European Journal of Plant Pathology **108:** 385–389, 2002.

Effect of film-forming polymers on infection of barley with the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*

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Accepted 9 January 2002

Key words: film-forming polymers, powdery mildew

Abstract

The effects of three film-forming compounds, Ethokem, Bond and Vapor Gard, on infection of barley by the powdery mildew fungus *Blumeria graminis* f. sp. *hordei* were examined in glasshouse and field experiments. The three compounds provided significant control of powdery mildew infection when applied as pre- or post-inoculation treatments in the glasshouse. Such treatment had no effect on plant growth. Bond and Vapor Gard reduced the germination of conidia of *B. graminis* by 78% and 85% respectively, and reduced the subsequent formation of appressoria (73% and 85% respectively) and haustoria (75% and 79% respectively). The three compounds were less effective in field experiments, although they provided significant control of mildew infection and had no impact on plant growth and grain yield.

Introduction

Film-forming polymers are widely used as antitranspirants and as spray adjuvants within the agricultural and horticultural industries. As adjuvants their main use is as filming agents used to reduce weathering and extend pesticide efficacy, and as stickers/spreaders to improve distribution and adherence of agrochemicals (Backman, 1978). Polymer antitranspirants form a film over the stomata, increasing resistance to water vapour loss (Gale and Hagan, 1966). They are used to decrease water stress and wilting, particularly of seedlings and transplants, and to improve water use efficiency in arid conditions (Quarles, 1991).

During field trials of the effect of an antitranspirant on the water balance of sugar beet, Gale and Poljakoff-Mayber (1962) found that the incidence of powdery mildew was reduced in treated plots. Subsequent studies on film-forming polymers have found several to be effective in controlling foliar pathogens of a variety of plants including cereals (Ziv and Frederiksen, 1987; Walters, 1992), vegetables (Han, 1990; Marco, et al., 1994), fruit (Han, 1990) and ornamentals (Ziv and Hagiladi, 1984; Kamp, 1985). There is very little information on the effects of film-forming polymers on the control of foliar pathogens in the field. The aim of this study was to determine the effect of three commercially available film-forming compounds on powdery mildew infection of barley in glasshouse and field experiments. As part of this work, a detailed examination was made of growth and yield of barley following treatment with the film-forming compounds.

Materials and methods

Powdery mildew infection of barley seedlings

Seeds of barley (*Hordeum vulgare* L. cv Delibes) were sown in 36 cm seed trays in Fisons Levington M3 compost and grown in a glasshouse under natural daylight supplemented for 16 h daily by 400 W mercury vapour lamps. The minimum temperature was 9 °C at night rising to a maximum of 24 °C during the day. Plants at growth stage 12 (second leaf unfolded) were used. The compounds used were Ethokem (ethoxylated tallow amine: surfactant, Midkem Agrochemicals, UK), Bond (synthetic latex: sticker-spreader, Newman Agrochemicals Ltd, UK) and Vapor Gard (polyterpene: antitranspirant, Miller Chemical & Fertilizer Corporation, USA), which were sprayed to run-off at 0.1% (Ethokem) and 3% (Bond and Vapor Gard) concentrations using a Shandon spray unit. Pre-inoculation treatments were applied 2 h, 1 day and 3 days before inoculation, while post-inoculation treatments were applied 1, 2 and 7 days after inoculation. Plants were inoculated with conidia of the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei* (mixed inoculum) using the 'rolling' method (Nair and Ellingboe, 1962) to give an inoculation density of 15 spores mm^{-2}. Plants were assessed for intensity of infection by estimating the % leaf area infected using a standard area diagram at 7 days after inoculation.

Germination of powdery mildew conidia and development of infection structures

Barley was sown (6 seeds per pot) in 1 l pots. Plants at growth stage 12 were sprayed to run off with a 0.1% solution of Ethokem and 3% solutions of Bond and Vapor Gard. When dry, plants were inoculated. Leaf sections were removed, fixed with ethanol and glacial acetic acid and cleared in lactoglycerol. The leaf sections were placed on microscope slides and stained with lacto-fuchsin. Percentage germination, and formation of appressoria and haustoria were assessed for 100 conidia per leaf segment at 48 hours after inoculation. Appressorial formation was calculated as percentage of germinated spores with an appressorium, while haustorium formation was calculated as percentage of appressoria that formed an haustorium. Significance of differences between individual treatments and untreated controls was determined using Student's t-test based on variation among mounts. An arcsine transformation was applied to percentage data before statistical analysis to ensure normality of data. This approach to quantifying germling development results in a decreasing fungal population from germination through to haustorium formation. As a result, the reliability of the data are not the same throughout the data cells.

