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# The influence of IgG, proteases and glycosidases on adhesion to, and infection of soybean plants by *Phytophthora megasperma* f.sp. *glycinea*

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**Abstract**

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Adhesion of *Phytophthora megasperma* f.sp. *glycinea* to plastic tissue culture plates is inhibited by cations and surfactants implicating the involvement of electrostatic and hydrophobic interactions. Adhesion to plastic and to the soybean host surface is completely inhibited by proteases and partially by some glucanases and glycosidases. In the presence of these enzymes no appressoria develop and no penetration of the intact, hydrophobic cuticular host surface is observed. These enzymes appear to specifically affect adhesion by removing glycoproteins acting as adhesins, since they do not substantially inhibit germination and growth of the pathogen. IgG, ConA and D-mannose which inhibit adhesion to – hydrophilic – cell walls and infection through open (non-cutinized) wounds only partially inhibit adhesion to hydrophobic surfaces and did not prevent infection of the hypocotyls through the cuticle (D-mannose was not tested). It is concluded that adhesion is a prerequisite for infection, and that adhesion to *hydrophilic* and *hydrophobic* host surfaces is mediated by different surface molecules (glycoproteins) or parts thereof.

## Introduction

The questions how fungal pathogens adhere to their hosts, and whether or not adhesion is essential for fungus-host plant interactions has been a topic of rising interest in plant pathology (Nicholson and Epstein 1991, Hardham 1992, Gareth Jones 1994). Most of these investigations concerned adhesion to the cuticle, i.e., a *hydrophobic* surface. In previous papers (e.g. Hohl and Balsiger 1986a, Hohl 1991, Guggenbühl and Hohl 1992, Ding et al. 1994) we have shown that proteases, D-mannose and IgG-molecules inhibit adhesion of the fungus *Phytophthora megasperma* f.sp. *glycinea* to *hydrophilic* surfaces of the host (cell walls) and that 65 kDa glycoproteins present on host and pathogen surfaces probably act as adhesins in this process. Remarkably, inhibition of adhesion in turn prevented infection of the host through open wounds. In the present paper we examined the influence of salts, detergents, lectins and sugars on adhesion, and of proteases, glycosidases, the lectins ConA and WGA, and IgG molecules on adhesion to hydrophilic and hydrophobic surfaces, and on infection through the cuticle. The aim of the study was to determine whether or not adhesion to hydrophilic and hydrophobic surfaces varies, to gain more information on the adhesive forces and molecules involved, and on the importance of adhesion for infection.

## Materials and Methods

1. *Host plants.* – Soybean [Glycine max (L.) Merr.] seeds, cv. Harosoy (susceptible to *Phytophthora megasperma* f.sp. *glycinea* race 1), were obtained from Dr. R. I. Buzzell (Agriculture Research Station, Harrow, Ontario NOR IGO, Canada) and grown in vermiculite moistened with tap water. The temperature was 20 °C for the 8 h dark period and 25 °C for the 16 h light period (400 $\mu E \cdot m^{-2} \cdot s^{-1}$, 30 klx). Relative humidity was maintained at 80% (Bhattacharyya and Ward 1986).

2. *Pathogen.* – *Phytophthora megasperma* f.sp. *glycinea* Kuan and Erwin, race 1, was used and maintained on Borlotti bean agar. To obtain the latter 30 g of Borlotti beans were autoclaved for 8 min in 500 ml of distilled water and then filtered through nylon cloth. 15 g of Difco agar were added to the filtrate which was filled up with distilled water to make 1 L and reautoclaved (Ward et al. 1979, Hohl and Balsiger 1986 a).

Zoospores and cysts were produced according to Eye et al. (1978). Mycelial disks (5 mm diameter) were transferred to Difco lima bean agar. After 6 d of growth at 24 °C the plates were flooded six times at 30 min intervals with sterile distilled water to induce the formation of sporangia and zoospores, then incubated in darkness at 24 °C for 16 h. The supernatant was collected and cysts were obtained by shaking the zoospore suspension for 1 min on a Vortex mixer.

3. *Inoculation of plants.* – Etiolated plants grown for 5 d in the dark were placed horizontally in plastic boxes, and the roots were covered with moistened cellucotton. Drops of inoculum ($5 \cdot 10^4$ cysts $\cdot$ $ml^{-1}$ of distilled water or test solutions) were placed on the upper third of each hyopcotyl. The boxes were sealed with plastic film and incubated in the dark at 22 °C (Odermatt et al. 1988).

