Influence of Carbon Source on Attachment and Germination of Macroconidia of *Fusarium solani* f. sp. *phaseoli* on Roots of *Vigna radiata* Grown in Hydroponic Nutrient Solution

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ABSTRACT

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Twenty carbon sources were tested for their ability to induce or inhibit the agglutination of macroconidia of *Fusarium solani* f. sp. *phaseoli* in hydroponic nutrient solution. Carbon sources were added individually to fresh nutrient solution or to a root homogenate from *Vigna radiata*. An unknown factor present in the root homogenate, and believed to be of plant origin, consistently induced the agglutination of macroconidia within 20 min at 25 C and pH 5.5. Twenty carbon sources did not induce spore agglutination when tested at 50-mM concentrations in fresh nutrient solution, and they did not inhibit spore agglutination when tested at 50- or 100-mM concentrations in root homogenate. In a separate experiment, eight hapten sugars of plant lectins did not inhibit spore attachment to roots when tested at 50- or 100-mM concentrations in fresh nutrient solution. Observations did not support the hypothesis that plant lectins were involved in spore attachment to roots. Macroconidia germinated within 1.5–2.0 h in root homogenate or on root surfaces but required up to 5 h to germinate in 50-mM solutions of D-glucose, D-mannose, or sucrose. Spore germination was not observed when macroconidia were incubated for 24 h in 50-mM solutions of D-fucose, D-galactose, or fresh nutrient solution. Furthermore, macroconidia germinated primarily from the tips of terminal and foot cells when attached to roots or incubated in root homogenate but primarily from lateral walls of intercalary cells when incubated in various carbon sources. Spore germination during in vitro tests with various carbon sources differed greatly from spore germination on the root surface or in root homogenate.

Additional keywords: morphometrics, mung bean, SEM, spore mucilage.

Lectins were defined by Goldstein et al (9) as sugar-binding proteins or glycoproteins of nonimmune origin that agglutinate cells or precipitate glycoconjugates. Lectins, also referred to as phytoagglutinins, occur in many plant species from several families of flowering and nonflowering plants (6). These compounds may be involved in recognition and attachment of nitrogen-fixing bacteria to roots of leguminous plants (20,22), protection of plants against fungal attack by inhibiting fungal growth (26) or fungal polysaccharases (21), mediation of fungal attachment to plant surfaces (23), mycoparasitism of pathogenic fungi (5), and mediation of attachment of predatory fungi to nematodes (17,27).

A phytoagglutinin has been isolated from hypocotyl cell walls (12,18,19), seeds (13), and leaves (14) of mung beans (*Vigna radiata* (L.) Wilcz.). The phytoagglutinin apparently was a carbohydrate-binding protein (13,18) that had specific enzymatic activity (α-galactosidase) (12,13) and agglutinated rabbit erythrocytes (13,18). Agglutination was inhibited by D-galactose and required calcium or manganese ions for binding activity (18). In addition, the activity of the phytoagglutinin from *V. radiata* was sensitive to changes in hydrogen ion concentration (H^+) (18,19). Although no literature was found that described phytoagglutinins from roots of mung bean, the presence of phytoagglutinins in roots and root hairs of other leguminous plants (4,8) suggests that these lectins may be present in roots of *V. radiata*.

Macroconidia of *Fusarium solani* (Mart.) Appel. & Wr. f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans. can be agglutinated by the lectins concanavalin A and *Helix pomatia* L. agglutinin, indicating that α-D-glucosyl, α-D-mannosyl, and *N*-acetyl-α-D-galactosaminyl residues probably were exposed on the outer surfaces of the mucilaginous layer of the macroconidium walls (3,20). Consequently, agglutination of macroconidia in the presence of the lectins was blocked by the addition of the hapten sugars D-glucose, D-mannose, and *N*-acetyl-D-galactosamine, respectively (3).

In previous studies, macroconidia of *F. s. phaseoli* attached preferentially to root hairs of *V. radiata* (30) and were agglutinated by a crude extract from roots of *V. radiata* (31). Based on these results and on the literature, we hypothesized that a lectin was involved in attachment of macroconidia of *F. s. phaseoli* to roots of *V. radiata*. Therefore, the first objective of this study was to determine if specific hapten sugars of previously reported plant lectins (6,13,22) could block spore attachment to roots or macroconidium agglutination in root extracts of *V. radiata*. Inhibition of spore attachment by a specific hapten sugar might indicate that constitutive properties of both the fungus and the host were involved in attachment and agglutination. An alternative hypothesis was that constitutive properties were not involved in spore attachment but rather that the macroconidia became adhesive and germinated after spores were incubated in nutrient solutions containing carbon and nitrogen sources. Chlamydospores of *F. s. phaseoli* require an exogenous source of carbon and nitrogen for germination (2), and adhesion of spores of *Nectria haematococca* (anamorph: *F. s.* f. sp. *cucurbitae*) to surfaces is an inductive process that requires respiration and protein synthesis (15). Thus, the second objective of this study was to determine the effects of different carbon sources on the agglutination and germination of macroconidia of *F. s. phaseoli*.

