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[1]Nees-Institute for Biodiversity of Plants, University of Bonn, Germany
[2]Institute for Crop Science and Resource Conservation (INRES – Phytomedicine), University of Bonn, Germany

Efficiency of self-cleaning properties in wheat (*Triticum aestivum* L.)

A.K. Stosch[1], A. Solga[1], U. Steiner[2], E.-C. Oerke[2], W. Barthlott[1], Z. Cerman[1]

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Summary

An experimental study was carried out to assess the efficiency of self-cleaning properties of three wheat cultivars and their potential in the protection against *Blumeria graminis* f. sp. *tritici*, a fungus that causes powdery mildew. Leaf samples with intact epicuticular structure were compared to such with wiped wax crystals. Contact angles were determined and the surfaces were subjected to a standardized contamination test with hydrophobic fluorescence powder. Another set of samples was inoculated with conidia of *B. graminis* and, after various time intervals, exposed to artificial fog or rain.

For the intact surfaces of all cultivars contact angles of about 165° were measured. It is therefore suggested that wheat should be termed superhydrophobic. The wiping of the wax crystals led to a significant decrease of contact angles. This fact underlines the importance of surface roughness for achieving extreme water-repellency. In the standardized contamination test significantly more particles remained on the wiped surfaces than on those who had been left intact. This result was ascribed to increased adhesion on the smoothed samples.

The inoculation with subsequent precipitation revealed a significantly better removal effect of conidia from intact than from wiped surfaces. This was irrespective of the wheat cultivar. In general, conidia were more effectively removed by rain than by fog. This was probably due to the higher kinetic energy and the greater amount of water when using rain. If fog application was delayed by 3 hours a higher percentage of conidia remained on the surface. As possible causes are discussed increased adhesion by conidia secretions or the development of primary germ tubes.

Despite its highly efficient self-cleaning properties proved here, wheat is frequently infected by *Blumeria graminis*. We conclude that the high water content of the mildew conidia, the ability of *Blumeria graminis* to germinate at very low humidities and its rapid irreversible adhesion are effective adaptations in order to overcome the barrier of a superhydrophobic self-cleaning surface.

Introduction

Most higher plant species from major systematic groups do not have smooth but rough, micro- and sometimes also nanostructured surfaces. For the description of the surface characters four different categories are usually distinguished (cf. BARTHLOTT, 1981): 1. the cellular pattern, 2. the shape of the epidermal cells, 3. the relief of outer cell walls, 4. superimposed epicuticular secretions. The latter are termed epicuticular waxes and occur in a remarkable variety (e.g. JEFFREE, 1986; BARTHLOTT et al., 1998). Micro- and nanostructures of plant surfaces have several functions which, so far, only partially have been proved while others are still being discussed. They affect resistance to UV radiation and probably have an impact on the air flow at the surface (BARTHLOTT and WOLLENWEBER, 1981; PFÜNDEL et al., 2006). Furthermore, the structures change the degree of wettability and make a surface either more hydrophilic or more water-repellent. The combination of a microstructure and superimposed hydrophobic epicuticular wax crystals leads to extreme water-repellency with contact angles exceeding 150° ('superhydrophobicity', cf. MARMUR, 2004). Examples of common plant species with such extremely water-repellent surfaces are nasturtium (*Tropaeolum majus*), lily of the valley (*Convallaria majalis*) and the sacred lotus (*Nelumbo nucifera*). The superhydrophobicity is linked with an amazing phenomenon: The surfaces are self-cleaning, i.e. various contaminants as well as pathogenic microorganisms are washed away by water droplets rolling off (SCHWAB et al., 1995; BARTHLOTT and NEINHUIS, 1997). Regarding the colonization of pathogens the superhydrophobicity has an additional effect: As the availability of water on the surface is strongly reduced, the majority of spores is unable to germinate. However, this holds not for some specialists causing powdery mildew (JUNIPER, 1991).

