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

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

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 resistance 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., 2016; 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 in 2010, 2011 and 2012. Spring barley was sown in randomised 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. In 2010 the spring barley variety Optic was sown at the Bush Estate, Edinburgh, Scotland. The polymer (0.002 L ha⁻¹) was applied at growth stages (GS) GS24, GS31, GS49 and GS59 based on the scale of Zadocks et al. (1974), with some treatments repeating the application at multiple GS. Disease control was evaluated by visually scoring powdery mildew (*Blumeria graminis* f. sp. *hordei*) symptoms throughout the growing season and calculating the area under the disease progress curve (AUDPC; Shaner and Finney, 1977) for statistical analysis. cv. Optic has a resistance rating of 5 for powdery mildew based on the AHDB (Agricultural and Horticultural Development Board) recommended list 2011-12 (<http://cereals.ahdb.org.uk/varieties.aspx>). Yield was assessed in each plot at 85% dry matter. The effects of the polymer treatments on mildew control and yield were compared to a series of different fungicide treatments typical of local disease control programmes (Table 2).

In 2011 and 2012 trials were conducted at the Bush estate and at Lanark, Scotland, UK. At Bush Estate four spring barley varieties were assessed. The varieties were selected based on disease resistance ratings against Rhynchosporium scald (*Rhynchosporium commune*): NFC Tipple (Rhynchosporium resistance rating 4), Panther (4), Quench (8), Shuffle (6). Disease symptoms for Rhynchosporium and Ramularia leaf spot (RLS; *Ramularia collo-cygni*) were visually assessed throughout the growing season and used to calculate AUDPC for statistical analysis. Yield was calculated for each plot at 85% dry matter at the end of the trial. 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).

Two spring barley varieties were assessed in the field trials at Lanark in 2011 and 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). RLS resistance ratings for UK spring barley varieties were not released until 2013 and are therefore not reported as part of this study. Rhynchosporium and

mildew symptom development and plot yields were determined as in the trials at Bush Estate. RLS was scored in the Lanark trial in 2011 only. Plots were sprayed with various polymer treatments based on number of applications, timing of applications and applications with full and reduced fungicides programmes. Treatments containing the polymer were compared to untreated controls and a standard fungicide programme (Table 2). All treatments were applied using a knapsack sprayer in a volume equivalent to 200 L ha⁻¹ of water (Walters et al., 2011a).

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. 1A; $P < 0.05$) 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. 1B; $P < 0.001$).

In 2011 at Bush Estate higher levels of *Rhynchosporium* were observed on cv. NFC Tipple and cv. Panther (Fig. 2A) which both have lower resistance rating for this disease whereas NFC Tipple had lower levels of RLS (Fig. 2C). The polymer treatment had no effect on *Rhynchosporium* development or on yield in any of the varieties tested in this trial (Fig. 2A). A significant reduction in RLS was only observed on cv. Quench plots treated with the polymer (Fig. 2C; $P = 0.008$). The fungicide treatment significantly reduced *Rhynchosporium* levels (Fig. 2A) on cv. NFC Tipple ($P < 0.001$) and Panther ($P = 0.018$) and lowered RLS levels (Fig. 2C) 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. 2E) 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. 2B). The fungicide treatment was only effective at lowering *Rhynchosporium* on cv. NFC Tipple ($P = 0.045$) whereas fungicide application significantly reduced RLS (Fig. 2D) 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. 2F).

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. 3A; $P < 0.001$). The variety effect can be explained by the presence of the mutant *mlo* allele, which confers immunity to 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. 3B; $P < 0.001$). *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. Concerto with the polymer applications at GS31+GS59 (T3; $P = 0.031$) and cv. Optic following the polymer treatments at GS31 (T3; $P = 0.040$) and at GS31+GS59 (T9; $P = 0.039$).

RLS levels were significantly affected by both treatment and variety ($P < 0.001$) with higher levels of this disease typically observed on cv. Concerto compared to cv. Optic (Fig. 3C). The standard fungicide programme significantly reduced RLS levels in both varieties (T16; $P < 0.05$). All polymer applications that included full or reduced fungicide treatments also significantly reduced RLS on cv. Concerto ($P < 0.01$) as did the polymer treatments at GS31+GS39 (T9; $P = 0.034$). On cv. Optic only the polymer treatments that included fungicides were effective at reducing RLS (T11, T12, T14; $P < 0.05$) although not all polymer plus fungicide treatments significantly reduced the disease on this variety.

