Effect of Film-Forming Compounds on the Development of Leaf Rust on Wheat Seedlings

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ABSTRACT

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The effect of film-forming antitranspirants and glues on the development of leaf rust (caused by *Puccinia recondita* f. sp. *tritici*) was assessed on wheat seedlings (*Triticum aestivum* 'Ceeon'). When applied before rust inoculation, the compounds Bio-Film, Folicote, and Vapor Gard markedly reduced the number of pustules per square centimeter. Postinoculation application had a significantly smaller effect on suppression of rust development. The efficacy of each compound depended on its concentration. More than 80% suppression of rust development was achieved by application of 0.5% Bio-Film, whereas equivalent suppression was obtained with either 2% Folicote or Vapor Gard. Although germination of urediniospores was not affected, the number of appressoria formed was reduced by 20% with Folicote and by more than 70% with either Bio-Film or Vapor Gard. Only 30% of the appressoria were formed above the stomata. The results suggest that film-forming compounds alter the topography of the leaf surface, thus interfering with adhesion of the germ tube and recognition of penetration sites.

Certain passive defense mechanisms of plants against plant pathogens are related to the structure of the plant surface (i.e., the cuticle) and provide an effective barrier against direct penetration by parasites (9). The insoluble polymeric compounds of the cuticle constitute the main physical obstacle for fungal penetration (15). Such barriers, together with other measures, may be instrumental in limiting the host range of particular species of crop pathogens (9).

Plant surfaces may be a determinant in the recognition and attachment of fungal spores, which can affect the spores' association with stomata (7,8). According to Wynn and Staples (19), the steps involved in the tropism of germ tubes from urediniospores are adherence, directional growth, appressorium formation over stomates, directional emergence of infection pegs, and adherence of haustorial mother cells. Surface features of plants are important in controlling tropism and interferences with topography. The removal of epicuticular waxes may induce tropism-related mistakes and, subsequently, reduce infection frequencies (18). Surface waxes on leaves of eceriferum mutants of barley (*Hordeum vulgare* L.) had an altered physical structure and chemical composition (20). Abnormal appressoria were more frequently observed on mutants than on normal waxy plants. Removal of epicuticular wax caused disturbances in the formation of appressoria of germinating urediniospores of *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. on wheat (*Triticum aestivum* L.) leaves (1).

Various coating polymers such as oils, waxes, polyterpenes, alcohols, and silicons were used as artificial barriers over leaf surfaces to reduce water losses (4), protect plant organs against invading microorganisms, and prevent the development of certain plant diseases (5,13,16, 23–25). These coating polymers used as protective barriers were nonphytotoxic, permeable to gases, resistant to changing environmental conditions and penetration of solar irradiation, and biodegradable (23).

The objective of the present study was to evaluate the effect of several film-forming polymers applied to leaves of wheat seedlings on processes associated with the development of leaf rust.

MATERIALS AND METHODS

Seedling test. Pots of 20 seedlings (two-leaf stage) of the susceptible semidwarf spring wheat cv. Ceeon (*Yt*54A*3//*Nrn*10/*Bvr*) were inoculated with a suspension of urediniospores (5 mg in 20 ml of water + 0.1 ml of Tween 20) of *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* isolate ISR1010. Seedlings were divided into two groups: 1) preinoculation treatment, in which coating polymers were sprayed before inoculation, and 2) postinoculation treatment, in which the wheat seedlings were inoculated with urediniospores, placed in a humidity chamber for 24 hr, dried, and sprayed with coating polymers. Inoculated seedlings were placed for 24 hr in a humidity chamber (100% RH) in the dark at 17 ± 1 C and transferred to a temperature-controlled greenhouse (20 ± 1 C) for 10 days before assessing the number of rust pustules per square centimeter of leaf area. Means were based on four replicates each composed of 20 wheat seedlings.

