| | |
|---|---|
| **Project title:** | Use of Plant Defence Elicitors to Provide Induced Resistance Protection in Brassica and Allium Crops |
| **Project number:** | FV 417 |
| **Project leader:** | Nicola Holden, James Hutton Institute |
| **Report:** | Annual report, March 2016 |
| **Previous report:** | Annual, May 2015 |
| **Key staff:** | Nicola Holden<br>Adrian Newton<br>Dale Walters<br>Graham McGrann |
| **Location of project:** | Disease system 1 (Brussels sprouts):<br>Three grower sites Fife and St Andrews (Kettle Produce), and Borders (KP Drysdale)<br><br>Disease system 2 - 5 (broccoli, cabbage, radish, onion):<br>The James Hutton Institute, Errol Road, Invergowrie, DUNDEE, DD2 5DA |
| **Industry Representative:** | Disease system 1 (Brussels sprouts):<br>Name: Matt Rawson<br>Address: MRR Vegetable Agronomy Ltd, Pasture Farm, West End, Kilham, Driffield, East Yorkshire, YO25 4RR<br>Disease system 2 (broccoli)<br>Name: Alistair Ewan<br>Address: East of Scotland Growers, Prestonhall Industrial Estate, Cupar, Fife, KY15 4RD |

Disease system 3 (cabbage):

Name: Euan Alexander

Address: Kettle Produce, Balmalcolm, Cupar, Fife, KY15 7TJ

Disease system 4 (radish):

Name: Liz Johnson

Address: L J Technical Consultancy Ltd, 35 West Fen Road, Ely, Cambridge, CB6 1AN

Disease system 5 (onion):

Name: Andy Richardson

Address: Allium and Brassica Agronomy, Wash Road, Kirton, Boston, Lincs, PE20 1QQ

**Date project commenced:** 1st April 2013

**Date project completed (or expected completion date):** 30th April 2016

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.

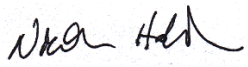
## AUTHENTICATION

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

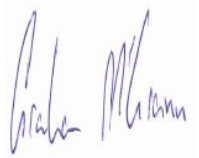
The James Hutton Institute

Signature .....  ..... Date .....13/04/2016.....

Graham McGrann

[Position]

SRUC

Signature .....  ..... Date .....11/04/2016.....

**Report authorised by:**

[Name]

[Position]

[Organisation]

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

Adrian C Newton

James Hutton Institute

Signature  ... Date ...27th March 2016.....

# CONTENTS

*Contents*

# GROWER SUMMARY

## Headline

Harpin applied on its own is as effective as standard fungicides in controlling bacterial disease of cabbage (*Xanthomonas campestris* pv. *campestris*) and red onions (*Burkholderia gladioli* pv. *alliicola*).

SiTKO-SA also provided a degree of protection against Bga on red onion bulb and Amistar was beneficial for yield on broccoli.

Targeted or appropriate application of elicitors needs to be considered since certain elicitors either alone or in combinations have the potential to cause negative effects on plant health.

Trials on the Brussels sprout varieties Cobus, Aurelius and Petrus at different sites over two growing seasons show that elicitors reduce development of Light Leaf Spot significantly and in particular the elicitor Bion® shows 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. Their effectiveness is influenced by the timing of application, weather conditions and the rate of plant development. Trials have been initiated to test whether plant defence elicitors can be used to provide protection against four bacterial and one fungal pathogen in five different horticultural crops for commercially important diseases:

Head rot in broccoli caused by a number of bacteria including *Pseudomonas fluorescens, Ps. marginalis* and *Pectobacterium atrosepticum*

Black rot in cabbage caused by *Xanthomonas campestris* pathovar *campestris* (Xcc)

Leaf blight on radish leaves caused by *Pseudomonas cannabina* pv. *alisalensis* (Pca)

Soft rot in red onion bulbs caused by *Burkholderia gladioli* pv. *alliicola* (Bga)

Light leaf spot on Brussels sprouts caused by the fungus *Pyrenopeziza brassicae*

The Brussels sprout area in the UK in 2013 was 3,363 ha, with the 51,000 tonnes produced having a farmgate value of £56.4 million (Basic Horticultural Statistics 2013). 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 (*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 light leaf spot 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, including those with Amistar 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 thought to be 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 3,380 ha, producing around 140,000 tonnes with a farmgate value of £33.2 million (Basic Horticultural Statistics 2013). It is estimated that severe disease outbreaks in some years can lead to production losses amounting to 15-20% or £7-10 million.

The radish production in the UK is about 5,800 tonnes, with a market value of around £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

Plant defence elicitors have the potential to aid in the treatment and control of bacterial and fungal diseases of Brassica and Allium species. Trials at three sites over two growing seasons (2013-14: Blackness Falkirk, and Tyninghame, East Lothian; 2015-16: St Andrews, East Fife) using early, mid- and late-season Brussels sprout varieties Cobus Aurelius and Petrus demonstrated that the elicitors Bion®, Regalia®, Softguard®, Companion and SiTKO-SA could reduce light leaf spot development substantially on leaves and sprouts. The degree of reduction visible symptoms was as much as 3-fold, although there was some variation

depending on the tissue type, variety and geographical location. Of particular interest was the elicitor Bion®: when used either on its own, or in combination with other elicitors, frequently gave significant reductions in light leaf spot development when applied just three times during the growing season.

Reproducible positive effects were seen for the elicitors on bacterial diseases. The effects were compared to fungicides that are normally applied to the crops as a means to control fungal pathogens. Harpin applied on its own was as effective as, or more so, than standard fungicides in controlling bacterial disease of cabbage (*Xanthomonas campestris* pv. *campestris*) and red onions (*Burkholderia gladioli* pv. *alliicola*). Glasshouse trials on radish showed a significant reduction in the severity of *Pseudomonas cannabina* pv. *alisalensis* - associated leaf blight symptoms, following application of SiTKO-SA on var. Celesta, whereas chitosan and seaweed extract showed some control in polytunnel grown plants. Application of Regalia increased the yield of broccoli, although this was correlated with an increase in hollow-stem disorder.

Most elicitors interacted with fungicides, which means that due consideration needs to be given to the whole system of the crop species, varieties, disease causing agents and environment. For example, Harpin was generally only seen to be beneficial when applied on its own and not when mixed with standard fungicides for cabbage or red onion. The same was true for SiTKO-SA on radish (glasshouse-grown), whereas the opposite effect was seen for chitosan plus seaweed extract on cabbage and red onion. Although fungicides are designed to specifically target fungi and not bacteria, their application alters the microbial community associated with the plants, which may then impact the likelihood of bacterial disease. The effects could be positive, i.e. in some way help to also reduce the pathogenic bacteria, but they may be negative, by removing competition for nutrients from pathogenic fungi, thereby providing pathogenic bacteria the opportunity to grow and cause disease. Therefore, it is anticipated that elicitors will be most useful as part of an integrated disease management programme.

## Financial Benefits

Losses from light leaf spot on Brussls Sprouts are thought to account for 10 – 15 % per annum. Under optimal conditions, the average yield of Brussels sprouts in the UK is 8 ton / acre; however, current yields are impacted by effects of disease and climatic events meaning that yields are closer to 6.5 ton / acre. Application of Bion® without additional fungicides resulted in a 4 to 8-fold reduction in disease symptoms on sprout heads (depending on variety), and reduced frequency of application of Bion®, i.e. three compared to six, also

reduced symptomatic disease. Therefore, its application could reduce treatment with standard fungicides both in frequency and volume, resulting in increased profitability.

Application of some of the elicitors (Harpin, SiTKO-SA, Amistar) appear as beneficial or more so for the bacterial diseases on red onion, cabbage and broccoli than application of standard fungicide regimes for cabbage (Amistar, Nativo, Rudis, Signum), red onion (Dithane NT, Invader, Olympus, Unicur, Valbon), or copper oxychloride for broccoli. This is likely to increase saleable crop and therefore financial benefit.

## Action Points

Due consideration must be given to how the various fungicides applied as standard to horticultural crops interact with elicitors, especially for bacterial diseases. One of the major findings of the project so far is that interactions occur between different treatment types (fungicides, elicitors), the local environmental conditions and crop variety which inevitably have consequences on the outcome of disease. Therefore, an important action is to use our knowledge of the underlying ecology of crops to help improve plant health.

Environmental factors have an important impact on the development of bacterial disease, which was clearly demonstrated by comparing radish grown under glasshouse or poly-tunnel conditions. Furthermore, under the conditions used here, broccoli was not particularly susceptible to head-rot. For both disease-systems, multiple bacteria are involved. Therefore, it is necessary to determine which pathogens are responsible for causing the disease, and whether their complement changes under different environments. This will in turn, allow more targeted applications for control.

# 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; (Holden, 2010; 2011)) that assessed the use of elicitors on broccoli and red onion, respectively, and extending to include light leaf spot 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 was to test products that are either readily available in the UK, or have a good chance of being so (Table 1).

Defence elicitors activate the plants natural defence mechanisms to protect the plants from pathogens (Walters *et al.,* 2013; Walters *et al.*, 2014). Different elicitors activate specific pathways resulting in the defence being constantly turned on, constitutive activation, or the defence system being geared up for a rapid response should pathogens attack, termed defence priming. Plant defence responses can be broadly classified based on the type of pathogen that they are activated against. Defence against biotrophic pathogens that feed on living plant tissue is typically mediated through pathways involving the plant hormone salicylic acid (SA). Some Brassica diseases, such as light leaf spot, and powdery mildew are caused by biotrophic pathogens. Necrotrophic Brassica pathogens include *Botrytis cinerea, Alternaria spp and Sclerotinia sclerotiorum.* Defence against necrotrophic pathogens that kill the plant tissue to release nutrients is typically regulated through pathways involving the plant hormones jasmonic acid (JA) and ethylene (ET). These pathways often interact with one another and can be antagonistic resulting in trade-offs in disease resistance where one type of pathogen is controlled at the expense of the plant becoming more susceptible to another type of pathogen (Glazebrook, 2005). Some elicitors work by targeting these plant hormone pathways. Bion® is an elicitor consisting of Acibenzolar-S-Methyl (ASM) also known as benzothiadiazole which is an analogue of SA. Bion® has been shown to reduce disease in many different pathosystems including Alternaria blight (*Alternaria brassicae*) severity on Brassica crops (Thakur et al., 2014) and powdery mildew (*Blumeria graminis*) on cereals (Görlach et al., 1996).

Other elicitors are composed of compounds that activate the basal defence system of plants. Chitin is a component of fungal cell wall and insect exoskeleton. Plants have evolved receptors that recognise chitin and its derivatives and switch on defence systems when the molecule is detected. Using products based on chitin perception has been shown to reduce downy mildew (*Sclerospora graminicola*) in pearl millet when applied as either a foliar spray

or seed treatment (Sharathchandra et al.2004). Harpin is another example of a microbially-derived molecule that induces basal defence in plants. It is a bacterial protein and so most likely to be effective against biotrophic bacterial phytopathogens, which normally trigger the SA pathway. Extracts from plants can also be used to elicit the plant defence response. Plant extracts such as those from seaweed or from *Reynoutria sachalinens* enhance the plant's defence system through non-systemic induced resistance mediated by increasing phenolics, antioxidants, and strengthening cell walls (Wurms et al., 1999; Fofana et al., 2002). There are also microbial 'pesticide' or biocontrol agents that can act as elicitors. *Bacillus subtilis* is more commonly associated as a biological control agent due to its antimicrobial effects but can also act as a defence elicitor inducing systemic resistance that can reduce disease in plants (Lowe et al., 2012).

**Table 1. Elicitors used in FV 417**

| ***Product & Supplier*** | ***Elicitor activity*** | ***Current use*** | ***Prospects for approval*** |
|---|---|---|---|
| Amistar | Strobilurin | Brassicas: control of White Blister, Ring Spot, Alternaria<br>Onion and Radish: control of Downy Mildew | Good |
| Signum | Strobilurin | Brussels sprouts, cabbage, broccoli and radish: control of Downy mildew | Good |
| Bion®<br>(Syngenta) | ASM – salicylic acid mimic | Actiguard (US) Label approved for various including Brassicas for Xanthomonas (black rot) | Fair |
| SiTKO-SA<br>(Growth Products USA) | Salicylic acid and phosphite | Sold as a fertilizer in the USA. Not currently sold in UK, but can be shipped. | Fair. |
| Softguard<br>(Travena, UK) | Chitosan | Sold as a plant health-care or growth promoter product (fertiliser) in the UK | Good |
| Alga600 products<br>(Travena, UK) | Seaweed extracts, laminarin | Sold as a nutritional supplement in the UK, often combined with Softguard. | Good |
| Harpin<br>(Plant Health Care, USA) | Secreted protein derived from *hrpN* of *E. amylovora*. | Sold as a plant health promoter, available in the UK via Plant Health Care, UK office. | Fair |
| Reysa / Regalia / Milsana<br>(Syngenta) | Knotweed extract | To be marketed in Europe by Syngenta. Used on range of crops to control wide range of pathogens. | Fair |

| | | | |
|---|---|---|---|
| Companion (Growth Products USA) | *Bacillus subtilis* GB03 | Sold as a liquid biological fungicide in the USA. Not currently sold in UK, but can be shipped. | 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, GMcG, 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. *alliicola* - Bga) in onion bulbs (NH, JHI lead)

In 2013, trials were established for broccoli, cabbage, Brussels sprouts and radish and in 2014, trials were repeated for the bacterial-infected crops plus for red onions. Light leaf spot trials were carried out in 2013-14 and were repeated in 2015-16. The work has been split into five sections for each of the five crops, for ease of reading.

## 1. Brussels spouts

The Brussels sprout trials were carried out on two grower sites in 2013-14: Blackness in Falkirk (Kettle) and Tyninghame in East Lothian (Drysdale); and in 2015-16 at one site in St Andrews, East Fife (Kettle). Three varieties were used: Cobus (early season), Aurelius (mid-season) and Petrus (late season) and transplants were planted out on 21 May 2013 at Tyninghame, on 24 May 2013 at Blackness and on 20th May 2015 at St Andrews. The varieties were selected to include those of high commercial relevance in Scotland where the field trials were located. Petrus has a market share of approximately 60% compared to Aurelius that varies between 5-10% of the commercial land used for Brussels sprout production. The variety Cobus was included as a light leaf spot susceptible variety even though it is no longer commonly grown in Scotland.

