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## In-field assessment of an arabinoxylan polymer on disease control in spring barley

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1 In-field assessment of an arabinoxylan polymer on disease control in spring barley

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18

19 Keywords:

20 Film-forming polymer; Integrated crop management; Powdery mildew; Rhynchosporium  
21 scald; Ramularia leaf spot.

22 Abbreviations:

23 GS = growth; GLM = general linear model; GzLM = generalized linear model; RLS =  
24 Ramularia leaf spot; AUDPC = area under disease progress curve

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31

32 **Abstract**

33 With the threat of certain plant protection products becoming ineffective due to reduced  
34 pathogen sensitivity to fungicides or through the removal of products due to changes in  
35 legislation, alternative compounds are sought for use in disease management programmes.  
36 The effects of an arabinoxylan film-forming polymer derived from maize cell walls to control  
37 crop diseases of spring barley was assessed in field experiments. Control of powdery  
38 mildew, *Rhynchosporium* scald, and *Ramularia* leaf spot on barley was achieved with the  
39 polymer but control was inconsistent between trials. However, good levels of disease control  
40 were observed when the polymer was applied with a reduced fungicide programme. No yield  
41 penalties were associated with use of the polymer in any trial irrespective of the level of  
42 disease control. Alternative plant protection products such as this arabinoxylan polymer may  
43 be useful components in future integrated disease management strategies aimed at reducing  
44 fungicide inputs without any cost to disease control.

45

46 **Highlights**

- 47 • Disease management using an arabinoxylan polymer were assessed
- 48 • Polymer-mediated control varied between sites, year, crop variety and disease
- 49 • Combined polymer plus reduced fungicide application offered more consistent control
- 50 • No yield penalties were associated with polymer applications
- 51 • Polymers may be useful as an early treatment in integrated disease management

52

## 53 **1. Introduction**

54 Managing the levels of disease in crops is essential to maintain the high yield and quality  
55 required to feed the growing global population. Disease control is often achieved by  
56 integrating different methods including the use of specific agricultural practices to lower the  
57 risk of disease occurring combined with varietal resistance and plant protection products such  
58 as fungicides (Walters et al., 2012). Control offered by varietal resistance based on race-  
59 specific resistance genes can breakdown due to the emergence of newly virulent races of  
60 plant pathogens (Brown, 2015). Similarly, prolonged use of fungicides to control crop  
61 pathogens can lead to the evolution of fungicide insensitive isolates. Fungal isolates  
62 exhibiting insensitivity to fungicides have been characterised for many important crop  
63 pathogens including the major pathogens on spring barley one of the most important crops in  
64 Scotland. Isolates insensitive to different fungicide active ingredients have been reported for  
65 *Rhynchosporium commune* (Phelan et al., 2016), *Ramularia collo-cygni* (Matusinsky et al.,  
66 2011; Piotrowska et al., 2016) and *Blumeria graminis* f. sp *hordei* (Bäumler et al., 2003;  
67 Wyand and Brown, 2005), the fungal pathogens responsible for Rhynchosporium scald,  
68 Ramularia leaf spot (RLS) and powdery mildew diseases of barley, respectively. Use of  
69 fungicides to control crop diseases is also at risk from EU legislation which aims to reduce  
70 fungicide inputs and may result in the removal of important active ingredients from use in  
71 agriculture (Hillocks, 2012).

72 With the effectiveness of varietal resistance eroding and the risk of reduced efficacy and  
73 potentially availability of fungicides to control crop pathogens, alternative options for disease  
74 control are required. The use of compounds that elicit the plants defence response has been  
75 shown to provide control in crops against different plant pathogens although this control can  
76 often be inconsistent and dependent on the crop variety and environment (McGrann et al.,  
77 2017; Oxley and Walters, 2012; Walters et al., 2008; 2011a; 2011b). Another alternative

78 type of plant protection product are film-forming polymers. The waxy cuticle of the leaf  
79 surface acts as the primary barrier to pathogen invasion but also contains features that act as  
80 cues for attachment and germination of fungal spores, and for subsequent germ tube growth  
81 and pathogen invasion (Ringelmann et al., 2009; Kolattukudy et al., 1995). Applying film-  
82 forming polymers that coat the leaf surface can suppress foliar infection by pathogens and  
83 consequently provide disease control (Walters, 2006). Sutherland and Walters (2001)  
84 initially demonstrated that film forming polymers could inhibit *in vitro* growth of  
85 *Pyrenophora avenae* and *Magnaporthe oryzae* and then reported that these polymers reduced  
86 *in planta* infection by the obligate biotroph *B. graminis* f. sp. *hordei* on barley under  
87 controlled environment conditions and in the field (Sutherland and Walters, 2002). Percival  
88 and Boyle (2009) showed that film-forming polymers could reduce the development of  
89 *Venturia inaequalis* and the severity of scab disease on apple. However, it was noted that the  
90 control conferred by the various polymers tested was not as effective as a typical fungicide  
91 treatment. Disease control provided by film-forming polymers is usually mediated by the  
92 polymer acting as a physical barrier to penetration, interfering with the processes involved in  
93 spore adhesion, hydration and germination or by disguising the topography of the leaf surface  
94 to prevent host recognition during germ tube growth (Walters, 2006). As these compounds  
95 usually do not act directly against the pathogens, the efficacy of film-forming polymers to  
96 control crop diseases is not likely to be at risk from insensitive fungal isolates evolving that  
97 reduces the effectiveness of the polymers.

98 Here we report the effects of foliar application of an arabinoxylan polymer to reduce disease  
99 in field grown spring barley. Arabinoxylans are one of the main cell wall polysaccharides in  
100 cereals (Fincher, 2009) and could provide a novel, cost-effective and environmentally benign  
101 plant protection product to be used in disease management programmes to reduce reliance on  
102 fungicides for disease control in crops.

103

## 104 **2. Materials and methods**

### 105 2.1 Plant protection products

106 An arabinoxylan polymer, derived from maize cell walls, was obtained from Cambridge  
107 Biopolymers Ltd., Cleveland, UK. Initial studies on barley seedlings indicated that the  
108 polymer forms a film coating on the leaf surface (Rätsep et al., 2012). The polymer was  
109 applied in field trials in an unmodified form. Arabinoxylan was dissolved in deionised water  
110 to obtain a 2% w/v solution and polymerised by adding 3% hydrogen peroxide and 100  
111 purpuroallin units of horseradish peroxidase. The polymerisation solution was mixed by  
112 shaking and incubated at 25°C for 10 minutes. Following the incubation step, a firm gel was  
113 formed, which was dissolved in water and diluted to a working concentration of 0.08%  
114 arabinoxylan. The efficacy of the polymer to control disease in spring barley was tested in  
115 field trial experiments and compared against various fungicides typically used for plant  
116 protection. Details of the different fungicides used in this work are presented in Table 1.

### 117 2.2 Spring barley field trial experiments

118 The effect of the arabinoxylan polymer treatment on lowering disease levels on spring barley  
119 was assessed in field trials conducted at the Bush Estate in 2010, 2011 and 2012 and at  
120 Lanark, Scotland, UK in 2011 and 2012. Spring barley was sown in a randomised block  
121 design in plots of 10 x 2 m at a seed rate of 360 seeds m<sup>-2</sup>, with a minimum of three replicates  
122 per treatment in each trial. Local standard agronomic practices were applied to each trial  
123 except for fungicide applications which are trial specific. All treatments were applied using a  
124 knapsack sprayer in a volume equivalent to 200 L ha<sup>-1</sup> of water (Walters et al., 2011a).

