Effects of Temperature and Hydrogen Ion Concentration on Attachment of Macroconidia of *Fusarium solani* f. sp. *phaseoli* to Mung Bean Roots in Hydroponic Nutrient Solution

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Portion of a Ph.D. dissertation submitted by the senior author to the University of Florida Graduate School.

We thank Stephen B. Linda, Institute of Food and Agricultural Sciences, Department of Statistics, University of Florida, Gainesville, and Julie Wildman-Pepe and Mark E. Johnson, Department of Statistics, University of Central Florida, Orlando, for their suggestions and assistance in the analysis of experimental data.

This research was supported in part by the Biomedical Operations and Research Office, National Aeronautical and Space Administration, John F. Kennedy Space Center, FL 32899.

Florida Agricultural Experiment Station Journal Series Paper R-02022.

Accepted for publication 17 July 1992.

ABSTRACT

Schuerger, A. C., and Mitchell, D. J. 1992. Effects of temperature and hydrogen ion concentration on attachment of macroconidia of *Fusarium solani* f. sp. *phaseoli* to mung bean roots in hydroponic nutrient solution. Phytopathology 82:1311-1319.

Hydroponically grown mung bean seedlings were inoculated with macroconidia of *Fusarium solani* f. sp. *phaseoli* to evaluate the effects of temperature (15, 20, 25, 30, and 35 C) and hydrogen ion concentration (pH 3, 4, 5, 6, and 7) on spore attachment to roots of *Vigna radiata*. Macroconidia of *F. s. phaseoli* attached to second-order roots with root hairs in greater numbers than to those without root hairs or to roots of other orders. Attachment of macroconidia to second-order roots was greatest at 20–30 C and pH 4 but decreased by up to two orders of magnitude when the temperature of the nutrient solution was increased to 35 C or the pH elevated to 7. The binding reaction of macroconidia to roots was observed to be reversible when plants inoculated at 25 C and pH 5 were transferred to nutrient solutions maintained at 35 C or pH 7. Plant fresh weights of *V. radiata* decreased with increasing inoculum density when plants were inoculated and maintained at 20 or 25 C but not at 30 C. Differences in plant fresh weights of *V. radiata* between inoculated and uninoculated plants were greatest at 20 C, decreased at 25 C, and were not observed at 30 C. In a separate experiment, plant roots were exposed to inoculum for 24 h at 24 C and pH 4, 5, 6, or 7. The nutrient solutions of each treatment were then adjusted to and maintained at pH 6 for an additional 13 days. Disease was greatest when roots were inoculated at pH 4 as compared to pH 5 or 6. Plants inoculated at pH 7 were not different from uninoculated plants. Differences in disease among plants inoculated at different hydrogen ion concentrations are explicable when based on the effects of hydrogen ion concentration on the attachment of macroconidia to root surfaces. Differences in disease among plants inoculated at different temperatures between 20 and 30 C are not explicable when based on the effects of temperature on spore attachment to roots or on growth of the pathogen. We propose that differences in disease among plants inoculated between 20 and 30 C are due to the effects of temperature on host resistance.

Additional keywords: morphometrics, multiphase regression.

Attachment of fungal structures to plant surfaces has been defined to include all mechanisms, both mechanical and chemical, through which fungi maintain initial contact with the host tissue (23). A conventional view of fungal attachment to foliar surfaces includes entrapment of the infective propagules on leaf projections followed by the secretion of adhesive materials by the fungus (23). Attachment of fungal spores to foliar surfaces may be a prerequisite for pathogenesis (24). In soils, however, physical entrapment or attachment of an infective propagule to host tissue may not be required for pathogenesis, because the proximity of the propagule to the infection court is maintained by the stability of the soil matrix.

The process of spore attachment to plant roots grown in hydroponic nutrient solutions may be conceptually similar to the process of spore attachment to aboveground plant surfaces. Fluid dynamics of nutrient solution and air flowing past plant surfaces should be similar. The principles of laminar flow, turbulence, fluid-shearing forces, eddy formation, convective heating, and boundary-layer dynamics described for air movement though crop canopies (26) should be applicable to fluid movement around root systems in liquid matrices. Considerable differences certainly exist between roots grown in nutrient solution and plant surfaces in the ambient air environment, but spore attachment to root surfaces in nutrient solution also may be a prerequisite for pathogenesis. However, no studies were found in the literature that described the attachment of nonmotile fungal spores to plant roots grown in hydroponic nutrient solution.

