

**Project title:** Use of Plant Defence Elicitors to Provide Induced Resistance Protection in Brassica and Allium Crops

**Project number:** FV 417

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**Previous report:** None

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**Date project commenced:** 1<sup>st</sup> April 2013

**Date project completed**  
**(or expected completion date):** 31<sup>st</sup> March 2015

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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.

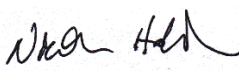
## ***AUTHENTICATION***

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.

Nicola Holden

Project lead

The James Hutton Institute

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## **GROWER SUMMARY**

### **Headline**

- Plant defence elicitors have the potential to aid in the treatment and control of bacterial and fungal diseases of Brassica and Allium species.
- Initial trials show positive results for two elicitors in control of leaf blight (Pca) in radish and black rot (Xcc) in cabbage.
- Some elicitors increased head-weight of broccoli, coupled with presence of hollow-stem disorder.
- Trials on Brussels sprout varieties at two sites show that elicitors reduce severity of Light Leaf Spot significantly and two elicitors in particular, Bion® and Regalia®, show the most promise.

### **Background**

Brassica and Allium crops suffer from a number of important fungal and bacterial diseases. Bacterial pathogens are a serious concern because available control options are very limited in choice and their efficacy depends on appropriate application. Trials have been initiated to test whether plant defence elicitors can be used to provide protection against bacterial and fungal pathogens in five different horticultural crops for commercially important diseases.

The Brussels sprout area in the UK in 2011 was 3,045ha, with the 45,000 tonnes produced having a farmgate value of £41 million (Basic Horticultural Statistics 2012). The disease Light Leaf Spot (LLS) (*Pyrenopeziza brassicae*) is a particular problem in the wetter north of England and in Scotland, and has become established further south in Nottinghamshire and Lincolnshire. It is estimated that annual losses due to LLS are in the region of 10-15% or around £4-6 million.

Head rot is a major disease of broccoli (*Brassica oleracea* L. var. *italica* Plenck) that can cause 30-100% crop losses, estimated to cost the UK industry £10-15 million annually - up to 30% of the market value (Harling & Sutton, 2002). The disease is caused by the soft rotting bacteria, predominantly *Pseudomonas fluorescens*, *Ps. marginalis* and *Pectobacterium carotovorum* (Cui & Harling, 2006). Previous work (FV 378) tested whether plant defence elicitors were able to reduce or prevent head-rot symptoms in a broccoli trial

and indicated that application of some combinations could reduce the incidence of symptomatic disease.

Black Rot is a major bacterial disease of cabbage throughout the world and can cause significant losses in UK winter cabbage, with Savoy and Savoy x White hybrids particularly susceptible. The disease is probably introduced by infected seed and is now endemic in production fields in these areas and although the preventative use of copper and strobilurin fungicides can minimise disease outbreaks there is little that can be done to control established disease. Winter cabbage area in the UK is around 2,900ha, producing around 147,000 tonnes with a farmgate value of £54 million (Basic Horticultural Statistics 2012). It is estimated that severe disease outbreaks in some years can lead to production losses amounting to 15-20% or £7-10million.

The radish production in the UK is ~ 5800 tonnes, with a market value of ~ £11 million. Approximately 15% of the production is sold as a bunched product, and although radish leaves are not intended for consumption, there has been an increase in demand for radish bulbs sold in bunches with the leaves attached. The presence of bacterial blight and development of scorched-leaf symptoms caused by *Pseudomonas* species renders the crop unmarketable, despite the absence of disease symptoms on the roots. The disease has been observed in crops over the past few seasons particularly during spells of wet weather. It has been estimated that during a high infection period there could be up to 6% losses.

## Summary

The initial trials have shown some promising results for the application of elicitors to control bacterial and fungal pathogens of brassica and allium species. Year two of the project aims to repeat these trials.

Trials at two sites using early, mid- and late-season Brussels sprout varieties demonstrated that a range of elicitors reduced LLS severity substantially on leaves and sprouts. Of particular interest was the elicitor Bion®, which used either on its own, or in combination with the elicitor Regalia®, gave significant reductions in LLS severity when applied just 3 times during the growing season.

Glasshouse trials on radish showed a significant reduction in the severity of *Pseudomonas cannabina* pv. *Alisalensis* (Pca) associated leaf blight symptoms, following application of SitKO-SA on var. Celesta. Trials on cabbage showed a significant decrease in Xcc-associated symptoms for Harpin-treated plants compared to the other treatments, although the difference was not significant when compared to the fungicide-treated control. Some

elicitors increased the yield of broccoli, although this tended to correlate with an increase in hollow-stem disorder.

Year 2 of the project aims to repeat the initial findings. It is anticipated that elicitors will be most useful as part of an integrated disease management programme.

## **Financial Benefits**

There are no financial benefits, as yet, from this project.

## **Action Points**

Action points will be developed once the project has been completed at the end of year 2.



## SCIENCE SECTION

### Introduction

This project was initiated to test whether products that have the potential to induce the plant defence response can reduce or prevent symptomatic disease on selected brassica and allium crops. The work is a logical extension of two previous projects (FV378, FV393) investigating the efficacy of elicitors and has been extended to LLS on Brussels sprouts. This project involves trials on four brassica: broccoli, radish, cabbage and Brussels sprouts, and one allium: red onion. One of the key drivers of the project is to test products that either readily available in the UK, or have a good chance of being so (Table 1).

Product & Supplier	Elicitor activity	Current use	Prospects for use
Amistar	Strobilurin	Brassicas: control of White Blister, Ring Spot, Alternaria Onion and Radish: control of Downy Mildew	Good
Signum	Strobilurin	Brussels sprouts, cabbage, broccoli and radish: control of Downy mildew	Good
Bion (Syngenta)	ASM – salicylic acid mimic	Actiguard (US) Label approved for various including Brassicas for Xanthomonas (black rot)	Good - Fair
Sitko-SA (Growth Products USA)	Salicylic acid and phosphite	Sold as a fertilizer in the USA. Not currently sold in UK, but can be shipped.	Good - Fair.
Softguard (Travena, UK)	Chitosan	Sold as a plant health-care or growth promoter product (fertiliser) in the UK	Good
Algal 600 products (Travena, UK)	Seaweed extracts, laminarin	Sold as a nutritional supplement in the UK, often combined with Softguard.	Good
Harpin (Plant Health Care, USA)	Secreted protein derived from <i>hrpN</i> of <i>E. amylovora</i> .	Sold as a plant health promoter, available in the UK via Plant Health Care, UK office.	Good - Fair
Reysa / Regalia /Milsana (Syngenta)	Knotweed extract	To be marketed in Europe by Syngenta. Used on range of crops to control wide range of pathogens.	Good – Fair
Companion (Growth Products USA)	<i>Bacillus subtilis</i> GB03	Sold as a liquid biological fungicide in the USA. Not currently sold in UK, but can be shipped.	Good-fair

The work has been divided into five work packages based on the disease system:

- 1: Light Leaf Spot fungi (*Pyrenopeziza brassicae*) on Brussels sprouts (DW, SAC Commercial Ltd lead)
- 2: Head rot bacteria (*Pseudomonas fluorescens*, *Ps. marginalis*, *Pectobacterium atrosepticum*) on broccoli (NH, JHI lead)
- 3: Black rot bacteria (*Xanthomonas campestris* pathovar *campestris* - Xcc) on cabbage (NH, JHI lead)
- 4: Leaf blight bacteria (*Pseudomonas cannabina* pv. *alisalensis* - Pca) on radish (NH, JHI lead)
- 5: Soft rot bacteria (*Burkholderia gladioli* pv. *alliiicola* - Bga) in onion bulbs (NH, JHI lead)

In 2013, trials were established for broccoli, cabbage, Brussels sprouts and radish. (Red onion sets trials will be initiated in 2014).

