Initial Events in the Establishment of Cereal Powdery Mildew Infection

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Abstract

Like spores of many fungi, conidia of *Blumeria graminis*, the powdery mildew fungus of cereals, release extracellular material. It is released within seconds where conidial surface projections touch a leaf. This ECM is probably adhesive since centrifugation showed that forces greater than those due to normal wind speeds are needed to displace conidia. Also, ECM release is probably involved in rapid sensing of substratum contact, leading to germ tube emergence close to the contact site. Thus, ECM release apparently confers at least two benefits to pathogen survival.

Keywords: adhesion; *Blumeria graminis*; cereals; conidia; extracellular material; germ tubes; germling development; powdery mildew

The fungus, the disease and the context

Blumeria graminis DC Speer (syn. *Erysiphe graminis* DC) is a biotrophic fungus that causes cereal powdery mildew. It is dispersed by asexual conidia. Current understanding of processes following spore germination was reviewed recently (e.g. GREEN *et al*. 2002). In summary, the first signs of germination are not evident until some time after spore deposition when conidia sequentially produce two germ tubes (Figure 1A), the primary (PGT) and appressorial germ tube (AGT). The short (5–10 μm) PGT emerges after 1–3 h and contacts the host surface forming a penetration peg but no haustorium. Its functions include adhesion, accessing host water and recognising host features. Correct PGT function permits elongation of the AGT (30–40 μm) which adheres to and recognises the leaf, leading to apical lobe differentiation (9–10 h). An infection peg then attempts to penetrate the host cell (12–15 h) and, if successful, a haustorium is formed and colony growth commences.

Here, we review our recent studies of events occurring before germination. Like many other foliar pathogens, ungerminated *B. graminis* conidia are vulnerable to displacement by the direct action of wind and rain and by their indirect effects in causing leaf shake. It is clear, therefore, that events occurring within minutes or even seconds of spore deposition can be crucial to pathogen survival and development.

Conidia have the biological potential to release extracellular material (ECM)

On certain artificial substrata, ECM forms large, pad-like deposits (Figure 1B) and its release can be observed within seconds of conidium deposition (CARVER *et al*. 1999; ROBERTS & MIMS 1988). Kunoh, Nicholson and co-workers (see WRIGHT *et al*. 2002a) showed that this ECM contains various esterase enzymes including cutinase, and hydrolytic activity associated with the ECM is detectable within 3 min of deposition. Esterase activity is released in two phases, the first within 2 min of contact and the second ca 15 min later. The first is not inhibited by cycloheximide treatment, implying release of preformed enzyme, but the second can be inhibited, indicating dependence on active, *de novo* synthesis. Conidial ECM collected from artificial substrata can erode the barley leaf surface.