MATERIALS AND METHODS

Effects of carbon sources on agglutination of macroconidia. Macroconidia of *F. s. phaseoli* (isolate F28A [28], obtained from A. J. Anderson, Utah State University, Logan) were prepared

for the spore-agglutination and -attachment tests by the following procedure. Macroconidia from modified carnation leaf agar (MCLA) cultures (31) were collected in sterile deionized water (SDIW). Macroconidia were washed three times by first collecting them on 5-μm cellulose-nitrate filters (MSI Micron Separations, Inc., Westboro, MA) and then resuspending them in SDIW. Macroconidia were subjected to various treatments at final densities of 5–10 $\times$ 10^3 spores per milliliter.

Methods for preparation of root-homogenate and -leachate solutions were described previously (31). Seedlings of *V. radiata* used to produce root homogenate and leachate were grown in seed-germination trays (Fig. 1) to minimize the wounding of roots encountered in preliminary tests when plants were grown in sand or vermiculite (28). Fresh nutrient solutions (29) were prepared for each experiment. Nitrogen was provided as nitrate ions at a concentration of 175 mg/L. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO), except D-cellobiose and D-melibiose, which were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). All solutions were adjusted to pH 5.5 with 0.01 M HNO_3 and 0.02 M KOH.

Agglutination tests were conducted at room temperature (approximately 23–25 C) in separate 50-ml volumes of the specific solutions. Spore suspensions were gently agitated on rotary shakers or magnetic stirrers. Incubation periods differed between experiments and are described below. After incubation, two 100-μl samples were withdrawn from each solution and placed on acid-washed glass slides. Acid-washed glass slides were essential for determining spore agglutination because macroconidia attached to debris on uncleaned slides, forming aggregates of spores that appeared similar to agglutinated spores in root homogenate. Samples were viewed by bright-field microscopy, and the levels of spore agglutination for each 100-μl sample were rated by the following scale: 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.

Twenty carbon sources were tested for their ability to agglutinate macroconidia of *F. s. phaseoli* in fresh nutrient solution. Macroconidia from 10- to 12-day-old cultures of MCLA were incubated for 1.5 h in 50-mM aqueous solutions of each of the following carbon sources: α-methyl-D-glucoside, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *N*-acetylneuraminic acid, L-arabinose, D-asparagine, D-cellobiose, D-fructose, D-fucose, L-fucose, D-galactose, D-gluconic acid, D-glucose, lactose, maltose, D-mannose, D-melibiose, methyl-α-D-mannopyranoside, L-rhamnose, or sucrose. Separate 50-ml samples of root homogenate, root leachate, and fresh nutrient solution were included in each test as the controls. The experiment was conducted four times, except for *N*-acetylneuraminic acid, which was tested twice.

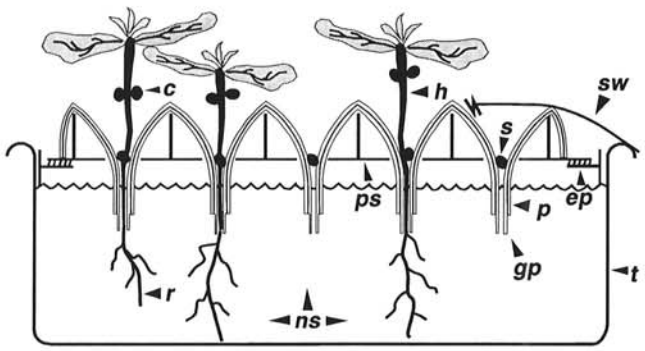

Fig. 1. Schematic drawing of a cross section through a seed-germination tray (t) used for the production of seedlings of *Vigna radiata*. Extruded plastic struts (ps) were assembled by joining overlapping sections with two-part epoxy (ep). A single layer of fiber-reinforced plastic sheeting (p) was cut to fit through gaps between plastic struts and extended low enough to be submersed 1–1.5 cm below the surface of the nutrient solution (ns). Seed-germination paper (gp) was cut and fit over the plastic sheeting to function as a wick for the nutrient solution. Seeds (s) were placed with hilums down between opposing surfaces of adjacent sections of seed-germination paper. Saran Wrap (sw) was placed over the seed-germination tray for 3 days to enhance seed germination and hypocotyl (h) extension. Cotyledons (c) often would be dislodged from the stem before seedlings were transplanted. Root (r) growth consisted of first- and second-order roots.

