INTRODUCTION

Surfaces of microbial pathogens and symbionts contribute to host and tissue specificity in a variety of ways (Smith, 1977; Giles & Atherly, 1981). Of particular interest are those microbial, surface-borne macromolecules which enable pathogens to adhere specifically or selectively (Garrod & Nicol, 1981) to host tissues. There are many examples for bacterial and viral diseases (Klenk, 1980; Smith, 1980; Tardieu et al., 1982), but not any extensively researched example for fungal pathogens. J. Gould & D. H. Northcote (unpublished work) showed that adhesins of fungal pathogens can reside asymmetrically on their surfaces and that their expression is subject to developmental regulation. The character, developmental stage and proportion of fungal surfaces present in vitro was fixed by establishment of conditions for semi-synchronous germination of populations of fungal conidia. This simultaneously overcame the difficulty of definition of a unit of mycelial pathogen. Two different classes of maize (Zea mays) pathogens were shown, with fluorescently labelled maize root mucilage, to have different distributions of adhesions that bound mucilage selectively. Mucilages are produced by root-cap cells and root epidermal cells and form the outermost layers of roots. They are hydrophilic, lubricate the passage of roots through soil and aid water uptake (Gould & Northcote, 1985). Phialophora radicicola P29 is a highly specialized, ectotrophic root-infecting fungus (infection is often asymptomatic) of the roots of species of Gramineae only. It bound mucilage solely at hyphal apices. Fusarium moniliforme 173 211 is a vascular-wilt fungus capable of attacking a greater variety of tissues, such as roots, culms and aerial parts of plants. This fungus bound mucilage with both conidial and hyphal surfaces.

The work presented here examines quantitatively the adsorption of mucilage by two pathogenic, ascomycetous fungi, P29 and 173 211, used in other studies (J. Gould & D. H. Northcote, unpublished work), so that optimum conditions and mechanisms for adsorption could be determined.

MATERIALS AND METHODS

Reagents and biological material

All chemicals were of analytical-reagent grade or of the most pure grade available and, unless stated to the contrary, were obtained from BDH, Poole, Dorset, U.K., or from Sigma, Poole, Dorset, U.K. All solutions were made with water that had been distilled twice (firstly with a metal still and secondly with a glass still). Fucose-binding lectins were isolated from seeds of Lotus tetragonolobus (Thompson and Morgan, Ipswich, Suffolk, U.K.) by affinity chromatography (Sundberg & Parath, 1974; Vretblad, 1976). Yeast mannanprotein (from Saccharomyces cerevisiae) was prepared as described by Northcote & Horne (1952) and desulphated (2%, w/w, SO4 remaining) fucoidan by the method of Wright et al. (1976). Heparin (from porcine mucosa) was from Evans Medical Ltd., Speke, Liverpool, U.K., pectin 250 grade (analysis by g.l.c.: Rha/Fuc, 4%; Ara, 9%; Xyl, 4%; Man, 2%; Gal, 16%; Glc, 18%; GalAe, 47%) and dextran (from Leuconostoc) grade A (Mw 200–275000) were from BDH. Chondroitin 6-sulphate (from Shark, pure) and cetylpyridinium chloride were from Koch–Light Laboratories, Colnbrook, Bucks., U.K. Chitin oligomers [di-, tri-, and tetra-β(1–4)-N-acetylglucosamine] were prepared as described by Read & Northcote (1983). The preparation and purification of L-[1-14C]arabinose (300 TBq/mol) has been described (Fry, 1983).
Fusarium hydrochloride 3H[glucosamine from Amersham International. L-[35S]Methionine (1166.5 Ci/mmol) was obtained from New England Nuclear.

Caryophses of maize (Zea mays L. var. Caldera 535, F1) were obtained from Elsom Seeds, Spalding, Lincs., U.K. Fusarium avenaceum (Fr.) Sacc., F6, Philalophora graminicola, P56; P. radiicicola (Cain), Deacon, P29; P. radiicicola, P53; and Trichoderma sp., Pers., ex. Fr., T1 were obtained from the Botany School, University of Cambridge, Cambridge, U.K. F. graminearum 160 243 and F. moniliforme Sheldon (Giiberella fujikuroi Sarado, Ito) 173 211 were obtained from the Commonwealth Mycological Institute, Kew, Surrey TW9 3AF, U.K. Philalophora sp. Medlar R5; P. graminicola K9 and P. graminicola P4 were obtained from J. W. Deacon, Department of Microbiology, School of Agriculture, Edinburgh EH9 3JG, Scotland, U.K.

Collection and radioactive labelling of mucilage

Apices of maize roots were incubated aseptically with [3H]Methionine (200 µCi), [3H]Glucosamine (200 µCi), L-[3H]arabinose (5 Ci) or L-[3H]fucose (1 × 10^-4 Ci) (J. Gould & D. H. Northcote, unpublished work). The specific radioactivity of the mucilage produced and percentage efficiency of incorporation for each of these labels was 102 d.p.m./µg and 0.17%, 66 d.p.m./µg and 0.11%, 2.9 × 10^3 d.p.m./µg and 0.9%, and 118 d.p.m./µg and 0.8% respectively. Unincorporated radioactivity was removed by dialysis and checked by gel filtration (Sephadex G-25, 1 cm × 30 cm column, 0.2 M-disodium tetraborate buffer, pH 8.0) and paper chromatography by using the descending method (Whatman no. 1 paper; ethyl acetate/pyridine/water, 8:2:1, by vol.). The identity of radioactive sugars in polymers was determined by acid hydrolysis and paper chromatography (Wright & Northcote, 1975).

