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McGrann, GRD; Yoxall, T; Paterson, LJ; Taylor, JMG; Birmpilis, IG; Walters, DR; Havis, ND

*Published in:*

European Journal of Plant Pathology

*DOI:*

[10.1007/s10658-016-1103-7](https://doi.org/10.1007/s10658-016-1103-7)

First published: 26/11/2016

*Document Version*

Peer reviewed version

[Link to publication](#)

*Citation for published version (APA):*

McGrann, GRD., Yoxall, T., Paterson, LJ., Taylor, JMG., Birmpilis, IG., Walters, DR., & Havis, ND. (2016). Control of light leaf spot and clubroot in brassica crops using defence elicitors. *European Journal of Plant Pathology*, 148(2), 447 - 461. <https://doi.org/10.1007/s10658-016-1103-7>

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1 Control of light leaf spot and clubroot in brassica crops using defence elicitors  
2 Graham RD McGrann, Tracy Yoxall, Linda J Paterson, Jeanette MG Taylor, Ioannis G  
3 Birmopilis, Dale R Walters, Neil D Havis  
4 Crop Protection Team, Crop and Soil Systems, SRUC, West Mains Road, Edinburgh, EH9  
5 3JG, UK

6

7 Keywords:

8 Bion

9 Disease management

10 Elicitor

11 Induced resistance

12 *Plasmodiophora brassicae*

13 *Pyrenopeziza brassicae*

14

15 **Abstract**

16 Plant defence elicitors are compounds that can induce host defence responses against plant  
17 pathogens and offer a novel strategy for disease management. Disease control by elicitors  
18 can be inconsistent and is often dependent on the crop, the variety and the environment. The  
19 use of foliar application of defence elicitors to control light leaf spot (LLS) disease caused by  
20 *Pyrenopeziza brassicae* in the brassica crops winter oilseed rape (WOSR) and Brussel sprouts  
21 was evaluated in field trials across multiple years. Elicitor responses in WOSR varied  
22 between years. Yield benefits were also inconsistent and did not reflect the level of disease  
23 control. Results with Brussel sprouts were more consistent although variation between  
24 variety, trial site and year were observed. In particular the salicylic acid analog Acibenzolar-  
25 S-Methyl, in the commercial product Bion®, demonstrated good disease control across the  
26 field trial sites in the early maturing Brussel sprout variety Cobus. Levels of LLS were  
27 consistently reduced when Bion® was alternated within a standard fungicide programme,  
28 applied as an individual spray or in combination with other defence elicitors. When applied  
29 as a root drench or seed soak Bion® also reduced symptom development of the soil-borne  
30 brassica disease caused by *Plasmodiophora brassicae*, clubroot, in WOSR. These results  
31 indicate that defence elicitors such as Bion® can be used as an additional disease  
32 management tool alongside host resistance and standard fungicide programmes to protect  
33 brassica crops.

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36

## 37 **Introduction**

38 The *Brassica* genus contains a range of different crop species that have multiple uses  
39 including food for human consumption, animal fodder and vegetable oils. Oilseed rape  
40 (OSR; *Brassica napus*) is grown throughout the world as an important source of oil and  
41 proteins for animal feed. Furthermore, OSR is important as a break crop in intensive cereal  
42 rotations which has resulted in rapeseed oil production increasing more than two-fold in the  
43 last ten years in Europe (<http://faostat.fao.org/>). Vegetable brassica crops are often high  
44 value commodities grown for their edible leaves and roots. *Brassica oleracea* is the principle  
45 vegetable Brassica crop encompassing cabbages, broccoli, Brussel sprouts and cauliflowers  
46 (Rakow, 2004). Frequently OSR and brassica vegetables are grown in close proximity to one  
47 another with OSR and vegetable brassica crops grown over approximately 15% of the land in  
48 use for arable and horticultural production in the UK in 2014 (Anon., 2015). This can pose  
49 issues to the health of these crops as both OSR and vegetable brassica crops are susceptible to  
50 many of the same disease threats such as light leaf spot and clubroot.

51 Light leaf spot (LLS) is an economically important disease of OSR and vegetable brassica  
52 crops across Northern Europe (Rawlinson et al., 1978). The disease is caused by the splash-  
53 borne fungal pathogen *Pyrenopeziza brassicae* which can lower crop yields by reducing  
54 photosynthetic leaf area as well as affecting the quality of vegetable brassica crops (Boys et  
55 al., 2007). Varietal resistance to LLS is available in different brassica crops but this is often  
56 not sufficient to control the disease (Maddock et al., 1981; Simons and Skidmore, 1988; Boys  
57 et al., 2007; Karolewski, 2010) resulting in the widespread use of synthetic fungicides to  
58 protect the crops. Fungicides of the methyl benzimidazole carbamate (MBC) and the  
59 demethylation inhibitor (DMI) classes have been used alone or in combination to manage  
60 LLS. The widespread use of MBC and DMI fungicides to control LLS has resulted in the  
61 evolution of *Pyrenopeziza brassicae* isolates that are insensitive to these chemicals (Carter et  
62 al., 2013; 2014). The threat posed to LLS control in brassica crops by fungicide insensitive  
63 isolates has led to the suggestion that alternative control strategies should be sought for this  
64 disease (Carter et al., 2014).

65 Clubroot is a disease of global importance that affects both broad acre and vegetable brassica  
66 crops. Caused by the soil-borne protist, *Plasmodiophora brassicae*, the disease typically  
67 results in yield losses of 10-15% although complete crop failures have been associated with  
68 severe infection (Dixon, 2009; Hwang et al., 2012). Clubroot symptoms result from

69 hypertrophic growth of the roots leading to gall formation and deformed roots which can  
70 affect the quality value of some brassica vegetables (Dixon, 2009). *Plasmodiophora*  
71 *brassicae* survives as long lived resting spores which can remain viable in the absence of a  
72 host for more than fifteen years (Wallenhammar, 1996). The average resting spore half-life  
73 of approximately 3.5 years (Wallenhammar, 1996) means control of the disease by rotation is  
74 often not a viable option. Different fungicides, biological control agents and soil  
75 amendments (Tremblay et al., 2005; Kowata-Dresch and May-De Mio, 2012; Peng et al.,  
76 2014; McGrann et al., 2016) have been tested for the control of clubroot but despite some  
77 showing activity against *Plasmodiophora brassicae*, their field efficacy is inconsistent and  
78 often ineffective (Donald and Porter, 2009). Varietal resistance has provided an effective  
79 method of controlling clubroot in different brassica crops but pathogen evolution has resulted  
80 in *Plasmodiophora brassicae* populations that can overcome the resistance mechanism  
81 (Diederichsen et al., 2009; McGrann et al., 2016; Strelkov et al., 2016).

82 With fungicides either no longer effective, or declining in efficacy and varietal resistance not  
83 completely reliable, alternative control measures for both clubroot and LLS are urgently  
84 required. Defence elicitors are compounds that trigger the plants natural defence  
85 mechanisms, a process called induced resistance (Walters et al. 2013), and have been shown  
86 to provide control against diseases in field grown cereals (Walters et al., 2009; 2011a; 2001b)  
87 and brassica crops (Thakur et al., 2014). Oxley and Walters (2012) demonstrated that a  
88 combination of the defence elicitors acibenzolar-S-methyl (ASM), cis-jasmone (CJ) and  $\beta$ -  
89 aminobutyric acid (BABA) was able to reduce LLS levels in winter oilseed rape (WOSR)  
90 more effectively than traditional fungicides. Different elicitors can activate distinct plant  
91 defence pathways often regulated by the action of specific plant hormones such as salicylic  
92 acid (SA), jasmonic acid (JA) and ethylene (ET) (Walters et al., 2013). Application of the  
93 plant hormone SA has been reported to reduce clubroot levels in broccoli (Lovelock et al.,  
94 2013) and *Arabidopsis thaliana* (Agarwal et al., 2011) but with negative plant growth effects.  
95 These data indicate that compounds that can alter plant hormones and associated defence  
96 responses may have potential for use in LLS and clubroot control in brassica crops. The  
97 study reported here examined the potential of various defence elicitors to control disease in  
98 brassica crops. Field trial experiments assessed the effectiveness of defence elicitors to  
99 control LLS in WOSR and Brussel sprouts, whereas glasshouse trials were used to test the  
100 effect on clubroot in WOSR.

101

## 102 **Methods and materials**

### 103 *Defence elicitors and fungicides*

104 A range of commercially available products were tested for their ability to induce disease  
105 resistance in brassica crops. Details of the elicitors and fungicides used in the field trial and  
106 glasshouse experiments are provided in Table 1.

### 107 *Winter oilseed rape (WOSR) field trials*

108 Field trials were used to assess the effects on defence eliciting compounds on reducing LLS  
109 disease levels in 2012-13, 2013-14 and 2014-15. Trial design and management was as  
110 previously described (Oxley and Walters, 2012) with WOSR varieties sown in a randomised  
111 block design at a rate of 60 seeds m<sup>-2</sup> for a target population size of 50 plants m<sup>-2</sup> in two  
112 adjacent 10 m x 2 m plots with eight rows spaced at 11.5 cm intervals. Local standard  
113 practice was followed for all agronomic inputs except for fungicide and elicitor applications.  
114 Elicitor and fungicide treatments were applied using a knapsack sprayer in 200 L ha<sup>-1</sup> of  
115 water. There were four replicates of each variety and treatment combination. The 2012-13  
116 trial, at the Bush Estate, Edinburgh, Scotland, examined the effects of Bion® (0.175 g L<sup>-1</sup> –  
117 active ingredient (a.i.) acibenzolar-S-methyl/benzothiadiazole [500 g Kg<sup>-1</sup>]), and BABA (0.5  
118 g L<sup>-1</sup> - a.i. DL-b-aminobutyric acid [>95%]) in comparison to the fungicide Folicur (0.5 L ha<sup>-1</sup>  
119 <sup>1</sup> Bayer Crop Science, Cambridge, UK) and untreated control plants of four different WOSR  
120 varieties (Table 2). LLS disease resistance ratings from the AHDB (Agricultural and  
121 Horticultural Development Board) recommended list  
122 (<http://cereals.ahdb.org.uk/varieties.aspx>) of the four varieties were similar and are noted in  
123 parentheses; Excaliber (6), Fashion (6), Flash (5) and Mendel (5). Each treatment was  
124 applied twice, once in November 2012 and again in March 2013. In 2013-14 a single WOSR  
125 variety, Castille (5), was tested at a trial site in Aberdeen, Scotland. Thirteen treatments were  
126 assessed, each applied in November 2013 and again in March 2014 (Table 2). The 2014-15  
127 trial was also located at the Bush Estate, Edinburgh, Scotland and used two WOSR varieties  
128 Camelot (5) and PR46W21 (5) and five treatments (Table 2). Treatments were applied in  
129 October 2014 and March 2015. Disease levels in all trials were recorded as the percent leaf  
130 area infected with LLS four times during the season (Oxley and Walters, 2012). Plot yields  
131 at 91% dry matter and the average height of the WOSR plants in each plot were also  
132 measured. In 2012-13 and 2014-15 plots were also observed for phytotoxic effects of elicitor  
133 or fungicide treatments. Phytotoxicity was visually assessed at 7 and 14 days post

134 application using a percentage scale where: 0% = no phytotoxicity and 100% = complete  
135 foliar necrosis. LLS disease assessments were used to calculate the area under the disease  
136 progress curve (AUDPC; Shaner and Finney, 1977) and this value was used for statistical  
137 analysis.