In a preliminary experiment of the current study, low numbers of macroconidia of *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W. C. Snyder & H. N. Hans. attached to roots of *Vigna radiata* (L.) R. Wilcz. at temperatures greater than 30 C, which coincided with temperatures that suppressed the severity of root disease in pathogenicity tests reported in a parallel study (30). Furthermore, hydrogen ion concentration (H^+) was reported to alter the severity of root disease of plants grown in nutrient solution (30). On the basis of these observations, it was hypothesized that temperature or H^+ can alter the inoculum loads on plant roots in hydroponic solutions by affecting spore attachment, and that these effects might correlate to differences in the severity of disease among plants exposed to different temperatures or H^+. If this hypothesis is confirmed, the manipulation of temperature or H^+ might be a practical method for managing root diseases in hydroponic systems by suppressing spore attachment of the pathogen to roots.

The objectives of this study were to determine if macroconidia of *F. s. phaseoli* attached preferentially to different root orders of *V. radiata*; to evaluate the effects of temperature and H^+ on spore attachment to, and germination on, roots of *V. radiata*; to determine the influence of inoculum density on plant disease at different temperatures; and to determine if the effects of temperature or H^+ on the severity of disease are correlated to the effects of temperature and H^+ on spore attachment to roots in hydroponic solutions. A preliminary report has been published (29).

MATERIALS AND METHODS

Effects of temperature and H^+ on radial growth and germ tube elongation. Inoculum of *F. s. phaseoli* (isolate F 28A [28], obtained from A. J. Anderson, Utah State University, Logan) was prepared on modified carnation leaf agar (MCLA) (28). Four in vitro experiments were conducted to determine the effects of temperature or H^+ on the radial growth rate of fungal colonies and on the elongation of macroconidium germ tubes. Tests were conducted on either nutrient solution agar (NSA) (28) or MCLA. The H^+ of culture media and all nutrient solutions were adjusted with 0.01 N HNO_3 and 0.02 N KOH. Unless otherwise stated, chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

When temperature was the treatment variable (15–35 C), the pH was adjusted to 6–6.5. When H^+ was the treatment variable (pH 2.5–11), temperature was maintained at 25 C. The H^+ in the media were adjusted to predetermined levels prior to autoclaving such that the pH of separate batches of solidified media were within the range of 2.5–11.

Radial growth rates of *F. s. phaseoli* were determined by placing one 2-mm^3 block of agar from a 14-day-old MCLA culture onto each of 10 plates of fresh MCLA per temperature or H^+. Cultures were maintained for 10 days in an incubator (23L, Rheem Scientific, Asheville, NC) under a 12-h diurnal period. A photon flux of 70 $\mu mol \cdot s^{-1} \cdot m^{-2}$ was maintained during the day by fluorescent illumination. When effects of temperature were tested, growth rates of the pathogen were measured at 15, 20, 22, 25, 27, 30, 32, or 35 C; the experiment was conducted twice. The experiment on the effects of H^+ on radial growth of *F. s. phaseoli* was conducted three times.

To determine the effects of temperature or H^+ on germ tube elongation, macroconidia from 14-day-old MCLA cultures were collected in sterile deionized water. Inoculum was adjusted to a density of 4.0×10^4 macroconidia per milliliter. A hemacytometer was used to quantify inoculum in this and all subsequent experiments. One plate of NSA was preincubated for 1 h at each temperature (15, 20, 22, 25, 27, 30, 32, or 35 C); in the experiment testing the effects of H^+ (pH 2.5–11), all plates were incubated at 25 C. Ten 1-μl drops of a macroconidium suspension were placed on the surface of each NSA plate. Cultures were incubated for an additional 6 h, and then 7 ml of a 0.05% trypan blue stain (30) was dispensed onto the agar surface of each plate. The lengths of 90 germ tubes of macroconidia on individual plates of each treatment were measured with a Sony color video camera (model DXC 3000) mounted on a Nikon Optiphot compound microscope. The video image was electronically combined with the Microcomp Integrated Video Image Analysis System (Southern Micro Instruments, Inc., Atlanta, GA) (28). Mean lengths of 90 germ tubes per treatment were calculated, and each experiment was conducted three times.

