of high pH and salinity treatments on basil stem and 1st internode length in Experiment 2. dps = days after first treatment. Error bars indicate +/- one standard error of the mean.

Overall biomass

No significant effects of the treatments on total wet or dry biomass or % dry weight were found in this experiment (data not shown).

After the two preliminary experiments to determine the correct experimental setup, the following alterations to the experimental design were implemented: Each species was arranged separately to eliminate the problem of taller species shading the smaller ones. 6 rather than 5 plants were used per pot to allow for occasional seedling death and seedlings of similar size selected as far as possible. 10 pots per treatment rather than 5 were used to obtain more robust data. Treatments were made weekly to increase the chance of effects being seen. A minimum day and night temperature of 18°C was used to more closely match commercial growth conditions and a lack of supplementary lighting used to enhance elongation rates in order to observe larger treatment effects. Plants would also only be measured once, with different plants being used for each time point as handling during the measuring process caused the plants to fall over and so could affect their growth.

Experiment 3 – Optimised Design

Plant Elongation

A number of statistically significant effects of treatments were recorded in this experiment. The largest effects were noted for the NaCl salt drench treatment. This treatment led to an average reduction in 1st petiole length of 16% for parsley after 4 weeks and 11% for coriander after 2 weeks versus the water spray control (Figures 6 and 7). In addition, the pH12 foliar spray of $KHCO_3$ led to an average reduction in the length of parsley 1st petioles by 14% versus the water spray control after 4 weeks (Figure 6). Interestingly the $KHCO_3$ pH 12 foliar spray also caused a slight but significant increase in stem length for this species after 4 weeks.

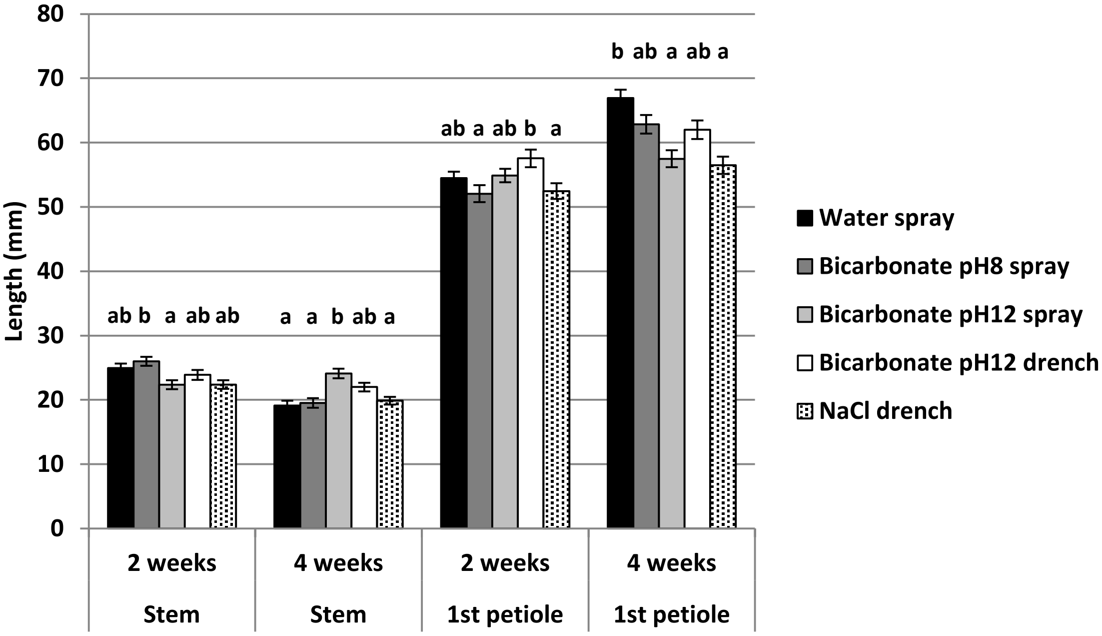

Figure 6. Effect of high pH and salinity treatments on flat leaf parsley stem and 1st petiole length after 2 and 4 weeks in Experiment 3. Error bars indicate +/- one standard error of the mean.

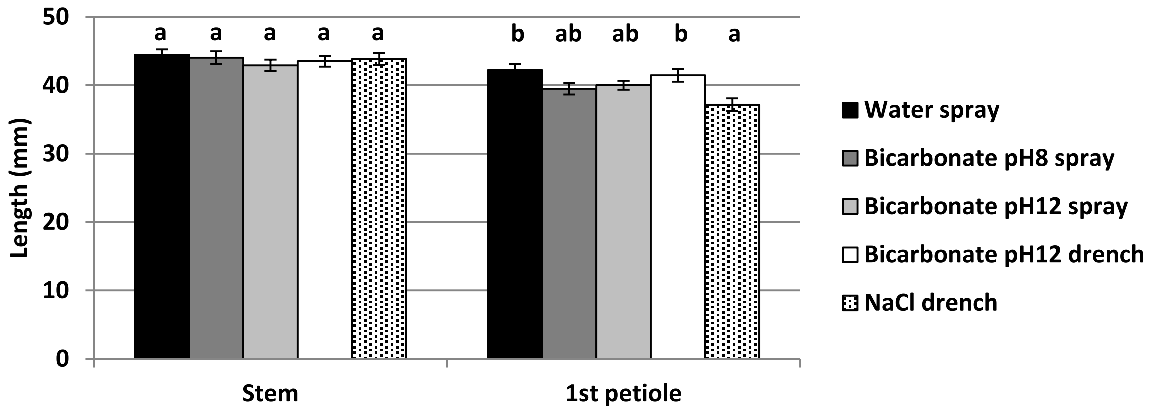

Figure 7. Effect of high pH and salinity treatments on coriander stem and 1st petiole length after 2 weeks in Experiment 3. Error bars indicate +/- one standard error of the mean.

Overall biomass

For overall plant biomass, the NaCl drench appeared to cause the largest alterations, but significant effects were only noted for parsley. For parsley, this treatment led to an average reduction of 32% in wet weight and 27% in dry weight after 4 weeks versus the water spray (Figure 8). Interestingly, the NaCl drench also led to an increase in the per cent dry weight of mint plants versus the water spray control by 15% after 4 weeks (Figure 9). This effect was not seen with parsley (data not shown). The bicarbonate pH12 spray and drench also significantly reduced the wet weight of parsley plants (by 21% and 18%, respectively), while the pH12 drench also reduced the dry weight by 25%. The bicarbonate treatments did not significantly affect coriander biomass in this experiment.

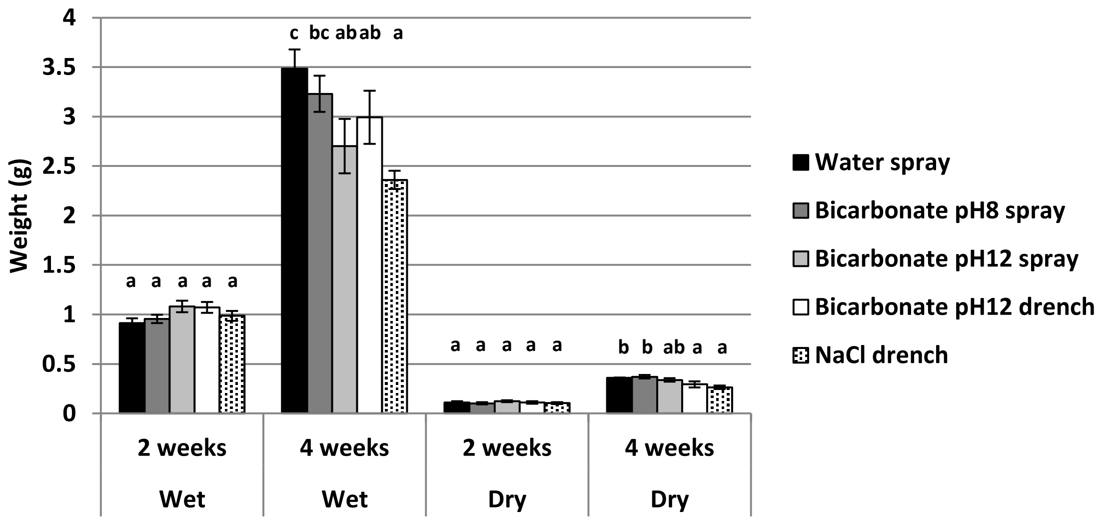

Figure 8. Effect of high pH and salinity treatments on flat leaf parsley biomass in Experiment 3. Error bars indicate +/- one standard error of the mean.

