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

Ratsep, J; Havis, ND; Loake, GJ; Walters, DR; McGrann, GRD

Published in:
Crop Protection

DOI:
10.1016/j.cropro.2018.03.003

First published: 30/03/2018

Document Version
Peer reviewed version

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

Jaan Rästep[1]

Neil D Havis[1]

Gary J Loake[2]

Dale R Walters[1]

Graham RD McGrann[1, *]

[1] Crop Protection Team, Crop and Soil Systems Group, SRUC, West Mains Road, Edinburgh, EH9 3JG

[2] Institute of Molecular Plant Sciences, School of Biological Sciences, University of Edinburgh, King's Buildings, Edinburgh, EH9 3BF, UK

[*] Present address: Potato Branch, Science & Advice for Scottish Agriculture (SASA), Roddinglaw Road, Edinburgh, EH12 9FJ

Corresponding author:

Dr Graham McGrann

Email: grahammcgrann@googlemail.com

Telephone: +44 (0) 131 535 4000

Keywords:

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

Abbreviations:

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

Declarations of interest:

The authors declare they have no competing interests.

Funding:

This research was funded by the Grain Research Development Corporation (GRDC) project No. SAC00001 and the Scottish Government funded work package 'Epidemiology of disease' 2011-16.

Abstract

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

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

1. Introduction

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

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

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

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

2. Materials and methods

2.1 Plant protection products

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

2.2 Spring barley field trial experiments

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

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^{-1}) 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 3rd 2010. Grain from each experimental
141 plot was collected and weighed as kg $plot^{-1}$. 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 21st 2011 and March 15th 2012. Disease

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

2.2.3 Spring barley trials at Lanark in 2011 and 2012

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

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

2.3 Meteorological data collection

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

2.4 Statistical analysis

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

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

3. Results

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

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

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

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

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

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

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

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

3.3 Environmental variation between field trials

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

4. Discussion

As alternatives to traditional disease management options such as fungicides and varietal resistance are sought compounds that can induce the plant defence response have received a lot of attention as potential plant protection products with mixed results on disease control (McGrann et al., 2017; Oxley and Walters, 2012; Walters et al., 2008; 2011a; 2011b; 2013). 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 in 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

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

5. Conclusions

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

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

Acknowledgments

We thank Cambridge Biopolymers Ltd., Cleveland, UK for the arabinoxylan polymer and Rodrigo Alegria (SRUC) for technical assistance. This research was funded by the Grain Research Development Corporation (GRDC) project No. SAC00001. This work is kindly supported by the Scottish Government funded work package 'Epidemiology of disease' 2011-16. SRUC receives support from Scottish Government RESAS.

References

Baumler, S.S., Umler, B., Felsenstein, F.G., Schwarz, G.G., 2003. CAPS and DHPLC analysis of a single nucleotide polymorphism in the cytochrome b gene conferring resistance to strobilurins in field isolates of *Blumeria graminis* f. sp. *hordei*. J. Phytopathology. 151, 149–152.

Brown, J.K.M., 2015. Durable desistance of crops to disease: A Darwinian perspective. Annu. Rev. Phytopathol. 53, 513–539. doi:10.1146/annurev-phyto-102313-045914

Elad, Y., Ayish, N., Katan, J., 1990. Control of grey mould (*Botrytis cinerea*) with film-forming polymers. Plant Pathol. 39, 249–254. doi:10.1111/j.1365-3059.1990.tb02500.x

Faralli, M., Grove, I.G., Hare, M.C., Boyle, R.D., Williams, K.S., Corke, F.M.K., Kettlewell, P.S., 2016. Canopy application of film antitranspirants over the reproductive phase enhances yield and yield-related physiological traits of water-stressed oilseed rape (*Brassica napus*). Crop Pasture Sci. 67, 751–765. doi:10.1071/CP15421

391 Fincher, G.B., 2009. Update on cell wall biology in the grasses revolutionary times in our
392 understanding of cell wall biosynthesis and remodeling in the grasses. Plant Physiol.
393 149, 27–37. doi:10.1104/pp.108.130096

394 Haggag, W.M., 2002. Application of epidermal coating antitranspirants for controlling
395 cucumber downy mildew in greenhouse. Plant Pathol. Bull. 11, 69–78.