Film-forming polymers. The following film-forming polymers were evaluated: Bio-Film (spreader-sticker containing alkylaryl-polyethoxyethanol, free and combined fatty acids, glycol ethers, dialkyl benzenedicarboxylate, and isopropanol; Callo Agricultural Chemical Inc., Overland Park, KS), Folicote (wax emulsion; Crystal Soap and Chemical Co., Inc., Lansdale, PA), Nu-Film P (poly-1-*p*-menthene antitranspirant; Miller Chemical and Fertilizer Co., Hanover, PA), Plyac (spreader-sticker containing octylphenoxy polyethoxyethanol; Hopkins Agricultural Chemical Co. Madison, WI), Vapor Gard (di-1-*p*-menthene antitranspirant; Miller Chemical), and Wilt Pruf (β-pinene antitranspirant; Nursery Speciality Products, Greenwich, CT). The systemic fungicide propiconazole (TILT 250EC; 1-(2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl)-*1H*-1,2,4-triazole) (Ciba-Geigy Ltd., Basel, Switzerland) was used as a control treatment.

In most studies, the film-forming compounds were applied at a concentration of 2% (v/v) from the commercial preparation suspended in water. A 20-ml suspension of each compound was applied to a group of four pots. For control, a 15-ml suspension of the fungicide at a concentration of 125 g a.i./100 L of water was used.

Fluorescence microscopy. Segments (1 cm long) from the midsection of 10 treated leaves were fixed in 99% ethanol and acetic acid (1:3, v/v) 24 hr after inoculation. Staining was performed according to Rhoringer et al (14) and a modification by Kuck et al (11). The

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segments were boiled for 1.5 min in ethanol with lactophenol (1:1, v/v) and incubated in the same solution for 24 hr, after which they were washed for 15 min with 50% ethanol followed by two washes, 15 min each, in 0.05 M NaOH before final washing in distilled water. The segments were then transferred to 0.1 M Tris/HCl buffer (pH 8.5) for 30 min before staining with 0.3% Calcofluor solution in Tris/HCl buffer solution for 5 min. After staining, the leaf segments were rinsed four times in distilled water, followed by a 30-min wash with 25% glycerol. The segments were mounted in 25% glycerol and observed with a Zeiss Universal microscope.

Scanning electron microscopy. Leaf segments were sampled at various times and fixed in 5% glutaraldehyde in phosphate buffer (pH 7.0). Segments were dehydrated in an ethanol series and dried to the critical point. Preparations were observed and photographed with a JEOL JSM 35 scanning electron microscope (SEM).

RESULTS

Pre- and postinoculation application. Application of film-forming compounds at a concentration of 2% before inoculation with urediniospores significantly reduced the number of pustules per square centimeter (Table 1, experiment I). Pre- and postinoculation application of the fungicide propiconazole completely prevented formation of leaf rust pustules. The spreader-sticker Bio-Film, which was added later to the trials, was compared with those film-forming compounds that did not express suppression of pustule development when applied after inoculation with rust urediniospores (Table 1, experiment II). In both experiments, Folicote and Vapor Gard expressed marked suppression (52–99%) of pustule development when applied preinoculation but suppression of less than 19% when applied postinoculation. Bio-Film was almost as effective as the fungicide propiconazole when applied before inoculation but did not differ statistically from the film-forming compounds in the postinoculation treatment (Table 1, experiment II).

Table 1. Effect of film-forming compounds and a fungicide on leaf rust incidence on wheat seedlings[u]

Treatment	Preinoculation[v] Pustules/cm² (no.)	Preinoculation[v] Percent reduction[x]	Postinoculation[w] Pustules/cm² (no.)	Postinoculation[w] Percent reduction
Experiment I				
Untreated	39.8 a[y]	...	39.8 a	...
Folicote	19.1 b	52.0	40.2 a	−1.0
Nu-Film P	0.6 d	98.5	23.5 c	40.9
Plyac	9.0 c	77.4	28.5 c	28.4
Vapor Gard	0.4 d	98.9	35.2 b	11.6
Wilt Pruf	2.2 d	94.5	32.7 b	17.8
TILT[z]	0.0 d	100.0	0.0 d	100.0
Experiment II				
Untreated	43.3 a	...	43.3 a	...
Bio-Film	0.2 bc	99.5	28.4 b	34.4
Folicote	8.5 b	80.4	35.1 b	18.9
Vapor Gard	6.3 b	85.4	37.4 ab	13.6
TILT	0.0 c	100.0	0.0 c	100.0