Treatments included the elicitors Bion®, Regalia®, SoftGuard, SiTKO-SA and Companion in 2013-14 which was replaced with Alga 600® in 2015-16 due to issues with availability. Fungicides used in the trial were Signum (BASF), Rudis (Bayer) and Nativo (Bayer). In total, 22 different treatments were applied (summarised in Table 2 and in Table A1.1 of the Appendix for full details of treatment combinations).These treatments were split into the following groups:

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 (various) applied at end July, early September, mid October.

Elicitors were applied at the rates listed in Table 3:

Application rates for the elicitors were those which gave consistent disease control in previous SRUC field work on spring barley, oilseed rape, potato and raspberries. Fungicides were applied at the manufacturers' recommended rate (Table 3).

Three plots were used per treatment, with 20 plants per plot in a randomised block design. Treatments were applied randomly to the 22 plots in each block.

Light leaf spot assessments were carried out in July, August, September, October, November, December, January, February, and March. Percentage of affected leaf area covered with symptoms of light leaf spot was determined on lower leaves, top leaves and sprouts on 10 randomly selected plants per plot. Disease levels were analysed using the area under disease progress curve (AUDPC) to evaluate the effects of various treatments on light leaf spot development over the growing season.

**Table 2. Crops, treatment schedules and elicitors used**.

| ***Crop*** | ***Application and timing in days (date)*** | | ***Elicitors*** |
|---|---|---|---|
| Brussels sprouts (vars. Cobus, Aurelius, Petrus) | Plant transplants | | ○ Bion |
| | Blackness: | 24/05/2013 (Y1) | ○ Regalia |
| | Tyninghame: | 21/05/2013 (Y1) | ○ Softguard |
| | St Andrews | 20/05/2015 (Y2) | ○ SiTKO-SA |
| | Treatment groups | See text above: 3 groups including Single, Combination, Alternate. | ○ Companion (2013)/Alga 600 (2015) |

**Table 3. Concentration of elicitor and fungicide treatments used**:

| ***Elicitor*** | ***Working concentration, application rate*** |
|---|---|
| Bion (ASM = 50%) | 0.175g / L |
| Regalia | 2.5 kg / Ha |
| SoftGuard | 1:500 * |
| SiTKO-SA | 5 L / Ha |
| Companion | 6 L / Ha |
| Alga 600 | 0.6 g / L |
| Tween-20 | 0.01 % |
| Activator-90 wetter | 0.05 % |
| **Fungicides (main a.i.)** | **Working concentration** |
| Nativo (trifloxystrobin) | 0.4 L / Ha |
| Rudis (prothioconazole) | 0.4 L / Ha |
| Signum (pyraclostrobin) | 1 kg / Ha |

* applied to run-off

## Results

### *Year 1 Results*

Crop growth at both sites was uniform. Very little light leaf spot was observed until January 2014 and in both trials, little light leaf spot was detected on the variety Petrus. Levels of light leaf spot on the varieties Aurelius and Cobus varied with site with disease development greater at the Tyninghame site than at Blackness. Moreover, varietal differences were observed, since light leaf spot development on Cobus was consistently greater than on Aurelius. The highest levels of light leaf spot were observed on Cobus at the Tyninghame site.

Since many of the treatments applied had no significant effect on light leaf spot development, particularly on the varieties Aurelius and Petrus, only the results from treatments on cv. Cobus where substantial and significant disease control compared to the untreated control plots were achieved are presented. The full data set showing the light leaf spot development on lower leaves, top leaves and sprouts on the varieties Aurelius, Cobus and Petrus from all 22 treatments can be found in the appendix (Fig. A1 and Fig. A2).

At the Tyninghame site, although the full fungicide programme (T2) significantly reduced light leaf spot development on lower leaves and sprouts of the early season variety Cobus compared to untreated control plants, the largest reductions in light leaf spot severity were obtained with treatments containing elicitors (Figure 1). Application of Bion® alternating with fungicides (T4) also reduced light leaf spot significantly, although the reduction was no greater than that achieved using the standard fungicide treatment only. Of particular interest are treatments T12 (Bion® only) and T21 (Bion® + Regalia®), since here the treatments were only applied three times in the season, compared to the usual six applications for most other treatments. Alternating some of the other elicitor treatments including Regalia®, Softguard or SiTKO-SA with fungicides also reduced light leaf spot development on lower leaves and sprouts although the effects were not typically as strong as observed for treatments containing Bion® (Figure 1). Only the six spray Bion® treatment (T7) significantly reduced light leaf spot development on the top leaves at Tyninghame whereas the Regalia® plus SiTKO-SA treatment (T22) significantly increased disease development (Fig. A1). Some of the elicitor treatments also provided improved disease control compared to the standard fungicide programme at Tyninghame. On lower leaves and sprouts of the variety Cobus all five treatments that contained Bion® as a component (T4, T7, T12, T19, T21) significantly reduced light spot levels more than the standard fungicide programme. Only the six spray Bion® treatment (T7) had a significantly improved disease control performance on the top leaves compared to the standard fungicide programme (Fig. A1)

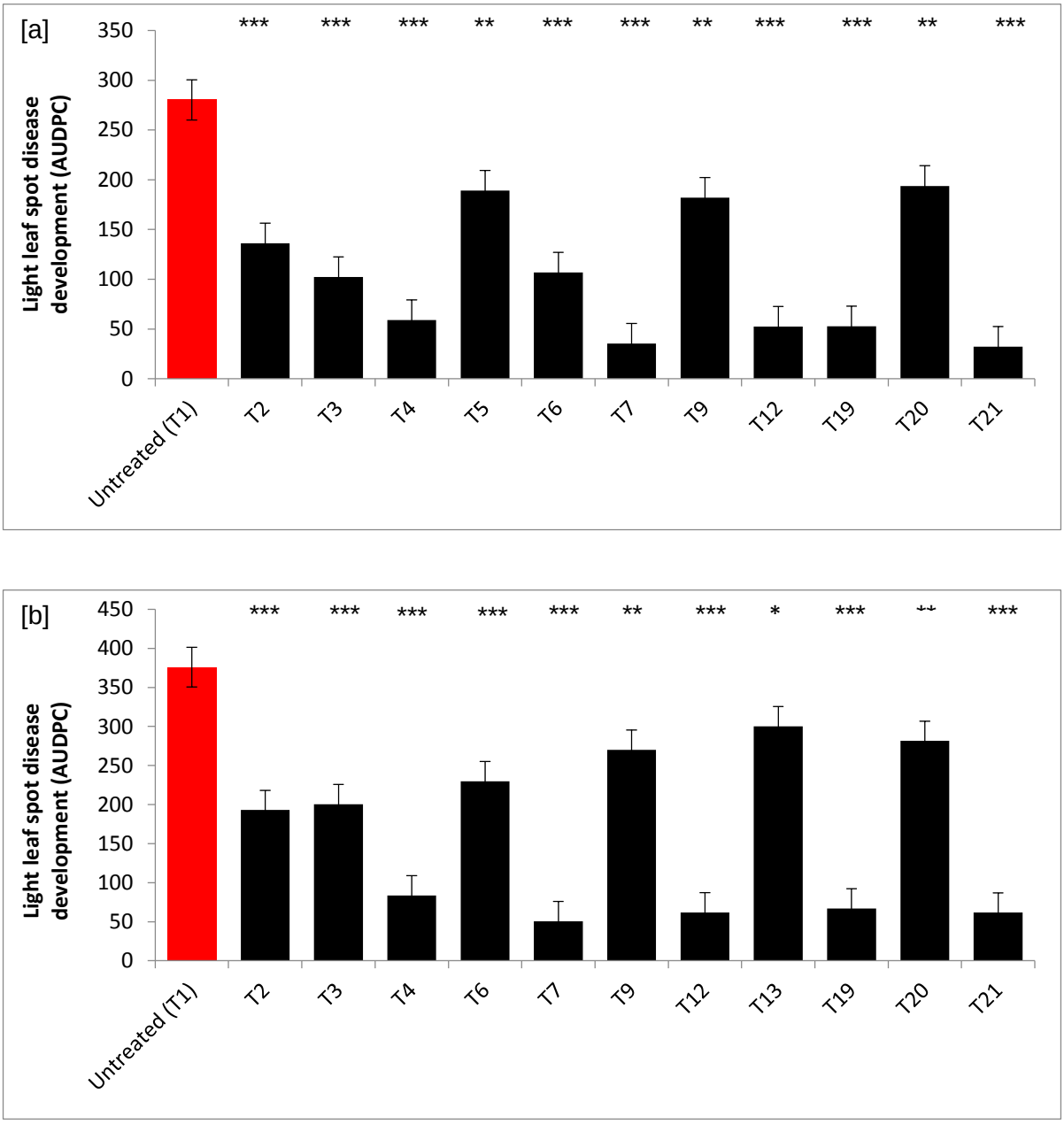



**Figure 1.** Development of Light Leaf Spot on the Brussels sprout variety Cobus at Tyninghame in the 2013-14 season [a] light leaf spot on lower leaves [b] light leaf spot on sprouts Treatments shown are: T2 = fungicide programme ; T3 = alternate Regalia + fungicides; T4 = alternate Bion + fungicides; T5 = alternate Softguard + fungicides; T6 = alternate SiTKO-SA + fungicides; T7 = Bion only – 6 applications; T9 = Softguard only – 6 applications; T12 = Bion only – 3 applications; T13 = Regalia only – 3 applications ; T19 = Bion + Companion– 3 applications; T20 = SiTKO-SA + Companion– 3 applications; T21 = Bion + Regalia– 3 applications. Significant differences compared to the untreated control plots at P<0.001 = ***, P<0.01 = **, P<0.05 = * (Generalised linear model analysis).

At the Blackness site, light leaf spot development was lower than at the Tyninghame site, but here too, the elicitor treatments were most effective although statistically significant effects were only observed on cv. Cobus. On lower and top leaves and on sprouts of Cobus, similar to the Tyninghame site the most consistently effective treatments contained the elicitor Bion® applied in different rate and/or combinations (Figure 2). It should be noted that light leaf spot development was increased on both lower leaves and top leaves of cv. Cobus plants treated with the elicitor combination of Regalia® and SiTKO-SA (T22; Figure 2a, b) and on sprouts treated with SiTKO-SA (T16; Figure 2c) indicating that certain elicitors either alone or in combinations have the potential to cause negative effects on plant health. On the mid season variety Aurelius, light leaf spot levels were even lower and although many treatments reduced symptom development, most of these differences were not statistically significant (results not shown). However there was a general trend that treatments containing the elicitor Bion® typically showing lower levels of disease compared to the untreated plots (Fig. A1 and Fig. A2). None of the elicitor treatments showed significantly improved disease control compared to the standard fungicide programme at Blackness (Fig. A2).

A second trial was run in 2015-2016 (Year 2) at a single site in St Andrews. Light leaf spot levels at the St. Andrews site in 2015-16 were similar to those observed at Tyninghame in 2013-14 with particularly high levels of disease recorded on the sprouts at the late assessments dates. The various fungicide and elicitor treatments applied in this trial significantly reduced disease on the different plant parts in cv. Aurelius and cv. Cobus but not on cv. Petrus. Only the results from treatments on cv. Cobus and Aurelius where statistically significant differences in light leaf spot control were observed are presented. The full data set can be found in the appendix (Fig. A3).

On cv. Cobus disease levels were significantly reduced on the lower leaves, top leaves and sprouts (Figure 3) by alternating Bion® within the fungicide programme (T4), six Bion® sprays alone (T7), three Bion® sprays (T12), combining Bion® and Alga 600 sprays (T19) and combining Bion® and Regalia® sprays (T21). The traditional fungicide programme (T2) and three SiTKO-SA applications (T16) reduced light leaf spot on the lower leaf and sprouts but not on the top leaves. Other elicitor treatments such as alternate Regalia® (T3), Softguard (T5) or SiTKO-SA (T6) sprays within the fungicide programme, six Softguard (T9) or three Softguard sprays alone (T14) and combining Softguard and Alga 600 sprays (T17) also decreased light leaf spot on the sprouts but the level of control was generally not as high as that observed for the treatments that contained Bion ® (Figure 3c).