Data from the experiments on the effects of temperature on radial growth and germ tube elongation were analyzed by standard regression models (32). Data from the experiments on the effects of H^+ on radial growth and germ tube elongation were analyzed by three-phase regression (19,33).

Spore attachment to morphologically distinctive roots. In order to quantify the differences in spore attachment to different portions of root systems, plants of *V. radiata* cv. Berken were first grown for 7–8 days in a hydroponic nutrient solution (30). Three plants were then placed into a 600-ml beaker containing 500 ml of nutrient solution. Macroconidia were added to 500 ml of fresh nutrient solution in a second beaker, and the density of the spore suspension adjusted to 4,000 propagules per milliliter after microscopically determining the numbers of spores in 10 1-μl samples transferred from the macroconidium suspension to NSA. A third beaker contained 500 ml of fresh nutrient solution and was used to rinse loosely attached macroconidia from roots following inoculation. Beakers were placed in a Magni-Whirl water bath (Blue M Co., Blue Island, IL), and the nutrient solutions were maintained at 25 C and pH 5. Compressed air was passed though a 0.45-μm Mini-Capsule cartridge filter (Gelman Sciences, Inc., Ann Arbor, MI) and then bubbled though the nutrient solution containing *V. radiata* seedlings. Plants and inoculum were incubated for 1 h under six 20W fluorescent lamps, which provided a light intensity of 180 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at the level of the plant foliage. Inoculum was then transferred to 600-ml beakers wrapped with 4 mm of closed-celled foam tape (Macklenburg-Duncan, Oklahoma City, OK). Plants were inoculated individually for 5 min by transferring each seedling to the insulated beakers, in which the macroconidium suspension was gently agitated with a magnetic stirrer. Inoculated plant roots were rinsed for 30 s in fresh nutrient solution. Roots were immersed for 5 min in 0.05% trypan blue stain and then transferred to a clearing solvent of lactic acid, water, and glycerol (1:1:2). Morphologically distinct root types, termed *root orders* in accordance with the morphometric method of root classification described by Fitter (7), plus the lower hypocotyl of each plant were severed from the shoot and mounted on microscope slides. The numbers of macroconidia attached to the lower hypocotyl, second-order roots with root hairs, second-order roots without root hairs, first-order roots, and root tips were estimated by counting macroconidia present on 11 randomly selected root segments of each root order. The upper half of root surfaces were examined by bright-field microscopy, and the data were adjusted to represent the numbers of macroconidia present on one linear centimeter of root tissue. Each plant was considered a replicate, and the experiment was conducted five times.

Two different statistical analyses were used to determine the quantitative differences in spore attachment among root orders. In the first analysis, unadjusted data representing the numbers of macroconidia attached per linear centimeter of each root order were transformed to $\log_{10}$ and then subjected to an analysis of variance followed by Fisher's least significant difference test ($P \leq 0.05$). In the second analysis, data were adjusted to compensate for the increased area of root hairs present on second-order roots and to compensate for the different diameters of the root orders. The area of an "average" root hair was estimated as that of a cylinder with a length and radius equal to the mean length and radius of 150 root hairs measured with the video image analysis system. The numbers of root hairs per unit area of root surface were estimated from scanning electron microscope photographs. The increased surface area created by root hairs on 1 mm^2 of second-order roots was calculated. The area of root and hypocotyl segments were calculated in a similar manner. Adjusted data were analyzed in a manner identical to that used for unadjusted data.

Effects of temperature and H^+ on spore attachment and germ tube elongation on roots. To determine the effects of H^+ and temperature on spore attachment to roots of *V. radiata*, plants were inoculated as described for the previous experiment, except that temperature and H^+ were altered during inoculation. After inoculation, roots were rinsed for 30 s, stained in 0.05% trypan blue, and cleared in solvent. The second-order roots were then excised from other root and stem tissues and mounted in solvent on microscope slides. The upper half of root surfaces were examined by bright-field microscopy. Macroconidia were counted in 20 fields of view; 10 of the fields were second-order roots with root hairs, and 10 fields were second-order roots without root hairs. The sum of the counts of all 20 fields of view per root system were adjusted to represent the numbers of macroconidia attached per linear centimeter of root.