To determine if the various carbon sources would inhibit spore agglutination in root homogenate, macroconidia from 10- to 12-day-old MCLA cultures were collected and washed in SDIW as described above. Macroconidia then were added to separate 50-ml samples of root homogenate, each of which contained one of the 20 carbon sources tested above. Macroconidia were incubated for 1.5 h in 50- or 100-mM solutions of each carbon source in root homogenate. Tests with 50-mM solutions were conducted three times, and tests with 100-mM solutions were conducted twice.

Induction of agglutination of macroconidia. A procedure was developed in which macroconidia were stimulated to begin agglutination, but before large aggregates of macroconidia formed, macroconidia were removed from the root homogenate and transferred to solutions of potentially competitive hapten sugars. The following procedure was used to stimulate the agglutination of macroconidia. Macroconidia from 10-, 14-, and 21-day-old MCLA cultures were washed as described above and separately placed in 50-ml samples of root homogenate for 1, 5, 10, 15, or 20 min. After incubation in root homogenate, macroconidia were collected on 5-μm cellulose-nitrate filters and washed once by resuspending each sample in 50 ml of fresh nutrient solution. Macroconidia were again collected on 5-μm filters and resuspended in 50 ml of fresh nutrient solution. Macroconidia were incubated in fresh nutrient solution for 1 h, and then two 100-μl subsamples of the spore suspensions were viewed by bright-field microscopy. The experiment was conducted three times.

Effects of hapten sugars on agglutination and attachment of macroconidia to roots. Eight hapten sugars were selected to test their ability to block spore agglutination after macroconidia had been preincubated in root homogenate. Macroconidia from 21-day-old cultures were incubated for 20 min in eight separate 50-ml samples of root homogenate. Macroconidia from each sample were collected separately on 5-μm cellulose-nitrate filters and washed once with 50 ml of fresh nutrient solution. Macroconidia were resuspended in separate 50-ml samples of fresh nutrient solution, each of which contained one of the following sugars at 50 mM: D-fucose, D-galactose, D-glucose, D-mannose, *N*-acetylneuraminic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, or sucrose. Macroconidia were agitated gently for 1 h, and then two 100-μl subsamples of the spore suspensions were viewed by bright-field microscopy. Controls included untreated macroconidia incubated for 1 h in 50 ml each of freshly prepared root homogenate or nutrient solution, and homogenate-treated macroconidia (20 min) were incubated in fresh nutrient solution. The experiment was conducted three times.

To determine if the hapten sugars used in the previous experiment would block spore attachment to root surfaces, single 7- to 8-day-old seedlings of *V. radiata* were placed in 200 ml of 50- or 100-mM solutions of D-fucose, D-galactose, D-glucose, D-mannose, *N*-acetylneuraminic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, or sucrose. Macroconidia were collected from 10- to 12-day-old MCLA cultures and washed three times by the filtration procedure described above. Plants were preincubated for 15 min in each sugar solution, and then inoculum was added to each solution so the final spore densities were 4–5 $\times$ 10^3 propagules per milliliter. Inoculated plants were agitated gently for 10 min on a rotary shaker. Plants inoculated in fresh nutrient solution without the hapten sugars served as controls. At the end of the inoculation period, roots were severed at the crown of each plant and stained for 5 min in 0.05% trypan blue stain in a lactic acid/water/glycerol (1:1:2) solvent (29). Second-order roots (sensu Fitter [7]) with root hairs were mounted on glass slides and viewed with bright-field microscopy. The experi-

ment was conducted twice for each sugar concentration.