#### 125 2.2.1 Spring barley field trial at Bush Estate 2010

126 In 2010 the spring barley variety Optic was sown at the Bush Estate, Edinburgh, Scotland on  
127 March 6th. The polymer (0.002 L ha<sup>-1</sup>) was applied as single application at growth stages  
128 (GS) GS24, GS31, GS49 and GS59 based on the scale of Zadocks et al. (1974), as a double  
129 application at GS25 and GS31 and as a triple application at GS25, GS31 and GS49 (Table 2).  
130 For each treatment three replicate plots were assessed. Disease control was evaluated by  
131 visually scoring powdery mildew (*Blumeria graminis* f. sp. *hordei*) symptoms as a proportion  
132 of leaf area covered averaged across the upper three leaf layers. Mildew symptoms were  
133 scored at GS39, GS49, GS73 and GS83 at a minimum of three points across the length of the  
134 plot. Disease score data was used to calculate the area under the disease progress curve  
135 (AUDPC; Shaner and Finney, 1977) for statistical analysis. cv. Optic has a resistance rating  
136 of 5 for powdery mildew based on the AHDB (Agricultural and Horticultural Development  
137 Board) recommended list 2011-12 (<http://cereals.ahdb.org.uk/varieties.aspx>). The effects of  
138 the polymer treatments on mildew control and yield were compared to a series of different  
139 fungicide treatments typical of local disease control programmes (Table 2). Plots were  
140 harvested using a research combine on September 3<sup>rd</sup> 2010. Grain from each experimental  
141 plot was collected and weighed as kg plot<sup>-1</sup>. Moisture content was assessed on a 1 kg  
142 subsample collected from each plot which was oven dried at 103°C for 24 hours and used to  
143 standardise the yield in each plot to 85% dry matter (Walters et al. 2011c).

#### 144 2.2.2 Spring barley field trials at Bush Estate 2011 and 2012

145 At Bush Estate in 2011 and 2012 the effect of the polymer on disease control on four spring  
146 barley varieties was assessed. The varieties were selected based on disease resistance ratings  
147 against *Rhynchosporium* scald (*Rhynchosporium commune*): NFC Tipple (*Rhynchosporium*  
148 resistance rating 4), Panther (4), Quench (8), Shuffle (6). RLS resistance ratings for UK  
149 spring barley varieties were not released until 2013 and are therefore not reported as part of  
150 this study. The trials were sown on March 21<sup>st</sup> 2011 and March 15<sup>th</sup> 2012. Disease



151 symptoms for *Rhynchosporium* and *Ramularia* leaf spot (RLS; *Ramularia collo-cygni*) were  
152 visually assessed as a proportion of leaf area covered with disease lesions averaged across the  
153 upper three leaf layers. In 2011 both diseases were first scored at a point when the GS of the  
154 four varieties varied between GS32-49. The two further scores date saw all four varieties at  
155 the same GS when scored at GS63 and GS76. Disease was scored at a minimum of three  
156 points across the length of the plot. In 2012 disease was scored at three dates corresponding  
157 to GS31, GS39 and GS72. Disease score data was used to calculate AUDPC for statistical  
158 analysis. The polymer treatment was applied at GS24, GS31 and GS49 and compared to  
159 untreated control plots and plots treated with a fungicide programme of Siltra Xpro (0.5 L ha<sup>-1</sup>)  
160 at GS31 and Proline 275 (0.175 L ha<sup>-1</sup>) and Bravo (0.5 L ha<sup>-1</sup>) at GS49 (Table 2). Yield  
161 was calculated for each plot at 85% dry matter following harvest of the trials on August 30<sup>th</sup>  
162 2011 and September 4<sup>th</sup> 2012 as described for the 2010 trial. Three replicate plots were  
163 assessed per treatment for each variety.

### 164 2.2.3 Spring barley trials at Lanark in 2011 and 2012

165 Two spring barley varieties were assessed in the field trials at Lanark in 2011 and 2012. The  
166 trials were sown on March 24<sup>th</sup> 2011 and March 22<sup>nd</sup> 2012. Spring barley cv. Concerto has  
167 high resistance against mildew (8) but low resistance against *Rhynchosporium* (4) and cv.  
168 Optic has low resistance to both mildew (5) and *Rhynchosporium* (4). In 2011 disease  
169 symptoms were scored at GS32 and GS76. In 2012 only *Rhynchosporium* was scored and it  
170 was assessed three times at dates when it was noted that the two varieties were at different  
171 growth stages. cv. Optic was scored at GS32, GS57 and GS79 whereas cv. Concerto was  
172 scored when the crop was between GS35-37 and then again at GS62 and GS82. Diseases  
173 were visually assessed as a proportion of leaf area covered with disease lesions averaged  
174 across the upper three leaf layers at a minimum of three points across the length of the plot.  
175 Plots were sprayed with a range of different polymer treatments based on number of

176 applications (x1, x2, x4), timing of applications (GS24, GS31, GS39, GS59) and applications  
177 with full and reduced fungicides programmes. Full details of the different treatments used in  
178 this trial are presented in Table 2. Treatments containing the polymer were compared to  
179 untreated controls and a standard fungicide programme (Table 2). Yield was calculated for  
180 each plot at 85% dry matter following harvest of the trials on September 15<sup>th</sup> 2011 and  
181 September 19<sup>th</sup> 2012 as described for the 2010 trial. Three replicate plots were assessed per  
182 treatment for each variety.

### 183 2.3 Meteorological data collection

184 Local meteorological data was recorded at the Bush and Lanark trial sites using automatic  
185 weather recording stations (Delta-T Devices, Cambridge, UK). located *in situ*. Sensors were  
186 used to monitor air temperature and rainfall. Mean local temperature (°C) and rainfall (mm)  
187 was collected for each 24 hour period and used to calculate the monthly averages for each  
188 parameter. No data was recorded by the weather station at the Bush site February 2<sup>nd</sup> to 13<sup>th</sup>  
189 2012 nor at the Lanark site April 18<sup>th</sup> to May 1<sup>st</sup> 2011

### 190 2.4 Statistical analysis

191 Data were analysed using GenStat v15 (Payne et al., 2009). Variation in mildew  
192 development on spring barley cv. Optic at Bush Estate in 2010 was assessed using a  
193 generalized linear model (GLzM) with the canonical link function transformation to  
194 approximate normality. Block and treatment were used as factors in the GLzM. The same  
195 factors were also used in a general linear model (GLM) to assess variation in yield in this  
196 trial. Generalized linear modelling was used to assess variation in the different disease levels  
197 in the 2011 and 2012 field trials at both Bush Estate and Lanark. AUDPC data was square  
198 root transformed to approximate normality. Variation attributed to block, variety, treatment  
199 and the interaction between variety and treatment was assessed within the GLzM. Effects on

200 yield were assessed with a GLM with using the same factors as the GLzM. Variability in  
201 local environmental conditions was assessed between sites, years and months using a GLM  
202 for mean local temperatures (°C) and a GLzM with the logarithmic link function  
203 transformation for average rainfall (mm).