4. *Mechanical isolation of soybean mesophyll cells.* – The method of Schwenk (1981) was modified as follows. Primary leaves from 14 day old plants were collected, the midrib was removed and the remainder minced into fine slices with a razor blade. The minced tissue was suspended in 15 ml sterile distilled water in an Erlenmeyer flask. The slices were vortexed for 1 min at top speed. The suspension was filtered through a 30 µm nylon mesh to separate the isolated cells from the remaining tissue. The latter was resuspended in 10 ml of sterile distilled water, revortexed for 1 min and again filtered. This process was repeated twice to give a total of 45 ml of cell suspension.

The suspension was then centrifuged for 1 min at 120 *g*, the supernatant discarded and the pellet resuspended in 10 ml of sterile distilled water, and recentrifuged. Cells were thus washed three times. The final pellet was resuspended with 1 ml of sterile distilled water and the number of cells determined with a Neubauer chamber. Fluorescein diacetate was used to determine cell viability (Widholm 1972) which was around 70%. Yields ranged from 1.5 to 2.5 million cells $\cdot$ $g^{-1}$ fresh weight (equaling about 6 leaves).

5. *Adhesion of germinating cysts to plastic and glass.* – Cysts ($5 \cdot 10^5$ $ml^{-1}$) were germinated for 2 h at 23 °C in sterile distilled water. Average germination rate was 65–75%, average germ tube length about 12 µm. Their concentration was adjusted to $2 \cdot 10^4$ $ml^{-1}$. Of this suspension 0.2 ml was spread evenly over an area of 1.2 $cm^2$ of the test surface (see below) and incubated up to 4 h at 24 °C. Non-adhering germlings were then removed by rinsing the surfaces carefully three times with distilled water. To determine the number of adhering germlings, the surface was divided into 8 sectors of which 4 were randomly sampled using a Nikon inverted microscope.

Test material: *Polystyrene* was used in form of polystyrene petri dishes 3 cm in diameter, *coated polystyrene* in form of 24-hole tissue culture plates coated with collagen/cellulose (hydrophilic). *Glass* was used as glass coverslips 12 mm in diameter. Prior to use the coverslips were immersed for 1 h in ethanol/ether 1:1, then cooked for 1 h, immersed for 1 h in 25% sulfuric acid, and finally washed with distilled water to neutrality. The air-dried coverslips were kept in 96% ethanol and oven-sterilized at 160 °C before use.

6. *Inhibition of pathogen adhesion by enzymes.* – To study the effect of enzymes on fungal adhesion to plastic and glass, the germinating cysts were suspended in the enzyme solutions to give a final concentration of $2 \cdot 10^4$ $ml^{-1}$. This suspension was then used for the adhesion test as described above (section 5). For concentrations of enzymes used, see Table 4.

7. *Lectins.* – The following lectins were obtained from SIGMA and used in the adhesion experiments (all at 1 mg · $ml^{-1}$): Concanavalin A (ConA), *Lens culinaris* agglutinin (LCA), *Pisum sativum* agglutinin (PSA), *Ricinus communis* toxin (RCA I), *Arachis hypogaea* (peanut) agglutinin (PNA), *Glycine max* (soybean) agglutinin (SBA), *Phaseolus vulgaris* agglutinin (PHA V, eryhtroagglutinin), *Triticum vulgaris* (wheat germ) agglutinin (WGA), *Tetragonolobus purpureus* (asparagus pea) agglutinin (TPA) and *Ulex europaeus* agglutinin (UEA I). For the adhesion test (section 5) the cysts were suspended in lectin solutions instead of distilled water.

8. *Metabolic inhibitors.* – Cycloheximide, actinomycin D, tunicamycin, and cytochalsin B were purchased from SIGMA. The compounds were used as aqueous solutions with the exception of tunicamycin which was dissolved in 10% ethanol. For determining the effect of inhibitors on adhesion, inhibitor solutions instead of distilled water were used in the adhesion test (section 5).

9. *Adhesion of mesophyll cells to fungal mycelium* (Hohl and Balsiger 1986a, Guggenbühl and Hohl 1992, Ding et al. 1994). – $7 \cdot 10^3$ cysts suspended in 0.1 ml of 20% pea broth were placed into each well of FALCON 3072 96-well (Becton Dickinson Labware, New Jersey, USA), flat bottom tissue culture plates. The fungal cultures were incubated overnight (16 h) at room temperature. During this period a regular network of hyphae had developed which was rinsed twice with sterile distilled water before adding the cells.

$2.5 \times 10^4$ soybean cells (see section 4) suspended in 50 µl of distilled water were pipetted into the wells containing the hyphal network. The plates were incubated at room temperature (22–23 °C) without shaking and the adhesion determined after various time intervals. To measure adhesion, the plates were washed three times with sterile distilled water. This washing procedure had to be standardized and was critical for reproducibility of the test. The wells were kept moist by the addition of sterile distilled water until the number of adhering cells was determined.