## Materials and methods

### Experimental trials

Experimental field trials for broccoli and cabbage, and glasshouse trials for radish were established at the James Hutton Institute, Dundee, Scotland, and at two grower sites, Blackness and Tynningame, for Brussels sprouts. Treatments were tested in replicate plots of three, and 20 replicate plants were assessed per treatment. Broccoli and cabbage were grown on 100 m x 25 m sites in open-ended poly-tunnels. Radish was grown from seed for four-five weeks in compost, in a glasshouse, at ambient temperature.

### Applications

Elicitors were applied as the sole treatment for broccoli and either applied independently or in conjugation with fungicides for cabbage and radish. The timing of application was dependent on plant development and all treatments were applied with hand-held sprayer. Two applications of elicitors were applied to broccoli at 14-day intervals, 40 days after the transplants were established; four to cabbage at one-month intervals; two to radish at 7 days intervals between 7 and 10 days after seedling emergence. The treatment schedules and elicitors used are listed in Table 1.

Elicitor and fungicides were applied at the same rates and concentrations as previous trials (FV378, FV393), (application rates in Table 2) and included the following: Amistar, Signum (stobulurin); ASM (Bion); Regalia; Softguard (chitosan); Algal600, (seaweed extracts); Harpin; SitKO-SA; Probenazole. Controls included the no treatment control (NTC), no bacteria control (NBC) and no treatment no bacteria control (NBNTC); standard fungicide programme (SFP). Additional information on the treatments is provided in Appendix 1.

**Table 1**

Crops, treatment schedules and elicitors used.

Crop	Application and timing in days (date)		Elicitors
Broccoli (var. Parthenon)	Plant transplants	0 (21/06/2013)	○ SitKO-SA
	Treatment 1 (elicitors)	39	○ Harpin
	Apply bacteria 1	48	○ Chitosan & Seaweed extract
	Treatment 2 (elicitors)	54	○ Amistar
	Apply bacteria 2	58	○ Probenazole & Amistar
	Disease assessment	80	○ Coded product DM31 ○ Regalia
Cabbage (var. Tundra)	Plant transplants	0 (08/07/2013)	○ Bion
	Apply bacteria	28	○ Harpin
	Treatment 1 (elicitor +/- Signum)	60	○ Chitosan & Seaweed extract
	Treatment 2 (elicitor +/- Amistar Top)	91	<i>applied (i) alone; (ii) + fungicide; (iii) alternating with fungicide</i>
	Treatment 3 (elicitor +/- Rudis)	122	
	Treatment 4 (elicitor +/- Nativo)	151	
	Disease assessments	122 – 191	
Radish (vars. Celesta and Expo)	Sow from seed	0 (various dates)	○ SitKO-SA
	Treatment 1 (elicitor +/- Amistar)	7 – 14	○ Harpin
	Apply bacteria	10 - 17	○ Chitosan & Seaweed extract
	Treatment 2 (elicitor +/- Signum)	14 – 21	○ Bion
	Disease assessment	23 – 35 (varied dependent on growth rate)	○ Regalia <i>applied (i) alone; (ii) + fungicide</i>
Brussels sprouts (vars. Cobus, Aurelius, Petrus)	Plant transplants	24/05/2013 (Blackness) 21/05/2013 (Tynninghame)	○ Bion
	Treatment groups	See Table 2	○ Regalia
	Assessment dates	Blackness 2/12/2013; 20/12/2013; 22/01/2014; 19/02/2014  Tynninghame 3/12/2013; 19/12/2013; 30/01/2014; 18/02/2014; 17/03/2014	○ Softguard ○ SiTKO-SA ○ Companion
			For a complete list of treatments, see Table 1 in Appendix

**Table 2**

Concentration of elicitor and fungicide treatments used:

<b>Elicitor</b>	<b>Working concentration, application rate</b>
Bion (ASM = 50%)	1 mM; 0.175g/l for Brussels sprout trials
Probenazole	0.2 mM
Regalia	4.9 L / Ha (2.5 kg/ha for Brussels sprout trials)
SoftGuard	1:600 *
Algal 600	1:500 *
SitKO-SA	5 L / Ha
ProAct (Harpin)	0.15 kg / Ha
Companion	6 L / Ha
Tween-20	0.01 %
Activator-90 wetter	0.05 %
<b>Fungicides</b>	<b>Working concentration</b>
Signum	1 kg / Ha
Cuprokylt	5 kg / Ha
Amistar	1 L / Ha

\* applied to run-off

Where required (radish, broccoli, cabbage) bacteria inoculum was applied at  $10^6$  cfu/ml by foliar spray, until run-off. Broccoli were infected with a cocktail of *Pseudomonas fluorescens*, *Ps. marginalis* and *Pectobacterium carotovorum* (collectively known as head-rot bacteria); cabbage were infected with *Xanthomonas campestris* pv. *campestris* (Xcc) and radish was infected with *Pseudomonas cannabina* pv. *alisalensis* (Pca) / radish blight bacteria. It should be noted that the speciated isolate indicated as Pca (NCPPB1820, originally classified as *Ps. syringae* pv. *maculicola*) was in fact a different species and 16S sequence analysis indicated closest homology to *Pantoea agglomerans* (strain DSM 3493). Therefore, a pathogenic *Pseudomonas* species obtained directly from infected radish plants (supplied by Liz Johnson) was used in the trials. Its identity was confirmed by 16S sequence determination. All bacteria were grown initially in rich media at 28 °C to saturation and sub-cultured into defined media designed to optimise expression of virulence factors (at 25 °C). PCR amplification was used to detect pseudomonads (Spasenovski et al. 2009) from inoculated plant material.