138

### 139 *Brussel sprout field trials*

140 Field trials were conducted in Scotland in 2013-14 at sites in Tynninghame, East Lothian and  
141 Blackness, Falkirk, and in 2015-16 at a site in St Andrews, East Fife. In both years trials  
142 were sown in the month of May with three Brussel sprout varieties Cobus, Aurelius and  
143 Petrus representing early, mid and late maturing crops, respectively. A series of elicitor  
144 treatments were used to assess the effectiveness of five different elicitors applied in four  
145 different spray programmes. The five elicitors used were Bion®, Regalia® (a.i. extract of  
146 *Reynoutria sachalinensis* [5%]), Softguard® (a.i. chitosan) [2.6%], SiTKO-SA (a.i.  
147 Salicylic acid [4%] and Silica [5%]), and Companion (a.i. *Bacillus subtilis* GB03 [0.03%]),  
148 although Companion was replaced with Alga 600 (soluble seaweed extract powder [40-55%  
149 organic matter]) in the 2015-16 trial (Table 1; Supplementary Table 1). Elicitor treatments  
150 were compared to untreated control plants and to a standard fungicide programme consisting  
151 of fungicide applications in mid July, mid August, early September, end of September, mid  
152 October and early November. Elicitor treatments were applied as a six spray programme  
153 following the same spray timings as for the standard fungicide programme; a three spray  
154 programme applied at end July, early September, mid October; a six spray programme where  
155 elicitor and fungicide were alternated e.g. elicitor (end July), fungicide (mid August), elicitor  
156 (early September), fungicide (end September), elicitor (mid October), fungicide (early  
157 November); a three spray programme with elicitors applied in combination applied at end  
158 July, early September, mid October. Full details of all 22 treatments are provided in  
159 Supplementary Table 1. Local standard practice was followed for all agronomic inputs  
160 except for fungicide and elicitor applications. A total of three blocks per treatment with 20  
161 plants in each block were sown. Sprays were applied by knapsack sprayer in 500 L ha<sup>-1</sup> of  
162 water. LLS assessments were made on the lower and top leaves as well as the sprouts on a  
163 monthly basis from September until February. During the early assessments (September-  
164 November) LLS levels were typically too low for in field scoring. Therefore five samples for  
165 each tissue type for all variety and treatment combinations were collected from the field and

166 incubated in plastic bags for 48 hours at room temperature. Symptoms were scored visually  
167 as the percentage of the surface area that was diseased. From December onwards symptoms  
168 were visually assessed in the field.

169

#### 170 *Clubroot glasshouse trials*

171 An isolate of *Plasmodiophora brassicae* was collected from clubroot infested soil from  
172 Cupar, Scotland and maintained by serial passage through the susceptible OSR cv. Fashion.  
173 Infected galls were collected from susceptible plants, washed free of contaminating soil and  
174 stored at -20°C until required. Resting spore suspensions were prepared from frozen galls as  
175 previously described (McGrann et al., 2016) and 50 mL of the spore suspension at  $2 \times 10^5$   
176 spores mL<sup>-1</sup> added to each 9 x 9 x 8 cm pot filled with compost (John Innes No. 3 compost,  
177 John Innes Manufacturers Association, Berkshire, UK) to give a final concentration of  $10^4$   
178 spores mL<sup>-1</sup>. WOSR cv. Fashion seeds were sown directly into inoculated soil. Elicitors  
179 were applied as a foliar spray using a hand held pump-action sprayer, to run off, or as a 50  
180 mL root drench when WOSR plants were at the 2-3 leaf stage (approximately two weeks  
181 after sowing). Elicitors tested included Bion® (0.175 g L<sup>-1</sup>), Regalia® (5 mL L<sup>-1</sup>), BABA (1  
182 mM), Companion® (12 mL L<sup>-1</sup>), SiTKO-SA (10 mL L<sup>-1</sup>) and Softguard (2 mL L<sup>-1</sup>). Clubroot  
183 was scored on a 0-5 scale based on the severity of galling; 0 = no galling; 1 = small clubs  
184 present, most of fibrous root still healthy; 2 = galls visible around tap root and crown; 3 =  
185 moderately severe galling with healthy roots still visible; 4 = severe galling with few healthy  
186 fibrous roots present; 5 = severe galls with root system now rotten. Clubroot gall fresh  
187 weights were also measured in elicitor and control treated plants. The elicitor screen was  
188 assessed in three independent experiments. Bion® was also applied as a seed soak to WOSR  
189 cv. Fashion by soaking seeds in a solution of Bion® (0.175 g L<sup>-1</sup>) for 24 hours at 4°C. After  
190 24 hours the seeds were rinsed with distilled water for 10 mins. The effect of soaking seeds  
191 with Bion® prior to planting was assessed in two independent experiments and compared  
192 control plants grown from seeds soaked in water for 24 hours. Clubroot infection and gall  
193 weights were assessed as above 5-8 weeks post inoculation. Plants were observed throughout  
194 each experiment for potential growth defects associated with elicitor treatments.

195

#### 196 *Gene expression assays*



197 Transcripts levels of *Pathogenesis-related 1* (PR1), a known marker of the salicylic acid (SA)  
198 defence pathway, were assessed in two distinct experiments following WOSR cv. Fashion  
199 treatment with BION®. In the first experiment seeds were sown in pots in clubroot-free  
200 compost as described previously and seedlings were treated with a solution of Bion® (0.175  
201 g L<sup>-1</sup>) as either a foliar spray or as a root drench at the two-three leaf stage approximately two  
202 weeks after sowing. Two days later (day 0) each pot was inoculated with a 50 mL (2 x 10<sup>5</sup>  
203 spores mL<sup>-1</sup>) *Plasmodiophora brassicae* resting spore suspension. Leaf samples were  
204 collected from three individual plants prior to *Plasmodiophora brassicae* inoculation (day 0)  
205 and at 1 and 2 days post inoculation (dpi). For the second experiment clubroot-free compost  
206 was inoculated with *Plasmodiophora brassicae* resting spores prior to sowing WOSR seeds,  
207 as described previously. Plants were treated with Bion® as in the first experiment and leaf  
208 samples collected from three individuals at 1, 2 and 7 days following elicitor treatment. In  
209 both experiments samples were also collected from control plants that were not treated with  
210 elicitors. In a third experiment WOSR cv. Mendel plants were treated at the two-three leaf  
211 stage with Bion® as a foliar spray and one or six days later plants were inoculated with a  
212 *Pyrenopeziza brassicae* isolate collected from a WOSR plot in Aberdeen in 2011.  
213 *Pyrenopeziza brassicae* was grown on malt extract agar at 16°C for 21-28 days, before spores  
214 were collected by flooding the plate with water containing 0.01% Tween 20 and scraping the  
215 culture with a spreader. Spores were counted on a haemocytometer and diluted to give a final  
216 concentration of 1 x 10<sup>6</sup> spores mL<sup>-1</sup> and sprayed on to plants to run-off using a hand held  
217 pump-action sprayer. Plants were transferred to clear polythene bags post inoculation and  
218 incubated at 16°C for 24 hours in the dark followed by 24 hours under a 12 h light:dark  
219 photoperiod. The polythene bags were removed 48 hours post inoculation. PR1 expression  
220 was assessed in leaf samples 1 and 2 dpi with *Pyrenopeziza brassicae* and compared to  
221 untreated control plants.

222 For gene expression analysis leaf samples were snap frozen in liquid nitrogen after sampling  
223 and total RNA extracted using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany)  
224 followed by Turbo I DNase (Ambion, Austin, Texas, USA) treatment to remove  
225 contaminating genomic DNA. cDNA was synthesised from 1 µg total RNA using the  
226 Superscript III first strand cDNA synthesis kit system following the manufacturers  
227 instructions (Invitrogen, Carlsbad, CA, USA) and diluted 1 in 50 with sterile distilled water.  
228 PR1 transcript levels were measured by quantitative reverse transcription-PCR (qRT-PCR)  
229 using the Brilliant II Sybr ® Green qPCR low ROX master mix kit (Agilent Technologies,

230 Stockport, UK) and gene specific primers PR1\_for CACTACACTCAAGTTGTTTGA and  
231 PR1\_rev TAGTATGGCTTCTCGTTCACAT. PR1 transcript levels across samples were  
232 normalised using primers that amplify the reference gene elongation factor 1 $\alpha$  (EF1a)  
233 EF1a\_for TGAGCACGCTCTTCTTGCTTTCA and EF1a\_rev  
234 GGTGGTGGCATCCATCTTGTTACA. Relative expression of PR1 in elicitor treated plants  
235 was calculated using the  $2^{-\Delta\Delta C_q}$  method (Livak and Schmittgen, 2001) with EF1a as the  
236 reference gene and control plants not treated with elicitors as the calibrator. qRT-PCR  
237 reactions were run using MxPro-Mx3000P 4.10 QPCR System (Agilent Technologies). Each  
238 reaction contained 12.5  $\mu$ L of 2 $\times$  Brilliant II SYBR Green QPCR Low ROX master mix and  
239 5  $\mu$ L of cDNA. Final primer concentrations varied on the target gene with PR1\_for at 200  
240 nm and PR1\_rev at 100 nm whilst EF1a\_for and EF1a\_rev were both at 300 nm. Reactions  
241 were made up to a final volume of 25  $\mu$ L with sterile distilled water. Thermocycler  
242 conditions were as follows: an initial 10 min denaturation step at 95 $^{\circ}$ C followed by 40 cycles  
243 of 30s at 95 $^{\circ}$ C; 45 sec at 55 $^{\circ}$ C and 30 sec at 72 $^{\circ}$ C. A dissociation curve was run at the end of  
244 each run to confirm that primers were amplifying a single target.

245

#### 246 *Statistical analyses*

247 All data were analysed using GenStat v15 (Payne et al., 2009). Variation in LLS AUDPC,  
248 yield, plant height and phytotoxicity in WOSR field trials was assessed using general linear  
249 modelling between years and within years using experimental replicate, treatment and variety  
250 as factors where appropriate. Disease symptom data from the Brussel sprout field trials were  
251 converted into AUDPC and analysed using a generalized linear model (GLzM) following  
252 square root transformation of the AUDPC data to approximate normality. The GLzM  
253 assessed variation attributed to block effect, the variety and treatments and any interaction  
254 between specific varieties and treatments. Data from the clubroot glasshouse experiments  
255 were analysed using general linear modelling using experiment and treatment as factors.