396 Han, J.-S., 1990. Use of antitranspirant epidermal coatings for plant protection in China.
397 Plant Dis. 74, 263–266.

398 Hillocks, R.J., 2012. Farming with fewer pesticides: EU pesticide review and resulting
399 challenges for UK agriculture. Crop Prot. 31, 85–93. doi:10.1016/j.cropro.2011.08.008

400 Jørgensen, I.H., 1992. Discovery, characterization and exploitation of *Mlo* powdery mildew
401 resistance in barley. Euphytica 63, 141–152. doi:10.1007/BF00023919

402 Kettlewell, P.S., Heath, W.L., Haigh, I.M., 2010. Yield enhancement of droughted wheat by
403 film antitranspirant application: rationale and evidence. Agric. Sci. 1, 143–147.
404 doi:10.4236/as.2010.13017

405 Kolaitukudy, P.E., Rogers, L.M., Li, D., Hwang, C.-S., Flaishman, M.A., 1995. Surface
406 signaling in pathogenesis. P. Natl. Acad. Sci. USA. 92, 4080–4087.

407 Matusinsky, P., Svobodova-Leisova, L., Marik, P., Tvaruzek, L., Stemberkova, L., Hanusova,
408 M., Minarikova, V., Vysohlidova, M., Spitzer, T., 2011. Frequency of a mutant allele of
409 cytochrome b conferring resistance to QoI fungicides in the Czech population of
410 *Ramularia collo-cygni*. J. Plant Dis. Protect. 117, 248–252.

411 McGrann, G.R.D., Yoxall, T., Paterson, L.J., Taylor, J.M.G., Birmpilis, I.G., Walters, D.R.,
412 Havis, N.D., 2017. Control of light leaf spot and clubroot in brassica crops using

413 defence elicitors. Eur. J. Plant Pathol. 148, 447-461 doi:10.1007/s10658-016-1103-7

414 Oxley, S.J.P., Walters, D.R., 2012. Control of light leaf spot (*Pyrenopeziza brassicae*) on
415 winter oilseed rape (*Brassica napus*) with resistance elicitors. Crop Prot. 40, 59–62.
416 doi:10.1016/j.cropro.2012.04.028

417 Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B., Soutar, D.M. 2009. GenStat for
418 Windows (12th Edition) Introduction. *VSN International, Hemel Hempstead.*

419 Percival, G.C., Boyle, S., 2009. Evaluation of film forming polymers to control apple scab
420 (*Venturia inaequalis* (Cooke) G. Wint.) under laboratory and field conditions. Crop Prot.
421 28, 30–35. doi:10.1016/j.cropro.2008.08.005

422 Percival, G.C., Keary, I.P., Marshall, K., 2006. The use of film-forming polymers to control
423 Guignardia leaf blotch and powdery mildew on *Aesculus hippocastanum* L. and *Quercus*
424 *robur* L. Arboric. Urban For. 32, 100–107.

425 Phelan, S., Barthe, M.-S., Tobie, C., Kildea, S., 2016. Detection of the cytochrome b
426 mutation G143A in Irish *Rhynchosporium commune* populations using targeted 454
427 sequencing. Pest. Manag. Sci. doi:10.1002/ps.4434

428 Piotrowska, M.J., Fountaine, J.M., Ennos, R.A., Kaczmarek, M., Burnett, F.J., 2016.
429 Characterisation of *Ramularia collo-cygni* laboratory mutants resistant to Succinate
430 Dehydrogenase Inhibitors. Pest Manag. Sci. doi:10.1002/ps.4442

431 Rätsep, J., Havis, N.D., Loake, G., Jeffrey, C., Walters, D.R. 2012. Use of arabinoxylan
432 polymers for plant defence. Proceedings Crop Protection Northern Britain 2012, p167-
433 172

