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Release of the extracellular matrix from conidia of *Blumeria graminis* in relation to germination

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The release of extracellular matrix (ECM) and the emergence of germ tubes from conidia of *Blumeria graminis* were studied by light microscopy and micromanipulation. More prompt and frequent ECM release was confirmed on an artificial hydrophobic substratum than on an artificial hydrophilic substratum. Conidia initially incubated on the hydrophilic substratum were transferred by micromanipulation to either the hydrophobic or the hydrophilic substrata. Immediately after transfer onto the hydrophobic substratum, 75% of conidia released ECM, whereas only 16% did so upon transfer to the hydrophilic substratum. Conidia transferred onto the hydrophobic substratum produced a primary germ tube (PGT) more promptly and frequently than those transferred to the hydrophilic substratum. Thus, conidia recognize and respond to substratum hydrophobicity perhaps immediately after contact. When inoculated onto either isolated barley cuticle or the hydrophobic artificial substratum, 2/3 of the conidia produced a PGT from their polar regions. By contrast, on the hydrophilic substratum 2/3 of conidia did so from the side region. These results show that substratum hydrophobicity affects the location of PGT emergence from conidia. Furthermore, the study indicates that very rapid recognition of surface hydrophobicity by conidia promotes ECM release and this in turn may influence the location of PGT emergence.

Key Words——*Blumeria graminis*; conidia contact; extracellular matrix; germination.

Many investigations of the fungal infection process deal with events that occur after germination. However, evidence shows that phenomena essential to the success of infection very often occur in advance of germination. The release of extracellular materials from ungerminated propagules is perhaps the most readily apparent of these phenomena (Carver et al., 1999; Nicholson, 1996).

Kunoh et al. (1988) first demonstrated that the surface of conidia of *Blumeria graminis* D. C. Speer (syn. *Erysiphe graminis* D. C.) is covered with a network of minute reticulate ridges interspersed with more obvious spine-like wall protrusions. Within 10 min of contacting a host barley leaf or a cellophane membrane, the conidium released material that obscured the reticulate network, and components of this material appeared to be deposited onto the contact surface moving outwards of the conidium. This process occurred in advance of germination and was completed within 30 to 60 min. Recently, Carver et al. (1999) studied the time course of extracellular matrix (ECM) release from *B. graminis* conidia by cryo-scanning electron and light microscopy. Conidia released ECM within 20 s of contacting certain hydrophobic artificial substrata, although no such rapid ECM release could be detected when conidia were deposited onto the hydrophilic surface of clean glass. Light microscopy and micromanipulation showed that the ECM was a liquid present at the contact interface of the conidium and substratum.

Kunoh et al. (1977) showed that conidia of *B. graminis* have a unique germination pattern whereby they form at least two germ tubes. A short, non-appressorial germ tube always emerged about 2 h before the appressorial germ tube, and the two types of germ tube were readily distinguished soon after emergence (Kunoh et al., 1977). The first remained short (ca. 5–10 μm) and rather thin although it swelled slightly towards the tip, while the appressorial germ tube elongated (ca. 30–40 μm) and thickened markedly towards the apex. Also, the first germ tube remained aseptate, while the appressorial germ tube formed a septum close to the conidium. Generally, conidia of *B. graminis* f. sp. *hordei* and f. sp. *tritici* produce one short non-appressorial germ tube, but a small proportion formed two or more (13–16%) short germ tubes in addition to the appressorial germ tube. Kunoh et al. (1978) showed that the short, non-appressorial germ tube breached the host epidermal wall and induced a papilla response, while the others often appeared not to make host surface contact. Based on these findings, non-appressorial germ tubes capable of stimulating a host response were considered functional and described as "primary germ tubes (PGTs)". Additional short germ tubes that caused no host cell response were

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considered non-functional, and described as "subsidiary germ tubes" (Kunoh, 1982).

Current evidence indicates that PGTs have several important functions. Firstly, they attach strongly and rapidly to the host surface, holding the germling in place during growth and differentiation of the appressorial germ tube (Carver et al., 1995; Kunoh, 1982). Secondly, PGTs can gain access to inorganic and possibly organic host components (Kunoh and Ishizaki, 1981) and to host water (Carver and Bushnell, 1983) via a short penetration peg (Kunoh et al., 1978) prior to appressorium formation. Thirdly, they recognize certain host surface characteristics and this drives subsequent elongation of the appressorial germ tube (Carver and Ingerson, 1987; Carver et al., 1995). Obviously, an absolute prerequisite to fulfilling these important functions is that the PGT makes contact with the host surface. Since the first-formed germ tube is relatively short, it must emerge from part of the conidial wall close to the host surface in order to make host contact. The fact that most first-formed germ tubes successfully make host surface contact in turn implies the existence of processes that control the position of germ tube emergence. Very recent evidence (Hall and Gurr, 2000) implicates cAMP and PKA in intracellular signaling processes controlling *B. graminis* germ tube emergence and differentiation. Since the PGT emerges within 30 min to 2 h after conidium deposition (Kunoh et al., 1979), it is clear that these processes must be engaged very rapidly following contact (Carver et al., 1999). Using conidia incubated on cellulose membrane, Kinane et al. (2000) demonstrated that an increase in endogenous cAMP levels was detectable 15 min after inoculation. Since this preceded PGT emergence, it may well be part of the control system.