Storage, sporogenesis and germination of fungi

Storage of all fungi and the sporulation of ectotrophic root-infecting fungi and F. moniliforme was by the method of J. Gould & D. H. Northcote (unpublished work). Macrogomidia of other Fusarium sp. were produced by growth in a liquid, carboxymethylcellulose medium (J. Gould & D. H. Northcote, unpublished work) that was shaken (100 rev./min, 5 cm lateral displacement). Trichoderma sp. sporulated on Emerson yeast extract and soluble starch agar (Tuite, 1969). Conidia of all fungi were germinated in Armstrong Fusarium medium at 5 × 10^5 conidia/ml and 25 °C in the dark (J. Gould & D. H. Northcote, unpublished work).

Surface areas of germinant conidia were measured with photomicrographs. Hyphae and non-spherical conidia were assumed to be cylindrical. Widths of conidia were estimated at approximately one-third of the distance from apices. Measurements did not account for the contribution of hyphal or conidial capsules (J. Gould & D. H. Northcote, unpublished work).

Adsorption of radioactive mucilage by fungi

The process of separation of unbound radioactive mucilage from that bound to fungal surfaces was optimized. Aliquots (0.5 ml) of an aqueous solution of mucilage labelled with [3H]arabinose (7.6 × 10^4 d.p.m./ml, 2.6 µg of carbohydrate/ml) were placed on pre-wetted Whatman GF/C (2.5 cm diameter) filters. Filters were washed with various quantities of buffer (10 mM-phosphate, pH 7.0, or 10 mM-Hepes, pH 7.0). Filters (three per treatment) were dried overnight at 60 °C.

The standard adsorption assay mixture contained germinant conidia (5 × 10^7), which were harvested from germination media (100 ml) by filtration (filter pore size 0.45 µm). Fungi were resuspended in one of a variety of buffers (2 ml), containing radioactive mucilage, in Beckman Bio-Vials. Several different batches of radioactive mucilage were used. There was slight variation in composition amongst batches of mucilage (Wright & Northcote, 1975) and therefore data obtained with different batches must be compared with caution. The concentrations of mucilage used were chosen to provide about 200 c.p.m. bound above background radiation levels (80 c.p.m.) per 1 × 10^7 germinant conidia. For mucilage labelled with L-[3H]arabinose, which was used extensively, this corresponded to a final concentration in assay cocktails of 3.37 × 10^4 d.p.m./ml with 2.9 µg of carbohydrate/ml and for another batch to 2.6 × 10^4 d.p.m./ml and 4 µg of carbohydrate/ml. Vials that contained mucilage and fungi were capped and placed on a rotating wheel (32 rev./min) in the dark for various times and at different temperatures. Results presented are the averages for aliquots (4 × 0.4 ml) of complexes of germinant conidia and radioactive mucilage. Controls (4 × 0.4 ml), which lacked germinant conidia and which were run in parallel, have been subtracted. Data plotted graphically show means for independent experiments (two or three) with vertical bars to indicate ranges of results. Data in Tables are expressed as percentages of controls ± S.D. Assay conditions for fractions obtained from mucilage labelled with L-[3H]arabinose were similar, except: (1) mucilage precipitated with 0–55% (v/v) ethanol contained 1 × 10^4 d.p.m./ml, 12 µg of carbohydrate/ml; (2) mucilage precipitated with 55–80% (v/v) ethanol contained 1.4 × 10^4 d.p.m./ml, 4.6 µg of carbohydrate/ml; (3) mucilage soluble in 80% (v/v) ethanol contained 1.8 × 10^4 d.p.m./ml, 6 µg of carbohydrate/ml; (4) mucilage that was soluble in detergent contained 1.9 × 10^4 d.p.m./ml and 1.2 µg of carbohydrate/ml; (5) fraction 1 from an ion-exchange column contained 2.1 × 10^4 d.p.m./ml, 5.5 µg of carbohydrate/ml; (6) fraction 2 contained 2.3 × 10^4 d.p.m./ml, 4.2 µg of carbohydrate/ml. The effects of the addition of lactose, maltose, cellobiose, heparin, yeast mannoprotein, de- sulphated fucoidan, dextran, apple pectin, chondroitin sulphate, concanavalin A and a fucose-binding lectin were determined. Non-radioactive mucilage (600 µg/ml), mucilage precipitated with cetylpyridinium chloride (1.24 mg/ml), and chitin oligomers (3 mg/ml) were also used. The effects of enzymic pretreatment of germinant conidia (2 ml of 2.5 × 10^5/ml) was assessed. Fungi were pretreated for 1 h at 25 °C on a wheel (32 rev./min) with Pronase at 10 mg/ml, or at 5% (w/v) Driselase, lactase, which contained pectinase (0.5%, w/v). Suspensions of germinant conidia were washed with 20 ml of buffer (10 mM-Hepes, pH 7.0) collected by filtration (filter pore size 0.45 µm), resuspended in assay buffer and then assayed under standard conditions. Interspecific and intergeneric comparisons of total adsorption and specific adsorption capacities also used standard assay conditions. For these experiments the standard assay contained germinant conidia (5 × 10^7) in buffer (50 mM-Mes (pH 6.5)/5 mM-Mes, pH 7.0).
Adsorption of mucilage by fungal pathogens

CaCl₂, 2 ml] that contained mucilage labelled with [³H]arabinose (4 µg of carbohydrate/ml, 2.6 × 10⁴ d.p.m./ml). Radioactive mucilage and germinant conidia were incubated for 2 h at 25°C on a wheel (32 rev./min) in the dark. The mucilage bound was separated from unbound mucilage by washing with 10 ml of buffer (10 mM-Hepes, pH 7.0).

