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Mutual adhesion and interactions of isolated soybean mesophyll cells and germ tubes of *Phytophthora megasperma* f. sp. *glycinea*

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Abstract

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Physical contact and interactions between *Phytophthora megasperma* f. sp. *glycinea* and soybean were analyzed with *in vitro* systems, involving germlings or young hyphae of the pathogen and mechanically isolated, walled mesophyll cells of the host plant. Callose-containing wall appositions formed in the host cells at 20 to 25% of contact or penetration sites with the pathogen. Rarely, appressoria were induced at the host cell surface. Maximum adhesion between host cells and pathogen occurred after 4 h. The adhesive material was elaborated by the fungus after contact with the host cell had been established. Lectins and oligosaccharide residues are likely to play a role in the adhesion process since the addition of D-mannose but not of L-mannose or a series of other monosaccharides tested reduced adhesion significantly. Using lectin-mediated agglutination, mannose/glucose and, to a lesser extent, fucose residues were localized at the surface of isolated soybean cells. With the aid of beads covalently coated with specific sugar residues, the presence of a mannose-binding receptor could be detected on the surface of the mechanically isolated soybean cells. This receptor possibly binds to the mannose/glucose residues previously demonstrated at the surface of the pathogen, and this bond most likely is responsible for that part of the adhesion which is abolished in the presence of D-mannose.

Key words: adhesion – appressoria – cell wall – *Glycine max*-lectins – *Phytophthora*

Introduction

Initial interactions between fungal parasites and their host may involve signal molecules (elicitors: Albersheim et al. 1986, Keen 1986, 1990, Callow et al. 1988). Alternatively, they might be the result of direct physical contact between the two organisms involving unspecific attachment phenomena as well as specific interactions between surface molecules (Nicholson and Epstein 1991, Hohl 1991).

Attachment *per se* is often very unspecific. Fungal parasites of plants adhere to a variety of biotic or abiotic (glass, teflon, cellophane) surfaces (Hamer et al. 1988; Mims et al. 1988, Nicholson et al. 1988, Gubler et al. 1989). Yet, attachment may also involve parasite recognition of the specific surface topography of the host (Hoch et al. 1987) or specific binding between complementary molecules (Érsek et al. 1985, Mendgen et al. 1985, Hoch and Staples 1987, Hohl 1989, 1991).

The literature on adhesion during fungal infection of higher plants deals mainly with attachment of propagules, germ tubes or appressoria to the cuticle of plant leaves (Mendgen and Dressler 1983, Epstein et al. 1987, Beckett and Porter 1988, Freytag et al. 1988; Hamer et al. 1988, Jones and Epstein 1989). Little is known about adhesion of fungal plant pathogens to cell walls or plasma membranes of the host (Hohl 1991), an event frequently occurring during infection once the pathogen has breached the outermost barriers of the host surface and grows among and into host cells. These internal contacts might be important for the development of infection structures, recognition between host cell and pathogen, and triggering interactions between the two organisms.

In previous studies adhesion (Hohl and Balsiger 1986 b) and interaction of isolated protoplasts of host origin (soybean) with germ tubes of *Phytophthora megasperma* f. sp. *glycinea* (Odermatt et al. 1988) were analyzed. It was found that lectin-ligand type of interactions were involved in the mutual adhesion of the two organisms, and that some but not all interactions observed *in planta* could be reproduced with this simplified system. However, no hypersensitive reactions and no differences between protoplasts from resistant and susceptible cultivars could be observed. Using suspension-cultured lettuce cells infected by *Bremia lactucae*, Fagg et al. (1991) did obtain differential resistance reactions but no rapid hypersensitive responses. This suggested that the cell wall might be involved in these differential reactions.

In the present study experiments were performed using isolated, walled mesophyll cells instead of protoplasts. The major aim was to see how walled cells differed in their reactions to germ tubes from non-walled protoplasts and, thus, to gain a better understanding of the role of the cell wall in these interactions.

Material and methods

Host plants

Soybean [*Glycine max* (L.) Merr.] seeds, cv. Harosoy (susceptible to *Ph. megasperma* f. sp. *glycinea* race 1) and Harosoy 63 (resistant to *Ph. 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 kept moistened by 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).

Pathogen

Ph. megasperma f. sp. *glycinea* Kuan and Erwin, race 1, were grown and cysts prepared as described elsewhere (Eye et al. 1978, Ward et al. 1979, Hohl and Balsiger 1986 b).

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 a 100 ml 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 a total of 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).

Embedding of cysts and cells in agarose

The method of Odermatt et al. (1988) was employed. Cysts and cells were prepared and used in experiments on the same day. The cysts were resuspended in sterile distilled water and their concentration adjusted to 10^6 cysts $\cdot$ ml^{-1}. Suspensions of the mechanically isolated cells contained about $5 \cdot 10^5$ cells $\cdot$ ml^{-1}.

Adhesion of mesophyll cells to fungal mycelium

$7 \cdot 10^3$ cysts suspended in 0.1 ml of 20% pea broth (Hohl and Balsiger 1986 b) were placed into each well of 96-well flat-bottom tissue culture plates (FALCON 3072, Becton Dickinson Labware, New Jersey, USA). 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.

$2.5 \cdot 10^4$ soybean cells suspended in 0.05 ml test solution 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. With this procedure only very few cells adhered directly to the plastic or glass.

The number of cells attached to the mycelium was determined either microscopically (Hohl and Balsiger 1986 b), or enzymatically by measuring the activity of α-mannosidase in the following way: The solution in the wells was replaced with 50 µl of *p*-nitro-phenyl-α-D-mannopyranoside (2 mg $\cdot$ ml^{-1} in 0.2 M sodium acetate buffer of pH 5.0). The suspension was incubated at 37 °C for 3 h, 50 rpm. The reaction was stopped with 150 µl of 0.2 M sodium carbonate solution and the extinction was 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. To transform enzyme activities into cell numbers, the enzyme activities of a dilution series with known numbers of cells were determined and used as standard. A linear relationship existed between cell numbers ranging from 0 to $25 \cdot 10^3$ with the extinction measured at 410 nm. In cases where the mannosidase test might have been influenced by the test compounds, e.g., when measuring the influence of mannose on adhesion, adhesion was also determined microscopically.