Fungal morphogenesis is commonly affected by the external environment. This is true for the powdery mildew fungi, where much evidence shows that, in addition to environmental factors such as relative humidity and temperature, the substratum on which conidia are incubated can have a great influence on morphogenesis. Thus, the conidia of *Uncinula necator* (Schwein.) Burrill (Delp, 1954) and *B. graminis* (Carver and Ingerson, 1987; Manners and Hossain, 1963; Kobayashi et al., 1991) generally produce only multiple short germ tubes on glass slides, whereas they form a long appressorial germ tube on leaves of their host plants. Furthermore, *B. graminis* conidia generally produced only multiple short germ tubes when they were held in a stimulated airborne state on microthreads (Carver and Ingerson, 1987) or deposited on agar (Carver and Ingerson, 1987; Carver et al., 1995). By contrast, incubation on hydrophobic artificial substrata (silanized glass, perspex, or polystyrene) stimulated appressorial germ tube formation (Carver et al., 1995). This suggests that physical and/or chemical characteristics of substrata, including their hydrophobicity, strongly affect germination and differentiation processes. Hydrophobicity is now known to affect not only the development of appressoria, but also the release of conidial ECM. Carver et al. (1999) demonstrated that while *B. graminis* conidia released little or no ECM when deposited on clean glass, copious ECM was released on glass or plastic surfaces silanized to render them hydrophobic.

The present study tested the idea that the position and timing of germ tube emergence, particularly of the first-formed tube, relates to ECM release from conidia of *B. graminis.* This was done by comparing the time course of ECM release and emergence of these short germ tubes from conidia incubated on hydrophobic and hydrophilic artificial substrata.

Materials and Methods

Fungus *Blumeria graminis* (DC.) Speer f. sp. *hordei* Marchal race I was maintained on seedlings of barley (*Hordeum vulgaris* L. cv. Kobinkatagi) grown in a chamber illuminated at 34.8 W $m^{-2}sec^{-1}$ for 12 h/d at 22°C. One day before conidia were used for experiments, leaves bearing conidial chains were shaken to remove older conidia.

Test substrata were inoculated in a spore-settling tower by blowing young conidia from infected leaves directly into the tower with a carbon dioxide jet. Inoculum density was adjusted to approximately 100–120 conidia mm^{-2}. The substrata were supported on glass slides coated with a film of 2% water agar and surrounded on all sides by small pieces of wet filter paper to ensure high humidity at the substratum surface. The glass slides were transferred to a plastic incubation box in which they were held over 2% water agar so that conidia were incubated at 100% RH at 20°C in the dark.

Preparation of artificial substrata

Clean glass Pieces of glass microscope slides, 0.5 cm^2, were immersed in chromic acid (a saturated solution of potassium dichromate in conc. sulfuric acid) for 1 h, followed by five washes in deionized water and drying in a laminar air-flow chamber. Goniometer measurements of 0.5 ml water droplets placed on this clean glass showed a contact angle of 38°, indicating that the surface was relatively hydrophilic. These glass pieces provided the hydrophilic substratum used throughout.

Silanized plastic Plastic coverslips (Thermanox, Nunc Inc., Raperville, Ill., USA) cut into 0.5 cm^2 pieces were immersed for 5 min in dimethyldichloro-silane (DMS: a 2% solution in octamethylcyclotetra-siloxane; Repelcote VS, BDH, Poole, UK), followed by washing in absolute ethanol, rinsing three rinses in deionized water and then drying. Such silanized plastic pieces gave a water droplet contact angle of 80°, indicating that they were relatively hydrophobic. These provided the hydrophobic substratum used throughout.