Effects of hapten sugars on germination of macroconidia. Macroconidia of *F. s. phaseoli* were incubated in separate 50-mM samples of D-fucose, D-galactose, D-glucose, D-mannose, *N*-acetylneuraminic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, or sucrose. Sugars were dissolved in separate 50-ml samples of fresh nutrient solution. Nitrogen was provided as nitrate ions in the nutrient solution at a concentration of 175 mg/L. One additional nutrient-solution sample contained a mixture of the eight sugars (10 mM each). Control treatments consisted of 50 ml each of fresh nutrient solution or root homogenate. Macroconidia were collected from 10- to 12-day-old MCLA cultures and washed three times in SDIW as described above. Macroconidia were added to each solution, and the solutions were incubated for 24 h with continuous agitation at room temperature. Two 100-μl samples were withdrawn from each solution at 1.5, 5, and 24 h; samples were mounted on acid-washed glass slides and viewed by bright-field microscopy. After 1.5, 5 and 24 h, macroconidia also were vacuum-filtered onto Whatman No. 42 filter paper (Whatman, Maidstone, England). Filter-paper disks were transferred to fresh nutrient solution and cut into 4-mm^2 pieces. Sections of filter paper from each solution were prepared for scanning electron microscopy (SEM) by fixation in 1% unbuffered osmium tetroxide containing 0.003% Photo-flo (Eastman Kodak Company, Rochester, NY) (31). Samples were photographed with Polaroid 4 × 5 instant film, type N55 (Polaroid Corp., Cambridge, MA) with a scanning electron microscope (model S-530, Hitachi Instruments, Inc., Danbury, CT). Tests in which spore germination was studied with bright-field microscopy were conducted four times, and tests with SEM were conducted twice.

RESULTS

Effects of carbon source on agglutination of macroconidia. Agglutination of macroconidia of *F. s. phaseoli* was not observed in nutrient solutions containing 50-mM concentrations of the various carbon sources. Conversely, agglutination was observed in all 50- or 100-mM solutions of carbon sources in root homogenate. Results in the agglutination test were consistent among the repetitions of the experiment, and all treatments were rated as 2 or 3. Throughout this and all subsequent experiments, agglutination occurred consistently in control root-homogenate preparations (rating = 2–4) but always was absent in control nutrient-solution preparations. In contrast, agglutination of macroconidia in root leachate was variable; either negative or weakly positive (rating = 1) results were observed in separate repetitions of separate experiments.

Induction of agglutination of macroconidia. Spore agglutination did not occur with 10- or 14-day-old macroconidia, but spore agglutination was induced with 21-day-old macroconidia incubated in root homogenate for 1–20 min before spores were transferred to fresh nutrient solution (Table 1). Weak positive reactions were observed with 10- and 14-day-old macroconidia incubated for 20 min in root homogenate, but aggregates broke up and dispersed during the 1-h incubation period in fresh nutrient solution. Reactions were inconsistent with 10- and 14-day-old macroconidia incubated for 5–15 min in root homogenate; both negative and weakly positive (rating = 1) reactions were observed among different repetitions of the experiment, but when aggregates were formed, they dispersed during subsequent incubation of macroconidia in fresh nutrient solution.

Effects of hapten sugars on agglutination and attachment of macroconidia to roots. None of the eight hapten sugars inhibited spore-tip to spore-tip agglutination when macroconidia were first stimulated to agglutinate in root homogenate and then incubated in fresh nutrient solutions containing competitive sugars. In addition, the eight hapten sugars did not inhibit macroconidium attachment to root surfaces of *V. radiata* in fresh nutrient solution at either 50- or 100-mM concentrations of the sugars. Macroconidia attached to roots equally well (greater than 200 macroconidia per linear centimeter of root) in all solutions, including the sugar-free control nutrient solutions.

Effects of hapten sugars on germination of macroconidia. Macroconidia germinated within 1.5–2.0 h in root homogenate or when attached to root surfaces of *V. radiata*. Germ tubes were not observed on macroconidia incubated for 1.5 h in solutions of any hapten sugars. Germ tubes were observed after 5 h on a very low number of macroconidia (less than 0.1% of the spores present in a 100-μl sample) incubated in D-glucose, D-mannose, and sucrose. Germ tubes emerged from lateral walls of intercalary cells when macroconidia were incubated in hapten-sugar solutions (Fig. 2A), but germ tubes emerged primarily from the tips of terminal and foot cells when macroconidia were incubated in root homogenate (Fig. 2B).

An amorphous material, termed spore mucilage (31), was associated with germ tube emergence from terminal and foot cells when macroconidia were incubated in root homogenate (Fig. 2B). Spore mucilage was observed at the sites of germ tube emergence at spore tips but was not observed on the elongating germ tubes. Spore mucilage was not observed when germ tubes emerged from lateral walls of intercalary cells of macroconidia incubated in hapten sugars, and germ tubes appeared to physically break through lateral walls of intercalary cells, leaving ragged edges in macroconidium walls (Fig. 2C and D).