Inhibition of adhesion of mesophyll cells to fungal mycelium

In some experiments the following compounds were added to the wells harboring hyphae 1 h prior to the addition of the cells and maintained during the test: snail digestive enzyme (Boehringer, Mannheim, FRG) 5 µl $\cdot$ ml^{-1}, cellulase (Onozuka R-10, Serva, Heidelberg, FRG) 4 mg $\cdot$ ml^{-1}, proteinase K (Merck, Darmstadt, FRG) 4 mg $\cdot$ ml^{-1}, cycloheximide (SIGMA Corp.) 2 µg $\cdot$ ml^{-1}, actinomycin D (SIGMA Corp.) 50 µg $\cdot$ ml^{-1} tunicamycin (SIGMA Corp.) 5 µg $\cdot$ ml^{-1}, 2-deoxy-D-glucose (Fluka, Buchs, Switzerland) 10 mM, and the following oligo-saccharides (0.1 M): D-galactose (Gal), N-acetyl-galactosamine (GalNAc), chitobiose (β-D-GlcNAc)$_2$, D-glucose (Glc), N-acetyl-glucosamine (GlcNAc), methyl-D-glucoside (MeGlc), D-lactose (Lac), D-mannose (D-Man), L-mannose (L-Man), methyl-mannoside (MeMan), D-sucrose (Suc), L-fucose (Fuc); all dissolved in water.

To kill cells and mycelium by chemical fixation the material was fixed for 15 min at room temperature with 3% paraformaldehyde in H_2O, washed and resuspended in H_2O. The mycelium adhering to the bottom of the wells was fixed similarly, washed and then exposed to the cell suspension.

Agglutination of mechanically isolated mesophyll cells by lectins

The method of Hohl and Balsiger (1986a) was modified as follows. Agglutination tests were made in 96-well tissue culture plates (Falcon 3072, Becton Dickinson Labware, New Jersey, USA) which allowed the use of small quantities of lectins and cells (100 µl total volume). The lectins were diluted in phosphate buffered saline (0.01 M PBS, pH 7.2, 0.15 M NaCl) at concentrations ranging from 62.5 $\mu g \cdot ml^{-1}$ to 500 $\mu g \cdot ml^{-1}$, and 10^4 in 10 µl of water cells were added per well. For controls the lectins were preincubated with the corresponding sugar ligands during 30 min at room temperature. Mixing and shaking of the different compounds were critical steps and performed with a gyratory rocking table. Incubation occurred at room temperature. The degree of agglutination was estimated every 30 min for 2 h using an inverted light microscope.

The following lectins were obtained from SIGMA Corp. and used in the agglutination experiments: 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, erythroagglutinin), *Triticum vulgaris* (wheat germ) agglutinin (WGA), *Tetragonolobus purpureus* (asparagus pea) agglutinin (TPA) and *Ulex europaeus* agglutinin (UEA I).

Histochemistry

To label germ tubes with fluorescein-isothiocyanate (FITC) lectins, the cysts were pregrown for 4 h on coverslips in wells and washed with 0.01 M PBS of pH 7.2. 0.1 ml of FITC-labelled lectin solution (from SIGMA Corp., 1 $mg \cdot ml^{-1}$) was added for 30 min. The coverslips were washed three times with PBS and mounted upside down in a drop of glycerol-PBS (1:1) on a microscope slide.

Mechanically isolated cells and hand sections of the upper third of 6 d old hypocotyls of soybean were immersed in 200 µl FITC-lectin solution (1 $mg \cdot ml^{-1}$) diluted in PBS (0.01 M, 0.15 M NaCl, pH 7.2) for 30 min. The specimens were washed three times with cold borate buffer (pH 8.4) and mounted in a drop of glycerol-PBS (1:1) on a microscope slide. For controls the lectins were preincubated with the corresponding sugar (50 mM) ligands during 30 min at room temperature.

Wall appositions (collars or papillae) formed by the host cells were visualized with the synthetic aniline blue fluorochrome (Biosupplies Australia, Parkville, Victoria, Australia). This compound shows superior contrast and higher specificity for callose (a β-1,3-glucan) than decolourized aniline blue (Stone et al. 1984). A stock solution of 0.1 $mg \cdot ml^{-1}$ was prepared in distilled water and stored at 4 °C in the dark. This solution was diluted 1:3 with PBS just prior to use. The probes were incubated for 30 min at 20 °C, washed in water and examined by fluorescence microscopy.

Microscopy

All microscopical work was done with a Zeiss Photomicroscope II equipped with a HBO 50W mercury lamp and epifluorescence optics. Observations were made with Neofluar objectives 6.3 ×, 16 ×, 40 ×, and two Neofluar oil immersion objectives (40 × and 100 ×). *Fluorescent probes:* (a) For fluorescein-isothiocyanate the excitation filter was a band-pass interference filter (546/12 nm) and the barrier a long-pass interference filter (590 nm). (b) Aniline blue stained probes were observed with an excitation band-pass interference filter (405/6 nm) and a long-pass interference barrier filter (435 nm). Photomicrographs were taken with Ilford PanF (50 ASA/18° DIN) and with Kodak Ektachrome 160 Tungsten film (160 ASA/23° DIN).

Adhesion to carbohydrate-coated agarose beads (Hohl and Balsiger 1988)

The following carbohydrate-coated agarose beads were purchased from SIGMA Chemicals Comp. (St Louis, USA): N-acetyl-D-galactosamine-agarose, N-acetyl-D-glucosamine-agarose, L-fucose-agarose, β-D-glucose-agarose, α-lactose-agarose. Caproyl-galactosamine-agarose was

purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), and α-D-mannopyranoside-agarose from PL Biochemicals, Inc. (Milwaukee, USA).

For agglutination 2 µl of beads in distilled water were pipetted into 96-wells tissue culture plates (Falcon 3072, Becton Dickinson Labware, New Jersey, USA). Approximately $5 \cdot 10^3$ cysts pregerminated for 1 h, or $5 \cdot 10^3$ mechanically isolated mesophyll cells were mixed in with the beads to give a final volume of 100 µl. Incubation occurred at room temperature on a gyratory rocking table. Agglutination was checked after 30 min. For controls cysts or cells were preincubated for 60 min and maintained during the test in up to 1 M of the corresponding sugar.

Abbreviations

Con A = concanavalin A, FITC = fluorescein-isothiocyanate, PBS = phosphate buffered saline

Results

Interactions between isolated host cells and pathogen in flat-embedded cultures

Cocultures of isolated soybean mesophyll cells and germinating cysts of *Phytophthora megasperma* f. sp. *glycinea*, race 1 were microscopically examined and quantified after 18 h. There was no obvious long-distance attraction of the growing hyphae to the cells. Quite often hyphae passed by cells at close range without displaying any apparent reaction or change in growth direction (Fig. 1 a). Even in cases where a cyst was germinating on the surface of a cell, growth of the germ tube was not necessarily towards the cell. In a few instances a hypha was turning towards a cell or grew along the periphery of the cell for a short distance. Occasionally penetration occurred (Fig. 1 b).