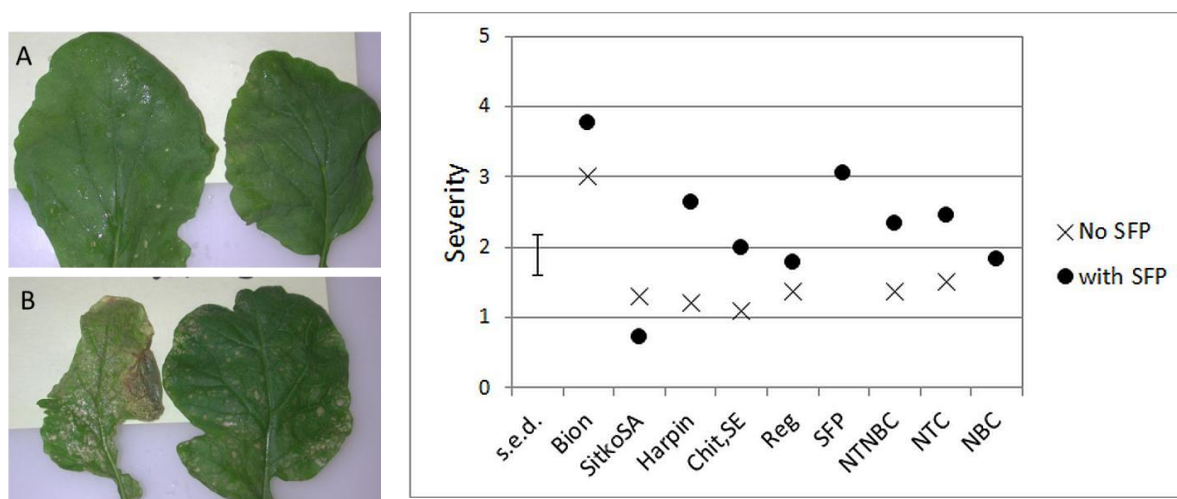
**Disease assessment and analysis**

Disease was assessed visually for all crops *in situ*: the incidence of symptomatic disease was scored as 'Healthy' or 'Diseased' and the extent assessed on 5-point scale of symptoms for radish, cabbage and broccoli. The amount of symptomatic LLS disease on Brussels sprouts was scored as a percentage. Broccoli heads were harvested at maturity (~ 80 days after transplant establishment) and yield determined from fresh weight (stem length was ~ 5 -7 cm below the lower set of florets). Analysis of variance was carried out using Excel (Microsoft) or Genstat (VSN International) computer programmes.

## Results

### Radish

Radish leaves spray-inoculated with 'Pca' developed blight-like symptoms on the leaves that in some instances became necrotic (Figure 1). Elicitors were tested on radish either independently, or incorporated into a fungicide programme. Preliminary results show a decrease in the extent of symptomatic disease with the application of SitKO-SA (Figure 1). The decrease was significant for radish var. Celesta, where there was a high extent of disease, but not for var. Expo, where the extent of disease was lower (not shown). The elicitors tended to have a greater beneficial effect on disease in the absence of fungicide treatments.



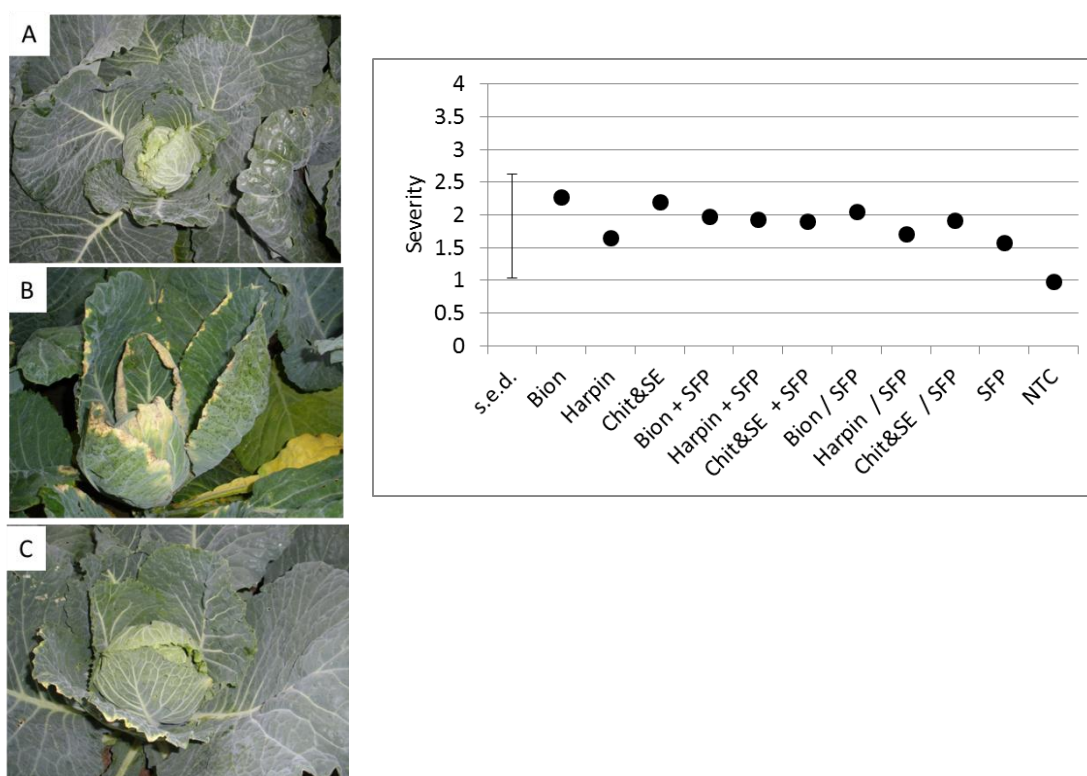
**Figure 1** Radish.

Left: 'Pca' symptoms on radish (var.Celesta) leaves. Symptoms of low (A) and high (B) severity. Right: Disease assessment, showing the disease severity level per treatment for Celesta. Disease severity was measured on a 0 (no disease) to 5 (maximum disease) scale. The average severity is shown for elicitor treatments incorporated into the fungicide

programme (circles) or used independently (crosses). The error bar represents the standard error of the difference. Values are also provided for the controls (SFP; NTNBC; NTC; NBC).

## Cabbage

Cabbage inoculated with Xcc developed characteristic lesions along the leaf margins and small black lesions on the leaves (Figure 2). Elicitors were applied to cabbage, either alone, in combination with the SFP or alternating with the SFP. Disease severity was significantly reduced with the addition of Harpin compared to the other two treatments used in the absence of SFP. The effect was also seen when the elicitor was alternated with the SFP, although not to a significant level. No difference was seen when the elicitors were used together with the fungicides.

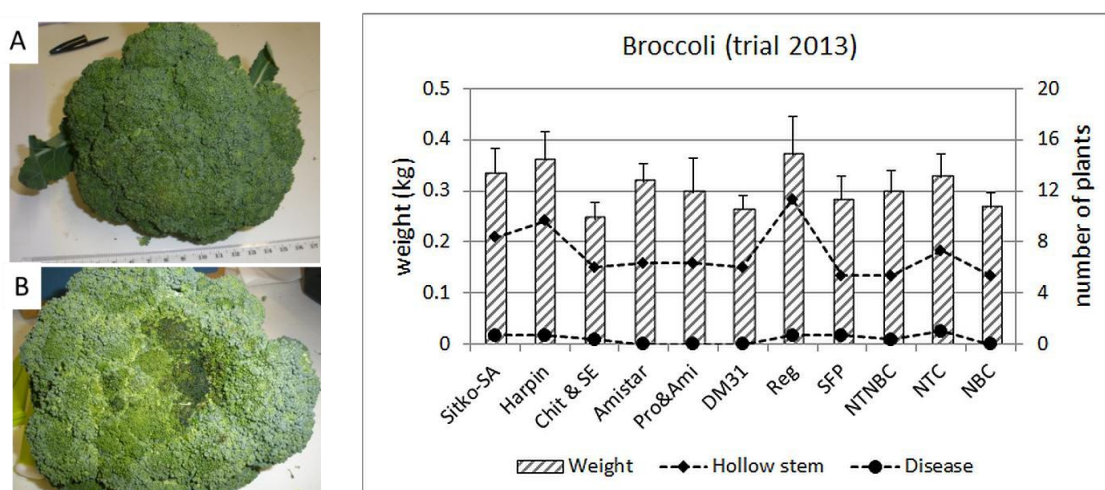


**Figure 2** Cabbage.

Left: Xcc symptoms on cabbage (Tundra) leaves. An uninfected control (A) compared to plants showing severe symptoms (B) or low level of severity (C). Right: Disease assessment, showing the level of disease severity per treatment. Disease severity was measured on a 0 (no disease) to 5 (maximum disease) scale and the average shown. The error bar represents the standard error of the difference. Values are also provided for the controls (SFP; NTC).