Most macroconidia germinated within 24 h when incubated in D-glucose, D-mannose, *N*-acetylneuraminic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, or sucrose solutions. However, most macroconidia did not germinate when incubated for 24 h in D-fucose, D-galactose, or fresh nutrient-solution samples (e.g., D-galactose; Fig. 3A). Macroconidia incubated in D-glucose, D-mannose, or sucrose exhibited severely swollen intercalary cells that often produced conidiophores and secondary macroconidia (Fig. 3B and C). The immediate production of secondary macroconidia from germinating spores of *F. s. phaseoli* was not observed when macroconidia were incubated in root homogenate or on root surfaces. Surfaces of germ tubes and conidiophores of macroconidia incubated for 24 h in D-glucose, D-mannose, or sucrose were covered with an amorphous material that appeared to be a flocculent of uncertain origin (Fig. 3C and D). Macroconidia incubated in the mixture of hapten sugars germinated in a manner consistent with the processes observed with D-glucose, D-mannose, or sucrose, except germination appeared to progress slightly more rapidly.

TABLE 1. Effect of macroconidium age on the agglutination of spores of *Fusarium solani* f. sp. *phaseoli* in nutrient solution after incubation of macroconidia in root homogenate

Time in root homogenate (min)[a]	Agglutination rating of macroconidia					
	10 days[b]		14 days		21 days	
	0 h	1 h	0 h	1 h	0 h	1 h
1	0,0,0[c]	0,0,0	0,0,0	0,0,0	0,0,0	2,2,2
5	0,0,0	0,0,0	1,0,0	0,0,0	0,0,0	2,2,2
10	0,1,1	0,0,0	1,0,0	0,0,0	0,0,0	3,2,2
15	0,1,1	0,0,0	1,1,0	0,0,0	1,1,0	3,3,2
20	1,1,1	0,0,0	1,1,2	1,0,0	1,1,1	3,4,2
Controls						
60 (NS)	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
60 (RH)	0,0,0	2,2,2	0,0,0	3,3,2	0,0,0	3,4,4

[a] Macroconidia were incubated in root homogenate for 1–20 min, transferred to fresh nutrient solution, and incubated for an additional hour. For the controls, macroconidia were incubated in fresh nutrient solution (NS) and root homogenate (RH) for 1 h.

[b] Spore agglutination was determined 0 and 1 h after macroconidia were transferred from root homogenate to fresh nutrient solution. Macroconidia were taken from 10-, 14-, and 21-day-old modified carnation leaf agar cultures.

[c] Agglutination in 100-μl samples was rated for each repetition of the experiment (tests 1, 2, and 3) 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.