Callose-containing deposits were detected by staining with the fluorochrome aniline blue. 25% of the embedded plant cells produced callose even when not contacting fungal structures. 80% of these cells showed small numbers (less than 10) of patches stained with aniline blue and 20% had their surfaces studded with numerous small, yellow patches probably representing callose-plugged plasmodesmata (Fig. 1 d).

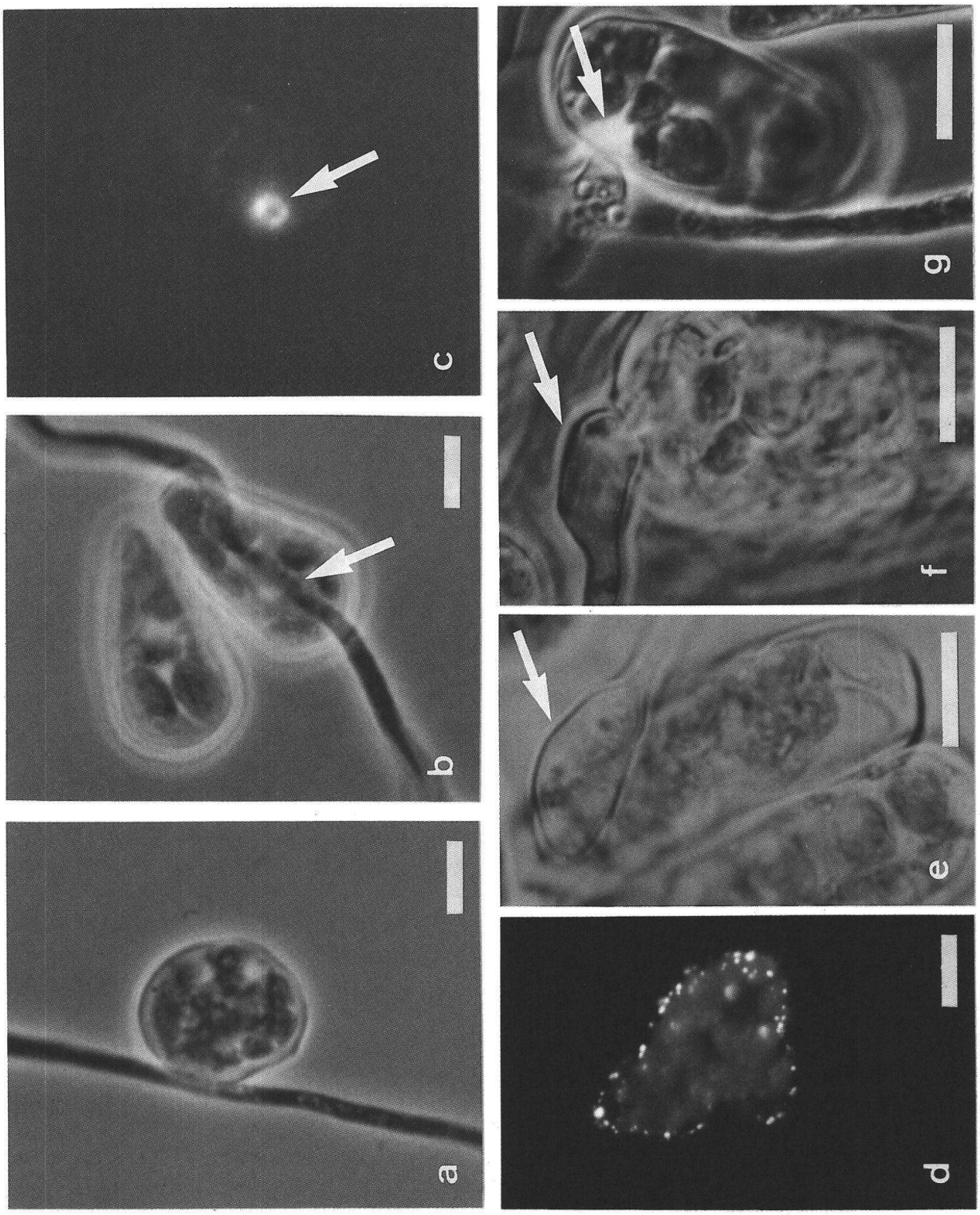
Twenty to 25% of the cells in physical contact with a fungal hyphae formed collars or papillae of callose. The cells isolated from the resistant soybean cultivar (Harosoy 63) formed callose at the same rate as those from the susceptible cultivar (Harosoy).

In a small number of encounters the hyphae penetrated, traversed the host cell and continued growth after exiting (Fig. 1 b). Callose formation could be detected in half of the cells being penetrated in this manner (Fig. 1 c). The cells from both cultivars never showed a hypersensitive browning reaction or collapse of their protoplasts.

Occasionally, where contacts occurred, the fungus grew along the cell surface and broadened its tip to form an appressorium (Fig. 1 e, f). Appressoria formation by the fungal hyphae was observed in about 1% of the contact sites studied. The morphological criteria used to identify appressoria were: broadened hyphal elements directed towards the cell wall of the host cell and apposed to it, often highly vacuolized. In a few cases the host cell had formed a papilla or a collar of callose containing material directly beneath the appressorium (Fig. 1 g).

Adhesion of soybean mesophyll cells to the mycelium

Results obtained with the enzyme test were comparable to those from microscopic observation. Fig. 2 shows the kinetics of adhesion of cells to the fungal mycelium. There was no obvious lag period, and adhesion increased linearly before reaching a plateau between 3–4 h.


a
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g

Effect of various treatments or compounds on adhesion of cells to the mycelium

In a first approach, a series of potential inhibitors of adhesion was tested. Pretreatment of the hyphae with snail digestive enzyme, proteinase K and cellulase inhibited adhesion of soybean mesophyll cells to the mycelium. In addition, chemical fixation of the hyphae prevented attachment as did addition of inhibitors of protein synthesis (cycloheximide), RNA synthesis (actinomycin D) or glycosylation (tunicamycin, deoxy-D-glucose), indicating that adhesion involves biosynthetic processes. Since chemical fixation of hyphae but not of mesophyll cells inhibited adhesion, the latter must be accomplished by the fungal pathogen. Preincubation of the fungal mycelium with D-mannose reduced adhesion of soybean cells significantly. Maximum inhibition was obtained with concentrations above 37.5 mM of D-mannose (Fig. 3). L-mannose and the other sugars tested did not inhibit adhesion (D-galactose, N-acetyl-galactosamine, chitobiose, D-glucose, N-acetyl-glucosamine, methyl-D-glucoside, D-lactose, methyl-mannoside, D-sucrose, L-fucose).