The number of cells attached to the mycelium was determined enzymatically by measuring the activity of α-mannosidase. The solution in the wells was replaced with 50 µl of *p*-nitro-phenyl-α-D-mannopyranoside (2 mg · $ml^{-1}$ in 0.2 M sodium acetate buffer of pH 5.0), followed by an incubation at 37 °C for 3 h, on a rotary shaker at 50 rpm. The reaction was stopped with 150 µl of 0.2 M sodium carbonate and the extinction measured at 410 nm with a MR 250 Microplate Reader (Dynatech Laboratories, CH-8423 Embrach-Embraport, Switzerland). The values obtained were subtracted from the control well with only the hyphal network incubated with the test solution. A standard curve of cell concentration/enzyme activity was used to transform enzyme activities into cell numbers, if this was desired.

10. *Enzymes and enzyme inhibitors.* – The following enzymes (and inhibitors) were used: lipase in phosphate buffer pH 7.1, lysozyme in phosphate buffer pH 6.3, α-glucosidase in phosphate buffer pH 6.8, β-glucosidase in phosphate buffer 5.0, α-mannosidase in phosphate buffer 5.0, β-mannosidase in phosphate buffer 5.0, β-galactosidase in phosphate buffer 7.3, β-glucuronidase in phosphate buffer pH 6.8, trypsin in Tris-HCl buffer pH 7.6 (trypsin inhibitor in Tris-HCl buffer pH 7.5), proteinase K in Tris-HCl buffer pH 7.5, and protease E in Tris-HCl buffer pH 7.5 (protease inhibitor in Tris-HCl buffer pH 7.6). All enzymes were purchased from SIGMA. Enzyme concentrations used are given in Table 4.

11. *IgG.* – Rat IgG was purchased from SIGMA, rabbit IgG from Bio-Science. Both were diluted 1:100 with 0.01 M PBS.

12. *SEM.* – 2 mm sections of inoculated soybean hypocotyls were fixed for 24 h at room temperature in 70% acetone containing 1% glutaraldehyde, then critical-point dried, shadowed with gold/palladium, and viewed in a Hitachi model S-4000 field emission scanning electron microscope.

## Results

### *1. Adhesion to plastic (tissue culture plates) and glass surfaces*

### *1.1 Adhesion kinetics*

As is shown in Fig. 1, adhesion kinetics for the three different surfaces were almost identical, indicating that *Phytophthora megasperma* f.sp. *glycinea* adheres equally well to hydrophobic and hydrophilic surfaces. Hyphal tips remained cylindrical on all three surfaces, i.e. hardly ever broadend to form an appressoria-like structure.