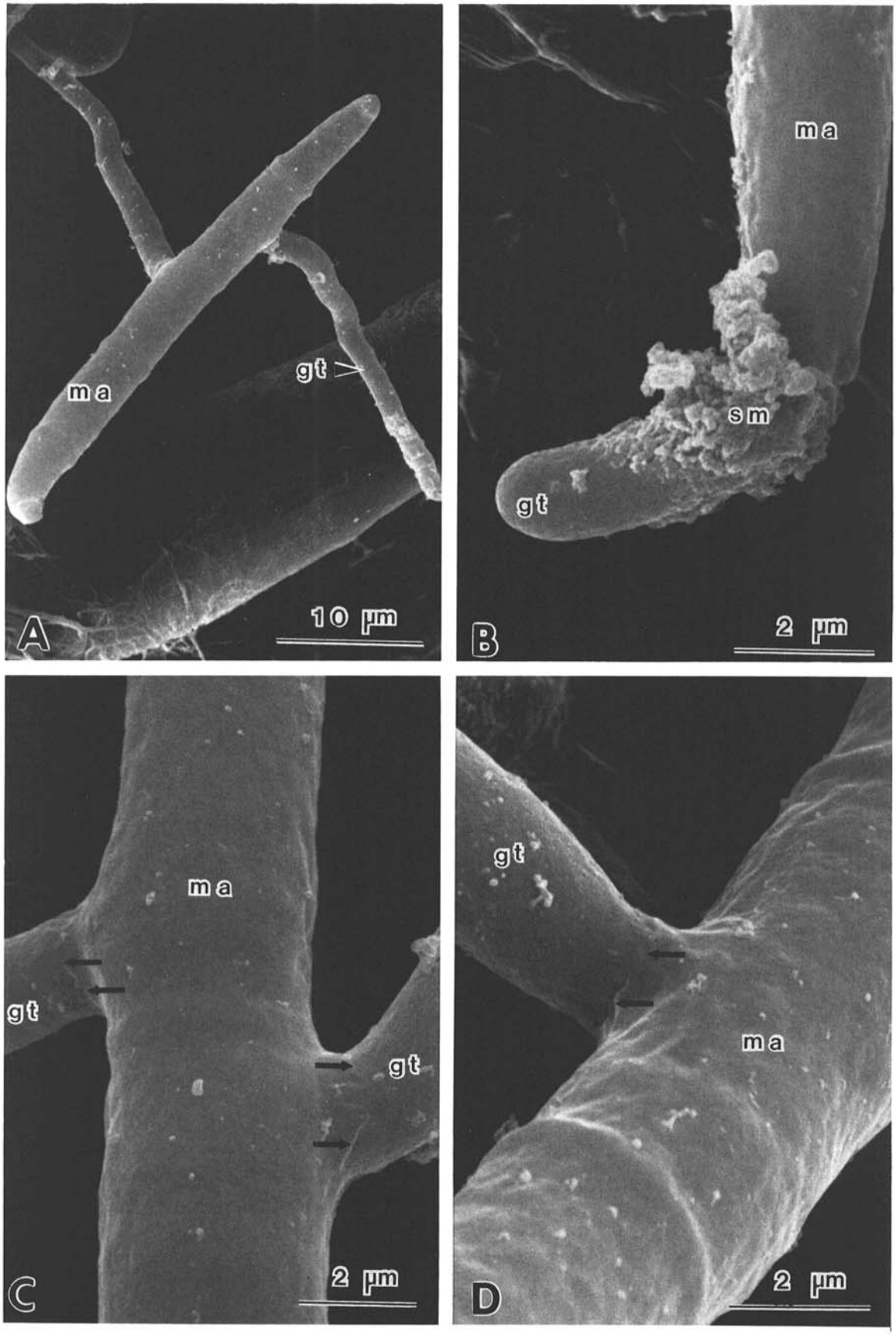

Fig. 2. Macroconidia of *Fusarium solani* f. sp. *phaseoli* incubated for 1.5–5 h in 50-mM solutions of hapten sugars and root homogenate. **A,** Germ tubes (gt) emerging from intercalary cells of a macroconidium (ma) incubated for 5 h in D-glucose. **B,** A germ tube emerging from a foot cell of a macroconidium incubated for 1.5 h in root homogenate; spore mucilage (sm) was present at the site of germ tube emergence. **C and D,** Germ tubes emerging from intercalary cells of macroconidia incubated in D-glucose (**C**) or sucrose (**D**); germ tubes mechanically ruptured macroconidium walls during germination (arrows).