Characterization of the surface of mechanically isolated soybean mesophyll cells

Lectin-mediated agglutination. – Of the 10 lectins tested five (ConA, LCA, PSA, PHA V, TPA) agglutinated mesophyll cells Table 1). Agglutination was inhibited by the presence of the corresponding carbohydrate hapten (0.1 M were used). A quantitative comparison by end titer determination revealed that glucose- and mannose-binding lectins had agglutinating activity at a concentration of 250 $\mu g \cdot ml^{-1}$ and above. Galactose-N-acetyl-glucosamine-mannose- and fucose-binding lectins agglutinated the cells only at higher concentrations (500 $\mu g \cdot ml^{-1}$).

Lectin-histochemistry. – Cells incubated with ConA-FITC (Fig. 4a) were labelled irregularly over the entire cell surface and WGA-FITC-labelled cells displayed a few fluorescent patches. This could explain why agglutination requires high lectin concentrations. No qualitative or quantitative differences in lectin-mediated agglutination were detected between the near isogenic lines Harosoy and Harosoy 63.

Hand-sections of leaf and hypocotyl tissue were strongly labelled with ConA-FITC and WGA-FITC (Fig. 4c, e) indicating that the lining of the intercellular spaces is particularly rich in glucose/mannose and N-acetyl-glucose residues.

Adhesion to monosaccharide-coated beads. – Mechanically isolated mesophyll cells adhered within 30 min to beads coated with α-D-mannopyranoside but to no other sugar residue tested (N-acetyl-galactosamine, N-acetyl-glucosamine, fucose, glucose, lactose, caproyl-galactosamine). Adhesion was substantially inhibited in the presence of 1 M D-mannose. The result is shown in Table 2, together with results from other studies.

Fig. 1. Interactions between host cells and pathogen. Phase contrast microscopy. (a) Hypha passing a host cell at close range without changing its direction. (b) Host cell completely traversed by a fungal hypha. The protoplast does not collapse. (c) Same cell as (b) stained with aniline blue and examined by fluorescence microscopy. A collar (→) has formed around the penetrating hypha. (d) Fluorescence of a soybean cell stained with aniline blue 18 h after mechanical isolation. (e, f) Development of appressoria (→) from the fungal hypha. (g) Phase contrast illumination combined with fluorescence excitation. Sample stained with aniline blue. A branch of the hypha is directed towards a host cell which reacted with the production of a callose-containing papilla (→). Bars represent 10 μm.

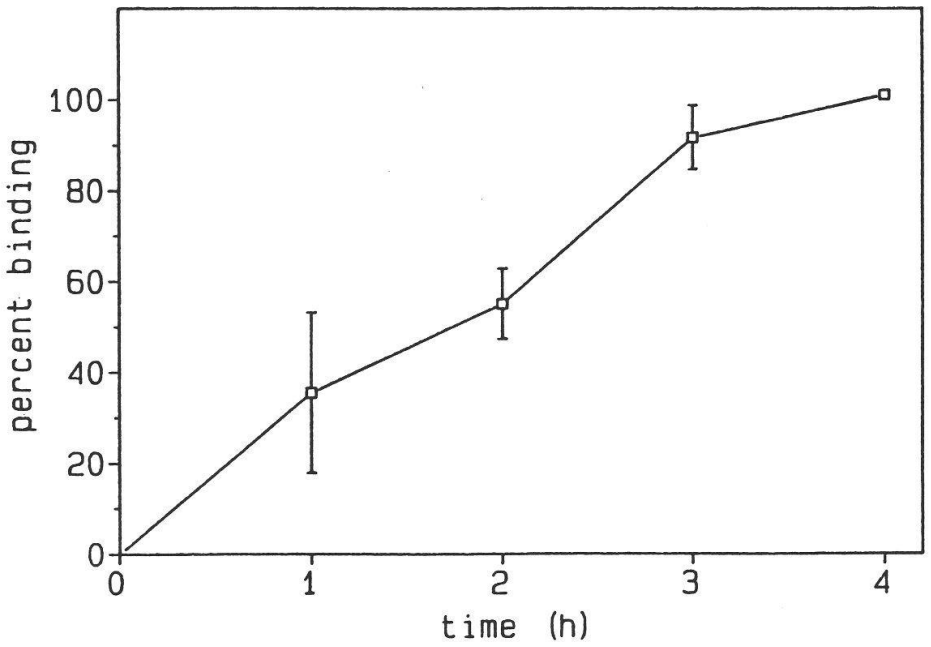

Fig. 2. Adhesion of isolated soybean cells to fungal mycelium. Binding is expressed as a percentage of the maximum quantity of adhering cells. Error bars represent standard deviation based on a minimum of 3 replicates.

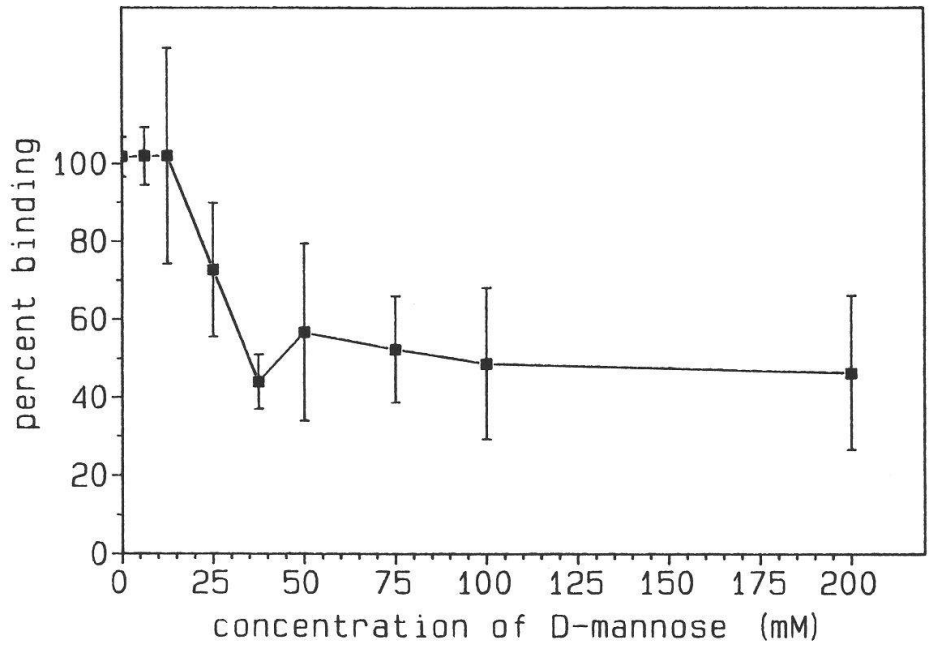

Fig. 3. Influence of various concentrations of D-mannose on adhesion of soybean cells to fungal mycelium. Maximal inhibition is obtained with concentrations above 37.5 mM. Error bars represent standard deviation based on a minimum of 3 replicates.