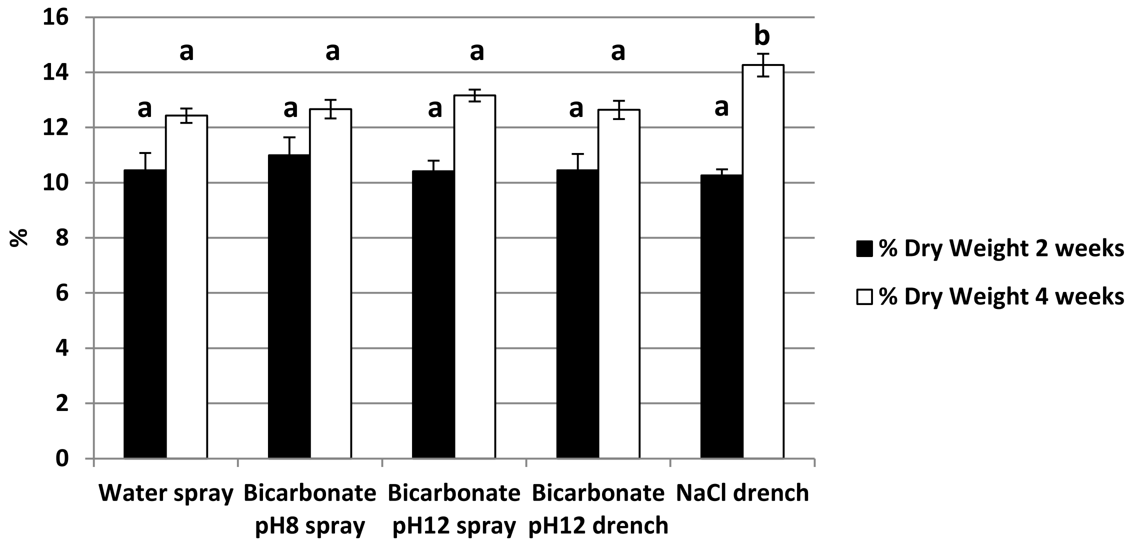

Figure 9. Effect of high pH and salinity treatments on mint per cent dry weight in Experiment 3. Error bars indicate +/- one standard error of the mean.

Unfortunately, basil results were hard to interpret for this experiment due to the failure of a number of pots, possibly due to disease, while coriander dry weights were too small to analyse accurately.

The discrepancies between the experiments may result from the differing conditions and protocols used as the method was optimised and conditions were changed to more closely mimic those in a commercial facility. For this reason we consider the results of Experiment 3 to possibly be the most representative of treatment effects under commercial conditions.

From the results of Experiment 3, it was decided to proceed with parsley and coriander as the species of choice for further evaluation due to a number of reasons. Firstly, the magnitude of the effects of the treatments was higher for parsley than for the other species. Secondly, the effect of treatments on elongation of parsley was greatest for a high pH spray and high salinity drench, which suggests the possibility of different modes of action of the two types of treatment. Additionally, coriander is a much higher volume species for commercial growers than parsley, and frequently suffers from weak stems, so this species was also included for further evaluation. Finally, parsley and coriander showed good reliability/consistency of growth during the experiment.

The salt drench and bicarbonate pH12 treatments appeared to be the most effective in Experiment 3 and so were used as the basis for the treatment design for Experiment 4, which investigated the effect of altering treatment frequency and combining treatments on the herbs.

Experiment 4- Looking for a Combinatorial Effect of High pH and High Salinity

Plant elongation

This experiment investigated a new group of treatments applied once weekly for four weeks investigating both salt stress and the effect of combining salt and high pH treatments. The salt drench treatments were again the most effective. Measurements of stem and petiole 1 (oldest) to 3 (younger) length were made for each plant.

For coriander, petiole 2 length was reduced by 15% and 19% by NaCl drench once and twice weekly respectively (versus water drench once per week). Petiole 3 length was reduced by 21% and 28% by NaCl drench once and twice weekly respectively (versus water drench once per week) (Figure 10). No significant effects on elongation were noted for bicarbonate sprays alone.

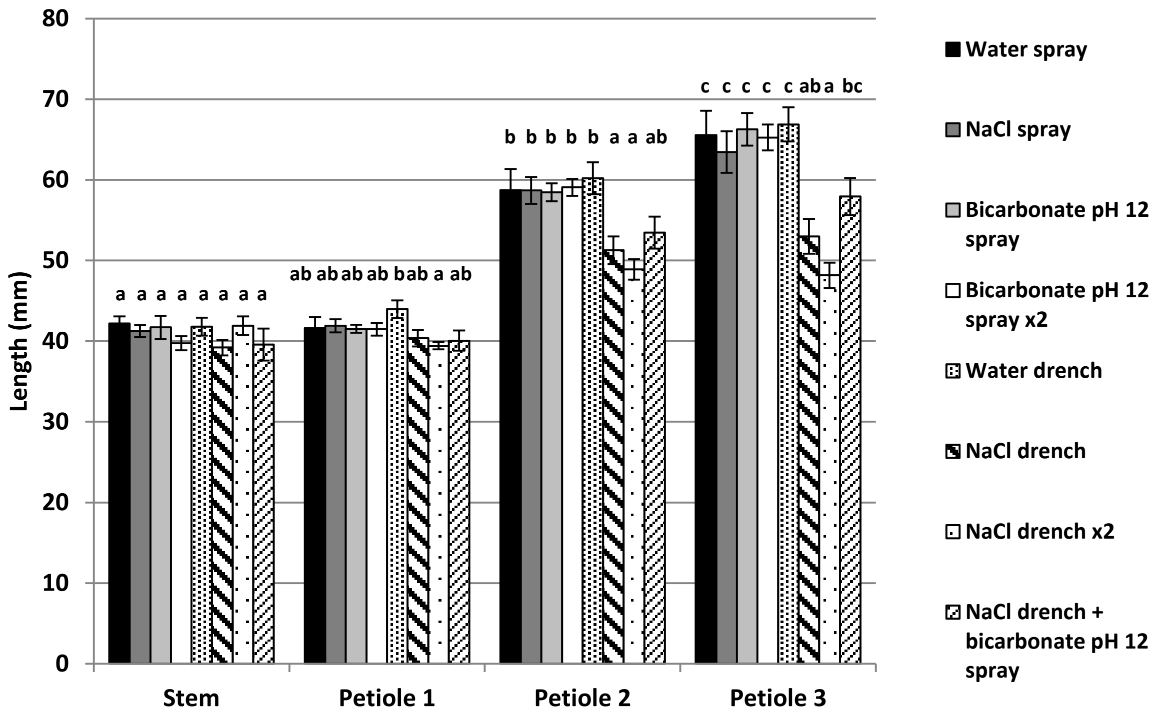

Figure 10. Effect of salt and high pH treatments on elongation of coriander plants in Experiment 4. Error bars indicate +/- one standard error of the mean.

For parsley, petiole 2 length was reduced by 13% by NaCl drench twice weekly (versus water drench once per week). Petiole 3 length, meanwhile, was reduced by 22% by NaCl drench twice weekly (versus water drench once per week) (Figure 11).