434

435 Ringelmann, A., Riedel, M., Riederer, M., Hildebrandt, U., 2009. Two sides of a leaf blade:
436 *Blumeria graminis* needs chemical cues in cuticular waxes of *Lolium perenne* for
437 germination and differentiation. Planta 230, 95–105. doi:10.1007/s00425-009-0924-4
438
439 Shaner, G., Finney, R.E., 1977. The effect of nitrogen fertilization on the expression of slow-
440 mildewing resistance in knox wheat. Phytopathology 77, 1051–1056.
441 doi:10.1094/Phyto-67-1051
442 Sutherland, F., Walters, D.R., 2002. Effect of film-forming polymers on infection of barley
443 with the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*. Eur. J. Plant Pathol.
444 108, 385–389. doi:10.1023/A:1016077914741
445 Sutherland, F., Walters, D.R., 2001. *In vitro* effects of film-forming polymers on the growth
446 and morphology of *Pyrenophora avenae* and *Pyricularia oryzae*. J. Phytopathol. 149,
447 621–624.
448 Walters, D.R., 2006. Disguising the leaf surface: The use of leaf coatings for plant disease
449 control. Eur. J. Plant Pathol. 114, 255–260. doi:10.1007/s10658-005-5463-7
450 Walters, D.R., 1992. The effects of three film-forming polymers, with and without a
451 polyamine biosynthesis inhibitor, on powdery mildew infection of barley seedlings.
452 Ann. Appl. Biol. 120, 41–46. doi:10.1111/j.1744-7348.1992.tb03401.x
453 Walters, D.R., Ratsep, J., Havis, N.D., 2013. Controlling crop diseases using induced
454 resistance: Challenges for the future. J. Exp. Bot. 64, 1263–1280. doi:10.1093/jxb/ert026
455 Walters, D.R., Avrova, A., Bingham, I.J., Burnett, F.J., Fountaine, J., Havis, N.D., Hoad,
456 S.P., Hughes, G., Looseley, M., Oxley, S.J.P., Renwick, A., Topp, C.F.E., Newton,
457 A.C., 2012. Control of foliar diseases in barley: towards an integrated approach. Eur. J.

458 Plant Pathol. 133, 33–73

459 Walters, D.R., Havis, N.D., Sablou, C., Walsh, D.J., 2011a. Possible trade-off associated with
460 the use of a combination of resistance elicitors. Physiol. Mol. Plant P. 75, 188–192.
461 doi:10.1016/j.pmpp.2011.02.001

462 Walters, D.R., Paterson, L., Sablou, C., Walsh, D.J., 2011b. Existing infection with
463 *Rhynchosporium secalis* compromises the ability of barley to express induced resistance.
464 Eur. J. Plant Pathol. 130, 73–82. doi:10.1007/s10658-010-9733-7

465 Walters, D.R., Havis, N.D., Paterson, L., Taylour, J., Walsh, D.J., 2011c. Cultivar effects on
466 the expression of induced resistance in spring barley. Plant Dis. 95, 595–600. doi:
467 10.1094/PDIS-08-10-0577

468 Walters, D.R., Paterson, L., Walsh, D.J., Havis, N.D., 2008. Priming for plant defense in
469 barley provides benefits only under high disease pressure. Physiol. Mol. Plant P. 73,
470 95–100. doi:10.1016/j.pmpp.2009.03.002

471 Wyand, R.A., Brown, J.K.M., 2005. Sequence variation in the *CYP51* gene of *Blumeria*
472 *graminis* associated with resistance to sterol demethylase inhibiting fungicides. Fungal
473 Genet. Biol. 42, 726–735. doi:10.1016/j.fgb.2005.04.007

474 Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of
475 cereals. Weed Res. 14, 415–421. doi:10.1111/j.1365-3180.1974.tb01084.x

476 Ziv, O., Zitter, T.A., 1992. Effects of biocarbonates and film-forming polymers on cucurbit
477 foliar diseases. Plant Dis. 76, 513–517.