### 204 **3. Results**

#### 205 3.1 Field trial assessment of the arabinoxylan polymer on disease control in spring barley at 206 Bush Estate, Scotland, UK

207 At Bush Estate in 2010 none of the polymer treatments significantly reduced mildew  
208 development on spring barley cv. Optic whereas all of the fungicides treatments significantly  
209 reduced disease development (Fig. 1;  $P < 0.05$ ) except the application of Fandango and  
210 Flexity at GS25 alone ( $P = 0.064$ ). All treatments except the application of the polymer at  
211 both GS25 and GS31 ( $P = 0.062$ ) or at GS59 only ( $P = 0.779$ ) significantly increased yield  
212 compared to the untreated control (Fig. 2A;  $P < 0.001$ ).

213 In 2011 at Bush Estate higher levels of Rhynchosporium were observed on cv. NFC Tipple  
214 and cv. Panther (Fig. 3A) which both have lower resistance rating for this disease whereas  
215 NFC Tipple had lower levels of RLS (Fig. 3C). The polymer treatment had no effect on  
216 Rhynchosporium development or on yield in any of the varieties tested in this trial (Fig. 3A).  
217 A significant reduction in RLS was only observed on cv. Quench plots treated with the  
218 polymer (Fig. 3C;  $P = 0.008$ ). The fungicide treatment significantly reduced  
219 Rhynchosporium levels (Fig. 3A) on cv. NFC Tipple ( $P < 0.001$ ) and Panther ( $P = 0.018$ ) and  
220 lowered RLS levels (Fig. 3C) on cv. Panther ( $P = 0.004$ ), Quench ( $P = 0.020$ ) and Shuffle ( $P$   
221  $< 0.001$ ). Significant yield increases were only observed in fungicide treated (Fig. 2B) cv.  
222 NFC Tipple ( $P = 0.001$ ), cv. Quench ( $P < 0.001$ ) and cv. Shuffle ( $P = 0.003$ ).

223 The polymer treatments had no effect on reducing Rhynchosporium or RLS development or  
224 on yield in the trials at Bush Estate in 2012. Similar to the 2011 trial Rhynchosporium  
225 development was highest on cv. NFC Tipple (Fig. 3B). The fungicide treatment was only  
226 effective at lowering Rhynchosporium on cv. NFC Tipple ( $P = 0.045$ ) whereas fungicide  
227 application significantly reduced RLS (Fig. 3D) in all four varieties ( $P < 0.001$ ). However,  
228 yields were significantly increased in fungicide treated cv. NFC Tipple ( $P = 0.003$ ) and cv.  
229 Quench ( $P = 0.029$ ) only (Fig. 2C).

230

### 231 3.2 Field trial assessment of the arabinoxylan polymer on disease control in spring barley at 232 Lanark, Scotland, UK

233 In the 2011 trial at Lanark a significant effect on mildew development was observed for both  
234 variety and treatment (Fig. 4A;  $P < 0.001$ ). The variety effect can be explained by the  
235 presence of the mutant *mlo* allele, which confers immunity to powdery mildew (Jørgensen,  
236 1992), in cv. Concerto. Therefore, no treatment effect was observed on cv. Concerto. There  
237 were treatment effects on cv. Optic with polymer applications at GS24+GS31 ( $P = 0.021$ ;  
238 Treatment 6 [T6]) or GS24+GS39 ( $P = 0.002$ ; T7) as well as all polymer treatments that  
239 included either a full or reduced fungicide programme ( $P < 0.001$ ; T11-15). The full  
240 fungicide programme also significantly reduced mildew in this trial ( $P < 0.001$ ; T16).

241 No effect of variety was observed on Rhynchosporium levels at Lanark in 2011 ( $P = 0.635$ )  
242 but there was a significant treatment effect (Fig. 4B;  $P < 0.001$ ). Rhynchosporium was  
243 significantly reduced on both varieties by the standard fungicide programme (T16), polymer  
244 application at GS59 ( $P < 0.05$ ; T5) and with all polymer plus fungicide treatments ( $P < 0.05$ )  
245 except the polymer at GS24 plus Proline ® 275 at GS39 (T13) on cv. Concerto. Significant  
246 reductions in Rhynchosporium levels compared to control plants were also seen on cv.

247 Concerto with the polymer applications at GS31+GS59 (T3;  $P = 0.031$ ) and cv. Optic  
248 following the polymer treatments at GS31 (T3;  $P = 0.040$ ) and at GS31+GS59 (T9;  $P =$   
249  $0.039$ ).

250 RLS levels were significantly affected by both treatment and variety ( $P < 0.001$ ) with higher  
251 levels of this disease typically observed on cv. Concerto compared to cv. Optic (Fig. 4C).  
252 The standard fungicide programme significantly reduced RLS levels in both varieties (T16;  $P$   
253  $< 0.05$ ). All polymer applications that included full or reduced fungicide treatments also  
254 significantly reduced RLS on cv. Concerto ( $P < 0.01$ ) as did the polymer treatments at  
255 GS31+GS39 (T9;  $P = 0.034$ ). On cv. Optic only the polymer treatments that included  
256 fungicides were effective at reducing RLS (T11, T12, T14;  $P < 0.05$ ) although not all  
257 polymer plus fungicide treatments significantly reduced the disease on this variety.

258 Yield was significantly affected (Fig. 2D) by both variety and treatment ( $P < 0.001$ ) with a  
259 significant interaction between these two factors also observed ( $P = 0.032$ ). Significant yield  
260 responses were recorded on cv. Concerto following polymer application at GS31+GS59 (T9;  
261  $P = 0.040$ ), polymer at GS24 followed by the standard fungicide programme (T11;  $P <$   
262  $0.001$ ), polymer at GS24 (T12;  $P = 0.040$ ) or at GS24+GS31 plus the reduced fungicide  
263 programme (T14;  $P = 0.021$ ) as well as the standard fungicide programme ( $P < 0.006$ ; T16).  
264 On cv. Optic yield responses were observed on plants that received the full fungicide  
265 programme plus those polymer applications that included a full or reduced fungicide  
266 treatment (T11-16;  $P < 0.05$ ).

267 The 2012 trial at Lanark exhibited very high levels of *Rhynchosporium* such that the  
268 observed levels of mildew were too low to deduce any accurate conclusions from and  
269 therefore not presented. *Rhynchosporium* development was significantly affected by  
270 treatment ( $P < 0.001$ ) but not variety ( $P = 0.066$ ). Only the polymer treatments that were

271 applied in combination with either a full or reduced fungicide programme (T11-15) or the full  
272 fungicide programme (T16) alone had a significant effect on reducing *Rhynchosporium*  
273 development (Fig. 4D) on cv. Concerto ( $P < 0.01$ ) or cv. Optic ( $P < 0.01$ ). Yield was not  
274 significantly affected by either variety ( $P = 0.154$ ) or treatment ( $P = 0.764$ ) despite the observed  
275 disease control (Fig. 2E).

### 276 3.3 Environmental variation between field trials

277 Crops were slightly forward at Bush Estate in 2011 compared to 2010 and 2012 with GS25  
278 recorded more than one week earlier than in the other two years. However, the crops reached  
279 GS39 at approximately the same time in each season during the first week of June (Fig. S1A).  
280 Spring barley development was typically slower in crops grown at Lanark compared to those  
281 grown at Bush Estate (Fig. S1A). In particular crop development was slower in the 2012  
282 season at Lanark with crop growth stages at least one week behind in 2012 compared to  
283 2011. There was no significant difference in mean local temperatures (Fig. S1B) between the  
284 Bush and Lanark sites ( $P = 0.063$ ) but 2011 was on the whole warmer than 2010 or 2012 ( $P <$   
285  $0.05$ ). There was significantly more rainfall at the Bush site ( $P < 0.001$ ) over the duration of  
286 the trials. Significantly more rainfall was recorded in 2011 and 2012 (Fig. S1C;  $P < 0.05$ ).