To determine the effects of temperature and H^+ on germ tube elongation, plants were inoculated as described above for the spore attachment experiment, except that all plants were inoculated at 25 C and pH 5. After exposure to inoculum, roots were rinsed in fresh nutrient solution maintained at 25 C and pH 5, and then individual plants were transferred to 600-ml beakers containing 500 ml of fresh nutrient solution adjusted to various combinations of temperature and H^+. Plants were placed under six 20W fluorescent bulbs at a light intensity of 180 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and incubated for 6 h. Second-order roots were excised from other root and stem tissues, stained in trypan blue, and then mounted on microscope slides. The lengths of 30

randomly selected germ tubes per root system were measured with the integrated video image analysis system.

A split-plot design with temperature (15, 20, 25, 30, or 35 C ± 1 C) as the main plot and H^+ (pH 3, 4, 5, 6, or 7 ± 0.1) as the subplot was used in both experiments. Tests of each combination of temperature and H^+ were conducted three times for each experiment. Data from the spore attachment experiment were transformed to 0.25-powers and then subjected to an orthogonal polynomial contrast analysis. Data from the germ tube elongation experiment were transformed to square roots and then analyzed with two-phase regression for the effects of H^+ within each temperature (8,19,33) and with orthogonal polynomial contrasts for the effects of temperature within each H^+ (32).

Effects of inoculum density and temperature on root disease. An experiment was conducted to determine the effects of inoculum density and temperature on disease severity, which was measured as the differences in fresh weights between inoculated and uninoculated plants. Plants 7–8 days old were transplanted into 800-ml flasks containing 750 ml of fresh nutrient solution and were held in place by open-celled, polyurethane foam plugs that were heat-treated and autoclaved (30). Flasks were transferred to temperature control tanks in plant growth chambers (model M-13, Environmental Growth Chambers, Chagrin Falls, OH). Temperature of the plant nutrient solution was regulated by controlling the water temperature in the tanks via closed-loop recirculating systems connected to water baths (30). Compressed air was passed though carbon filters and distributed to each flask to oxygenate the nutrient solution. Plants were placed in the flasks 24 h prior to inoculation.

Inoculum was prepared from 14-day-old MCLA cultures. Appropriate volumes of inoculum were added to each of seven flasks per inoculum density per growth chamber to provide 0, 100, 500, 1,000, 2,000, or 3,000 propagules per milliliter. Temperature was maintained at 20, 25, or 30 C ± 1 C; vapor pressure deficit was maintained between 6 and 7 mm Hg for each temperature. Plants were provided a photon flux of 275 $\mu mol \cdot s^{-1} \cdot m^{-2}$ by a mixture of fluorescent and incandescent lamps. The pH was adjusted in each flask to 5.5 just prior to exposure of the plants to inoculum, but after 24 h the pH was allowed to fluctuate between 6 and 7. After 14 days, plants were harvested for fresh weights.

A completely randomized split-plot design with temperature as the main plot and inoculum density as the sub-plot was utilized for this experiment. Plants receiving different inoculum levels were randomized within each of two growth chambers. Each temperature was replicated twice per repetition, and the experiment was conducted twice. Linear and two-phase regressions were used to analyze untransformed fresh weights in conjunction with inoculum densities transformed to $\log_{10}$.

Effects of H^+ on root disease. An experiment was conducted to determine whether the H^+ during inoculation would affect the severity of disease by altering the inoculum load on each plant. Experimental units consisted of 4-L tanks. Root temperature was controlled by closed-loop water recirculating systems (30). Combinations of different H^+ and inoculum levels were replicated three times per repetition, and the experiment was conducted three times.

Four 7- to 8-day-old seedlings were transplanted into each of 24 experimental units arranged on tables within a 2.5- × 4.5-m tissue culture clean room. High-intensity discharge lamps were suspended over the plants (30). A mixture of 250W high-pressure sodium and 400W metal halide lamps provided uniform illumination at 275 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at the tops of the plant canopies. Root and air temperatures were maintained at 24 C ± 1.5 C, and humidity was maintained between 7 and 9.5 mm Hg vapor pressure deficit. Plants were grown for 14 days and then harvested to determine fresh weights. Second- and first-order roots were obtained from two plants per experimental unit and stained in 0.05% trypan blue. Roots were then mounted on glass slides and viewed under bright-field microscopy to determine if conidiophores and secondary macroconidia were produced on inoculated plants.

Nutrient solutions within experimental units were adjusted to pH 4, 5, 6, or 7 ± 0.2, and then plants were transplanted into the containers. After 24 h, the H^+ was readjusted to specific treatment levels, and 50% of the plants at each H^+ were inoculated by dispensing macroconidia from 14-day-old MCLA cultures into the nutrient solutions to provide a density of 500 propagules per milliliter. After an additional 24 h, the H^+ in all treatments was adjusted to pH 6 and thereafter readjusted to pH 6 daily.