Wheat plays a vital role as food, feed and, in the future, probably also as an energy source. With an annual production of about 550 million tonnes the cereal is among the three most important crops worldwide (FAO, 2004). Moreover, it is the staple for nearly 40% of the world's population (WIESE, 1991). A large portion of the wheat harvest is destroyed by pests and diseases every year. For the period from 1988 to 1990 the damage caused by those factors has been estimated at 244 billion US-Dollars (OERKE et al., 1994; AGRIOS, 1997). From the economical point of view, powdery mildew of wheat is one of the most important diseases (BRAUN, 1995). Powdery mildews are capable to infect wheat despite the strong water-repellency of its surface. This water-repellency is caused by a dense cover of wax crystals which are predominantly flat ('platelets', BARTHLOTT et al., 1998) but which, depending on the part of the plant and the stage of growth, also occur as tubules and rodlets (TROUGHTON and HALL, 1967). Consequences of a mildew infection for the host are nutrient deficiency, a reduction of photosynthesis and an increase of respiration and transpiration. Yield losses up to 40% have been recorded (WIESE, 1991).

In the majority of studies on the initial stages of mildew infection conducted yet the focus was on specific adaptations of the pathogen enabling it to stick rapidly to a surface and to colonize the host (KUNOH, 1981; CARVER et al., 1995a; CARVER et al., 1995b; CARVER et al., 1996; CARVER et al., 1999). On the other hand, relatively few studies gave priority to the properties of the host surface preventing it from mildew formation and to environmental parameters influencing the colonization success of the pathogen (e.g. MERCHÁN and KRANZ, 1986b; MERCHÁN and KRANZ, 1986a; HOLTHUIS et al., 1990). The results of these studies indicate that precipitation is a key factor in removing conidia from the wheat surface and hence in reducing powdery mildew infection. However, no survey which has been carried out so far focused on the potential self-cleaning properties of wheat and the evaluation of their efficiency.

The present experimental study attempts to fill this gap: a) For the first time, it investigates whether wheat can be considered as superhydrophobic and self-cleaning; b) It attempts to elucidate the relationship between the epicuticular structure of wheat as the outermost line of defence and the plant's susceptibility to mildew infection; c) It examines the effect of time between deposition and precipitation on the success of a powdery mildew in colonizing the wheat surface. We confined our investigations to the physical aspects of superhydrophobicity and self-cleaning properties, physiological processes were not taken into consideration. In order to demonstrate independence of hydrophobicity phenomena on plant surface from resistance due to specific genes, wheat cultivars both with and without resistance genes were used in the tests.

Material and methods

Biological material

The experiments were conducted with the three winter wheat cultivars Kanzler, Kris and Ludwig. Cultivar Kris is known to have the resistance genes *Pm2* and *Pm6* against powdery mildew, whereas Kanzler and Ludwig lack such genes (BUNDESSORTENAMT, 2002). The wheat seedlings were grown in 9x9 cm pots containing organic soil (Klasmann-Deilmann, Geeste-Groß Hesepe, Germany) in a greenhouse at 20-23°C and 70-80% relative humidity. A photoperiod of 16 h (>300 µE m^{-2} s^{-1}) per day was ensured by supplementary lights (Philips SGR 140, Hamburg, Germany). Pots were watered daily with tap water avoiding water splashes to wet the plants. For all tests the second leaves of 14-days-old seedlings (growth stage GS 12) were used.

The obligate, host-specific and ubiquitous parasite *Blumeria graminis* (DC.) Speer form species *tritici* Em. Marchal was chosen as the pathogen in all inoculation experiments. The fungus was maintained on wheat (*Triticum aestivum* cv. Kanzler). Since only young and vigorous conidia should be used in the tests, host plants were shaken at the day before conidia harvest. In this way, old conidia were removed from the plants and a harvest of a homogeneous inoculum was ensured (cf. MOUNT and SLESINSKI, 1971).

Preparation and treatment of samples

Both for the standardized contamination test and for the inoculation with mildew conidia wheat leaves with intact wax crystals and such with destroyed epicuticular structure were required. The latter were obtained by wiping the leaf surfaces with cotton (cf. SCHWAB et al., 1995). Though the wax crystals were destroyed by this method the surface chemistry was not affected because a thin amorphous wax film remained on the leaves. The success of the procedure was controlled by scanning electron microscopy (SEM, Fig. 1). Below, surfaces that have been wiped will be called ‘wiped surfaces’. As preparation for the processes of contamination, inoculation and microscopical investigation of wheat surfaces, pieces of 1x2 cm were cut off the leaves and were fixed on SEM aluminium stubs with double-sided adhesive tape.

