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Orientation of the primary infectious structures of powdery mildew fungi (*Blumeria graminis*) and their adhesion to the surface of infected wheat (*Triticum aestivum*) leaves

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ABSTRACT

The anisotropic nature of the surface of highly elongated cereal leaves means that longitudinally and transversely oriented germinating conidia of phytopathogenic fungi could have different biological properties. We measured the angles between the long axis of the conidium, the axis of the plant leaf and the direction of the appressorial germ tube of the powdery mildew fungus *Blumeria graminis* f. sp. *tritici* on wheat leaves. Two main distribution patterns were observed. Normal appressorial germ tubes were associated with the longitudinal microrelief of the wheat leaves, whereas abnormal germ tubes tended to have a random distribution. The increased rotational mobility of these abnormal germ tubes may result from a reduction in their adhesion to the surface of plant cells.

1. Introduction

Many fungal phytopathogens have quite a long phase of growth on the surface of the host plant before penetrating its tissues. Physiological heterogeneity of the plant surface means that some of the areas may be more susceptible to penetration than others, and thus, some time is consumed for finding the best entry point. Thus, the primary infectious structures of fungi should follow an adaptive strategy based on the local susceptibility to infection and the microtopography of the host plant's epidermis.

Induced resistance and susceptibility appear to play an important role in the pathogenesis of powdery mildew fungi [15]. At least in the early stages of the infection process, altered resistance is localised within the attacked cells and generally not transferred to the adjacent cells [19]. Thus, a second lobe of the appressorium attempts to penetrate an adjacent cell to avoid the resistance induced by the failure of the first attack [27].

Even before germination, the spores of many fungal pathogens can recognise surface properties of the plant. For example, surface hydrophobicity and surface rigidity have been shown to affect germination and appressorium formation [8,21,26]. Furthermore, contact of the barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) conidia with a substratum almost immediately triggers the release of an extracellular proteinaceous matrix from the conidia [6,7,18].

In most cases, the germinated conidium of barley powdery mildew produces a primary germ tube and an appressorial secondary germ tube [13]. Only the latter forms an appressorium and penetration peg. One of the most common anomalies of infectious structures of cereal powdery mildew is the formation of undifferentiated thin and elongated tubes instead of the usual short and slightly thickened appressoria. The distal parts of these long germ tubes, which correspond to the appressoria blades, very rarely attach to the host epidermal cells and are unable to form haustoria.

The number of anomalies in wheat powdery mildew was correlated with the degree of plant resistance [24,25]. Treatment with cytokinins or hydrogen peroxide has changed the proportion of normal and abnormal propagules [1,2]. This variability in the infectious structures may be at least partially due to local variation in the resistance of the epidermal cells. For example, a hydrophilic surface is apparently perceived by conidia as unfavourable, leading an increased proportion of anomalies [28].

The topographic aspects of pathogenesis are of great interest, but remain poorly understood [16]. Plant pathogenic fungi often exhibit thigmotropic responses. On the artificial substrata with

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multiple ridges, topographical signals alone were sufficient to trigger appressorium differentiation in several cereal rusts [9]. On the host surface, some fungi trace the depressions over the anticlinal cell walls, while others grow across the junctions between epidermal cells [3]. The epidermal cells of cereal leaves are strongly elongated along the main leaf veins. Therefore, longitudinally and transversely oriented germ tubes of phytopathogenic fungi could have different biological properties in the pathogenesis. It has previously been found that the germ tubes of several rust fungi, which penetrate the stomata, grow perpendicular to the long axis of the epidermal cells and towards the stomata in susceptible varieties [22]. In contrast, wheat powdery mildew propagules were generally observed growing parallel to the leaf axis, with the proportion of propagules growing with a transverse orientation significantly increasing after treatment with cytokinins [23] or hydrogen peroxide [1].

In previous studies, the proportion of visually longitudinal appressoria have been counted, which includes appressoria with a <30° angle to the leaf axis [1,23]. Although visual assessment is a high-performance technique, more accurate quantitative data and consideration of the orientation of other morphological structures of the germinating conidia are required to better understand the infection process.

In this study, we used quantitative angular parameters to determine the orientation patterns of germinating conidia of powdery mildew [*Blumeria graminis* f. sp. *tritici* (DC.) Speer (syn. *Erysiphe graminis* f. sp. *tritici* Marchal)] on the anisotropic surface of elongated wheat (*Triticum aestivum* L., cvs. Khakasskaya and Zarya) leaves.