Liquid-scintillation counting

The method of Wright & Northcote (1974) was used to estimate the radioactivity of chromatograms. Fungi with bound radioactive mucilage were collected on Whatman GF/C filters, washed, dried at 60°C overnight, cooled and Triton X-100 toluene scintillant was added (3 ml). Samples were left (12 h, dark, 4°C) before radioactivity was determined. Results were converted into d.p.m. with the signal-channels-ratio method for heterogeneous samples and standard curves.

Fractionation and analyses of mucilage

Mucilage was fractionated with ethanol and cetylpyridinium chloride (Barrett & Northcote, 1965). Detergent was removed by precipitation three times with ethanol and dissolution in KCl (Kennedy et al., 1981). CsCl-density-gradient ultracentrifugation of mucilage was performed as described by Green & Northcote (1979). High-voltage electrophoresis on Whatman GF/A was performed in pyridine (0.8 litre), acetic acid (0.024 litre), water (7.56 litres), pH 6.5 (Barrett & Northcote, 1965).

Mucilage was fractionated by ion-exchange chromatography. Ion exchange of polysaccharides with cellulose as the solid phase has problems of hydrogen-bonding and incomplete recovery of samples (Selvendran & Dupont, 1984). Therefore a derivative of small polycrylamide beads (Bio-Gel P2, 40–80 µm diameter) was used as an adsorbent for mucilage. The beads were allowed to react with 1,2-diaminoethane (Imman, 1974) to produce 2 mm-aminoethyl groups/g dry wt. of original gel. The reaction was confirmed with 2,4,6-trinitrobenzenesulphonic acid. A sample of gel was placed in saturated aqueous Na₂B₆O₁₅·10H₂O andaq. 3% (w/v) trinitrobenzenesulphonic acid (5 drops) was added. Primary amines turn orange within 30 s. Radioactive mucilage was diluted with an equal volume of buffer (50 mM-Mes, pH 6.5) and applied to a column (2.5 cm × 20 cm) that had been pre-equilibrated with buffer (25 mM-Mes, pH 6.5) at 4°C. Fractions (2 ml) were collected and aliquots (0.1 ml) assayed for radioactivity. The column was developed with a linear gradient of KCl (0–2 M) in buffer. The column was developed further with: (1) 25 mM-sodium citrate, pH 3, with or without 1 mM-KCl; (2) 25 mM-HCl; (3) 25 mM-sodium hydrogen carbonate, pH 11; (4) 40 mM-Na₂B₆O₁₅·10H₂O, pH 11. Single peaks were pooled, neutralized and dialysed.

Germinant conidia (5.5 × 10⁹) of Phialophora radicola P29 were incubated with mucilage labelled with [³H]arabinose (4.1 µg of carbohydrate, 2.6 × 10⁴ d.p.m., 1 ml) in buffer (50 mM-Mes, pH 6.5; 5 mM-CaCl₂, 7 ml) for 2 h at 25°C in the dark on a rotating wheel (32 rev./min). Germinant conidia were then washed on Whatman GF/C filters to remove unbound radioactivity (10 mM-Hepes, pH 7.0, 50 ml), and resuspended in buffer [50 mM-Mes (pH 6.5)/5 mM-CaCl₂, 1 ml]. The suspension was loaded into a Pasteur pipette and washed with the same buffer until germinant conidia were well packed. This column was developed with buffer (50 mM-Mes, pH 6.5; 25 mM-Na₄EDTA), with a fraction volume of 0.2 ml, and then a linear gradient of KCl (0–1 M in 15 ml) in the same buffer. Scintillant was added directly to the fractions, which had been collected in plastic scintillation vials (Beckman Bio-Vials).

Hydrolsates (Wright & Northcote, 1975) of mucilage were analysed by g.l.c. of alditol acetate derivatives (Sloneker, 1972). Recoveries were calculated after hydrolysis losses, and molar response factors relative to inositol were determined. These were rhamnose, 73.7%; fucose, 66.2%; ribose, 58.3%; arabinose, 74%; xylose, 70.2%; mannose, 83.6%; galactose, 83.6%; glucose, 88.1%. Hexosamines in mucilage were analysed qualitatively as O-methylxime acetate derivatives (Mawhinney et al., 1979). The uronic acid content of mucilage was quantified by reduction of carboxy groups (Taylor et al., 1976). Total carbohydrate was estimated with sulphonated α-naphthol (Dische, 1962). Uronic acids were assayed with m-hydroxybiphenyl (Blumenkranz & Asboe-Hansen, 1973). Protein concentration was determined with modified Bradford reagent (Read & Northcote, 1981).

Details of unpublished methods referred to above can be obtained from D.H.N. on request.

RESULTS

Adsorption of radioactive maize root mucilage by fungi

Radioactive mucilages were compared at similar concentrations (50 µg of carbohydrate/ml) in an adsorption assay in vitro with germinant conidia of Fusarium moniliforme. Of the various radioactive maize root mucilages tested only that sample with the highest specific
radioactivity labelled with [3H]arabinose registered that adsorption had occurred. Other radioactive mucilages may not have shown that adsorption had occurred either because they were insufficiently radioactive or their radioactivity was predominantly in polymers that did not participate in adsorption.