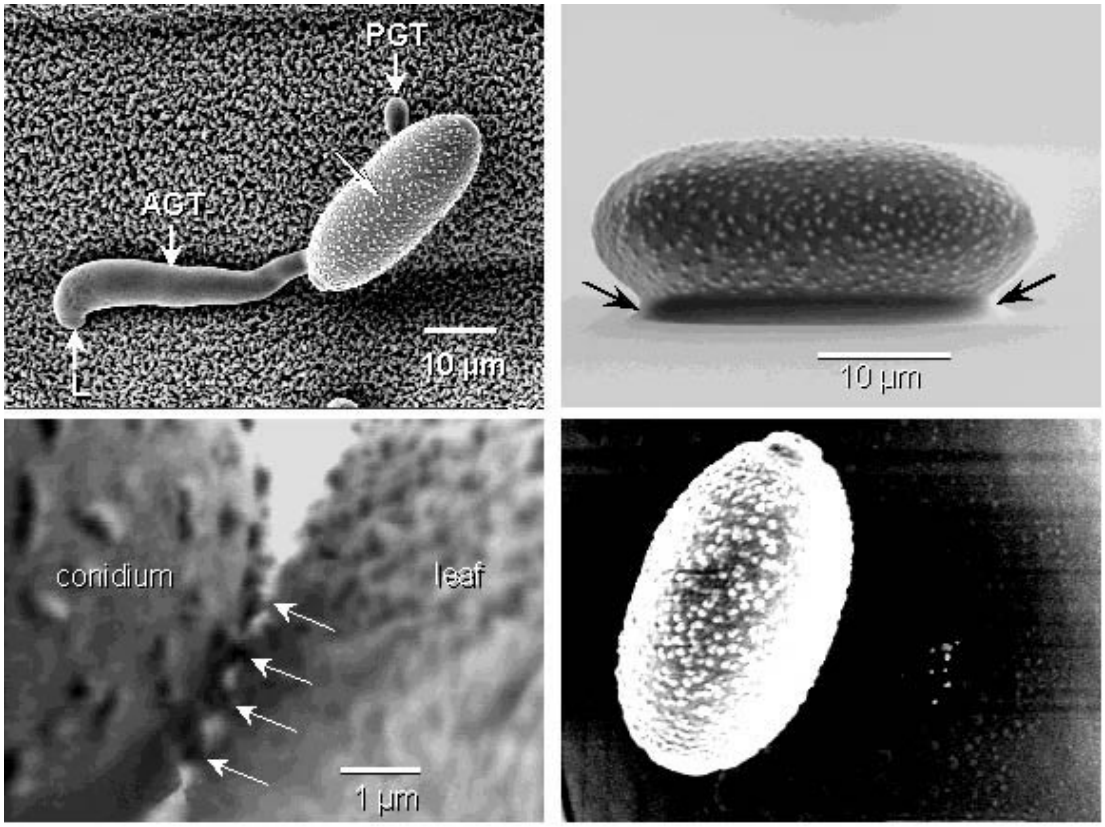

Figure 1. Scanning electron microscope images of cryofixed *B. graminis* specimens

A. A fully mature *B. graminis* germling on a barley leaf. The short PGT and elongated AGT with a hooked, apical lobe (L), have a smooth featureless surface but the conidial wall is covered with distinct surface projections (arrow)
B & **C**. Low angle observations (from CARVER *et al.* 1999). B. After 15 min on a hydrophobic artificial substratum a large, pad-like ECM deposit (black arrows) is evident at the conidium/substratum interface. **C**. After 60 min on a barley leaf, the tips of a few conidial surface projections touch leaf wax crystals (white arrows) but no conidial ECM can be seen and intervening spaces between contact points are open
D. After 1 min incubation on a de-waxed barley leaf the conidium was displaced by micro-manipulation to the left of its original contact site with the leaf. A small number of conidial ECM deposits are exposed at the site of original contact (arrows) (from WRIGHT *et al.* 2002a)

Until recently, however, it was not possible to ascribe a role for conidial ECM in pathogenesis because it could not be detected on host leaves even using low angle, low temperature scanning electron microscopy (Figure 1C) (CARVER *et al.* 1999). This led to the conclusion that if ECM is released on leaves, the quantity is very small.

Micro-manipulation shows rapid but limited conidial ECM release on leaves

Because conidial ECM could not be seen when conidia were in place on leaves, we developed a low temperature method that allowed us to move conidia by micro-manipulation and expose original contact sites on leaves (WRIGHT *et al.* 2002a). When cryofixed conidia on de-waxed barley leaves were displaced, a few small, discrete ECM deposits were revealed. These were visible within 1 min of inoculation (Figure 1D). Up to 12 h, deposits remained similar in form and amount indicating that little, if any, further ECM was released. The size and spatial distribution of deposits indicated that they were released from conidial surface projections that touched the curved leaf epidermal cell surface. This also appeared true on the at surface of glass, but here much more ECM was present and it formed relatively large pad-like deposits. The geometric relationships between conidia and at (glass) or curved surfaces (leaf cells) can explain the different forms of ECM deposit. On planar surfaces, more conidial surface projections

touch the substratum and release ECM droplets. The combined capillary force exerted by these droplets may pull the spore towards the substratum causing droplet coalescence and pad formation. On curved leaf cells, where fewer conidial surface projections make contact, capillary force is apparently insufficient to pull conidia and droplets remain discrete.

Do ungerminated conidia adhere to host leaves?

The relative strength of adhesion by conidia and germlings to barley leaves was assessed using centrifugation (WRIGHT *et al*. 2002b). Ungerminated conidia were incubated for 10 or 30 min before centrifugation, while extended incubation for up to 12 h allowed germlings to reach different developmental stages before treatment. After incubation, material was subjected to relative centrifugal force (RCF) up 26.00×10^3 g. Although adhesion was far stronger after germination (because of adhesion by germ tubes), about 80% of ungerminated conidia remained in place even when subjected to displacement force of about 4.2×10^{-9} N. This is far greater (*ca* 1000×) than the forces likely to be generated by wind acting to displace deposited conidia from leaves in a barley crop (BAINBRIDGE & LEGG 1976). Thus, adhesion, presumably involving conidial ECM, is sufficient to hold the majority of ungerminated conidia in place even under extreme conditions.

Is conidial ECM involved in the accurate and rapid sensing of leaf contact?

