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Influence of Mucilage Secreted by Macroconidia of Fusarium solani f. sp. phaseoli on Spore Attachment to Roots of Vigna radiata in Hydroponic Nutrient Solution

A. C. Schuerger and D. J. Mitchell

The Land, EPCOT Center, Walt Disney World Co., P.O. Box 10,000, Lake Buena Vista, FL 32830; and Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

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ABSTRACT


Macroconidia of Fusarium solani f. sp. phaseoli agglutinated after incubating 1.5 h in a crude root extract called root homogenate. Spore agglutination was not observed in deionized water, nutrient solution, or root leachate. Spore agglutination in root homogenate was consistent with spore attachment to roots of Vigna radiata at different combinations of temperature and hydrogen ion concentration. At 25 C and pH 3 or 7, macroconidia did not agglutinate in root homogenate nor attach to intact roots. At 25 C and pH 4, 5, or 6, macroconidia agglutinated in root homogenate and readily attached to roots. A spore mucilage was secreted by terminal and foot cells of germinating macroconidia incubated for 1.5 h in root homogenate at 25 C and pH 4, 5, or 6 but not when macroconidia were incubated at 25 C and pH 3 or 7. Furthermore, the spore mucilage was not secreted by macroconidia when they were incubated in root homogenate at 35 C. Spore mucilage was not observed at the time of spore attachment to roots but was observed during germ tube emergence when macroconidia were incubated in root homogenate for 1.5 h under conducive conditions of temperature and hydrogen ion concentration. Macroconidia age affected agglutination in root homogenate. The strongest agglutination response was observed with macroconidia from 14- to 37-day-old cultures. A weak agglutination response was observed with macroconidia from 7- to 12-day-old and from 40- or 60-day-old cultures. Macroconidia from cultures older than 60 days did not agglutinate in root homogenate.

Additional keywords: agglutinin, lectin, mung bean, scanning electron microscopy.

The soilborne, pathogenic fungus, Fusarium solani (Mart.) Appel. & Wr. f. sp. phaseoli (Burkholder) W.C. Snyder & H.N. Hans., can infect hypocotyl and root tissues of bean plants grown in soil (3,4). Infection can occur through stomata or via direct penetration of host tissues (5,24). In contrast, F. s. phaseoli caused few hypocotyl lesions when mung bean seedlings (Vigna radiata (L.) R. Wilez.) are inoculated in a hydroponic nutrient solution (26). In soil, infective propagules of nonmotile phytopathogenic fungi may be maintained by the stability of the soil matrix in positions favorable for root infection. However, a solid matrix is absent in hydroponic nutrient solution and infective propagules of nonmotile pathogenic fungi must attach directly to root tissues before pathogenesis can be initiated. If spore attachment is impaired, plant infection and disease will be suppressed. For example, disease caused by F. s. phaseoli in mung bean was suppressed when plants were inoculated at pH 7, compared to those inoculated at pH 4–6; disease suppression at pH 7 was attributed to a decrease in the numbers of infective propagules attached to root surfaces (27).

Various mechanisms have been proposed that may mediate fungal attachment to plant surfaces (6,21,22). The secretion of fungal adhesives appears to be involved in the attachment of motile zoospores of Phytophthora (29) and Pythium spp. (12) to root surfaces and in the attachment of nonmotile conidia of Magnaporthe grisea (14) and Cladosporium cucumerinum (23) to foliar and hypocotyl surfaces. The release of adhesive materials appears to be a prerequisite for spore attachment with several pathogenic fungi (14,22,23). In addition, different mechanisms may account for release of adhesive materials by fungal propagules. For example, the secretion of spore mucilage by zoospores of Pythium aphanidermatum occur during zoospore encystment and require no more than 1–3 min (12). In contrast, spore mucilage is released from the spore tips of M. grisea by the rupture of...
Fig. 1. Effects of culture age (10-102 days) on the internal cell structure of macroconidia of *Fusarium solani* f. sp. *phaseoli* (arrows indicate vacuoles; ch = chlamydospores).
the conidium walls during hydration; release of the spore mucilage is rapid and independent of spore contact with a surface (14).

