Lectin-Detectable Differences in Carbohydrate-Containing Surface Moieties of Macroconidia of Fusarium roseum 'Avenaceum' and Fusarium solani

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ABSTRACT

Relative qualitative and quantitative aspects of the structural-chemical nature of the mucilaginous layers of the spores of *Fusarium roseum* 'Avenaceum' and *Fusarium solani* f. sp. *phaseoli* are revealed as differences in spore agglutination by lectins which bind to specific carbohydrate-containing receptor sites. Available receptors for Concanavalin A (Con A) were most numerous on the nontreated spore surfaces of either species. Receptors for wheat germ agglutinin (WGA) and ricin were not detected on nontreated surfaces. Trypsin-treated *F. roseum* macroconidia indicated moderate amounts of ricin receptors but few Con A and WGA receptors. Trypsin-treated *F. solani* macroconidia suggested massive quantities of WGA receptors and moderate amounts of Con A and ricin receptors. Macroconidia of *F. roseum* stripped of the mucilaginous coat by KOH treatment revealed moderate quantities of Con A, WGA, and ricin receptors. Similarly treated *F. solani* spores showed massive amounts of Con A receptors and no detectable WGA or ricin receptors at this structural level.

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The complementary molecules responsible for the specific associative and compatible behavior of cells involved in cellular recognition, aggregation, histocompatibility, and other immunologically based cellular phenomena are most often carbohydrate-containing surface moieties such as glycoproteins, glycolipids, and polysaccharides (13, 20). These cell surface moieties, particularly carbohydrates, have also recently been implicated in the specificity of plant host-parasite relationships (1). However, information regarding the chemistry and architecture of the outermost layer of different fungal spore walls intimately involved in specific pathogenicities is incomplete. Initial investigations by Marchant (12) showed the outermost layer of the macroconidium of *Fusarium roseum* 'Culmorum' (Link) emend. Snyd. & Hans. to be mucilaginous in nature and probably composed principally of xylan. No other species of *Fusarium,* however, were investigated. Preliminary studies by Kleinschuster and Baker (8) of the macroconidia of *F. roseum* 'Avenaceum' using enzymatic and chemical surface alteration and a technique employing several plant lectins (which agglutinate cells in a fashion resembling immunological reactions by binding to specific carbohydrate-containing receptor sites on cell surfaces) revealed in part the relative qualitative and quantitative carbohydrate composition of the outermost layer of this spore.

The present study investigated the lectin-detectable carbohydrate composition of normal and altered macroconidial surfaces of the carnation pathogen, *F. roseum* 'Avenaceum' and the bean pathogen, *Fusarium solani* f. sp. *phaseoli* (Mart.) Appel & Wr. emend. Snyd. & Hans., in an attempt to demonstrate a chemical-structural difference in the outer cell surface of two related, but host-distinct, pathogens.

MATERIALS AND METHODS.—Macroconidia of *F. solani* f. sp. *phaseoli* and *F. roseum* 'Avenaceum' were

obtained from 4-wk-old slant cultures by introduction of 5 ml of phosphate buffered saline (PBS) (NaCl, 8 g; KH_2PO_4, 0.34 g; K_2HPO_4, 1.21 g; H_2O, 1 liter; pH 7.3) into the culture tubes, gentle shaking and filtering through several layers of cheesecloth to remove mycelial fragments, followed by washing three times in PBS. The concns of all conidia used in experiments were adjusted to approximately 1.5×10^6/ml of PBS.

Spore surface alteration.—1) Partial degradation.—The partial degradation of spore surfaces was accomplished enzymatically. Trypsin (Armour Pharmaceutical Co.) was used to partially digest the mucilaginous layer of the spore wall, thus exposing its inner architecture and carbohydrate-containing moieties. This was achieved by a 20-min exposure of the spores to a 1% trypsin/Tyrode's CMF (Ca- and Mg-free) solution at 37 C followed by reaction inhibition with soybean trypsin inhibitor (Worthington Biochem. Co.), three washings in PBS, and resuspension in PBS to the final concn.

—2) Total degradation.—Chemical treatment with 4.5% KOH for 2 h reportedly removes the mucilaginous coat totally, thus exposing the next inner layer of the spore wall (12). The present experiments employed a similar treatment at 37 C, after which the spores were washed three times with PBS and resuspended in PBS to the final concn.