287

## 288 **4. Discussion**

289 As alternatives to traditional disease management options such as fungicides and varietal  
290 resistance are sought compounds that can induce the plant defence response have received a  
291 lot of attention as potential plant protection products with mixed results on disease control  
292 (McGrann et al., 2017; Oxley and Walters, 2012; Walters et al., 2008; 2011a; 2011b; 2013).  
293 Less attention has been directed towards the use of film-forming polymers as plant protection

294 products. This study examined the potential of an arabinoxylan polymer derived from maize  
295 to control fungal diseases in spring barley. Treatment with the polymer did provide disease  
296 control on spring barley but the results were variable and dependent on environmental  
297 conditions associated with different trial sites and year of study. Applications of the polymer  
298 as the sole plant protection product were able to reduce the development of powdery and  
299 *Rhynchosporium* of spring barley at Lanark in 2011 but there was no consistency in the  
300 number or timing of polymer applications associated with disease control (Fig. 4B).

301

302 Polymers have previously been shown to significantly reduce the development of fungal  
303 disease on a number of different crops. Application of film-forming polymers prior to fungal  
304 inoculation in glasshouse experiments tends to result in better levels of disease control  
305 (Haggag, 2002; Walters, 1992) although treatment post inoculation can also provide adequate  
306 disease control (Sutherland and Walters, 2002). On spring barley Walters (1992)  
307 demonstrated that three different film-forming polymers were able to reduce powdery mildew  
308 development in glasshouse trials. However, Sutherland and Walters (2002) showed that the  
309 control of mildew on spring barley provided by polymers was not as effective in field grown  
310 crops compared to glasshouse plants. Based on the evidence from our experiments the  
311 arabinoxylan polymer is unlikely to be suitable as a plant protection if used as a single active  
312 ingredient, at least at the dose rate used in this study. Where film-forming polymers have  
313 been tested as plant protection products in almost all cases the disease control afforded by  
314 these compounds is not as strong as that provided by more traditional synthetic fungicides  
315 (Percival et al., 2006; Percival and Boyle, 2009; Sutherland and Walters, 2002). Film-  
316 forming polymers can offer protection against invading pathogens by forming a physical  
317 barrier on the plant to prevent fungal colonisation but the efficacy of these compounds to  
318 control fungal disease varies (an, 1990; Elad et al., 1990; Walters, 1992; Ziv and Zitter,

319 1992). Based on the different chemical and physical properties of these compounds, each  
320 film-forming polymer is likely to function differently under the changing environmental  
321 conditions crops encounter each growing season. However, the barriers formed by polymers  
322 do not stretch as the crops grows and therefore differences in crop development between sites  
323 and years may affect the efficacy of the arabinoxylan polymer to control disease as observed  
324 between the trials reported here (Fig. S1). This level of inconsistent disease control is similar  
325 to that observed for plant defence elicitors that can effectively reduce disease but are not as  
326 reliable as fungicides (Walters et al., 2013). However, whether or not using increased dose  
327 rates of the polymer would improve the consistency of disease control when used as a single  
328 active ingredient remains to be determined.

329 Promising results were observed when the polymer was used in combination with fungicide  
330 applications where more consistent levels of disease control were recorded. Of particular  
331 interest is the potential to use the arabinoxylan polymer with reduced rates of fungicides.  
332 Significant levels of disease control were observed when the polymer was used as an early  
333 treatment to the crop and the GS31 fungicide application was omitted from the disease  
334 control programme (Fig. 4). Reduced fungicide applications are preferable, where possible,  
335 in modern agriculture to not only protect the environment but to also lower the risk of fungal  
336 isolates becoming insensitive to the active ingredients and therefore reducing the efficacy of  
337 the chemical control measures. Research with defence elicitor compounds when used with  
338 reduced fungicide applications has also showed potential for providing effective disease  
339 control (McGrann et al., 2017; Oxley and Walters, 2012). Employing alternative crop  
340 protection products such as this arabinoxylan polymer within reduced fungicide application  
341 programmes may allow fungicides to be used in a more sustainable way.

342 To fully utilise the arabinoxylan polymer as a component of integrated disease control  
343 programmes in crops a better understanding of the mechanisms through which this compound



344 reduces disease is required. Preliminary electron microscopy showed that the polymer forms  
345 a film on the leaf surface (Rätsep et al., 2012). This may indicate the arabinoxylan  
346 compound could act by altering surface hydrophobicity or thickness to prevent spore  
347 attachment or fungal penetration to the crop (Walters, 2006). The film-forming properties of  
348 polymers has led to these products also being used as anti-transpirants to protect plants from  
349 water loss (Faralli et al., 2016; Kettlewell et al., 2010). This can lead to yield penalties  
350 caused by blocked transpiration and photosynthesis particularly if the timing of the  
351 application is incorrect (Kettlewell et al., 2010). No yield penalties were observed in plots  
352 treated with the arabinoxylan polymer in any of the trials presented here (Fig. 2) suggesting  
353 that at the dose rate used in these experiments the polymer has no negative effect on yield.  
354 Increased yields were observed in the Lanark trials in 2011 for most of the polymer  
355 applications that included a full or reduced fungicide programme (Fig. 2D). At the Bush  
356 Estate in 2010 mildew development was not significantly affected by any of the  
357 treatments that included a GS25 fungicide application combined with at least one polymer  
358 application. However, despite the lack of disease control in this trial spring barley yields were  
359 improved except when the polymer was applied at GS49 (Fig. 2A). This contrasts with the  
360 spring barley trial at Lanark site in 2012 where despite significant disease lowering effects no  
361 yield response was observed in the crop (Fig. 4D+Fig. 2E). Detailed analysis of the  
362 mechanism through which the arabinoxylan polymer operates in disease control may provide  
363 insights for the optimum deployment of this compound in crop protection.

## 364 **5. Conclusions**

365 The arabinoxylan polymer is unlikely to be an effective plant protection product when used  
366 as an individual active ingredient. However, using this polymer within a fungicide  
367 programme may allow lower fungicide dose rates to be used, potentially slowing the risk of  
368 fungicide insensitive isolates evolving. Integrating film-forming polymers within crop

369 protection programmes may offer a means to help protect crops against disease and  
370 safeguarding the efficacy of available chemical control options whilst also reducing water  
371 loss.