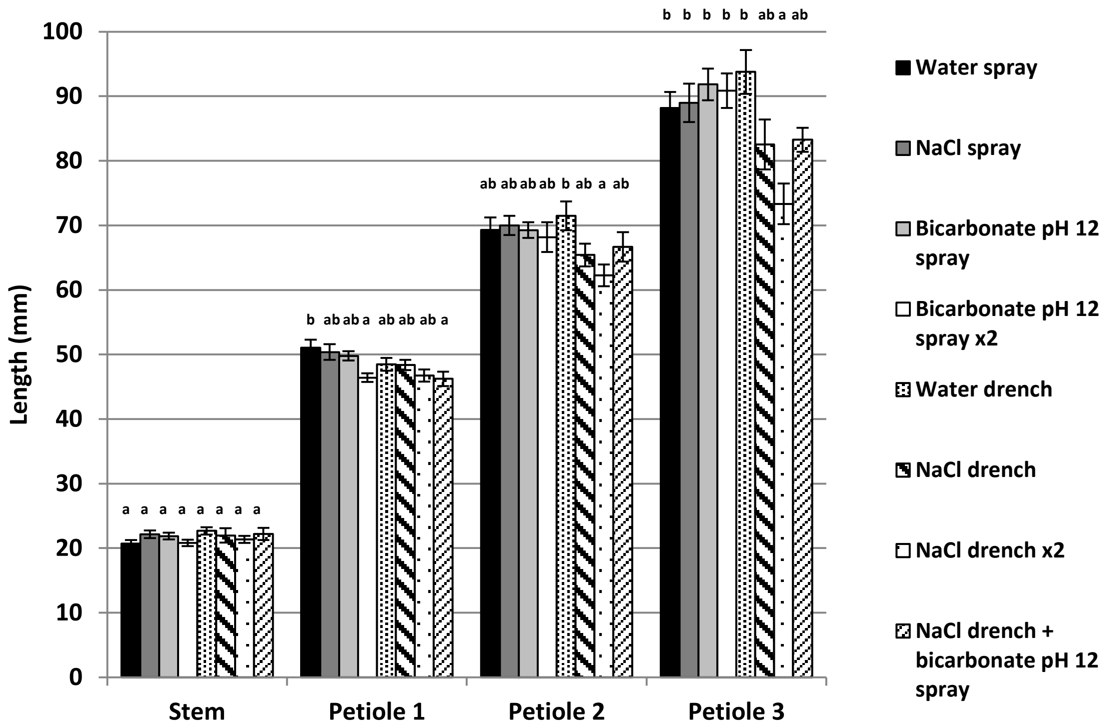

Figure 11. Effect of salt and high pH treatments on elongation of parsley plants in Experiment 4. Error bars indicate +/- one standard error of the mean.

Overall biomass

Effects on biomass were less noticeable in this experiment, possibly due to water application times. No significant effects were noted for parsley (Figure 12), while for coriander, the twice weekly NaCl drench led to a 31% and 42% reduction in wet and dry weight respectively (versus once weekly water drench) (Figure 13).

It should be noted that the plants were much larger than at this stage in experiment 2. This could be due to the provision of additional lighting. They also required more frequent watering and showed more rapid substrate drying. A twice weekly water drench control was added for Experiment 5 as twice weekly-treated salt drench pots were damper than those of other treatments. Some scorching of salt drench coriander plants was noted, particularly for those treated twice weekly.

From Experiment 4 it can be seen that the salt drench is still effective in reducing elongation, in this case for petiole 2 and 3. Parsley needed two applications per week for Tukey's test to detect an effect but only one application per week was required for coriander. The bicarbonate spray, however, was only effective for reducing elongation of petiole 1 of parsley. No additive effect of combining NaCl drench with bicarbonate spray was noted.

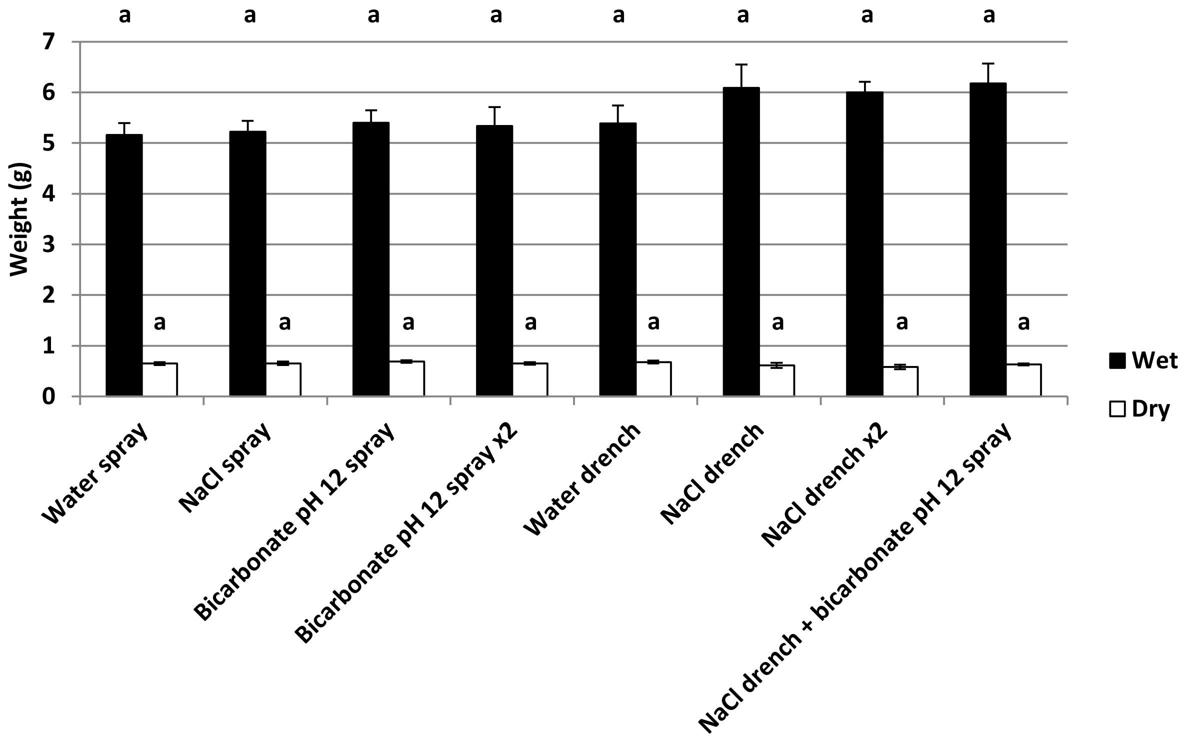

Figure 12. Effect of salt and high pH treatments on biomass of parsley plants in Experiment 4. Error bars indicate +/- one standard error of the mean.

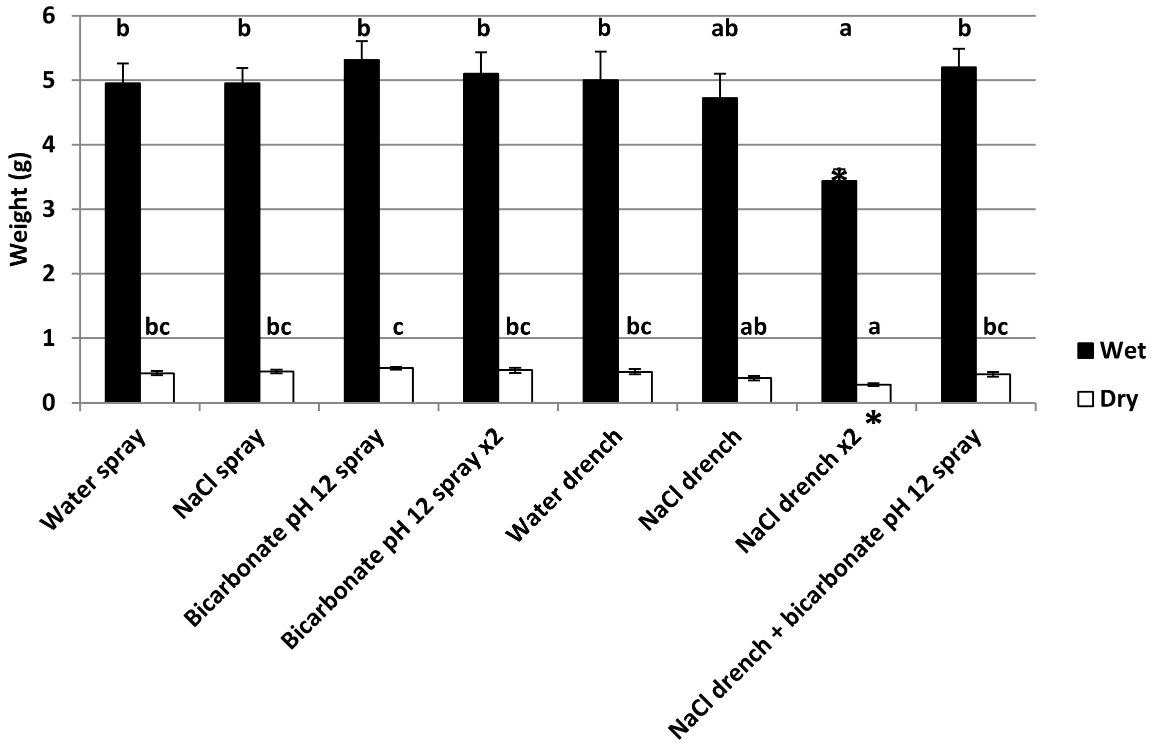

Figure 13. Effect of salt and high pH treatments on biomass of coriander plants in Experiment 4. Error bars indicate +/- one standard error of the mean.