A standardized contamination test was performed to assess the self-cleaning properties of the leaf surfaces (FÜRSTNER, 2002; FÜRSTNER et al., 2005). Five samples with intact epicuticular structure and five with wiped crystals of each cultivar were put in a contamination chamber. Furthermore, four SEM-stubs with conducting tabs (W. Plannet GmbH, Wetzlar, Germany) were added which served as reference and which were required for the determination of the density of deposited particles. In the contamination chamber 0.5 g of a hydrophobic fluorescence powder (Osram Leuchstoff F20, Osram GmbH, Schwabmünchen, Germany) were dispersed with a ventilator. The particle size of the powder varied between 2 and 30 µm. After a sedimentation period of 60 minutes, the stubs were taken out and all except the reference stubs were moved to a fog chamber. In this chamber they were arranged on a plate inclined 45°. A fine mist of deionized water with droplet sizes between 8 and 20 µm, produced by a high pressure fog system (Osberma, Engelskirchen-Osberghausen, Germany), was injected into the chamber. The process took 5 minutes, corresponding to a precipitation amount of 1.5 mm. After the treatment the samples remained in the chamber for 60 minutes and were subsequently dried in an oven.

The inoculation with conidia was carried out in a home-made inoculation chamber. Four samples with intact epicuticular structure, four with wiped crystals and four SEM-stubs with conducting tabs were randomly placed on a plate at the bottom of the chamber. The latter were required to monitor inoculum density. Freshly harvested conidia were put in a dish close to the top of the apparatus and were dispersed by an air blast. Afterwards the samples were left in the chamber until further processing. The inoculation was follwed by an exposition of the leaf samples either to a fine mist or to artificial rain. The fog treatment has already been described above. For simulation of rain the method described by Fürstner (2002) was applied. In this procedure each sample was sprinkled with 50 ml deionized water which fell from a container furnished with 24 cannulas and installed 15 cm above the sample holder. The droplet size of the artificial rain was approximately 2 mm. In different experimental series the treatment with fog and that with artificial rain were carried out one, two and three hours after inoculation, respectively.

Sample analysis

In order to determine wettability, static contact angles were measured. For this purpose, samples of 1x3 cm were cut off the leaves and were fixed on glass slides, using double-sided adhesive tape. The measurement was conducted with a contact angle meter (OCA 30, Dataphysics Instruments GmbH, Filderstadt, Germany), using 15µl droplets and the Young-Laplace-Fit. Prior to further examination by scanning

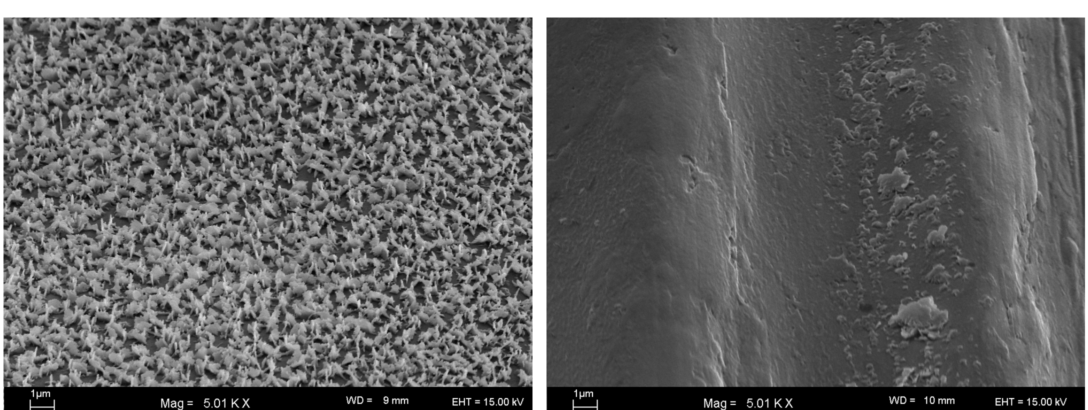


Fig. 1: SEM images of *Triticum aestivum* cv. Kanzler leaf surfaces. Left: wax crystals intact, right: amorphous wax film after wiping.

electron microscopy, all samples were coated with gold in a sputter coater (Balzer Union SCD 040, Balzer-Pfeifer GmbH, Asslar, Germany). The samples of the standardized contamination test were investigated in a Cambridge Stereoscan 200 (Leica, Bensheim, Germany) coupled with a cathodoluminescence-detector. The fluorescence images taken by this method were analyzed with the program Scion Image (Scion Corporation, Maryland, USA). Then the coverage of the plant samples with particles relative to that of the reference stubs was calculated. The samples of the inoculation with conidia were examined in a LEO 440i SEM (Carl Zeiss SMT, Oberkochen, Germany). Ten SEM micrographs were taken of each sample and the total number of conidia displayed in each micrograph was counted.