To determine the fluctuations in the numbers of propagules of *F. s. phaseoli* detected in the nutrient solutions of treatments that received inoculum, one 1-ml sample was withdrawn from each experimental unit at 30 min, and at 1, 5, 10, and 14 days after inoculation. The samples were dispensed onto Difco potato-dextrose agar supplemented with 50 mg of chlortetracycline hydrochloride and 1 ml of tergitol NP-10 per liter of medium. Plants were harvested 14 days after inoculation for fresh weights.

A split-plot design with H^+ (pH 4, 5, 6, or 7) as the main plot and inoculum level (0 or 500 propagules per milliliter) as the sub-plot was used for this experiment. Plots were arranged in a randomized block design within the tissue culture room. Nontransformed plant fresh weight data were subjected to an orthogonal polynomial contrast analysis. The experiment was conducted four times. Data from the study on the fluctuations in the numbers of propagules recovered from nutrient solutions from different treatments were adjusted with square root transformations. The transformed data were subjected to orthogonal polynomial contrast analyses when testing the effects of H^+ within each day and analyses of variance followed by least-squares mean separation procedures for repeated measures when testing the effects of days within each H^+. Treatments analyzed with orthogonal polynomial contrast analyses were considered significantly different if the confidence intervals (95%) failed to overlap.

Statistical analyses. Statistical analyses were conducted with the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Experimental designs were balanced except for the in vitro tests on the effects of H^+ on radial growth and germ tube elongation of *F. s. phaseoli*. Unless otherwise stated, the measured responses were similar among separate repetitions of individual experiments. Treatment means were considered different if their 95% confidence intervals (CI) failed to overlap; CIs in tables are half-width intervals (mean, ± CI).

RESULTS

Effects of temperature and H^+ on radial growth and germ tube elongation. The effects of temperature on the radial growth and germ tube elongation of *F. s. phaseoli* were similar (Fig. 1). Radial growth of the pathogen did not occur at 35 C, but did occur

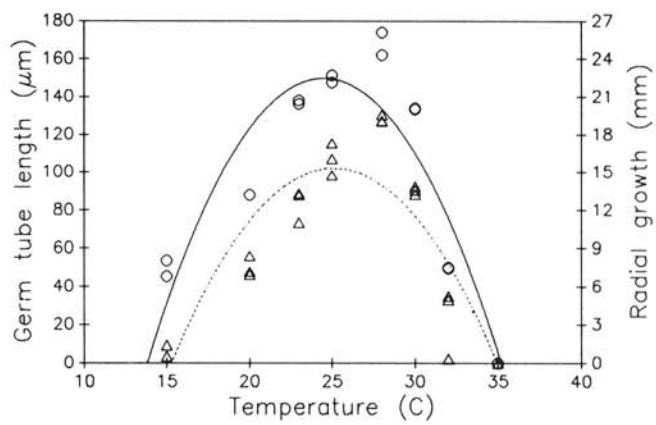

Fig. 1. Effects of temperature on radial growth of mycelia and germ tube elongation of macroconidia of *Fusarium solani* f. sp. *phaseoli* during in vitro tests. Radial growth was measured after 10 days on modified carnation leaf agar, and germ tube elongation was measured after 6 h on nutrient solution agar. (○—○ = Radial growth; △—△ = germ tube length.)

at all other temperatures tested. Quadratic polynomial equations best described the effects of temperature on radial growth [$Y = -95.7 + 9.64(T) - 0.19678(T^2)$, $R^2 = 0.83$] and germ tube elongation [$Y = -564.5 + 53.1(T) - 1.0572(T^2)$, $R^2 = 0.78$]. The optimum temperature for radial growth and germ tube elongation was predicted at 25 C. Macroconidia germinated at the tips of the terminal and foot cells between 15 and 30 C; but at 32 C, germ tubes emerged from the lateral walls of terminal, foot, and intercalary cells.