256

## 257 **Results**

### 258 *Effect of elicitor treatments on light leaf spot (LLS) in winter oilseed rape (WOSR) field trials*

259 Disease levels were significantly different between the three years ( $P < 0.001$ ) of WOSR field  
260 trials with the highest levels of LLS spot recorded in 2014-2015 and the lowest in 2013-14.

261 In 2012-13 none of the treatments ( $P = 0.054$ ) nor varieties ( $P = 0.393$ ) significantly affected  
262 LLS AUDPC (Fig. 1a). However, both treatment ( $P < 0.001$ ) and variety ( $P < 0.001$ )  
263 significantly affected yields at 91% dry matter (Fig. 1b). Overall the application of the  
264 fungicide Folicur ( $P = 0.011$ ) and the elicitor Bion® ( $P < 0.001$ ) significantly increased yields  
265 compared to control plots. Yields were highest in cv. Flash and Excalibur and lowest in cv.  
266 Mendel (Fig. 1b). No significant interaction effect on yield was observed between treatment  
267 and variety ( $P = 0.052$ ). There was no significant effect of treatment on plant height ( $P =$   
268  $0.091$ ) but there were differences noted between varieties ( $P < 0.001$ ) with plants of cv. Flash  
269 taller than the others (Supplementary Fig. S1a). There were very low levels of phytotoxicity  
270 associated with any of the treatments following either of the application dates. None of the  
271 treatments had a significant effect on symptoms of phytotoxicity following the Autumn ( $P =$   
272  $0.118$ ) or Spring ( $P = 0.984$ ) applications. There were significantly more phytotoxicity  
273 symptoms on the variety Mendel ( $P = 0.048$ ) following application of the Autumn treatments  
274 but this was not linked to any of the treatments and no significant interaction between  
275 treatment and variety was observed ( $P = 0.561$ ).

276 In 2013-14 there was significant effect of treatment on LLS AUDPC ( $P < 0.001$ ) with all of  
277 the treated plots, including those treated with just the adjuvant Warrior, showing lower  
278 disease levels compared to the controls (Fig. 1c). There was a significant effect of treatment  
279 on yield ( $P = 0.048$ ), however, despite the reductions in LLS AUDPC associated with all of  
280 the treatments tested, only the Bion® ( $0.175 \text{ g L}^{-1}$ ) plus Proline ( $0.35 \text{ L ha}^{-1}$ ) treatment  
281 conferred a significant increase in yield ( $P = 0.006$ ; Fig. 1d). Treatment also significantly  
282 effected plant height ( $P < 0.001$ ) with treatments that included the fungicide Folicur as a  
283 component significantly shorter than control and treatments without Folicur (Supplementary  
284 Fig. S1b).

285 In the 2014-15 field trial disease levels appeared lower in treated plots compared to untreated  
286 controls. However no significant effect of treatment ( $P = 0.287$ ) or variety ( $P = 0.332$ ) on  
287 LLS AUDPC was observed (Fig. 1e). There was no effect of treatment on yield in the 2014  
288 trial ( $P = 0.052$ ) however variety did significantly affect this trait with cv. Camelot typically  
289 yielding significantly higher than cv. PR46W21 ( $P < 0.001$ ; Fig. 1f). Plant height was  
290 significantly affected by variety ( $P < 0.001$ ) with cv. Camelot plants taller than cv. PR46W21  
291 plants (Supplementary Fig. S1c). Treatment also significantly affected plant height ( $P <$   
292  $0.001$ ) although there was a significant interaction between variety and treatment ( $P <$   
293  $0.001$ ) with all four treatments increasing the height of cv. PR46W21 plants ( $P < 0.001$ ) but having

294 no significant effect on cv. Camelot plants. There were no signs of phytotoxicity observed on  
295 any of the plants following either the Autumn or Spring application of the treatments (results  
296 not shown).

297 *Effector of elicitor treatments on light leaf spot (LLS) in Brussel sprouts field trials*

298 Significant effects ( $P < 0.001$ ) on LLS levels attributable to variety, site and treatment were  
299 observed on the lower leaves, top leaves and sprouts in the 2013-14 trial. Disease  
300 development was highest on cv. Cobus, followed by cv. Aurelius with little LLS observed on  
301 cv. Petrus for all three plant parts scored. LLS development was highest at the Tynninghame  
302 site (Supplementary Fig. S2) for the lower leaves and sprouts but disease developed more  
303 extensively on the top leaves at Blackness (Supplementary Fig. S3). The different treatments  
304 had variable effects of LLS depending on the site, variety and plant part scored. Disease  
305 levels scored on either the lower or top leaf or the sprout were not affected by any of the  
306 treatments on cv. Aurelius or cv. Petrus. Results on the cv. Cobus were more promising  
307 although no single treatment consistently reduced LLS levels on all three scored plant parts at  
308 both sites. The standard fungicide programme (treatment (T) 2) provided effective LLS  
309 control on lower leaves at both sites but disease control was only observed on the top leaves  
310 at Blackness and sprouts at Tynninghame. Of the twenty elicitor-based treatments there was a  
311 trend of reduced LLS for treatments that contained Bion® as one of the active ingredients.  
312 Alternating Bion® within the fungicide programme (T4) or applied in combination with the  
313 elicitors Companion® (T19) or Regalia® (T21) significantly reduced disease in all plant  
314 parts at both sites except top leaves at Tynninghame (Fig. 2; Supplementary Fig. S2).  
315 Furthermore, the six (T7) and three Bion® spray (T12) programmes were also both effective  
316 at lowering LLS on lower leaves at both sites with T7 also effective on top leaves whilst T12  
317 was effective on the sprout at Tynninghame. Although some of the other elicitor treatments  
318 had positive effects on LLS control no single ingredient had such a consistent effect as  
319 Bion® (Supplementary Fig. S2, S3). Combined Regalia® and SiTKO-SA treatment (T22)  
320 increased disease on top leaves at both sites and on lower leaves at Blackness (Supplementary  
321 Fig. S2, S3). The three spray SiTKO-SA (T16) programme also increased LLS on sprouts at  
322 Blackness (Supplementary Fig. S3).

323 In the 2015-16 trial, variety and treatment had a significant effect ( $P < 0.001$ ) on LLS  
324 AUDPC observed on lower leaf, top leaf and sprouts. Various treatments reduced disease on  
325 the different plant parts in cv. Aurelius and cv. Cobus but not on cv. Petrus (Supplementary

326 Fig. S4). Alternating Bion® within the fungicide programme, six or three spray Bion®  
327 treatments or combining Bion® with either Alga® or Regalia® all significantly reduced LLS  
328 development in both cv. Aurelius and cv. Cobus (Fig. 2) on all three plant parts assessed  
329 (Supplementary Fig. S4). This contrasts with the standard fungicide programme which  
330 reduced LLS levels on the sprouts of both varieties but was only effective in significantly  
331 lowering disease on the top leaves of cv. Aurelius and the lower leaves of cv. Cobus. In  
332 addition six Regalia® sprays (T8) significantly increased disease on the lower leaves. As  
333 was seen in 2013-14 some of the other elicitor treatments provided significant reductions in  
334 LLS but this control was overall more inconsistent between variety and plant part than seen  
335 for the treatments where Bion® was an active ingredient (Supplementary Fig. S4). At all  
336 three sites the Bion®-based treatments (T4, T7, T12, T19, T21) were more effective in  
337 controlling LLS on the different plant parts in cv. Cobus than the traditional fungicide  
338 programme (T2) (Fig. 2).

339 *Effect of elicitor treatments on clubroot in winter oilseed rape (WOSR) glasshouse*  
340 *experiments*

341 Elicitors used against LLS in field trial experiments were assessed in glasshouse assays as  
342 potential control agents against clubroot disease of WOSR. Each elicitor was applied as a  
343 foliar spray or a root drench. Significant differences in clubroot levels were observed between  
344 treatments and experiments and the interactions between the two factors ( $P < 0.001$ ). Across  
345 the experiments significant reductions in clubroot symptoms were observed on plants treated  
346 with Bion® ( $P < 0.001$ ) and SiTKO-SA ( $P = 0.003$ ) drench treatments (Fig. 3a). However,  
347 only the Bion® drench treatment significantly reduced the fresh weights of the clubroot galls  
348 compared to control plants ( $P = 0.003$ ; Fig. 3b). No obvious growth defects were observed  
349 on WOSR plants treated with any elicitor in the experiments (results not shown). Separate  
350 experiments were used to assess whether applying Bion® as a seed soak treatment prior to  
351 planting could also effect clubroot development in WOSR. Clubroot symptoms were  
352 significantly reduced in plants grown from seeds soaked in Bion® ( $P < 0.001$ ) compared to  
353 control plants (Fig. 3c) and fresh weights of the clubroot galls were significantly lower in the  
354 Bion® treated plants ( $P = 0.003$ ; Fig. 3d).

355

356 *Measurements of Pathogenesis-related protein 1 (PR1) transcript levels following Bion®*  
357 *treatment*

358 PR1 transcript levels were increased greater than 10-fold in the leaves of plants two days  
359 after treatment with Bion® (day 0) as both a foliar spray and soil drench (Fig. 4a) compared  
360 to control plants. This indicates Bion® constitutively activates PR1 expression in WOSR.  
361 PR1 expression remained high in Bion® treated plants 1 and 2 dpi with *Plasmodiophora*  
362 *brassicae* resting spores (Fig. 4a). In plants grown for 14 days in *Plasmodiophora brassicae*  
363 infested soil prior to Bion® treatment PR1 expression was increased 1, 2 and 7 days  
364 following foliar spray or soil drench elicitor treatment compared to untreated controls (Fig.  
365 4b). Plants treated with Bion® either one or six days prior to pathogen inoculation also  
366 showed increased PR1 expression 1 and 2 dpi with *Pyrenopeziza brassicae* (Fig. 4c).

367

368 **Discussion**

369 Sustainable crop productivity is currently under threat due to losses of available disease  
370 control options. Chemical control of fungal diseases is at risk from loss of efficacy due to the  
371 evolution of fungicide insensitive pathogen isolates (Leadbeater, 2011; Hollomon, 2015).  
372 Varietal resistance is limited by a lack of effective, novel sources of resistance against  
373 multiple pathogens and the evolution of virulent pathogen isolates (Brown, 2015). The range  
374 of crop species in intensive production systems is often limited which exacerbates the disease  
375 burden from trash and soil-borne diseases due to close rotations. As such, alternative disease  
376 management strategies are required to help maintain adequate levels of control. Defence  
377 elicitors that induce plant resistance have been proposed as one control option that could be  
378 used to manage disease threats in crops (Walters et al., 2013).