When unbound radioactive mucilage was separated from fungi, a proportion of it was bound non-specifically by the glass-fibre filters used. To ensure that this non-specific adsorption by filters was minimal, a variety of washing procedures were compared (Fig. 1). Phosphate and Hepes buffer (both 10 ml, 10 mM, pH 7.0) gave similar results and were used as washing solutions.

The time courses of adsorption of [3H]arabinose-labelled mucilage by germinant microconidia of Fusarium moniliforme and germinant allantoid conidia of Phialo- phora radicicola are shown in Fig. 2.

The amount of mucilage bound increased with increased time of incubation to a maximum. An incubation period of 2 h was used subsequently. The inability of germinant conidia to bind greater amounts of radioactive mucilage after increased times of incubation reflected either saturation of sites of adsorption on fungal surfaces or selective and exhaustive removal of components from the complex of polymers of mucilage. Various suspension densities of germinant conidia of F. moniliforme or P. radicicola were incubated with fixed quantities of radioactive mucilage (Figs. 3a and 3b respectively).

The proportion of mucilage bound by F. moniliforme was limited solely by the availability of one fraction of the mucilagenous polymers (Fig. 3a). In contrast, the plot of adsorption against suspension density for P. radicicola was biphasic (Fig. 3b). At low suspension densities the proportion of radioactivity bound per conidium was greater than that bound per conidium at higher suspension densities. This probably represented the presence of site(s) on the surface of the pathogen that had different affinities for particular fractions of radioactive mucilage. This feature of P. radicicola was accentuated when this experiment was performed in the presence of CaCl₂.

The proportion of mucilage adsorbed by germinant conidia may have been limited by availability of sufficient sites on fungal surfaces. This was investigated by incubation of various concentrations of radioactive mucilage with a fixed suspension density of germinant conidia (Fig. 4).

At the ratios of mucilage to suspension densities of germinant conidia employed, the proportion of mucilage bound was limited by selective and exhaustive removal of particular components of mucilage and not by the availability of sites for adsorption on fungal surfaces.

**Ability of conidia to bind mucilage**

Germination of conidia re-establishes the vegetative phase of growth that is actively pathogenic and results in
Adsorption of mucilage by fungal pathogens

Fig. 4. Effects on adsorption by germinant conidia of *F. moniliforme* (●) and *P. radicicola* (○) of increased concentration of mucilage in 10 mM-phosphate buffer, pH 7.0, at 25 °C

Fig. 5. Ability of conidia of *F. moniliforme* (●) and *P. radicicola* (○) to bind mucilage in 10 mM-L-Heps, pH 7.0, at 25 °C

![Graph 1](image1)

![Graph 2](image2)

The synthesis of new types of fungal walls. J. Gould & D. H. Northcote (unpublished work) showed, with fluorescently labelled mucilage, that there were differences between the adhesive abilities of conidial and hyphal walls of the ectotrophic root-infecting fungi but not the vascular-wilt fungi. A comparison was made of the ability of conidia (Fig. 5) and germinant conidia (Figs. 3a and 3b) of these fungi, at various suspension densities, to bind radioactive mucilage.

*F. moniliforme* microconidia bound mucilage at all but the lowest suspension densities. The maximum proportion of mucilage bound by conidia of *F. moniliforme* (1.1%) did not increase with vastly increased numbers of conidia present (Fig. 5). This suggested that only one fraction, of the several present in mucilage, was bound by microconidial surfaces. This was also the case with germinant conidia of *F. moniliforme* (Fig. 3a), where the maximum proportion of mucilage bound was similar (1.1%). Allantoid conidia of *P. radicicola* did not bind mucilage at comparable suspension densities. Only at the highest suspension density of 25% examined was there an indication that these conidia bound mucilage (Fig. 5). This may be attributed to the presence of a small proportion of nascent hyphae, because conidial populations were approx. 2% germinant at the time of preparation. The inability of allantoid conidia and ability of germinant allantoid conidia to bind mucilage reflected differences in the character of spore and hyphal surfaces and developmental regulation of expression of adhesins. This confirmed patterns of adsorption of fluorescently labelled polymers (J. Gould & D. H. Northcote, unpublished work).

**Dependence of adsorption on pH**

Zwitterionic buffers were chosen in preference to others because they are non-toxic, not metabolized, buffer over the physiological range required and do not chelate metal ions. The effects of pH on the adsorption of [3H]arabinose-labelled maize root mucilage by germinant conidia of *F. moniliforme* and *P. radicicola* are illustrated in Fig. 6.

*F. moniliforme* and *P. radicicola* have similar pH optima (about pH 6.5) for adsorption of mucilage.

**Effect of buffer strength on adsorption**

The presence of charged groups on and within the fungal wall endowed them with a buffering capacity. An increase in buffer strength to counteract this effect may have adversely affected the capacity of fungi to bind mucilage. The titration of germinant conidia of *F. moniliforme* with 10 mM-L-Heps, pH 7.0, is shown in Fig. 7. The effect of buffer strength on adsorption of radioactive mucilage by germinant conidia of *P. radicicola* is illustrated in Fig. 8.

Suspensions of germinant conidia of *F. moniliforme* and *P. radicicola* (results not shown) had a pH below the optimum (pH 6.5) for adsorption. At higher buffer
Fungi were suspended (with a magnetic stirrer) in degassed water in a small beaker and the pH was monitored as portions of buffer were added. The pH was allowed to stabilize after addition of buffer (10 mM-Hepes, pH 7).