Experiment 5 –Variation in High Salinity Treatments

It was decided to concentrate efforts on the high salinity treatments as these appeared most promising from the previous experiments. Three different salt treatments were used with varying NaCl concentration, treatment duration and treatment frequency each with a corresponding water control treatment (see materials and methods and below). Treatments were applied for four weeks.

Elongation

In this experiment, interestingly, no significant effects on parsley petiole elongation were detected by Tukey's test (Figure 14).

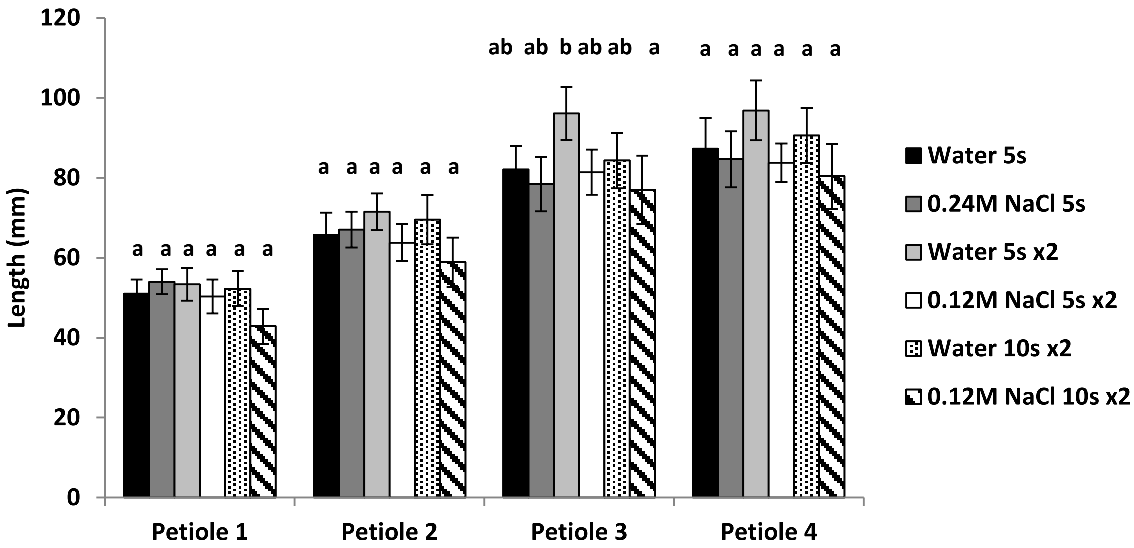

Figure 14. Effect of high salinity treatments on elongation of parsley plants in Experiment 5. Error bars indicate +/- one standard error of the mean.

For coriander, however, the 0.24M NaCl 5s once weekly treatment significantly reduced the length of petiole 1 by 9%, the 0.12M NaCl 5s twice weekly treatment reduced the lengths of petioles 3 and 4 by 11% and 12%, respectively, while the 0.12M NaCl 10s twice weekly treatment significantly reduced the lengths of petioles 1 to 4 by 9%, 12%, 17% and 19%, respectively (in each case versus the corresponding water control) (Figure 15).

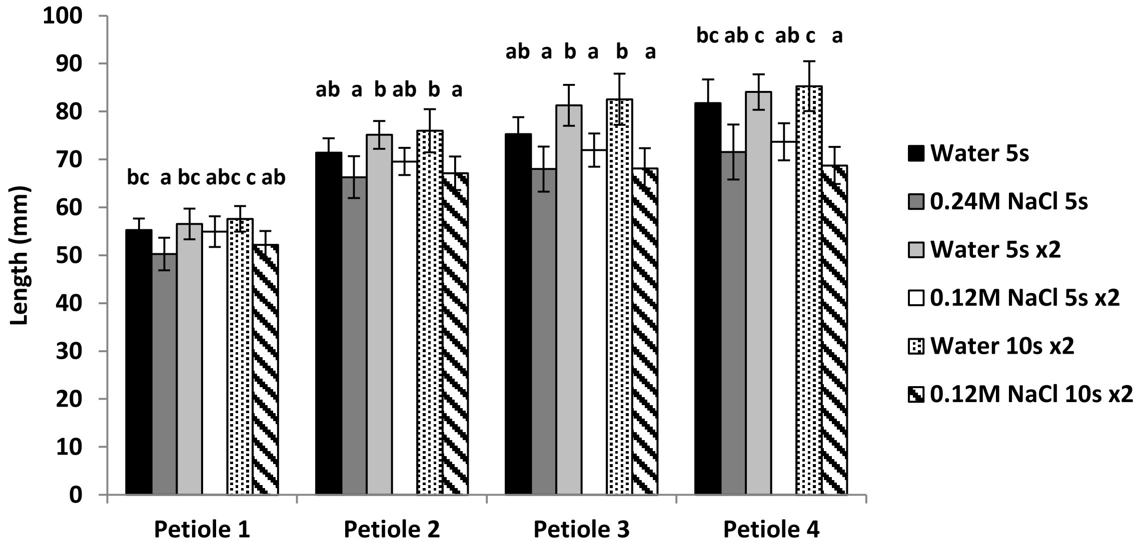

Figure 15. Effect of high salinity treatments on elongation of coriander plants in Experiment 5. Error bars indicate +/- one standard error of the mean.

Leaf Area

We also wished to investigate the effect of the treatments on growth of other parts of the plant, to determine whether stem/petiole elongation alone was affected. We compared the area of the third leaf of the plants. For both parsley and coriander, no significant effects were detected (Figure 16).

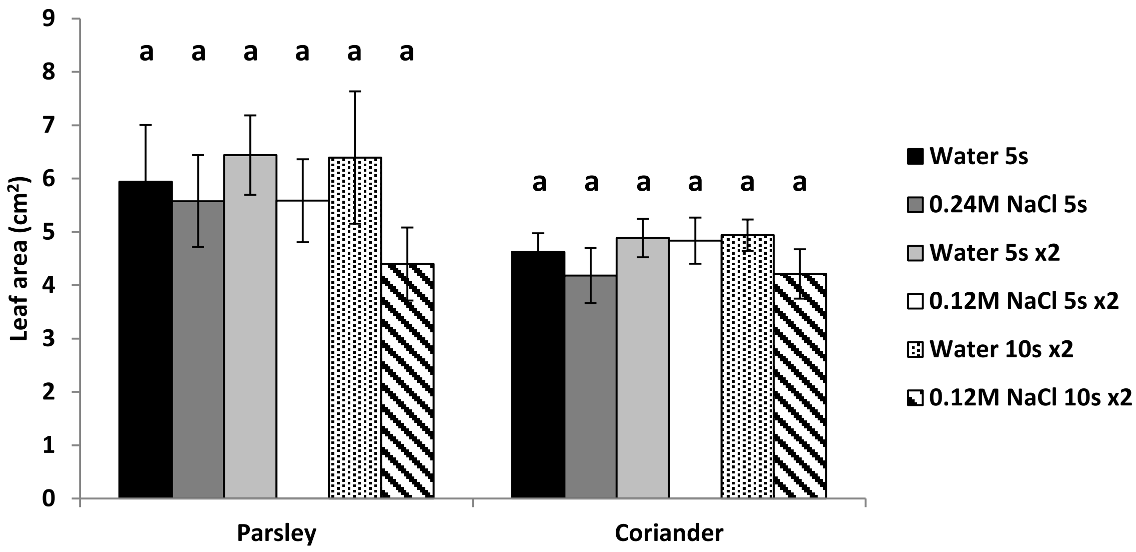

Figure 16. Effect of high salinity treatments on leaf area of parsley and coriander plants in Experiment 5. Error bars indicate +/- one standard error of the mean.

Leaf Colour

As a measure of the effect of treatment on plant appearance, leaf colour measurements were also recorded in this experiment (Table 10). No significant effects on colour of the third leaf were found for the parsley and coriander plants in this experiment. However, unlike the majority of previous experiments, there was evidence of some foliar damage (scorching, yellowing) due to salinity treatment on the coriander plants, particularly when using the 0.12M NaCl 10s twice weekly treatment. This could be due to the increased light intensity present at the time of year of this experiment compared to earlier experiments. The increased growth rate or rate of soil drying for the plants could have led to these symptoms which were only very mild or absent entirely in previous experiments.

Table 10. Effect of high salinity treatments on leaf colour of parsley and coriander plants in Experiment 5. Asterisks indicate an overall significant difference between salt and water treated plants ($p<0.05$).