379 Despite the potential for induced reduced in disease management, a major concern with the  
380 use of elicitors is inconsistent levels of disease control and resulting yield benefits (Walters et  
381 al., 2013). In the WOSR trials the effects of elicitors on yield did not always correspond to  
382 the level of LLS control. In 2012-13 no significant effect of treatment, including the  
383 fungicide Folicur, was observed on LLS development yet plots treated with either Folicur or  
384 the elicitor Bion® showed an increase in yield (Fig 1a,b). The opposite occurred in 2013-14  
385 where LLS levels were significantly reduced in treated plots compared to the controls but no  
386 yield benefit was recorded. Elicitor induced resistance is typically sensitive to varietal  
387 variation and environmental conditions (Walters and Fountaine, 2009; Walters et al., 2011a).  
388 Such variation in LLS control by various elicitor treatments was observed in both WOSR and  
389 Brussel sprouts and was dependent on year, sites and varieties. In particular the late maturing  
390 Brussel sprout variety cv. Petrus showed limited response to elicitors compared to the early  
391 maturing variety cv. Cobus. This may be related to the lower disease levels observed on  
392 Petrus as elicitor-mediated defence typically confers benefits to the crop under high disease  
393 pressure (Walters et al., 2009). However, this was not the case in the WOSR trials where the  
394 biggest reduction on LLS symptoms was observed in 2013 when disease levels were lowest.  
395 It is unclear as to whether or not this discrepancy in elicitor response relates specifically to  
396 how these two brassica crops respond to elicitors or is a result of varietal variation in LLS  
397 susceptibility (Walters et al., 2011a).

398 The elicitor that showed the most consistent effects across crops and trials was Bion®.  
399 Bion® contains 50% (w/w) acibenzolar-S-methyl (ASM) also called benzothiadiazole (BTH)

400 which is reported to function as a structural analogue of SA and activate pathways mediated  
401 by this plant hormone (Görlach et al., 1996; Ryals et al., 1996). Experiments measuring the  
402 expression of the SA marker gene PR1 indicate that treatment with Bion® before or after  
403 inoculation with *Plasmodiophora brassicae* or *Pyrenopeziza brassicae* increased levels of  
404 PR1 transcript suggesting activation of the SA pathway contributes to induced resistance  
405 against these pathogens. SA mediated defence pathways typically function against pathogens  
406 with biotrophic development stages (Glazebrook, 2005). *Plasmodiophora brassicae* is an  
407 obligate biotroph (Hwang et al., 2012) whereas *Pyrenopeziza brassicae* is classified as a  
408 hemibiotroph, with an initial biotrophic infection stage followed by necrotrophic  
409 development (Boys et al., 2007). ASM/BTH has been reported to reduce phoma  
410 (*Leptosphaeria maculans*) lesions in OSR (Borges et al., 2003; Liu et al., 2006) in glasshouse  
411 experiments and Alternaria blight (*Alternaria brassicae*) severity on field grown OSR and  
412 Indian mustard (Thakur et al., 2014). Similar to *Pyrenopeziza brassicae*, *Leptosphaeria*  
413 *maculans* and *Alternaria brassicae* have a hemibiotrophic growth habit indicating that  
414 ASM/BTH can be effective against fungi with an initial biotrophic growth stage as well as  
415 though pathogens that develop specifically biotrophic in nature (Görlach et al., 1996).  
416 Additionally ASM/BTH in combination with the defence elicitors cis-jasmone and BABA  
417 was shown to effectively lower LLS symptoms in OSR (Oxley and Walters, 2012).  
418 Application of SA to brassica plants can reduce clubroot symptoms (Agarwal et al., 2011;  
419 Lovelock et al., 2013; Lemarié et al., 2015) whereas recent evidence indicates the  
420 *Plasmodiophora brassicae* genome contains a secreted methyltransferase, PbBSMT that can  
421 methylate SA. This suggests that *Plasmodiophora brassicae* may be able to modify host SA-  
422 mediated defence responses to facilitate pathogen colonisation (Ludwig-Müller et al., 2015).  
423 The role of host SA-related pathways in clubroot resistance is further supported by the  
424 finding that another SA containing elicitor, SiTKO-SA, also reduced clubroot levels in  
425 WOSR. SiTKO-SA contains 4% SA as well as silica which can not only induce defence  
426 response but also act as a barrier against pathogen infection (Cai et al., 2009). More  
427 effective clubroot control in terms of reduced galling and lower gall weights were observed  
428 with Bion®, which contains the high concentration of the SA analog active ingredient  
429 compared to SiTKO-SA. High application levels of SA reduce clubroot development in  
430 brassica crops but can have detrimental growth effects on the crop (Lovelock et al., 2013).  
431 However, no growth defects were observed on the WOSR crops treated with Bion® or  
432 SiTKO-SA at the application rates used in these experiments (results not shown).



433 Clubroot control is inherently difficult due to the soil-borne nature of the disease and the  
434 longevity of the resting structures of *Plasmodiophora brassicae*. This is compounded by  
435 limited varietal resistance which is under threat from more virulent pathotypes (McGrann et  
436 al., 2016; Strelkov et al., 2016). Despite trialling many potential control products, with some  
437 showing activity against *Plasmodiophora brassicae* (Kowata-Dresch and May-De Mio, 2012;  
438 Peng et al., 2014), the use of chemical control for clubroot is generally inconsistent and often  
439 ineffective (Donald and Porter, 2009). Inconsistent clubroot control is also an issue for soil  
440 amendments which increase soil pH and soil calcium levels (Tremblay et al., 2005; McGrann  
441 et al., 2016). The potential of defence elicitors which affect the host SA pathway opens up  
442 new opportunities for the management of clubroot. Although neither Bion® nor SiTKO-SA  
443 completed prevented clubroot development, both reduced symptom formation and should be  
444 considered as another management tool that can be integrated alongside varietal resistance,  
445 rotation and the use of pH raising soil amendments to control this disease.

446 Other defence elicitors tested in these experiments had more variable effects across the  
447 different crops and years particularly on LLS development on Brussel sprouts. Regalia® is a  
448 produced from an extract from the plant *Reynoutria sachalinensis* with biofungicidal  
449 properties. The extract enhances the plant's defence system through non-systemic induced  
450 resistance mediated by increasing phenolics, antioxidants, and strengthening cell walls  
451 (Wurms et al., 1999; Fofana et al., 2002). Regalia® and other products formulated from *R.*  
452 *sachalinensis* extracts such as Milsana® are able to effectively control damping-off disease  
453 (*Pythium* spp.) in glasshouse produced lettuce (Baysal-Gurel and Miller, 2013), and have  
454 shown potential as a component of integrated disease control programmes in organic cucurbit  
455 production to reduce powdery mildew (*Podosphaera xanthii*) and downy mildew  
456 (*Pseudoperonospora cubensis*) development (Wurms et al., 1999; Fofana et al., 2002; Everts,  
457 2014). Softguard contains chitosan, the deacetylated form N-acetylchitooligosaccharide, a  
458 polymer present in fungal cell walls known to trigger defence responses. Products containing  
459 chitosan can reduce downy mildew (Sharathchandra et al.2004) in pearl millet when applied  
460 as either a foliar spray or seed treatment. The biofungicide Companion® contains *Bacillus*  
461 *subtilis*, which is more commonly associated as a biological control agent due to  
462 antimicrobial effects of the bacterium (Fravel, 2005). However, reports have indicated *B.*  
463 *subtilis* can also act as a defence elicitor inducing systemic resistance that can reduce disease  
464 in plants (Ongena et al., 2007; Lowe et al., 2012). Despite the reported efficacy of these  
465 various defence elicitors to control disease in different crop-pathosystems, results in our

466 experiments were inconsistent and highly dependent on the trial, variety and part of the plant  
467 scored.

468 In limited cases some of the defence elicitors had a negative impact on disease control.  
469 Treatments containing a combination of SiTKO-SA and Regalia® increased LLS on the top  
470 leaves of Brussel sprout cv. Cobus plants at both sites in the 2013-14 trial. Combinations of  
471 elicitors can result in trade offs in disease resistance against different pathogens. Walters et  
472 al. (2011b) demonstrated that a combination of three elicitors, Bion®, BABA and cis-  
473 jasmone, was able to effectively reduce the levels of powdery mildew (*Blumeria graminis* f. sp.  
474 *hordei*) and scald (*Rhynchosporium commune*) but significantly increased levels of *Ramularia*  
475 leaf spot (*Ramularia collo-cygni*) in field trials of two barley varieties. Why the combined  
476 treatment of SiTKO-SA and Regalia® or these two elicitors as individual components  
477 increased LLS in some of the trials is unclear. Antagonism between defence pathways is  
478 well known in plants, particularly the pathways regulated by plant hormones SA and JA/ET  
479 involved in defence against biotrophic and necrotrophic pathogens (Glazebrook, 2005). As it  
480 is likely that different elicitors target specific induced resistance pathways then altered cross-  
481 talk between signalling pathways within the plant could lead to antagonistic interactions such  
482 that combining certain elicitors may result in potential trade offs in disease resistance and  
483 increased pathogen development (Walters et al., 2011b).

484 Declines in fungicide efficacy against LLS in brassica crops (Carter et al., 2013; 2014)  
485 together with the threat of reduced fungicide availability in the long term (Leadbeater, 2011)  
486 has resulted in a need to protect those fungicides that still effectively control disease.  
487 Integrated disease management strategies focussed on using alternative control measures to  
488 manage crop diseases in order to lower fungicide inputs can help prolong the effective shelf  
489 life of fungicides by reducing the risk of fungicide insensitivity and limit evolution in  
490 pathogen populations (Hollomon, 2015). Alternating Bion® within the fungicide programme  
491 in the Brussel sprout field trials (treatment 4) or using a reduced fungicide rate in  
492 combination with Bion® in the 2013-14 WOSR trial effectively reduced LLS levels.  
493 Furthermore, treatments which alternated other elicitors, such as Regalia®, Softguard and  
494 Companion with fungicides, were able to lower disease levels, although not as consistently as  
495 Bion®. Together these results suggest that elicitors may have a useful role as plant  
496 protection products going forward to help delay development of fungicide insensitivity.  
497 Furthermore, using elicitors in conjunction with clubroot resistant brassica crops may also  
498 help prevent erosion of the limited resistance sources to this disease which are under threat

499 from resistance-breaking isolates of *Plasmodiophora brassicae* (McGrann et al., 2016;  
500 Strelkov et al., 2016). The results presented here are promising but to realise the full  
501 potential of these compounds and to implement defence elicitors within disease management  
502 programmes a more comprehensive understanding of how specific defence elicitors affect  
503 plant defence pathways that operate against different pathogens and how induced resistance is  
504 influenced by environmental conditions and host genetics is required.

## 505 **Acknowledgments**

506 This project was funded by a research grant, HDC FV417, from AHDB-Horticulture (then  
507 HDC). SRUC receives support from Scottish Government RESAS.