Barley leaf cuticles with and without epicuticular waxes Primary leaves taken from 9-d barley seedlings were mounted onto glass microscope slides, abaxial surface up. To prepare isolated cuticle without epicuticular wax (wax-minus), the abaxial leaf surface was painted with a solution of cellulose acetate in acetone, the acetone was allowed to evaporate (ca. 5 min), and the dry acetate film was peeled away, removing the epicuticular waxes

(Carver and Thomas, 1990). Epidermal strips from leaves either with (wax-plus) or without epicuticular waxes (wax-minus) were peeled from the abaxial surfaces or floated on a solution of cellulase (Cellulase Onozuka R-10, Yakult Honsha Co. Ltd., Japan), abaxial surface up, at room temperature for 1 h to allow cell walls to be degraded. Floating epicuticular layers were washed with deionized water several times (10 min each) and mounted on small pieces of cellulose dialysis membrane (Sanko Junyaku Co. Japan), abaxial surface up, and then air-dried in a laminar flow chamber. Wax-plus and -minus cuticle layers gave a water droplet contact angle of 134° and 102°, respectively.

Observation and micromanipulation Throughout the experiments, observation and manipulation were restricted to conidia that settled so that their long axis lay parallel to the substratum.

In time course experiments, slides bearing small pieces of inoculated substratum were removed from the incubation box and observed with a Zeiss interference contrast microscope. Populations of conidia were examined to determine whether they had released ECM. Where conidial ECM had been released, it was clearly evident as a bright droplet at the conidium-substratum interface (Carver et al., 1999; Fig. 1). The same conidia were also examined to determine whether they had produced a PGT. Some conidia formed two or more short germ tubes. In these cases, careful focusing showed that generally only one of these germ tubes made substratum contact, and the apex of this tube was slightly swollen. This is characteristic of a PGT on a host surface (Carver et al., 1999), and such tubes were designated as PGTs in the current studies. Where no substratum contact was made, the short germ tube apex was not swollen. This is typical of "subsidiary germ tubes" (Carver et al., 1999; Kunoh, 1982), and these germ tubes were regarded as such for the current studies. In these experiments, 110–120 conidia were observed on each substratum in each of five replicates. Microscope observations were made at hourly intervals up to 4 h after inoculation starting immediately after inoculation (within 1 min). Each observation was completed within 5 min.

In an experiment to determine the relationship between conidial ECM release and location of germ tube emergence, clean glass, silanized plastic strips and wax-plus and -minus cuticular layers were inoculated with fresh conidia and incubated as above. The release of ECM and the location of germ tube emergence from individual conidia were recorded during 2–4 h, as appropriate (see Results).

To examine further the relationship between conidial ECM release and germ tube emergence on hydrophilic and hydrophobic substrata, conidia were deposited and pre-incubated on the hydrophilic substratum before they were transferred to a second substratum. Thus, they were pre-incubated on clean glass for 0–10, 11–20 or 21–30 min after inoculation and then transferred individually by micromanipulation to either the hydrophobic or the hydrophilic substratum. Following transfer, individual conidia were examined every 10 min to determine when and if conidial ECM was released and the characteristics of emerging germ tubes.

In transfer experiments, 5–10 conidia were transferred in each trial. Some conidia were collapsed by the end of the observation (within 60 min), perhaps because of mechanical damage by micromanipulation. These conidia were disregarded. Trials were repeated until at least 50 acceptable conidia were transferred for each treatment.

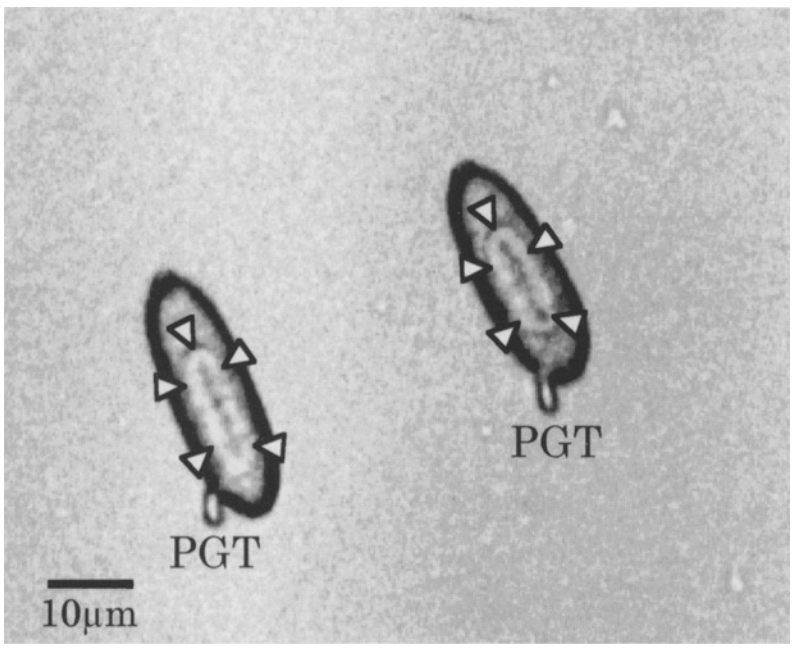

Fig. 1. ECM (arrowheads) released underneath germinated conidia with a PGT.