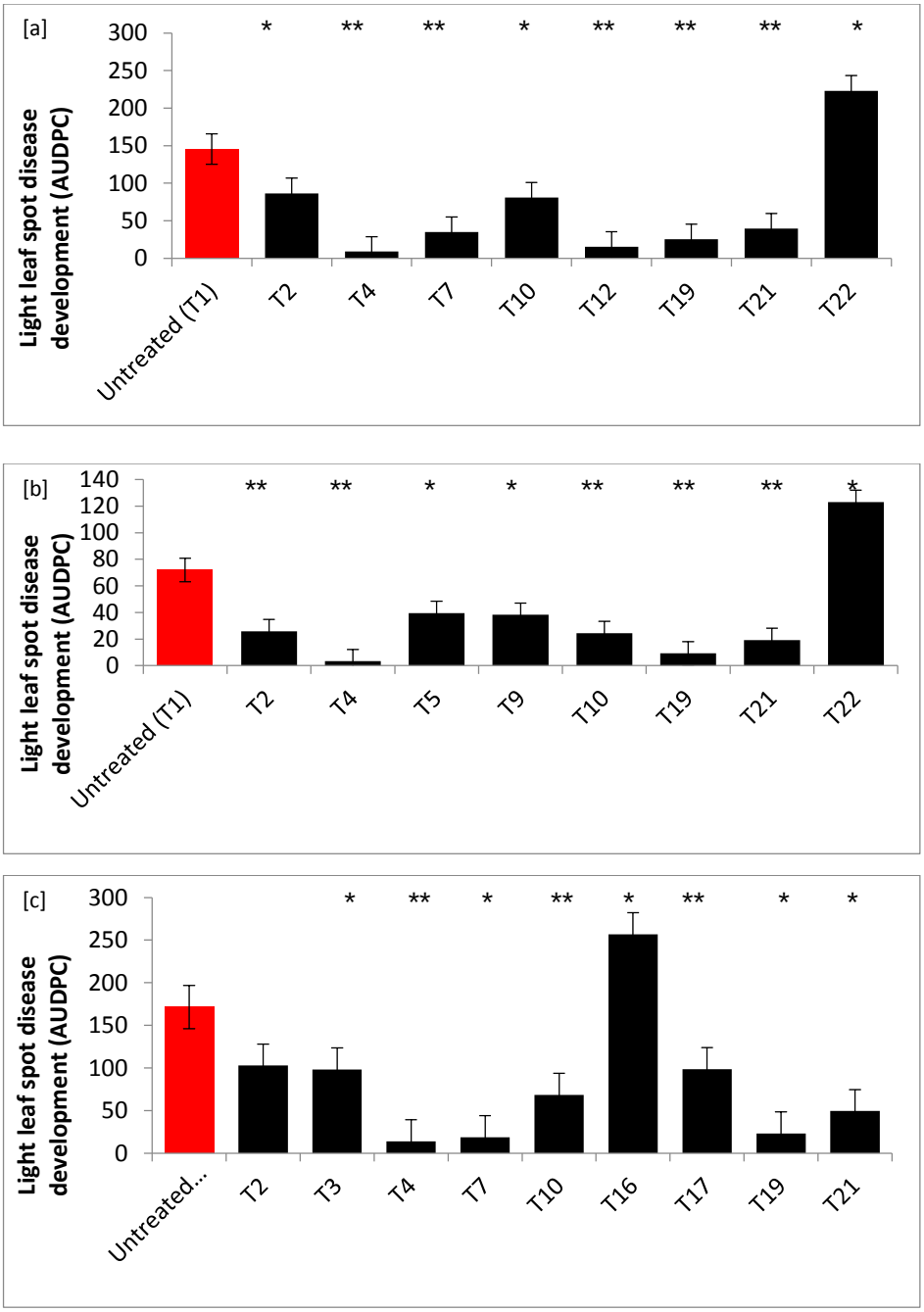



**Figure 2.** Development of Light Leaf Spot on the Brussels sprout variety Cobus at Blackness in the 2013-14 season. [a] light leaf spot on lower leaves [b] light leaf spot on top leaves [c] light leaf spot on sprouts. Treatments shown are: T2 = fungicide programme ; T3 = alternate Regalia + fungicides; T4 = alternate Bion + fungicides ; T5 = alternate Softguard + fungicides; T7 = Bion only – 6 applications ; T9 = Softguard only – 6 applications; T10 = Companion only – 6 applications; T12 = Bion only – 3 applications; T16 = SiTKO-SA only – 3 applications; T17 = Softguard + Companion – 3 applications; T19 = Bion + Companion – 3 applications; T21 = Bion + Regalia only – 3 applications; T22 = Regalia + SiTKO-SA – 3 applications. Significant differences compared to the untreated control plots at P<0.001 = ***, P<0.01 = **, P<0.05 = * (Generalised linear model analysis).

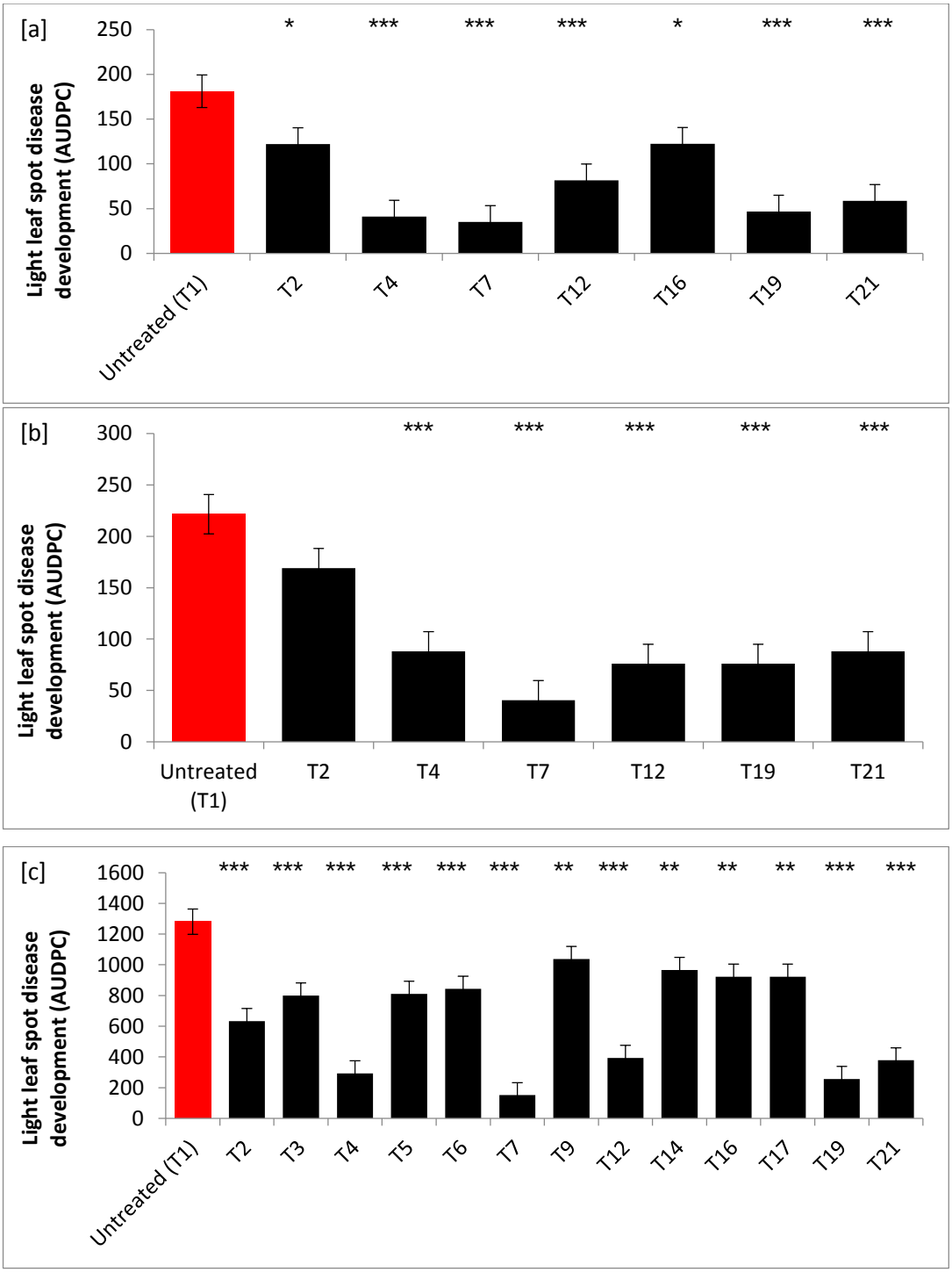



**Figure 3.** Development of Light Leaf Spot on the Brussels sprout variety Cobus at St Andrews in 2015-16 season. [a] light leaf spot on lower leaves [b] light leaf spot on top leaves [c] light leaf spot on sprouts. Treatments shown are: T2 = fungicide programme ; T3 = alternate Regalia + fungicides; T4 = alternate Bion + fungicides ; T5 = alternate Softguard + fungicides; T6 = alternate SiTKO-SA + fungicides; T7 = Bion only – 6 applications ; T9 = Softguard only – 6 applications; T12 = Bion only – 3 applications; T14 = ; Softguard only – 3 applications; T16 = SiTKO-SA only – 3 applications; T17 = Softguard + Alga 600 – 3 applications; T19 = Bion + Alga 600– 3 applications; T21 = Bion + Regalia– 3 applications. Significant differences compared to the untreated control plots at P<0.001 = ***, P<0.01 = **, P<0.05 = * (Generalised linear model analysis).

Light leaf spot development on lower leaves, top leaves and sprouts was significantly controlled on cv. Aurelius (Figure 4) by alternate fungicide and Bion® (T4) within the fungicide programme, by either six (T7) or three Bion® sprays (T12) alone, combining Bion® and Alga 600 sprays (T19) and combining Bion® and Regalia® sprays (T21). Light leaf spot AUPDC on the top leaf and sprout was also significantly reduced by the traditional fungicide programme (T2) or with alternate Softguard (T5) or SiTKO-SA (T6) sprays within the fungicide programme. As observed with cv. Cobus treatments that contain Bion® generally conferred a higher level of control compared to the other elicitor treatments. Treatment 8 with six Regalia® sprays alone significantly increased light leaf spot on the lower leaves (Figure 4a).

On both Cobus and Aurelius some of the elicitor treatments that contained Bion® as a component significantly improved disease control over the standard fungicide programme although the responses were not always consistent between the plant structures scored for disease on the two varieties. Treatments T4 (alternate fungicide and Bion®) and T7 (six Bion® sprays) significantly improved disease control over the standard fungicide programme on lower leaves, top leaves and sprouts on both varieties whereas T12 (three Bion® sprays) and T19 (Three Bion® plus Alga600 sprays) also significantly more effective on the top leaves of Cobus and Aurelius. On the lower leaves T12 was more effective than the standard fungicide programme on Aurelius unlike T19 and T21 (three Bion® plus Regalia® sprays) which reduced light leaf development further than the fungicide programme on Cobus. Three treatments (T12, T19, T21) also improved disease control over the fungicide programme on sprouts of the variety Cobus (Fig. A3).

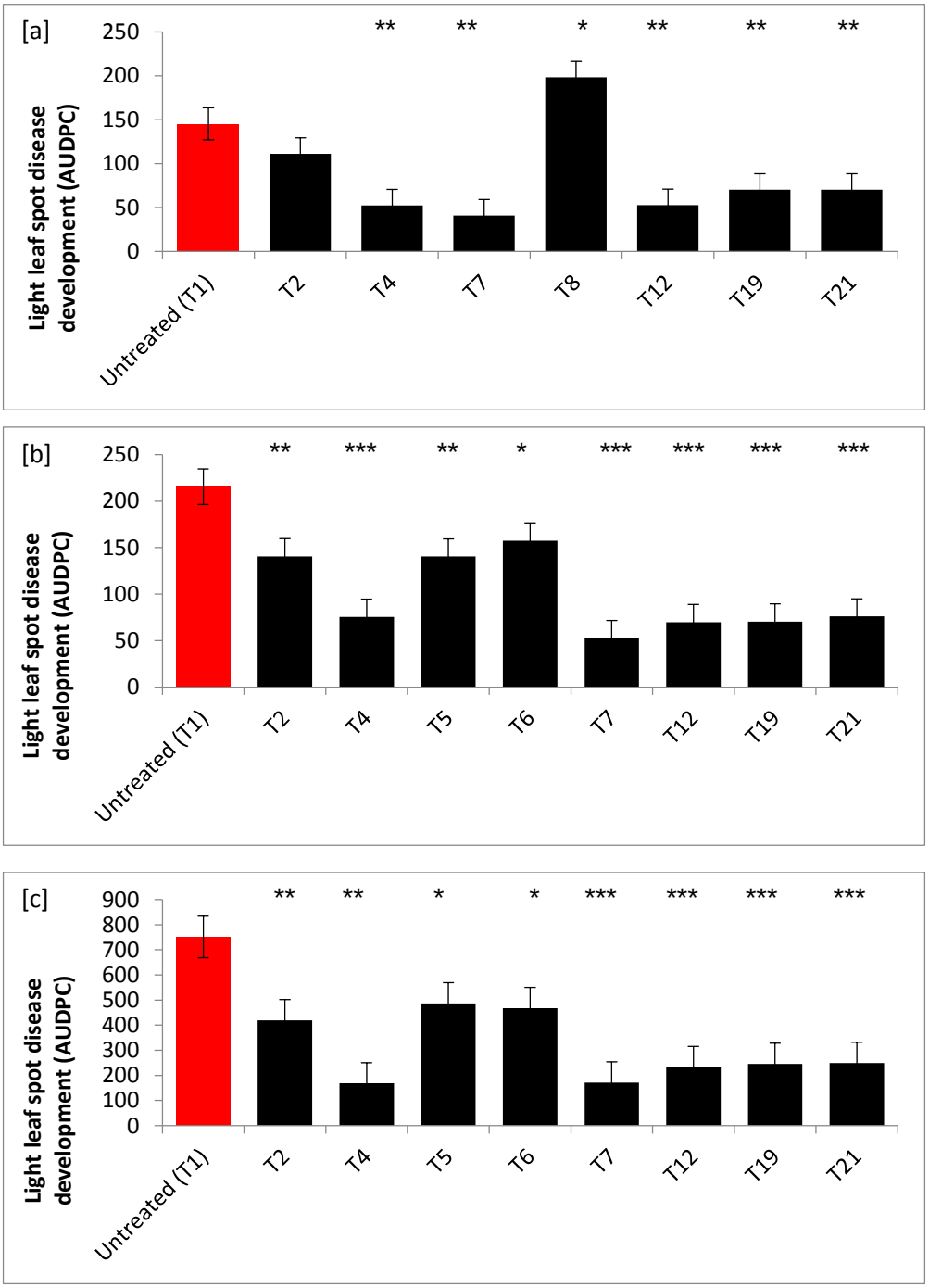



**Figure 4.** Development of Light Leaf Spot on the Brussels sprout variety Aurelius at St Andrews in 2015-16 season. [a] light leaf spot on lower leaves [b] light leaf spot on top leaves [c] light leaf spot on sprouts. Treatments shown are: T2 = fungicide programme; T4 = alternate Bion + fungicides ; T5 = alternate Softguard + fungicides; T6 = alternate SiTKO-SA + fungicides;T7 = Bion only – 6 applications ; T8 = Regalia only 6 sprays; T12 = Bion only – 3 applications; T19 = Bion + Alga 600– 3 applications; T21 = Bion + Regalia– 3 applications. Significant differences compared to the untreated control plots at $P<0.001$ = ***, $P<0.01$ = **, $P<0.05$ = * (Generalised linear model analysis).

## 2. Broccoli

### Materials and methods

#### Experimental trials

Experimental field trials for broccoli were established at the James Hutton Institute, Dundee, Scotland. Parthenon was used as a representative variety that is relevant to East Scotland and is susceptible to head-rot. Treatments were tested in replicate plots of three using a randomised design, and 20 replicate plants were assessed per treatment. Broccoli (2013, 2014) was grown in open-ended poly-tunnels on 100 m x 25 m sites in an attempt to control the environmental conditions in order to induce disease. Mist irrigation was used on a time system, 3-times daily for 15 minutes each time. Once the transplants established, growth appeared uniform.