## 508 **Conflicts of interest**

509 The authors declare that they have no conflict of interest.

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653

654



655 **Tables**

656 Table 1 Details of defence elicitors and fungicides used in this study

	<b>Active ingredient</b>	<b>Company</b>
<b><u>Defence elicitors</u></b>		
Bion®	Acibenzolar-S-methyl/benzothiadiazole (500 g Kg <sup>-1</sup> )	Syngenta, Jealott's Hill, UK
Regalia® Biofungicide	Extract of <i>Reynoutria sachalinensis</i> (5%)	Syngenta, Jealott's Hill, UK
BABA	DL-b-aminobutyric acid (>95%)	Sigma, Dorset, UK
Companion ®	Bacillus subtilis GB03 (0.03%)	Growth products, USA
SiTKO-SA	Salicylic acid (4%), Silica (5%)	Growth products, USA
Softguard	Chitosan (2.6%)	Travena, UK
Alga 600	Soluble Seaweed extract powder (40-55% organic matter)	Travena, UK
<b><u>Fungicides</u></b>		
Folicur ®	250 g L <sup>-1</sup> (25.9% w/w) tebuconazole	Bayer CropScience, Cambridge, UK
Proline 275 ®	275 g L <sup>-1</sup> (27.5% w/w) prothioconazole	Bayer CropScience, Cambridge, UK
Signum ®	26.7% w/w boscalid and 6.7% w/w pyraclostrobin	BASF,
Rudis ®	480 g/L (40 % w/w) prothioconazole	Bayer CropScience, Cambridge, UK
Nativo 75WG ®	250 g Kg <sup>-1</sup> (25.0 % w/w)trifloxystrobin and 500 g Kg <sup>-1</sup> (50.0% w/w) tebuconazole	Bayer CropScience, Cambridge, UK
<b><u>Adjuvant</u></b>		
Warrior	192 g L <sup>-1</sup> primary alcohol ethoxylate	Intracrop ©, Gloucestershire, UK

657

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659 Table 2 Treatments used in WOSR field trials 2012-13, 2013-14 and 2014-15

		<b>Treatments</b>		
		<b><u>2012-2013</u></b>	<b><u>2013-14</u></b>	<b><u>2014-15</u></b>
1		Untreated (control)	Untreated (control)	Untreated (control)
2		Folicur (0.5 L ha <sup>-1</sup> )	Folicur (0.7 L ha <sup>-1</sup> )	Folicur (0.7 L ha <sup>-1</sup> )
3		Bion® (0.175 g L <sup>-1</sup> )	Bion® (0.175 g L <sup>-1</sup> )	Bion® (0.175 g L <sup>-1</sup> )
4		BABA (0.5 g L <sup>-1</sup> )	BABA (0.5 g L <sup>-1</sup> )	+ Warrior (25 mL 100 L <sup>-1</sup> ) BABA (0.5 g L <sup>-1</sup> )
5			Folicur (0.5 L ha <sup>-1</sup> )	+ Warrior (25 mL 100 L <sup>-1</sup> ) Regalia (2.5 L ha <sup>-1</sup> )
6			Bion® (0.175 g L <sup>-1</sup> )	+ Warrior (25 mL 100 L <sup>-1</sup> )
7			+ Folicur (0.5 L ha <sup>-1</sup> )	
8			BABA (0.5 g L <sup>-1</sup> )	
9			+ Folicur (0.5 L ha <sup>-1</sup> )	
10			Folicur (0.5 L ha <sup>-1</sup> )	
11			+ Warrior (25 mL 100 L <sup>-1</sup> )	
12			Bion® (0.175 g L <sup>-1</sup> )	
13			+ Proline (0.35 L ha <sup>-1</sup> )	
			Proline (0.35 L ha <sup>-1</sup> )	
			Proline (0.5 L ha <sup>-1</sup> )	
			Bion® (0.175 g L <sup>-1</sup> )	
			+ Folicur (0.7 L ha <sup>-1</sup> )	
			Warrior (25 mL 100 L <sup>-1</sup> )	

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## Figure legends

**Fig. 1** Field performance of elicitor treatments on winter oilseed rape (WOSR) crops. Effects of elicitors on light leaf spot development measured as the area under the disease progress curve (AUDPC) in 2012-13 (a), 2013-14 (c), 2014-15 (e) and WOSR yield at 91% dry matter in 2012-13 (b), 2013-14 (d), 2014-15 (f). Bars indicate standard error. \*\*\* =  $P < 0.001$ ; \*\* =  $P < 0.01$ ; \* =  $P < 0.05$ .

**Fig. 2** Field performance of elicitor treatments containing Bion® as a component on the Brussel sprout cv. Cobus. Effects of elicitor treatments on light leaf spot development measured as the area under the disease progress curve (AUDPC) on the lower leaves (a), top leaves (b) and sprouts (c). Trials were run at sites in Scotland at Blackness and Tynninghame in 2013-14 and in St Andrews in 2015-16. Treatments were T1 = Untreated controls; T2 = standard fungicide programme; T4 = Bion® alternated within the standard fungicide programme; T7 = six Bion® sprays; T12 = three Bion® sprays; T19 = three Bion + Companion/Alga 600 (2013-14/2015-16) sprays; T21 = three Bion® + Regalia® sprays. Bars indicate standard error. \*\*\* =  $P < 0.001$ ; \*\* =  $P < 0.01$ ; \* =  $P < 0.05$ .

**Fig. 3** Effect of elicitor compounds on clubroot development in glasshouse conditions. Elicitors were applied as a foliar spray or root drench and the effect on clubroot symptom development (a) and gall fresh weight (b) was assessed after 5-8 weeks growth infested soil. Effect of Bion® as a seed soak on clubroot development (c) and gall fresh weight (d). Bars indicate standard error. \*\*\* =  $P < 0.001$ ; \*\* =  $P < 0.01$ ; \* =  $P < 0.05$ .

**Fig. 4** Effect of Bion® on *Pathogenesis-related 1* (PR1) transcript levels in winter oilseed rape (WOSR). (a) PR1 levels in Bion treated WOSR plants at 0, 1 and 2 days post inoculation (dpi) with *Plasmodiophora brassicae*. (b) PR1 levels in WOSR grown in clubroot infested soil and then treated with Bion®. Transcript levels measured 1, 2, and 7 days post Bion® treatment. (c) PR1 levels in WOSR plants treated with Bion® either one or six days prior to inoculation with *Pyrenopeziza brassicae*. Transcript levels measured 1 and 2 dpi with *Pyrenopeziza brassicae*. PR1 transcript levels are normalised to the reference genes elongation factor 1- $\alpha$  and data is presented as fold change relative to the elicitor control (water)-treated samples. Bars indicate standard error.

## **Electronic Supplementary Material**

Supplementary Table 1 Treatments used in Brussel sprout field trials 2013-14 and 2015-16

Supplementary Fig. 1. Effects of elicitors on winter oilseed rape height in field trials in 2012-13 (a), 2013-14 (b), 2014-15 (c). Bars indicate standard error.

Supplementary Fig. 2. Field performance of elicitor treatments on light leaf spot development on Brussel sprout varieties at Tynninghame site in 2013-14.

Supplementary Fig. 3. Field performance of elicitor treatments on light leaf spot development on Brussel sprout varieties at Blackness site in 2013-14.

Supplementary Fig. 4. Field performance of elicitor treatments on light leaf spot development on Brussel sprout varieties at Blackness site in 2015-16.