[u] All film-forming compounds were applied at 2% (v/v) concentration in water.
[v] Compounds applied before inoculation with urediniospores.
[w] Compounds applied after inoculation with urediniospores.
[x] [(Untreated-treated)/untreated] × 100.
[y] Within each column, values followed by the same letter do not differ significantly at $P < 0.05$, according to Duncan's multiple range test.
[z] Propiconazole fungicide.

Effect of concentration. Increasing concentrations of Bio-Film, Folicote, and Vapor Gard, ranging from 0.05 to 5% in preinoculation application, had a corresponding effect in reducing the amount of rust pustules (Fig. 1A). Preinoculation application of wheat seedlings with a concentration of 2% resulted in significant reductions in rust pustules by Bio-Film (99%), Folicote (82%), and Vapor Gard (85%) (Fig. 1A). At a concentration of 5%, the effectiveness of the three compounds was over 90%; however, Vapor Gard and Bio-Film did not differ statistically from one another at this concentration. Application of the three film-forming compounds at a concentration of 2% postinoculation resulted in reductions of 34, 19, and 14% in leaf rust pustules by Bio-Film, Folicote, and Vapor Gard, respectively, with Bio-Film significantly different from the other three treatments (Fig. 1B). At a concentration of 5%, Bio-Film reduced infection by 45%, whereas Folicote and Vapor Gard reduced infection by 0 and 14%, respectively. Bio-Film was statistically different from the other three treatments.

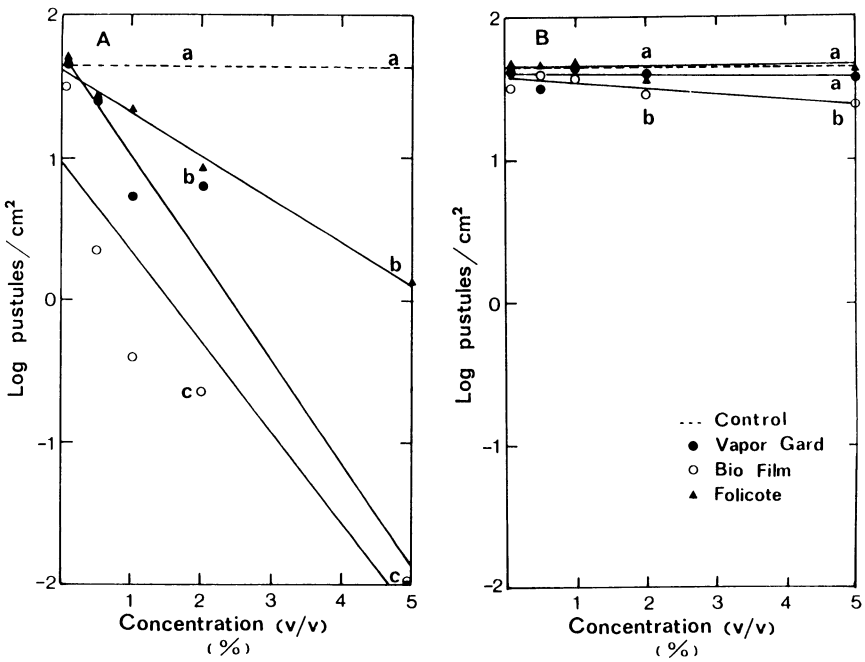

Fig. 1. The effect of different concentrations of film-forming compounds on the development of leaf rust pustules when applied **(A)** preinoculation or **(B)** postinoculation with urediniospores. Means (at concentrations of 2 and 5%) not followed by the same letter differ ($P = 0.05$) according to Duncan's multiple range test.