Lectins.—Three plant hemagglutinins or lectins were used in the present experiments. Concanavalin A (Con A), binding with high specificity to α-D mannopyranosyl residues and related sugars (5, 10, 19), was used at a concn of 500 μg/0.2 ml of culture medium. Wheat germ agglutinin (WGA), binding preferentially to a n-acetyl glucosamine (2), and ricin (castor bean agglutinin), reportedly binding to terminal nonreducing galactose (15), were also used at final concns of 500 μg/0.2 ml of culture medium (PBS).

Agglutination.—Large Maximov tissue-culture slides were used as the culture vessels for agglutination reactions. To each of these was added 0.05 ml of a conidial suspension and 0.15 ml of lectin solution dissolved in PBS. Control vessels contained only spore suspension and PBS. Vessel contents were placed in an incubator for 10 min at 37 C. During this time, vessels were gently swirled every 30 s to assure maximum cellular contact. At the end of this period, cultures were removed from the incubation chamber and the resulting conidial agglutinations were photographed and arbitrarily scored from 0-7.

Concanavalin A was obtained from Calbiochem. Wheat germ agglutinin was isolated from wheat germ lipase according to Burger and Goldberg (2). Ricin was obtained from Dr. E. Goldwasser, Department of Biochemistry, University of Chicago.

RESULTS.—*Agglutination reactions of F. roseum.*—Figures 1 and 2 show the degree of agglutination of lectin-treated macroconidia of *F. roseum* either nontreated or treated with trypsin or KOH. There was no conidial agglutination in the control, WGA, or ricin cultures of nontreated spores. This reflects the paucity and/or unavailability of receptor sites for these lectins on the outermost surface of the nontreated macroconidium. Conidia of *F. roseum* in the Con A treatments, however, showed a small degree of agglutination indicative of the relative abundance and/or availability of Con A receptors on the surface of the nontreated spore.

Following trypsinization and challenge by the lectins, changes in the agglutination reactions of *F. roseum* macroconidia were readily apparent (Figs. 1 and 2). Proteolytic treatment of *F. roseum* with trypsin rendered available moderate amounts of terminal galactose as indicated by the agglutination reaction of these ricin and trypsin-treated spores (Figs. 1 and 2). Thus, the failure of the nontreated conidia of *F. roseum* to be agglutinated by ricin may not be due to the total lack of receptors for this lectin on the outer surfaces, but rather to receptors being specifically masked by a trypsin-sensitive material or to their being generally located deeper within the cell surface and therefore unavailable for reaction with ricin until unmasked by proteolytic treatment. The agglutination reactions of trypsin-treated spores challenged with Con A and WGA, however, are seen to change only slightly, with Con A and WGA reactions minimally decreasing and increasing, respectively. This indicates the paucity of receptors for these lectins at this newly exposed level of spore surface.

Total hydrolysis and removal of the mucilaginous layer of the *F. roseum* macroconidium by KOH treatment revealed agglutination patterns differing from those seen in nontreated or trypsin-treated spores (Figs. 1 and 2). There was no agglutination of the control cultures. However, moderate increases in the numbers or availability of receptors for Con A and WGA at this conidial architectural level, exposed after removal of the mucilaginous layer, were indicated by the increased agglutination responses to these lectins when compared to the corresponding trypsin-treated spore reactions. Also, a decrease in the numbers or availability of ricin

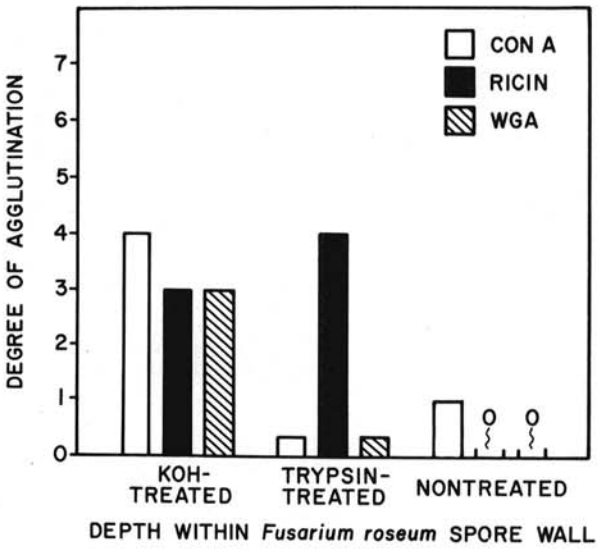

Fig. 1. Agglutination reactions of nontreated, trypsin-treated, and KOH-treated macroconidia of *Fusarium roseum* after 10 min incubation with Concanavalin A (Con A), wheat germ agglutinin (WGA), and ricin indicating relative differences in available receptors for each lectin at each architectural level of the spore wall.