## 372 **Acknowledgments**

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478 Table 1 List of fungicides used in field trial experiments

| <b>Trade name</b> | <b>Active Ingredient</b>   | <b>Company</b>                   |
|-------------------|--|----------------------------------|
| Fandango ®        | 100 g L <sup>-1</sup> prothioconazole plus 100 g L <sup>-1</sup> fluoxastrobin | Bayer CropScience, Cambridge, UK |
| Flexity ®         | 300 g L <sup>-1</sup> metrafenone.   | BASF, Cheshire, UK               |
| Bravo ® 500       | 500 g L <sup>-1</sup> chlorothalonil   | Syngenta, Jealott's Hill, UK     |
| Tracker ®         | 233 g L <sup>-1</sup> boscalid plus 67 g L <sup>-1</sup> epoxiconazole.        | BASF, Cheshire, UK               |
| Pentangle ®       | 500 g L <sup>-1</sup> chlorothalonil plus 180 g L <sup>-1</sup> tebuconazole.  | Nufarm, Victoria, Australia      |
| AmiStar ® Opti    | 100 g L <sup>-1</sup> azoxystrobin plus 500 g L <sup>-1</sup> chlorothalonil   | Syngenta, Jealott's Hill, UK     |
| Proline ® 275     | 275 g L <sup>-1</sup> prothioconazole  | Bayer CropScience, Cambridge, UK |
| Siltra ® Xpro     | 60 g L <sup>-1</sup> bixafen plus 200 g L <sup>-1</sup> prothioconazole        | Bayer CropScience, Cambridge, UK |

479

Table 2 Fungicide and elicitor treatments used in spring barley field trials 2010-2012

| <b>Bush Estate 2010</b>  | <b>Bush Estate 2011 and 2012</b>   | <b>Lanark 2011 and 2012</b> |
|--|--|-----------------------------|
| -Untreated   | -Untreated   | -Untreated                  |
| -Fandango (1.0 L ha <sup>-1</sup> ) + Flexity (0.25 L ha <sup>-1</sup> ) GS25 <sup>a</sup><br>(1.0 L ha <sup>-1</sup> )  | -Polymer GS24 and GS31 and<br>GS49   | -Polymer GS24               |
| -Fandango (1.0 L ha <sup>-1</sup> ) + Flexity (0.25 L ha <sup>-1</sup> ) GS25+<br>Bravo (1.0 L ha <sup>-1</sup> ) GS49 <sup>a</sup>  | -Siltra Xpro (0.5 L ha <sup>-1</sup> ) GS31<br>and Proline 275 (0.175 L ha <sup>-1</sup> ) +<br>Bravo GS49 (0.5 L ha <sup>-1</sup> ) | -Polymer GS31               |
| -Fandango (1.0 L ha <sup>-1</sup> ) + Flexity (0.25 L ha <sup>-1</sup> ) GS25+<br>Pentangle (1.0 L ha <sup>-1</sup> ) GS49 <sup>a</sup>                                    |  | -Polymer GS39               |
| -Fandango (1.0 L ha <sup>-1</sup> ) + Flexity (0.25 L ha <sup>-1</sup> ) GS25+<br>Tracker (1.0 L ha <sup>-1</sup> ) GS49 <sup>a</sup>                                      |  | -Polymer GS59               |
| -Fandango (1.0 L ha <sup>-1</sup> ) + Flexity (0.25 L ha <sup>-1</sup> ) GS25+<br>AmiStar Opti (1.0 L ha <sup>-1</sup> ) GS49 <sup>a</sup>                                 |  | -Polymer GS24 and GS31      |
| -Fandango (1.0 L ha <sup>-1</sup> ) + Flexity (0.25 L ha <sup>-1</sup> ) GS25+<br>Proline 275 (0.4 L ha <sup>-1</sup> ) +Bravo (1.0 L ha <sup>-1</sup> ) GS49 <sup>a</sup> |  | -Polymer GS24 and GS39      |
| -Polymer GS25  |  | -Polymer GS31 and GS59      |
| -Polymer GS25 and GS31   |  | -Polymer GS31 and GS39      |



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|                                 |   |
|---------------------------------|---|
| -Polymer GS25 and GS31 and GS49 | -Polymer GS24 and GS31 and GS39 and GS59  |
| -Polymer GS49                   | -Polymer GS24 and Siltra Xpro (0.5 L ha <sup>-1</sup> ) GS31 and Proline 275 (0.175 L ha <sup>-1</sup> ) + Bravo GS39 (0.5 L ha <sup>-1</sup> ) |
| -Polymer GS59                   | -Polymer GS24 and Proline 275 (0.175 L ha <sup>-1</sup> ) + Bravo GS39 (0.5 L ha <sup>-1</sup> ) and Polymer GS59                               |
|                                 | -Polymer GS24 and Proline 275 (0.175 L ha <sup>-1</sup> ) + Bravo GS39 (0.5 L ha <sup>-1</sup> )  |
|                                 | -Polymer GS24 and GS31 and Proline 275 (0.175 L ha <sup>-1</sup> ) + Bravo GS39 (0.5 L ha <sup>-1</sup> )                                       |
|                                 | -Polymer GS24 and GS31 and Proline 275 (0.175 L ha <sup>-1</sup> ) + Bravo GS39 (0.5 L ha <sup>-1</sup> ) and Polymer GS59                      |
|                                 | -Siltra Xpro (0.5 L ha <sup>-1</sup> ) GS31 and Proline 275 (0.175 L ha <sup>-1</sup> ) + Bravo GS39 (0.5 L ha <sup>-1</sup> )                  |

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## Figure legends

Fig.1 Field trial assessment of the effect of an arabinoxylan polymer and fungicide treatments at Bush Estate, Scotland in 2010 on A, Powdery mildew development on spring barley cv. Optic. Polymers were applied as single application or multiple applications at different growth stages (GS). All fungicide treatments received Fandango (1.0 L ha<sup>-1</sup>) + Flexity (0.25 L ha<sup>-1</sup>) at GS25, labelled Fungicide GS25 on x-axis, followed by different fungicide products at GS49 as indicated on the x-axis. \* = P < 0.05, \*\* = P < 0.01, \*\*\* = P < 0.001.

Fig. 2 Field trial assessment of the effect of an arabinoxylan polymer and fungicide treatment on yield. Yield is assessed at 85% dry matter at A, trials at Bush Estate, Scotland in 2010 on cv. Optic. cv. Optic. Polymers were applied as single application or multiple applications at different growth stages (GS). All fungicide treatments received Fandango (1.0 L ha<sup>-1</sup>) + Flexity (0.25 L ha<sup>-1</sup>) at GS25, labelled Fungicide GS25 on x-axis, followed by different fungicide products at GS49 as indicated on the x-axis. B, in spring barley at Bush Estate, Scotland in 2011, C, in spring barley at Bush Estate, Scotland in 2012 assessed on four spring barley varieties that were untreated (light grey bars; controls), treated with the fungicide (black bars) Siltra XPro (0.5 L ha<sup>-1</sup>) at GS31 and GS49 Proline 275 (0.175 L ha<sup>-1</sup>) plus Bravo (0.5 L ha<sup>-1</sup>) or with the polymer (dark grey bars) at GS24, GS31 and GS49 (0.002 L ha<sup>-1</sup>). D, in spring barley at Lanark, Scotland in 2011 and E, in spring barley at Lanark, Scotland 2012 was assessed on cv. Concerto (grey bars) and cv. Optic (black bars). Treatments used in the Lanark trials: T1 = untreated; T2 = Polymer GS24; T3 = Polymer GS31; T4 = Polymer GS39; T5 = Polymer GS59; T6 = Polymer GS24+31; T7 = Polymer GS24+39; T8 = Polymer GS31+59; T9 = Polymer GS31+39; T10 = Polymer GS24+31+39+59; T11 = Polymer GS24 and Siltra Xpro (0.5 L ha<sup>-1</sup>) GS31 and Proline 275 (0.175 L ha<sup>-1</sup>) + Bravo GS39 (0.5 L ha<sup>-1</sup>);

T12 = Polymer GS24 and Proline 275 (0.175 L ha<sup>-1</sup>) + Bravo GS39 (0.5 L ha<sup>-1</sup>) and Polymer GS59; T13 = Polymer GS24 and Proline 275 (0.175 L ha<sup>-1</sup>) + Bravo GS39 (0.5 L ha<sup>-1</sup>); T14 = Polymer GS24 and GS31 and Proline 275 (0.175 L ha<sup>-1</sup>) + Bravo GS39 (0.5 L ha<sup>-1</sup>); T15 = Polymer GS24 and GS31 and Proline 275 (0.175 L ha<sup>-1</sup>) + Bravo GS39 (0.5 L ha<sup>-1</sup>) and Polymer GS59; T16 = Siltra Xpro (0.5 L ha<sup>-1</sup>) GS31 and Proline 275 (0.175 L ha<sup>-1</sup>) + Bravo GS39 (0.5 L ha<sup>-1</sup>). \* = P < 0.05, \*\* = P < 0.01, \*\*\* = P < 0.001.