Table 1. Effects of various ions on the adsorption of radioactive mucilage by germinant conidia of *Fusarium moniliforme* 173 211 and *Phialophora radicicola* P29

<table>
<thead>
<tr>
<th>Salt (5 mM)</th>
<th><em>Fusarium moniliforme</em> 173 211</th>
<th><em>Phialophora radicicola</em> P29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 13</td>
<td>100 ± 12</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>290 ± 30</td>
<td>188 ± 13.4</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>72 ± 11</td>
<td>121 ± 7.5</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>170 ± 15</td>
<td>128 ± 12</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>129 ± 10</td>
<td>144 ± 15</td>
</tr>
<tr>
<td>BaCl₂</td>
<td>92.5 ± 19.5</td>
<td>128 ± 16</td>
</tr>
<tr>
<td>SrCl₂</td>
<td>132 ± 3</td>
<td>91 ± 14</td>
</tr>
<tr>
<td>KCl</td>
<td>ND</td>
<td>72 ± 12</td>
</tr>
<tr>
<td>NaCl</td>
<td>ND</td>
<td>109 ± 3</td>
</tr>
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</table>

Results are means ± s.d. Abbreviation used: ND, not determined.

attributable to the anion present (Cl⁻). Other bivalent cations from Group 2 (Ba²⁺, Mg²⁺, Sr²⁺), Mn²⁺ and univalent cations did not have as pronounced an effect as Ca²⁺ or were without effect entirely. When NO₃⁻, rather than Cl⁻, was the counter-ion to Ca²⁺, the adsorption of mucilage by germinant conidia was not enhanced to as great an extent. This may have been due to secondary effects of this anion, e.g. indirectly via nutrition.

The concentration of CaCl₂ in assay cocktails was optimized (Fig. 9).
Table 2. Composition (mol%) of samples of whole and fractionated maize root mucilage

Table 2. Composition (mol%) of samples of whole and fractionated maize root mucilage

<table>
<thead>
<tr>
<th>Sugar</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Fuc</td>
<td>20.4 ± 5.19 (7)</td>
<td>11.6</td>
<td>36.6</td>
<td>22.75</td>
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<td>22.75</td>
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<tr>
<td>Ara</td>
<td>17.5 ± 3.27 (7)</td>
<td>17.6</td>
<td>14.25</td>
<td>13.75</td>
<td>17.5</td>
<td>19.25</td>
</tr>
<tr>
<td>Xyl</td>
<td>15.0 ± 7.57 (7)</td>
<td>22.5</td>
<td>6</td>
<td>13</td>
<td>18</td>
<td>11.75</td>
</tr>
<tr>
<td>Man</td>
<td>3.2 ± 1.33 (7)</td>
<td>5.5</td>
<td>5</td>
<td>4.5</td>
<td>3</td>
<td>3.5</td>
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<tr>
<td>Gal</td>
<td>28.9 ± 3.05 (7)</td>
<td>20.25</td>
<td>35.25</td>
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<td>15</td>
<td>15.25</td>
</tr>
<tr>
<td>Glc</td>
<td>15.0 ± 8.29 (7)</td>
<td>22.6</td>
<td>3</td>
<td>18</td>
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</tr>
<tr>
<td>Protein</td>
<td>1.5% (w/w)</td>
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</table>

The proportion of mucilage bound increased with increased concentration of CaCl₂, to a maximum at 5 mM. A CaCl₂ concentration of 5 mM was used subsequently.

Effect of nutrition during conidial germination on adsorption

The ability of symbionts and pathogens to bind to host surfaces can be modified nutritionally (Smith, 1977; McCourtie & Douglas, 1981; Girardeau et al., 1982; Sherwood et al., 1984). Conidia of *P. radicicola* P29 were germinated in *Arbustum Fusarium* medium with different carbon sources that were at similar concentrations, or in media that lacked vital nutrients. These media did not affect germination. Germinant conidia were then tested for their ability to bind radioactive mucilage.

Conidia germinated in the control medium (sucrose) bound 100 ± 18.8%. With arabinose as carbon source, 81 ± 11.5% was bound; with celllobiose, 85.5 ± 11%; with glucose, 78.5 ± 7%; with lactose, 62 ± 23%; and with maltose, 91 ± 21%. If other constituents of the medium were omitted except the carbon and nitrogen sources, 77% ± 12.4 was bound. These media had very little effect, detectable at this level of sensitivity on the ability of germinant conidia to bind mucilage. This suggested that sites on fungal surfaces for the adsorption of mucilage were present at similar densities on these nutritionally induced states. If the expression of sites for adsorption was subject to modulation by nutrition, then carbon source, iron concentration and the various salts present in germination media were not contributory.

Identification of the fraction of mucilage that was active in adsorption

The identification of those polymers of maize root mucilage that were bound by fungal pathogens was determined by assay of individual, radioactive fractions of mucilage. If a fraction could not be prepared to a sufficiently high specific radioactivity to assay adsorption by fungal surfaces, a similar non-radioactive fraction was prepared. Non-radioactive fractions were tested for ability to inhibit adsorption. Mucilage labelled with [³H]arabinose was precipitated fractionally with ethanol and cetylpyridinium chloride. Fractions were not composed of identical molecules but only enriched, with regard to whole mucilage, by a common physical property such as charge or solubility. The composition of neutral sugars of whole mucilage and fractions obtained from it is illustrated in Table 2.