The effects of H^+ on radial growth and germ tube elongation were best described by three-phase regression models (Table 1 and Fig. 2), in which the growth responses of the pathogen increased rapidly between pH 3 and 4, were level (radial growth) or increased slightly (germ tube elongation) between pH 4 and 8, and decreased rapidly between pH 8 and 11. Spline points (the pH at which the plateau was reached) between the first- and second-phase and between the second- and third-phase portions of the response surfaces were similar for both radial growth and germ tube elongation (Table 1). Regression coefficients (R^2) for the three-phase models for radial growth and germ tube elongation were 0.91 and 0.90, respectively.

Germ tube elongation did not occur, and macroconidia failed to attach to the surface of NSA, below pH 3 and above pH 10. Macroconidia germinated at the tips of the terminal and foot cells at all H^+ tested between pH 3 and 10 but did not germinate at any pH below 3 or above 10.

Spore attachment to morphologically distinctive roots. When analyzed on the basis of the numbers of macroconidia attached per linear centimeter of root, the numbers of macroconidia were different among distinctive root orders (Table 2) ($P \leq 0.05$). The numbers of macroconidia attached to the lower hypocotyls were less than those attached to roots in any of the root orders. The highest numbers of macroconidia attached per linear centimeter of root occurred on second-order roots with root hairs. When data were adjusted to represent spore attachment on the basis of area, the numbers of macroconidia attached to second-order roots without root hairs and to first-order roots were not different. However, the numbers of macroconidia attached to second-order roots with root hairs remained higher than those for all other root orders. Macroconidia attached to the following rhizoplane sites, in decreasing order of abundance: root hairs, mucigel, sloughed root cap cells, junction points for the emergence of first-order roots, root epidermis, and root tips.

TABLE 1. Parameter estimates of the three-phase regression models for the effects of hydrogen ion concentration on growth of *Fusarium solani* f. sp. *phaseoli* during in vitro tests

Experiment	Parameter[w]	Estimate[x]	95% Confidence interval[y]
Radial growth	μ	−36.92	9.06
	β_1	13.68	2.47
	β_2	0.09	0.53
	β_3	−9.61	2.59
	t_1	4.42	0.20
	t_2	8.82	0.40
	R^2	0.91	...[z]
Germ tube elongation	μ	−205.91	65.93
	β_1	71.89	18.20
	β_2	4.21	9.90
	β_3	−64.91	17.62
	t_1	4.60	0.50
	t_2	8.60	0.40
	R^2	0.90	...

[w]Parameter estimates were significant at $P \leq 0.05$; μ = y-intercept of the first-phase regression line, β_1 = slope of the first-phase regression line, β_2 = slope of the second-phase regression line, β_3 = slope of the third-phase regression line, t_1 = spline point (point at which the plateau was reached) for the first- and second-phase regression lines, t_2 = spline point for the second- and third-phase regression lines, and R^2 = correlation coefficient.
[x]Parameter estimates are presented as generated from untransformed data.
[y]Half-width confidence interval (estimate, ± confidence interval).
[z]Parameter was not estimable.

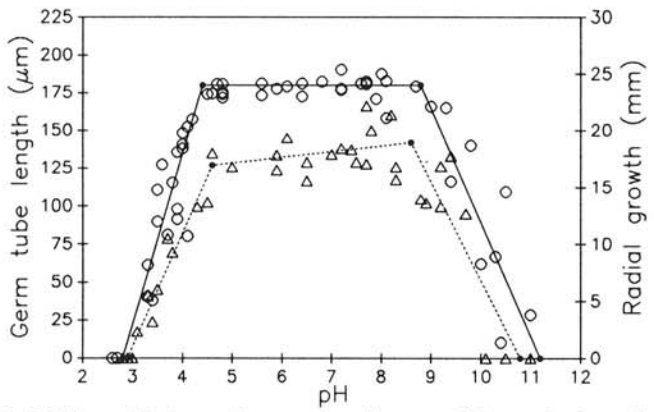

Fig. 2. Effects of hydrogen ion concentration on radial growth of mycelia and germ tube elongation of macroconidia of *Fusarium solani* f. sp. *phaseoli* during in vitro tests. Radial growth was measured after 10 days on modified carnation leaf agar, and germ tube elongation was measured after 6 h on nutrient solution agar. (O—O = Radial growth; Δ—Δ = germ tube length.)