Table 1. Agglutination (after 90 min) of mechanically isolated soybean cells by lectins

Lectin[a]	Sugar hapten(s)[b]	Agglutination[c]
Con A	α-D-Man, α-D-Glc	+
LCA	α-D-Man, α-D-Glc	+
PSA	α-D-Man, α-D-Glc	(+)
RCA I	D-GalNAc, β-D-Gal	–
PNA	β-D-Gal(1–3)-D-GalNAc	–
SBA VI	α-D-GalNAc, β-D-GalNAc	–
PHA V	β-D-Gal-β-D-GlcNAc-D-Man	(+)
WGA	$(\beta\text{-D-GlcNAc})_2$	–
TPA	α-L-Fuc	(+)
UEA I	α-L-Fuc	–

[a] Con A = concanavalin A, LCA = *Lens culinaris* agglutinin, PSA = *Pisum sativum* agglutinin, RCA = *Ricinus communis* toxin, PNA = *Arachis hypogea* agglutinin, SBA = *Glycine max* agglutinin, PHA = *Phaseolus vulgaris* agglutinin, WGA = *Triticum vulgaris* agglutinin, TPA = *Tetragonolobus purpureus* agglutinin, UEA = *Ulex europaeus* agglutinin.
[b] sugars bound by corresponding lectin. Man = mannose, Glc = glucose, GlcNAc = N-acetyl-glucosamine, Gal = galactose, GalNAc = N-acetyl-galactosamine, Fuc = fucose.
[c] – = no agglutination, (+) = agglutination requires at least 500 $\mu l \cdot ml^{-1}$ of lectin, + = agglutination requires at least 250 $\mu l \cdot ml^{-1}$.

Table 2. Summary of sugar binding sites and sugar residues exposed on the surfaces of soybean cells and protoplasts, and of germinated cysts of *Phytophthora megasperma* f. sp. *glycinea*, race 1

	Presence of binding site (receptor) for	Type of sugar residues (ligands) present
Cysts[a]	GalNCap[d]	–
	Fuc	–
	–	Glc/Man
Protoplasts[b]	β-Glc	Glc/Man
	Gal, GalNAc	Gal, GalNAc
Walled cells[c]	Man	–
	–	Glc/Man
	–	(Fuc)

[a] Hohl and Balsiger (1988); [b] Hohl (1991); [c] present study; [d] GalNCap = caproyl-galactosamine, Fuc = fucose, Glc = glucose, Man = mannose, Gal = galactose, GalNAc = N-acetyl-galactosamine.

Discussion

We have used a model system to study adhesion of *Phytophthora megasperma* f. sp. *glycinea* to the cell wall of soybean host cells and the cytological reactions ensuing from the interaction between the two organisms. The system used consists of isolated, walled mesophyll cells of the host and germlings or young hyphae of the pathogen. The model system allows analysis of some of the reactions observed *in planta* with methods not applicable in the latter. Some of the results obtained are: (1) Isolated mesophyll cells

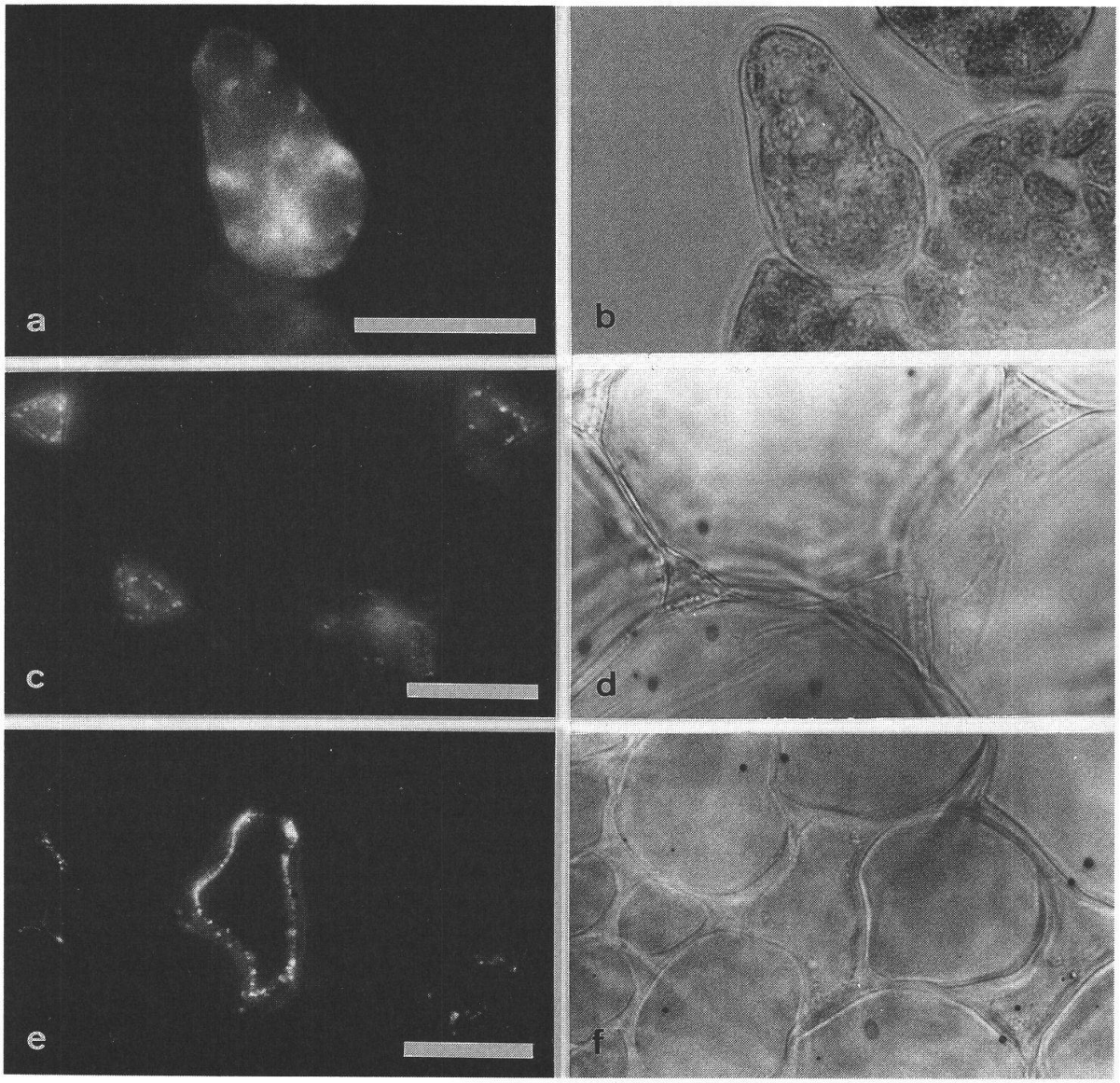

Fig. 4. Lectin-labelling of mechanically isolated soybean cells and hand sections of hypocotyls. (a) ConA-FITC binds irregularly to the surface of soybean cells, (b) same cells as in (a), phase contrast. (c) ConA-FITC labels intercellular spaces, (d) same picture taken with phase contrast, (e) WGA-FITC labels the periphery of the intercellular space, (f) same area as in (e), differential interference contrast. Bars represent 20 μm.