Data analysis

Differences between pairs of means were investigated by using student's t-test. If normality and homogenity of variances were not given and could not be achieved by data transformation, the non-parametric Mann-Whitney U-test was applied. Two-way analyses of variance were carried out to test the influence of the factors wheat cultivar and epicuticular structure on the variable number of conidia. In order to meet the assumptions of ANOVA or at least to improve homocedasticity, three different transformations were utilized: log x, log x+1 and 1/x. The effect of time between conidia deposition and precipitation (fog, rain) on the penetration success with intact wheat surfaces was studied by calculating the ratio of remaining conidia to the amount of conidia on the reference stubs. Afterwards multiple comparison tests (Tukey HSD) were conducted to reveal differences between the time intervals (1, 2 and 3 hours). All statistical analysis was carried out with SPSS 12.0, graphs were created with SigmaPlot 9.0 (both SPSS Science, Chicago, USA).

Results

Surface wettability

Contact angles around 165° were determined for the intact surfaces of all three cultivars (Tab. 1). The contact angles of the samples with wiped epicuticular waxes ranged from 105° to 110°. Variability was distinctly higher with the wiped surfaces. The difference between intact and disturbed structure was always highly significant ($p < 0.001$).

Tab. 1: Surface wettability of the three wheat cultivars with intact and wiped wax crystals. Means of 15 measurements with single standard deviation.

	Contact angles [°]	
Wheat cultivar	Waxes intact	Waxes wiped
Kanzler	165.8 ± 3.5	109.7 ± 6.5
Kris	166.8 ± 2.8	105.6 ± 8.4
Ludwig	163.0 ± 2.9	106.2 ± 6.2

Standardized contamination test

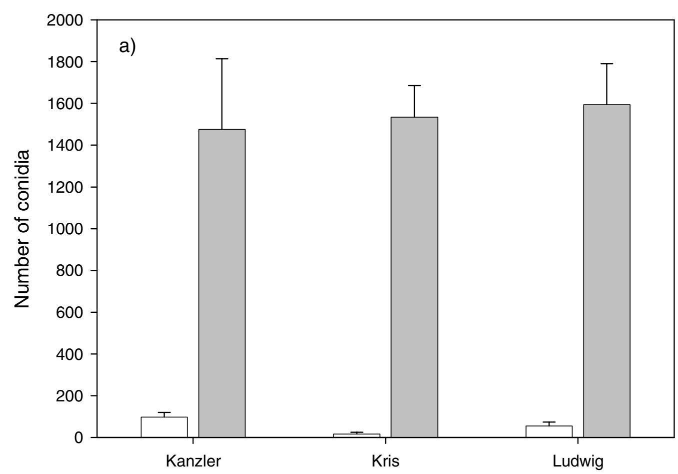
The analysis of the fluorescence images revealed relative coverages with particles below 1% for the surfaces with intact and around 10% for the samples with disturbed structure, respectively (Tab. 2). In all three cultivars differences between the samples with intact and those with wiped waxes were highly significant ($p < 0.001$). On the other hand, differences in coverage between the cultivars were negligible.

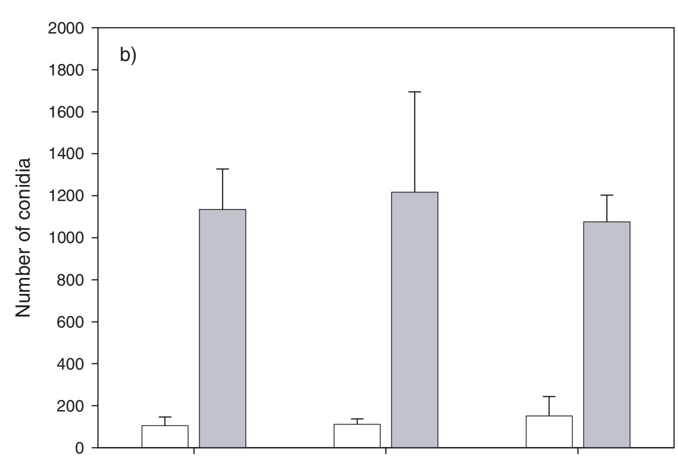
Tab. 2: Coverage of three wheat cultivars with fluorescence powder particles. Comparison between surfaces with intact and wiped wax crystals. Means of five replicates with single standard deviation.