478 Table 1 List of fungicides used in field trial experiments

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

479

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

Bush Estate 2010	**Bush Estate 2011 and 2012**	**Lanark 2011 and 2012**
-Untreated	-Untreated	-Untreated
-Fandango (1.0 L ha^{-1}) + Flexity (0.25 L ha^{-1}) GS25[a]	-Polymer GS24 and GS31 and GS49	-Polymer GS24
(1.0 L ha^{-1})		
-Fandango (1.0 L ha^{-1}) + Flexity (0.25 L ha^{-1}) GS25+	-Siltra Xpro (0.5 L ha^{-1}) GS31	-Polymer GS31
Bravo (1.0 L ha^{-1}) GS49[a]	and Proline 275 (0.175 L ha^{-1}) +	
	Bravo GS49 (0.5 L ha^{-1})	
-Fandango (1.0 L ha^{-1}) + Flexity (0.25 L ha^{-1}) GS25+		-Polymer GS39
Pentangle (1.0 L ha^{-1}) GS49[a]		
-Fandango (1.0 L ha^{-1}) + Flexity (0.25 L ha^{-1}) GS25+		-Polymer GS59
Tracker (1.0 L ha^{-1}) GS49[a]		
-Fandango (1.0 L ha^{-1}) + Flexity (0.25 L ha^{-1}) GS25+		-Polymer GS24 and GS31
AmiStar Opti (1.0 L ha^{-1}) GS49[a]		
-Fandango (1.0 L ha^{-1}) + Flexity (0.25 L ha^{-1}) GS25+		-Polymer GS24 and GS39
Proline 275 (0.4 L ha^{-1}) +Bravo (1.0 L ha^{-1}) GS49[a]		
-Polymer GS25		-Polymer GS31 and GS59
-Polymer GS25 and GS31		-Polymer GS31 and GS39

-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^{-1}) GS31 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1})
-Polymer GS59	-Polymer GS24 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1}) and Polymer GS59
	-Polymer GS24 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1})
	-Polymer GS24 and GS31 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1})
	-Polymer GS24 and GS31 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1}) and Polymer GS59
	-Siltra Xpro (0.5 L ha^{-1}) GS31 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1})

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^{-1}) + Flexity (0.25 L ha^{-1}) 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^{-1}) + Flexity (0.25 L ha^{-1}) 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^{-1}) at GS31 and GS49 Proline 275 (0.175 L ha^{-1}) plus Bravo (0.5 L ha^{-1}) or with the polymer (dark grey bars) at GS24, GS31 and GS49 (0.002 L ha^{-1}). 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^{-1}) GS31 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1});

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

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^{-1}) at GS31 and GS49 Proline 275 (0.175 L ha^{-1}) plus Bravo (0.5 L ha^{-1}) or with the polymer (dark grey bars) at GS24, GS31 and GS49 (0.002 L ha^{-1}). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

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^{-1}) GS31 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1}); T12 = Polymer GS24 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1}) and Polymer

GS59; T13 = Polymer GS24 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1}); T14 = Polymer GS24 and GS31 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1}); T15 = Polymer GS24 and GS31 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 L ha^{-1}) and Polymer GS59; T16 = Siltra Xpro (0.5 L ha^{-1}) GS31 and Proline 275 (0.175 L ha^{-1}) + Bravo GS39 (0.5 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 (°C) per month, (C) mean 24 hour rainfall (mm) per month