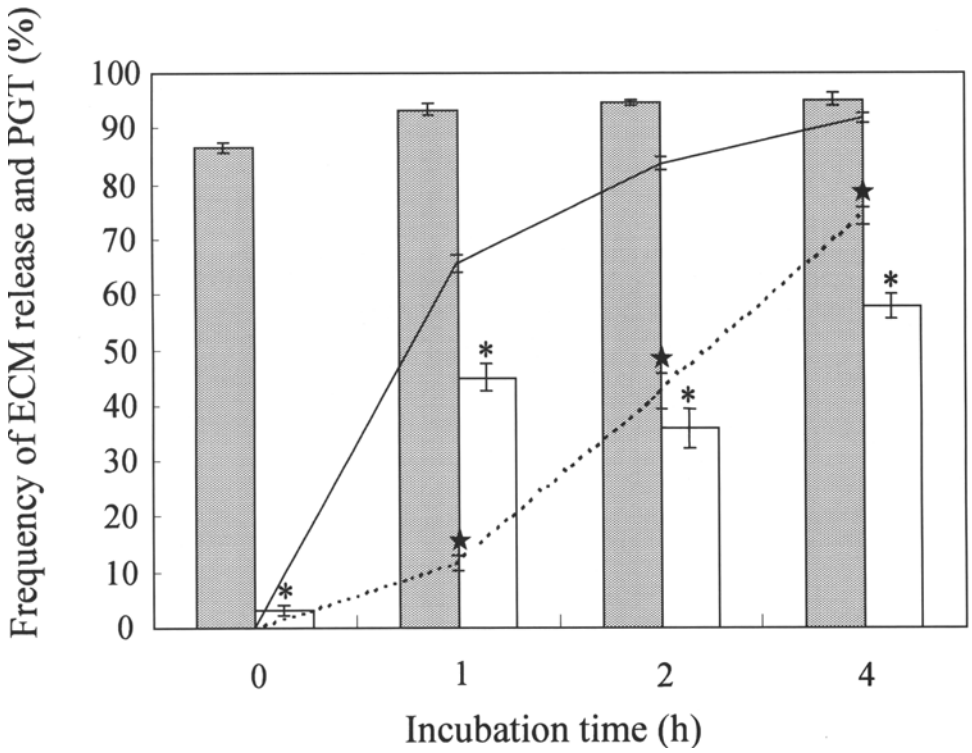

Fig. 2. Frequencies of ECM release and PGT emergence from conidia incubated on a hydrophobic and a hydrophilic substratum. ▒ ECM, hydrophobic, —— PGT, hydrophobic, □ ECM, hydrophilic, ····· PGT, hydrophilic.
Vertical lines represent standard errors.
Counts obtained within the same incubation time were compared by χ^2-test.
* and ★ over a value in the hydrophilic data set indicate significant difference ($P<0.05$) with equivalent datum in the hydrophobic data set.

In all experiments, contingency χ^2 analyses were applied to count data in order to assess the significance of treatment effects.

Results

Time course of ECM release and germination on hydrophobic and hydrophilic substrata When conidia were deposited with their long axis parallel to the substratum, conidial ECM, if present, was clearly visible by light microscopy as a bright ellipsoidal ring at the interface with the substratum (Fig. 1). On the hydrophobic substratum (Fig. 2), ECM was released from 87% of conidia immediately after inoculation (within 1 min), i.e., by the time the first microscope observations were made, and from more than 93% by 1 h. By contrast, on the hydro-

Table 1. Percentages* of conidia that released ECM at various times after transfer to either a hydrophobic or a hydrophilic substratum after initial pre-incubation for various periods on a hydrophilic substratum.