Secrecion of spore mucilage by several *Fusarium* spp. has been reported (16, 19, 20), but the secretion of spore mucilage has not been defined as essential for spore attachment to plant surfaces. Furthermore, only limited work has been reported on the effects of hydrogen ion concentration (H⁺) on adhesion of nonmotile fungal propagules to plant roots; for example, conidia of *F. moniliforme* and *Phialophora graminicola* both responded to different H⁺ in a similar manner, with optimum adsorptions to root mucilage at pH 6.5 (11).

The objectives of this study were to determine if the timing and location of the secretion of spore mucilage are significant factors in the attachment of macroconidia of *F. s. phaseoli* to roots of *V. radiata* and to determine if the combinations of temperature and H⁺ that suppressed spore attachment in a previous study (27) would also suppress the secretion of spore mucilage.

**MATERIALS AND METHODS**

**Effects of culture age on the internal cell structure and agglutination of macroconidia.** Macroconidia of *F. s. phaseoli* (isolate F28A [25], obtained from A. J. Anderson, Utah State University, Logan) were collected in sterile deionized water (SDIW) from 7- to 102-day-old cultures on modified carnation leaf agar (MCLA). MCLA was prepared by adding 15 g of agar (Difeo Laboratories, Detroit, MI) to 1 L of nutrient solution (26) and then floating 8-12, gamma-irradiated carnation leaf pieces onto the liquid surface of the medium in individual 9-cm petri dishes prior to the solidification of the agar. Spores were stained for 5 min in 100 μl of a 0.05% trypan blue stain (26), transferred to the surface of nutrient solution agar (prepared in a manner similar to MCLA but lacking carnation leaves), overlaid with coverslips, and photographed with Polaroid 4 × 5 instant film, type N55 (Polaroid Corp., Cambridge, MA) with a Nikon Optiphot compound microscope (Garden City, NY).

To determine the effects of culture age on spore agglutination, macroconidia were grown on MCLA for 7-102 days, harvested in SDIW, and dispersed in 50 μl of root homogenate (described below) to achieve a final spore density of 5–10 × 10⁷ macroconidia per milliliter. Macroconidia were agitated gently with a magnetic stirrer for 1 or 1.5 h, and 100-μl samples were mounted on acid-washed glass slides and viewed under bright-field microscopy.

Macroconidia were grown on MCLA for 7, 10, 12, 14, or 21 days and prepared for scanning electron microscopy (SEM) to determine the effects of culture age on spore fixation. Two different methods of fixation were tested in which the fixation procedures and buffer rinses were conducted at 4°C. In the first procedure, carnation leaf pieces possessing abundant conidiphores and macroconidia were fixed in 3% glutaraldehyde (Ted Pella, Inc., Tustin, CA) in 0.07 M phosphate buffer (10) at pH 6.8 for 4 h. Samples were rinsed three times at 20-min intervals in fresh buffer and then postfixed in a similarly buffered 1% osmium tetroxide solution (Ted Pella) for 12–18 h. Samples were rinsed three times in buffer at 20-min intervals. After the final buffer rinse, samples were washed three times at 20-min intervals in double-deionized water at 4°C; samples were allowed to warm to room temperature (approximately 23–25°C). In the second procedure, macroconidia of similar ages were fixed for 18–24 h in a 1% nonbuffered osmium tetroxide solution containing 0.003% Photo-flo (Eastman Kodak Company, Rochester, NY), as described by Brown and Brotzman (2). Samples from both fixation procedures were dehydrated in 10% incremental ethanol series. Carnation leaf pieces were critical-point dried in CO₂ and then sputter-coated with gold. Samples were photographed with Polaroid 4 × 5 instant film in a Hitachi S-530 scanning electron microscope (Hitachi Instruments, Inc., Danbury, CT).