#### Applications

Elicitors were applied as the sole treatment for broccoli and either applied independently or in conjunction with fungicides for cabbage, radish and onion. The timing of application was dependent on plant development and all treatments were applied with hand-held sprayer until run-off (Hozelock, 1.25 L, 20-35psi). Two applications of elicitors were applied to broccoli at 14-day intervals. The treatment schedules and elicitors used are listed in Table 4 and the application concentrations and rates for elicitors, fungicides and additives are listed in Table 5. 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 to directly compare all of the different disease systems in FV 417.

Disease was assessed on a 5-point scale from no disease (0) to extensive spread of symptomatic disease over > 60 % of the head (4). Incidence of disease was scored as the presence or absence of any symptoms. Broccoli heads were harvested at maturity (~ 80 days after transplant establishment) and yield determined from fresh weight in Year 1 (stem length was ~ 5 -7 cm below the lower set of florets); and from head width in Year 2. As a direct correlation was found between fresh harvested weight and head width, head width was used a suitable indicator without the need for harvest. Hollow stem disorder was scored as presence / absence. Analysis of variance was carried out using Excel (Microsoft) or Genstat (VSN International) computer programmes.

*Pseudomonas fluorescens, Ps. marginalis* and *Pectobacterium carotovorum* (collectively known as head-rot bacteria) were routinely grown initially in rich media (Luria Bertani) at 28 $^{o}$C to saturation. Prior to inoculation of broccoli plants, they were sub-cultured into defined

media (HMM, Hrp Minimal Media for the *Pseudomonas* species and MOPS supplemented with amino acids and g for *Pectobacterium*) designed to optimise expression of virulence factors (at 25 $^{o}$C). A bacterial inoculum containing a mixture of all three species was applied at $10^6$ cfu/ml by foliar spray, until run-off. PCR amplification was used to detect pseudomonads (Spasenovski et al. 2009) from inoculated plant material. The potential for head-rot bacteria to cause disease on their respective hosts was verified under laboratory conditions, by firstly surface-sterilising retail broccoli heads with 200 ppm hypochlorite and inoculating directly with bacteria. Symptomatic disease was assessed 7 days, at which point, characteristic soft-rot symptoms became evident.

**Table 4. Crops, treatment schedules and elicitors used**.

| ***Crop*** | ***Application and timing in days (date)*** | | ***Elicitors*** |
|---|---|---|---|
| Broccoli (var. Parthenon) | Plant transplants | Day 0: 21/06/2013 (Y1), 01/06/2014 (Y2) | ○ SiTKO-SA<br>○ Harpin |
| | Treatment 1 (elicitors) | 39 | ○ Chitosan & seaweed extract |
| | Apply bacteria 1 | 48 | ○ Amistar |
| | Treatment 2 (elicitors) | 54 | ○ Probenazole & Amistar |
| | Apply bacteria 2 | 58 | ○ Coded product DM31 |
| | Disease assessment | 80 | ○ Regalia |

**Table 5. Concentration of elicitor and fungicide treatments used**:

| ***Elicitor / additive*** | ***Working concentration, application rate*** |
|---|---|
| Bion (ASM = 50%) | 1 mM; |
| Probenazole | 0.2 mM |
| Regalia | 4.9 L / Ha |
| SoftGuard + Alga600 | 1:600 *; 1:500 |
| SiTKO-SA | 5 L / Ha |
| ProAct (Harpin) | 0.15 kg / Ha |
| Coded product DM31 | 0.5% v/v (2L/ha) |
| Tween-20 | 0.01 % |
| ***Wetting Agent*** | ***Working concentration*** |
| Activator-90 wetter | 0.05 % |
| ***Fungicides (main a.i.)*** | ***Working concentration*** |
| Amistar (azoxystrobin) | 1 L / Ha |
| Cuprokylt (copper oxychloride) | 5 kg / Ha |

Application rate at 200L / Ha unless specified * applied to run-off

## Results

Application of the head-rot cocktail of bacteria to broccoli resulted in the presence of characteristic soft-rot (Figure 5). The least amount of symptomatic head-rot was seen with application of Amistar, which was also found in previous trials (FV378). The incidence of disease was not sufficiently high to carry out a statistical analysis of the effect of the elicitors. Attempts to increase the likelihood of disease included irrigation with a mist irrigation system to raise the local humidity of the canopy. In addition, in Year 2 (2014) a herbicide, Aramo®, was applied to disrupt the waxy cuticle on the well-developed florets and so provide greater access for the head-rot bacteria. Neither strategy appeared to significantly increase head-rot incidence.

Yield measurements were taken because different treatments appeared to affect head development. Yield varied significantly between treatments and the effect was reproduced in both years (fresh weight of the broccoli heads in Year 1, head width in year 2). The combination of chitosan and seaweed extract had the most detrimental effect on yield, although those that increased yield (Harpin, Years 1 & 2 and Regalia, Year 1 tended to be associated with ‘hollow stem’.

Phytotoxic damage was observed with application of two of the elicitors (Bion and Regalia) on mature leaves of broccoli. However, the effect was limited to the affected leaf and did not appear to be systemic (not shown).

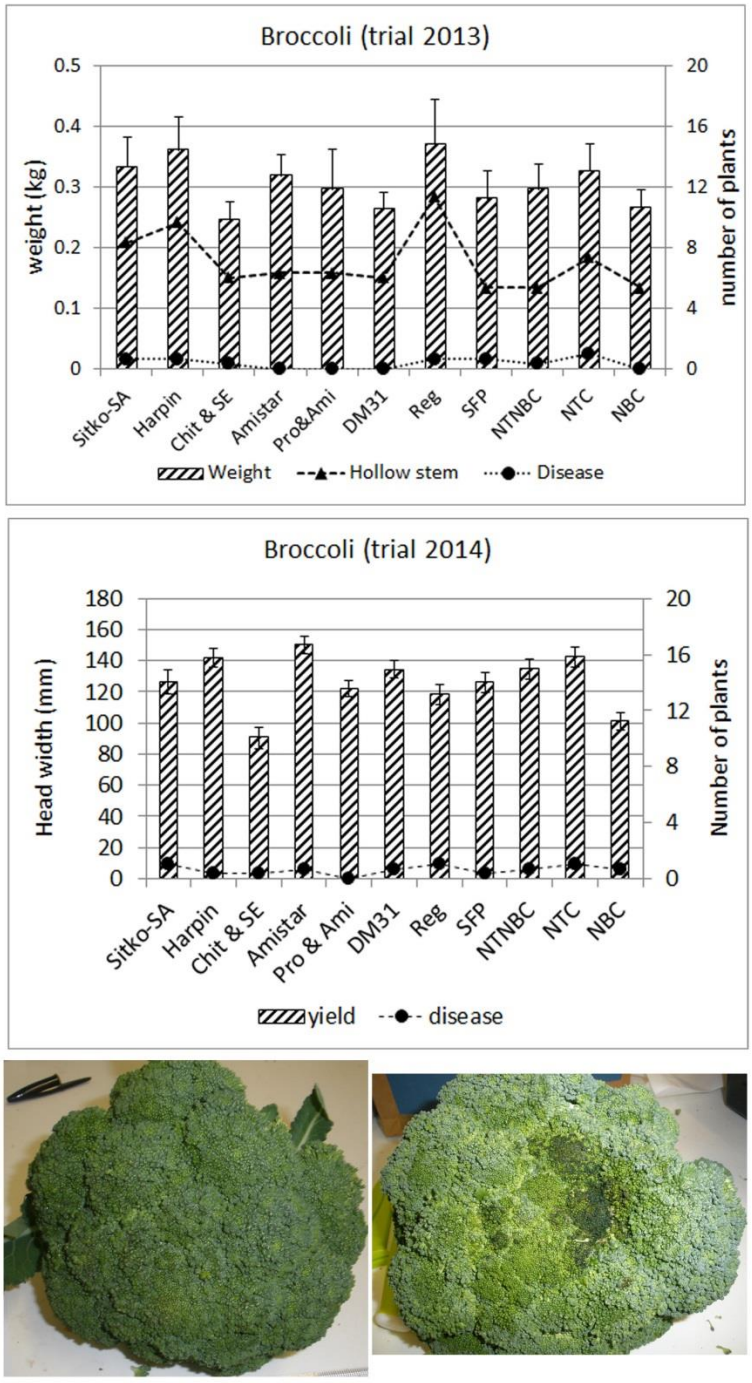



**Figure 5**. Broccoli

Top: Yield and number of diseased plants in Year 1 (2013) and Year 2 (2014). The chart shows the average yield (hatched bars) with standard deviation; and symptomatic disease (circles), together with the number of plants showing hollow stem disorder (triangles) for Year 1

Bottom: head-rot symptoms on broccoli heads (Parthenon). An uninfected control (A) compared to plants showing severe symptoms (B).

## 3. Cabbage

## Materials and methods

### Experimental trials

Experimental field trials for cabbage were established at the James Hutton Institute, Dundee, Scotland. Treatments were tested in replicate plots of three using a randomised design, and 20 replicate plants were assessed per treatment. Cabbage (2013, 2014) was grown in open-ended poly-tunnels on 100 m x 25 m sites. Tundra (a Savoy x White cross) was selected as the most relevant variety for the region and one that is susceptible to *Xanthomonas*. Poly-tunnels allowed for some degree of control over climatic conditions. The crop was irrigated with a mist irrigation system, 3-times daily for 15 minutes each time.

### Applications

Elicitors were applied either applied independently or in conjunction with fungicides. The timing of application was dependent on plant development and all treatments were applied with hand-held sprayer until run-off (Hozelock, 1.25 L, 20-35psi). Four applications of elicitors were applied to cabbage at one-month intervals. The treatment schedules and elicitors used are listed in Table 6 and the concentration and application rates of the elicitor, additives and fungicides are listed in Table 7. Controls included the no-treatment control (NTC) and the standard fungicide programme (SFP). Information to allow comparison of the treatments for the different disease systems in FV 417 is provided in Appendix 1.

Disease was assessed visually *in situ*: the incidence of symptomatic disease was scored as 'Healthy' or 'Diseased' and the extent assessed on 5-point scale of symptoms, from no symptoms (0) to symptoms across > 60 % leaf (4). Incidence of disease was scored as presence / absence of symptoms. Analysis of variance was carried out using Excel (Microsoft) or Genstat (VSN International) computer programmes.

*Xanthomonas campestris* pv. *campestris* (Xcc) were routinely grown initially in rich media (Luria Bertani) at 28 $^{o}$C to saturation. Prior to inoculation of cabbage plants, they were sub-cultured into defined media (NYGB, Nutrient Yeast Glucose Broth) designed to optimise expression of virulence factors (at 25 $^{o}$C). A bacterial inoculum was applied at $10^{6}$ cfu/ml by foliar spray, until run-off. The potential for Xcc to cause disease was verified under laboratory conditions, by firstly surface-sterilising Savoy cabbage leaves with 200 ppm hypochlorite and inoculating directly with bacteria. Symptomatic disease was assessed after ~ 7 days, at which point, characteristic disease symptoms became evident.

**Table 6. Concentration of elicitor and fungicide treatments used**

| ***Elicitor*** | ***Working concentration, application rate*** |
|---|---|
| Bion (ASM = 50%) | 1 mM |
| SoftGuard + Algal 600 | 1:600 * ; 1:500 |
| ProAct (Harpin) | 0.15 kg / Ha |
| Tween-20 | 0.01 % |
| ***Wetting Agent*** | ***Working concentration*** |
| Activator-90 wetter | 0.05 % |
| ***Fungicides (main a.i.)*** | ***Working concentration*** |
| Amistar (azoxystrobin) | 1 L / Ha |
| Nativo (trifloxystrobin) | 0.4 L / Ha |
| Rudis (prothioconazole) | 0.4 L / Ha |
| Signum (pyraclostrobin) | 1 kg / Ha |

Application rate at 200L / Ha unless specified

* applied to run-off

**Table 7 Crops, treatment schedules and elicitors used**

| ***Crop*** | ***Application and timing in days (date)*** | | ***Elicitors*** |
|---|---|---|---|
| Cabbage (var. Tundra) | Plant transplants | Day 0: 08/07/2013 (Y1), 08/07/2014 (Y2) | ○ Bion<br>○ Harpin<br>○ Chitosan & seaweed extract<br>*applied (i) alone; (ii) + fungicide; (iii) alternating with fungicide* |
| | Apply bacteria | 28 | |
| | Treatment 1 (elicitor +/- Signum) | 60 | |
| | Treatment 2 (elicitor +/- Amistar Top) | 91 | |
| | Treatment 3 (elicitor +/- Rudis) | 122 | |
| | Treatment 4 (elicitor +/- Nativo) | 151 | |
| | Disease assessments | 122 – 191 | |

## Results

Cabbage inoculated with *Xanthomas campestric* pathovar *campestris* (Xcc) developed characteristic lesions along the leaf margins and small black lesions on the leaves (Figure 6). Elicitors were applied to cabbage, either alone, mixed with and in combination with the standard fungicide program (SFP) or alternating with the SFP. Harpin used in the absence of SFP has a beneficial effect on the level of disease compared to the other two treatments,

Bion and chitosan + seaweed Extract. Both disease severity (i.e. the extent of symptoms) and incidence (the number of plants showing symptoms) was significantly lower with Harpin application in the 2014 trial. The same effect was also evident in the 2013 trial, but to a lesser and not significant extent. The effect was also seen when the Harpin application was alternated with the SFP, but not when Harpin was used together with the standard fungicide treatments. A no-bacteria control was not included because cabbage transplants are known to carry a degree of inoculum, indeed disease was observed in the un-infected plants in the guard plots (not shown). It is notable that application of any treatment appeared to induce greater disease symptoms and severity, as the lowest level of disease occurred in the no-treatment control. This may have occurred as a consequence of altering the native microflora through the addition of fungicide treatments.