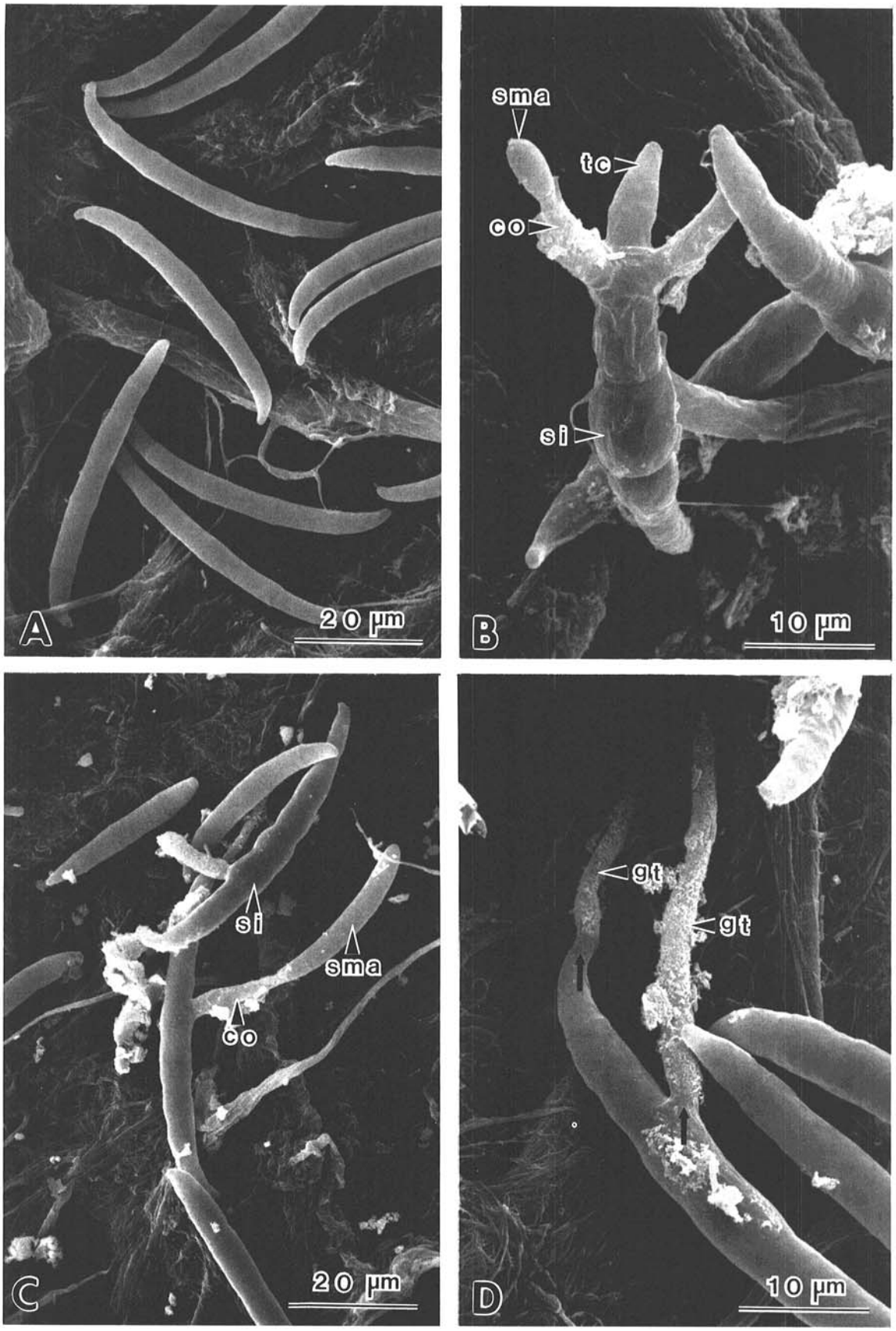

Fig. 3. Macroconidia of *Fusarium solani* f. sp. *phaseoli* incubated for 24 h in 50-mM solutions of hapten sugars in fresh nutrient solution. **A,** Macroconidia incubated in D-galactose. **B and C,** Conidiophores (co) and secondary macroconidia (sma) developing from swollen intercalary cells (si) of macroconidia incubated in D-glucose; tc = terminal cells. **D,** Germ tubes (gt) covered with a flocculent material emerging from a macroconidium incubated in D-glucose. (Arrows = points of germ tube emergence.)

DISCUSSION

The hypothesis that a root lectin might be involved in spore attachment by macroconidia of *F. s. phaseoli* to roots of *V. radiata* was not supported by the results from the current study. Plant phytoagglutinins (4,6,8,22,26), and specifically the phytoagglutinin previously reported for *V. radiata* (12,13,18,19), do not appear to be involved in the attachment of macroconidia of *F. s. phaseoli* to roots, because 20 carbon sources did not block agglutination of macroconidia in root homogenate and eight hapten sugars did not block spore attachment to roots. Furthermore, the phytoagglutinin from *V. radiata* had the greatest hemagglutinating activity at pH 7–8, which decreased as H^+ increased (18,19). In contrast, attachment to roots of *V. radiata* and agglutination in root homogenate by macroconidia of *F. s. phaseoli* were suppressed at pH 7 and increased as the H^+ increased to pH 4 (30,31).

In the current study, macroconidia were induced to agglutinate in nutrient solution by pretreatment in root homogenate. Results of the agglutination-induction experiment support the conclusion that macroconidia probably received a signal from the root homogenate that initiated the process leading to agglutination. We propose that an inducer of plant origin present in the root homogenate was the factor that led to spore agglutination. The inducer may be a structural component of the root surface released during the preparation of root homogenate or a soluble component of the root apoplast or symplast. Agglutinins have been described on root mucilage (10), in hypocotyl cell walls of leguminous plants (12), and as soluble extracellular proteins or glycoproteins in plant roots (1). Furthermore, based on the involvement of the macroconidium tips in attachment, agglutination, accretion of refuse, and secretion of mucilage during spore germination, the reactive site for the inducer is postulated to be on or near the tips of macroconidia. The induction signal may involve the adsorption or binding of the inducer to the tips of macroconidia during spore attachment to roots of *V. radiata*.

In a preliminary test of the current study, a strong spore-agglutination response was observed in a root-leachate solution prepared by incubating severed roots of *V. radiata* in fresh nutrient solution. Subsequently, the procedure was modified so only intact plants were used for preparing root leachate, and little or no agglutination occurred. The result of the preliminary test may indicate that symplastic fluids of *V. radiata* roots contain either the inducer or a chemically similar compound. In addition, the inconsistent agglutination response observed in the current study with different batches of root leachate prepared from intact plants may indicate that plant roots were wounded to differing degrees during separate preparations of root leachate, even though seedlings of *V. radiata* were produced in trays specially designed to minimize root damage. Caution should be exercised when root leachates are used in phytoagglutinin studies because the leachate may contain symplastic fluids that normally would not be secreted by the roots.

