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Mini-review

Disguising the leaf surface: the use of leaf coatings for plant disease control

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Abstract

The ability of fungal pathogens to develop resistance to fungicides and to overcome genetic resistance in their hosts, coupled with growing public concern for the environment, means that there is an urgent need for novel methods of disease control. The leaf surface provides the first barrier that fungi must overcome in order to gain access to the leaf, but it also provides chemical and physical cues that are necessary for the development of infection structures for many fungal pathogens. Film-forming polymers can coat the leaf surface, acting not just as an extra barrier to infection, but also disguising the cues necessary for germling development. Kaolin particle films can envelop the leaf in a hydrophobic particle film barrier that prevents spores or water from directly contacting the leaf surface and as a result, can suppress infection. Adhesion of fungal spores to the leaf surface, which is important to keep spores on the leaf surface and for appropriate development of the fungus on the leaf surface, can be inhibited, leading to reduced infection and lesion development. Polymer and particle films have been shown to provide disease control in the field, while research on agents that inhibit spore adhesion on leaf surfaces is still in its infancy. There is an urgent need for research on the practicality of using these novel methods under field conditions and on ways of integrating them into current crop protection programmes.

Introduction

In order to gain access to the nutrients available in the apoplast and within plant cells, pathogens must get through the outer layers of the plant. On the leaf surface, the first of these layers is the cuticle. Indeed, the insoluble polymeric compounds of the cuticle constitute the main physical obstacle to penetration by fungal pathogens (Royle, 1975). Plant surfaces may also be a determinant in the recognition and attachment of fungal spores. Thus, evidence suggests that chemical interactions on the barley leaf surface are required for adhesion of powdery mildew conidia, with consequences for subsequent development of the powdery mildew germling (Wright et al., 2002). For rust fungi, germinated uredospores 'sense' the leaf surface and several steps are involved in germ tube tropism on such surfaces: adhesion, directional growth, appressorium formation over stomatal openings, directional emergence of infection pegs and adherence of haustorial mother cells (Wynn and Staples, 1981). Surface features of the leaf are important in controlling this germ tube tropism and any alteration of the leaf surface can alter topography and influence the tropism of uredospore germ tubes. Thus, removal of epicuticular waxes may induce tropism related mistakes and result in reduced infection frequencies (Wynn, 1981). More recently, evidence was provided for the involvement of both topographical and chemical signals associated with the wheat stomatal complex, in the induction of appressoria by the stem rust fungus *Puccinia graminis* f.sp. *tritici* (Collins et al., 2001).

The importance of leaf surface features in the early development and establishment of foliar pathogens suggests that interference with leaf topography will disrupt pathogen development and lead to reductions in infection. Research on a variety of agents which coat the leaf surface has shown that interference with leaf topography can indeed lead to reductions in pathogen infection. This article is the first review of disease control provided by agents, which act by coating the leaf surface. It focuses on fungal pathogens and does not cover insect pests, which are dealt with in other, specific reviews (e.g. Vincent et al., 2003).

Disease control provided by film-forming polymers

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 or 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). Film-forming polymers used as antitranspirants include waxes, silicons and a variety of plastic polymers.