Effect of polymers on infection structures. The effect of Bio-Film, Folicote, and Vapor Gard on the formation of appressoria and their association with wheat stomata is presented in Table 2. The three polymers had no effect on urediniospore germination compared with untreated wheat leaves. Fewer than 20% of the germinating urediniospores formed appressoria after preinoculation treatment with either Bio-Film or Vapor Gard; the effect of Folicote on appressoria formation was less. Only a few of the appressoria formed on leaves treated with Bio-Film were observed on the stomata, whereas the effect of Folicote and Vapor Gard on orientation of germ tubes was somewhat

less significant.

Observation of the structure and film-coating properties of the three polymers by SEM revealed discontinuity of the film layer of the waxy polymer Folicote (Fig. 2). The effect of Bio-Film on appressorial formation and their association with leaf stomata is shown in Figure 3. Aberrant appressorial formation and disassociation with stomata (Fig. 3A,B) were observed as compared with appressoria formation (Fig. 3C) and the final formation of a leaf rust pustule (Fig. 3D) in untreated leaves.

DISCUSSION

The outer layers of plant tissue provide physical and/or chemical barriers against the establishment of plant pathogens. Film-coating polymers have been reported to provide additional protection against various foliar pathogens (10,12, 15,21–24). Our results showed marked reduction in disease with the use of polymers. It is assumed that the polymers either provide an impenetrable surface associated with their thickness or are resistant to enzymic degradation. It was also suggested (4) that the coated surface created a low water potential at infection sites because of its hydrophobicity. Some of the polymers tested here also greatly reduced rust infection following postinoculation treatment, which might indicate chemical rather than physical protection. Conversely, film-coating polymers that suppressed the formation of leaf rust pustules after preinoculation treatment did not affect pustule development after postinoculation treatment. They probably protect host tissue via a physical barrier.

Increasing concentrations of Bio-Film, Folicote, and Vapor Gard resulted in a progressive reduction in the number of pustules per square centimeter. These findings may be related to the thickness or the uniformity of the coat, although attempts to measure coat thickness were unsuccessful. Discontinuity in the uniformity of coated surfaces by Folicote resulted in less effective protection. This may be attributable to the ability of the rust fungus to penetrate through the interruptions in the coated surface. The film-forming polymers did not affect urediniospore germination, thus, failure in germination does not explain the significant reductions in the number of formed pustules.

Fluorescence microscopy and SEM revealed that the coated surfaces interfered with postgermination processes active in penetration. Both the orientation of the germinating urediniospore toward the stomata and the formation of appressoria were affected. It is thought that the leaf surface provides certain physical stimuli that orient the germinating urediniospore toward the leaf stomata (2,12,18,19). The stimulus can be associated in part with chemical factors originating in the stomata, such as CO_2 concentration (21), pH gradients (6), or identified compounds (3). It seems probable that the formation of an appressorium over a stoma requires an additional stimulus, which may be associated with the shape of the guard cells (17). It is suggested that the significant reduction in the number of appressoria and their distribution on the coated leaf surfaces are associated with disruption of the mechanism(s) associated with orientation of the germinating urediniospores toward the stomata and formation of appressoria. Wherever the coating layer is incomplete (e.g., Folicote), the formation of appressoria and their orientation are not as severely affected. The film-forming polymers may exclude the fungus by preventing physical contact between the invading pathogen and the

Table 2. Effect of film-forming compounds on the germination of urediniospores of *Puccinia recondita* f. sp. *tritici* and on the formation of appressoria and their association with wheat stomata

Treatment[x]	Germination (%)	Formation of appressoria (%)[y]	Appressoria on stomata (%)[y]
Untreated	94 a[z]	72.2 a	60.8 a
Bio-Film	98 a	17.6 c	5.4 c
Folicote	94 a	57.8 b	30.1 b
Vapor Gard	96 a	20.5 c	15.9 b

[x] All film-forming compounds were applied at a concentration of 2% (v/v) in water.
[y] Calculated as percentage of germinated urediniospores; assessed 28 hr after inoculation.
[z] Within each column, values followed by the same letter do not differ significantly at $P < 0.05$, according to Duncan's multiple range test.