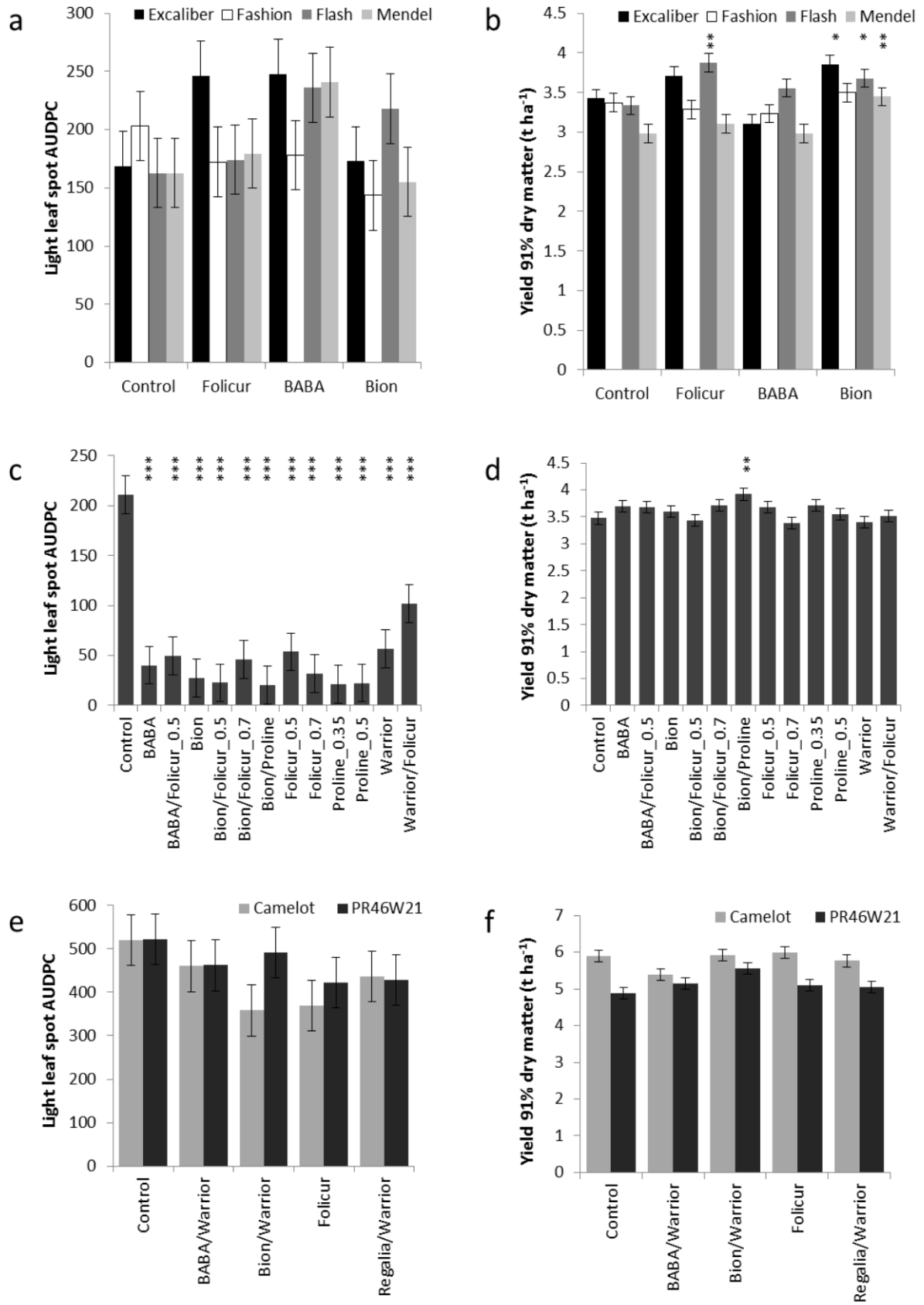


Fig. 1

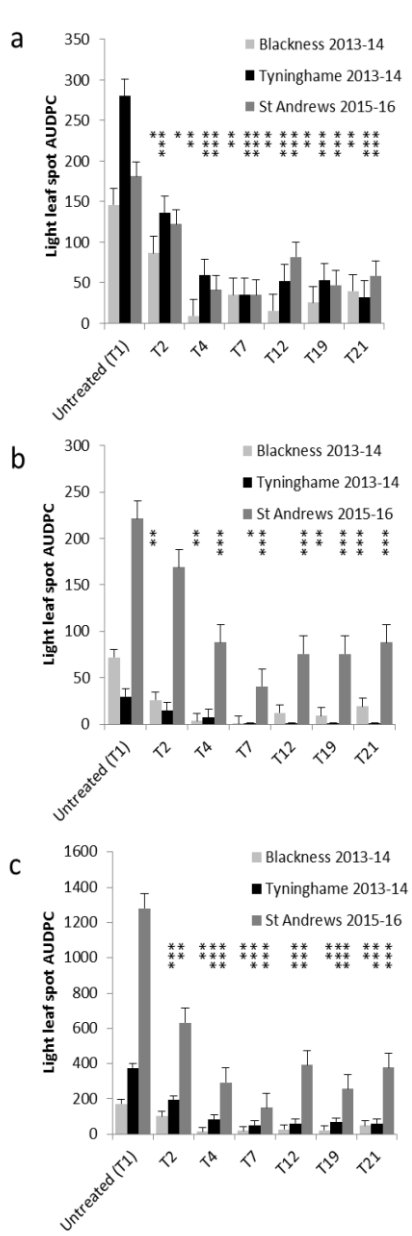


Fig. 2

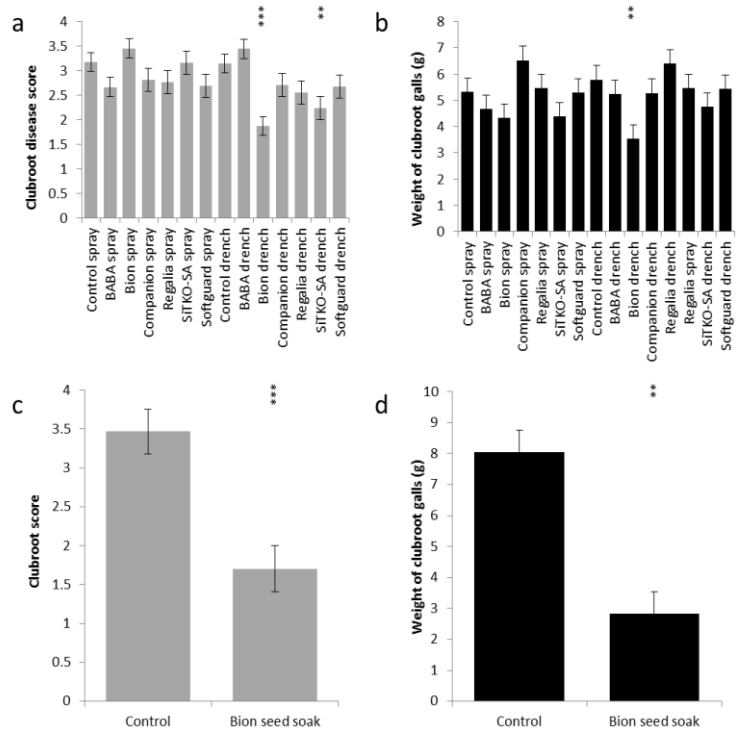


Fig. 3

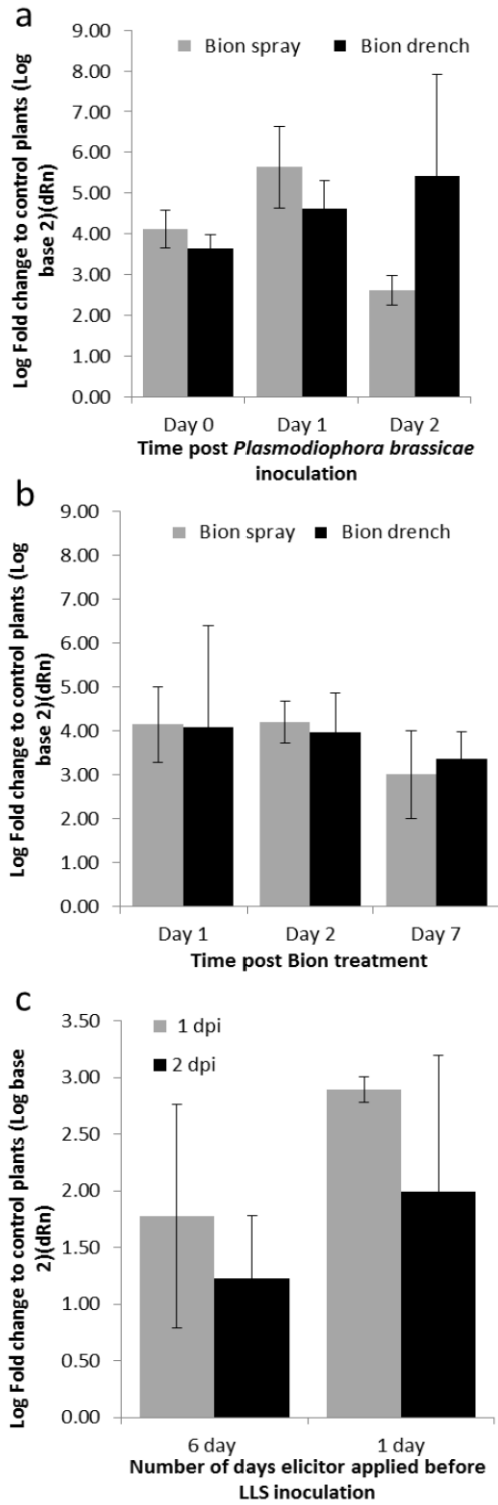


Fig. 4



Table S1 Treatments used in Brussel sprout field trials 2013-14 and 2015-16

Treatment	Mid July	End July	Mid August	Early September	End September	Mid October	Early November
1	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated
2	Signum 1 Kg ha <sup>-1</sup>						
3		Regalia 2.5 L ha <sup>-1</sup>	Rudis 0.4 L ha <sup>-1</sup>	Nativo 0.4 Kg ha <sup>-1</sup>	Signum 1 Kg ha <sup>-1</sup>	Rudis 0.4 L ha <sup>-1</sup>	Nativo 0.4 Kg ha <sup>-1</sup>
4		Bion 0.175 g L <sup>-1</sup>	Rudis 0.4 L ha <sup>-1</sup>	Bion 0.175 g L <sup>-1</sup>	Signum 1 Kg ha <sup>-1</sup>	Bion 0.175 g L <sup>-1</sup>	Nativo 0.4 Kg ha <sup>-1</sup>
5		Softguard 10 mL 5L <sup>-1</sup>	Rudis 0.4 L ha <sup>-1</sup>	Softguard 10 mL 5L <sup>-1</sup>	Signum 1 Kg ha <sup>-1</sup>	Softguard 10 mL 5L <sup>-1</sup>	Nativo 0.4 Kg ha <sup>-1</sup>
6		SiTKO-SA 5 L ha <sup>-1</sup>	Rudis 0.4 L ha <sup>-1</sup>	SiTKO-SA 5 L ha <sup>-1</sup>	Signum 1 Kg ha <sup>-1</sup>	SiTKO-SA 5 L ha <sup>-1</sup>	Nativo 0.4 Kg ha <sup>-1</sup>
7		Bion 0.175 g L <sup>-1</sup>	Bion 0.175 g L <sup>-1</sup>	Bion 0.175 g L <sup>-1</sup>	Bion 0.175 g L <sup>-1</sup>	Bion 0.175 g L <sup>-1</sup>	Bion 0.175 g L <sup>-1</sup>
8		Regalia 2.5 L ha <sup>-1</sup>	Regalia 2.5 L ha <sup>-1</sup>	Regalia 2.5 L ha <sup>-1</sup>	Regalia 2.5 L ha <sup>-1</sup>	Regalia 2.5 L ha <sup>-1</sup>	Regalia 2.5 L ha <sup>-1</sup>
9		Softguard 10 mL 5L <sup>-1</sup>	Softguard 10 mL 5L <sup>-1</sup>	Softguard 10 mL 5L <sup>-1</sup>	Softguard 10 mL 5L <sup>-1</sup>	Softguard 10 mL 5L <sup>-1</sup>	Softguard 10 mL 5L <sup>-1</sup>
10		Alga 600 3 g 5L <sup>-1</sup> <sup>a</sup>	Alga 600 3 g 5L <sup>-1</sup>	Alga 600 3 g 5L <sup>-1</sup>	Alga 600 3 g 5L <sup>-1</sup>	Alga 600 3 g 5L <sup>-1</sup>	Alga 600 3 g 5L <sup>-1</sup>
11		SiTKO-SA 5 L ha <sup>-1</sup>	SiTKO-SA 5 L ha <sup>-1</sup>	SiTKO-SA 5 L ha <sup>-1</sup>	SiTKO-SA 5 L ha <sup>-1</sup>	SiTKO-SA 5 L ha <sup>-1</sup>	SiTKO-SA 5 L ha <sup>-1</sup>
12		Bion 0.175 g L <sup>-1</sup>		Bion 0.175 g L <sup>-1</sup>		Bion 0.175 g L <sup>-1</sup>	
13		Regalia 2.5 L ha <sup>-1</sup>		Regalia 2.5 L ha <sup>-1</sup>		Regalia 2.5 L ha <sup>-1</sup>	
14		Softguard 10 mL 5L <sup>-1</sup>		Softguard 10 mL 5L <sup>-1</sup>		Softguard 10 mL 5L <sup>-1</sup>	
15		Alga 600 3 g 5L <sup>-1</sup>		Alga 600 3 g 5L <sup>-1</sup>		Alga 600 3 g 5L <sup>-1</sup>	
16		SiTKO-SA 5 L ha <sup>-1</sup>		SiTKO-SA 5 L ha <sup>-1</sup>		SiTKO-SA 5 L ha <sup>-1</sup>	
17		Softguard 10 mL 5L <sup>-1</sup>		Softguard 10 mL 5L <sup>-1</sup>		Softguard 10 mL 5L <sup>-1</sup>	
		Alga 600 3 g 5L <sup>-1</sup>		Alga 600 3 g 5L <sup>-1</sup>		Alga 600 3 g 5L <sup>-1</sup>	
18		Regalia 2.5 L ha <sup>-1</sup>		Regalia 2.5 L ha <sup>-1</sup>		Regalia 2.5 L ha <sup>-1</sup>	
		Alga 600 3 g 5L <sup>-1</sup>		Alga 600 3 g 5L <sup>-1</sup>		Alga 600 3 g 5L <sup>-1</sup>	
19		Bion 0.175 g L <sup>-1</sup>		Bion 0.175 g L <sup>-1</sup>		Bion 0.175 g L <sup>-1</sup>	
		Alga 600 3 g 5L <sup>-1</sup>		Alga 600 3 g 5L <sup>-1</sup>		Alga 600 3 g 5L <sup>-1</sup>	
20		SiTKO-SA 5 L ha <sup>-1</sup>		SiTKO-SA 5 L ha <sup>-1</sup>		SiTKO-SA 5 L ha <sup>-1</sup>	
		Alga 600 3 g 5L <sup>-1</sup>		Alga 600 3 g 5L <sup>-1</sup>		Alga 600 3 g 5L <sup>-1</sup>	
21		Bion 0.175 g L <sup>-1</sup>		Bion 0.175 g L <sup>-1</sup>		Bion 0.175 g L <sup>-1</sup>	
		Regalia 2.5 L ha <sup>-1</sup>		Regalia 2.5 L ha <sup>-1</sup>		Regalia 2.5 L ha <sup>-1</sup>	
22		Regalia 2.5 L ha <sup>-1</sup>		Regalia 2.5 L ha <sup>-1</sup>		Regalia 2.5 L ha <sup>-1</sup>	
		SiTKO-SA 5 L ha <sup>-1</sup>		SiTKO-SA 5 L ha <sup>-1</sup>		SiTKO-SA 5 L ha <sup>-1</sup>	

<sup>a</sup> In 2013-14 Treatment 10, 15, 17, 18, 19, 20 Companion 6L ha<sup>-1</sup> was used in place of Alga 600 3 g 5L<sup>-1</sup>.