Fig. 3 Field trial assessment of the effect of an arabinoxylan polymer and fungicide treatment on disease development in spring barley at Bush Estate, Scotland in 2011 and 2012.

Rhynchosporium scald in A, 2011 and B, 2012; Ramularia leaf spot in C, 2011 and D, 2012 were assessed on four spring barley varieties that were untreated (light grey bars; controls), treated with the fungicide (black bars) Siltra XPro (0.5 L ha<sup>-1</sup>) at GS31 and GS49 Proline 275 (0.175 L ha<sup>-1</sup>) plus Bravo (0.5 L ha<sup>-1</sup>) or with the polymer (dark grey bars) at GS24, GS31 and GS49 (0.002 L ha<sup>-1</sup>). \* = P < 0.05, \*\* = P < 0.01, \*\*\* = P < 0.001.

Fig. 4 Field trial assessment of the effect of an arabinoxylan polymer and fungicide treatment on disease development in spring barley at Lanark, Scotland in 2011 and 2012. In 2011 the effects of different polymer and fungicide applications on powdery mildew, A,

Rhynchosporium scald, B, Ramularia leaf spot, C, were assessed on spring barley cv.

Concerto (grey bars) and cv. Optic (black bars). In 2012 the effects of the different polymer and fungicide treatments were assessed on Rhynchosporium scald, D, in spring barley cv.

Concerto and cv. Optic. \* = P < 0.05, \*\* = P < 0.01, \*\*\* = P < 0.001. Treatments used in the

Lanark trials: T1 = untreated; T2 = Polymer GS24; T3 = Polymer GS31; T4 = Polymer

GS39; T5 = Polymer GS59; T6 = Polymer GS24+31; T7 = Polymer GS24+39; T8 = Polymer

GS31+59; T9 = Polymer GS31+39; T10 = Polymer GS24+31+39+59; T11 = Polymer GS24

and Siltra Xpro (0.5 L ha<sup>-1</sup>) GS31 and Proline 275 (0.175 L ha<sup>-1</sup>) + Bravo GS39 (0.5 L ha<sup>-1</sup>);

T12 = Polymer GS24 and Proline 275 (0.175 L ha<sup>-1</sup>) + Bravo GS39 (0.5 L ha<sup>-1</sup>) and Polymer

GS59; T13 = Polymer GS24 and Proline 275 ( $0.175 \text{ L ha}^{-1}$ ) + Bravo GS39 ( $0.5 \text{ L ha}^{-1}$ ); T14 = Polymer GS24 and GS31 and Proline 275 ( $0.175 \text{ L ha}^{-1}$ ) + Bravo GS39 ( $0.5 \text{ L ha}^{-1}$ ); T15 = Polymer GS24 and GS31 and Proline 275 ( $0.175 \text{ L ha}^{-1}$ ) + Bravo GS39 ( $0.5 \text{ L ha}^{-1}$ ) and Polymer GS59; T16 = Siltra Xpro ( $0.5 \text{ L ha}^{-1}$ ) GS31 and Proline 275 ( $0.175 \text{ L ha}^{-1}$ ) + Bravo GS39 ( $0.5 \text{ L ha}^{-1}$ ).

### **Supplementary material**

Fig. S1 Site and year dependent temporal variation in spring barley crop development and environmental conditions observed in field trials at Bush Estate (2010, 2011, 2012) and Lanark (2011, 2012), Scotland, UK. (A) Spring barley growth stages, (B) mean 24 hour temperature ( $^{\circ}\text{C}$ ) per month, (C) mean 24 hour rainfall (mm) per month

Fig. 1

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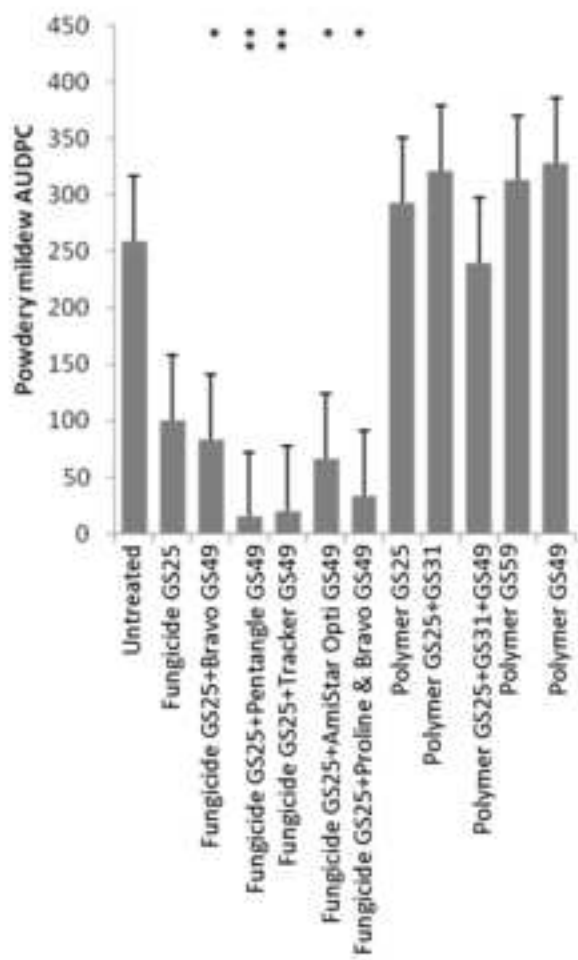