## Broccoli

Application of the head-rot cocktail to broccoli resulted in the presence of characteristic soft-rot (Figure 3). The incidence of disease was not sufficiently high to carry out a statistical analysis of the effect of the elicitors, although the least amount of disease was seen with application of Amistar, which was also found in previous trials (FV378). Yield, measured as the weight of the broccoli heads, was found to vary significantly between treatments. However, those with the greatest head weight also showed symptoms of 'hollow stem' disorder most frequently.



**Figure 3.** Broccoli

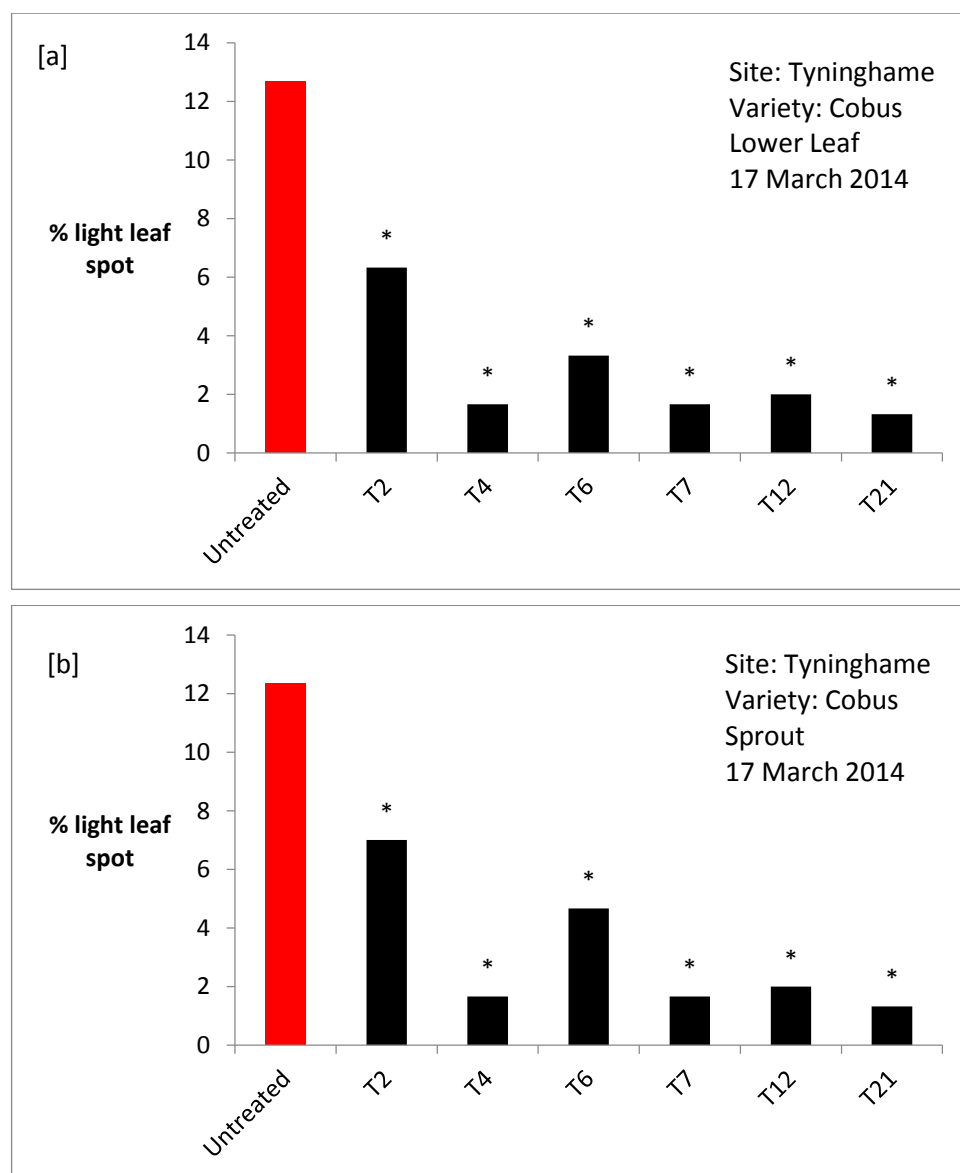
Left: head-rot symptoms on broccoli heads (Parthenon). An uninfected control (A) compared to plants showing severe symptoms (B). Right: Yield, incidence of hollow stem and diseased plants. The chart shows the average yield of fresh weight (hatched bars) with standard deviation; together with the number of plants showing hollow stem disorder (diamonds) and symptomatic disease (circles).

Phytotoxic damage was observed with application of two of the elicitors on radish and broccoli. However, the effect was only apparent on the cotyledons of radish and not on the true leaves. The effect was observed on mature leaves of broccoli, but was limited to the affected leaf and did not appear to be systemic (not shown).

## Brussels sprouts

At the Tynninghame site, although the full fungicide programme reduced Light Leaf Spot (LLS) severity on lower leaves and sprouts of the early season variety Cobus significantly,

the largest reductions in LLS severity were obtained with treatments containing elicitors (Figure 4). Of particular interest are treatments T12 (Bion only) and T21 (Bion + Regalia), since here the treatments were only applied 3 times in the season, compared to the usual 6 applications for most other treatments. Significant reductions in LLS severity were also obtained on the mid-season variety Aurelius at the Tynninghame site (Figure 5). Again, the best treatments were those containing elicitors, especially on the sprouts, where T4 (Bion + fungicides) and T21 (Bion + Regalia applied 3 times) were particularly effective (Figure 5 b).