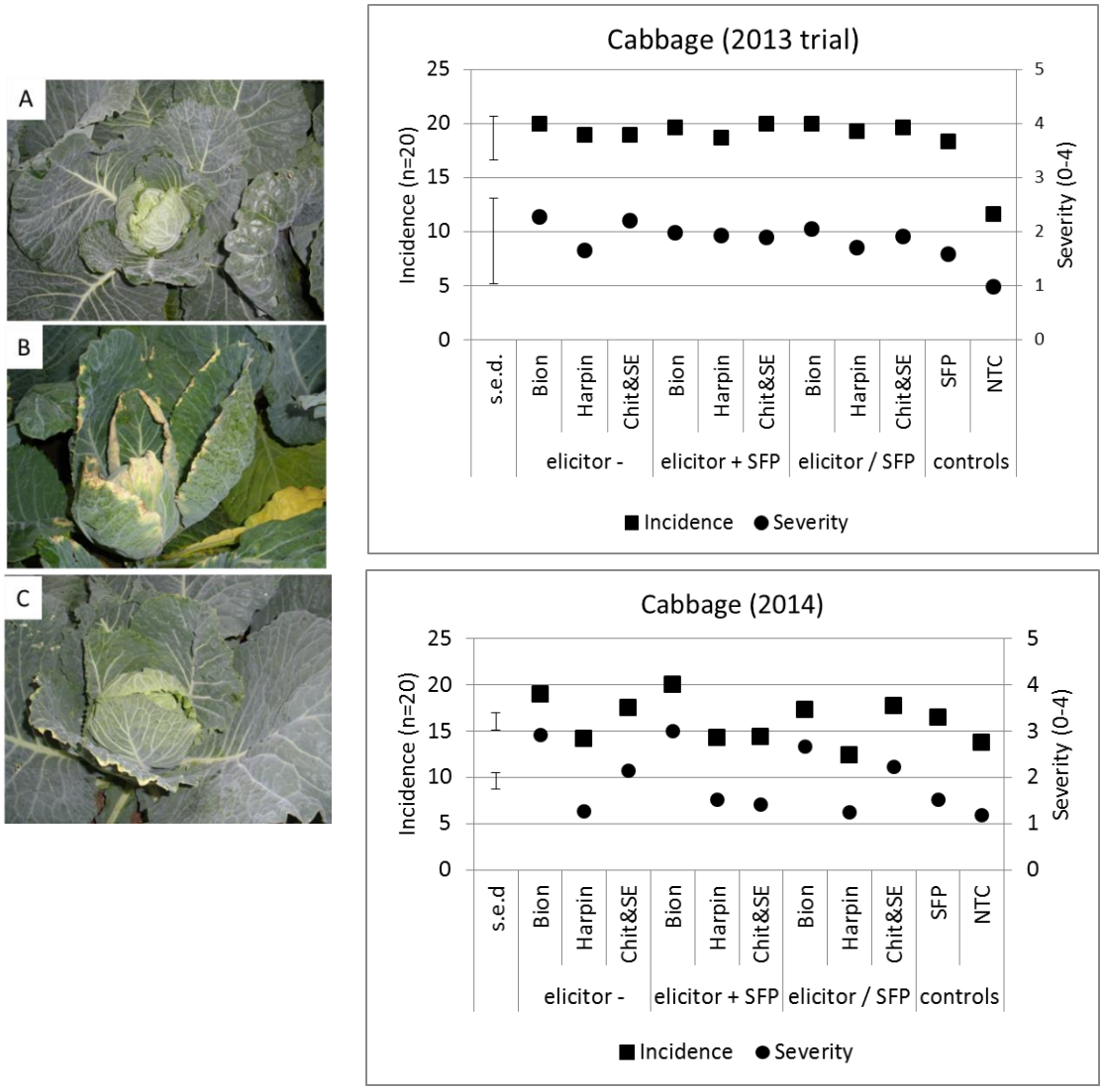



**Figure 6.** Cabbage.

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 for Year 1 (top, 2013) and Year 2 (bottom, 2014). Disease severity was measured on a 0 (no disease) to 5 (maximum disease) scale and the average shown. Disease incidence relates to the number of plants that showed symptoms in each plot (averaged for n=20). The error bar represents the standard error of the difference. Values are provided for the controls (SFP; NTC).

## 4. Radish

### Materials and methods

#### Experimental trials

Experimental field trials for radish were established at the James Hutton Institute, Dundee, Scotland. Treatments were tested in replicate plots of three in a randomised design, and 20 replicate plants were assessed in the glasshouse per plot, or 40 plants in 1.3 m x 0.25 m plots in the polytunnel. In 2013, radish was grown from seed for four to five weeks in compost, in a glasshouse, at ambient temperature and in 2014, radish was grown from seed.

#### Applications

Elicitors were applied either applied independently or in conjunction with fungicides for radish. The timing of application was dependent on plant development and all treatments were applied with hand-held sprayer until run-off (Hozelock, 1.25 L, 20-35psi). Two applications of elicitors were applied to radish at 7 days intervals between 7 and 10 days after seedling emergence. The treatment schedules and elicitors used are listed in Table 8 and the application rates and concentrations are listed in Table 9. Controls included the no-treatment control (NTC), no-bacteria control (NBC), no-treatment and no-bacteria control (NBNTC) and the standard fungicide programme (SFP). Information to allow comparison of the treatments with other crops is provided in the Appendix.

Disease was assessed visually for all crops *in situ*: the incidence of symptomatic disease was scored as 'Healthy' or 'Diseased' and the severity assessed on 5-point scale of symptoms. Analysis of variance was carried out using Excel (Microsoft) or Genstat (VSN International) computer programmes.

It should be noted that the 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, pathogenic *Pseudomonas* species obtained directly from infected radish plants (supplied by Liz Johnson, in June 2013) were used in the trials, for which the species identity was confirmed by 16S sequence determination. This came to light during the initial glasshouse-based studies; all data presented in the Results is representative of the combined cocktail of two radish-pathogenic *Pseudomonas* species. Radish blight bacteria were routinely grown initially in rich media (Luria Bertani) at 28 $^{o}$C to saturation. Prior to radish plant inoculation, they were sub-cultured into HMM (Hrp-minimal media) designed to optimise expression of virulence factors (at 25 $^{o}$C). A bacterial inoculum was applied at $10^{6}$ cfu/ml by foliar spray, until run-off. PCR amplification was used to verify the presence of pseudomonads (Spasenovski et al.

2009) from inoculated plant material. The potential for radish-blight bacteria to cause disease was verified under laboratory conditions, by firstly surface-sterilising leaves of radish plants grown in our glasshouse with 200 ppm hypochlorite and inoculating directly with bacteria. Symptomatic disease was assessed after a defined time 3 – 5 days, after which characteristic disease symptoms became evident.

**Table 8. Crops, treatment schedules and elicitors used**

| ***Crop*** | ***Application and timing in days (date)*** | | ***Elicitors*** |
|---|---|---|---|
| Radish | Sow from seed | 03/07/2014 – 10/10/2014 | SiTKO-SA |
| (vars. Celesta and Expo) | 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 | ○ Regalia |
| | | (varied dependent on growth rate *) | *applied (i) alone; (ii) + fungicide* |

***** Timing for treatments and disease assessment was dependent on when the plants were grown: the intervals were of 1 week for plants sown in the summer months and 2 weeks for plants sown in the autumn months, to account for differences in growth rate.

**Table 9. Concentration of elicitor, additives and fungicide treatments used**

| ***Elicitor*** | ***Working concentration, application rate*** |
|---|---|
| Bion (ASM = 50%) | 1 mM |
| Regalia | 4.9 L / Ha |
| SoftGuard | 1:600 * |
| Alga600 | 1:500 * |
| SiTKO-SA | 5 L / Ha |
| ProAct (Harpin) | 0.15 kg / Ha |
| Tween-20 | 0.01 % |
| ***Wetting Agent*** | ***Working concentration*** |
| Activator-90 wetter | 0.05 % |
| ***Fungicides (main a.i.)*** | ***Working concentration*** |
| Amistar (azoxystrobin) | 1 L / Ha |
| Signum (pyraclostrobin) | 1 kg / Ha |

Application rate at 200L / Ha unless specified

* applied to run-off

## Results

Two sets of trials were established to assess elicitors on radish plants. Glasshouse trials allowed preliminary testing under very controlled conditions in Year 1, and the experiments were then repeated outdoors, in poly-tunnel grown plants in Year 2. Radish leaves spray-inoculated with a cocktail of two pathogenic radish isolates of *Pseudomonas* developed blight-like symptoms on the leaves that in some instances became necrotic (Fig. 7). The *Pseudomonas* species isolates were used in combination to ensure disease since they were isolated together from the leaves of a single infected radish plant (June 2013) together. Elicitors were tested on radish either independently, or applied with fungicide.

Disease severity and incidence was extensive on glass-house grown plants (Year 1). The high degree of variation in disease severity meant that most treatments were not significantly different from each other (Fig. 8). In general, greater disease severity was observed in Celesta and the addition of SFP had a negative effect. Application of SiTKO-SA provided the highest level of protection, for both varieties and in the presence or absence of SFP. In contrast, application of Bion had a negative effect for Celesta, less so for Expo. Symptomatic disease occurred on the NBC controls, but this was attributed to spray application in an enclosed glasshouse cubicle with organisms that are known to persist in aerial water droplets for extended periods of time.

Repeating the trial in a poly-tunnel (Year 2) markedly reduced levels of disease, despite using the same bacterial inoculum and treatment conditions (Fig. 9). Furthermore, variation was seen between experimental repeats, suggesting that environmental factors played a significant influence on occurrence of disease. However, some similarities to the glasshouse trial were seen: e.g. there were variety-dependent differences in the level of disease (99.9 % confidence); application of chitosan and seaweed extract resulted in reduced disease incidence for both varieties; and Bion provided the least protection for Celesta. It should be noted that although the differences between the combination of treatment and variety were not significant to the 95% confidence level, they were marginal at 94.6 %. In the second year experiments, Regalia provided protection for Celesta applied in combination with the SFP, with a disease severity of 1.5, compared to a disease severity of 3 for Celesta treated with SFP only in the glasshouse trial and complete protection, i.e. 0 compared to 0.5 for the polytunnel trial, indicating that the effect was as a result of the elicitor. SiTKO-SA did not appear to provide any noticeable protection for poly-tunnel grown plants compared to those in the glasshouse.

To confirm that the symptomatic disease was caused by the strains used to inoculate the plants, bacteria were collected from lesions present on symptomatic tissue (Fig. 7). Bacteria

were isolated on Pseudomonas-selective medium (Oxoid code CM0559, similar to Kings' A medium) and subject to DNA fingerprinting. A BOX PCR approach was used because it provides a sub-species specific signature based on the presence of repetitive DNA sequences in a particular isolate. The BOX PCR amplicons were compared to the strains used as the inoculum and other library strains. Strains with the same BOX PCR signatures as the inoculum strains were present, proving that the inoculating strains were able to cause symptomatic disease in the field. However, multiple other strains were also recovered, indicating that the lesions contain multiple different pseudomonads. Questions as to which strains are pathogenic and whether any dominate on radish leaves was beyond the scope of this project. However, the occurrence of multiple potentially pathogenic isolates should be borne in mind for antibacterial treatment options.

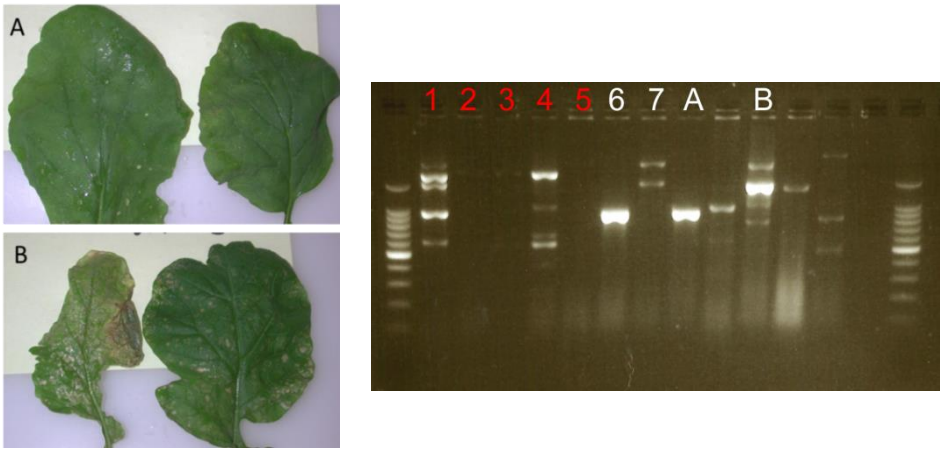
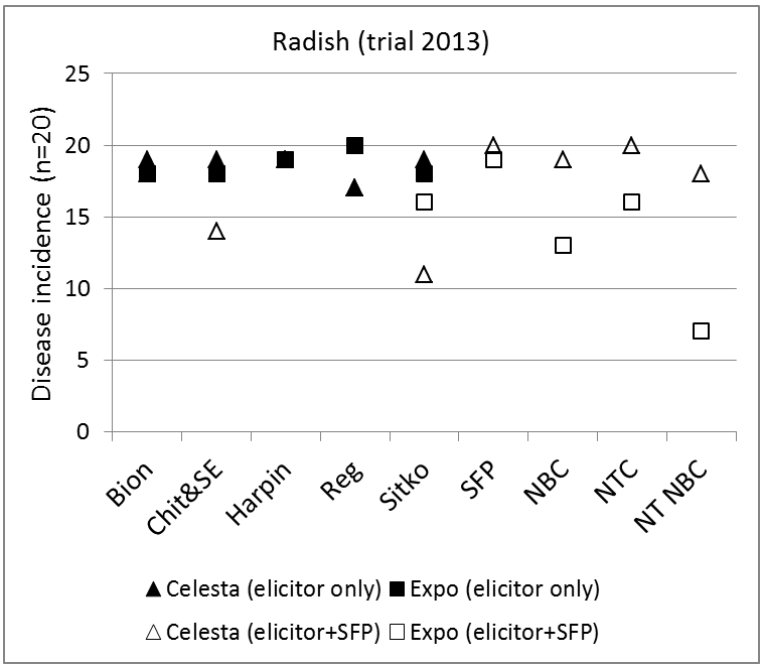



**Figure 7.** 'Pca' symptoms on radish (var.Celesta) leaves. Symptoms of low level (A) and high level (B) of disease severity, i.e. 1 and 4 on the disease severity scale. BOX PCR signatures of bacteria recovered from symptomatic lesions (1 to 7) and used in the infecting inoculum (A, B). Those in red letters were distinct from the strains used for inoculation.