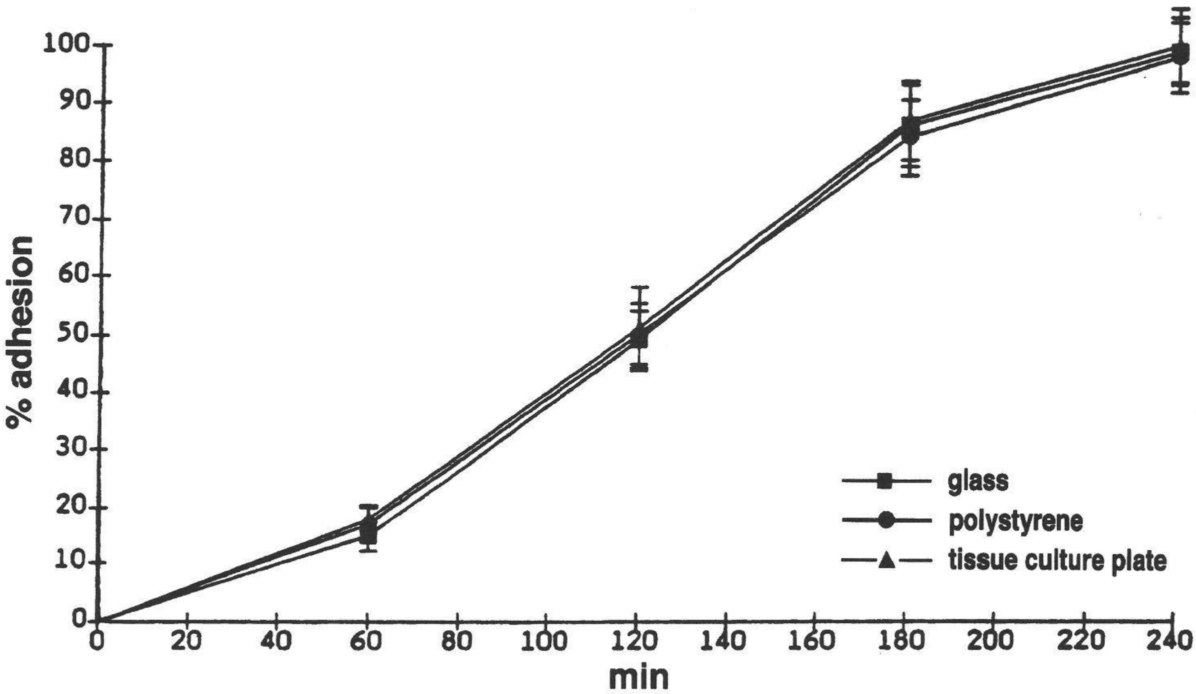


Fig. 1. Adhesion of *Phytophthora megasperma* f.sp. *glycinea* to glass, polystyrene and tissue culture plates. Bars represent standard error of the mean ($N = 9$).

### *1.2 Influence of pH*

To assess the influence of pH on adhesion to tissue culture plates, the following four buffer systems with overlapping range were used: 0.1 M citric acid/phosphate buffer (pH range 2.5–8.0), 0.2 M acetate buffer (pH range 4.0–5.5), 0.07 M phosphate buffer (pH range 5.0–7.5), and 0.2 M Tris-HCl-buffer (pH range 7.5–9.0).

Combining the data from the different buffers the following results were obtained (– no adhesion, +/– 1–10% adhesion, + 11–40% adhesion, + + 41–70% adhesion, + + + 71–100% adhesion):

| pH | adhesion |
|---|---|
| 2.5 | – |
| 3.0 | – |
| 4.0 | – |
| 4.5 | +/– |
| 5.0 | + |
| 5.5 | + + |
| 6.0 | + + |
| 6.5 | + + + |
| 7.0 | + + + |
| 7.5 | + + + |
| 8.0 | + + |
| 8.5 | + + |
| 9.0 | – |

Thus, the lower limit for adhesion was around pH 4.5, optimal pH from 6.5–7.5, maximal pH between 8.5–9.0.

### *1.3 Influence of salts and some other compounds*

The results are summarized in Table 1. Tween 20 at 0.001 M, SDS, Triton X100, LiCl, saponine, EDTA, EGTA, and acetic acid at 0.01 M inhibited adhesion of *Phytophthora megasperma* f.sp. *glycinea* to coated polystyrene (tissue culture plates). Salts had little effect. Even at 0.1 M only NaCl and LiCl reduced adhesion to + (not shown).

Table 1. Influence of some compounds on adhesion of *Phytophthora megasperma* f.sp. *glycinea* to plastic tissue culture plates.

| Compound | 100 mM | 10 mM | 1 mM | 0.1 mM |
|---|---|---|---|---|
| Water | + + + [1] | + + + | + + + | + + + |
| SDS [2] | – | – | + | + + |
| Tween20 | – | – | – | + |
| Triton X100 | – | – | + | + + |
| NaCl | + | + + | + + | + + + |
| LiCl | – | – | + | + + |
| $MgCl_2$ | + | + + | + + | + + + |
| $CaCl_2$ | + + | + + | + + | + + + |
| Urea [3] | + | + | + + | + + + |
| EDTA | – | – | + | + + |
| NaOH | – | + | + + | + + + |
| Acetic acid | – | – | + | + + |
| Ethanol | + + | + + | + + + | + + + |

[1] – no adhesion, +/– 1–10%, + 11–40%, + + 41–70%, + + + 71–100% adhesion
[2] sodium dodecyl sulfate
[3] 600 mM, 60 mM, 6 mM, 0.6 mM, respectively.

### 1.4 *Influence of sugars*

The following sugars were tested (at 0.1 M, all from Fluka Comp., Switzerland): L-arabinose, cellobiose, N,N′-diacetyl-D-chitobiose, D-fucose, D-galactose, N-Acetyl-D-galactosamine, gentobiose, D-glucose, N-Acetyl-D-glucosamine, 2-deoxy-D-glucose, methyl-α-D-glucopyranoside, D-lactose, D-maltose, D-mannose, L-mannose, methyl-α-D-mannopyranoside, D-sucrose. None of these inhibited adhesion to tissue culture plates markedly.

### 1.5 *Influence of lectins*

Of a number of lectins tested (ASP, UEA, PPA, RCA-I, PNAS, SBA, WGA, PAA, STA, ConA, LCA, PSA, PHA-E, and PHA-L, all at 1 mg · ml$^{-1}$), none inhibited adhesion of germlings of *Phytophthora megasperma* f.sp. *glycinea* to tissue culture plates completely, except for RCA-I which killed the germlings (Table 2). Some (UEA, SBA, PAA, PHA-E) greatly reduced adhesion which, however, in the cases of SBA and PAA might be due to a corresponding inhibition of growth.