Conidia transferred to:	Pre-incubation period before transfer (min)	Total No. of conidia transferred	Incubation time after transfer (min)						
			0–1	10	20	30	40	50	60
Hydrophobic	0–10	52	75.0%	88.9	90.4	90.4	90.7	90.7	90.7
	11–20	69	66.7	76.8	81.2	87.0	88.4	88.4	88.4
	21–30	52	67.3	73.1	78.8	78.8	80.8	82.7	82.7
Hydrophilic	0–10	63	15.9***	44.4***	63.5***	73.0**	81.0^{NS}	82.5^{NS}	87.3^{NS}
	11–20	68	8.8***	31.0***	50.0***	51.5***	58.8***	63.2***	63.2***
	21–30	50	10.0***	22.0***	22.0***	38.0***	40.0***	50.0***	52.0***

*: % = (No. of conidia with ECM/Total No. of transferred conidia) × 100.
Counts obtained within the same pre-incubation/time after transfer combinations were compared between the hydrophobic and hydrophilic substrata by contingency χ^2-test: *** and ** after a value in the hydrophilic data set indicate significant difference ($P<0.001$ & $P<0.05$, respetively) with equivalent datum in hydrophobic data set. NS: No significant difference.

Table 2. Percentages* of conidia with a PGT at various times after transfer to either a hydrophobic or a hydrophilic substratum after initial pre-incubation for various periods on a hydrophilic substratum.

Conidia transferred to:	Pre-incubation period before transfer (min)	Total No. of conidia transferred	Incubation time after transfer (min)					
			10	20	30	40	50	60
Hydrophobic	0–10	52	0.0%	15.4	36.5	50.0	51.9	59.6
	11–20	69	1.4	14.5	26.1	37.7	42.0	52.2
	21–30	52	15.4	23.1	25.0	34.6	38.5	40.4
Hydrophilic	0–10	63	0.0^{NS}	1.6***	3.2***	11.1***	22.2***	28.6***
	11–20	68	0.0^{NS}	2.9***	11.8**	17.6***	22.1***	22.1***
	21–30	50	0.0***	0.0***	0.0***	0.0***	2.0***	6.0***

*: % = (No. of conidia with PGT/Total No. of transferred conidia) × 100.
Counts obtained within the same pre-incubation/time after transfer combinations were compared between the hydrophobic and hydrophilic substrata by contingency χ^2-test: *** and ** after a value in the hydrophilic data set indicate significant difference ($P<0.02$ & $P<0.05$, respetively) with equivalent datum in hydrophobic data set. NS: No significant difference.

philic substratum (Fig. 2) less than 3% of conidia released ECM within 1 min, and although this percentage increased subsequently, only 58% of conidia were associated with ECM by the final sample time (4 h). Thus, ECM was released from a far higher proportion of conidia, and its release was much more rapid, on the hydrophobic than hydrophilic substratum ($P<0.05$).

Figure 2 shows the timing and frequency of PGT formation on the different substrata. On the hydrophobic substratum, ca. 66% of conidia had formed a PGT by 1 h after inoculation and ca. 84% and ca. 92% had done so by 2 and 4 h, respectively. By contrast, on the hydrophilic substratum only ca. 12 % of conidia had formed a PGT by 1 h, and even by 4 h less ca. 74% had done so. Thus, PGTs were formed by a far higher proportion of conidia, and their formation was much more rapid, on the hydrophobic than the hydrophilic substratum ($P<0.05$).

In summary, the data from these experiments indicate a correlation between the occurrence and rapidity of conidial ECM release, the formation of PGTs and hydrophobicity of the substratum.

ECM release and PGT emergence *from* conidia transferred from a hydrophilic to a hydrophobic substratum

The results in Fig. 2 led us to assume that ECM release could result from recognition and response to substratum hydrophobicity almost immediately after conidial deposition. To test this idea further, conidia deposited and pre-incubated for various initial periods (Table 1) on the hydrophilic substratum were transferred by micro-manipulation to either the hydrophobic or the hydrophilic substratum for further incubation and subsequent observation. It proved possible to transfer conidia successfully for up to 30 min after their initial deposition on the hydrophilic substratum. If, however, conidia were pre-incubated for longer than this, they tended to collapse immediately after transfer regardless of whether transfer was to a hydrophobic or hydrophilic substratum, suggesting that extended incubation on the hydrophilic substratum could make conidia sensitive to mechanical shock. Care was taken to transfer only conidia that showed no sign of having released ECM during initial pre-incubation on the hydrophilic substratum. We also attempted to transfer conidia pre-incubated on the hydrophobic substratum, but this proved impossible because they could not be picked up with the micro-needle. This was probably because capillary forces, associated with liquid ECM release on the hydrophobic substratum, attached conidia to its surface.

Broadly, results of this transfer experiment supported the view, indicated by the previous experiment (Fig. 2), that contact with a hydrophobic substratum promoted the release of ECM. Table 1 shows that when conidia were transferred to a hydrophobic substratum within 10 min of initial deposition on the hydrophilic substratum, ECM was released from 75% of conidia within 1 min after transfer. A maximum of ca. 90% of conidia had released ECM by 10 min after transfer. Similar results were obtained when conidia were pre-incubated

Table 3. Categories of the order of ECM release and PGT emergence from conidia transferred from a hydrophilic substratum to a hydrophobic or a hydrophilic substratum.