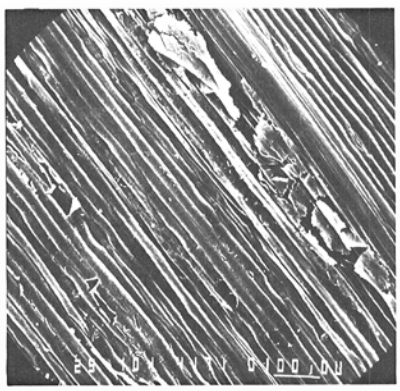

Fig. 2. Discontinuity of film layer on the surface of a wheat leaf coated with Folicote. Bar = 100 μm. (×100)

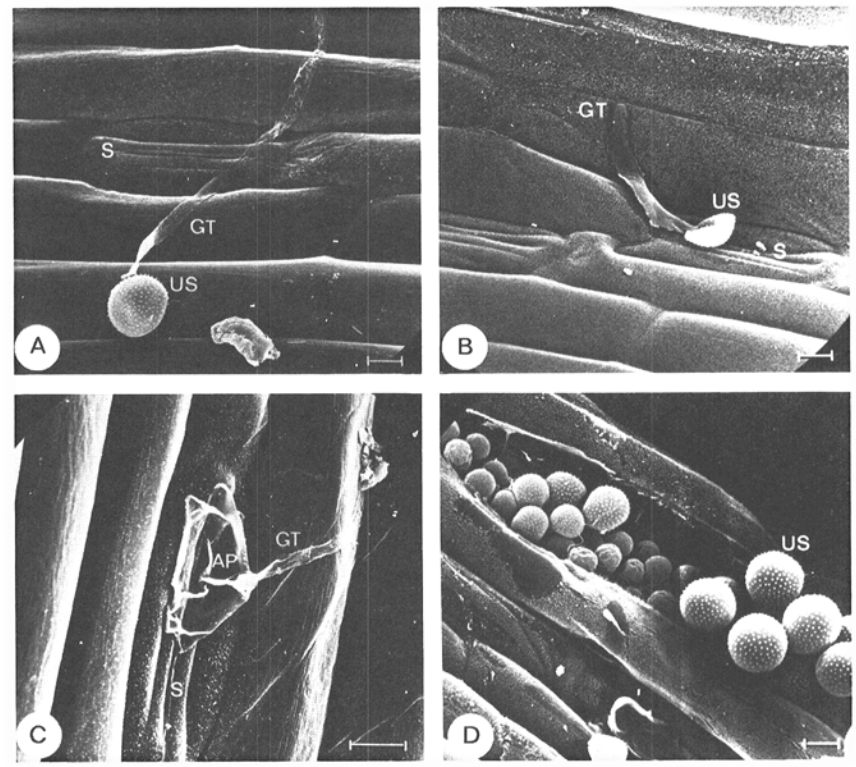

Fig. 3. (A) Disorientation of germinating urediniospores on wheat leaf coated with Bio-Film (24 hr after inoculation) and **(B)** germinating urediniospore without formation of appressorium on wheat leaf coated with Bio-Film (10 days after inoculation). **(C)** Appressorium formation over stoma on untreated wheat leaf (24 hr after inoculation) and **(D)** leaf rust pustule on untreated wheat leaf (10 days after inoculation). AP = appressorium, GT = germ tube, S = stoma, US = urediniospore. Bar = 10 μm.

host tissue, which apparently can stimulate the formation of infection structures in as yet undefined ways.

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