### 1.6 *Influence of inhibitors*

The results are shown in Table 3. Cycloheximide at 10 µg · ml$^{-1}$, actinomycin D at 100 µg · ml$^{-1}$ and tunicamycin at 100 µg · ml$^{-1}$ but not cytochalasin B inhibited adhesion of *Phytophthora megasperma* f.sp. *glycinea* to coated polystyrene. None of the compounds severely slowed growth of the germ tubes.

### 1.7 *Influence of enzymes*

The effect of enzymes on adhesion to tissue culture plates and soybean cells was determined as outlined in the material and methods section. The result are summarized in Table 4 and will be discussed in more detail in the next section together with the results on infection. The most prominent results are: chymotrypsin, trypsin, proteinase K, and protease E inhibited adhesion to polystyrene and coated polystyrene (results for polystyrene not shown). Partial inhibition was caused by a series of glycosidases and other carbohydrate degrading enzymes.

To check the regeneration capacity for adhesion, germlings were pretreated for 2 h with trypsin and protease E, then washed free of the enzyme, maintained in suspension for 4 h and then plated onto tissue culturee plates and checked for adhesion. In both instances adhesion was restored partially, reaching from 10–40% of controls not treated with enzymes.

## 2. *Influence of sugars, enzymes and IgG on adhesion to and infection of host cells and tissues*

Of the sugars tested only D-mannose but not L-mannose reduced adhesion of *Phytophthora megasperma* f.sp. *glycinea* to isolated soybean mesophyll cells substantially (to 41 ± 2.7%). Minor effects were observed with fucose (77 ± 2.6%) and N-acetyl-galactosamine (77 ± 2.1%). This confirms results published before (Guggenbühl and Hohl 1992).

ConA and WGA even at 500 µg · ml$^{-1}$ did not inhibit adhesion to the cuticle and delayed infection by one day only.

Table 2. Influence of lectins on adhesion to tissue culture plates, and on growth of *Phytophthora megasperma* f.sp. *glycinea*.

| Lectin [1] | Sugar ligand [2] | Adhesion [3] | Growth [4] |
|---|---|---|---|
| Control (water) | | +++ | +++ |
| ASP | α-L-fucose | ++ | ++ |
| UEA | α-L-fucose | + | ++ |
| PPA | α-D-galactose | ++ | ++ |
| PNA | β-D-gal(1–3)-D-galNAc | ++ | ++ |
| SBA | D-galNAc | + | + |
| WGA | $(D\text{-glcNAc})_2$ | ++ | ++ |
| PAA | $(D\text{-glcNAc})_3$ | + | + |
| STA | $(D\text{-glcNAc})_3$ | +++ | ++ |
| ConA | α-D-man, α-D-glc | ++ | ++ |
| LCA | α-D-man | ++ | ++ |
| PSA | α-D-man | ++ | ++ |
| PHA-E | Oligosaccharide | + | ++ |
| PHA-L | Oligosaccharide | ++ | ++ |

[1] $mg \cdot ml^{-1}$
[2] gal = galactose, glc = glucose, man = mannose, NAc = N-acetyl... amine
[3] no adhesion, +/− 1–10%, + 11–40%, ++ 41–70%, +++ 71–100% adhesion
[4] determined after 6 h.

Table 3. Influence of cycloheximide, actinomycin D, tunicamycin and cytochalasin B on adhesion of *Phytophthora megasperma* f.sp. *glycinea* (*P. meg.*) to tissue culture plates. For comparison, the data for *P. infestans* (*P. inf.*) from Hollenstein (1993) are also provided.

| Inhibitor | Conc. ($\mu g \cdot ml^{-1}$) | Adhesion *P. inf.* | Adhesion *P. meg.* | Growth both sp. |
|---|---|---|---|---|
| Control | | +++ | +++ | +++ |
| Cycloheximide | 100 | +++ | − | ++ |
| | 10 | +++ | − | +++ |
| Actinomycin D | 100 | +++ | +/− | ++ |
| | 10 | +++ | + | ++ |
| Tunicamycin [1] | 100 | ++ | +/− | ++ |
| | 10 | +++ | + | ++ |
| Cytochalasin B | 100 | +++ | +++ | +++ |
| | 10 | +++ | +++ | +++ |

[1] dissolved in 10% ethanol which itself reduces adhesion from +++ to ++.

Table 4 summarizes the influence of enzymes and IgG on adhesion and infection. The most striking result was obtained with several proteolytic enzymes which completely prevented adhesion and infection of the pathogen. Protease inhibitors strongly reduced the inhibitory action of these enzymes. Heat-inactivated proteases also did not show the inhibitory activity of the active enzymes, indicating specificity of the enzyme reaction.