**Agglutination of macroconidia in plant-root extracts.** Agglutination of macroconidia of *F. s. phaseoli* was determined in four solutions prepared from components of the plant-hydroponic system. Seedlings of *V. radiata* were grown in a specially designed seed-germination tray (28) to minimize root damage. A root-leachate solution was prepared by incubating 40–50 7- to 8-day-old seedlings of *V. radiata* in 600 ml of nutrient solution for 18 h at room temperature. A root-homogenate solution was prepared by triturating 10 g (fresh weight) of 7- to 8-day-old *V. radiata* roots sever 5 mm below the crown in 30 ml of a hydroponic nutrient solution (26). The root-homogenate extract was added to 1 L of nutrient solution and allowed to stand at room temperature for 60 min. Double-deionized water and fresh nutrient solution were used as control treatments. The root leachates and homogenates were filtered through four layers of cheesecloth and separate 0.45-μm Gelman mini-capsule cartridge filters (Gelman Sciences, Inc., Ann Arbor, MI). Prior to specific tests, root leachate and homogenate and control solutions were adjusted to pH 5.5. The H⁺ of each solution was adjusted in this and all subsequent experiments with 0.01 M HNO₃ and 0.02 M KOH.

Macroconidia from 14-day-old MCLA cultures were rinsed with 40 ml of SDIW. Macroconidia were washed three times by first collecting them on a 5-μm cellulose-nitrate filter (MSI Micron

![Fig. 2. Effects of culture age (A, 7 days; B, 14 days) on fixation of macroconidia of *Fusarium solani* f. sp. *phaseoli* for scanning electron microscopy.](image-url)
Separations, Inc., Westboro, MA) and resuspending them in 40 ml of SDIW. Spore agglutination was determined by dispersing washed macroconidia into 50 ml of each test solution at densities of 5–10 x 10⁶ macroconidia per milliliter. Spore suspensions were gently agitated for 5 h with magnetic stirrers; 100-μl samples were withdrawn from each suspension at 20, 30, 60, 180, or 300 min, mounted on acid-washed glass slides, and observed under bright-field light microscopy. Acid-washed glass slides were essential for observing spore agglutination because macroconidia adhered to debris on unwashed slides.

Spore agglutination was defined as the aggregation of macroconidia in which spore-to-spore contact occurred between terminal or foot cells. Spore agglutination in each 100-μl sample was rated as follows: 0 = no agglutination; 1 = four to 12 small aggregates, with each aggregate composed of four to eight macroconidia; 2 = 12-50 aggregates, with each aggregate composed of four to 12 macroconidia; 3 = 12-50 aggregates, with each aggregate composed of 12-100 macroconidia; and 4 = multiple large aggregates that contained hundreds of macroconidia within each aggregate.

Attachment of macroconidia to roots. Macroconidium attachment to roots of V. radiata was studied with SEM and the buffered glutaraldehyde and osmium tetroxide fixation procedure described above, except that glutaraldehyde fixation was extended to 12–18 h. Seedlings 7–8 days old were transferred to 500 ml of nutrient solution maintained at 25 C and pH 5.5. Plants were supported in the nutrient solution by closed-cell, polyurethane foam plugs that were heat-treated and autoclaved (26). Inoculum was prepared by suspending macroconidia from 10- to 12-day-old MCLA cultures in nutrient solution at a density of 3–5 x 10⁶ propagules per milliliter. Roots were inoculated for 5 or 10 min and briefly rinsed in fresh nutrient solution. Second-order roots (sensu Fitter [9]) were excised for SEM preparation and bright-field microscopy. Roots prepared for bright-field microscopy were stained in 0.95% trypan blue for 5 min and cleared in a lactic acid/water/glycerol (1:1:2) solvent (26). Fifteen root

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Fig. 3. Agglutination of macroconidia of *Fusarium solani* f. sp. *phaseoli* incubated in A, nutrient solution, or B and C, root homogenate (rf = refuse).