Uronic acid was measured after reduction. This resulted in an increase (11.5 mol%) in the proportion of galactose. The proportion of glucose in mucilage did not increase. Thus galacturonic acid was the sole uronic acid present. The presence of a trace of glucosamine was expected because the contents of autolysed cells are released into mucilage. The protein content of mucilage was about 1.5% (w/w). This protein did not migrate in polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate, and mucilage labelled with [³H]methionine was not precipitated by saturated (NH₄)₂SO₄. These properties are consistent with protein that was highly glycosylated.

Wright & Northcote (1974, 1975) showed that mucilage was composed of three fractions, one neutral, one weakly acidic and one strongly acidic. The proportion of the most acidic fractions was subject to great quantitative variation. A similar composition was suggested by results for mucilage labelled with [³H]arabinose. Neutral and weakly acidic material were labelled to far higher specific radioactivities than the strongly acidic material. Great clarity of electrophoretic separation, such as that obtained by Wright & Northcote (1974) with whole mucilage labelled with [¹⁴C]glucose, was not found for [³H]arabinose-labelled mucilage. Greater definition of peaks on electrophoretograms was not obtained with increased times of development or with increased applied voltages. Fractional precipitation with ethanol was insufficient to produce single, electrophoretically homogeneous fractions. However, the fraction precipitated by 50% (v/v) ethanol contained the greatest proportion of acid material. Fractional precipitation with detergent produced two fractions. One fraction was soluble in detergent. This fraction was composed of both neutral and weakly acidic polymers. Mucilage precipitated with detergent was by definition highly acidic and had a low specific radioactivity.

Analytical ultracentrifugation of mucilage labelled with [³H]arabinose in continuous gradients of CsCl were consistently not defined as clearly as those profiles obtained by Green & Northcote (1979) with other isotopes. It was demonstrated that mucilage labelled with [³H]arabinose contained both radioactive glycoprotein (density 1.36 g cm⁻³) and polysaccharide.
Table 3. Ability of *P. radicicola* P29 (P29) to bind various neutral (N), weakly acidic (WA) and strongly acidic (SA) fractions of radioactive mucilage

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Specific radioactivity (d.p.m./μg of carbohydrate)</th>
<th>Amount bound by P29 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole mucilage</td>
<td>3 × 10⁴</td>
<td>2.2</td>
</tr>
<tr>
<td>Ethanol (0-55%, v/v)-insoluble (SA)</td>
<td>8 × 10⁴</td>
<td>7</td>
</tr>
<tr>
<td>Ethanol (55-80%, v/v)-insoluble (N, WA, SA)</td>
<td>3 × 10⁴</td>
<td>2.5</td>
</tr>
<tr>
<td>Ethanol (80%, v/v)-soluble (N, WA)</td>
<td>2.9 × 10⁴</td>
<td>0.6</td>
</tr>
<tr>
<td>Cetylpyridium chloride-soluble (N, WA)</td>
<td>1.5 × 10⁴</td>
<td>0</td>
</tr>
<tr>
<td>Fraction 1 material from ion-exchange column (N)</td>
<td>3.9 × 10⁴</td>
<td>0</td>
</tr>
<tr>
<td>Fraction 2 material from ion-exchange column (WA)</td>
<td>5.4 × 10⁴</td>
<td>0</td>
</tr>
</tbody>
</table>

Three fractions were produced from mucilage labelled with [³H]arabinose by ion-exchange chromatography. Fraction 1 (10% of applied radioactivity) was uncharged. Fraction 2 (52%) was eluted with 0.2 m-KCl and therefore weakly acidic. Fraction 3 (10%) had the greatest avidity for the ion-exchange matrix and could only be eluted with 40 mm-disodium tetraborate, pH 11. This caused transelimination.

Fractions of mucilage were compared in ability to be bound by *P. radicicola* (Table 3).

All fractions that were produced by fractional precipitation with ethanol were bound, in part, by germinant conidia. Of these fractions, mucilage precipitated with 0-55% (v/v) methanol had the greatest proportion of material bound. Mucilage that was soluble in cationic detergent, and fractions 1 and 2 from anion-exchange chromatography, were unable to be bound by germinant conidia. Therefore, by deduction, fraction 3, which could not be isolated in an undegraded form, or material that remained bound to the ion-exchange column and mucilage that was precipitated by detergent and which was able, when non-radioactive (see below), to inhibit the adsorption of whole radioactive mucilage, contained those polymers that were bound by P29.

**Effects of haptens, lectins and enzymic modification of fungal surfaces on adsorption**

The effects of monosaccharides, amino sugars and uronic acids (at 1 mg/ml) on adsorption of radioactive mucilage by P29 were determined. The control bound 100 ± 15%. In the case of rhamnose, 87 ± 7.5% was bound; with fucose, 78.5 ± 14%; with ribose, 129 ± 14%; with arabinose, 85 ± 15%; with xylose, 83 ± 11.5%; with mannose, 100 ± 7%; with galactose, 85.6 ± 13%; with glucose, 92.5 ± 12%; with 2-deoxy-glucose, 112.5 ± 12.5%; with galacturonic acid, 100 ± 13%; with glucuronic acid, 92 ± 24%; with inositol, 120 ± 14%; with glucosamine, 105 ± 10%; with galactosamine, 101 ± 3%; and with N-acetylgalactosamine, 115 ± 14%. The constituent monosaccharides of mucilage were tested at the higher concentration of 10 mg/ml. None of these sugars, amino