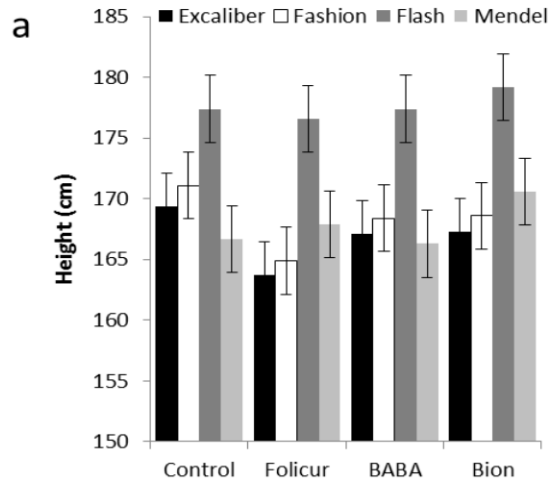
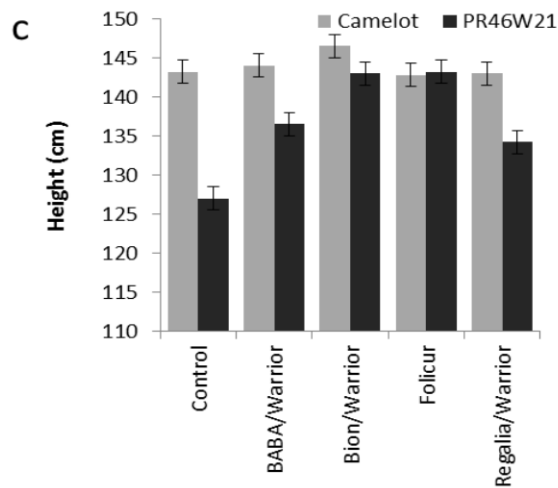
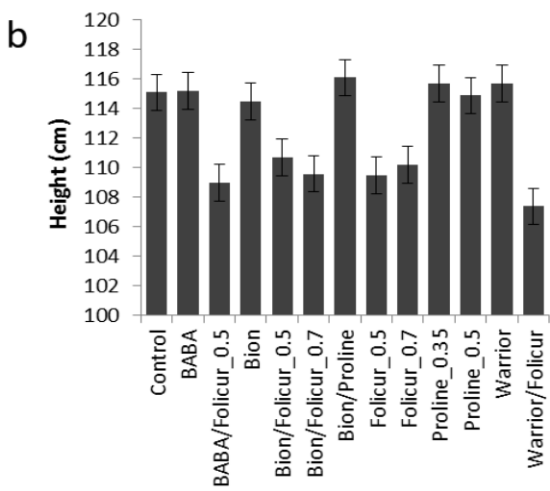


Figure S1. Effects of elicitors on winter oilseed rape height in field trials in 2012-13 (a), 2013-14 (b), 2014-15 (c). Bars indicate standard error.



Supplementary Figure 1

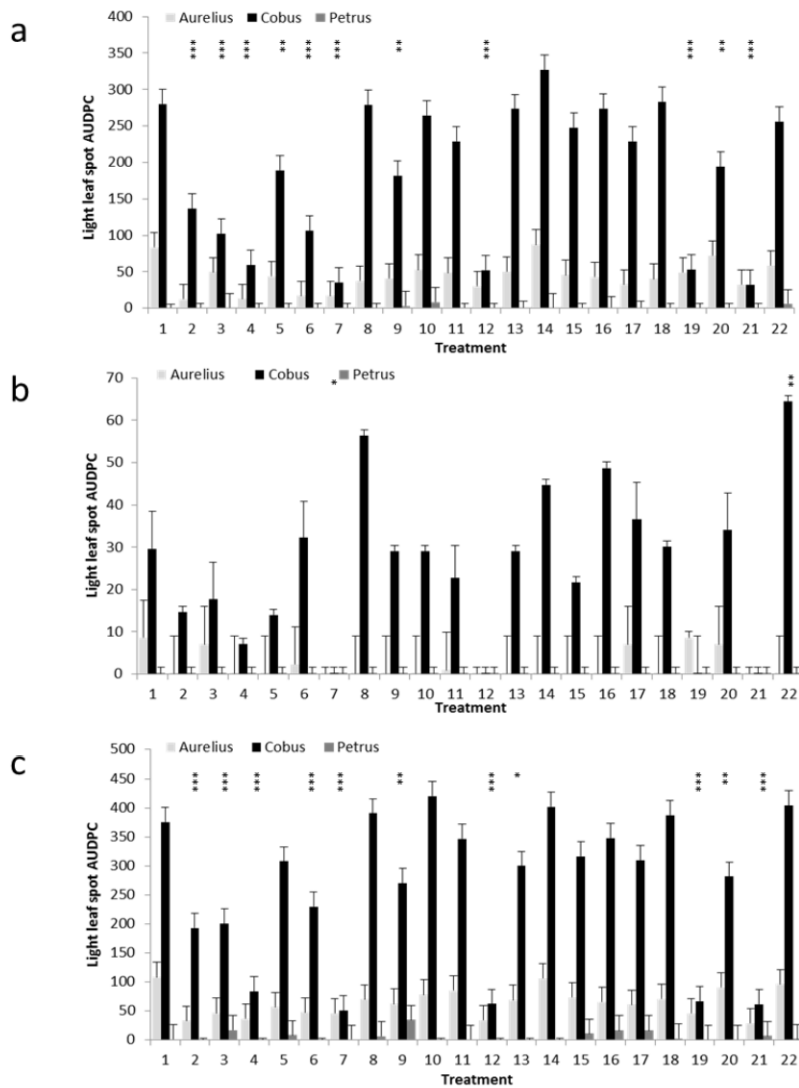


Figure S2. Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at Tynninghame site in 2013-14. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c). Treatments were as follows: Treatment 1 = untreated; Treatment 2 = Mid July Signum 1 Kg ha<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Nativo 0.4 Kg ha<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Rudis 0.4 L ha<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 3 = End July Regalia 2.5 L ha<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Regalia 2.5 L ha<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Regalia 2.5 L ha<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 4 = End July Bion 0.175 g L<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Bion 0.175 g L<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Bion 0.175 g L<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 5 = End July Softguard 10 mL 5L<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Softguard 10 mL 5L<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Softguard 10 mL 5L<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 6 = End July SITKO-SA 5 L ha<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September SITKO-SA 5 L ha<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October SITKO-SA 5 L ha<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 7 = End July, Mid August, Early September, End September, Mid October, Early November Bion 0.175 g L<sup>-1</sup>; Treatment 8 = End July, Mid August, Early September, End September, Mid October, Early November Regalia 2.5 L ha<sup>-1</sup>; Treatment 9 = End July, Mid August, Early September, End September, Mid October, Early November Softguard 10 mL 5L<sup>-1</sup>; Treatment 10 = End July, Mid August, Early September, End September, Mid October, Early November Companion 6L ha<sup>-1</sup>; Treatment 11 = End July, Mid August, Early September, End September, Mid October, Early November SITKO-SA 5 L ha<sup>-1</sup>; Treatment 12 = End July, Early September, Mid October, Bion 0.175 g L<sup>-1</sup>; Treatment 13 = End July, Early September, Mid October, Regalia 2.5 L ha<sup>-1</sup>; Treatment 14 = End July, Early September, Mid October, Softguard 10 mL 5L<sup>-1</sup>; Treatment 15 = End July, Early September, Mid October, Companion 6L ha<sup>-1</sup>; Treatment 16 = End July, Early September, Mid October, SITKO-SA 5 L ha<sup>-1</sup>; Treatment 17 = End July, Early September, Mid October, Softguard 10 mL 5L<sup>-1</sup> & Companion 6L ha<sup>-1</sup>; Treatment 18 = End July, Early September, Mid October, Regalia 2.5 L ha<sup>-1</sup> & Companion 6L ha<sup>-1</sup>; Treatment 19 = End July, Early September, Mid October, Bion 0.175 g L<sup>-1</sup> & Companion 6L ha<sup>-1</sup>; Treatment 20 = End July, Early September, Mid October, SITKO-SA 5 L ha<sup>-1</sup> & Companion 6L ha<sup>-1</sup>; Treatment 21 = End July, Early September, Mid October, Bion 0.175 g L<sup>-1</sup> & Regalia 2.5 L ha<sup>-1</sup>; Treatment 22 = End July, Early September, Mid October, Regalia 2.5 L ha<sup>-1</sup> & SITKO-SA 5 L ha<sup>-1</sup>. \*\*\* =  $P < 0.001$ ; \*\* =  $P < 0.01$ ; \* =  $P < 0.05$