Clearly, *B. graminis* development depends on fungal germ tubes making contact with the host surface. WRIGHT *et al*. (2000) showed that 80% or more of first-formed germ tubes emerged from conidia close to the host leaf surface and so made contact with it, allowing them to become functional PGTs. A statistical method was derived to predict the frequency of germ tube contact if emergence site was randomly determined. Predicted contact frequencies were far lower (ca three to eight times) than observed frequencies, indicating that germ tube emergence site is indeed determined in response to substratum contact. In part, this response appears to be non-specific because conidia also responded to various artificial substrata. Nevertheless, germ tube contact frequencies were greater on curved leaf cells that on flat artificial surfaces, suggesting that specific recognition of leaf surface characteristics promotes response. The area of contact required to stimulate response is apparently very small. On leaves, contact is limited to the tips of a few conidial wall projections that touch epicuticular leaf wax plates (Figure 1C), and conidia also responded to contact with a micro-needle tip or with spiders' suspension thread. Micro-manipulation to roll living conidia so that their original site of contact with a leaf was rotated away from it, led to the majority of first-formed germ tubes growing away from the leaf i.e. emerging close to the site of original contact (Figure 2). Data indicated that germ tube emergence site is determined within 1 minute of deposition. This again implicates conidial ECM release in recognition of the leaf surface contact site.

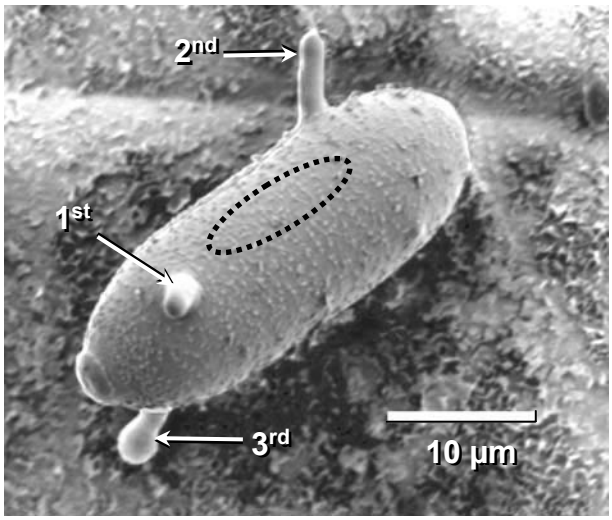

Figure 2. A *B. graminis* conidium that was rolled 15-20 min after inoculation onto a barley epidermal strip and then cryofixed after 3 h incubation. The spore was rolled by micro-manipulation through *ca* 180° so that the original site of contact with the epidermal strip (indicated approximately by the dotted line) faced away from the epidermal strip for the remainder of the incubation period. Repeated light microscope observation (prior to cryo-fixation) showed that the first- and second- (1st and 2nd) formed germ tubes emerged from the conidia wall close to its original site of contact, and therefore failed to make contact with the epidermis. The third-formed (3rd) germ tube successfully contacted the epidermal strip and swelled at its tip as is characteristic of a functional PGT (from WRIGHT *et al*. 2000).

Conclusions and speculations

While we can now be confident that conidial ECM is released rapidly on leaves, our arguments for its involvement in adhesion and surface sensing are based on deduction rather than direct evidence.

We know that the ECM contains hydrolytic enzymes and activity of such enzymes is necessary for function of adhesion pads formed by rust urediniospores (DEISING *et. al.* 1992). It has also been suggested that host surface components released by hydrolysis and taken up by the conidium may influence germ tube emergence site (NIELSEN *et al.* 2000). However, such factors cannot explain why conidia adhere strongly to, and respond to contact with, inert substrata such as glass (WRIGHT *et al.* 2000, 2002b). It is possible, therefore, that on leaves adhesion may be conferred both by physical (capillary) force and chemical bonding, while response to contact may be stimulated simply by physical release of the ECM in addition to uptake of leaf surface chemicals. Further work should resolve these possibilities.

The speed of ECM release clearly indicates that it is pre-formed and our observations show that conidial surface projections are made up, at least in part, of ECM (WRIGHT *et al.* 2002a). However, we do not know whether the ECM lies on the outermost spore surface or whether some form of external membrane overlies it. NICHOLSON *et al.* (1988) showed reduction in size of wall projection following repeated washing. We could not duplicate this finding when we used gentle immersion in water, but we did find a slight (but significant) reduction in the frequency of germ tubes making substratum contact after such immersion (unpublished). Together, these results suggest that some ECM is located on the spore surface. However, GAY *et al.* (1985) observed a cuticle-like structure investing the walls of pea powdery mildew conidia and in *B. graminis* we have recently observed (unpublished) a similar structure that follows the topography of conidial surface projections. In *B. graminis*, this structure lies outside an electron lucent region that is itself outside the conidial cell wall; we believe the lucent region is ECM. GAY *et al* (1985) considered the cuticle very vulnerable, and thought it probable that contact of conidia with a solid surface may cause 'minor breakage of the cuticle near areas of adherence'. It is attractive to speculate that while some ECM lies external to a 'cuticle', further material is contained within it. Following deposition, the cuticle may break at points where it projects from the spore surface and makes substratum contact. This could release ECM and lead to adhesion and signalling for directed germ tube emergence. Such a dual-function system would allow for rapid and efficient deployment of resources and increase the likelihood of successful subsequent pathogen development.

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