	Relative coverage [%]	
Wheat cultivar	Waxes intact	Waxes wiped
Kanzler	0.7 ± 0.6	11.9 ± 3.6
Kris	0.3 ± 0.2	10.4 ± 2.2
Ludwig	0.9 ± 0.7	10.9 ± 1.9

Effect of simulated fog

After inoculation and fog treatment the number of conidia on the surfaces with wiped wax crystals was always many times higher than that on intact wax structure (Fig. 2 a-c). The relationship between the surface structure and the number of conidia was significant irrespective of the interval between inoculation and artificial precipitation (Tab. 3). No influence of the cultivar on the number of remaining conidia could be detected which reflects the similar coverage of the different cultivars. Again, this held for all intervalls between inoculation and fog treatment.





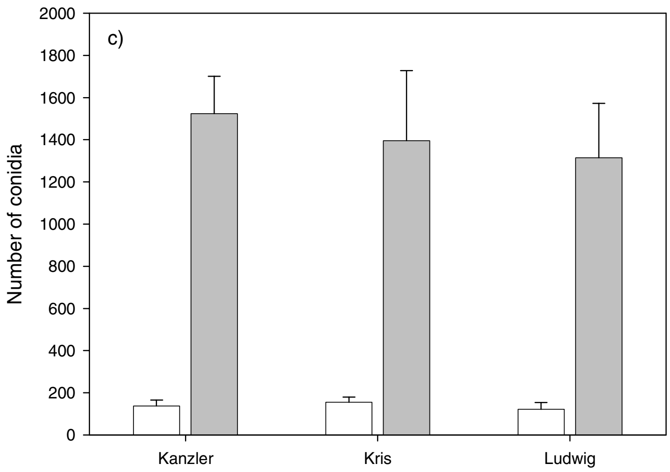


Fig. 2: Number of *Blumeria graminis* conidia remaining on the surfaces of three wheat cultivars with intact (open bars) and wiped (filled bars) wax crystals after inoculation and fog treatment. Intervals between inoculation and fog treatment: a) 1 hour, b) 2 hours, c) 3 hours. Means of four replicates with single standard deviation.

Tab. 3: Results of the two-way ANOVAs: effects on the variable number of conidia of the factors epicuticular structure and wheat cultivar and their interaction. Three different time intervals. *: $p \leq 0.05$, ***: $p \leq 0.001$, n.s. = not significant.

Interval	Structure	Cultivar	Structure x Cultivar
1 h	***	n.s.	*
2 h	***	n.s.	n.s.
3 h	***	n.s.	n.s.

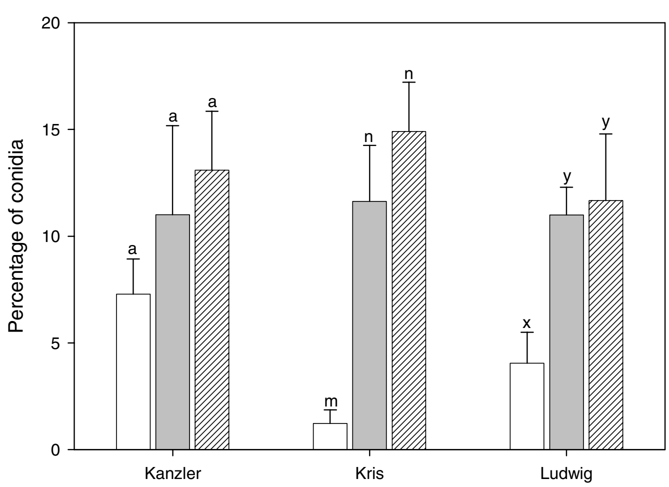


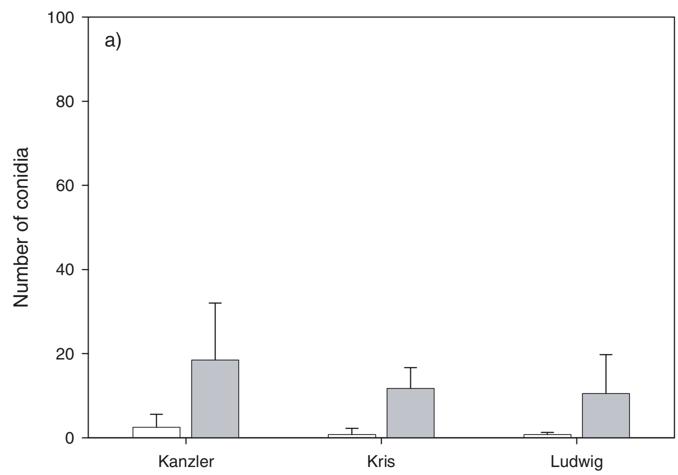
Fig. 3: Percentage of *Blumeria graminis* conidia remaining on the intact surfaces of three wheat cultivars after inoculation and fog treatment. Intervals between inoculation and fog treatment: 1 hour (open bars), two hours (filled bars), three hours (hatched bars). Means of four replicates with single standard deviation. Bars sharing the same letter are not significantly different ($p < 0.05$).