## Supplementary Figure 2

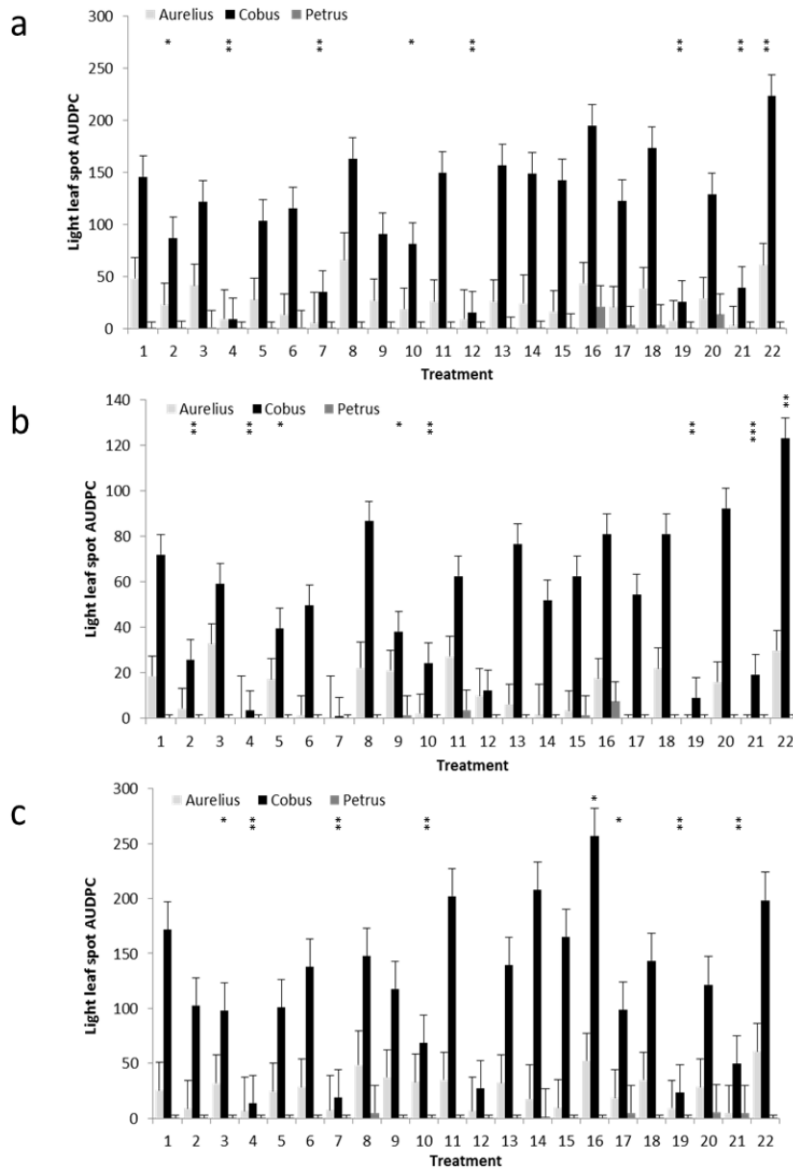


Figure S3. Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at Blackness site in 2013-14. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c). Treatments were as follows: Treatment 1 = untreated; Treatment 2 = Mid July Signum 1 Kg ha<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Nativo 0.4 Kg ha<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Rudis 0.4 L ha<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 3 = End July Regalia 2.5 L ha<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Regalia 2.5 L ha<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Regalia 2.5 L ha<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 4 = End July Bion 0.175 g L<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Bion 0.175 g L<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Bion 0.175 g L<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 5 = End July Softguard 10 mL 5L<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Softguard 10 mL 5L<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Softguard 10 mL 5L<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 6 = End July SITKO-SA 5 L ha<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September SITKO-SA 5 L ha<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October SITKO-SA 5 L ha<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 7 = End July, Mid August, Early September, End September, Mid October, Early November Bion 0.175 g L<sup>-1</sup>; Treatment 8 = End July, Mid August, Early September, End September, Mid October, Early November Regalia 2.5 L ha<sup>-1</sup>; Treatment 9 = End July, Mid August, Early September, End September, Mid October, Early November Softguard 10 mL 5L<sup>-1</sup>; Treatment 10 = End July, Mid August, Early September, End September, Mid October, Early November Companion 6L ha<sup>-1</sup>; Treatment 11 = End July, Mid August, Early September, End September, Mid October, Early November SITKO-SA 5 L ha<sup>-1</sup>; Treatment 12 = End July, Early September, Mid October, Bion 0.175 g L<sup>-1</sup>; Treatment 13 = End July, Early September, Mid October, Regalia 2.5 L ha<sup>-1</sup>; Treatment 14 = End July, Early September, Mid October, Softguard 10 mL 5L<sup>-1</sup>; Treatment 15 = End July, Early September, Mid October, Companion 6L ha<sup>-1</sup>; Treatment 16 = End July, Early September, Mid October, SITKO-SA 5 L ha<sup>-1</sup>; Treatment 17 = End July, Early September, Mid October, Softguard 10 mL 5L<sup>-1</sup> & Companion 6L ha<sup>-1</sup>; Treatment 18 = End July, Early September, Mid October, Regalia 2.5 L ha<sup>-1</sup> & Companion 6L ha<sup>-1</sup>; Treatment 19 = End July, Early September, Mid October, Bion 0.175 g L<sup>-1</sup> & Companion 6L ha<sup>-1</sup>; Treatment 20 = End July, Early September, Mid October, SITKO-SA 5 L ha<sup>-1</sup> & Companion 6L ha<sup>-1</sup>; Treatment 21 = End July, Early September, Mid October, Bion 0.175 g L<sup>-1</sup> & Regalia 2.5 L ha<sup>-1</sup>; Treatment 22 = End July, Early September, Mid October, Regalia 2.5 L ha<sup>-1</sup> & SITKO-SA 5 L ha<sup>-1</sup>. \*\*\* = P < 0.001; \*\* = P < 0.01; \* = P < 0.05

### Supplementary Figure 3

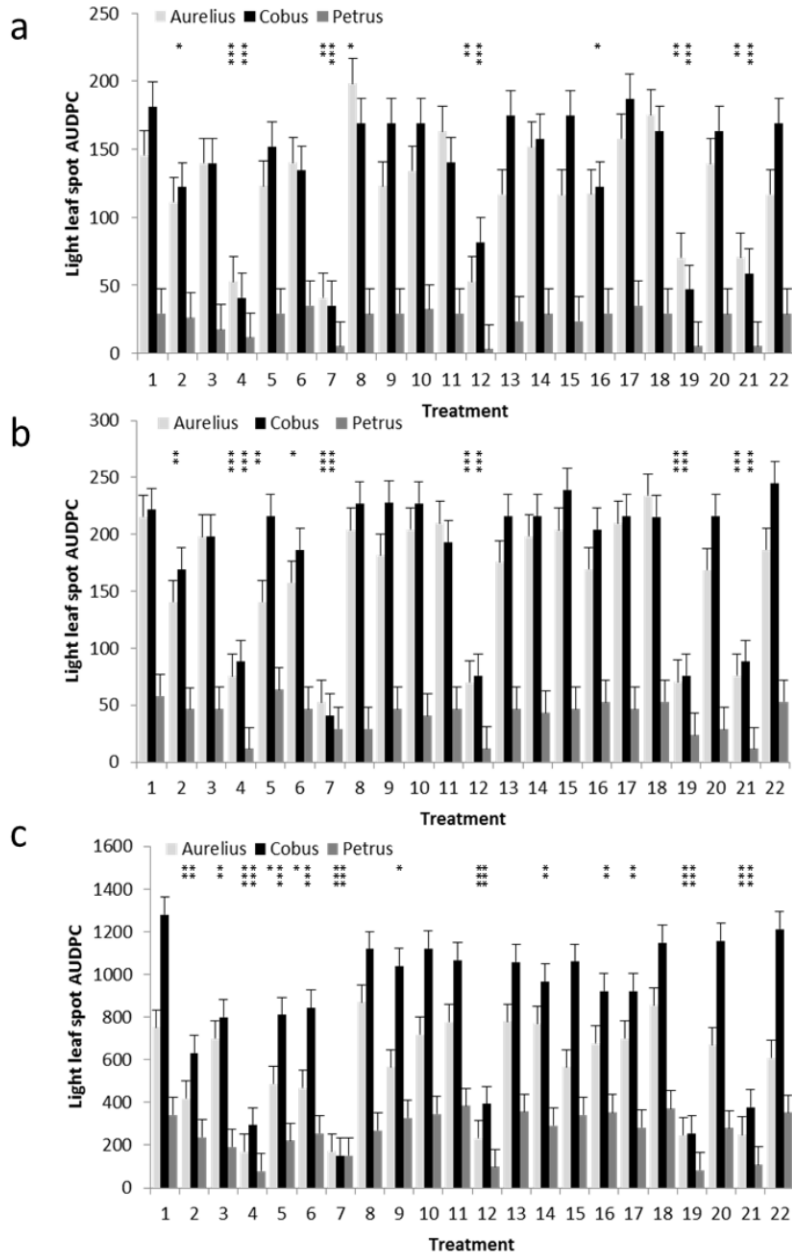


Figure S4. Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at St Andrews site in 2015-16. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c). Treatments were as follows: Treatment 1 = untreated; Treatment 2 = Mid July Signum 1 Kg ha<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Nativo 0.4 Kg ha<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Rudis 0.4 L ha<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 3 = End July Regalia 2.5 L ha<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Regalia 2.5 L ha<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Regalia 2.5 L ha<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 4 = End July Bion 0.175 g L<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Bion 0.175 g L<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Bion 0.175 g L<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 5 = End July Softguard 10 mL 5L<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September Softguard 10 mL 5L<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October Softguard 10 mL 5L<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 6 = End July SITKO-SA 5 L ha<sup>-1</sup>; Mid August Rudis 0.4 L ha<sup>-1</sup>; Early September SITKO-SA 5 L ha<sup>-1</sup>; End September Signum 1 Kg ha<sup>-1</sup>; Mid October SITKO-SA 5 L ha<sup>-1</sup>; Early November Nativo 0.4 Kg ha<sup>-1</sup>; Treatment 7 = End July, Mid August, Early September, End September, Mid October, Early November Bion 0.175 g L<sup>-1</sup>; Treatment 8 = End July, Mid August, Early September, End September, Mid October, Early November Regalia 2.5 L ha<sup>-1</sup>; Treatment 9 = End July, Mid August, Early September, End September, Mid October, Early November Softguard 10 mL 5L<sup>-1</sup>; Treatment 10 = End July, Mid August, Early September, End September, Mid October, Early November Alga 600 3 g 5L<sup>-1</sup>; Treatment 11 = End July, Mid August, Early September, End September, Mid October, Early November SITKO-SA 5 L ha<sup>-1</sup>; Treatment 12 = End July, Early September, Mid October, Bion 0.175 g L<sup>-1</sup>; Treatment 13 = End July, Early September, Mid October, Regalia 2.5 L ha<sup>-1</sup>; Treatment 14 = End July, Early September, Mid October, Softguard 10 mL 5L<sup>-1</sup>; Treatment 15 = End July, Early September, Mid October, Alga 600 3 g 5L<sup>-1</sup>; Treatment 16 = End July, Early September, Mid October, SITKO-SA 5 L ha<sup>-1</sup>; Treatment 17 = End July, Early September, Mid October, Softguard 10 mL 5L<sup>-1</sup> & Alga 600 3 g 5L<sup>-1</sup>; Treatment 18 = End July, Early September, Mid October, Regalia 2.5 L ha<sup>-1</sup> & Alga 600 3 g 5L<sup>-1</sup>; Treatment 19 = End July, Early September, Mid October, Bion 0.175 g L<sup>-1</sup> & Alga 600 3 g 5L<sup>-1</sup>; Treatment 20 = End July, Early September, Mid October, SITKO-SA 5 L ha<sup>-1</sup> & Alga 600 3 g 5L<sup>-1</sup>; Treatment 21 = End July, Early September, Mid October, Bion 0.175 g L<sup>-1</sup> & Regalia 2.5 L ha<sup>-1</sup>; Treatment 22 = End July, Early September, Mid October, Regalia 2.5 L ha<sup>-1</sup> & SITKO-SA 5 L ha<sup>-1</sup>. \*\*\* = P < 0.001; \*\* = P < 0.01; \* = P < 0.05

Supplementary Figure 4