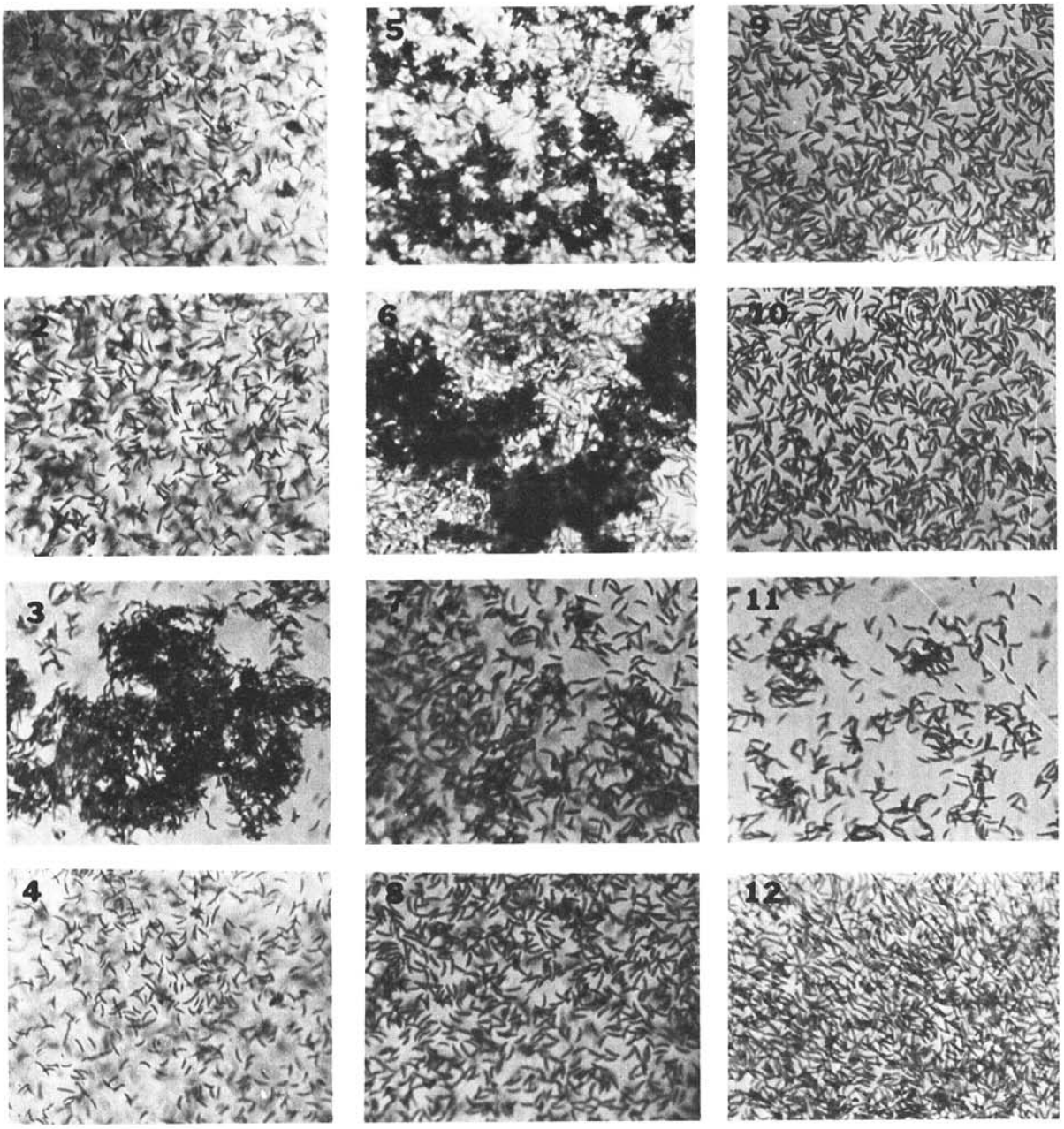

Fig. 2-(1 to 12). Agglutination reactions of nontreated (9-12), trypsin-treated (5-8), and KOH-treated (1-4) macroconidia of *Fusarium roseum* after 10 min incubation with Concanavalin A (Con A) (3, 7, 11), wheat germ agglutinin (WGA) (2, 6, 10), and ricin (1, 5, 9) indicating relative differences in available receptors for each lectin at each architectural level of the spore wall. Controls (4, 8, 12).

receptors at this structural level compared with the trypsinized level was seen.