Fig. 2

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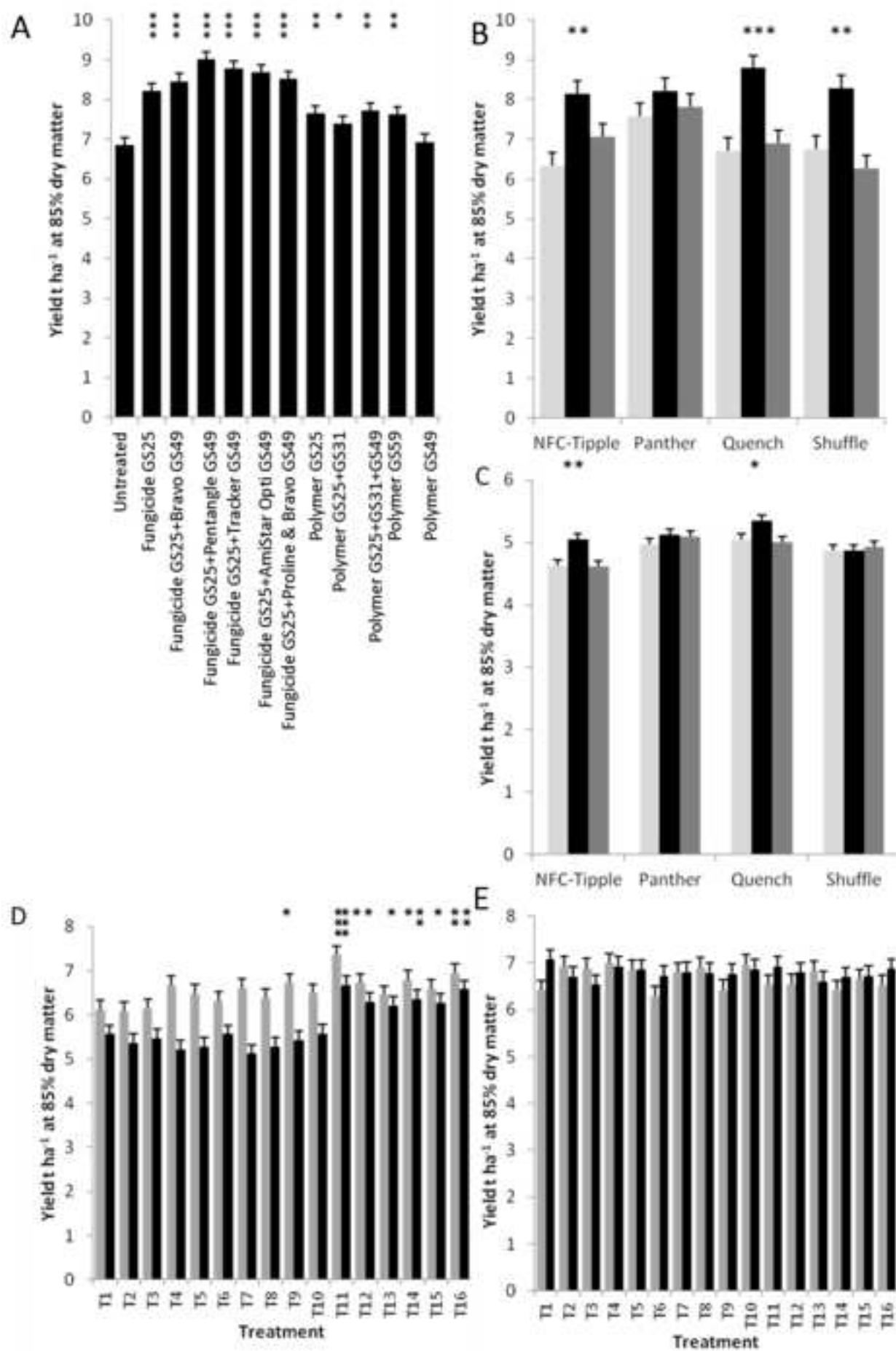


Fig. 3

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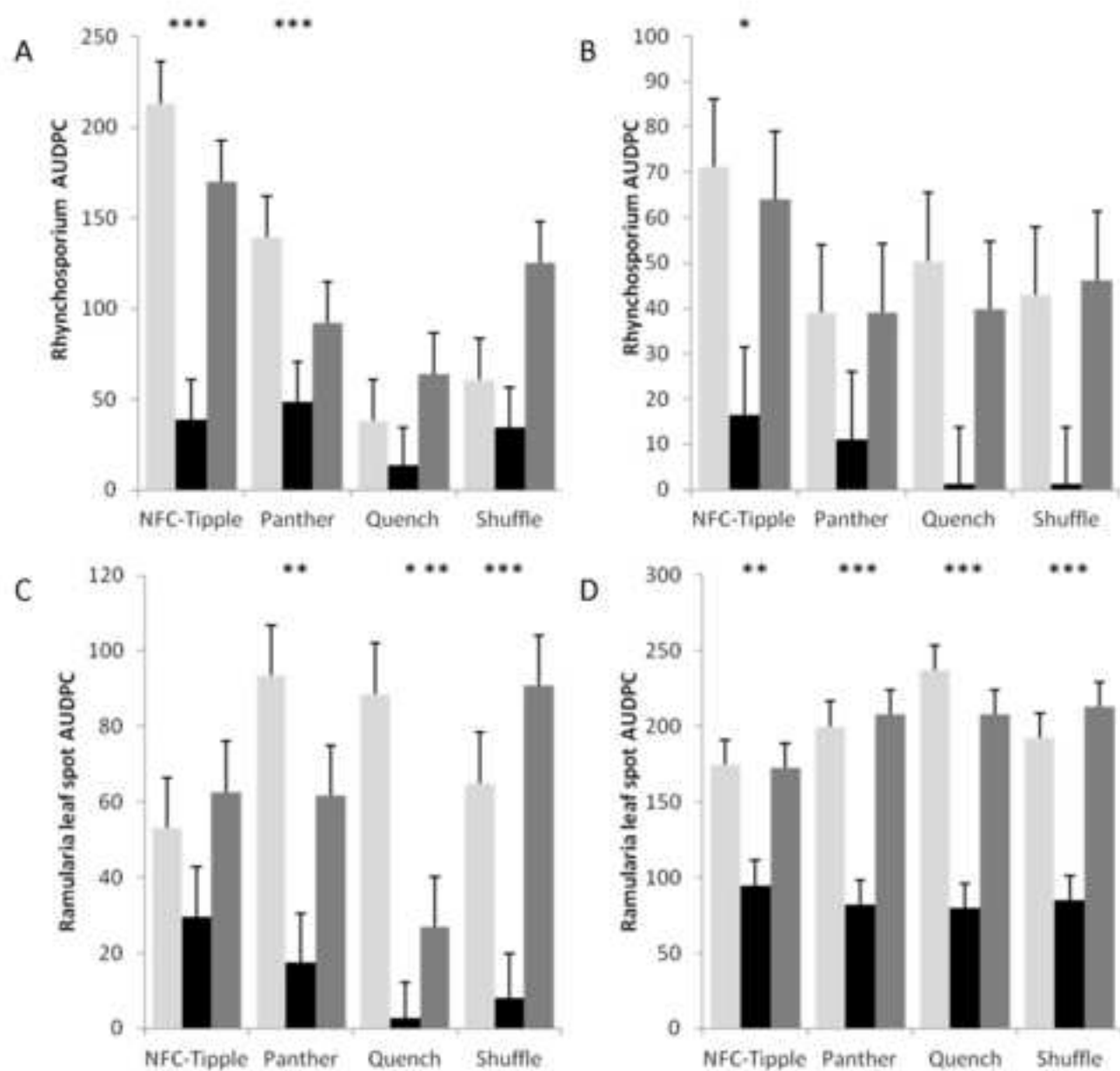