Fig. 1

Click here to download high resolution image

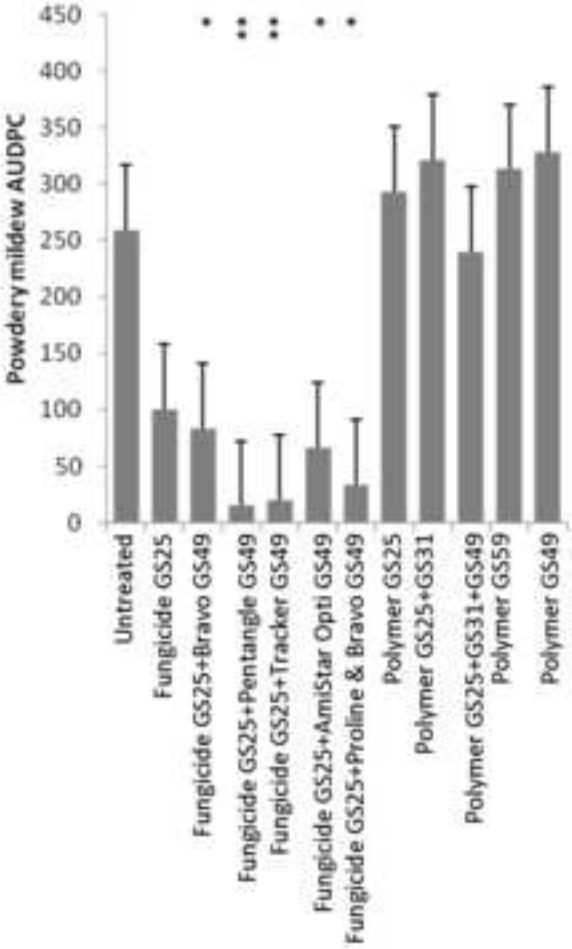


Fig. 2

Click here to download high resolution image