	Parsley			**Coriander**		
	Lightness (L)	**Hue Angle (H°)**	**Chroma (C)**	**Lightness (L)**	**Hue Angle (H°)**	**Chroma (C)**
Water 5s	**47.466**	**178.927**	**38.998**	**50.693**	**178.916**	**37.290**
0.24M NaCl 5s	**48.884**	**178.937**	**37.729**	**53.143**	**178.911**	**36.761**
Water 5s x2	**49.072**	**178.936**	**38.784**	**52.680**	**178.902**	**40.358**
0.12M NaCl 5s x2	**49.167**	**178.935**	**37.554**	**53.634**	**178.895**	**36.704**
Water 10s x2	**48.608**	**178.948**	**36.836**	**52.997**	**178.874**	**40.778**
0.12M NaCl 10s x2	**49.024**	**178.946**	**36.829**	**53.468**	**178.889**	**38.283**

Unfortunately, due to a temperature spike in the glasshouse facilities resulting from hot weather overcoming the environmental control unit, the plants were damaged by hot conditions and so petiole strength and electrolyte leakage could not be recorded for these plants. However, this data was able to be collected for the hydroponics experiment (see below).

Substrate Moisture Content

Salt stress can induce stomatal closure in plants (Zhu, 2001), which would reduce transpiration and so the rate of water uptake also. In this case, the rate of water uptake from the substrate in salt-treated plants would be expected to be slower than that for control plants and could be reflected by a higher substrate moisture content. As part of Experiment 5, plants were left for a drying period of 3 days after having been treated and watered as normal to investigate this effect. As shown in Figure 17, salt-treated plants showed a significantly higher substrate moisture content than their corresponding water-treated controls. This may suggest that the rate of water uptake in these plants has been reduced after stomatal closure in response to the high salinity treatments. Any effect on plant turgor however, would be dependent on the relative transpiration and water uptake rates of each plant.

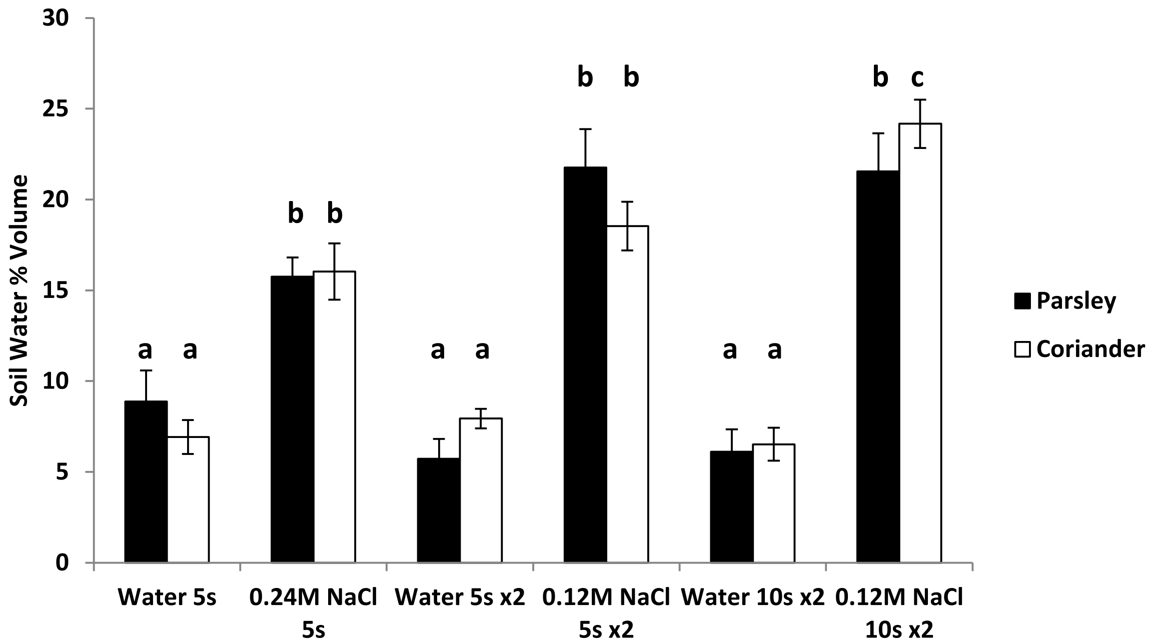

Figure 17. Effect of high salinity treatments on soil moisture content after a short period of drying for parsley and coriander plants in Experiment 5. Each species was analysed separately. Error bars indicate +/- one standard error of the mean.

Experiment 6 – Analysis of Storage Performance

This project also aimed to assess the impact of the treatments on the shelf life of herbs. Measurements of leaf colour can help indicate the impact of treatment on appearance, while petiole strength analysis can help give an indication of plant durability (see below). In addition, we also assessed the performance of plants placed in cold storage after a series of high salinity treatments, with an aim to reflect the conditions experienced by commercially-grown herbs prior to delivery to customers.

Plants were treated as for Experiment 5 except that the pots were not thinned out so as to maintain as close an appearance to the commercially grown pots as possible. Plants were scored for yellowing and scorching symptoms before and after a two week period of storage at 4°C and the difference in their scores before and after calculated. Plants were also given a general score of appearance after removal from cold storage (materials and methods and Figures 18-19). A higher score indicates more severe detrimental symptoms or a worse general appearance.

For parsley, there were significant differences in 'before' and 'general' scores between the treatments, with salt-treated plants appearing to have higher scores, indicating plants of a worse condition (Figure 18). For coriander, significant differences were detected between the treatments for all four scores (Figure 19). Salt-treated plants appeared to have higher 'before', 'after' and 'general' scores than water-treated plants, again indicating a worse plant condition. The 'change' score, however, appeared to be lower for the salt-treated plants, suggesting a smaller change in condition during storage than for water-treated plants.

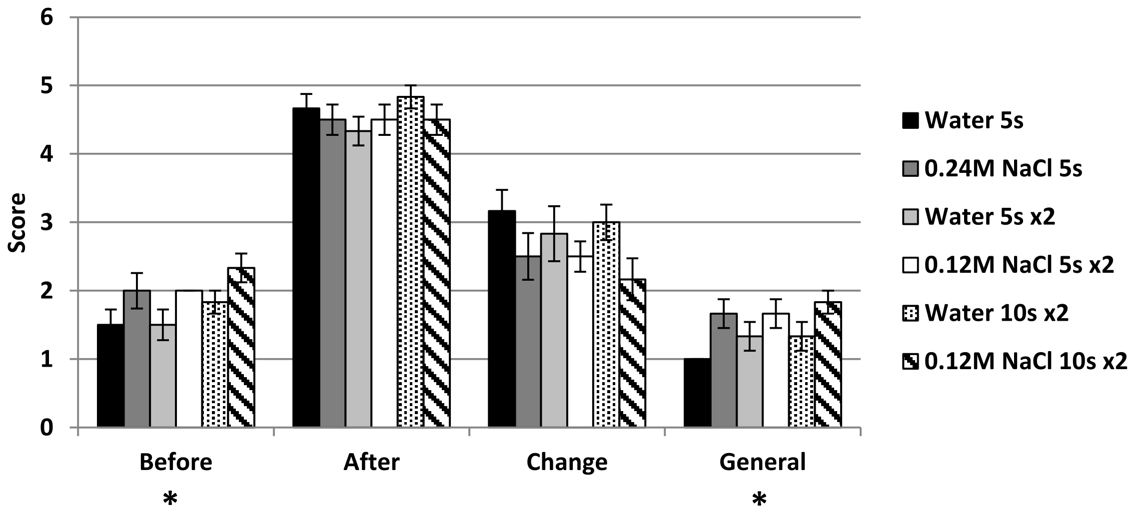

Figure 18. Effect of high salinity treatments on parsley cold storage performance in Experiment 6. Scores are indicated before and after storage and the change in score over

the storage period. A higher score indicates more severe yellowing/scorching. The general score is for overall appearance and colour with a lower score indicating a plant with better appearance. Error bars indicate +/- one standard error of the mean. Asterisks indicate a significant difference is detected between the treatments ($p<0.05$).