interact with germ tubes and hyphae of the fungal pathogen. This is shown by the induction of collars and papillae in the host and of appressoria in the pathogen. (2) Isolated host cells and pathogen adhere to each other. There is good evidence that lectins and their corresponding sugar ligands are implicated in this process.

Interactions between isolated mesophyll cells and the fungal pathogen

Appressorium formation is a regular and apparently essential step in the normal infection of the host (Nicholson and Epstein 1991). As shown in our study but in contrast

to the situation with protoplasts (Odermatt et al. 1988), walled mesophyll cells are capable of triggering the formation of appressoria in the pathogen. This indicates that some property of the wall acts as a signal for this morphogenetic event, since no appressoria formed when the fungus grew on glass or plastic surfaces. It should be noted, however, that only a small number of appressoria were induced by isolated cells, and that in most instances penetration of the cell occurred in the absence of appressoria. Possibly, the signal is modified or partially removed from the surface by the isolation procedure. Alternatively, an appressorium might not be needed for infection as pathogen and host cell probably are sufficiently anchored in the embedding agar to allow for mechanical penetration of the host wall, without the production of specialized adhesion and infection structures. That mechanical support might obliterate the need for appressoria is supported for *Ph. cinnamomi* by Tippett et al. (1976) who observed root penetration in eucalyptus without formation of appressoria, and for *Ph. palmivora* (now *P. capsici*) by Feuerstein and Hohl (1986) who noticed that appressoria were normally formed but absent if the fungus was wedged in narrow crevices around trichomes of the leaves. This notion that appressoria only form if required by the situation is also supported by the common observation that no typical appressoria form when infection hyphae grow from one host cell to another within the host tissue (e.g. Hohl and Suter 1976, for *Ph. infestans*).

We observed two types of structures stained with aniline blue, a dye which indicates the presence of callose, a β-1,3-glucan. Small dots were observed at the surface of mesophyll cells contacting or not contacting a fungal hypha. They possibly represent a wound response localized at sites of plasmodesmata induced by mechanical injury during the isolation process, an idea advanced before for similar reactions observed in infected tissue (Aist 1976, Aist and Israel 1977, Smith and McCully 1978).

The second type of aniline blue positive structure was localized at the contact zone between the fungal hypha and the host cell wall. Here, the structures correspond to wall appositions or to papillae observed during natural infections (Bracker and Littlefield 1973, Aist 1976, Stössel et al. 1980, Hächler and Hohl 1982). They possibly represent products of defence reactions (Hinch and Clarke 1982, Hächler and Hohl 1984, Allen and Friend 1983).

A comparison between reactions observed within infected host tissue (e.g. Hächler and Hohl 1982, 1984), or with infected isolated protoplasts (Odermatt et al. 1988) reveals the following:

(1) In common with infections of plant tissue, single, walled, isolated cells are capable of producing wall appositions or papillae upon challenge by the pathogen. In addition, they trigger formation of appressoria by the pathogen. In contrast to infections of entire tissues, no hypersensitive reactions were noticed with isolated cells. According to Davis and Currier (1986), however, a hypersensitive-like reaction was observed with potato tuber protoplasts treated with unsaturated fatty acids.

(2) In common with isolated protoplasts, isolated walled cells formed structures upon infection which were related to wall appositions and papillae. In the walled cells studied here, these structures were 'complete' and indistinguishable from those formed in the plant tissue (Hächler and Hohl 1982, 1984), while in protoplasts they looked incomplete with, probably, only glucans present (Odermatt et al. 1988). In contrast to walled cells, no appressoria were induced by protoplasts.

(3) No differences were found between cells from the resistant and the susceptible host. Possibly, differential reactions rely on factors changed or lost during the isolation procedure or require the coordinate effort of more than one host cell. In fact, Fagg et al.

(1991) did find compatibility and incompatibility expressed in small clumps of suspension cultured cells in some combinations, but again no rapid hypersensitive reaction was observed.

Adhesion of host cells and pathogen

Mechanically isolated, walled cells from both cultivars (Harosoy, susceptible, and Harosoy 63, resistant) adhere to hyphae of *Ph. megasperma* f. sp. *glycinea* with closely similar kinetics. Thus, adhesion *per se* is not a specific response in the system studied. Analogous results were obtained by Hohl and Balsiger (1986 b) with soybean protoplasts and enzymatically isolated cells from both cultivars. In contrast to the latter study, no clear-cut lag phase was observed using mechanically isolated mesophyll cells. Pectin present in the remaining middle lamellae might be responsible for the absence of a lag-phase in mechanically isolated cells, as this sticky material is probably removed in the case of enzymatically isolated cells.

Our results show that the fungus and not the cells produced the adhesive material and that the latter is not preformed (see also Hohl and Balsiger 1986 b). The microbial partner also plays the active part in the attachment of *Pseudomonas syringae* pv. *glycinea* (Érsek et al. 1985), of *Rhizobium* (Dazzo and Gardiol 1984), and of *Agrobacterium tumefaciens* (Matthysse 1984, Matthysse et al. 1982) to their respective host cells.

Tunicamycin and 2-deoxy-D-glucose inhibit adhesion. This might indicate that glycosylation is required for this process. However, the effect of these compounds has to be interpreted carefully, since our experiments were short-termed (2–5 h) which would imply a very high turnover of surface N-linked glycoproteins. Keen and Legrand (1980) showed that tunicamycin inhibited mycelial growth of *Phytophthora megasperma* and Datema et al. (1983) proved that 2-deoxy-D-glucose blocks the initiation of cellulose synthesis. The hyphal wall of *Ph. megasperma* is made primarily of two β-glucans, cellulose and $\beta(1-3)(1-6)$glucan (Bartnicki-Garcia and Wang 1983). Therefore, the inhibition of the synthesis of cellulose probably inhibits the growth of the fungus. Consequently, it is not possible to tell whether the formation of glucans or the growth of the fungus is important for adhesion, since the former is necessary for the latter. Nevertheless, cellulose or components of the cell wall which are embedded within the cellulose matrix might play a role in the adhesion process.