Table 4. Influence of enzymes and IgG on adhesion, and on infection of soybean hypocotyls by *Phytophthora megasperma* f.sp. *glycinea*.

| Compound | Germ.[1] | Growth[2] | Adhesion to | | | Infection (day) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Poly-styrene | Soybean cells | Cuticle | 1 | 2 | 3 |
| Control ($H_2O$) | 100 | 100 | + + +[3] | + + + | + + + | +[4] | + + | + + + |
| Lipase | 73 | 90 | + + | + + + | + + + | + | + + | + + + |
| β-glucuronidase[5] | 78 | 90 | + + + | + + + | + + + | + | + + | + + + |
| Chymotrypsin | 84 | 88 | – | – | – | – | – | – |
| Trypsin | 87 | 95 | – | – | – | – | – | – |
| Trypsin-inhibitor | 72 | 90 | + | + + + | + + + | – | + | + + |
| Trypsin & inhibitor | 78 | 90 | + + | + + + | + + + | – | + | + + |
| Proteinase K | 83 | 93 | – | – | – | – | – | – |
| Protease E | 88 | 98 | – | – | – | – | – | – |
| Protease E-inhibitor | 75 | 88 | + | + + + | + + + | – | + | + + |
| Protease E & inhib. | 77 | 90 | + + | + + + | + + + | – | + | + + |
| Lysozyme | 68 | 88 | + | + + | + + + | – | + | + + |
| α-glucosidase | 78 | 88 | + | n. t. | + + + | – | – | + |
| β-glucosidase | 80 | 90 | + | + + | + + + | – | – | + |
| α-mannosidase | 42 | 86 | +/– | + | + + + | – | – | + |
| β-mannosidase | 41 | 88 | + | n. t. | + + + | – | – | + |
| β-galactosidase | 68 | 88 | + | + + | + + + | – | – | + |
| Rat-IgG | 74 | 90 | + | + | + + + | – | + | + + |
| Rabbit-IgG | 77 | 88 | + | + | + + + | – | + | + + |

1 Germination rate of cysts in % of control
2 Growth of germlings in μm after 4 h, shown as % of control (42 μm). Standard deviation of the mean in all cases was between +/– 1.6–3.1
3 + + + 71–100%, + + 41–70%, + 11–40%, – no adhesion
4 + brown spot at site of inoculation, + + dark brown necrotic spots, + + + hypocotyls necrotized, tissue macerated
5 Enzymes: 5 U $ml^{-1}$ for adhesion to polystyrene, 20 U $ml^{-1}$ for adhesion to soybean cells, 2.5 U $ml^{-1}$ for adhesion to cuticle assay; IgG diluted 1:100 with 0.01 M PBS
n. t. not tested.

Some other enzymes tested (lysozyme, lipase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase, β-mannosidase) did not inhibit adhesion to the cuticle but partially inhibited adhesion to polystyrene, adhesion to soybean cells and/or polystyrene, and also fungal cyst germination. IgG reduced adhesion of *P. megasperma* f.sp. *glycinea* to polystyrene and soybean cells but not to the cuticle. Infection was not inhibited by IgG but delayed by about one day.

Fig. 2 illustrates some cytological aspects (TEM) of infection of soybean hypocotyls by *Phytophthora megasperma* f.sp. *glycinea* in the presence and absence of proteases. In essence it shows that proteolytic enzymes prevent adhesion and formation of appressoria and infection (penetration) pegs but do not inhibit germination and growth of the inoculum.