Fig. 4

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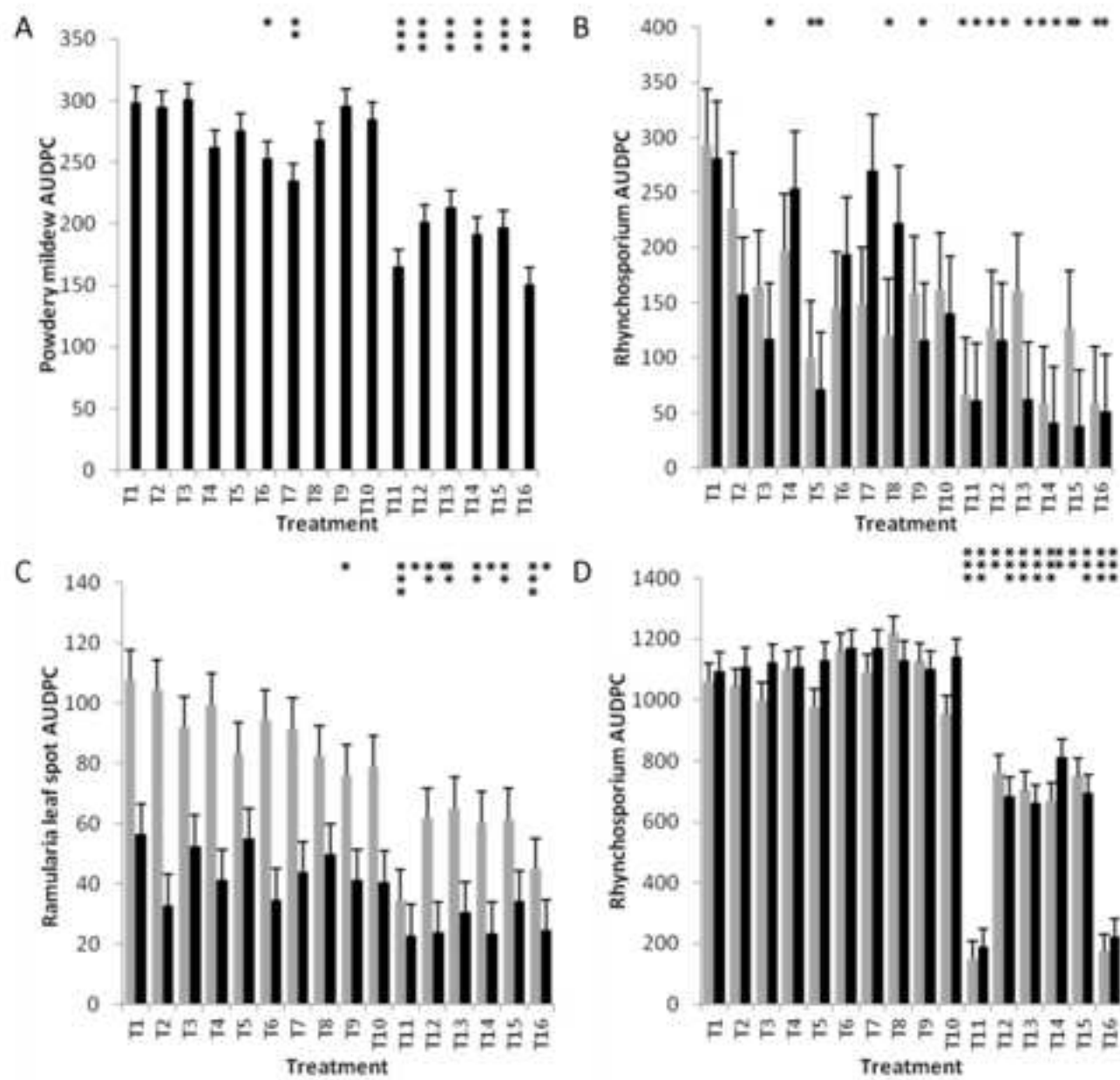
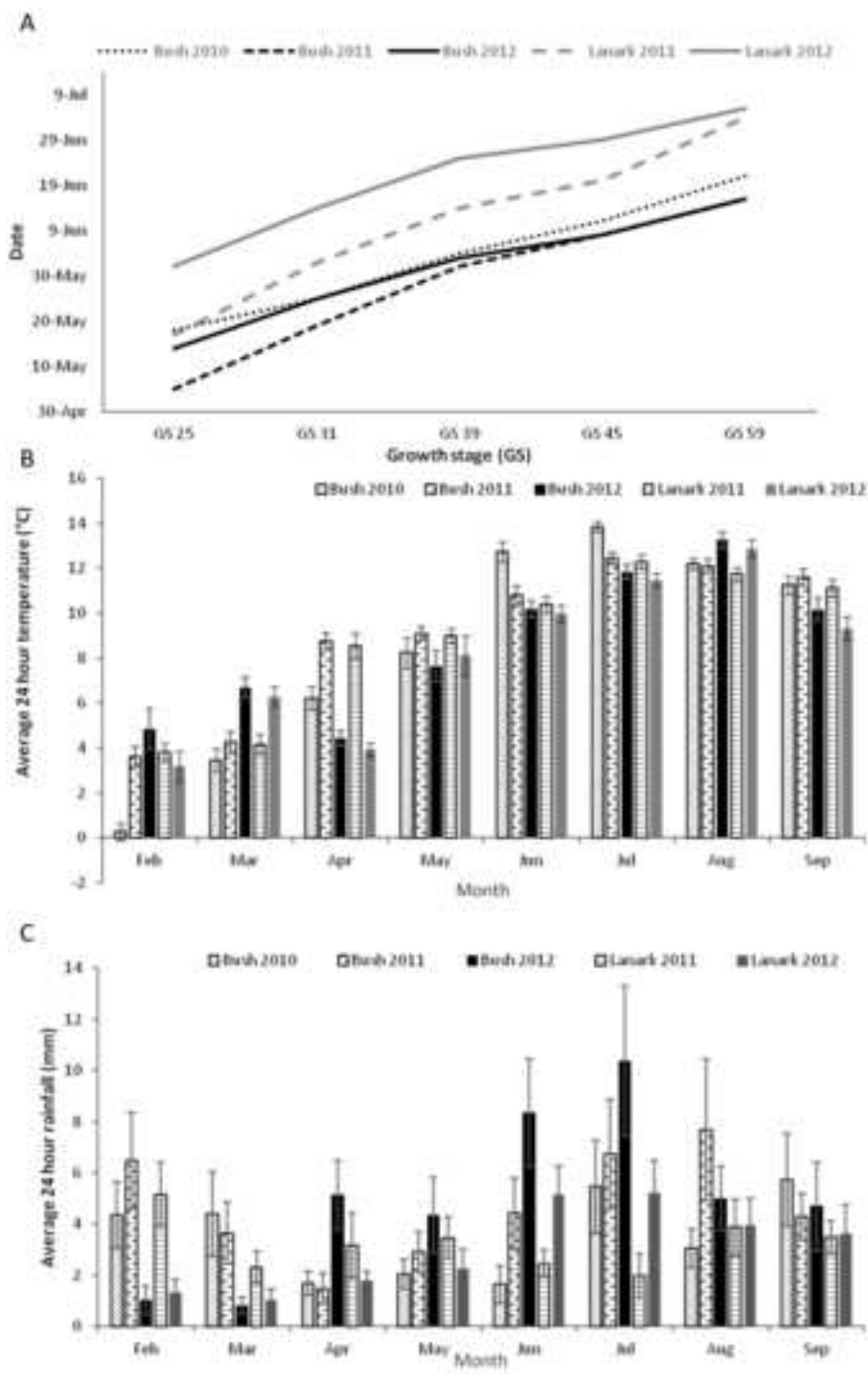




Fig. S1

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## Highlights

- Disease management using an arabinoxylan polymer were assessed
- Polymer-mediated control varied between sites, year, crop variety and disease
- Combined polymer plus reduced fungicide application offered more consistent control
- No yield penalties were associated with polymer applications
- Polymers may be useful as an early treatment in integrated disease management