The lectin-binding studies indicate that isolated walled cells carry glucosyl/mannosyl and some fucosyl residues at their surface. The binding of the lectins is inhibited by the appropriate sugar-ligand demonstrating that the lectin-binding is specific. Lectin-labelling of tissue sections indicates that the glucose/mannose and N-acetyl-glucose residues occur predominantly at or in the walls of the intercellular spaces. Interestingly, however, not all cells are labelled by the lectins pointing to possible qualitative or quantitative differences in surface carbohydrates present among the cell population isolated from the leaf mesophyll.

The same mesophyll cells appear to carry a receptor for mannose (Table 2) as shown by the binding of mesophyll cells to D-mannose-covered agarose beads but not to those covered by other sugars. Quite likely this receptor binds the mannose/glucose residues previously demonstrated on the surface of the pathogen (Hohl and Balsiger 1986 a). This protein-carbohydrate bond might thus be responsible for that part of the adhesion which is abolished in the presence of D-mannose, an inhibition which has properties characteristic of ligand-receptor interactions, such as saturation kinetics of inhibition (Yoshikawa et al. 1983) and specificity for a mono- or oligosaccharide.

The observation that D-mannose but not α-methyl-mannoside inhibits adhesion is at present puzzling and needs further study. However, the fact that L-mannose does not inhibit adhesion supports our hypothesis that a mannose receptor on the host cell wall binds to a mannosyl residue containing carbohydrate moiety on the surface of the fungal pathogen.

References

Aist J. R. 1976. Papillae and related wound plugs of plant cells. Annu. Rev. Phytopathol. 14: 145–163.

Aist J. R. and Israel H. W. 1977. Mechanically induced wall appositions of plant cells can prevent penetration by a parasitic fungus. Science 197: 568–571.

Albersheim P., Darvill A. G., Sharp J., Davis K. R. and Doares S. H. 1986. Studies on the role of carbohydrates in host-microbe interactions, 297–310. *In*: Recognition in microbe-plant symbiotic and pathogenic interactions, Proc. Nato Adv. Res. Workshop Vol. H4. Lugtenberg B., ed., Springer, Berlin.

Allen F. H. E. and Friend J. 1983. Resistance of potato tubers to infection by *Phytophthora infestans*: a structural study of haustorial encasement. Physiol. Plant Pathol. 22: 285–292.

Bartnicki-Garcia S. and Wang M. C. 1983. Biochemical aspects of morphogenesis in *Phytophthora*, 121–137. *In*: *Phytophthora*, Its biology, taxonomy, ecology, and pathology. Erwin D. C., Bartnicki-Garcia S. and Tsao P. H., eds., Amer. Phytopath. Soc., St. Paul, Minnesota.

Beckett A. and Porter R. 1988. The use of complementary fractures and low-temperature scanning electron microscopy to study hyphal – host cell surface adhesion between *Uromyces viciae-fabae* and *Vicia faba*. Can. J. Bot. 66: 645–652.

Bhattacharyya M. K. and Ward E. W. B. 1986. Expression of gene-specific and age-related resistance and the accumulation of glyceollin in soybean leaves infected with *Phytophthora megasperma* f. sp. *glycinea*. Physiol. Mol. Plant Pathol. 29: 105–113.

Bracker C. E. and Littlefield L. J. 1973. Structural concepts of host-pathogen interfaces, 159–318. *In*: Fungal Pathogenicity and the Plant's Response. Byrde R. J. W. and Cutting C. V., eds., Academic Press, London, New York.

Callow J. A., Ray T., Estrada-Garcia T. M., and Green J. R. 1988. Molecular signals in plant cell recognition, 167–191. *In*: Cell to cell signals in plant, animal and microbial symbiosis. Scannerini S., Smith D., Bonfante-Fasolo P. and Gianinazzi-Pearson V., eds., Springer, Berlin.

Datema R., Schwarz R. T., Rivas L. A. and Lezica R. P. 1983. Inhibition of β-1,4-glucan biosynthesis by deoxyglucose – the effect on the glucosylation of lipid intermediates. Plant Physiol. 71: 76–81.

Davis D. A. and Currier W. W. 1986. The effects of phytoalexin elicitors, arachidonic and eicosapentaenoic acids, and other unsaturated fatty acids on potato tuber protoplasts. Physiol. Mol. Plant Pathol. 28: 431–442.

Dazzo, F. B. and Gardiol, A. E. 1984. Host specificity in *Rhizobium* – legume interactions, 3–31. In: Genes involved in microbe – plant interactions. Verma D. P. S. and Hohn T., eds., Springer, Wien, New York.

Epstein L., Lacetti L. B., Staples R. C. and Hoch H. C. 1987. Cell-substratum adhesive protein involved in surface contact responses of the bean rust fungus. Physiol. Mol. Plant Pathol. 30: 373–388.

Érsek T., Gáborjányi R., Höltzl P. and Király, Z. 1985. Sugar-specific attachment of *Pseudomonas syringae* pv. *glycinea* to isolated single leaf cells of resistant soybean cultivars. Phytophatol. Z. 113: 260–270.

Eye L., Sneh B. and Lockwood J. L. 1978. Factors affecting zoospores production by *Phytophthora megasperma* var. *sojae*. Phytopathology 68: 1766–1768.

Fagg J., Woods-Tor A. and Mansfield J. 1991. Comparative study of interactions between *Bremia lactucae* and lettuce cells in suspension culture and in cotyledons. Physiol. Mol. Plant Pathol. 38: 105–119.