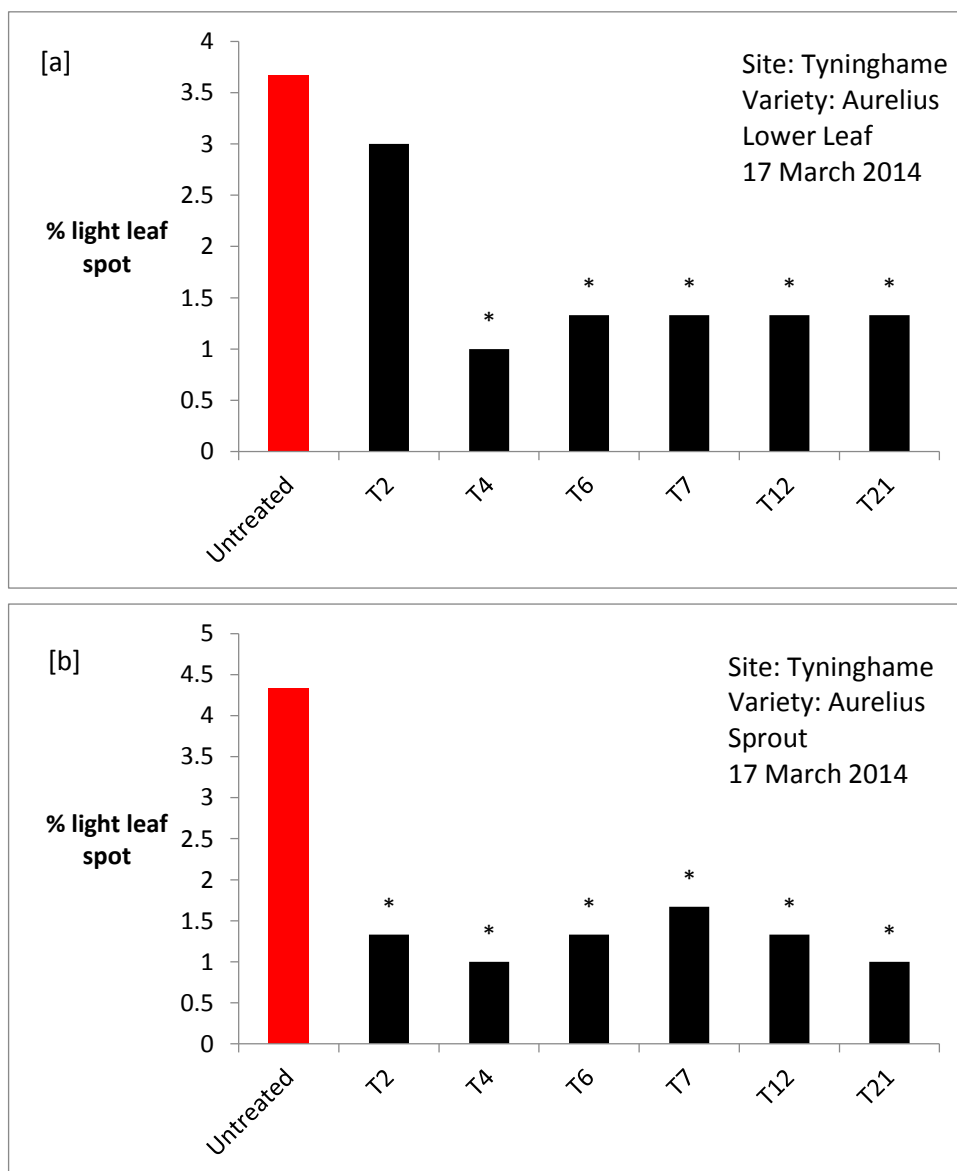


**Figure 4:** Severity of Light Leaf Spot on the Brussels sprout variety Cobus at Tynninghame on 17 March 2014. [a] LLS on lower leaves [b] LLS on sprouts. Treatments shown are:

T2 = fungicide programme ; T4 = alternate Bion + fungicides ; T6 = alternate SiTKO-SA + fungicides; T7 = Bion only – 6 applications ; T12 = Bion only – 3 applications; T21 = Bion + Regalia only – 3 applications

Significant differences at  $P < 0.001$  = \* (ANOVA)