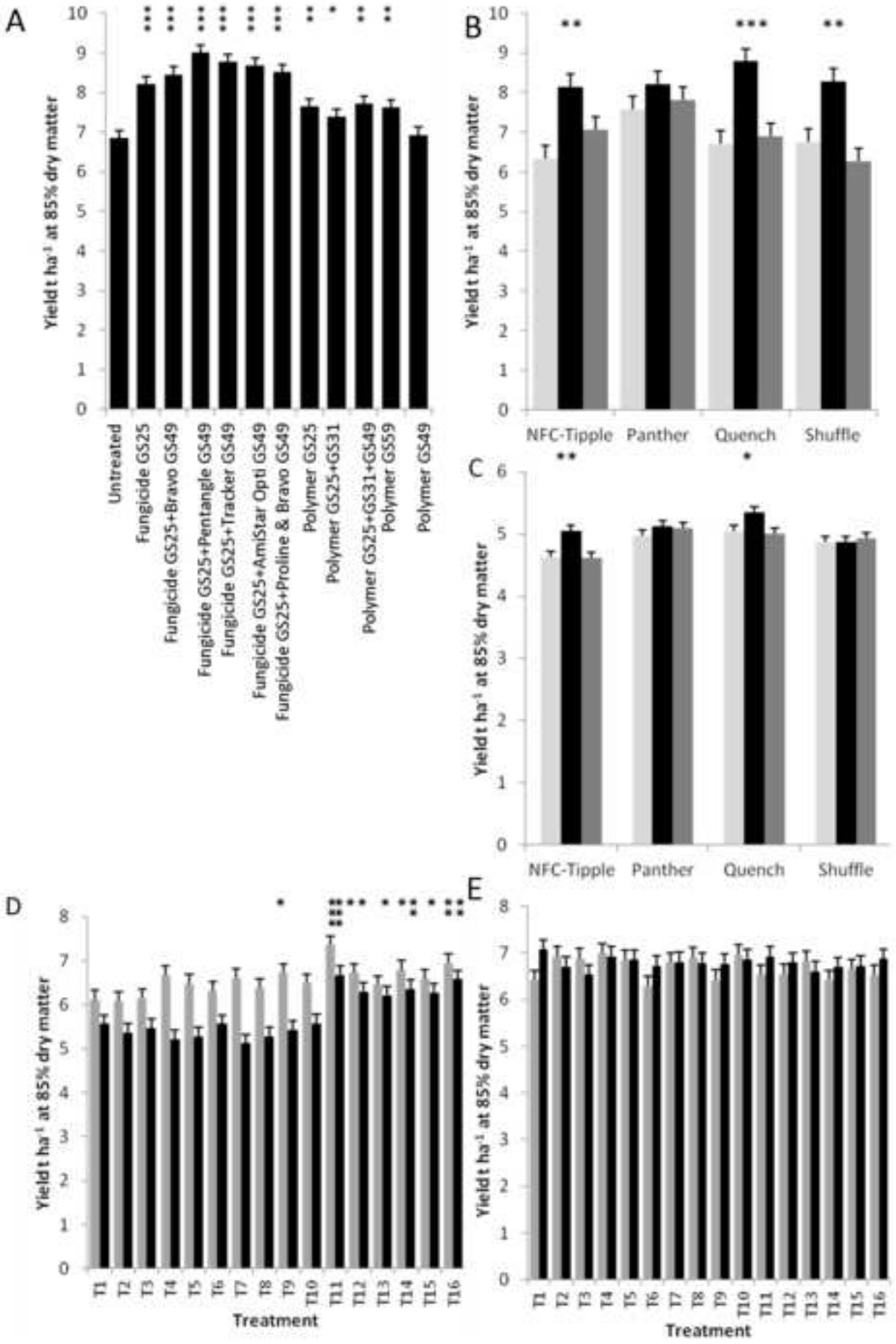


Fig. 3

Click here to download high resolution image

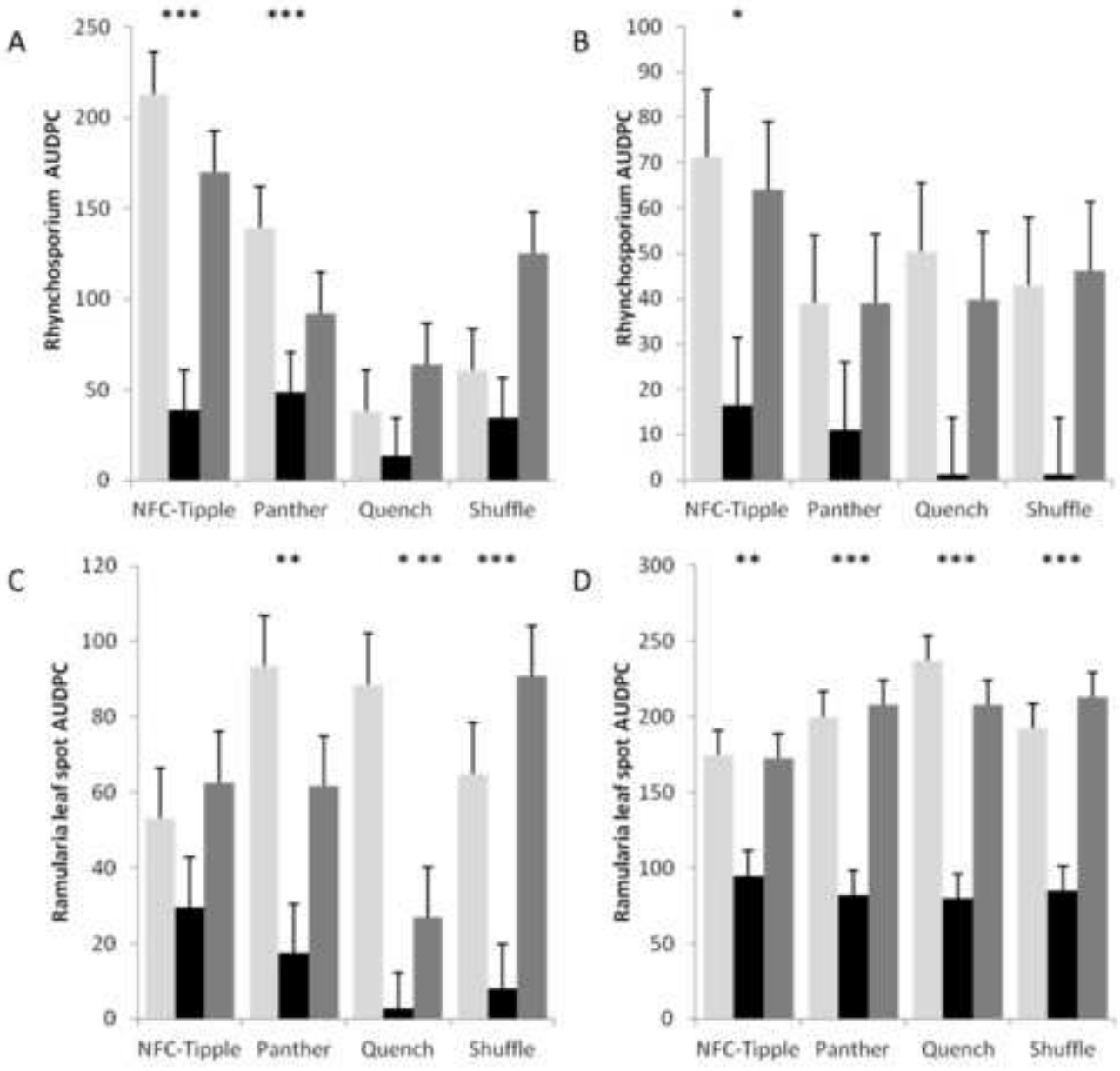