Field grown spring barley

Barley was sown in a randomised block design with 12 treatments and 4 replications. Bond and Vapor Gard at 3% concentration and Ethokem at 0.1% concentration were applied to run-off using an A20 compressed air sprayer at a rate of 1 l in 20 s with a boom width of 1.8 m. Two spray regimes were applied. Regime 1 consisted of one spray application at growth stage 32 and another at growth stage 59, while regime 2 had an additional spray application 2 weeks after growth stage 32. Alto SL 100 (Cyproconazole, Bayer) was applied as a commercial control at 240 g/l. Incidence of powdery mildew was assessed weekly. At growth stage 92, 10 plants and 100 ears per plot were sampled and plant height, dry weight and 100 ear grain dry weight were measured.

Growth analysis

Barley was sown in 1 l pots (1 plant per pot) in an equal mixture of sharp sand and John Innes No. 2 compost and grown under conditions described above. Plants at growth stage 12 were sprayed to run off with 3% solutions of Bond and Vapor Gard and a 0.1% solution of Ethokem and were inoculated with powdery mildew as described above. Shoots and roots were sampled at 3–4 day intervals for a total of 6 samplings. Shoot height, shoot fresh weight and leaf area were measured, and the samples were then dried at 60 °C for 7 days before obtaining shoot dry weights. Roots were washed and dried for 7 days at 60 °C before obtaining root dry weights.

Results

Control of powdery mildew on barley seedlings

Bond and Vapor Gard applied at a 3% concentration gave significant control of powdery mildew for all treatment timings (Table 1). Vapor Gard was the most effective treatment particularly when applied at days 1 and 2 after inoculation, during early stages of the infection process. Here, Vapor Gard reduced powdery mildew infection by 97% (Table 1). Ethokem used at 0.1% also reduced powdery mildew infection, with greatest control of infection achieved when the Ethokem was applied 1 day before inoculation (Table 1).

Germination and development of powdery mildew on the leaf surface

Bond and Vapor Gard significantly inhibited germination of powdery mildew conidia and subsequent formation of appressoria and haustoria. Vapor Gard was

Table 1. Effect of timing of application of film-forming compounds on infection of barley with powdery mildew in pot experiments

Treatment	% leaf area infected					
	Pre-inoculation treatment			Post-inoculation treatment		
	3 d	1 d	0 d	7 d	2 d	1 d
Control	44 ± 3.8	44 ± 3.8	44 ± 3.8	44 ± 3.8	44 ± 3.8	44 ± 3.8
Ethokem, 0.1%	9 ± 0.7	7 ± 0.9	9 ± 1.0	13 ± 1.1	11 ± 1.1	9 ± 0.8
Bond, 3.0%	13 ± 1.6	25 ± 3.7	10 ± 1.3	14 ± 1.4	12 ± 0.9	6 ± 0.8
Vapor Gard, 3.0%	6 ± 0.7	1 ± 0.1	4 ± 0.7	13 ± 1.6	1 ± 0.2	1 ± 0.1

All treatments significantly different from the control at $P \leq 0.001$.

Table 2. Effect of film-forming compounds on development of powdery mildew on barley leaves in pot experiments. Data were obtained 48 h after inoculation

Treatment	Germination[1] (%)	Appressoria[2] (%)	Haustoria[3] (%)
Control	66	58	51
Ethokem, 0.1%	54	55	49
Bond, 3.0%	15*	16*	13*
Vapor Gard, 3.0%	10*	9*	11*

[1]As percentage of spores.
[2]As percentage of germinated spores.
[3]As percentage of appressoria.
*Significantly different from the control at $P < 0.01$.

the most effective inhibitor of germination 8 h after inoculation (Table 2) and showed high levels of inhibition of all stages of development at all sampling times. Ethokem was the least effective inhibitor of germination, and did not reduce the percentage of conidia which formed appressoria, nor the percentage of appressoria that formed haustoria (Table 2).

Field grown spring barley

No significant control of powdery mildew was observed for any treatments applied under regime 1 (i.e. one spray at GS 32 and another at GS 59) (data not shown). However, plots which had received an additional application of the polymers Vapor Gard, Bond and Ethokem two weeks after the first application (at GS 32) showed significant control of powdery mildew, although levels of control were lower than the fungicide treatment (Table 3). There was no significant difference between control and treatments for plant heights, plant dry weights or 100 ear grain weights (Table 3).

Table 3. Effect of film-forming compounds on powdery mildew infection, plant height, dry weight and grain dry weight of barley in a field experiment

Treatment*	% powdery mildew infection	Plant height[1] (cm)	Plant dry weight[2] (g)	Grain dry weight[3] (g)
Control	17.5	58.8	6.8	53.2
Ethokem, 0.1%	9.6	59.2	6.6	51.0
Bond, 3.0%	6.6	58.1	6.8	55.0
Vapor Gard, 3.0%	8.4	61.5	7.1	56.5
Cyproconazole, 240 g/l	2.9	65.1	7.0	62.0
LSD ($P = 0.05$)	5.46	8.25	1.08	7.95

[1]Mean of 20 plants per plot.
[2]Mean of 20 plants per plot.
[3]Mean of 100 ears per plot.
*Sprays applied at GS 32, GS 32 + 2 weeks, and GS 59.