Category	To hydrophobic			To hydrophilic		
	0–10*	11–20	21–30	0–10	11–20	21–30
① Transfer, ECM, PGT	30	32	16	18	9	3
② ECM, PGT	0	1	3	0	3	0
③ ECM, PGT	0	1	1	1	1	0
④ PGT	2	2	2	2	7	5
Total No. of germinated conidia	32	36	22	21	20	8

*: Incubation time before transfer (min).

Table 4. Average times (min) between ECM release or transfer and PGT emergence from a hydrophilic substratum to a hydrophobic or a hydrophilic substratum after different incubation times on an original hydrophilic substratum.

Events	To hydrophobic			To hydrophilic		
	0–10*	11–20	21–30	0–10	11–20	21–30
ECM-PGT	36.3	31.3	24.4	35.0	44.4	nd
Transfer-PGT	37.0	37.2	27.5	54.4	53.3	nd

*: Incubation time before transfer (min).
nd: not determined because of the small number of observed conidia.

for 11–20 or 21–30 min before transfer to the hydrophobic substratum. In both cases, nearly 67% of conidia released ECM within 1 min after transfer and this increased to ca. 80% or more within 20 min after transfer.

Conidia transferred to the hydrophilic substratum behaved quite differently. Irrespective of how long they were pre-incubated, very few (9–16%) released ECM immediately after transfer (Table 1). In all cases, the proportion that released ECM increased with time after transfer, but when transferred after more than 10 min of pre-incubation, the proportion that released ECM remained significantly ($P<0.001$) lower than that for equivalent conidia transferred to the hydrophobic substratum. Interestingly, when conidia were pre-incubated for 10 min or less, a relatively high proportion eventually released ECM. Thus, from 40 min after transfer more than 80% had released ECM, and this proportion was indistinguishable from the equivalent on the hydrophobic substratum.

Table 2 presents detailed data regarding PGT formation. About 15% of conidia that were pre-incubated for less than 20 min formed a PGT within 20 min of transfer to the hydrophobic substratum. Thereafter, the percentages increased gradually, and by 60 min after transfer, 52–60% of conidia had formed a PGT. When pre-incubated for more than 20 min, about 15% of conidia

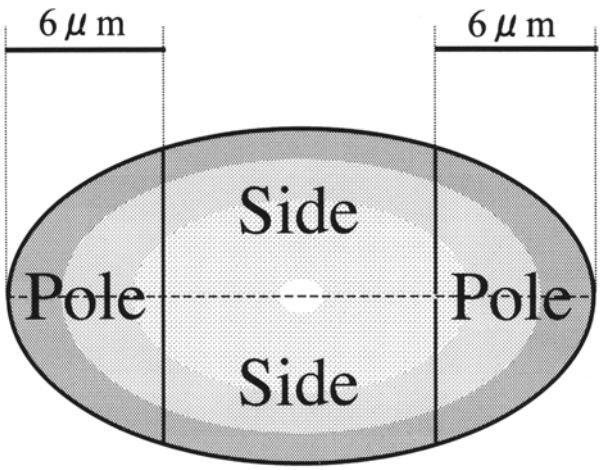

Fig. 3. Region of PGT emergence from a conidium.

Table 5. ECM released from conidia and percentages of PGTs emerged from polar region* on a hydrophobic or a hydrophilic substratum.

Substratum	ECM observable			ECM unobservable		
	A	B	C	A	B	C
Hydrophobic	423	354	61.6	29	11	36.4
Hydrophilic	147	93	33.3	183	110	35.5

*: Refer to Fig. 3.
A: Total No. of observed conidia.
B: Total No. of germinated conidia.
C: % of conidia with a polar PGT=No. of conidia with a polar PGT/B×100.

formed a PGT within 10 min of transfer but, ultimately, somewhat fewer formed a PGT than when pre-incubation periods were shorter. Nevertheless, 40% had formed a recognizable PGT by 60 min. By contrast, very low percentages of conidia transferred to the hydrophilic substratum produced a PGT. Even when transferred after only 10 min of pre-incubation, only about 29% of conidia ultimately produced a PGT, and when pre-incubated for longer than 20 min, PGT formation was extremely infrequent. Thus, in agreement with the previous experiment (Fig. 2), these data suggest that contact with a hydrophobic substratum promoted PGT formation.