Fig. 4

Click here to download high resolution image

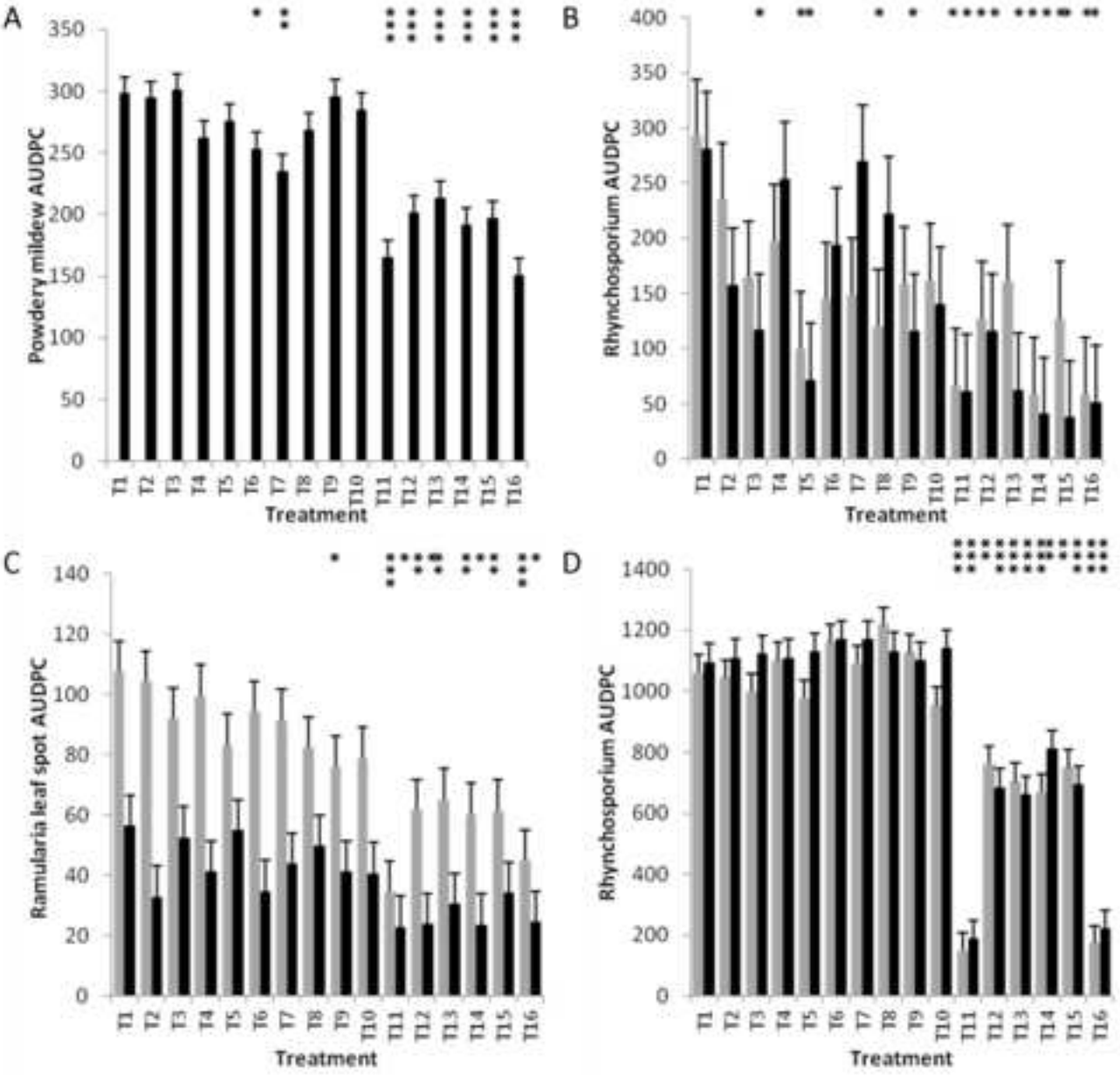