Yield was significantly affected (Fig. 3D) by both variety and treatment ($P < 0.001$) with a significant interaction between these two factors also observed ($P = 0.032$). Significant yield responses were recorded on cv. Concerto following polymer application at GS31+GS59 (T9; $P = 0.040$), polymer at GS24 followed by the standard fungicide programme (T11; $P < 0.001$), polymer at GS24 (T12; $P = 0.040$) or at GS24+GS31 plus the reduced fungicide programme (T14; $P = 0.021$) as well as the standard fungicide programme ($P < 0.006$; T16). On cv. Optic yield responses were observed on plants that received the full fungicide programme plus those polymer applications that included a full or reduced fungicide treatment (T11-16; $P < 0.05$).

The 2012 trial at Lanark exhibited very high levels of *Rhynchosporium* such that the observed levels of mildew were too low to deduce any accurate conclusions from and therefore not presented. *Rhynchosporium* development was significantly affected by

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. 3E) on cv. Concerto ($P < 0.01$) or cv. Optic ($P < 0.01$). Yield was not significantly affected by either variety ($P = 0.154$) or treatment ($P = 0.764$) despite the observed disease control (Fig. 3F).

3.3 Environmental variation between field trials

Film-forming polymers can offer protection against invading pathogens by forming a physical barrier on the plant to prevent fungal colonisation. However, these barriers do not stretch as the crops grows and therefore as differences in crop development between sites and years may affect the efficacy of the arabinoxylan polymer to control disease any variation in the timing of each GS when treatments were applied were noted. Crops were slightly forward at Bush Estate in 2011 compared to 2010 and 2012 with GS25 recorded more than 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. 4A). Spring barley development was typically slower in crops grown at Lanark compared to those grown at Bush Estate (Fig. 4A). 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. 4B) 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. 4C; $P < 0.05$).

4. Discussion

As the diversity and efficacy of available disease management tools are becoming more limited due to legislative issues regarding fungicide registrations (Hillocks, 2012) combined with changes in pathogen populations resulting in less effective chemical control (Phelan et al., 2016; Matusinsky et al., 2011; Piotrowska et al., 2016; Bäumler et al., 2003; Wyand and Brown, 2005) and varietal resistance (Brown, 2015) alternative plant protection products are needed to protect crop yields. Compounds that can induce the plant defence response have received a lot of attention with mixed results on disease control (McGrann et al., 2016; Oxley

and Walters, 2012; Walters et al., 2009; 2011a; 2011b; 2013) whereas less attention has been directed to the use of film-forming polymers as plant protection products.

Film-forming polymers can be used as anti-transpirants in agriculture and horticulture to protect plants from severe water loss (Farelli et al., 2016; Kettlewell et al., 2010). In addition to protecting plants from abiotic stress these polymers also showed promise as products for disease management in protected and field-grown crops against a number of fungal diseases (Elad et al., 1990; Han, 1990; Walters, 1992; Haggag, 2002; Percival et al., 2006; Percival and Boyle, 2009). This study examined the potential of an arabinoxylan polymer derived from maize to control fungal diseases in spring barley. Treatment with the polymer did provide disease control on spring barley but the results were variable and dependent on environmental conditions associated with different trial sites and year of study. Applications of the polymer as the sole plant protection product were able to reduce the development of powdery and *Rhynchosporium* of spring barley at Lanark in 2011 but there was no consistency in the number or timing of polymer applications associated with disease control (Fig. 3B).

Polymers have previously been shown to significantly reduce the development of fungal disease on numerous different crops. Application of film-forming polymers prior to fungal inoculation in glasshouse experiments tends to result in better levels of disease control (Haggag, 2002; Walters, 1992) although treatment post inoculation can also provide adequate disease control (Sutherland and Walters, 2002). On spring barley Walters (1992) demonstrated that three different film-forming polymers, Nu-Film P, Emerald or Vapor Gard, were able to reduce powdery mildew development in glasshouse trials. However, Sutherland and Walters (2002) showed that the control of mildew on spring barley provided by polymers, including Vapor Gard, was not as effective in field grown crops compared to glasshouse plants. Han (1990) reported that the anti-transpirant Gao-Zhf-Mo was effective against numerous diseases of a range of field grown and glasshouse crops. The efficacy of various film-forming polymers to control fungal disease differs (an, 1990; Elad et al., 1990; Walters, 1992; Ziv and Zitter, 1992) and, based on the different chemical and physical properties of these compounds, each film-forming polymer is likely to function differently under the changing environmental conditions crops encounter each growing season. This

level of inconsistent disease control is similar to that observed for plant defence elicitors that can effectively reduce disease but are not as reliable as fungicides (Walters et al., 2013).