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Fig. 4. Attachment of macroconidia of *Fusarium solani* f. sp. *phaseoli* to root surfaces of *Vigna radiata*. Macroconidia attached to A, intact and B, collapsed root hairs (rh) (rf = refuse). C, Macroconidia attached to mucigel (m) on the root epidermis (ep).
Fig. 5. Agglutination of macroconidia of *Fusarium solani* f. sp. *phaseoli* in root homogenate maintained at 25 C and at different hydrogen ion concentrations (a = spore-tip to spore-tip agglutination; fm = filter membrane; np = nutrient solution precipitates).
samples from six separately inoculated plants were observed with either SEM or bright-field microscopy.

Agglutination of macroconidia at different temperatures and \( H^+ \). Macroconidia from 10- to 12-day-old MCLA cultures were incubated at a density of \( 5-10 \times 10^6 \) macroconidia per milliliter for 1 or 1.5 h in 300 ml of root homogenate and then were collected on Whatman No. 42 filter paper (Whatman, Maidstone, England). Filter-paper disks were transferred to fresh nutrient solution and cut into 4-mm\(^2\) pieces for fixation. Macroconidia were fixed in unbuffered osmium tetroxide containing Photo-fluo and prepared for SEM as described above. In one experiment, root temperatures were maintained at 25 C with water baths (27), and separate batches of nutrient solution were adjusted to pH 3, 4, 5, 6, or 7. In a second experiment, the pH was maintained at 5, and separate batches of nutrient solution were adjusted to 15, 25, or 35 C. All experiments were conducted at least three times. The experiment on the effects of culture age on spore agglutination was conducted five times, and the experiments on the effects of temperature and \( H^+ \) on spore agglutination were conducted four times. Unless otherwise stated, the observed responses were similar among separate repetitions of individual experiments.

**RESULTS**

Effects of culture age on the internal cell structure and agglutination of macroconidia. The size and number of vacuoles increased as the age of macroconidia of *F. s. phaseoli* increased (Fig. 1). Vacuoles were not observed in 7- or 10-day-old macroconidia but were abundant in macroconidia older than 47 days. Chlamydospores generally developed from terminal and foot cells in macroconidia older than 60 days. The overall shape and dimensions of macroconidia did not appear to change as age increased.

Macroconidia older than 12 days exhibited wrinkled cell walls when prepared for SEM (Fig. 2). Macroconidia from 7- to 10-day-old MCLA cultures were smooth and uniform in shape. Septation in macroconidia from 7- to 10-day-old cultures was not observed as easily as it was in spores from cultures older than 12 days (Fig. 2). Both fixation procedures yielded similar results.

The age of macroconidia also influenced the intensity of agglutination when spores were incubated in root homogenate. Low numbers (four to 12) of small aggregates (between four and 10 macroconidia per aggregate) were observed when macroconidia from 7- to 12-day-old and from 40- to 60-day-old MCLA cultures were incubated in root homogenate (rating = 1). The strongest agglutination response in root homogenate (rating = 3-4) was observed with macroconidia from 14- to 37-day-old cultures, in which aggregates of several hundred macroconidia were observed. Macroconidia from 87- or 102-day-old cultures did not agglutinate in root homogenate.

**Agglutination of macroconidia in plant-root extracts.** Spore agglutination was not observed in deionized water or nutrient solution (Fig. 3A). Agglutination was weak (rating = 1) and inconsistent (rating = 0 or 1) in different batches of root leachate. A strong and consistent agglutination response (rating = 3-4) was observed when macroconidia of *F. s. phaseoli* were incubated for 20-30 min in root homogenate (Fig. 3B and C). In addition, refuse from MCLA cultures attached to spores when macroconidia were incubated in root homogenate (Fig. 3B). Washing spor suspensions on the cellulose-nitrate filters dramatically reduced, but did not eliminate, culture refuse in subsequent tests.

**Attachment of macroconidia to roots.** Macroconidia consistently attached to the following rhizoplane sites in decreasing order of abundance: root hairs, muigel, sloughed rootcap cells, junction points for the emergence of first-order roots, root epidermis, and root tips (A. C. Schuering, unpublished data). Macroconidia attached to root surfaces at terminal and foot cells of spores (Fig. 4). Macroconidia generally were not washed from roots when roots were rinsed in nutrient solution after inoculation. Macroconidia appeared to attach to both intact (Fig. 4A) and collapsed (Fig. 4B) root hairs of *V. radiata*. Refuse from MCLA cultures and plant roots was observed on the terminal and foot cells of macroconidia attached to root surfaces (Fig. 4). In addition, macroconidia readily attached to aggregates of muigel present on root surfaces (Fig. 4C).