Agglutination of treated or nontreated spores with Con A, WGA, or ricin could be reversed by the addition of equimolar or higher amounts of α-methyl mannopyranose, *n*-acetyl glucosamine, or galactose to the respective cultures. Inhibition of agglutination was seen if the hapten was added to the cultures prior to the addition of the lectin.

Agglutination reactions of F. solani.—Agglutination reactions of lectin-challenged, treated, and nontreated conidia of *F. solani* are shown in Figs. 3 and 4. Agglutination reactions of the nontreated spore surface of *F. solani* did not differ from those of *F. roseum* (Figs. 1 and 2), indicating similarity of numbers and availability of each lectin receptor on either nontreated spore.

The agglutination reactions of trypsin-treated *F. solani* spores, however, differ dramatically from those of either

nontreated *F. solani* or trypsinized *F. roseum* macroconidia. Although receptors for WGA were unavailable for lectin detection on nontreated spores of *F. solani* and only minimally detected on trypsinized *F. roseum* spores, they were massively exposed and available on trypsin-degraded surfaces of *F. solani* spores (Figs. 3 and 4). This is interpreted to indicate that n-acetyl glucosamine is cryptically present and proteolytically susceptible in the surface architecture of *F. solani*. It is interesting that the spore of *F. roseum* after proteolysis had little n-acetyl glucosamine at the same architectural level (Figs. 1 and 2). Similarly, whereas Con A receptors were barely detected on trypsinized *F. roseum* conidia and showed only slight numbers or availability on nontreated *F. solani* spores, they were moderately exposed and available for agglutination on *F. solani* spores following trypsinization (Figs. 3 and 4). Apparently, masked Con A receptors were present within the cell surface architecture of *F. solani* spores but were absent within the cell surface of *F. roseum* spores at the same structural level.

Ricin receptors of trypsinized *F. solani* spores, not detected on nontreated spores of either species, were rendered available and were exposed to the same moderate degree of *F. roseum* spores after similar treatment. Consequently, the interpretation of the agglutination reaction of trypsinized *F. roseum* spore challenged with Con A previously discussed applies to this situation as well.

As with *F. roseum,* total hydrolysis and removal of the mucilaginous layer of *F. solani* spores by KOH treatment revealed dramatic differences in the receptors of the spore surface at this level (Figs. 3 and 4). The numbers or availability of receptors for Con A on KOH-hydrolyzed *F. solani* spores, as indicated by the agglutination responses, increased greatly compared with those available on trypsinized *F. solani* spore surfaces. It also appears that the *F. solani* spores had many more Con A receptors available for detection following hydrolysis than did *F. roseum* spores (Figs. 1 and 2). This may reflect a compositional difference between the next, inner structural layer of *F. solani* and *F. roseum*.

Wheat germ agglutinin receptors, massively available and exposed on trypsinized spores of *F. solani,* were not detected on this same species after KOH hydrolysis. These results contrast with those indicating presence of WGA receptors on KOH-treated *F. roseum* spores, whose numbers or availability increased moderately from a minimal level after trypsinization (Figs. 1 and 2). This also may indicate inner, structural differences between these two species.

Following KOH hydrolysis, the numbers or availability of ricin receptors on the surfaces of *F. solani* spores decreased to the nondetectable level as indicated by the accompanying agglutination reaction (Figs. 3 and 4), whereas only a small decrease in these receptors were noted in similarly treated *F. roseum* spores (Figs. 1 and 2). These results again show a compositional difference of the next, inner layer of these two species.

Agglutination of treated or nontreated spores with Con A, WGA, or ricin could be reversed by the addition of equimolar or higher amounts of α-methyl mannopyranose, *n*-acetyl glucosamine, or galactose to the respective cultures. Inhibition of agglutination was seen if the hapten was added to the cultures prior to the addition of the lectin.