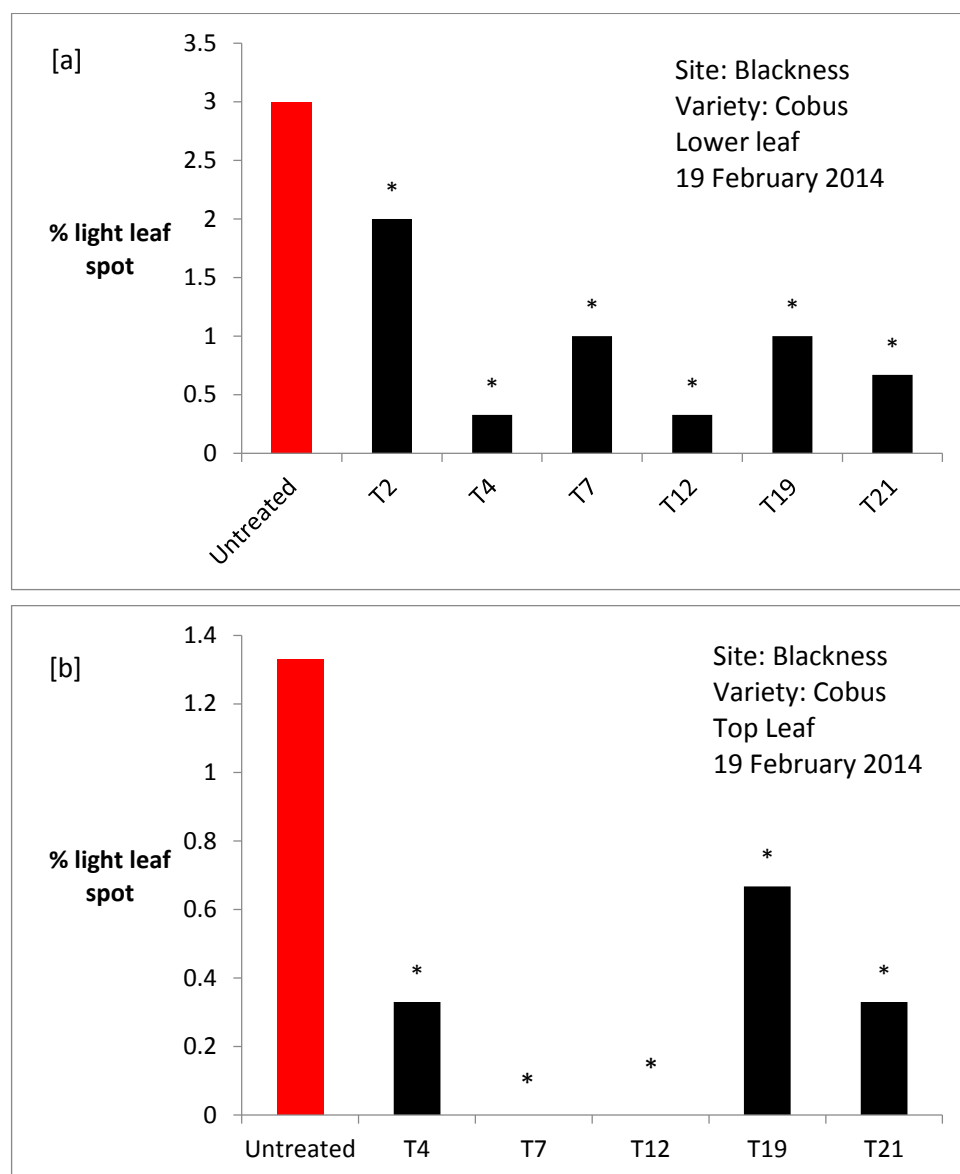


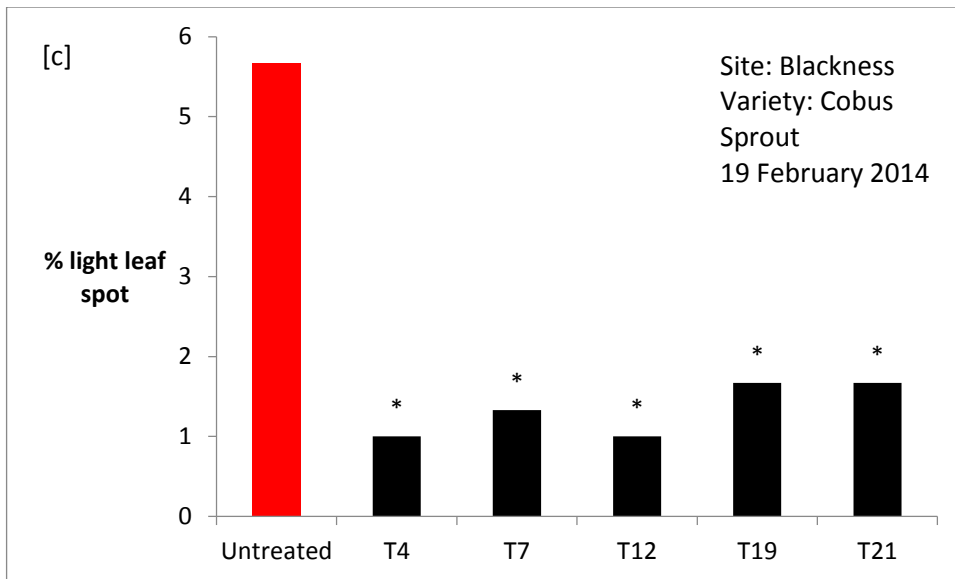
**Figure 5:** Severity of Light Leaf Spot on the Brussels sprout variety Aurelius at Tynninghame on 17 March 2014. [a] LLS on lower leaves [b] LLS on sprouts. Treatments shown are:

T2 = fungicide programme; T4 = alternate Bion + fungicides; T6 = alternate SiTKO-SA + fungicides; T7 = Bion only – 6 applications; T12 = Bion only – 3 applications; T21 = Bion + Regalia only – 3 applications

Significant differences at  $P < 0.001$  = \* (ANOVA)

At the Blackness site, LLS severity was lower than at the Tynninghame site, but here too, the elicitor treatments were most effective. Thus, on lower and top leaves and on sprouts of Cobus, a particularly effective treatment was Bion applied 3 times (Figure 6). On the mid-season variety Aurelius, LLS levels were even lower and although many treatments reduced symptom severity, most of these differences were not significant (Figure 7). Exceptions to this were on lower leaves, where treatments 7 (Bion only) and 21 (Bion + Regalia), reduced LLS significantly (Figure 7a).

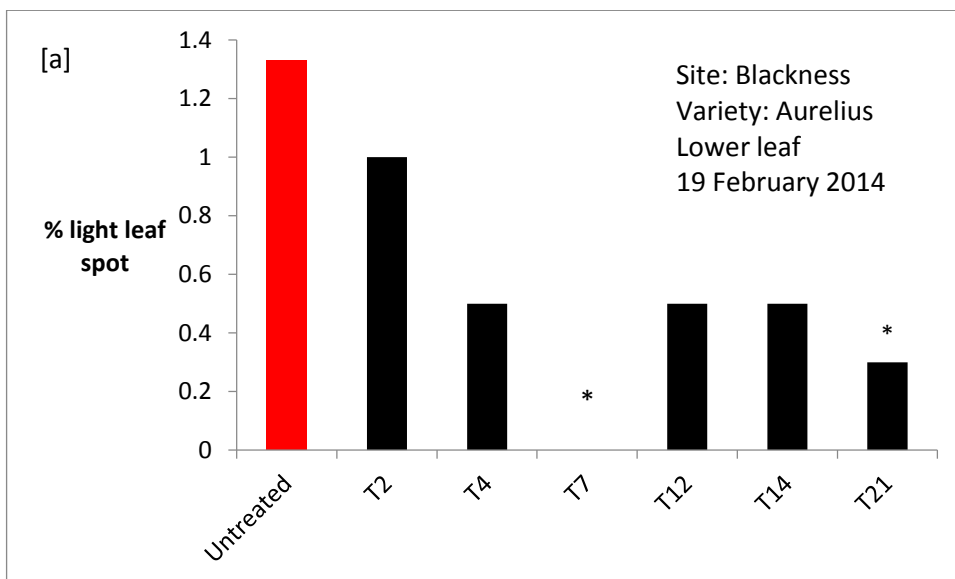


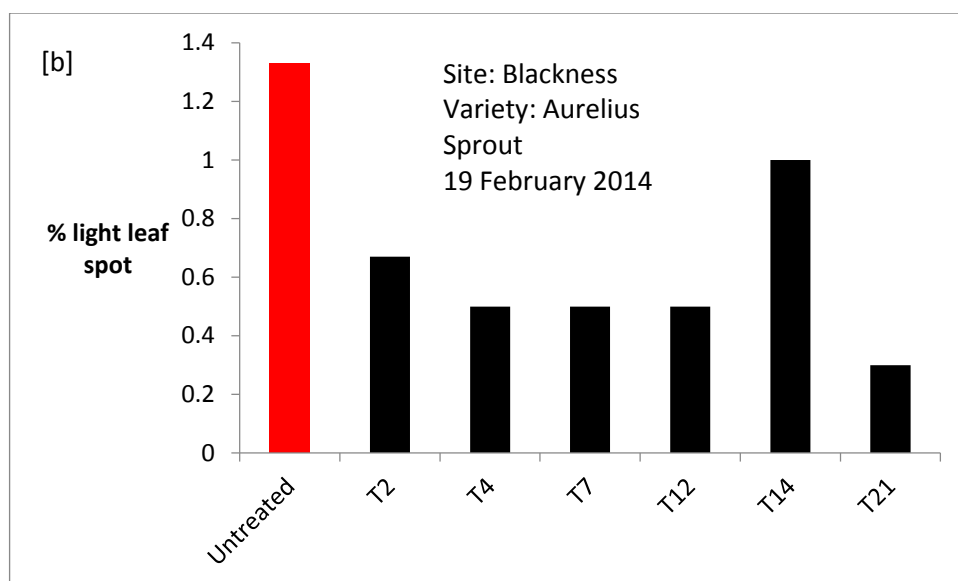


**Figure 6:** Severity of Light Leaf Spot on the Brussels sprout variety Cobus at Blackness on 19 February 2014. [a] LLS on lower leaves [b] LLS on top leaves [c] LLS on sprouts. Treatments shown are:

T2 = fungicide programme; T4 = alternate Bion + fungicides; T7 = Bion only – 6 applications; T12 = Bion only – 3 applications; T19 = Bion + Companion – 3 applications; T21 = Bion + Regalia only – 3 applications

Significant differences at  $P < 0.001$  = \* (ANOVA)