**Agglutination of macroconidia at different temperatures and \( H^+ \).** In a series of experiments, macroconidia of *F. s. phaseoli* were incubated in root homogenate under different temperature and \( H^+ \) environments. Agglutination of macroconidia (rating = 3-4) was observed at 25 C and pH 4, 5, or 6 but not at 25 C and pH 3 or 7 (rating = 0) (Fig. 5). At 25 C and pH 7, salts from the nutrient solution precipitated and subsequently adhered to macroconidium surfaces (Fig. 5); however, spor-tip agglutination of macroconidia was not observed at pH 7. When \( H^+ \) was maintained at pH 5 and temperature varied, agglutination of macroconidia was observed at 15 or 25 C (rating = 2-3 or 3-4, respectively) but not at 35 C (rating = 0) (data not shown).

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**Fig. 6.** Attachment of refuse from cultures of modified carnation leaf agar (MCLA) to macroconidia of *Fusarium solani* l. sp. phaseoli when macroconidia were incubated in root homogenate. A. Refuse (rf) from MCLA cultures (co = conidiophore). B. Refuse present as reactive centers for spore agglutination (te = terminal cell; fc = foot cell of macroconidia).
Fig. 7. Spore mucilage at the tips of macroconidia of *Fusarium solani* f. sp. *phaseoli* during germination of conidia in root homogenate. **A**, Spore mucilage (sm) at the sites of germ tube (gt) emergence from apical cells of macroconidia (ma). **B** and **C**, Spore mucilage exhibiting qualities of a soft viscous material. **D**, Spore mucilage bound to refuse (rf) present in the spore suspensions. Arrows delimit the extent of the spore mucilage.
Refuse from MCLA cultures and roots attached to macroconidia tips in temperature and H\(^+\) combinations in which spore agglutination was observed (Fig. 6B). In contrast, refuse was not observed on macroconidia tips in treatments in which spore agglutination failed to occur. The accretion of refuse on apical cells of macroconidia was observed only in root homogenate and when spores were attached to roots of *V. radiata*. Macroconidia incubated in nutrient solution, SDIW, or root leachate were free of refuse regardless of the temperature or H\(^+\). Refuse probably was composed of mycelial fragments, fungal cytoplasm, and shards of carnation leaves concomitantly harvested with macroconidia from MCLA cultures (Fig. 6A). Refuse appeared to be a center for spore attachment (Figs. 3 and 6). In addition, small fragments of refuse were attached to cell walls of terminal, intercalary, and foot cells of macroconidia (Fig. 6B) when spores were incubated in root homogenate at 15 or 25°C and pH 4.5, 5, or 6. Terminal and foot cells of *F. s. phaseoli* macroconidia appeared to be equally receptive to the attachment of refuse. Refuse was not observed on spore tips when macroconidia were incubated at 25°C and pH 3 or 7 or at 35°C and pH 5.

An amorphous material, termed spore mucilage, appeared to be secreted by terminal and foot cells of germinating macroconidia incubated for 1.5 h in root homogenate at 25°C and pH 4.5, but not when macroconidia were incubated at 25°C and pH 3 or 7 (Fig. 7). In a second experiment in which H\(^+\) was maintained at pH 5 and temperature was adjusted to different levels, spore mucilage was observed on macroconidia at 15 or 25°C but not at 35°C. When macroconidia were incubated in root homogenate, germ tubes emerged from the apices of terminal and foot cells. The spore mucilage was observed at the sites of germ tube emergence but not along the lengths of the elongating germ tubes (Fig. 7A). The spore mucilage was a soft, viscous material that appeared to flow away from spore tips on contact with other surfaces (Fig. 7B and C). Spore mucilage was observed on macroconidia incubated in root homogenate for 1.5 h but rarely after only 1 h. Mucilage was never observed on the spore tips of macroconidia that had attached to root surfaces. The accretion of refuse to spore tips did not appear to require spore mucilage (Fig. 6B); refuse apparently could attach to spore mucilage on macroconidia incubated in root homogenate, however (Fig. 7D).