Based on the evidence from our experiments the arabinoxylan polymer is unlikely to be suitable as a plant protection if used as a single active ingredient. Where film-forming polymers have been tested as plant protection products in almost all cases the disease control afforded by these compounds is not as strong as that provided by more traditional synthetic fungicides (Percival and Boyle, 2009; Sutherland and Walters, 2002). Polymers do not offer the systemic protection to new crop growth that many synthetic fungicides provide resulting in the need for additional application to ensure adequate disease control (Walters, 2006; Sutherland and Walters, 2002). Furthermore, it has been suggested that some polymer formulations are not able to stretch as the plant grows and therefore do not offer long term disease control (Percival et al. 2006). These limitations of polymers may explain why disease control observed in the field trials reported here was variable between trial sites with differences in crop development between trials associated with different environmental conditions at each site (Fig. 4) affecting the overall disease management potential of this compound.

More promising results were observed when the polymer was used in combination with fungicide applications where more consistent levels of disease control were observed. Of particular interest is the potential to use the arabinoxylan polymer with reduced rates of fungicides. Significant levels of disease control were observed when the polymer was used as an early treatment to the crop and the GS31 fungicide application was omitted from the disease control programme (Fig. 3). Reduced fungicide applications are preferable, where possible, in modern agriculture to not only protect the environment but to also lower the risk of fungal isolates becoming insensitive to the active ingredients and therefore reducing the efficacy of the chemical control measures. Research with defence elicitor compounds when used with reduced fungicide applications has also showed potential for providing effective disease control (McGrann et al., 2016; Oxley and Walters, 2012). Employing alternative crop protection products such as this arabinoxylan polymer within reduced fungicide application programmes may allow fungicides to be used in a more sustainable way.

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

reduces disease is required. Disease control by most film-forming polymers appears to operate by creating a physical barrier that prevents fungal penetration and masks surface cues that stimulate fungal spore adhesion and germination (Walters, 2006). However, some polymers also possess fungistatic effects (Elad et al., 1990; Sutherland and Walters, 2001). 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 (Farelli 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. 1-3). 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. 3D). At the Bush Estate in 2010 mildew development was not significantly affected by the treatments that included a GS25 fungicide application combined with various polymer applications but spring barley yields were improved except when the polymer was applied at GS49 (Fig. 1). 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. 3E+F). 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.

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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

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 (1.0 L ha ⁻¹)	-Polymer GS24 and GS31 and GS49	-Polymer GS24
-Fandango (1.0 L ha ⁻¹) + Flexity (0.25 L ha ⁻¹) GS25+ Bravo (1.0 L ha ⁻¹) GS49 ^a	-Siltra Xpro (0.5 L ha ⁻¹) GS31 and Proline 275 (0.175 L ha ⁻¹) + Bravo GS49 (0.5 L ha ⁻¹)	-Polymer GS31
-Fandango (1.0 L ha ⁻¹) + Flexity (0.25 L ha ⁻¹) GS25+ Pentangle (1.0 L ha ⁻¹) GS49 ^a		-Polymer GS39
-Fandango (1.0 L ha ⁻¹) + Flexity (0.25 L ha ⁻¹) GS25+ Tracker (1.0 L ha ⁻¹) GS49 ^a		-Polymer GS59
-Fandango (1.0 L ha ⁻¹) + Flexity (0.25 L ha ⁻¹) GS25+ AmiStar Opti (1.0 L ha ⁻¹) GS49 ^a		-Polymer GS24 and GS31
-Fandango (1.0 L ha ⁻¹) + Flexity (0.25 L ha ⁻¹) GS25+ Proline 275 (0.4 L ha ⁻¹) +Bravo (1.0 L ha ⁻¹) GS49 ^a		-Polymer GS24 and GS39
-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 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 and B, yield at 85% dry matter in 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 disease development and yield 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; and yield at 85% dry matter in E, 2011 and F, 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. 3 Field trial assessment of the effect of an arabinoxylan polymer and fungicide treatment on disease development and yield 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; and yield at 85% dry matter, D 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, E and yield at 85% dry matter, F in spring barley cv. Concerto and cv. Optic. * = P < 0.05, ** = P < 0.01, *** = P < 0.001. Treatments: 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).

Fig. 4 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