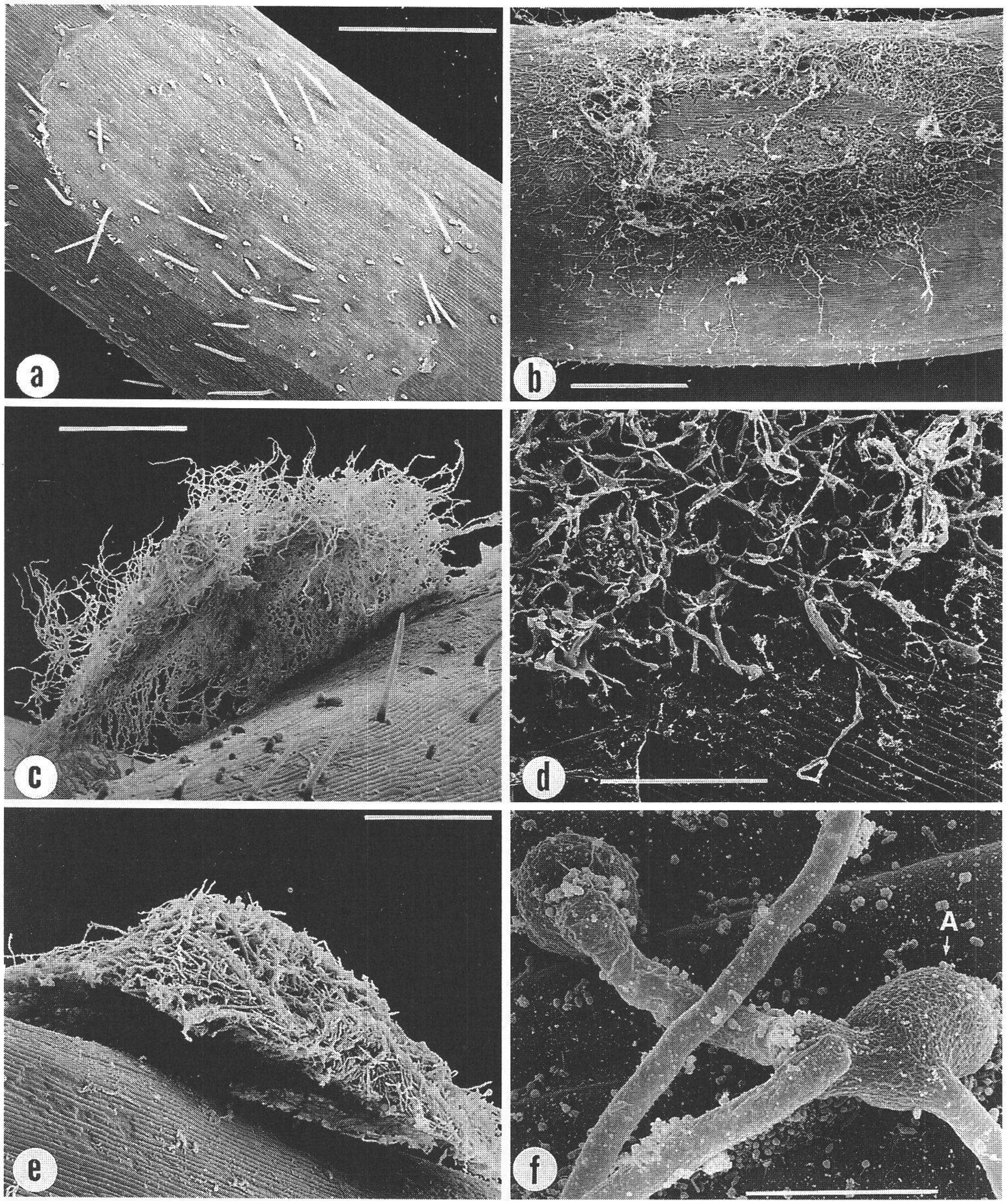


Fig. 2. Cytological aspects of infection of soybean hypocotyls by *Phytophthora megasperma* f.sp. *glycinea* in the presence and absence of proteases. In Figs. 2a, c, e the inoculum was applied in the presence of trypsin. The enzyme does not inhibit growth of the inoculum (cysts) but prevents formation of appressoria and adhesion. Brushing (Fig. 2a) or a slight blast from a pressurized air can (Fig. 2c, e) readily dislodges the inoculum and reveals the essentially unchanged surface of the host surface. Fig. 2b shows an inoculation site in the presence of lipase which has no effect on adhesion and infection, as do controls without added enzymes. Fig. 2d shows a situation similar to Fig. 2b at somewhat higher magnification. The presence of $\beta$-galactosidase has no effect on adhesion, infection or growth of the inoculum on the surface of the host. Fig. 2f is a detail from another inoculation site which shows formation of appressoria (A) at higher magnification.

## Discussion

In the first part we will discuss properties of adhesion of the fungal pathogen to artificial surfaces (glass, polystyrene). In the second part the role of adhesion to the cuticle for infection will be explored.

### (1) *Adhesion to artificial surfaces (glass and plastic)*

Adhesion of *Phytophthora megasperma* f.sp. *glycinea* to hydrophilic and hydrophobic surfaces appears to be mediated by proteinaceous material, most likely glycoproteins. This is indicated by the results that the protein degrading enzymes chymotrypsin, trypsin, proteinase K, and protease E completely inhibited adhesion to polystyrene and tissue culture plates. Several other enzymes (glycosidases, lysozyme) caused partial inhibition. The combined results are in favor of the model that adhesion in *Phytophthora* but also in a variety of other fungi is mediated by surface glycoproteins (e.g. Epstein et al. 1985, 1987, Chaubal et al. 1991, Hohl 1991, Sela-Buurlage et al. 1991).