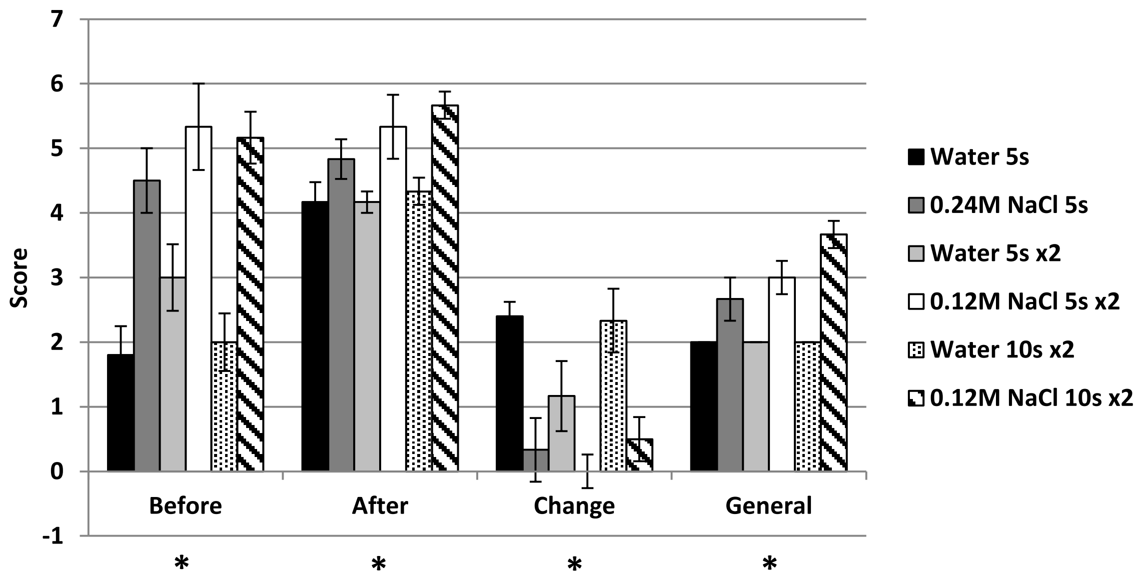

Figure 19. Effect of high salinity treatments on coriander cold storage performance in Experiment 6. Scores are indicated before and after storage and the change in score over the storage period. A higher score indicates more severe yellowing/scorching. The general score is for overall appearance and colour with a lower score indicating a plant with better appearance. Error bars indicate +/- one standard error of the mean. Asterisks indicate a significant difference is detected between the treatments ($p<0.05$).

Experiment 7 - Exploring a hydroponics approach

We also investigated the possibility of using the high pH and high salinity treatments in a hydroponics setup with flat leaf parsley. This would eliminate any buffering effects of the substrate, help ensure equal water provision for each plant and allow accurate determination of the period of exposure to high salinity treatments. High salinity treatments were administered via the nutrient solution bath, while high pH bicarbonate application remained as a spray application. Treatments were applied for four weeks.

Elongation

The high salinity treatment again gave the largest effect on plant elongation, reducing the length of petioles 2 and 3 by 10% and 19% respectively versus the nutrient solution control (Figure 20). Interestingly, stem length was slightly increased by high salinity treatment (13% versus water control) but the magnitude of this effect (in mm) was much smaller than for petioles. Bicarbonate pH 12 spray did not significantly affect elongation.

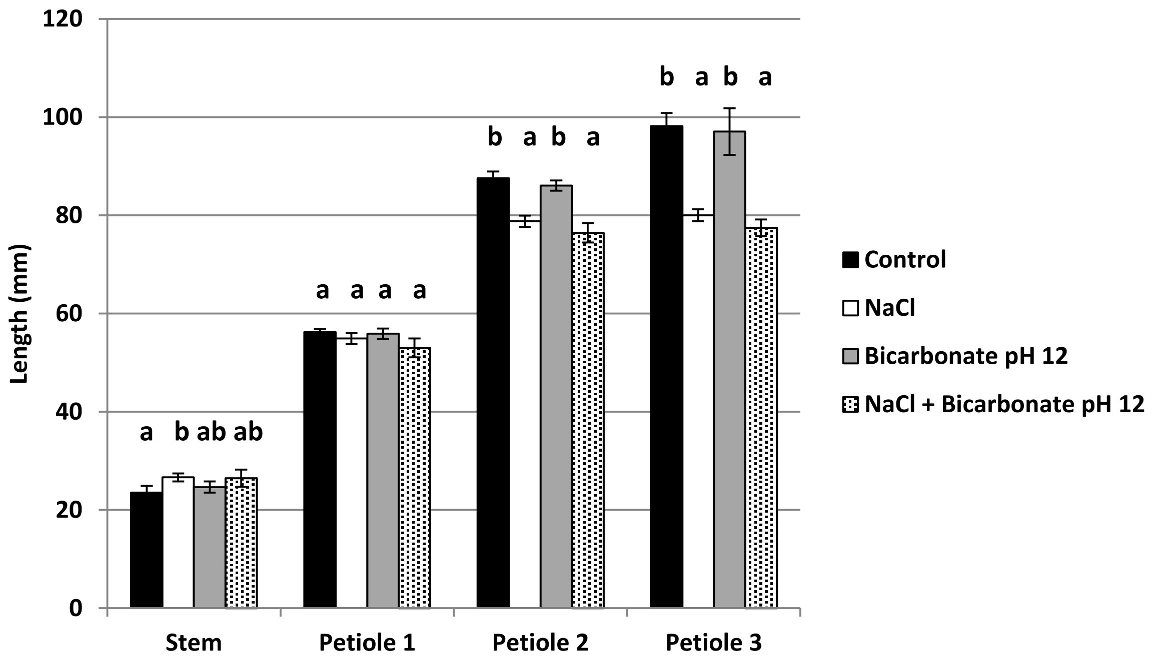

Figure 20. Effect of salt and high pH treatments on elongation of parsley plants in Experiment 7. Error bars indicate +/- one standard error of the mean.

Leaf Area

Leaf area was also examined for selected leaves of the hydroponics plants (Figure 21). No significant differences were found between the treatments.

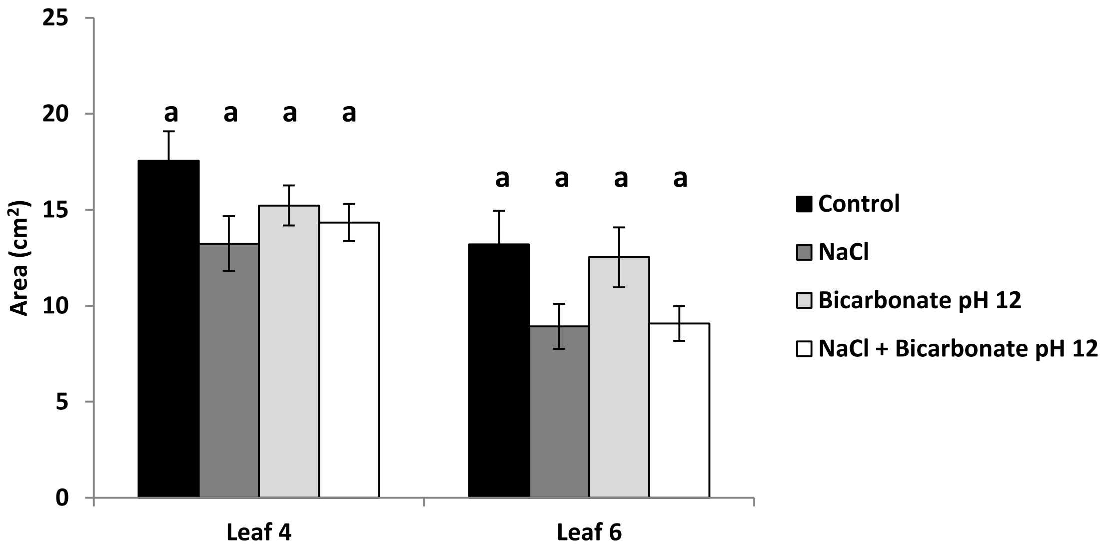

Figure 21. Effect of salt and high pH treatments on leaf area of parsley plants in Experiment 7. Error bars indicate +/- one standard error of the mean.

Overall biomass

High salinity treatments also showed a 39% decrease in both wet and dry biomass versus the nutrient solution control (Figure 22).