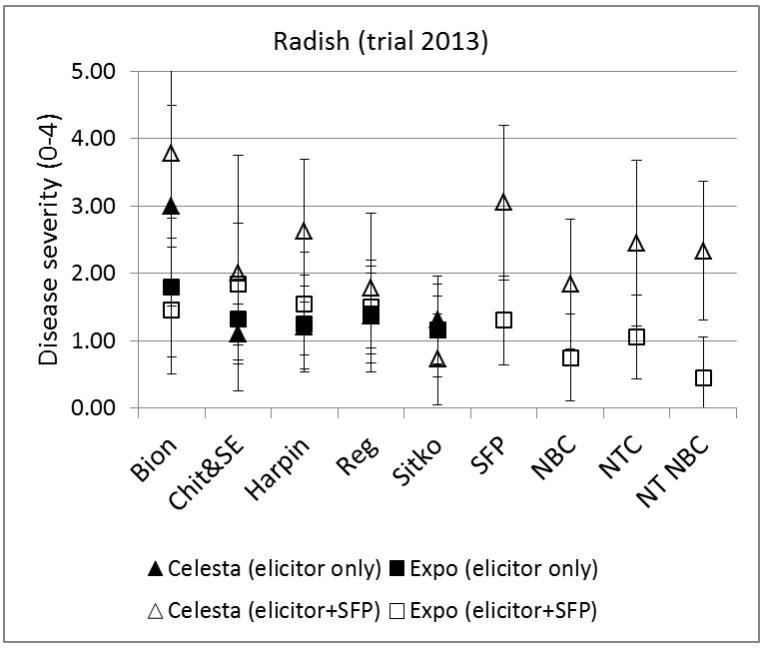




**Figure 8**. Radish disease assessment and severity for Year 1, Glasshouse trial (2013). Disease severity was measured on a 0 (no disease) to 4 (maximum disease) scale. The average severity is shown for elicitor treatments incorporated into the fungicide programme (open symbols) or used independently (closed symbols). Triangles represent var. Celesta and squares var. Expo. The error bar represents the standard error of the difference. Values are also provided for the controls (SFP; NTNBC; NTC; NBC).

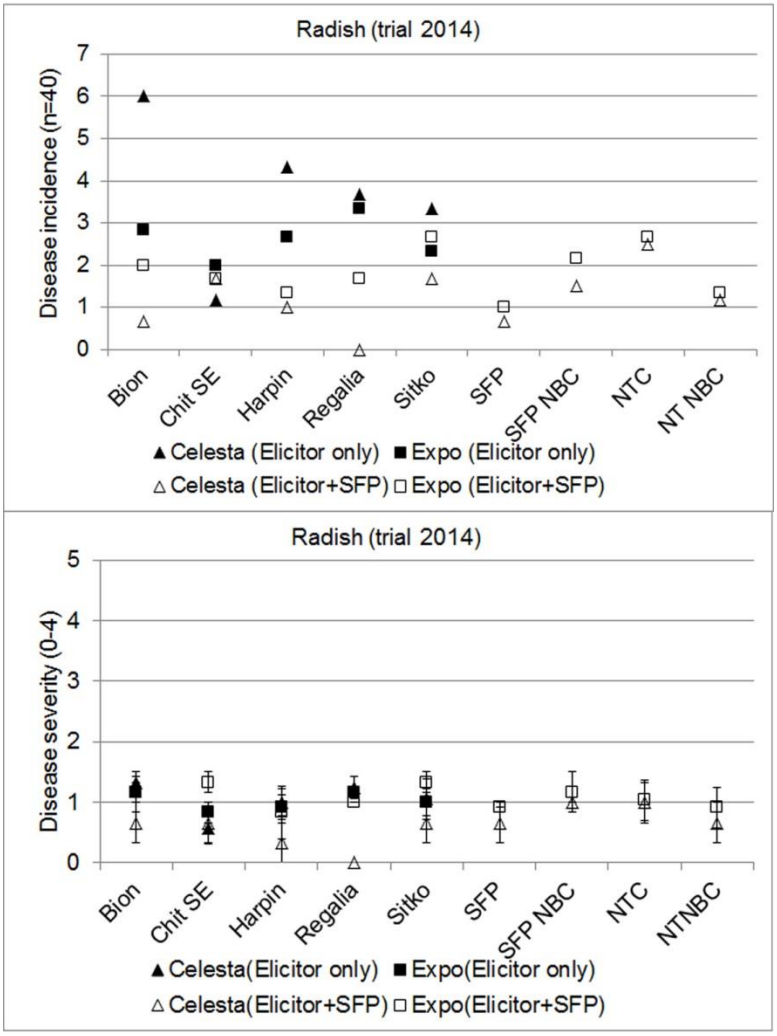



**Figure 9**. Radish disease assessment and severity for Year 2 Polytunnel trial ( 2014). Disease severity was measured on a 0 (no disease) to 4 (maximum disease) scale. The average severity is shown for elicitor treatments incorporated into the fungicide programme (open symbols) or used independently (closed symbols). Triangles represent var. Celesta and square var. Expo. The error bar represents the standard error of the difference. Values are also provided for the controls (SFP; NTNBC; NTC; NBC).

## 5. Red onion

### Materials and methods

#### Experimental trials

An experimental field trial for onion was established at the James Hutton Institute, Dundee, Scotland. Treatments were tested in replicate plots of three in a randomised design, and 20 replicate plants were assessed per plot. Onion (2014 only) was grown from seed in an open-ended poly-tunnel on 100 m x 25 m sites. Poly-tunnels were used to reduce the environmental variability from wind and rain, and the plants were irrigated with a mist irrigation system. Red Onion was selected as it is susceptible to soft rot in the bulbs from bacterial infection.

#### Applications

Elicitors were either applied independently or in conjunction with fungicides. The timing of application was dependent on plant development and all treatments were applied with a hand-held sprayer until run-off (Hozelock, 1.25 L, 20-35psi). Applications of elicitors were applied to onion at 9 days intervals, 11 weeks after sowing, after development of four to five true leaves. The treatment schedules and elicitors used are listed in Table 10 and the rates and concentrations are listed in Table 11. 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 the Appendix to allow comparison with other disease systems used in FV 417.

*Burkholderia gladioli* pathovar *allicola* (Bga) were routinely grown initially in rich media (Luria Bertani) at 28 $^{o}$C to saturation. Prior to plant inoculation, they were sub-cultured into defined media (MOPS supplemented with glycerol and amino acids) designed to optimise expression of virulence factors (at 25 $^{o}$C). Onion plants were damaged to mimic damage from hail stones by scrapping the leaves lightly with a plastic comb and a bacterial inoculum was applied at $10^{6}$ cfu/ml by foliar spray, until run-off. The potential for Bga to cause disease on red onion bulbs was verified under laboratory conditions, by firstly surface-sterilising purchased onion bulbs with 200 ppm hypochlorite, and stab-inoculating with bacteria. Symptomatic disease was assessed after 7 days, at which time, characteristic disease symptoms became evident on the onion scales. Bulbs were harvested, set to prevent bolting (28 °C for three weeks), and cold stored (1-3 °C for 4 weeks).

**Table 10. Concentration of elicitor and fungicide treatments used**

| ***Elicitor*** | ***Working concentration, application rate*** |
|---|---|
| Bion (ASM = 50%) | 1 mM; |
| Regalia | 4.9 L / Ha |
| SoftGuard | 1:600 * |
| Alga600 | 1:500 * |
| SiTKO-SA | 5 L / Ha |
| ProAct (Harpin) | 0.15 kg / Ha |
| Tween-20 | 0.01 % |
| ***Wetting Agent*** | ***Working concentration*** |
| Activator-90 wetter | 0.05 % |
| ***Fungicides (main a.i.)*** | ***Working concentration*** |
| Dithane NT (mancozeb) | 2.5 kg/ Ha |
| Invader (mancozeb) | 2.5 kg/ Ha |
| Olympus (azoxystrobin) | 2.5 L / Ha |
| Unicur (fluoxastrobin) | 1.25 L / Ha |
| Valbon (mancozeb) | 1.6kg / Ha |

Application rate at 200L / Ha unless specified

* applied to run-off

**Table 11 Crops, treatment schedules and elicitors used**

| ***Crop*** | ***Application and timing in days (date)*** | | ***Elicitors*** |
|---|---|---|---|
| Onion (var. Red Baron) | Sow seeds | 19/03/2014 (Y2) | |
| | Treatment 1 (elicitor +/- Olympus) | 77 | ○ Bion |
| | Treatment 2 (elicitor +/- Unicur, Dithane) | 86 | ○ SiTKO-SA |
| | Treatment 3 (elicitor +/- Valbon) | 95 | ○ Harpin |
| | Treatment 4 (elicitor +/- Unicur, Dithane) | 104 | ○ Chitosan & seaweed extract |
| | Apply bacteria | 111 | ○ Regalia |
| | Treatment 5 (elicitor +/- Valbon) | 114 | |
| | Treatment 6 (elicitor +/- Unicur) | 121 | |
| | Treatment 7 (elicitor +/- Invader) | 161 | |
| | Treatment 8 (elicitor +/- Invader) | 140 | |
| | Harvest and heat treat (28 $^{o}$C) | 161 | |
| | Cold store (0 – 4 $^{o}$C) | 182 | |
| | Biomass and disease assessment | 210 | |

Disease was not assessed visually for onion bulbs because it is not always an obvious measure of bacterial infection as bulbs can carry a relatively high inoculum without showing visible symptoms. Instead, the bacteria were quantified from onion bulb cores post-storage: heat treated for 21 days followed by cold stored for 28 days (Table 11). To assess the extent of disease, Bga bacteria were quantified from representative samples of each bulb. A sterile cork borer was used to take two samples (total ~ 1 g) from the bulb, suspended in 2.5 ml PBS media and vortex'ed for 30 seconds. The suspension was left at room temperature for 2 hours to allow the bacteria to migrate from the onion tissue to the PBS. Serial dilutions of the suspension were made, plated on Bga selective media (Salles*, et al.*, 2006) and incubated at 28 $^{o}$C for 48 – 60 hours. White colonies were verified as Bga from PCR amplification of Bga-specific sequences (Whitby*, et al.*, 2000). No other contaminating bacteria were detected on the agar plates. Analysis of variance was carried out using Excel (Microsoft) or Genstat (VSN International) computer programmes.

## Results

Red onion was grown in a polytunnel from seed to maturity. Elicitors were applied either independently or incorporated into a standard fungicide programme. A bacterial inoculum of Bga was applied mid-way through the treatment schedule, between treatments # 4 and 5 (out of a total of eight), following light damage applied to the leaves. Damage in this manner was applied to mimic the likely route and timing of entry for the bacteria into damaged leaves, e.g. as a consequence of hailstone-damage. prior to the bacterial load being assessed. Visual disease was not used for the assessment because it can be subjective and is not always a measure of bacterial infection. However, disease was apparent and extensive, such that it was not possible to harvest some individual plants (Fig. 10). This also meant that biomass could not be assessed in a robust manner. Soft rot and the accompanying characteristic smell were apparent during storage and sampling.


**Figure 10**. Red Onion (var. Red Baron)

Various levels of symptomatic disease at the point of harvest (A, B) and after heat set and storage (C, D).

Application of elicitors significantly affected the bacterial load. Application of Harpin or Bion alone reduced the levels of bacteria compared to that seen in the standard fungicide programme (SFP) (Fig. 11). Once again, there was an interaction between the elicitors and fungicides, such that inclusion of Harpin or SiTKO-SA with SFP increased the number of bacteria significantly compared to the SFP control. Application of Chitosan and Seaweed extract or Regalia alone significantly increased the bacterial load ($p < 0.05$), although this effect was reduced with the inclusion of SFP. *Burkholderia* was also present in the NBC control plants. We think that this organism, like the pseudomonads, is able to persist in water droplets, where it can infect other plants via drift. It was also noted that as with cabbage, the process of treatment application appears to encourage infection.

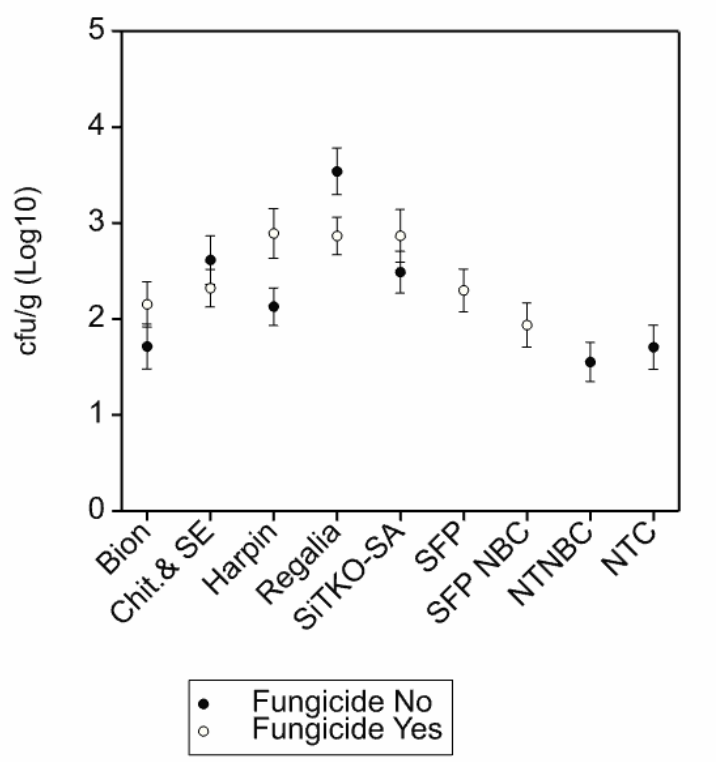



**Figure 11.** The average number of Bga bacteria recovered from treated plants (n=20 x 3 reps), expressed as cfu per gram of fresh tissue, with the standard error bars shown. The values for the elicitors are presented in the absence (filled circles) or presence (empty circles) of the SFP, here termed 'Fungicide'. Values are also provided for the controls (SFP; SFP NBC; NTNBC; NTC;). The limit of detection in this assay is ~ 1.25 $Log_{10}$ cfu/g.