*Phytophthora megasperma* f.sp. *glycinea* adheres equally well and with the same kinetics to a hydrophilic (glass) and a hydrophobic (polystyrene) surface. This contrasts with the situation in *P. infestans* which does not adhere to glass (Hollenstein 1993, Hollenstein and Hohl 1995). Most likely the fungus achieves adhesion by a combination of electrostatic and hydrophobic interactions, a proposal substantiated by the observation that salts at high concentrations but also detergents reduce or inhibit adhesion. Hydrophobic and/or electrostatic interactions have been proposed for other fungal adhesion systems (e.g., Klotz et al. 1985 for *Candida*, Sela-Buurlage et al. 1991 for *Colletotrichum musae*).

The effect of sugars and lectins was moderate and the interpretation remains ambiguous as there was little evidence for specificity of the interactions. In particular the total lack of adhesion-inhibiting activity of D-mannose, which strongly reduces adhesion to hydrophilic host cell walls (Guggenbühl and Hohl 1992), for the hydrophobic polystyrene, supports the view that adhesion to hydrophilic and hydrophobic surfaces is controlled by different surface molecules or different parts of these molecules. Additional evidence for this is derived from the observation that IgG and ConA which completely inhibit adhesion to hydrophilic host tissue (Ding et al. 1994), at most partially inhibited adhesion to tissue culture plates and had no visible inhibitors effect on adhesion of the pathogen to the host cuticle.

Cycloheximide and actinomycin D (both protein synthesis inhibitors), tunicamycin (inhibits glycosylation of proteins), but not cytochalasin B (inhibitor of actin microfibril polymerization), completely or substantially inhibited adhesion of *Phytophthora megasperma* f.sp. *glycinea* to tissue culture plates at 0.1 $\mu g \cdot ml^{-1}$. This supports the proposal by Hohl and Balsiger (1986a) and by Guggenbühl and Hohl (1992) that production of the adhesive material is at least in part induced upon contact of the fungus with a surface (there is a very marked lag phase for adhesion to protoplasts but hardly any for adhesion to walled cells). It contrasts sharply with the situation for *P. infestans*, the adhesion of which is not inhibited by these substances (see also Hollenstein 1993) and the adhesion kinetics of which is different from *Phytophthora megasperma* f.sp. *glycinea* in that adhesion starts immediately after deposition of the germlings and increases very rapidly thereafter (Hohl 1991).

### (2) *Adhesion and infection*

Turning now to the events on the host surface and preceding infection we recognize that the major effect observed is obtained with the proteases tested. These enzymes completely inhibited *adhesion* and *infection* of the host without substantially inhibiting germination and growth of the pathogen. That the effect is due to protease activity is substantiated by the observation that proteases in the presence of their inhibitor are hardly inhibitory. Inhibition by proteolytic enzymes of adhesion of fungal pathogens to the host cuticle has been demonstrated before (Epstein et al. 1985, 1987, Xiao et al. 1994) and seems to be a rather general phenomenon. Proteases most likely remove a proteinaceous component from the fungal surface which is essential for adhesion. This could involve proteins, glycoproteins, or lipoproteins, even though the fact that lipase does not show any effect on adhesion and infection does not support the latter possibility. Not completely excluded is the possibility that the adhesin is a component fastened to the wall by a (glyco)protein. In the absence of adhesion the fungus does not form appressoria on the host cuticle. Without adhesion and appressoria formation, inhibited by proteases, infection does not occur, indicating that these early events are essential for infection in the system studied.

In a previous paper (Ding et al. 1994) we have demonstrated that D-mannose, ConA and IgG inhibit adhesion of *Phytophthora megasperma* f.sp. *glycinea* to soybean cells and tissues, and also inhibit infection of soybean leaves through open wounds. IgG appears to accomplish this by binding to a 65 kDa glycoprotein present on the cell surface of both, the pathogen and the host. While in this hydrophilic situation IgG completely inhibited adhesion and infection (Ding et al. 1994) it (and ConA) hardly inhibited adhesion to the hydrophobic cuticle and delayed subsequent infections by only one day. In other words, IgG and ConA inhibit infection through hydrophilic but not through hydrophobic host surfaces. Thus, both vary in their effect on adhesion and infection depending on the degree of hydrophobicity of the host surface.

From these observations we conclude that adhesion is a prerequisite for infection, and that adhesion to hydrophilic and lipophilic surfaces are mediated by different molecules or different parts of the same molecules. We propose that adhesion to the cuticle and other hydrophobic surfaces involves relatively unspecific hydrophobic interactions while adhesion between hydrophilic surfaces (walls of host and pathogen) is mediated by more specific lock-and-key type interactions as exemplified by the 65 kDa glycoprotein discussed above (Ding et al. 1994).

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