Individual, sequentially observed conidia from these transfer experiments were categorized in terms of the time at which they released ECM and formed a PGT (Table 3). Regardless of the length of time that conidia were pre-incubated on the hydrophilic substratum, after transfer to the hydrophobic substratum most conidia released ECM before they produced a PGT (category 1 in Table 3). On the hydrophobic substratum, PGT formation before, or simultaneously with, ECM release (categories 2 and 3) and PGT formation without ECM release (category 4) occurred only rarely. Of conidia that formed a PGT after transfer to the hydrophilic substratum, category 1 was again most common when pre-incubation was for 10 min or less. However, when pre-incubated for a longer time, category 4 occurred more frequently.

The average times between ECM release and PGT emergence and those between transfer and PGT emer-

Table 6. Percentage of PGT emergence from a polar region* of transferred conidia.

Transfer direction to:	Time before transfer (min)		
	0–10	11–20	21–30
Hydrophobic	90.6** (32)	63.9 (36)	50.0 (22)
Hydrophilic	45.0 (21)	30.0 (20)	nd

*: Refer to Fig. 3.
**: %=No. of conidia with a polar PGT/Total No. of transferred conidia with PGT×100.
( ): Total No. of transferred conidia with PGT
nd: not determined because of conidia crush

gence were calculated for individual conidia in category 1 of Table 3. As indicated in Table 4, in cases of transfer to the hydrophobic substratum, the time between ECM release and PGT emergence varied slightly according to pre-incubation treatment, but in all cases the mean times lay in the range 24.4–36.3 min. In these cases, the mean times between conidial transfer and PGT emergence covered a very similar range, i.e., 27.5–37.2 min. This similarity followed from the fact that, for many conidia, ECM release occurred very soon after their transfer to the hydrophobic substratum (Table 1). By contrast, when conidia were transferred to the hydrophilic substratum, the times between ECM release and PGT formation were longer, 35.0 and 44.4 min when conidia were incubated on an initial hydrophilic substratum for less and longer than 10 min, respectively (Table 4). In addition, the times between transfer and PGT emergence were also longer, 54.4 and 53.3 min, respectively in both cases. Again, these data were consistent with the finding (Table 1) that conidial ECM release was delayed following transfer to the hydrophilic substratum, particularly when pre-incubation was for more than 10 min. Since only a few transferred conidia germinated on a hydrophilic substratum (see Table 2), these times could not be determined when the pre-incubation time was beyond 20 min.

Location of PGT emergence from conidia inoculated on various substrata *Blumeria graminis* conidia are ellipsoidal bodies that are described as prolate spheroids (symmetrical egg-shaped bodies). As indicated in Fig. 3, the site of PGT emergence from a conidium could be classified as either from a 'pole' or a 'side' region of the spore. For our purpose we define a pole region as being within 6 μm from either end of the long axis of the ellipsoidal conidium. Thus, we classified the location of PGT emergence as polar or in the intermediate side region (Fig. 3). Again, observations were restricted to conidia deposited with their long axis lying parallel to the substratum surface.

Table 5 shows percentages of emergence of a polar PGT from ECM-released and -nonreleased conidia on a hydrophobic and a hydrophilic substratum. On the hydrophobic substratum, about 62% of conidia that released ECM prior to PGT formation produced PGT from the polar region. Conversely, only ca. 36% of conidia that failed to release ECM on this hydrophilic substratum produced a polar PGT. However, on the hydrophilic substratum, only *ca.* 33–36% of conidia produced the PGT from the polar region, regardless of whether they had released ECM. As reported by Carver et al. (1999), release of ECM was hardly ever observed on isolated barley cuticles. Neglecting ECM release from conidia, about 63% and 57% of PGTs emerged from a polar region, respectively, on wax-plus and wax-minus cuticle surfaces. Contingency χ^2 analysis showed that these values were not significantly different ($P>0.05$), but confirmed that regardless of the presence or absence of epicuticular wax, the majority of PGTs emerged from a polar region of conidia on isolated host leaf cuticles. These results demonstrated that the location of PGT emergence on a hydrophobic substratum was similar to that on the surface of host origin only when the conidia had released ECM.

In a subsequent experiment, conidia pre-incubated on the hydrophilic substratum were individually transferred onto either the hydrophobic or the hydrophilic substratum 0–10, 11–20 or 21–30 min after inoculation (Table 6). The transferred conidia were incubated for an additional 60 min before the site of PGT emergence was determined. For conidia transferred to the hydrophobic substratum, the majority (ca. 91%) produced a polar PGT if transfer was done within 10 min after inoculation, and *ca.* 64% did so if transfer was done within 11–20 min. However, if incubated for longer than 20 min, equal numbers emerged from pole and side regions. By contrast, for conidia transferred to the hydrophilic substratum, 55–70% of PGTs emerged from side regions irrespective of the length of the pre-incubation period. Thus, these transfer experiments again suggested that hydrophobicity of the surface stimulated polar PGT emergence, but that extended pre-incubation on the hydrophilic substratum negated this effect and led to an increase in frequency of emergence from the side region.