Fig. 3 compares the relative coverages of the intact surfaces that have been treated with fog one, two and three hours after inoculation, respectively. No significant differences were found for the cultivar Kanzler. In contrast, on Kris and Ludwig significantly more conidia were detected when fog treatment was performed two or three hours after inoculation as compared to a treatment already after one hour.

Effect of simulated rain

Since no remarkable difference between the two and the three hours interval in the fog treatment was found, the two hours interval was omitted in subsequent experiments. Thus, artificial rain was applied only one and three hours after inoculation.

In general, artificial rain produced a much higher reduction of conidia on the surfaces than fog (Fig. 4 a-b, notice the different scales of the y-axes). As with the fog treatment, significantly more conidia were found on wiped than on intact surfaces (Tab. 4), and no relationship between the wheat cultivar and the number of remaining conidia was detectable.



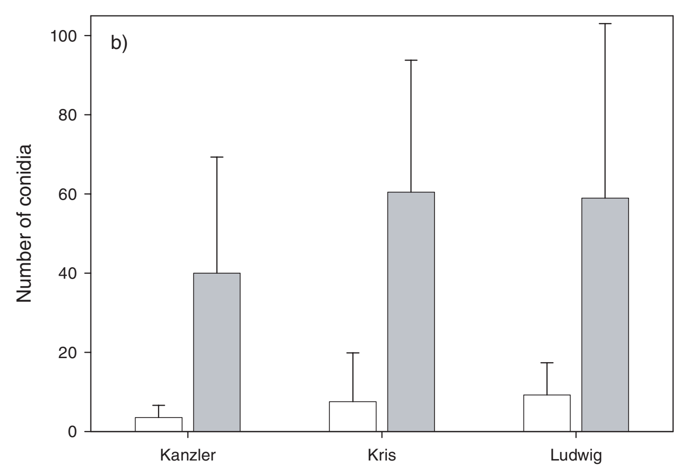


Fig. 4: Number of conidia remaining on the surfaces of three wheat cultivars with intact (open bars) and wiped (filled bars) wax crystals after inoculation and rain treatment. Intervals between inoculation and rain treatment: a) 1 hour, b) 3 hours. Means of four replicates with single standard deviation.

Tab. 4: Results of the two-way ANOVAs: effects on the variable number of conidia of the factors epicuticular structure, wheat cultivar and their interaction. Two different time intervals. ***: $p \leq 0.001$, n.s. = not significant.

Interval	Structure	Cultivar	Structure x Cultivar
1 h	***	n.s.	n.s.
3 h	***	n.s.	n.s.

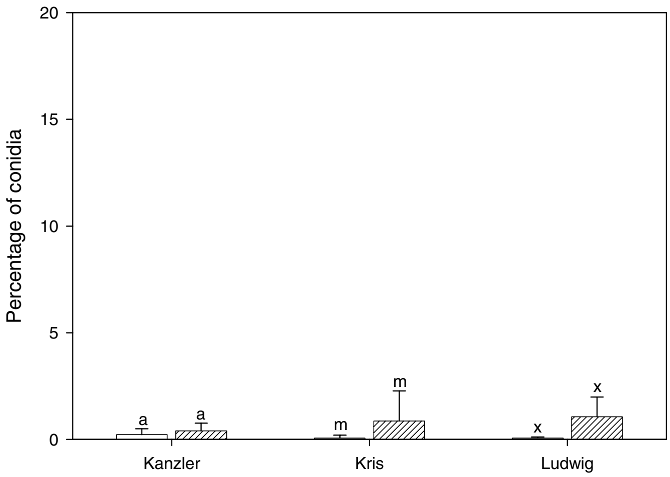


Fig. 5: Percentages of *Blumeria graminis* conidia remaining on the intact surfaces of three wheat cultivars after inoculation and rain treatment. Intervals between inoculation and rain treatment: 1 hour (open bars), three hours (hatched bars). Means of four replicates with single standard deviation. Bars sharing the same letter are not significantly different ($p < 0.05$).