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 on 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, 1983, 1987; Walters, 1992), vegetables (Osswald et al., 1984; Han, 1990) and fruit (Han, 1990). In studies of the control of powdery mildew on cucumber, Elad et al. (1989) examined the effects of Vapor Gard (1-*p*-menthene), Wilt Pruf (β-pinene), Safe Pack (a wax emulsion), Colfix (40% polyvinyl) and a number of commercial fungicides, including fenarimol. They found that in pot experiments, Vapor Gard and Wilt Pruf reduced powdery mildew on cucumber by up to 82% and 55%, respectively, while under cover, Safe Pack and the fungicide fenarimol reduced infection by up to 67% and 96%, respectively (Elad et al., 1989). Most effective control of powdery mildew on cucumber was provided by a mixture of Safe Pack and fenarimol. According to these authors, film-forming polymers can only form an effective barrier on the leaf surface if applied at concentrations greater than 3%. Since powdery mildew infection of cucumber was reduced by concentrations of polymers between 0.5 and 3%, Elad et al. (1989) suggested that the polymers were exerting a direct effect on the pathogen. Indeed, in their studies, the film-forming polymers reduced germination of powdery mildew conidia when used at concentrations as low as 0.5% (Elad et al., 1989). Similar effects were observed with *Botrytis cinerea*, with the film-forming polymers Wilt Pruf and Colfix reducing germination of conidia by 33% and 74% respectively, when used at a concentration of 0.2% (Elad et al., 1990). Hsieh and Huang (1999), working on lily leaf blight caused by the pathogen *B. elliptica*, demonstrated that six film-forming, cationic polyelectrolytes controlled disease under both glasshouse and field conditions. Some of the most active compounds were found to reduce conidial germination and inhibit germ tube growth and interestingly, to suppress esterase production by germ tubes (Hsieh and Huang, 1999). Esterases are thought to be important for infection by this pathogen and Hsieh and Huang (1999) observed a significant correlation between esterase production by germ tubes and lesion number on inoculated tissue. The authors suggested that the fungicidal activity of the polyelectrolytes was due, in part, to suppression of esterase production by the fungus.

More recently, Sutherland and Walters (2001) found that film-forming polymers altered the growth and morphology of two plant pathogenic fungi *in vitro*. Thus, Ethokem (an ethoxylated tallow amine) and Bond (a synthetic latex), completely inhibited linear growth of *Pyricularia oryzae* when incorporated into the growth medium at 1 and 2%, and strongly inhibited linear growth of *Pyrenophora avenae* at the same concentrations (Sutherland and Walters, 2001). Less of an effect on linear growth of these fungi was obtained with Vapor Gard, although all three polymers decreased cell lengths and led to gross changes in hyphal morphology, including swollen, shortened cells, granulation of cytoplasm, increased branching and collapsed empty cells (Sutherland and Walters, 2001). The mechanism(s) by which Bond and Vapor Gard alter hyphal morphology is not known. However, 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. The possibility that Ethokem may exert its effects on hyphal morphology by altering the permeability of fungal membranes is worthy of investigation.

In a comparison of three different film-forming polymers, Ethokem (ethoxylated tallow amine), Bond (synthetic latex) and Vapor Gard, Sutherland and Walters (2002) found that all three compounds provided significant control of powdery mildew infection on barley under controlled conditions. Under these conditions, Bond and Vapor Gard reduced germination of conidia, as well as the subsequent formation of appressoria and haustoria (Sutherland and Walters, 2002). The film-forming polymers performed less well under field conditions, with Bond used as a 3% spray reducing mildew infection by 63%, in comparison with the commercial fungicide cyproconazole, which reduced mildew infection by 84% (Sutherland and Walters, 2002).

Many of the data reported above demonstrate a direct effect of film-forming polymers on the fungal pathogen e.g. on spore germination. But what about the film-forming polymers acting as a physical barrier to infection or altering the topography of the leaf surface?