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Table 4. Effects of various temperatures, oligomers, polymers, lectins and enzymatic pre-treatments of germinant conidia on the adsorption of radioactive mucilage by *P. radicicola* P29

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc. (mg ml⁻¹)</th>
<th>Amount bound (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>100 ± 15</td>
</tr>
<tr>
<td>Lactose</td>
<td>1</td>
<td>96 ± 16</td>
</tr>
<tr>
<td>Maltose</td>
<td>1</td>
<td>115 ± 18</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>1</td>
<td>58 ± 20</td>
</tr>
<tr>
<td>Chitin oligomers</td>
<td>3</td>
<td>77 ± 7.5</td>
</tr>
<tr>
<td>Heparin</td>
<td>1</td>
<td>94 ± 21</td>
</tr>
<tr>
<td>Yeast mannoprotein</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fucoidan</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dextran</td>
<td>1</td>
<td>14 ± 12</td>
</tr>
<tr>
<td>Apple pectin</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Chondroitin sulphate</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Maize root mucilage</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>Detergent-insoluble</td>
<td>1.24</td>
<td>0</td>
</tr>
<tr>
<td>Mucilage</td>
<td>1</td>
<td>52.5 ± 18*</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>1</td>
<td>62 ± 13.†</td>
</tr>
<tr>
<td>Fucose-binding protein</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Driselase and pectinase</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>Proteinase</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>25 °C</td>
<td>---</td>
<td>100 ± 12</td>
</tr>
<tr>
<td>4 °C</td>
<td>---</td>
<td>52 ± 14</td>
</tr>
</tbody>
</table>

* Independent of 0.1 m-Man.
† Independent of 0.1 m-Fuc.
sugars or uronic acids affected appreciably the adsorption of radioactive mucilage, so that adsorption of mucilage by pathogens was probably not a result of interactions between fungal-borne lectins and sugar residues in mucilage. However, the variety of monosaccharides examined was not exhaustive, and fungal lectins with very high affinity for substrates would not have shown reversal or inhibition of binding at the concentrations of monosaccharides used.

The effects of inclusion of various substances in the adsorption assay and enzymic modification of fungal surfaces are illustrated in Table 4. Viability of germinant conidia was assessed before and after treatment with enzymes by staining with fluorescein diacetate. Appearance before and after enzymic modification, after staining with fluorescein diacetate, was indistinguishable. Therefore effects on adsorption were attributable directly to enzymic modification and not a secondary effect that resulted from metabolic debilitation.

**An interspecific comparison of adsorption amongst pathogens of maize, pathogens of other plants and non-pathogens**

In order to compare the ability of different genera, species and fungal surfaces to bind mucilage, data should be based upon specific capacity (mucilage bound/unit of fungal surface area). The effect of germination and growth on capacity of *Phialophora sp.* R5 to bind maize root mucilage is illustrated in Fig. 10. The capacity of germinant conidia of various species of pathogen and non-pathogen to bind mucilage is shown in Table 5.

**Preliminary identification of components of fungal cell walls that are involved in adsorption of mucilage**

The nature of the bonds formed between mucilage and the walls of a fungal pathogen was examined by chromatography of mucilage labelled with \(^{3}H\)arabinose on germinant conidia of *P. radicola* P29 (Fig. 11).

EDTA eluted 45% of the radioactive mucilage that remained associated with the germinant conidia after washing in an approximately symmetrical peak. A further quantity (15%) of mucilage was eluted as a rather less symmetrical peak by a salt concentration that corresponded to about 0.2 M-KCl. A large part (40%) of radioactive mucilage was not removed from germinant conidia.

The extent of metabolism of bound radioactive mucilage was indicated by comparison of chromatograms of hydrolysates of samples of mucilage that had and had not been bound by fungi. Radioactive profiles were similar for both samples. A great proportion (35% of total) of radioactivity remained immobile at the origin of the chromatogram (Wright & Northcote, 1975). A large part of radioactivity (32.5%) co-chromatographed with authentic l-arabinose, whereas a smaller proportion (12.5%) co-chromatographed with authentic xylose/fucose. Mucilage bound under these conditions was not metabolized.

**DISCUSSION**

The optimization of adsorption assumed that an increase in quantity of mucilage bound by germinant conidia represented a movement in assay conditions towards a single optimum. This was correct only if one fraction of the heterogeneous collection of polymers that constitutes mucilage was bound by a single site on surfaces of germinant conidia. The similar values of the adsorption optima for time, pH and Ca\(^{2+}\) for both *F. moniliforme* and *P. radicola* showed that there was at least one general method of adsorption. However, *P. radicola* bound a greater proportion and variety of the polymers of mucilage than did *F. moniliforme*. Sites for adsorption on *P. radicola* were present predominantly on surfaces formed by germination. This is in contrast with the situation in *F. moniliforme*, where...
mucilage was bound by both conidial and hyphal surfaces. These patterns of adsorption have also been shown with fluorescently labelled mucilage (J. Gould & D. H. Northcote, unpublished work).