Discussion

The present results confirmed the earlier report (Carver et al., 1999) that ECM release from *B. graminis* conidia occurred more promptly and frequently after contact with a hydrophobic than a hydrophilic artificial substratum. The transfer experiment (Table 3) provided additional proof that release of ECM was specifically stimulated by contact with a hydrophobic substratum and showed that conidia retain the ability to release ECM for some time after their deposition onto a non-stimulatory hydrophilic substratum.

The function(s) of *B. graminis* conidial ECM are a matter of ongoing research. Extracellular materials are released by spores of many fungi that attack plants and animals, and have biotrophic, hemibiotrophic or necrotrophic habits. It has been suggested that the release of such materials is involved with spore adhesion (e.g., Epstein and Nicholson, 1997; Nicholson and Epstein, 1991). It was demonstrated that conidial ECM possesses non-specific esterase activity (Nicholson et al., 1998) and contains cutinase (Pascholati et al., 1992) and cellulases and pectinases (Suzuki et al., 1998, 1999). Furthermore, Deising et al. (1992) demonstrated that the activity of non-specific esterases was necessary for the function of adhesion pads formed by urediniospores of *Uromyces viciae-fabae*. Such activity may be important as a factor conferring adhesive properties to conidial ECM of *B. graminis*. However, it is difficult with certainty to ascribe such a role to ECM release by *B. graminis* conidia, because release has proved extremely difficult to detect on leaf surfaces (Carver et al., 1995, 1999). Our current observations confirmed this finding.

A relatively recent suggestion is that ECM may be involved in rapidly sensing the site of conidial contact with the host surface (Nielsen et al., 2000). This in turn may

influence the site of germ tube emergence from the conidium so as to maximize the likelihood of germ tubes making contact with the host (Carver et al., 1999). Carver et al. (1999) suggested that small quantities of ECM might be released from the tips of the characteristic surface projections that are distributed over the wall of *B. graminis* conidia (Kunoh and Akai, 1967; Akai et al., 1968). Carver et al. (1999) showed that the only contact between a conidium and leaf surface is via the tips of a limited number of these projections, and proposed that light and scanning electron microscopy may not be able to resolve limited quantities of ECM produced at these sites. They speculated that a feedback mechanism from the host prevented the wasteful over-production of ECM, and that this may fail on hydrophobic artificial substrata where, as in the current experiments, large quantities of ECM are released to form a visible droplet at the conidium-substratum interface. Nielsen et al. (2000) demonstrated hydrolytic enzyme activity present within ECM released at the substratum interface and also showed that recognition of the substratum by conidia can stimulate uptake of anionic, low-molecular-weight materials before germination. They argued that hydrolysis of host surface components to release chemicals for uptake by the conidium, and facilitated transport of external chemicals into the conidium, could be involved in recognition of the host and determining the site of germ tube emergence.

The current experiments support the view that, on hydrophobic artificial substrata, ECM release may influence the site of PGT emergence. On the hydrophobic substratum, ECM was most often released prior to germ tube emergence, and where this happened PGTs generally emerged, around 30 min later, from a polar region of the conidium. This was similar to the emergence behavior on isolated host cuticles. By contrast, where conidia failed to release ECM, germ tubes emerged from a side region, and this was not normal for conidia on host tissues. On the hydrophilic substratum, ECM release was relatively uncommon and/or delayed. Here again, PGTs generally emerged from a side region, suggesting that failure of ECM release was associated with such 'abnormal' PGT emergence. Interestingly, the act of ECM release did not appear to be sufficient in itself to drive polar emergence. Thus, even where ECM was released on the hydrophilic substratum, most PGTs emerged from a side region. This suggests that although contact with a hydrophobic substratum stimulates ECM release, polar emergence is promoted by later response(s) that are also stimulated by hydrophobicity of the substratum.

Obviously, any process that influences the site of germ tube emergence from a conidium must be activated well in advance of that emergence. The present data show that ECM release is a suitable candidate. On the hydrophobic substratum, where PGTs were formed relatively rapidly and generally emerged from a polar region, ECM was generally released within 1 min of contact and around 30 min before the germ tube emerged. The data are therefore fully compatible with the idea that ECM release is involved in perception of substratum features that determine the site of germ tube emergence.

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