Two mechanisms have been proposed to account for the effects of film-forming polymers in reducing fungal infection on leaf surfaces (Gale and Poljakoff-Mayber, 1962; Ziv and Frederiksen, 1983; Osswald et al., 1984). First, it has been suggested that leaf surfaces coated with polymers are hydrophobic, leading to low water potential at infection sites (Gale and Hagan, 1966). Second, various workers have suggested that coated leaf surfaces may be impenetrable due to the thickness, hardness, or resistance to enzymic attack of the film-forming polymer (e.g. Ziv and Frederiksen, 1983). Working on the effects of film-forming polymers on development of the rust *Puccinia recondita* f.sp. *tritici* on wheat, Zekaria-Oren and Eyal (1991) found that polymers applied prior to inoculation had a greater effect on rust infection than compounds applied post-inoculation. They found that increasing the concentration of the film-forming polymers led to a progressive reduction in infection intensity, suggesting that efficacy was related to thickness and uniformity of the coat on the leaf surface. Using fluorescence microscopy and scanning electron microscopy, Zekaria-Oren and Eyal (1991) found that surfaces coated with film-forming polymers interfered with fungal penetration of the leaf. They observed that both the orientation of the germinating uredospore towards the stomata and the formation of appressoria were altered on coated surfaces. The leaf surface is known to provide various stimuli that orient the germinating uredospore towards a stoma (Wynn, 1981; Wynn and Staples, 1981) and induce appressorium formation (Collins et al., 2001). Zekaria-Oren and Eyal (1991) suggested that the significant reduction in appressorium formation and the distribution of appressoria on the coated leaf surface was associated with disruption of mechanisms responsible for orientation of the germinating uredospores towards stomata and triggering appressorium formation. In their work on the effects of film-forming polymers on powdery mildew infection of barley, Sutherland and Walters (2002) suggested that the polymers might have disrupted the 'first touch' phenomenon described by Nielsen et al. (2000). The 'first touch' of conidia of *Blumeria graminis* on the barley leaf surface is associated with conidial uptake of anionic, low-molecular weight materials before germination. Nielsen et al. (2000) suggest that this 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. There is an urgent need for work on the mechanisms by which film-forming polymers reduce fungal infection on leaf surfaces. Since the fungal pathogen on a leaf surface will capitalise on any break or weakness in the film produced by the polymer, research on polymers with increased stretching or 'self-healing' properties would also be useful. Polymer composites with 'self-healing' properties have been reported (White et al., 2001; Brown et al., 2004).

As an interesting aside, in an inspired piece of lateral thinking, a film-forming polymer was shown to control an important parasitic plant. Thus, Press et al. (1989) demonstrated that Wilt Pruf S600, an antitranspirant containing 23% di-*p*-menthene, controlled the parasitic plant

Striga hermonthica infesting sorghum. Parasitic plants like *S. hermonthica* maintain leaf temperatures several degrees below air temperature by means of high transpiration rates and consequent evaporative cooling. Application of the antitranspirant reduced rates of transpiration in the parasitic plant, leading to leaf temperatures increasing above the optimum for photosynthesis in the parasite. The result was death of *S. hermonthica* leaves within 4 h of antitranspirant application (Press et al., 1989) and the potential for using antitranspirants to control parasitic plants.

Particle films as agents for control of plant diseases

Glenn et al. (1999) introduced the concept of hydrophobic particle film technology for the control of pests and diseases. The hydrophobic particle film is based on the inert mineral, kaolin, which is treated with a water-repelling agent. Kaolin is a white, non-porous, non-swelling, non-abrasive, fine grained aluminosilicate mineral ($Al_4Si_4O_{10}(OH)_8$) that easily disperses in water and is chemically inert over a wide pH range. Kaolin particles can be made with varying degrees of hydrophobicity by coating them with waterproofing agents such as chrome complexes, stearic acid and organic zirconate. Glenn et al. (1999) obtained control of arthropod pests and fungal and bacterial pathogens on fruit trees by dusting with hydrophobic kaolin particles. The authors suggested that disease control was achieved because plants were enveloped in a hydrophobic particle film barrier that prevented pathogen propagules or water from directly contacting the leaf surface. However, hydrophilic kaolin particles can also provide plant disease control. Thus, Puterka et al. (2000) found that hydrophilic particle films controlled fabraea leaf spot on pear, while the hydrophilic kaolin based product Surround WP (Engelhard Corporation, Iselin, NJ, USA) was shown to control *Zygophiala jamaicensis* and *Gloeodes pomigena* on apple fruits and *Phoma* sp. on apple leaves, although it was not consistently effective against the cedar apple rust pathogen *Gymnosporangium juniperi-virginianae* (Thomas et al., 2004). In a recent four year study, hydrophobic kaolin particle films failed to control *Cladosporium carpophilum* or *Podosphaera leucotricha* on peach, although it did control *Monilinia fructicola* (Lalancette et al., 2005). In contrast, hydrophilic kaolin particle films did not control any of the peach pathogens, leading the authors to suggest that hydrophobicity and deposit density may be important factors for effective disease management (Lalancette et al., 2005).