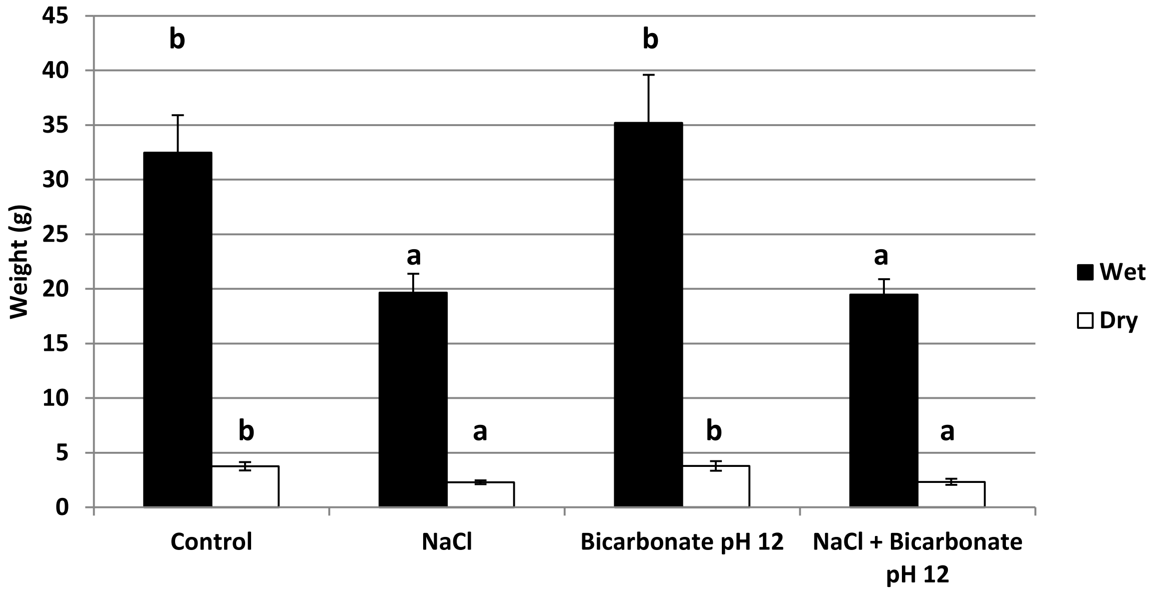

Figure 22. Effect of salt and high pH treatments on biomass of parsley plants in Experiment 7. Error bars indicate +/- one standard error of the mean.

Leaf Colour

None of the treatments had a significant effect on leaf lightness, hue angle or chroma (Table 11).

Table 11. Leaf colour in Experiment 7.

	Lightness (L)	**Hue Angle (H°)**	**Chroma (C)**
Control	**51.679**	**178.948**	**40.621**
NaCl	**53.460**	**178.938**	**41.463**
Bicarbonate pH 12	**51.163**	**178.952**	**39.446**
NaCl + Bicarbonate pH 12	**53.788**	**178.929**	**42.732**

Leaf Electrolyte Leakage

There was no significant effect of treatments on leaf electrolyte leakage versus the nutrient solution control (Figure 23).

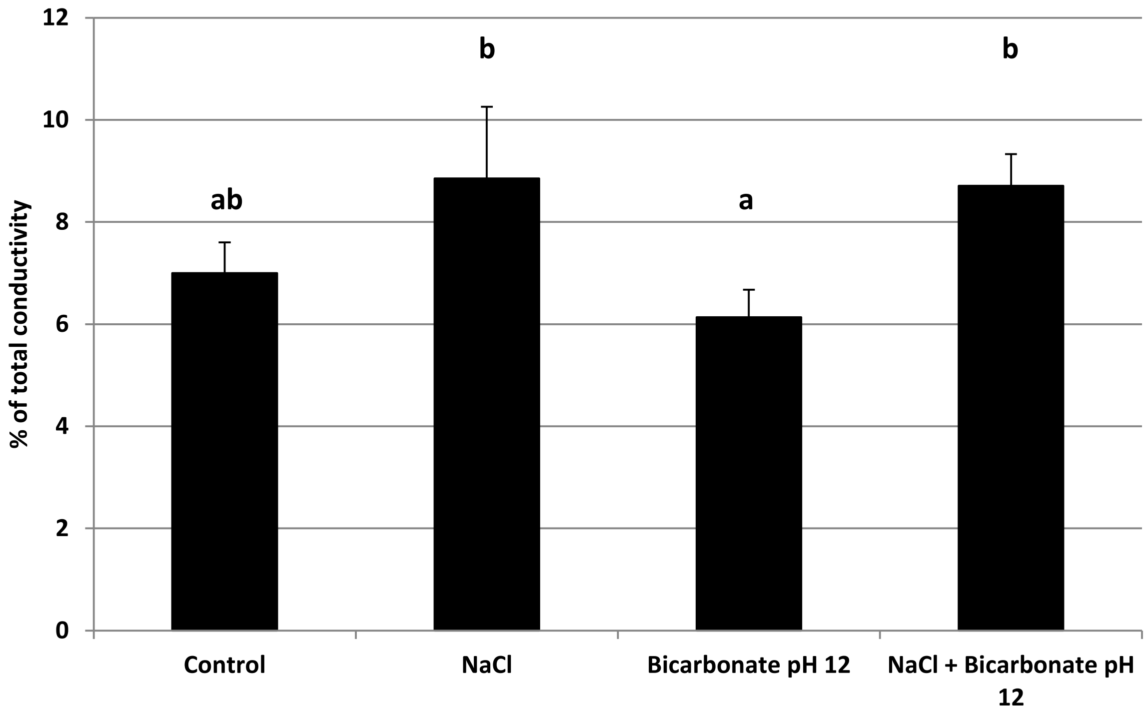

Figure 23. Effect of salt and high pH treatments on leaf electrolyte leakage of parsley plants in Experiment 7. Error bars indicate +/- one standard error of the mean.

Petiole Strength

A measure of petiole strength was also made by crushing selected petioles to determine their firmness (Figure 24). This indicated that there were no significant differences in petiole firmness between the treatments.

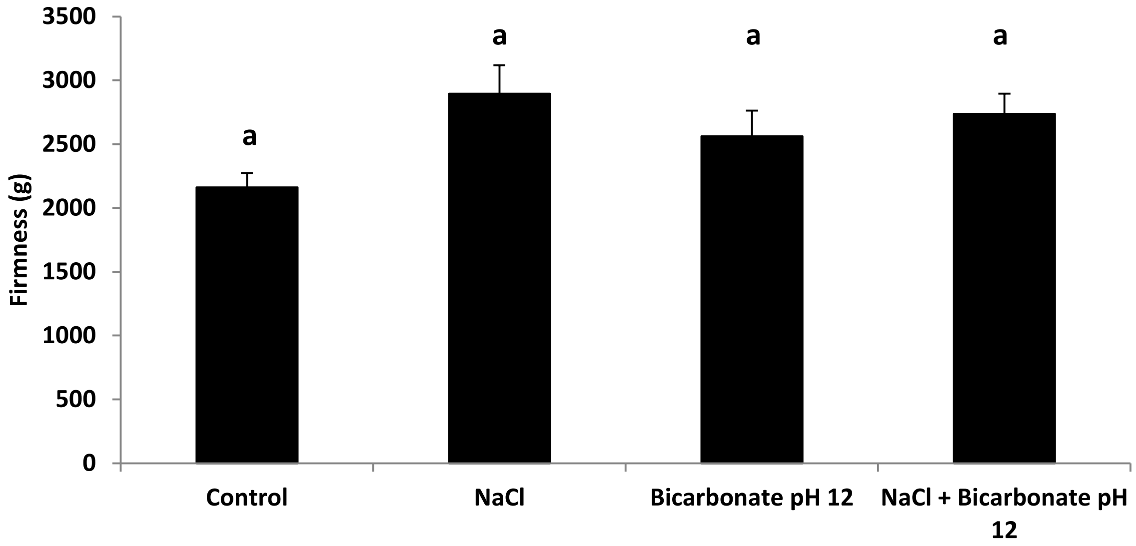

Figure 24. Effect of salt and high pH treatments on petiole firmness of parsley plants in Experiment 7. Error bars indicate +/- one standard error of the mean.

Discussion

We have investigated the effect of a number of high pH and high salinity treatments on the growth, appearance and shelf life of culinary herbs grown in glasshouse environments. Two preliminary experiments were used to optimise the experimental design and found that flat leaf parsley (*Petroselinum crispum* var. *neapolitanum*) and coriander (*Coriandrum sativum*) exhibited the greatest reductions in petiole length in response to treatments. These two species were used in subsequent experiments which investigated a wider range of high pH and salt treatments.