Growth analysis

Analysis of plant heights, fresh weights, leaf areas and dry weight of shoots and roots found no significant differences between treatments (data not shown). The results of growth analysis on disease free and powdery mildew infected barley suggest that the film-forming polymers do not have a detrimental effect on the growth of barley.

Discussion

The data show that three film-forming compounds, Ethokem, Vapor Gard and Bond, provided significant control of powdery mildew infection of barley in glasshouse experiments. This agrees with previous work which demonstrated control of barley powdery mildew infection in the glasshouse using menthene-based antitranspirants (Walters, 1992) and control of powdery mildew infection of wheat by menthene and wax emulsion based antitranspirants in the field (Ziv and Frederiksen, 1987). In addition, the application of the film-forming compounds to barley plants in the glasshouse produced no effects on plant growth. Indeed, detailed growth analysis showed no effect on leaf area ratio and net assimilation rate, suggesting that the compounds had no significant effect on rates of photosynthesis and leaf expansion.

Many previous studies suggested that the ability of film-forming polymers to control foliar pathogens is due to their enhancement of natural defences at the leaf surface (Gale and Poljakoff-Mayber, 1962; Osswald et al., 1984; Ziv and Frederiksen, 1983). Several mechanisms have been proposed. Osswald et al. (1984) suggested that adherence of urediniospores to the polymer coated surface prevented development of germ tubes. Other work suggested that film-forming polymers increase thickness, hardness and resistance to enzymatic degradation of the epicuticular wax layer, thus increasing impenetrability (Elad et al., 1990). Zekaria-Oren and Eyal (1991) found that film-forming polymers affected successful orientation of germinating urediniospores and development of appressoria. They suggested this could be due to suppression of stimuli by the formation of a physical barrier between the invading pathogen and the host plant. Whilst certain film-forming polymers may be biologically inert and act by purely physical mechanisms, there is evidence which suggests that some may have direct fungicidal or fungistatic effects (Elad et al., 1989; 1990; Zekaria-Oren and Eyal, 1991). In the present work, Bond and Vapor Gard produced reductions in the germination of powdery mildew conidia and in the subsequent formation of appressoria and haustoria. The data suggest that these two film-forming polymers have a direct effect on the fungus and agree with other work from this laboratory which showed that Bond and Vapor Gard both reduced mycelial growth of *Pyrenophora avenae* and *Magnaporthe grisea* in *in vitro* experiments (Sutherland and Walters, 2001). Recent work on the 'first touch' of conidia of *B. graminis* on the barley leaf surface provided evidence for conidial uptake of anionic, low-molecular weight materials before germination (Neilsen et al., 2000). The authors suggested that this process could be a mechanism for recognition of the host and determination of the direction of growth of the emerging germ tube toward the leaf surface. During the later stages of germling development, between the formation of the primary germ tube and differentiation of the appressorium, evidence suggests that perception of factors such as hydrophobicity of the substratum, release of cutin monomers by cutinase and possibly cellulose breakdown products could be important (Nielsen et al., 2000). However, such an explanation is unlikely to apply to Ethokem, which reduced powdery mildew infection without affecting germination and germling development. In recent work, Ethokem was shown to reduce growth of *P. avenae* and *M. grissea in vitro*, with gross changes in hyphal morphology in both fungi (Sutherland and Walters, 2001). Ethokem is a cationic surfactant and as such may be active at the cell membrane. Wade et al. (1993) reported that cationic surfactants such as ethoxylated tallow amines increase plasma membrane permeability in plants. Why Ethokem did not exert an effect on germling development is not known. It is possible, for example, that the germlings became more susceptible to Ethokem following formation of haustoria. Clearly, the mechanism(s) by which Ethokem, Vapor Gard and Bond exerted these effects are worthy of further investigation. Although we cannot rule out a direct toxic effect of the film-forming compounds on the mildew conidia, it is also possible that the film formed by these compounds on the leaf surface prevented the recognition of the leaf surface by the conidia and/or prevented the uptake of materials by the conidia prior to germination.

Ethokem, Bond and Vapor Gard were less effective in controlling powdery mildew infection in the field and although Vapor Gard reduced infection by 52%, the commercial fungicide treatment reduced mildew by 83%. This should not be surprising since the compounds were only applied three times during the season and new leaf area produced between treatments would not have been protected. There may be scope therefore for film-forming polymers with improved stretching properties and which, as a result, might protect leaf surfaces for a longer period. Given the increasing concern with the environmental fate of agrochemicals, pathogen resistance to pesticides, and food safety issues, combined with a growing interest in sustainable systems for crop production, there is an urgent need for new disease control agents. Viewed in this context, the development of novel types of film-forming polymers may provide an addition to existing methods of plant disease control.

Acknowledgements

We are grateful to MAFF for the award of a postgraduate research studentship to Fiona Sutherland. SAC receives grant-in-aid from SERAD.

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