Irrespective of the level of disease control provided by kaolin particle films, other, benefical effects of applying the particle films have been observed. Thus, liquid formulations of both hydrophobic and hydrophilic particle films were shown to double yields of pear in field trials, while delayed fruit maturation, increased fruit size, increased fruit number and increased fruit yield was obtained following use of kaolin particle films (Lalancette et al., 2005). In the latter case, these effects were attributed to a reduction in plant stress. Indeed, reduced plant stress during extreme temperature conditions was also observed following kaolin particle film applications to apple trees (Thomas et al., 2004).

Interfering with adhesion of spores to the leaf surface

Adhesion of fungal spores to the plant surface is thought to be the essential first step in the infection process (e.g. Epstein and Nicholson, 1997). Thus, in *Magnaporthe grisea*, the causal agent of rice blast, hydration of conidia leads to the release of an adhesive mucilage from the spore tip that forms a viscous pad for attaching the conidium to the plant surface (Hamer et al., 1988). Following successful adhesion, germ tube formation is rapid, whereas if conidia fail to adhere to the substrate, viability is rapidly lost (Talbot, 1995). Once germ tube formation has occurred, their adhesion to the plant surface is important in the perception of signals for further differentiation e.g. formation of the appressorium. The importance of spore adhesion to successful infection of plant surfaces suggests that disruption of this process could be useful in disease control. Indeed, some fungicides used for control of rice blast have been shown to interfere with spore adhesion (Inoue et al., 1987). More recently, Stanley et al. (2002) showed that adhesion of spores of phytopathogenic fungi on artificial and plant surfaces could be inhibited using zosteric acid. Zosteric acid [*p*-(sulphoxy) cinnamic acid] is a naturally occurring compound found in the eelgrass *Zostera marina* and which inhibits the attachment of marine bacteria and barnacle larvae (Todd et al., 1993). Stanley et al. (2002) showed that zosteric acid not only inhibited attachment of spores of *M. grisea* and *Colletotrichum lindemuthianum*, but also inhibited formation of appressoria, leading to a failure to infect leaves. Indeed, on intact plants, zosteric acid reduced lesion development on rice leaves caused by inoculation with *M. grisea* and delayed lesion development on bean leaves following inoculation with *C. lindemuthianum* (Stanley et al., 2002). These workers found that zosteric acid was not toxic to the fungi and that the inhibition of spore adhesion in *M. grisea* was reversible by washing. Although this could lead to transitory protection under field conditions, Stanley et al. (2002) point out that reduced adhesion could lower inoculum potential, either because non-attached spores could be more easily detached from leaf surfaces by wind or rain splash, or as in the case of *M. grisea*, the spores quickly lose viability. This work hints at the considerable potential for the design of environmentally benign strategies for plant disease control based on agents which inhibit adhesion (Stanley et al., 2002).

Conclusions

Fungal pathogens must breach the outer surfaces of their hosts if they are to gain access to the nutrient supply required for their continued growth and survival. Leaf surfaces for example, provide chemical and physical cues that are important factors in the development of infection structures of many plant pathogenic fungi. It is no surprise therefore that disrupting these processes, by coating the leaf with polymer films or applying agents that interfere with spore adhesion, can reduce infection and provide disease control.

Farmers and growers in many parts of the world have grown accustomed to very high levels of disease control achieved by using fungicides. The use of many novel disease control methods, like polymer or particle films, or adhesion inhibitors, is unlikely to provide equal levels of disease control. However, the problems of fungicide resistance, breakdown in host resistance and increased public concern for the environment, means that the development of new disease control methods cannot be ignored. Moreover, there are many diseases of agricultural and horticultural crops for which no adequate disease control measures currently exist. But the adoption of new disease control measures will not be easy, since, in some cases, it will require changes in crop protection practice. For the approaches described in this review, application before the pathogen arrives on the crop is important, since these approaches will act essentially as protectants. This in turn will require robust systems of disease forecasting. Some changes, for example alternating use of a novel method with fungicides in the spray programme, may be easier to integrate into current practice. However, since information in many of these areas is lacking, there is a clear need for research on integrating potential novel methods of disease control into crop protection programmes.

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