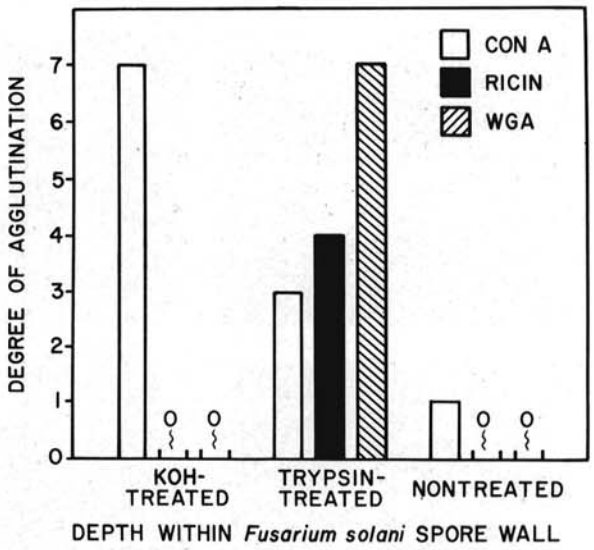

Fig. 3. Agglutination reactions of nontreated, trypsin-treated, and KOH-treated spores of *Fusarium solani* after 10 min incubation with Concanavalin A (Con A), wheat germ agglutinin (WGA), and ricin indicating relative differences in available receptors for each lectin at each architectural level of the spore wall.

DISCUSSION.—Under specified conditions, the surfaces of fungal conidia used in this investigation interacted with Con A, WGA, and ricin in a fashion resembling immunological reactions. Assuming the reported specificites for these lectins are valid for this system, our findings demonstrate that: (i) the carbohydrate-containing receptors for Con A, WGA, and ricin on the spore of a single fungal species are varied in numbers and/or availabilities at different levels of the glycocalyx of the spore surface; and (ii) the numbers or availabilities of receptors for Con A, WGA, and ricin are different at each subsurface level of the cell walls of the two host-distinct, but taxonomically related pathogens used in this study.

Marchant (12), on the basis of electron microscopy and chemical studies, proposed a model for the organization of the microconidial and hyphal wall of *F. roseum* 'Culmorum.' The basic structure of this model consisted of a central, nonfibrillar layer bounded on the inner and outer surface by a layer of randomly oriented chitin microfibrils. The wall of the conidium was reportedly modified by the addition to the outer surface (outer, chitinous microfibrillar layer) of a mucilaginous layer composed largely of xylan. Therefore, this report of the differential deposition of receptors for Con A, WGA, and ricin, presumably α-D methyl mannopyranosyl residues and related sugars, *n*-acetyl glucosamine, and terminal, nonreducing galactose, respectively, within the spore surface of *F. roseum* presents a more complete model of this outermost conidial layer and provides the first data on the outermost layer of the *F. solani* spore.