**DISCUSSION**

Previous studies (12, 14, 15, 22, 23, 29, 30) indicated that secretion of adhesives is associated with the ability of several fungi to attach to plant surfaces. We hypothesized that if a secreted spore mucilage was involved in the attachment of macroconidia of *F. s. phaseoli* to roots of *V. radiata*, then secretion of the spore mucilage should coincide with spore attachment under different temperature and H\(^+\) environments. In the current study, a spore mucilage was observed on macroconidia at the time of germination (approximately 1.5 h) in root homogenate but not during the initial stages of spore attachment to roots of *V. radiata*. Spore mucilage was always observed at the tips of macroconidia at the time of germ tube emergence. The secreted spore mucilage was similar to flocculents described for germ tubes of macroconidia of *F. s. phaseoli* incubated in sugar solutions (28) or of germinating conidia of *Erysiphe graminis* (18). Although spore tips were involved in macroconidium attachment to roots and as sites for agglutination in root homogenate, the spore mucilage may not adhere spores to roots because mucilage was not observed at the time of spore attachment. These results do not eliminate the possibility that an undetected, rapidly secreted, thin layer of adhesive material was involved in spore attachment to roots. For example, secretion of an adhesive cyst coat by *F. aphiderteratum* occurs 1–3 min (12).

Secretion of spore mucilage, accretion of refuse to spore tips, agglutination of macroconidia in root homogenate, and spore attachment to root surfaces affected similarly by different temperatures and H\(^+\). These processes were all suppressed at pH 3 or 7 and at 35°C, which confirmed other quantitative studies on the effects of temperature and H\(^+\) on spore attachment by macroconidia of *F. s. phaseoli* to roots of *V. radiata* (27). These processes probably are related because they respond to temperature and H\(^+\) in similar ways.

Results from the current and other (28) studies indicate that the secretion of spore mucilage probably occurs after spore attachment of macroconidia to roots. We propose that the process of germination begins with the contact of macroconidium tips to root surfaces. Furthermore, the first step in this process may be the adsorption or binding of a plant-associated inducing agent to macroconidium tips that initiates spore agglutination and germination (28). Agglutinins have been described on root mucilage (11), in hypocotyl cell walls of leguminous plants (13), and as soluble extracellular proteins or glycoproteins in plant roots (1).

Differences occurred in the internal cell structure of macroconidia, spore agglutination, and quality of SEM fixation as the age of macroconidia increased. Furthermore, macroconidium age affected the sensitivity of macroconidia to agglutination in root homogenate (28). These effects support the possibility that receptive sites for an inducer on spore tips change in either quality or quantity as macroconidia age.

Additional research is required to clarify the role of the spore mucilage in macroconidium attachment and agglutination. SEM studies utilizing cryo-scanning with a nitrogen flush to reduce artifacts of fixation (17) may be useful in determining whether a rapidly secreted, thin layer of adhesive material is present on spore tips early in the attachment process. Furthermore, histochemical staining and competitive inhibition studies may help characterize the chemical nature of the binding phenomenon. However, in preliminary tests of the current study, histochemical and fluorescent stains described for the visualization of mucilaginous hyphal sheaths of *Bipolaris* spp. (7, 8) proved inconclusive in staining secreted spore mucilage of *F. s. phaseoli* (A. C. Schuerger, unpublished data). The mucilaginous sheaths of *Bipolaris* spp. (7, 8) are composed of multiple layers extending several micrometers from germ tubes. In contrast, the secreted spore mucilage of *F. s. phaseoli* in the current study was limited in size and distribution. Thus, there may be a physical limitation to the resolution of the secreted spore mucilage on germ tubes of *F. s. phaseoli* with light microscopy.

**LITERATURE CITED**