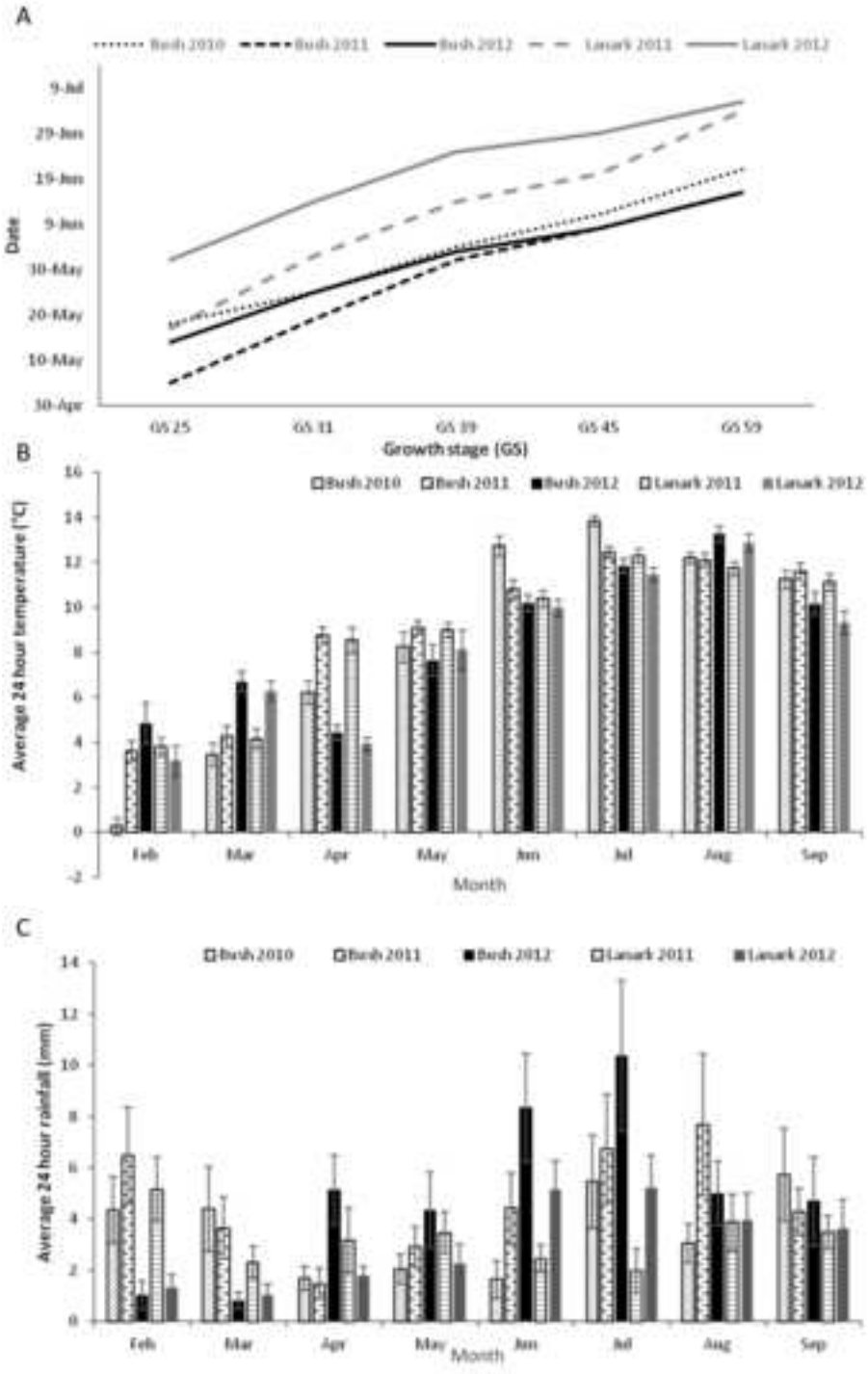


Fig. S1

Click here to download high resolution image



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