One objective of the current study was to determine if exogenously supplied carbon and nitrogen sources could induce spore agglutination or germination. Carbon and nitrogen sources appear to be required for the germination of macroconidia and chlamydospores of several *Fusarium* spp. (2,16,25). In addition, carbon and nitrogen sources may be required for the attachment of macroconidia of *N. haematococca* to plant surfaces (16). In the current study, however, spore germination in the different carbon sources differed greatly from spore germination in root homogenate or on roots of *V. radiata*. First, macroconidia germinated within 1.5 h when incubated in root homogenate or attached to roots of *V. radiata* but required at least 5 h to germinate in nutrient solutions containing D-glucose, D-mannose, or sucrose. In other studies, macroconidia of *F. s. phaseoli* germinated within 2 h when attached to root surfaces (30) but up to 12 h were required when incubated in distilled water containing glucose and KNO_3 (11). Second, severely swollen intercalary cells of macroconidia were consistently observed when spores were incubated in several of the sugar solutions but were not observed when macroconidia were incubated in root homogenate or on root surfaces. A similar swelling of intercalary cells of macroconidia of *F. culmorum* occurred when spores were incubated in distilled water containing glucose and a nitrogen source (25). Spore swelling by *F. culmorum* was described as an active process in which macroconidia had an enhanced capacity to take up water (25). However, spore swelling may be an exclusively osmotic effect. Third, germ tubes emerged primarily from intercalary cells of macroconidia when spores were incubated in sugar solutions. In contrast, germ tubes emerged primarily from spore tips when macroconidia were incubated in root homogenate or when they were allowed to germinate on root surfaces. Fourth, spore mucilage at germination sites was not observed when germ tubes emerged from lateral walls of macroconidia incubated in sugar solutions but was observed when germ tubes emerged from foot and terminal cells of macroconidia incubated in root homogenate. Fifth, conidiophores and secondary macroconidia developed directly from germinating spores incubated in nutrient solution containing D-glucose, D-mannose, or sucrose but were not observed in any tests in which macroconidia germinated on the root surface or in root homogenate. The production of conidiophores and secondary macroconida directly from germinating macroconida is not in agreement with the principle that fungal sporulation occurs when the available food base is depleted (24); the sugars appeared to stimulate the production of secondary macroconidia.

Although these results support the conclusion that a factor other than the simple availability of carbon and nitrogen is involved in spore attachment and germination by *F. s. phaseoli* on plant roots in hydroponic solution, several other possible mechanisms should be considered. First, a specific carbon source, or a combination of organic molecules, other than those tested in the current study may be involved in spore attachment and germination on root surfaces. Second, a region of a plant-associated lectin other than the hapten reactive site may be involved in spore attachment and germination. Kaplan et al (17) demonstrated that nematode trap formation by *Arthrobotrys dactyloides* was stimulated by several plant lectins, but heat treatment of the lectins and competitive inhibition studies with the lectin haptens did not significantly suppress the stimulative effects of the lectins on trap formation. Third, only trace amounts of a plant-associated lectin might be involved in the induction of spore adhesiveness or germination by *F. s. phaseoli*, and, thus, these processes would likely occur in the presence of hapten sugars.

In the current study, a secreted spore mucilage was observed at spore tips when germ tubes emerged from terminal and foot cells of macroconidia incubated in root homogenate, confirming results from a parallel study (31). However, an amorphous material that appeared to be a flocculent was observed on elongated germ tubes from macroconidia incubated for 24 h in D-glucose, D-mannose, and sucrose. The flocculent adhered to the full length of the germ tubes in contrast to spore mucilage, which was present only at spore tips of germinating macroconidia incubated in root homogenate (Figure 2B; [31]).

The mechanism that mediates spore attachment by *F. s. phaseoli* to roots of *V. radiata* is not known. Based on the results from the current and parallel indirect studies (31), however, we conclude that plant lectins are not involved in spore attachment by *F. s. phaseoli* to roots of *V. radiata* and that a compound is present in roots of *V. radiata* that induces agglutination of macroconidia. Furthermore, the processes of spore attachment to roots, agglutination in root homogenate, and macroconidium germination on roots may be related because secretion of spore mucilage, accretion of refuse to spore tips, agglutination of macroconidia in root homogenate, spore attachment to root surfaces, and germination of macroconidia were similarly affected by changes in temperature and H^+ (30,31).

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