The adsorption of mucilage by *P. radicicola* was abolished by either protease or polysaccharase digestion of fungi, so that adsorption was dependent upon the integrity of fungal molecules that contained both carbohydrate and protein. Other workers have suggested that pathogenic fungi bind to host surfaces with lectins (Nordbring–Hertz & Mattiasson, 1979; Hinch & Clarke, 1980, 1982; Barak et al., 1985). However, we found no evidence for the involvement of fungal lectins. Some lectins have stringent requirements for specific oligosaccharide sequences. These could possibly be determined for the maize–fungal interaction by the use of oligomers derived from mucilage. Chromatography of radioactive mucilage on germinant conidia suggested that there were at least three ways in which mucilage was bound. One of these was dependent on Ca\(^{2+}\) (Ca\(^{2+}\) bridges), another independent of Ca\(^{2+}\) and disrupted by salt (ionic interactions between charged polymers) and the third was independent of Ca\(^{2+}\) and resistant to high salt concentrations. Ca\(^{2+}\) may have acted by formation of ionic bridges between anions on fungal surfaces (J. Gould & D. H. Northcote, unpublished work) and in mucilage. Ca\(^{2+}\) could also form bridges between *vicinal* hydroxy groups and carboxy groups of neighbouring polymers (Schweiger, 1964).

*P. radicicola* bound only the most acidic of the various radioactive fractions of mucilage. The low specific radioactivity of mucilage that was precipitated by cationic detergent must have resulted from a comparative paucity of arabinose in the acidic moiety of mucilage. At a 310-fold excess (w/w, carbohydrate) this fraction of mucilage inhibited adsorption of radioactive mucilage completely. In general, adsorption was inhibited by other negatively charged polymers. However, there were two exceptions: heparin and dextran. Inhibition of adsorption by dextran and the polyanions fucoidan and chondroitin sulphate may have resulted simply from an increase in viscosity or by preferential formation of ionic associations with mucilage. None of these compounds when fluorescently labelled was bound by germinant conidia (J. Gould & D. H. Northcote, unpublished work). Heparin did not inhibit adsorption, and when labelled with fluorescein was not bound by fungi.

The effect on adsorption of apple pectin and unlabelled mucilage was predicted because both of these polymers were bound by fungal surfaces when labelled with fluorescein (J. Gould & D. H. Northcote, unpublished work). Complete inhibition was achieved with a 150-fold excess (w/w, carbohydrate) of mucilage and a 250-fold excess (w/w, carbohydrate) of apple pectin. The similarity between the biochemistries of pectins and mucilages (Gould & Northcote, 1985) suggested that inhibition of adsorption of mucilage by these polymers was by direct competition for sites for adsorption on fungal surfaces.

Yeast mannoproteins inhibited completely the adsorption of radioactive mucilage by *P. radicicola*. Mannoproteins have been implicated as the polymers responsible for the adsorption of the fungal pathogen *Candida albicans* to host epithelia (Centeno et al., 1983; Lee & King, 1983; Sobel et al., 1984). It is probable that similar glycoproteins are present on the surfaces of most other fungi (Ballou, 1982; Nakajima et al., 1984a,b) and possible that, like mannoproteins of *C. albicans*, they too are able to bind other polymers. Fluorescently labelled mannoprotein did not bind to fungal surfaces (J. Gould & D. H. Northcote, unpublished work). Therefore inhibition of adsorption of radioactive mucilage may have resulted from a pre-emptive interaction between dissolved mucilage and mannoprotein before adsorption by fungal surfaces.

Most of the \(\beta\)-linked di-, oligo- and polysaccharides tested did not inhibit appreciably the adsorption of mucilage. This indicated that arabinogalactan proteins (\(\beta\)-lectins), which may be present in mucilage (A. E. Clarke, personal communication) and which bind \(\beta\)-glycosyl residues (Fincher et al., 1983), did not play a major role in adsorption.

The inhibition of adsorption of mucilage with lectins was independent of the presence of their specific hapten. Hinch & Clarke (1980, 1982) examined the adsorption of zoospores of the phytopathogenic fungus *Pythophthora cinnamoni* to root surfaces of the non-host *Zea mays*. Adhesion was abolished by periodate oxidation of entire roots or enzymic removal of terminal fucose residues of mucilage and fucose-binding lectins. They suggested that adherence was mediated by fucose-binding proteins on the zoospore surface and fucose residues in maize root mucilage. However, fucose is rare in mucilages of other plants (Rougier, 1981; Northcote, 1982), and no direct correlation was made between adherence that was mediated by fucose and pathogenicity.

All fungi tested bound mucilage to some extent. There was no clear-cut gross distinction of capacity to bind mucilage with pathogens of roots, pathogens of other parts of plants and non-pathogens. However, the highly specialized ectotrophic root-infecting fungus *P. radicicola* P29 was capable of binding a greater proportion and variety of mucilagenous polymers than was the vascular-wilt fungus *F. moniliforme*, which has a more catholic host and tissue range. An ability to bind some fractions of mucilage may be unrelated to disease specificity. Other isolates of *Phialophora radicicola* bound less mucilage than P29. This may have resulted from a loss of pathogenic character in culture. These difficulties could be resolved by combination of the assay with a test for pathogenicity. The definition of degree of germination was insufficient for precise comparison of gross and specific adsorption capacities of the vascular-wilt fungi or the ectotrophic root-infecting fungi, the former because both conidial and hyphal surfaces bound mucilage and the latter because there was a linear increase in capacity of *Phialophora sp*. R5 to bind mucilage with increased time of growth which was unrelated to the percentage germination. This was unexpected, because the hyphal apices of the ectotrophic root-infecting fungi were solely responsible for adsorption of fluorescently labelled mucilage (J. Gould & D. H. Northcote, unpublished work).

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REFERENCES

Adsorption of mucilage by fungal pathogens


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