**Figure 7:** Severity of Light Leaf Spot on the Brussels sprout variety Cobus at Blackness on 19 February 2014. [a] LLS on lower leaves [b] LLS on sprouts. Treatments shown are:

T2 = fungicide programme; T4 = alternate Bion + fungicides; T7 = Bion only – 6 applications; T12 = Bion only – 3 applications; T19 = Bion + Companion – 3 applications; T21 = Bion + Regalia only – 3 applications

Significant differences at  $P < 0.001$  = \* (ANOVA)

## Discussion

The effect of elicitors was tested on cabbage, radish and broccoli plants infected with phytopathogenic bacteria, and on naturally LLS-infected Brussels sprouts. Preliminary data from the first of a two-year trial shows that some elicitors can have a beneficial effect in reduction of bacterial disease symptoms in radish and cabbage and fungal symptoms in sprouts. It is notable that different elicitors were effective for each disease system (SitKO-SA for radish, Harpin for cabbage, Bion (and others) for sprouts), indicative of specificity in the effect. Interactions are evident between dependent on the presence / absence of fungicides. It may be possible that the fungicides are affecting the plant response, the native micro-biota or a combination of both. In addition, variety also appeared to have an effect (Celesta vs Expo). Application of elicitors was also shown to affect yield of broccoli, although those treatments that showed the highest yield also suffered from the highest incidence of hollow stem, a disorder associated with rapid growth and prone to stem rot. Symptomatic disease was rare on broccoli, despite the addition of head-rot bacteria that were grown under disease-inducing conditions. Since the bacteria were able to cause symptomatic disease under laboratory conditions, it is most likely that the environmental

conditions were not conducive for disease, in this case. It is also possible that other microbes associated with the plants were able to compete with the head-rot bacteria.

On Brussels sprouts, it was encouraging to see that several elicitor treatments reduced LLS severity significantly. Of particular interest is effect of treatments containing Bion on its own, and Bion combined with Regalia, both applied just three times in the season, and yet providing very good control of LLS. It would be useful to determine the effects of these treatments on clubroot severity, since on-going work at SRUC has demonstrated highly significant effects of Bion and Regalia treatments (separately) on clubroot development on winter oilseed rape.

SitKO-SA contains a combination of SA and phosphite. There is a reasonable body of work reporting some success using salicylic acid mimics in experimental field trial, for example, the use of ASM in the control bacterial phytopathogens in orchard trees, lettuce, broccoli and tomato (Pajot and Silue 2005; Graham and Myers 2011; Yigit 2011; Balajoo et al. 2012). Furthermore, phosphite has also been shown to induce systemic resistance (Lobato et al. 2011). These studies support the hypothesis that the reduction of Pca symptoms on radish and LLS on Brussels sprouts may be as a direct result of SitKO-SA- / ASM-mediated induced defence.

Regalia is an extract of giant knotweed (*Reynoutria sachalinensis*) and although its mode of action is unclear, it is thought to induce multiple defence pathways in the host plant. It is recognised to have pharmaceutical properties and has been shown to induce phytoalexins which may aid in the control of fungal pathogens (La Torre et al. 2004; Peng et al. 2013). It is interesting that it had a significant growth effect on broccoli, although this was coupled with a trade-off in the incidence of hollow stem disorder, an undesirable property for producers that can also lead to stem rot.

Harpin is a protein derived from the secreted protein HrpN (from *Erwinia amylovora*), which acts as a virulence factor once it enters the plant tissue (Wei *et al.*, 1992). It is delivered by the type 3 secretion system, a mechanisms to inject manipulative 'effector' proteins into the plant cell by the bacterium. The protein is well conserved among related phytopathogenic *Enterobacteriaceae* and may even be perceived as a MAMP (microbe-associated molecular pattern) by the plant, and induce the defence response. The possibility that Harpin can possibly confer protection against a member of a different family, the *Xanthomonaceae* it interesting and suggests that the recognition may trigger a basal level of immunity.

On-going work aims to extend the trials to better assess the potential effect of elicitors in horticultural crops. There is a growing need to understand the molecular basis for the

action of elicitors, so that they can be used as part of an integrated programme of crop management, in a more targeted manner.

## Conclusions

The initial trial suggests that some of the elicitors may be effective against bacterial disease on Brassicas. There appears to be specificity in the response and interactions with other factors such as fungicides and plant variety. Year 2 aims to repeat the trials and extend the work to include an Allium (red onion sets).

Trials at both Tynningham and Blackness have demonstrated significant control of LLS on early and mid-season varieties of Brussels sprouts using elicitors. Bion and Regalia were found to be particularly effective when applied just 3 times during the season.

## Knowledge and Technology Transfer

British Soil Society, soil amendment meeting, 30/05/2013, SRUC, Edinburgh. Presentation “Microbial bio-effectors: boosting induced resistance in horticultural crops”, Holden. ...

Brassica Growers Association meeting, 29/01/2014, Ingliston, Edinburgh. Presentation “Using elicitors to control Brassica diseases”, Holden.

BGA annual meeting, 21/01/2014, Lincoln. Presentation, Self-help for brassicas: helping plants to help themselves, Walters.

Crop Protection in Northern Britain 2014 meeting, 25/02/2014, Dundee. Presentation and conference proceedings entry “Application of plant defence elicitors to control bacterial pathogens on horticultural crops”, Holden.

## References

- Balajoo, OM, Kesahavarzi, M, Zahabi, A, Danesh, YR, Haghjuyan, R. (2012) Protective effect of Acibenzolar-S-Methyl on fireblight severity in quince and characterization of the *Erwinia amylovora* strains involved. *Journal of Plant Pathology* **94**: 211-214
- Cui, X. and Harling, R. (2006) Evaluation of bacterial antagonists for biological control of broccoli head rot caused by *Pseudomonas fluorescens*. *Phytopathology* **96**, 408
- Graham, JH and Myers, ME (2011) Soil application of SAR inducers Imidacloprid, Thiamethoxam, and Acibenzolar-S-Methyl for citrus canker control in young grapefruit trees. *Plant Disease* **95**: 725-728

Harling, R and Sutton, M. (2002). Spear rot of Calabrese (Horticultural Development Company).

La Torre, A, G Spera and D Lolleti 2004. Activity of natural products against courgette powdery mildew. *Communications in Agriculture and Applied Biological Sciences* **69**: 671-678.

Lobato, MC, Machinandiarena, MF, Tambascio, C, Dosio, GAA, Caldiz, DO,, Daleo, GR, Andreu, AB, Olivieri, FP, (2011). Effect of foliar applications of phosphite on post-harvest potato tubers. *European Journal of Plant Pathology*. **130**: 155-163

Pajot, E and Silue, D. (2005). Evidence that DL-3-aminobutyric acid and acibenzolar-S-methyl induce resistance against bacterial head rot disease of broccoli. *Pest Management Science* **61**: 110-1114

Peng, W, R Qin, X Li and H Zhou 2013. Botany, phytochemistry, pharmacology, and potential application of *Polygonum cuspidatum* Sieb.et Zucc.: a review. *Journal of Ethnopharmacology* **148**: 729-745.