Potassium bicarbonate solutions were used to provide the high pH treatments in this study. Both artificial alkalinisation of xylem sap of detached seedlings and the application of alkaline solutions to a wide range of plant species has been shown to reduce growth rate (Bacon *et al.,* 1998; Fung and Wong, 2001; Kaya *et al.,* 2002; Shi and Sheng, 2005; Aronsson *et al.,* 2009; Afshari *et al.,* 2011; Kang *et al.,* 2011; Kering and Kaps, 2011; Kettlewell *et al.,* 2012; Zhao *et al.,* 2013) and lead to reduced height of plants (Afshari *et al.,* 2011; Zhao *et al.,* 2013). It has been suggested that bicarbonate may contribute to an increase in apoplastic pH (Mengel, 1994) and in maize (*Zea mays*), bicarbonate addition has been shown to increase xylem pH (Wegner and Zimmermann, 2004). However, we found that the effect of high pH treatments on petiole elongation of parsley and coriander was limited, reducing parsley 1st petiole length by 14% and wet and dry weight by 21% and 18% respectively in Experiment 3 but otherwise not significantly affecting plant growth. We do not know if the high pH treatments were able to alter pH of xylem sap in treated plants. This, together with differences between the species under study or the method of treatment could explain the limited response to high pH treatments in this study.

The results indicated that the length of petioles could be reduced most by using a soil drench application of NaCl. High salinity solutions were successful in reducing the petiole lengths of both parsley and coriander plants, frequently by around 10-20% compared to controls. In order to determine the effect on overall growth rate, we also recorded the change in plants biomass and leaf area. In some experiments, salt drenches also reduced the overall biomass of the plants by a greater degree than the change in petiole length, suggesting that additional parts of the plants could be reduced in size. An investigation of leaf size indicated that in Experiment 5, however, NaCl treatments significantly reduced coriander petiole elongation but not 3rd leaf area, suggesting a possible elongation-specific effect.

More frequent applications of the salt drench gave more drastic changes in elongation but more frequently led to phytotoxic symptoms such as leaf scorch and yellowing, especially for coriander plants which appeared to be more sensitive than parsley plants. Longer duration doses of 0.12M NaCl in Experiment 5 gave more drastic changes to plant parameters but also led to the most severe phytotoxicity. Spreading the NaCl dose out in Experiment 5 by using two 0.12M treatments compared to one of 0.24M only altered the effect on elongation for the occasional petiole.

The appearance of herbs is also an important consumer consideration and so we analysed the leaf colour of treated plants. In pot-grown herbs, the lightness, hue and chroma of the third leaf of both parsley and coriander appeared to be unaffected by the treatments, indicating that the elongation of plants may be successfully reduced without negatively impacting on leaf colour. However, some yellowing and scorching of coriander plants was also noted in this experiment.

An assessment of petiole strength of plants from Experiment 7 suggested that there is no change in petiole firmness after NaCl treatment, however, the shorter petioles of NaCl-treated plants may be able to resist bending better than the longer petioles of water-treated plants. In Experiment 7 (see below), NaCl treatment did not significantly increase parsley leaf electrolyte leakage compared to the water control, indicating that neither harder, unpalatable, nor weaker, more-damage-prone leaves are produced by this treatment.

To investigate the effect on shelf life of the herbs we scored plants for visible phytotoxic effects and general appearance before and after a period of cold storage designed to mimic that which may be encountered between packing and delivery to customers. The degree of yellowing and scorching of parsley plants was slightly increased by salt treatment prior to storage, however did not appear to be significantly different to water-treated plants treatments after storage. The general appearance of parsley plants colour and leaf firmness was slightly worse after storage compared to water-treated plants. The appearance of coriander plants was negatively impacted by salt treatment (although more drastically in the experiment used than in previous experiments) both before and after storage. Interestingly, however, the change in appearance during storage was less for salt-treated plants than for water-treated plants. These results could indicate that salt-treated plants deteriorate less quickly during storage than water-treated plants or, alternatively, that most deterioration had occurred for salt-treated plants prior to storage and cannot continue further, unlike for water treated plants. It should be noted that the condition of coriander plants in this experiment was worse than noted in previous experiments, perhaps due to ambient light levels.

We also investigated the response of parsley grown in a hydroponics setup to allow an accurate determination of treatment duration by avoiding the problems of substrate retention of salt and buffering. NaCl treatment again significantly reduced the elongation of petioles and overall biomass of the plants while not affecting leaf area or colour, as found for the pot-grown plants.

High salinity soil drenches also led to a slower rate of soil drying for both species, probably due to a reduced transpiration rate as plants close stomata in response to salt stress, perhaps combined with a reduced uptake of water by the roots.

The absolute magnitude of effects may be affected by factors such as ambient light levels and soil drying rate as the extent of changes varied slightly between experiments. The relative magnitude of effects for parsley and coriander also varied between experiments and is probably also affected by such factors. Phytotoxic symptoms appeared to be slightly worse under the higher ambient light conditions found in the spring months, such as March-April for Experiment 5 and 6 but were mostly absent under the lower levels found in winter months, such as November-December for Experiment 3. As this is the time of year when etiolation of herbs is most severe and when the need for the treatments would be highest this should help reduce issues of phytotoxicity. Provision of additional lighting in Experiment 4 appeared to reduce treatment effectiveness.

Changes in the xylem pH in response to drought likely form part of a drought stress signalling system in combination with the plant hormone abscisic acid (ABA) and ethylene (reviewed in Wilkinson and Davies, 2010). Drought conditions can lead to increases in the concentration of ABA within the xylem sap or leaves (Bahrun *et al.,* 2002; Hartung *et al.,* 1988; Stoll *et al.,* 2000) and increase the level of ABA synthesis in leaves (Christmann *et al.,* 2005).

The apoplastic pH of broad bean (*Vicia faba*) increased transiently by around 0.3 pH units after treatment with 20 mM potassium chloride or sodium chloride (Felle and Hanstein, 2002), suggesting that there may be some overlap between the signalling systems of drought and salt stress. Indeed, increasing salinity shows a concomitant increase in ABA concentration in some species, which is correlated with reduced leaf expansion (Cramer, 1994; He and Cramer, 1996; Montero et al., 1998; Babu *et al.,* 2012). In addition, ABA-deficient *Arabidopsis* mutants show an increased tolerance to salt stress during germination (Koornneef et al., 1984), while salt tolerant rice lines have been shown to contain inherently lower levels of ABA in their leaves than salt sensitive lines (Zhang *et al.,* 2006).

A previous study of sunflower seedlings, found that they showed reduced growth and leaf area at both higher salinity and pH (Shi and Sheng, 2005). When both stresses were combined together, the effect was much larger, raising the possibility of a combinatorial approach of salinity and pH modulation in an attempt to regulate herb growth. We therefore also investigated the possibility of an additive effect of the two treatments. In Experiment 4, however, we found no additive effect of combining the two treatments, plants appearing to respond only to the high salinity treatment in most cases. This could reflect a more limited overlap of the pH and salinity responses of the herbs or an additional response under high salinity that results in a more drastic response than that seen for high pH.

Conclusions

- Potassium bicarbonate high pH treatments show limited effectiveness in altering the growth of culinary herbs.
- High salinity NaCl treatments can be used to reduce the petiole elongation of parsley and coriander plants (by 10-20%) with no significant effects on leaf area.
- No additive effect on herb growth is seen by combining high pH and high salinity treatments.
- Plant overall biomass is sometimes reduced by salt treatment.
- NaCl treatment does not affect parsley leaf electrolyte leakage or colour, suggesting no change in leaf quality.
- Petiole firmness was also unaffected by NaCl treatment. However, the reduction of petiole length may mean that petioles resist bending more in NaCl-treated plants.
- NaCl treatment effectiveness and phytotoxicity appear to depend on environmental conditions and treatment frequency, concentration and duration, with phytotoxicity being reduced in low light in winter months and increased with higher frequency, higher duration treatments.
- NaCl treatment may reduce plant quality deterioration during storage.

Future Work

Based on the above discussion, further investigation in the following areas is essential for determining the most appropriate treatment regime for modifying herb growth in a commercial setting and enabling recommendations to growers to be made.

- Further investigation of different salt application regimes – concentration, frequency, duration and their application in a large-scale glasshouse environment, perhaps involving trials at Lincolnshire Herbs or VHB.
- Additional investigation of petiole strength parameters may be possible using alternative equipment such as an Instron Universal Materials Testing Machine for both parsley and coriander in a pot-based system.
- Further investigation of storage performance and shelf life using plants grown in lower light/temperature conditions to reduce damage from treatments. Includes investigation of leaf electrolyte leakage for non-hydroponics and coriander plants.
- Analysis of the flavour qualities of NaCl-treated plants by sensory panel.

- Consultation with Chemicals Regulation Directive on whether commodity substance approval is needed for sodium chloride drench as a plant growth retardant.

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