## Discussion

The effect of elicitors was tested on cabbage, radish, broccoli plants and onions bulbs infected with phytopathogenic bacteria, and on naturally-occurring light leaf spot-infected Brussels sprouts. Some elicitors had a beneficial effect in reduction of bacterial disease symptoms in radish, cabbage and onion, and on fungal symptoms in sprouts. It is notable that different elicitors were effective for each disease system (Harpin for cabbage and onion, chitosan and seaweed extract for radish, Bion (and others) for sprouts), indicative of specificity in their effect. Interactions occurred between elicitor treatments and standard fungicides for the bacterial infections. It is possible that the fungicides affect the plant response, the native microbiota or a combination of both, which in turn alters the ability of the bacteria to colonise and cause disease. In addition, variety also appeared to have an effect on elicitor treatment in radish plants (Celesta vs Expo) and Brussel sprouts. Furthermore, application of elicitors increased broccoli yield, 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 (in Hrp-minimal medium, to induce expression of bacterial virulence factors). 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, which may be because the experiments were conducted in mid-summer rather than in the autumn, when disease can be more prevalent. Attempts that were made to increase to disease incidence including use of a polytunnel with mist irrigation and application of a de-waxing agent, did not appear to be successful. It is also possible that other microbes associated with the plants were able to compete with the head-rot bacteria. Similarly, multiple pseudomonads were recovered from lesions in radish leaves, for polytunnel grown plants, which are likely to have come from an environmental source such as soil splash, since these are soil-borne bacteria. Together, this suggests that a multitude of causative organisms can be responsible for diseases associated with opportunistic pathogens. In contrast, *Xanthomonas campestris* pv. *campestris* (Xcc) and *Burkholderia gladioli* pathovar *allicola* (Bga) are thought to have a relatively narrow host range and demonstrate much more specificity towards cabbage and onion, respectively.

On Brussels sprouts, it was encouraging to see that several elicitor treatments reduced light leaf spot development significantly. The standard fungicide programme also reduced light leaf spot development, but not always significantly and in the data shown in this report, several

elicitor treatments consistently out-performed the fungicide treatment. Results of elicitor treatments varied between varieties with more consistent disease control responses observed on the more susceptible variety Cobus. There was also a trend for elicitor-mediated disease control on the variety Aurelius although this was only statistically significant at the St Andrews site in 2015-16. No response to the elicitors was observed on the variety Petrus which developed low levels of disease in all three trials. The high levels of resistance on Petrus may be sufficient to keep light leaf spot development at levels where defence elicitors have no additional effect on disease control. Of particular interest is effect of treatments containing Bion® on its own, and Bion® combined with other elicitors both applied just three times in the season, and yet providing very good control of light leaf spot. Bion® is known to control diseases on a range of crops and is used commercially in various parts of the world (Walters et al., 2013; 2014). In other work at SRUC, Bion® has been shown to provide effective control of diseases on various crops e.g. root rot on raspberry and clubroot on cabbage and winter oilseed rape (Walters, McGrann and colleagues, unpublished results), although it was less effective at controlling foliar diseases on spring barley. Interestingly, on spring barley and winter oilseed rape, more effective disease control was provided by a combination of elicitors, including Bion® (Walters et al., 2011; 2012; 2014). 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® treatment on clubroot development on winter oilseed rape. On Brussels sprouts, it seems likely that in treatments involving a combination of Bion® and other elicitors the major disease suppressing effect is the result of resistance induced by Bion®, since in most cases the other elicitors when applied without Bion had little effect on light leaf spot development. In line with work on other crops (e.g. Walters et al., 2011), differences were observed in varietal responses to the elicitor treatments. If elicitors are to be used to control light leaf spot on Brussels sprouts, it will be important to determine the responsiveness of particular varieties to the elicitor of choice.

How to optimally apply defence elicitors such as Bion® to integrate their use within a disease management programme requires further testing. Data from the Brussels sprouts trials suggests that as few as three Bion® sprays can reduce light spot levels during the growing season. However, as timing of application and plant age are important factors in priming the defence response it is important to know how persistent the defence response mediated by an elicitor application is. For module grown crops the possibility of applying an elicitor to modules prior to field transplantation could offer an initial priming of the defence response that could lower initial disease development in the field. Such an application would be more practical to apply and could subsequently be supported by an integrated disease

management strategy that can combine elicitor applications with a reduced fungicide doses to maintain disease levels below the economic thresholds.

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 belongs to a conserved family of harpin proteins in phytopathogenic bacteria. Their main role is as translocators, to facilitate delivery of effector proteins into host cells, although they have other functions and can be perceived as MAMPs (microbe-associated molecular pattern) by the plant (Choi *et al.*, 2013). Importantly, harpins from a number of diverse phytopathogenic bacteria have been shown to elicit a defence response. In our trial, application of Harpin conferred protection in multiple disease systems: Xcc in cabbage and Bga in onion. In addition, proteins of the harpin family have been shown to promote plant growth, which may explain the effect observed in broccoli.

SiTKO-SA contains a combination of salicylic acid (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 of 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 light leaf spot on Brussels sprouts may be as a direct result of SiTKO-SA- / ASM-mediated induced defence. Chitosan has been well characterised as an elicitor of plant defence as various forms of the polymer are found in fungal cell walls and are recognised by the plant as PAMPs (Trouvelot *et al.,* 2014). Chitosan triggers an alternative defence pathway, through jasmonic acid, which is required for recognition of nectrotrophic pathogens. However, there is feedback and cross-over into other pathways, which may explain the beneficial effect on opportunistic pseudomonads on radish leaves.

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.

The finding for Harpin in particular is extremely encouraging for the treatment of bacterial pathogens of horticultural crops, but more work is required to better understand the interaction with fungicides and how best to use Harpin alongside other pathogen control treatments.

## Conclusions

We have found that application of Harpin on its own is as effective as standard fungicides in controlling bacterial disease of cabbage and red onions, and it had a positive effect on broccoli yield. Furthermore, significant control of light leaf spot was demonstrated on early and mid-season varieties of Brussels sprouts using elicitors. Bion® and a combination of Bion® and other elicitors were found to be particularly effective when applied just three times during the season. Other elicitors that provided a degree of protection against opportunistic pathogens were chitosan and seaweed extract for radish (especially those grown in a glasshouse), and Amistar was beneficial for broccoli yield.

It was notable that there appeared to be specificity in the response to elicitor application, and interactions with other factors such as fungicides, plant variety and growth conditions. This indicates that due consideration must be given to the whole system: plant, disease agents, treatment strategies (nutrition and pesticides) and environment in order to best promote plant health.

## Knowledge and Technology Transfer

British Soil Society, soil amendment meeting, 30/05/2013, SRUC, Edinburgh. Presentation "Microbial bioeffectors: 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.

Crop Protection in Northern Britain 2016 meeting, 23/02/2016, Dundee. Presentation and conference proceedings entry "Harpin-mediated protection against bacterial pathogens of horticultural crops", Holden.

Dundee Food and Flower Festival, 05/09/2014, Dundee. Interactive stall "Putting microbes on the table", Holden

Vegetable Consultant Association annual meeting, 01/12/2014, Stilton. Invited presentation, "Plant Defence Elicitors to Control Brassica Pathogens", Holden

UK Brassica and Leafy Salad Conference, 28/01/2015, Peterborough. Presentation, 'Control of light leaf spot on Brussels sprouts using resistance elicitors'. Walters.

## 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

Choi, M.S., Kim, W., Lee, C., and Oh, C.S. (2013). Harpins, multifunctional proteins secreted by Gram-negative plant-pathogenic bacteria. *0894-0282* 26**,** 1115-1122. doi: 10.1094/mpmi-02-13-0050-cr.

Holden, N. (2010). "FV 378: Assessment of plant elicitors to induce resistance against head-rot in broccoli", in: *Final report.* (ed.) Agriculture and Horticulture Development Board.).

Holden, N. (2011). "FV 393: Reducing bacterial infection in seed onions through the use of plant elicitors", in: *Final Report.* (ed.) Agriculture and Horticulture Development Board.).

Cui X, Harling R. (2006) Evaluation of bacterial antagonists for biological control of broccoli head rot caused by *Pseudomonas fluorescens*. *Phytopathology* **96**: 408.

Fofana B, McNally DJ, Labbé C, Boulanger R, Benhamou N, Séguin A, Bélanger RR**.** 2002. Milsana-induced resistance in powdery mildew-infected cucumber plants correlates with the induction of chalcone synthase and chalcone isomerase. *Physiological and Molecular Plant Pathology* **61,** 121-132.

Glazebrook J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* **43,** 205-227.

Görlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel KH, Oostendorp M, Staub T, Ward E, Kessmann H. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *The Plant Cell* **8,** 629-643.

Graham JH, 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, Sutton M. (2002). Spear rot of Calabrese (Horticultural Development Company).

La Torre A, Spera G, Lolleti D (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.

Lowe A, Rafferty-McArdle SM, Cassells AC**.** 2012. Effects of AMF-and PGPR-root inoculation and a foliar chitosan spray in single and combined treatments on powdery mildew disease in strawberry. *Agricultural and Food Science* **21,** 28-38.

Oxley SJP, Walters DR (2012) Control of light leaf spot (Pyrenopeziza brassicae) on winter oilseed rape (Brassica napus) with resistance elicitors. Crop Protection 40, 59-62.

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

Salles, J.F., Samyn, E., Vandamme, P., Van Veen, J.A., and Van Elsas, J.D. (2006). Changes in agricultural management drive the diversity of *Burkholderia* species isolated from soil on PCAT medium. *Soil Biology and Biochemistry* 38**,** 661-673

Sharathchandra RG, Raj SN, Shetty NP, Amruthesh KN, Shetty HS. 2004. A chitosan formulation Elexa™ induces downy mildew disease resistance and growth promotion in pearl millet. *Crop Protection* **23,** 881-888.

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

Thakur M, Sohal BS, Sandhu PS. 2014. Impact of elicitor spray on Alternaria blight severity and yield of *Brassica juncea* and *Brassica napus* species. *Journal of Oilseed Brassica* **5,** 78-82.

Trouvelot S, Héloir M, Poinssot B, Gauthier A, Paris F, Guillier C, Combier M, Tdra L, Daire X, Adrian M. (2014) Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. *Frontiers in Plant Science* **5:** 00592

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

Walters DR, Havis ND, Paterson L, Taylor J, Walsh DJ (2011). Cultivar effects on the expression of induced resistance in spring barley. Plant Disease 95, 595-600.

Walters DR, Ratsep J, Havis ND (2013). Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany* **64,** 1263-1280.

Walters DR, Havis ND, Paterson L, Taylor JD, Walsh DJ, Sablou C (2014). Control of foliar pathogens of spring barley using a combination of resistance elicitors. Frontiers in Plant Science 5:241. doi:10.3389/fpls.2014.00241.

Walters DR, Newton AC, Lyon GD (2014). *Induced Resistance for Plant Disease Control: a sustainable approach to crop protection.* Second Edition. Wiley-Blackwell.

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**:85-88.

Whitby, P.W., Pope, L.C., Carter, K.B., Lipuma, J.J., and Stull, T.L. (2000). Species-specific PCR as a tool for the identification of *Burkholderia gladioli*. *Journal of Clinical Microbiology* 38**,** 282-285

Wurms K, Labbé, Benhamou N, Bélanger RR. 1999. Effects of Milsana and benzothiadiazole on the ultrastructure of powdery mildew haustoria on cucumber. *Phytopathology* **89,** 728-736.

## Appendix

Additional information on crop systems and treatments:

**Table A1.1 (Brussels sprouts)**

| ***Brussels sprouts*** | |
|---|---|
| Varieties: | Cobus, Aurelius, Petrus |
| | Planted from transplants (May*)* |
| Fungicides: | Signum (BASF), Rudis (Bayer), Nativo (Bayer) |
| Elicitors: | Bion®, Regalia®, SoftGuard, Companion®, 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 (various) applied at end July, early September, mid October |
| light leaf spot assessments | July, August, September, October, November, December, January, February, March |

## 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 |

Note in 2015/16 Companion was replaced by Alga600 is all treatments

**Table A2. (broccoli)**

| ***Broccoli*** | |
|---|---|
| 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)**

| ***Cabbage*** | |
|---|---|
| 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)**

| ***Radish*** | |
|---|---|
| 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) |

**Table A5. (onion)**

| ***Onion*** | |
|---|---|
| Varieties: | Red Baron |
| | Planted from seed (April) |
| Fungicides: | Olympus, Unicur + Dithane NT DF, Valbon, Invader |
| Elicitors: | Bion, chitosan and seaweed Extract, Harpin, Regalia, SiTKO-SA |
| Standard fungicide programme: | Applied every 9 days 15 weeks post seeding: (1) Olympus; (2) Unicur + Dithane NT DF; (3) Valbon; (4) Unicur + Dithane NT DF; (5) Valbon; (6) Unicur; (7) Invader; (8) Invader |
| Treatment 1: | Elicitors only in place of SFP |
| Treatment 2: | Elicitors plus SFP |
| Assessments | Bacterial load post cold storage (Nov) |

Figure A1. Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at Tyninghame site in 2013-14. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c). Treatments were as follows: Treatment 1 = untreated: Treatment 2 = standard fungicide programme: Treatment 3 = Alternate Regalia and fungicides: Treatment 4 = Alternate Bion and fungicides: Treatment 5 = Alternate Softguard and fungicides: Treatment 6 = Alternate SiTKO-SA and fungicides: Treatment 7 = Six Bion sprays: Treatment 8 = Six Regalia sprays: Treatment 9 = Six Softguard sprays: Treatment 10 = Six Companion sprays: Treatment 11 = Six SiTKO-SA sprays: Treatment 12 = Three Bion sprays: Treatment 13 = Three Regalia sprays: Treatment 14 = Three Softguard sprays: Treatment 15 = Three Companion sprays : Treatment 16 =Threes SiTKO-SA sprays: Treatment 17 = Three Softguard plus Companion sprays : Treatment 18 = Three Regalia plus Companion sprays : Treatment 19 = Three Bion plus Companion sprays : Treatment 20 = Three SiTKO-SA plus Companion sprays : Treatment 21 = Three Bion plus Regalia sprays: Treatment 22 = Three Regalia plus SiTKO-SA sprays. Significant reduction in disease development differences compared to the standard fungicide treated (T2) plots at $P<0.001$ = ***, $P<0.01$ = **, $P<0.05$ = * (Generalised linear model analysis).