The percentages of conidia remaining on the intact surfaces after artificial rain were several times lower than after fog treatment (Fig. 5). Moreover, none of the three cultivars showed significant differences between the one and the three hours interval.

Discussion

The formation of micro- and nanostructured superhydrophobic surfaces is only one among several options for plants to defend themselves against pathogens. Eliminating spores before they stick and germinate has the advantage that no further physiological efforts to resist a pathogen attack have to be made.

The first question was whether intact wheat surfaces can be termed superhydrophobic and self-cleaning. Taking into account recent studies on the topic wetting and non-wetting (e.g. Quéré, 2002; Yoshimitsu et al., 2002; Marmur, 2006), the contact angles of about 165° determined for all cultivars clearly match the criterium of superhydrophobicity. The values correspond to those reported in earlier studies on wheat and its surface properties (Troughton and Hall, 1967; Holloway, 1969). The significant decrease of contact angles after wiping points to the indispensability of surface roughness and heterogenous wetting with air in the cavities under droplets for the purpose of achieving extraordinarily high contact angles (cf. Dettre and Johnson, 1964). The enhanced variation found for the wiped surfaces is probably due to surface heterogenity caused by the treatment. Values around 110° were also found by Holloway (1969) who determined contact angles on smooth films of isolated wheat waxes on glass. Furthermore, it has been suggested that contact angles in this order of magnitude occur only if wax is involved at the cuticle surface (Holloway, 1970). In addition to the visual documentation (Fig. 1), this confirms that the wiping process in fact only destroyed the epicuticular structure and that it did not completely remove the wax layer.

Since particles were almost completely removed from the intact wheat surfaces in the standardized contamination test, it is justified to add *Triticum aestivum* the the group of plants with self-cleaning leaf surfaces. The elimination of particles was even more complete than in lotus (*Nelumbo nucifera*), the classic example for self-cleaning plants (cf. Barthlott and Neinhuis, 1997). This can be explained by considering the differences in surface sculpture between lotus and wheat: The leaves of lotus are papillose on the micrometer scale and the papillae are additionally covered with epicuticular wax crystals (Barthlott and Neinhuis, 1997; Wagner et al., 2003). The wheat surface, on the other hand, consists of elongate, slightly convex cells with a dense layer of wax platelets (Carver et al., 1995b; Koch et al., 2006). As a consequence, if small particles on lotus leaves are not reached and picked up by droplets with high kinetic energy they remain between the epidermal papillae. In contrast to this, on wheat leaves even small particles lie always on top of the wax layer and can be removed even by very fine droplets with low kinetic energy. The 10-30 times higher coverage of the samples with wiped wax crystals after fog treatment is probably due to increased adhesive forces between smoothed wax films and deposited particles (Burton and Bhushan, 2006). Furthermore, it is likely that lump-like wax accumulations produced by wiping prevented particles from getting removed.

The results of the inoculation with *Blumeria graminis* conidia and subsequent fog treatment agree well with the findings of the standardized contamination test. Again, the high efficiency of the self-cleaning mechanism in case of an intact epicuticular structure was apparent. Significantly less conidia on the wheat leaves with intact structure as compared to those with partially removed waxes have also been observed by Merchán and Kranz (1986b). As expected, artificial rain generally washed off much more conidia than fog. It is suggested that both the higher kinetic energy of the larger rain droplets and the amount of water which was about 150 times higher in the rain treatment led to this result. The stronger impact of rain in comparison to the fine mist probably also caused the less distinct differences between intact and disturbed surfaces (Fig. 4 a-b). The fact that neither in the fog nor in the rain treatment wheat cultivars had an influence on the results indicates similarity of surface texture within the experimental sets.