Spasenovski, T, Carroll, MP, Payne, MS, Bruce, KD (2009). Molecular analysis of diversity within the genus *Pseudomonas* in the lungs of cystic fibrosis patients. *Diagnostic Microbiology and Infectious Diseases* **63**: 261-267

Yigit, F. (2011). Acibenzolar-S-methyl induces lettuce resistance against *Xanthomonas campestris* pv. *Vitians*. *African Journal of Biotechnology* **10**: 9606-9612

Wei ZM, Laby RJ, Zumoff CH, Bauer DW, He SY, Collmer A, Beer SV. (1992). Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. *Science*. **257**:85-8.

## Appendices

Appendix 1: Additional information on crop systems and treatments:

Table A1.1 (Brussels sprouts)

Brussels sprouts	
Varieties:	Cumulus, Clodius, Millennium Planted from transplants (May)
Fungicides:	Signum (BASF), Rudis (Bayer), Nativo (Bayer)
Elicitors:	Bion®, Regalia®, SoftGuard, Vacciplant®, SiTKO-SA
Standard fungicide programme (SFP):	Signum (end July), Rudis (mid-August), Nativo (early September), Signum (end September), Rudis (mid October), Nativo (early November)
Treatment 1:	Elicitors applied (singly and in combination) at end July, mid-August, early September, end September, mid-October, early November
Treatment 2:	Elicitors applied (singly) at end July, early September, mid-October
Treatment 3:	Alternate elicitor and fungicide e.g. elicitor (end July), fungicide (mid-August), elicitor (early September), fungicide (end September), elicitor (mid October), fungicide (early November)
Treatment 4:	Elicitor combination (SoftGuard + Vacciplant) applied at end July, early September, mid-October
LLS assessments	to be carried out in July, August, September, October, November



Table A1.2 Treatments applied to Brussels Sprouts

Trts	Mid July	End July	Mid Aug	Early Sept	Late Sept	Mid Oct
1	Untreated					
2	Signum 1kg/ha		Rudis 0.4l/ha	Nativo 0.4kg/ha	Signum 1kg/ha	Rudis 0.4l/ha
3		Regalia 2.5kg/ha	Rudis 0.4l/ha	Regalia 2.5kg/ha	Signum 1kg/ha	Regalia 2.5kg/ha
4		Bion 0.175g/l	Rudis 0.4l/ha	Bion 0.175g/l	Signum 1kg/ha	Bion 0.175g/l
5		Softguard 10mls/5l	Rudis 0.4l/ha	Softguard 10mls/5l	Signum 1kg/ha	Softguard 10mls/5l
6		SiTKO-SA 5l/ha	Rudis 0.4l/ha	SiTKO-SA 5l/ha	Signum 1kg/ha	SiTKO-SA 5l/ha
7		Bion 0.175g/l	Bion 0.175g/l	Bion 0.175g/l	Bion 0.175g/l	Bion 0.175g/l
8		Regalia 2.5kg/ha	Regalia 2.5kg/ha	Regalia 2.5kg/ha	Regalia 2.5kg/ha	Regalia 2.5kg/ha
9		Softguard 10mls/5l	Softguard 10mls/5l	Softguard 10mls/5l	Softguard 10mls/5l	Softguard 10mls/5l
10		Companion 6l/ha	Companion 6l/ha	Companion 6l/ha	Companion 6l/ha	Companion 6l/ha
11		SiTKO-SA 5l/ha	SiTKO-SA 5l/ha	SiTKO-SA 5l/ha	SiTKO-SA 5l/ha	SiTKO-SA 5l/ha
12		Bion 0.175g/l		Bion 0.175g/l		Bion 0.175g/l
13		Regalia 2.5kg/ha		Regalia 2.5kg/ha		Regalia 2.5kg/ha
14		Softguard 10mls/5l		Softguard 10mls/5l		Softguard 10mls/5l
15		Companion 6l/ha		Companion 6l/ha		Companion 6l/ha
16		SiTKO-SA 5l/ha		SiTKO-SA 5l/ha		SiTKO-SA 5l/ha
17		Softguard 10mls/5l + Companion 6l/ha		Softguard 10mls/5l + Companion 6l/ha		Softguard 10mls/5l + Companion 6l/ha
18		Regalia 2.5kg/ha + Companion 6l/ha		Regalia 2.5kg/ha + Companion 6l/ha		Regalia 2.5kg/ha + Companion 6l/ha
19		Bion 0.175g/l + Companion 6l/ha		Bion 0.175g/l + Companion 6l/ha		Bion 0.175g/l + Companion 6l/ha
20		SiTKO-SA 5l/ha + Companion 6l/ha		SiTKO-SA 5l/ha + Companion 6l/ha		SiTKO-SA 5l/ha + Companion 6l/ha
21		Bion 0.175g/l + Regalia 2.5/ha		Bion 0.175g/l + Regalia 2.5/ha		Bion 0.175g/l + Regalia 2.5/ha
22		Regalia 2.5kg/ha + SiTKO-SA 5l/ha		Regalia 2.5kg/ha + SiTKO-SA 5l/ha		Regalia 2.5kg/ha + SiTKO-SA 5l/ha

**Table A2 (broccoli)**

<b>Broccoli</b>	
Varieties:	Parthenon Planted from transplant early May
Fungicides:	Fungicides are not routinely applied to broccoli
Elicitors:	SoftGuard & Algal600, SiTKO-SA, Harpin, Amistar (applied singly and in combination)
Standard fungicide programme (SFP):	Amistar & Cuprokylt at head initiation and 14 days later
Treatment 1:	Elicitors applied three times in ~ 10-day cycle mid June, late June and early July. Bacterial inoculum applied mid and late June
Head-rot assessments	July

Table A3 (cabbage)

<b>Cabbage</b>	
Varieties:	Tundra Planted from transplant early July
Fungicides:	Amistar Top, Rudis, Nativo
Elicitors:	SoftGuard & Algal600, Harpin, Amistar, Bion (applied singly and in combination)
Standard fungicide programme (SFP):	Signum, (Aug) Amistar Top (Sept), Rudis (Oct), Nativo (Nov)
Treatment 1:	Elicitor only, applied four times in place of SFP (Aug, Sept, Oct, Nov)
Treatment 2:	Elicitor + fungicide: elicitors included in SFP (above)
Treatment 3:	Elicitor alternating with fungicide: i.e. elicitor (Aug), fungicide (Sept), elicitor (Oct), fungicide (Nov)
Black-rot assessments	Sept - Dec

Table A4 (radish)

<b>Radish</b>	
Varieties:	Expo, Celesta Planted from seed as required (April – Oct)
Fungicides:	Amistar, Signum
Elicitors:	SitKO-SA, Harpin, Softguard+Algal 600, Bion, Regalia (applied singly and in combination)
Standard fungicide programme:	Amistar 7 (Summer) / 14 (Spring) days, Signum 14 (Summer ) / 21 (Spring) days
Treatment 1:	Elicitor only, applied (singly) at 7 (Summer) / 14 (Spring) and 14 (Summer ) / 21 (Spring) days Bacteria applied at 10 (Summer) / 17 (Spring) days
Treatment 2:	Elicitor + fungicide: elicitors included in SFP (above)
Blight assessments	At 23 days (Summer) / 35 days (Spring)