2. Materials and methods

2.1. Pathogen and plants

Isolates of *B. graminis* were collected in the Moscow (M) and Krasnodar (K) regions, Russia. The seedlings were cultivated in rolls of filter paper in Knop solution at 20°C–22 °C under a 16-h photoperiod. The first-formed and second-formed fully expanded leaves of 12-day-old seedlings were detached and inoculated with 70–80 conidia/mm^2 and were then incubated in a humidity chamber for 48 h. Different combinations of wheat cultivars, fungal isolates and the number of the leaf were tested to provide six experimental variants, as shown in Table 1.

2.2. Scanning electron microscopy (SEM)

One or two samples (10–15 mm) were taken from the middle part of each leaf blade and fixed in 4% glutaraldehyde followed by 2% osmium tetroxide. These were then dehydrated in a graded alcohol series and acetone, dried in a critical-point dryer, and coated with gold. The adaxial surfaces of samples from three leaves per variant were examined under a Zeiss scanning electron microscope LEO-1430 VP at a 20-kV accelerating voltage.

Inocula of *B. graminis* were collected in the Moscow (M) and Krasnodar (K) regions, Russia. Detached first-formed and second-formed fully expanded leaves of two wheat cultivars were used. Values are the mean and standard error (±SE) obtained for angles in the range of 0°–90°. The table shows the probability of error (P) as calculated by Watson's U^2 test for uniformity (for doubled angles in the range of 0°–180°). Values of $P < 0{\cdot}05$ indicate a significant difference from the uniform distribution for each experimental variant.

2.3. Angle measurement

Digital images were analysed in Image J. Germinated conidia with distinct appressorial germ tubes were classified as having either normal (Fig. 1) or abnormal (undifferentiated, long germ tubes; Fig. 2) growth patterns. During germination of the conidia, the production of appressorial germ tubes on the horizontal plane accompanies differentiation of the left and right versions—conventionally referred to as 'right' or 'left' conidia— that cannot be superimposed through rotation. Here, right versions are those in which the conidia are clockwise (right) from the appressoria (Fig. 3).

The angles between the long axis of the conidium and the main axis of the leaf blade (WC, angle COW in Fig. 3), the direction of the appressorium and the long axis of the conidium (AC, angle AOC in Fig. 3) and the direction of the appressorium and the main axis of the leaf blade (WA, angle AOW in Fig. 3) were measured. We counted positive angles clockwise from the leaf axis. We also noted the position of each of the arms of the measured angles (i.e. the axis

Table 1
Some angular parameters for the infectious units of *Blumeria graminis*.

Objects	Cultivar	Khakasskaya				Zarya		All variants
	Inoculum	M		K		K		
	Leaves	First	Second	First	Second	First	Second	
	Variants	1	2	3	4	5	6	
Ungerminated conidia	Sample volume	368	228	130	598	39	65	1428
	WC Mean	34 ± 1	37 ± 2	34 ± 2	38 ± 1	30 ± 4	34 ± 3	36 ± 1
	P	<0·005	<0·005	<0·005	<0·005	<0·005	<0·005	<0·005
Normal appressoria	Sample volume	56	214	17	720	29	84	1120
	WC Mean	41 ± 4	39 ± 2	58 ± 7	42 ± 1	47 ± 5	42 ± 3	42 ± 1
	P	>0·25	<0·01	>0·05	<0·005	>0·5	>0·25	<0·05
	WA Mean	28 ± 3	40 ± 2	46 ± 7	36 ± 1	31 ± 5	29 ± 3	36 ± 1
	P	<0·005	<0·005	>0·15	<0·005	<0·025	<0·005	<0·005
	AC Mean	36 ± 3	51 ± 2	45 ± 6	47 ± 1	42 ± 5	45 ± 3	47 ± 1
	P	>0·1	<0·005	>0·05	<0·005	>0·5	>0·5	<0·005
Abnormal appressoria	Sample volume	12	83	1	237	3	3	339
	WC Mean	40 ± 9	41 ± 3	41	44 ± 2	27 ± 5	37 ± 13	43 ± 1
	P	>0·5	>0·15	–	>0·5	–	–	>0·25
	WA Mean	27 ± 5	39 ± 3	79	43 ± 2	49 ± 14	75 ± 9	42 ± 1
	P	<0·05	>0·10	–	>0·25	–	–	<0·05
	AC Mean	33 ± 6	44 ± 3	66	45 ± 2	35 ± 26	52 ± 19	45 ± 1
	P	>0·15	>0·10	–	<0·05	–	–	<0·005

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