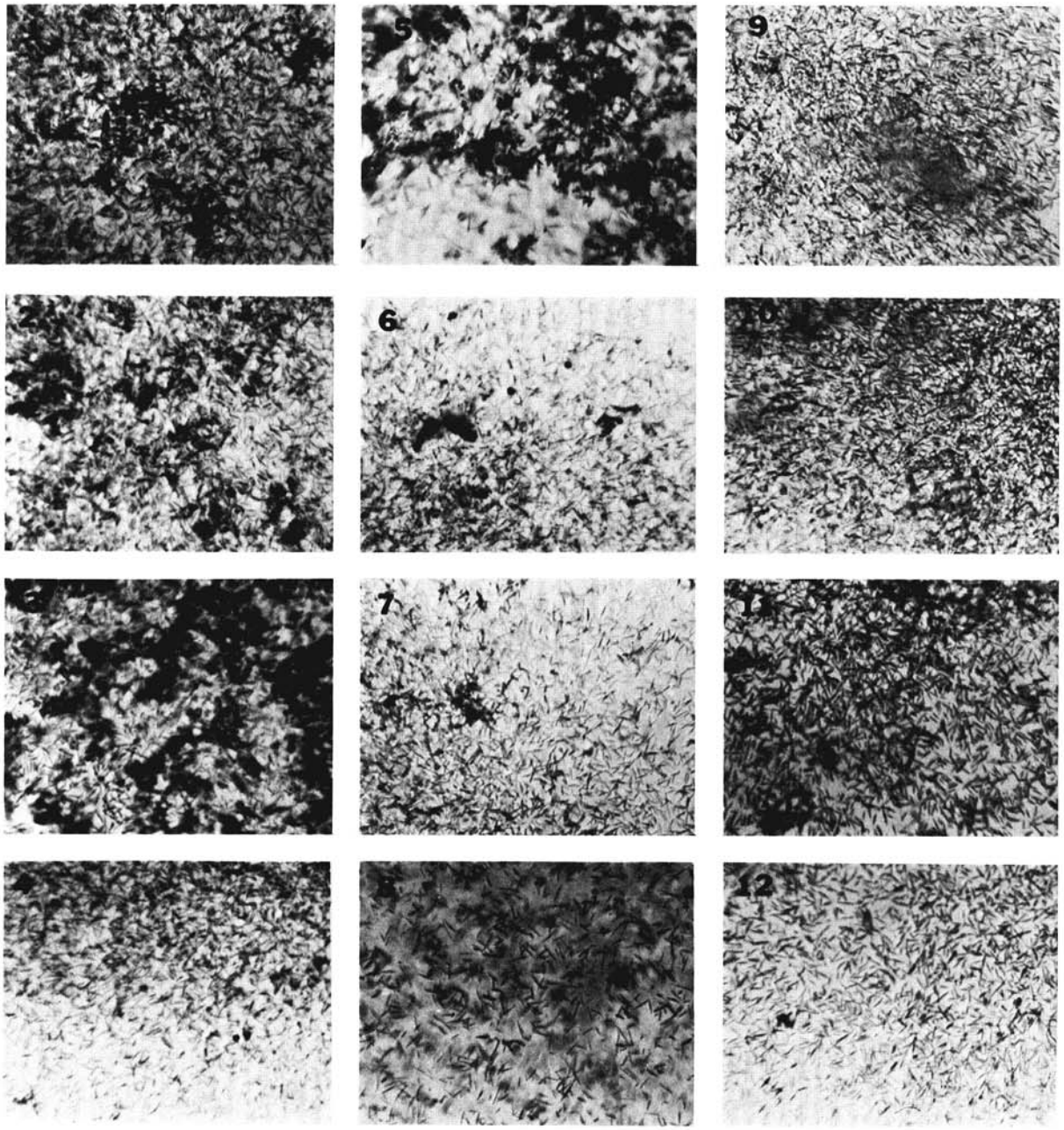

Fig. 4-(1 to 12). Agglutination reactions of nontreated (9-12), trypsin-treated (5-8) and KOH-treated (1-4) spores of *Fusarium solani* after 10 min incubation with Concanavalin A (Con A) (3, 7, 11), wheat germ agglutinin (WGA) (2, 6, 10), and ricin (1, 5, 9), indicating relative differences in available receptors for each lectin at each architectural level of the spore wall. Controls (4, 8, 12).

As noted above, the layer deep to the mucilaginous layer of the *F. roseum* 'Culmorum' spore wall is reported to be chitin, the monomer of which is n-acetyl glucosamine. In this study, receptors for WGA, presumably n-acetyl glucosamine, were found in moderate amounts after removal of the mucilaginous layer of the *F. roseum* spore but was not detected on similarly treated *F. solani* spores. In this context it is interesting to note that Skujins et al. (17) reported that the hyphal wall of *F. solani* probably contains a chitin-containing core, apparently masked by glucans and other substances. This proposed structure of the hyphal wall of *F. solani* is in contrast with the model of the hyphal or conidial wall of *F. roseum* 'Culmorum' proposed by Marchant (12), which contains a nonchitinous core surrounded by chitin. Assuming, as in the case of *F. roseum* 'Culmorum,' that the hyphal and conidial walls of *F. solani* (with the exception of the mucilaginous layer)

are similar, this report supports this structural contrast as the spore of *F. solani* (stripped of its mucilaginous layer) revealed no detectable receptors for WGA, presumably n-acetyl glucosamine. However, massive amounts of receptors for Con A, presumably α-D methyl mannopyranosyl residues or related sugars, were detected at this structural level.

In general, the peripheral components of cell surfaces, particularly carbohydrate-containing moieties (polysaccharides, glycoproteins, and glycolipids), have recently received increased interest. Most of this interest involving cells of animal origin has brought increased understanding of several hitherto unexplained biological phenomena. Through these studies, some employing the use of lectins, it has become evident that cell carbohydrate-containing surface components are intimately involved in the antigenicities and immuno-responses associated with blood-typing (7), gamete recognition in animal (13) and bacterial cells (17), tissue-typing and histocompatibility (11), tumor-specific antigens (6), differentiating neural retina tissue (9), reaggregation of dissociated tissue (14) and cell recognition (4). Host-parasite specificity, which must be considered cellular recognition and compatibility, may also depend on carbohydrate-containing surface antigens as the participative, complementary molecules. This suggestion is supported by the fact that complementary molecules of glycoprotein composition have been isolated from male and female strains of *Chlamydomonas* (18) and also from a sexually agglutinative yeast, *Hansenula wingei* (3).

The evidence reported here suggests the value of a promising investigative approach to this area of study. In this respect, similar research using other fungi in investigations dealing with the exact nature of masked receptors and their exposure following proteolysis should be conducted.

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