Powdery Mildew Pustules Supporting *Fusarium culmorum* Infection of Wheat Leaves

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ABSTRACT

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In greenhouse experiments, powdery mildew on wheat leaves was stopped 6 days after inoculation by a root application of benomyl. Three days after benomyl application, the plants were inoculated with a benomyl-tolerant mutant of *Fusarium culmorum*. Thus, the fungus was able to establish itself on the leaves, whereas neither wild-type nor mutant strains could infect healthy (powdery mildew-free) leaves. It is concluded that powdery mildew pustules allow *F. culmorum* to infect wheat leaves.

Fusarium spp. cause serious wheat diseases and can attack all parts of the plant. The most important diseases are foot rot and head blight, with reported losses between 20 and 25% (6,8). Depending on climatic conditions, the dominant species are *F. nivale* Ces. ex Sacc. (4,7,8), *F. culmorum* (W. G. Smith) Sacc. (1,5,12), and *F. graminearum* Schwabe (5,11).

In Western Europe, Schroeder and Christensen (14) assumed that infections of wheat leaves by *Fusarium* spp. do not cause economic losses but are an important source of inoculum for ear infection. Observation of *Fusarium* lesions collected in the field often revealed dark spots in the center caused by old pustules of *Erysiphe graminis* DC. ex Mérat f. sp. *tritici* (7) or mechanical damage (8,17).

Assistance of *E. graminis* to other pathogens has been described (2,3,7,10). Presence of the mildew pathogen was necessary for *Septoria tritici* Rob. ex Desm. to colonize wheat leaves. Necrotic lesions produced artificially by applying paraquat to simulate senescing mildew lesions were not colonized by this fungus (2). Broscious et al (3) found in field experiments with wheat that applications of triadimefon stopped powdery mildew and resulted in higher yields but increased *Septoria* severity.

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Powdery mildew can induce accessibility to secondary parasites in the infected and closely situated cells of wheat leaves (13). Field experiments showed that powdery mildew significantly influences the susceptibility of wheat to *F. graminearum* (11).

The objective of this study was to determine if powdery mildew can assist in the entry of *F. culmorum* into wheat leaves.

MATERIALS AND METHODS

A wild-type strain of *F. culmorum* (LD_{50} 1.4 ppm a.i. of benomyl 50WP) and a benomyl-tolerant mutant from it (LD_{50} 70 ppm of benomyl) obtained by methods described earlier (16) were employed. Both strains were maintained on potato-dextrose agar at 20 C and 12 hr of light (Philips TL 20W/29 and TL 20W/08 NUV alternately placed, distance 30 cm) per day. Conidial suspensions were obtained by washing the plates with sterile tap water. Five wheat plants of the mildew-susceptible cultivar Lita were grown in plastic pots (10 cm in diameter) in quartz sand by bottom-watering with Knop nutrition solution. The pots were placed in a greenhouse cabinet at 25 C for 15 hr with additional light (Osram L 36W/30) and at 20 C for 9 hr in darkness with 70 ± 10% RH. Each pot was treated as a statistical unit. Twenty-four pots were used for each treatment, and all tests were repeated twice.

Plants carrying the powdery mildew inoculum were tapped sharply to dislodge old conidia, and 18 hr later, they were gently shaken above the 7-day-old experimental plants. Pots containing the latter were inclined to expose the greater leaf area to the falling conidia. Six days after inoculation, powdery mildew, at that time covering 41 ± 7% of the leaf surface (assessment according to James [9]), was stopped by pouring 5 ml of a benomyl solution (200 ppm) into each pot. Three days later, a 5-ml conidial suspension of *F. culmorum* (2×10^6/ml in 0.05% Etalfix, Maag AG Switzerland) was sprayed onto the plants in each pot. The pots were placed in a closed plastic moist chamber with water-saturated atmosphere for 2 or 4 days (temperature and light period same as before).

The day after the humidity treatment, leaf samples were cleared and stained for microscopic observations following the method of Shipton and Brown (15). Macroscopic evaluation of the infection was done 4 and 7 days after the *Fusarium* inoculation by assessing the percentage of leaves with *Fusarium* lesions.

RESULTS AND DISCUSSION

Glasshouse experiments showed that the *F. culmorum* wild-type and the benomyl-tolerant mutant infected less than 2% of the leaves of untreated and benomyl-drenched wheat seedlings. Five of 240 plants (benomyl-treated) showed small (less than 5 mm in diameter) lesions caused by the *F. culmorum* mutant. This mutant heavily infected wheat leaves on which powdery mildew had been stopped with a benomyl drench (Table 1). A long moisture period after inoculation with the benomyl-tolerant strain shortened the latent period and increased the percentage of infected leaves (Table 2). Symptoms similar to those described by Wiese (18) for *F. nivale* appeared first on the distal halves of the primary leaves. (Only these parts were infected with powdery mildew.) Symptomatic leaves were distorted and covered with white aerial mycelium and yellowish sporodochia. One to 2 days after the wetness period, the mycelium disappeared and the *Fusarium* advanced toward the stem and killed the

Table 1. Effect of powdery mildew pustules combined with a benomyl drench on *Fusarium culmorum* infection of young wheat leaves

Fusarium strain	Percentage of leaves showing *Fusarium* lesions[a] (treatment of the plants)			
	None (healthy)	Benomyl drench	Mildew-infected	Mildew-infected + benomyl drench[b]
Wild-type	2	0	–[c]	0
Benomyl-resistant mutant	1	2	–	97

[a] Evaluation 7 days after inoculation, with a wetness period of 4 days after inoculation. Means of 24 replicates of five plants in two experiments.
[b] *Erysiphe graminis* was stopped with benomyl 6 days after inoculation of 7-day-old plants; 3 days after benomyl drench, plants were inoculated with *F. culmorum* conidia.
[c] No *Fusarium* lesions present, but data not assured because the mildew overgrew and killed the leaves.

Table 2. Effect of duration of wetness period on appearance of *Fusarium*[a] symptoms on benomyl-treated wheat leaves with mildew pustules[b]

Wetness period (days)	Percentage of leaves showing *Fusarium* symptoms (evaluation time in days after inoculation)	
	4	7
2	28 ± 18[c]	63 ± 30
4	93 ± 12	97 ± 10

[a] Benomyl-resistant strain.
[b] *Erysiphe graminis* was stopped with benomyl 6 days after inoculation of 7-day-old plants; 3 days after benomyl drench, plants were inoculated with *F. culmorum* conidia.
[c] Means of 24 replicates of five plants in two experiments. All treatments are significantly different from controls ($P = 0.01$) according to Student's unpaired t test. Standard deviations are indicated.

whole leaf.

Microscopic observations revealed that 58% of *Fusarium* conidia in or near powdery mildew pustules had germinated. The penetration mechanism of the *Fusarium* could not be detected. On leaves not inoculated with *E. graminis*, only a few (6%) *Fusarium* conidia formed short germ tubes.

F. culmorum is well known as a fungus with a high competitive saprophytic ability (1). Therefore, we presume that *Fusarium* can use powdery mildew hyphae or injured or infected leaf cells to establish itself as a saprophyte. Once established, the *Fusarium* spreads necrotrophically in the leaf.

Further work is needed to determine the importance of this secondary infection under more natural conditions and to measure yield losses resulting from this interaction. The subject is under further investigation.

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