The third aspect examined in this study was whether the amount of time passing from deposition of conidia on intact wheat surfaces to the start of artificial precipitation has an influence on the colonization success of *Blumeria graminis*. The significant relationship that was revealed in the fog treatment for the cultivars Kris and Ludwig and the tendency found for the cultivar Kanzler can be explained by initial processes that start right after the first contact between conidium and surface. Within the first few minutes the production of extracellular material (ECM) including proteins begins (Kunoh et al., 1988; Kunoh et al., 1990; Nicholson and Kunoh, 1995). 10 to 15 minutes after initial contact of the conidium with the surface a second phase of ECM release can be observed: cutinases are exuded which dissolve the wheat cuticle (Pascholati et al., 1992; Meguro et al., 2001). 45-50 minutes later the first primary germ tubes (PGTs) are formed, followed by secondary germ tubes emerging after 2-3 hours (Carver et al., 1995a; Green et al., 2002). The development of appressoria starts not until 8-9 hours and is therefore of minor importance here (cf. Carver et al., 1995b; Nicholson, 1996). Considering these first steps of the infection process it is suggested that sticking by ECM and/or the growth of PGTs caused increased adhesion which resulted in significantly higher percentages of remaining conidia on Kris and Ludwig (see also Nicholson and Epstein, 1991). The development of PGTs had also been observed in the SEM while counting remaining conidia. In the rain treatment no significant differences could be established. This result is most likely due to the generally much more effective removal of conidia by rain than by fog (see above). Referring to Holthuis et al. (1990), a substantial washing-off with rain is to be expected until the full development of appressoria.

In the present study no differences between the cultivar with resistance genes and the two cultivars without such genes could be observed. It is likely that the occurrence of resistance genes has an impact only in later stages of the infection process and that the integrity and functional efficiency of the surface microstructure is the decisive factor within the first hours after conidia deposition. Even though wheat can have different types of epicuticular waxes (TROUGHTON and HALL, 1967), the shape of the latter should be of minor importance for the self-cleaning efficiency (cf. BARTHLOTT and NEINHUIS, 1997).

It could be shown that the epicuticular waxes of wheat provide an efficient protection against the attachment of particles and conidia contamination. However, despite this effective self-cleaning mechanism proved here, fungi causing powdery mildew like *Blumeria graminis* are pathogens with a worldwide distribution and a strong impact on the global wheat harvest. Different reasons may account for this discrepancy. First of all, it is unlikely that the epicuticular waxes in a wheat canopy are completely intact. For example, rubbing of plants, mechanical impact by rain droplets and kinks of leaves and culms cause wax erosion and provide spots at which pathogens can start their attack (TROUGHTON and HALL, 1967; WILSON, 1984; BAKER and HUNT, 1986; CLEUGH et al., 1998). It has to be mentioned that such surface defects are partly repaired by regeneration processes (cf. WOLTER et al., 1988; NEINHUIS et al., 2001; KOCH et al., 2004). Secondly, there is evidence that surfactant-containing agrochemicals alterate the wax fine structure of wheat and other crops (WOLTER et al., 1988; NOGA et al., 1991). As shown in experiments with *Brassica oleracea* (NEINHUIS et al., 1992), such an alteration of micromorphology reduces the self-cleaning ability of the surface and thus facilitates powdery mildews to adhere to the plant surface. In addition to agrochemicals also airborne pollutants can cause a degeneration of epicuticular waxes (TURUNEN and HUTTUNEN, 1990). Thirdly, if precipitation occurs mainly as fog or dew with low kinetic energy, the interval between the deposition of a conidium and the precipitation event must be very short. As already discussed above, conidia of *Blumeria graminis* adhere rapidly after their first contact with the surface. In consequence, their removal is only possible when free water is available without delay. Moreover, it has been demonstrated that *Blumeria graminis* stores considerable quantities of water in its spores (YARWOOD, 1950). This property enables the mildew to germinate at low relative humidity (YARWOOD, 1978) without the presence of free water, i.e. under conditions that are unfavourable for most other fungal diseases (HARRISON et al., 1994). It is therefore suggested that pathogens causing powdery mildew have evolved a high degree of adaptation and that they have overcome the barrier of a superhydrophobic self-cleaning surface. A disturbance of the epicuticular structure does not inevitably increase mildew infestation. Recent studies suggest that particularly the epicuticular waxes provide chemical (CARVER et al., 1996) and/or topographical (WYNN, 1981) information which stimulates fungal development.

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Address of the authors:

Anne Kathrin Stosch, Dr. Andreas Solga, Prof. Dr. Wilhelm Barthlott and Zdenek Cerman (corresponding author), Nees-Institute for Biodiversity of Plants, University of Bonn, Meckenheimer Allee 170, D-53115 Bonn, Germany.

HD Dr. Ulrike Steiner and PD Dr. Erich-Christian Oerke, Institute for Crop Science and Resource Conservation (INRES – Phytomedicine), University of Bonn, Nussallee 9, D-53115 Bonn, Germany.