Feuerstein P. and Hohl H. R. 1986. Histologie des Blattes und des Blattbefalls durch *Phytophthora palmivora* bei *Piper nigrum* (Schwarzer Pfeffer). Bot. Helv. 96: 95–108.
Freytag S., Bruscaglioni L., Gold R. E. and Mendgen K. 1988. Basidiospores of rust fungi (*Uromyces* species) differentiate infection structures *in vitro*. Exp. Mycol. 12: 275–283.
Gubler F., Hardham A. R. and Duniec J. 1989. Characteristic adhesiveness of *Phytophthora cinnamomi* zoospores during encystment. Protoplasma 149: 24–30.
Hächler H. and Hohl H. R. 1982. Histochemistry of papillae in potato tuber tissue infected with *Phytophthora infestans*. Bot. Helv. 92: 23–31.
Hächler H. and Hohl H. R. 1984. Temporal and spatial distribution patterns of collar and papillae wall appositions in resistant and susceptible tuber tissue of *Solanum tuberosum* infected by *Phytophthora infestans*. Physiol. Plant Pathol. 24: 107–118.
Hamer J. E., Howard R. J., Chumley F. G. and Valent B. 1988. A mechanism for surface attachment in spores of a plant pathogenic fungus. Science 239: 288–290.
Hinch J. M. and Clarke A. E. 1982. Callose formation in *Zea mays* as a response to infection with *Phytophthora cinnamomi*. Physiol. Plant Pathol. 21: 113–124.
Hoch H. C. and Staples R. C. 1987. Structural and chemical changes among the rust fungi during appressorium development. Annu. Rev. Phytopathol. 25: 231–247.
Hoch H. C., Staples R. C., Whitehead B., Comeau J. and Wolf E. D. 1987. Signaling for growth orientation and cell differentiation by surface topography in *Uromyces*. Science 235: 1659–1662.
Hohl H. R. and Suter E. 1976. Host-parasite interfaces in a resistant and a susceptible cultivar of *Solanum tuberosum* inoculated with *Phytophthora infestans*: leaf tissue. Can. J. Bot. 54: 1956–1970.
Hohl H. R. and Balsiger S. 1986a. Probing the surfaces of soybean protoplasts and of germ tubes of the soybean pathogen *Phytophthora megasperma* f. sp. *glycinea* with lectins. Bot. Helv. 96: 289–297.
Hohl H. R. and Balsiger, S. 1986b. A model system for the study of fungus – host surface interactions: adhesion of *Phytophthora megasperma* to protoplasts and mesophyll cells of soybean, 259–272. *In*: Recognition in microbe – plant symbiotic and pathogenic interactions, Proc. Nato Adv. Res. Workshop Vol. H 4. Lugtenberg B., ed., Springer, Berlin.
Hohl H. R. and Balsiger S. 1988. Surface glycosyl receptors of *Phytophthora megasperma* f. sp. *glycinea* and its soybean host. Bot. Helv. 98: 271–277.
Hohl H. R. 1989. Cytology and morphogenesis of fungal cells. Progr. in Bot. 51: 4–15.
Hohl H. R. 1991. Surface-related host-pathogen interactions in *Phytophthora*, 70–89. *In*: *Phytophthora*. Lucas J. A., Shattock R. C., Shaw D. S. and Cooke L. R., eds., Cambridge Univ. Press, Cambridge.
Jones M. J. and Epstein L. 1989. Adhesion of *Nectria haematococca* macroconidia. Physiol. Mol. Plant Pathol. 35: 453–461.
Keen N. T. and Legrand M. 1980. Surface glycoproteins: evidence that they may function as the race specific phytoalexin elicitors of *Phytophthora megasperma* f. sp. *glycinea*. Physiol. Plant Pathol. 17: 175–192.
Keen N. T. 1986. Pathogenic strategies for fungi, 171–188. *In*: Recognition in microbe – plant symbiotic and pathogenic interactions, Proc. Nato Adv. Res. Workshop Vol. H 4. Lugtenberg B., ed., Springer, Berlin.
Keen N. T. 1990. Gene-for-gene complementarity in plant-pathogen interactions. Annu. Rev. Genet. 24: 447–463.
Matthysse A. G. 1984. Interaction of *Agrobacterium tumefaciens* with the plant cell surface, 33–54. In: Genes involved in microbe – plant interactions. Verma D. P. S. and Hohn T., eds., Springer, Wien.
Matthysse A. G., Holmes K. V. and Gurlitz R. H. G. 1982. Binding of *Agrobacterium tumefaciens* to carrot protoplasts. Physiol. Plant Pathol. 20: 27–33.
Mendgen K. and Dressler E. 1983. Culturing *Puccinia coronata* on a cell monolayer of the *Avena sativa* coleoptile. Phytopathol. Z. 108: 226–234.
Mendgen K., Lange M. and Bretschneider K. 1985. Quantitative estimation of the surface carbohydrates on the infection structures of rust fungi with enzymes and lectins. Arch. Microbiol. 140: 307–311.

Mims C. W., Richardson E. A. and Taylor J. 1988. Specimen orientation for transmission electron microscopic studies of fungal germ tubes and appressoria on artificial membranes and leaf surfaces. Mycologia 80: 586–590.

Nicholson R. L., Yoshioka H., Yamaoka N. and Kunoh H. 1988. Preparation of the infection court by *Erysiphe graminis*: II. Release of esterase enzyme from conidia in response to a contact stimulus. Exp. Mycol. 12: 336–349.

Nicholson R. L. and Epstein L. 1991. Adhesion of fungi to the plant surface – prerequisite for pathogenesis, 3–23. *In*: The fungal spore and disease initiation in plants and animals. Cole G. T. and Hoch H. C., eds., Plenum Press, New York London.

Odermatt M., Röthlisberger A., Werner C. and Hohl H. R. 1988. Interactions between agarose-embedded plant protoplasts and germ tubes of *Phytophthora*. Physiol. Mol. Plant Pathol. 33: 209–220.

Schwenk F. W. 1981. Callus formation from mechanically isolated mesophyll cells of soybean and sweet potato. Plant Sci. Lett. 23: 147–151.

Smith M. M. and McCully M. E. 1978. Enhancing aniline blue fluorescent staining of cell wall structures. Stain Technol. 53: 79–85.

Stone B. A., Evans N. A., Bonig I. and Clarke A. E. 1984. The application of sirofluor, a chemically defined fluorochrome from aniline blue for the histochemical detection of callose. Protoplasma 122: 191–195.

Stössel P., Lazarovits G. and Ward E. W. B. 1980. Penetration and growth of compatible and incompatible races of *Phytophthora megasperma* var. *sojae* in soybean hypocotyl tissues differing in age. Can. J. Bot. 58: 2594–2601.

Tippett J. T., Holland A. A., Marks G. C. and O'Brien T. P. 1976. Penetration of *Phytophthora cinnamomi* into disease tolerant and susceptible eucalypts. Arch. Microbiol. 108: 231–242.

Ward E. W. B., Lazarovits G., Unwin C. H. and Buzzell R. I. 1979. Hypocotyl reactions and glyceollin in soybeans inoculated with zoospores of *Phytophthora megasperma* var. *sojae*. Phytopathology 69: 951–955.

Widholm J. M. 1972. The use of fluorescein diacetate and phenosaffranine for determining viability of cultured plant cells. Stain Technol. 47: 189–194.

Yoshikawa M., Keen N. T. and Wang M.-C. 1983. A receptor on soybean membranes for a fungal elicitor of phytoalexin accumulation. Plant Physiol. 73: 497–506.