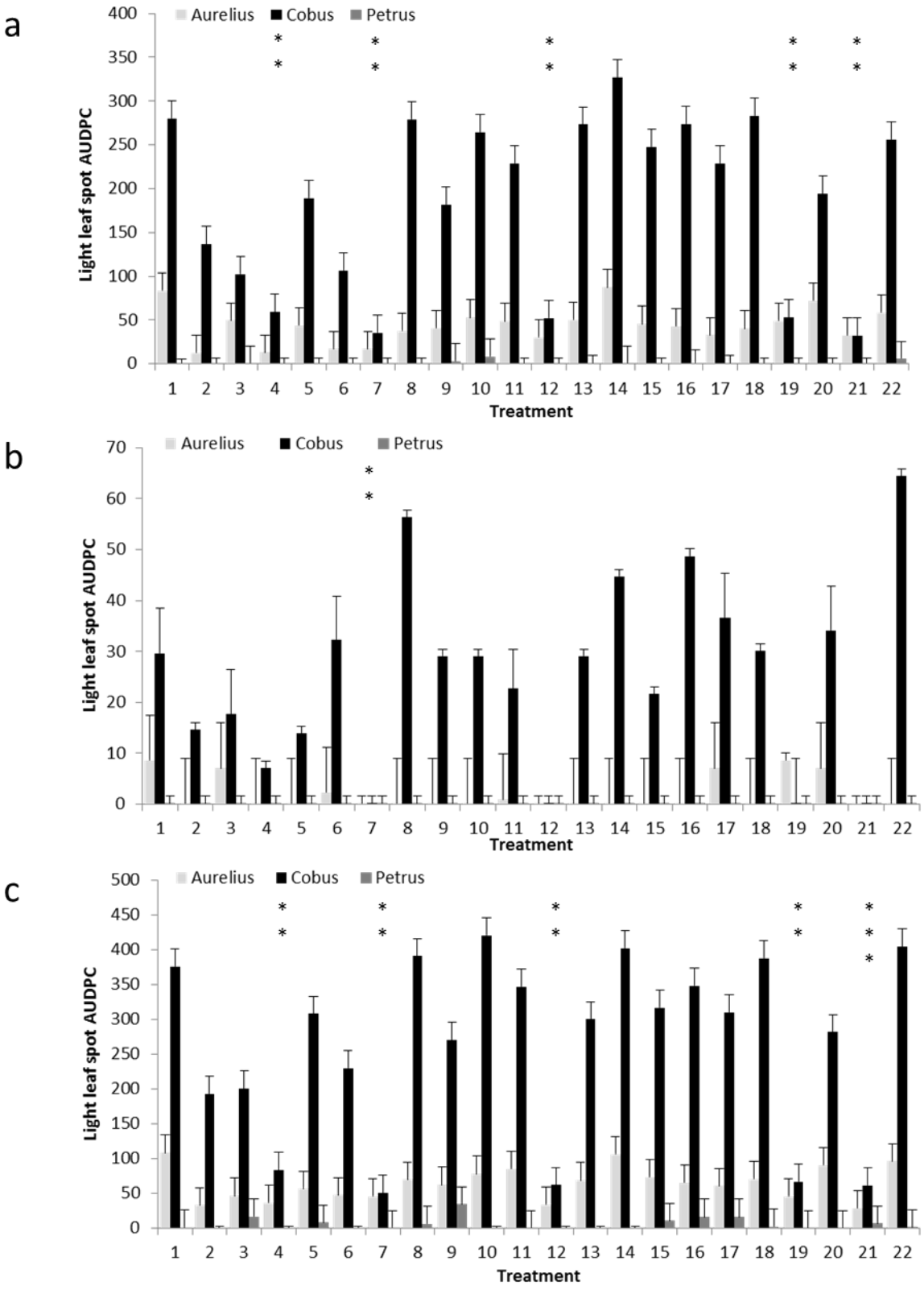



**Figure. A1.** Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at Tyninghame site in 2013-14. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c).

Figure A2. Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at Blackness site in 2013-14. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c). Treatments were as follows: Treatment 1 = untreated: Treatment 2 = standard fungicide programme: Treatment 3 = Alternate Regalia and fungicides: Treatment 4 = Alternate Bion and fungicides: Treatment 5 = Alternate Softguard and fungicides: Treatment 6 = Alternate SiTKO-SA and fungicides: Treatment 7 = Six Bion sprays: Treatment 8 = Six Regalia sprays: Treatment 9 = Six Softguard sprays: Treatment 10 = Six Companion sprays: Treatment 11 = Six SiTKO-SA sprays: Treatment 12 = Three Bion sprays: Treatment 13 = Three Regalia sprays: Treatment 14 = Three Softguard sprays: Treatment 15 = Three Companion sprays: Treatment 16 =Threes SiTKO-SA sprays: Treatment 17 = Three Softguard plus Companion sprays : Treatment 18 = Three Regalia plus Companion sprays : Treatment 19 = Three Bion plus Companion sprays : Treatment 20 = Three SiTKO-SA plus Companion sprays : Treatment 21 = Three Bion plus Regalia sprays: Treatment 22 = Three Regalia plus SiTKO-SA sprays. Significant reduction in disease development differences compared to the standard fungicide treated (T2) plots at $P<0.001$ = ***, $P<0.01$ = **, $P<0.05$ = * (Generalised linear model analysis).

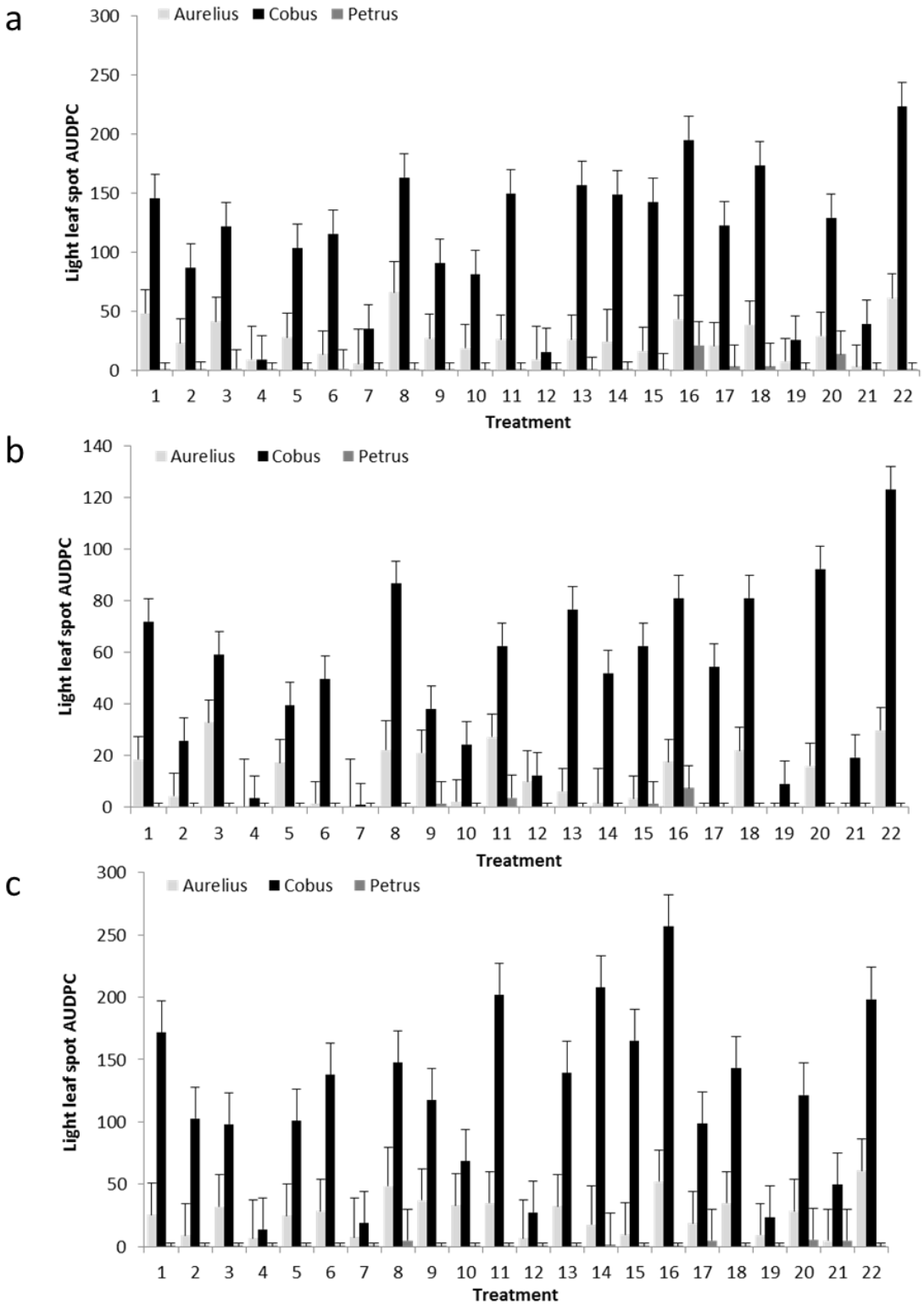



**Figure. A2.** Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at Blackness site in 2013-14. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c).

Figure A3. Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at Blackness site in 2015-16. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c). Treatments were as follows: Treatment 1 = untreated: Treatment 2 = standard fungicide programme: Treatment 3 = Alternate Regalia and fungicides: Treatment 4 = Alternate Bion and fungicides: Treatment 5 = Alternate Softguard and fungicides: Treatment 6 = Alternate SiTKO-SA and fungicides: Treatment 7 = Six Bion sprays: Treatment 8 = Six Regalia sprays: Treatment 9 = Six Softguard sprays: Treatment 10 = Six Alga sprays: Treatment 11 = Six SiTKO-SA sprays: Treatment 12 = Three Bion sprays: Treatment 13 = Three Regalia sprays: Treatment 14 = Three Softguard sprays: Treatment 15 = Three Alga sprays: Treatment 16 =Threes SiTKO-SA sprays: Treatment 17 = Three Softguard plus Alga sprays: Treatment 18 = Three Regalia plus Alga sprays : Treatment 19 = Three Bion plus Alga sprays: Treatment 20 = Three SiTKO-SA plus Alga sprays: Treatment 21 = Three Bion plus Regalia sprays: Treatment 22 = Three Regalia plus SiTKO-SA sprays. Significant reduction in disease development differences compared to the standard fungicide treated (T2) plots at $P<0.001$ = ***, $P<0.01$ = **, $P<0.05$ = * (Generalised linear model analysis).

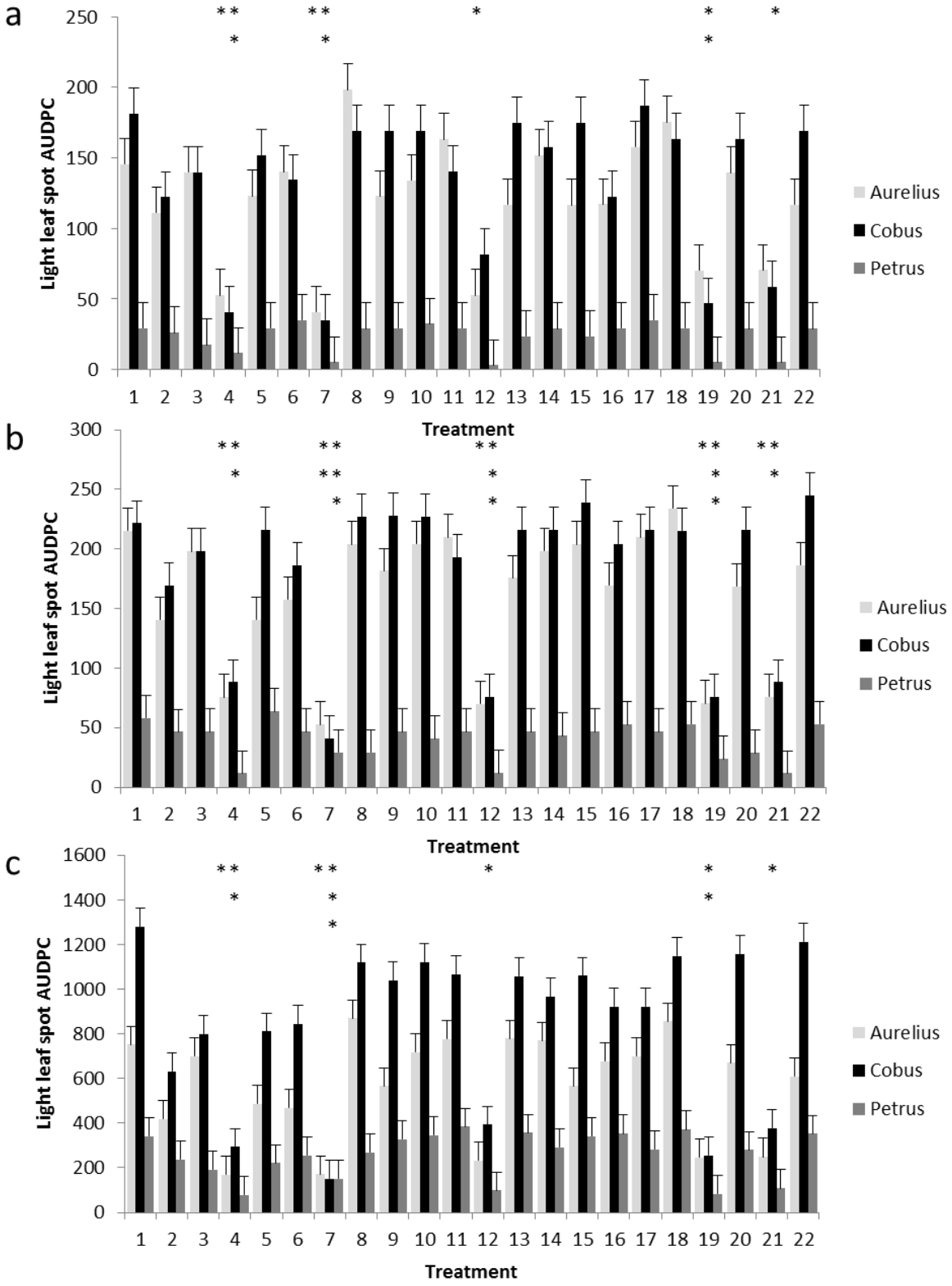



**Figure A3.** Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at Blackness site in 2015-16. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c).