TABLE 2. Attachment of macroconidia of *Fusarium solani* f. sp. *phaseoli* to four different root orders and to the lower hypocotyls of *Vigna radiata*

Root orders or hypocotyl segments	Numbers of macroconidia	
	Spores/cm[w]	Spores/mm^2[x]
Lower hypocotyls[y]	12 a[z]	19 a
Second-order roots with root hairs	396 e	252 d
Second-order roots without root hairs	131 d	158 c
First-order roots	61 c	131 c
Root tips	28 b	83 b

[w]Data were not adjusted prior to statistical analysis and represent the numbers of macroconidia counted per linear centimeter of the root orders or hypocotyl segments.
[x]Data were adjusted to compensate for differences in area among root orders and hypocotyl segments attributable to differences in the diameter of the root and hypocotyl segments. In addition, data were adjusted to compensate for the presence of root hairs on the second-order roots below the crown.
[y]Sections (1 cm long) of the hypocotyl, directly above the root crown, were scanned for the presence of macroconidia.
[z]Treatment mean of 15 replicates (three replicates per treatment in each of five repetitions of the experiment). Data were transformed to $\log_{10}$ and then subjected to an analysis of variance followed by Fisher's least significant difference test; table values are presented as detransformed numbers. Treatments in columns followed by the same letter were not different ($P > 0.05$).

TABLE 3. Effects of temperature and hydrogen ion concentration on the attachment of macroconidia of *Fusarium solani* f. sp. *phaseoli* to second-order roots of *Vigna radiata*

Temperature (C)	Numbers of macroconidia at pH				
	3	4	5	6	7
15	13 (6)[z]	128 (56)	174 (98)	94 (40)	14 (10)
20	20 (13)	587 (191)	227 (75)	111 (94)	8 (3)
25	63 (53)	355 (89)	166 (76)	101 (39)	11 (7)
30	45 (38)	320 (117)	188 (81)	122 (70)	10 (7)
35	7 (3)	7 (5)	3 (3)	5 (4)	3 (2)

[z]Treatment mean of nine replicates (three replicates per treatment in each of three repetitions of the experiment); values in parentheses are half the confidence intervals for each treatment (±95% confidence interval). Each mean corresponds to the numbers of macroconidia attached per linear centimeter of second-order roots. Data were transformed to 0.25-powers and then subjected to an orthogonal polynomial contrast analysis; table values are presented as detransformed numbers.

Effects of temperature and H^+ on spore attachment and germ tube elongation. Attachment of macroconidia of *F. s. phaseoli* to second-order roots of *V. radiata* was greatest at 20–30 C and pH 4 (Table 3 and Fig. 3). The numbers of macroconidia attached to roots decreased as temperature or H^+ either increased or decreased. The numbers of macroconidia attached per linear centimeter of root at 20–30 C and pH 4 decreased by nearly two orders of magnitude when the temperature was raised to 35 C or the pH elevated to 7. There was no effect of temperature at pH 7, nor was there an effect of the H^+ at 35 C. Macroconidia were misshapen and internal cell structures disrupted at pH 3, but they appeared to be normal at all other H^+.

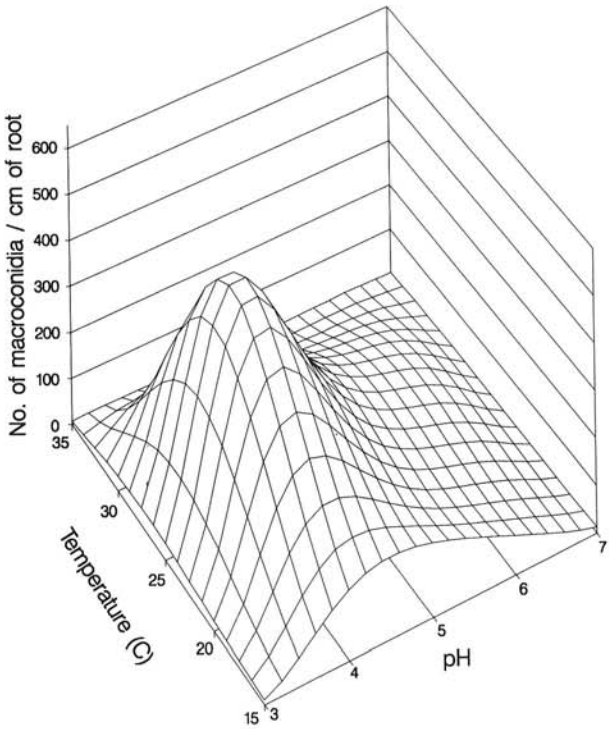

Fig. 3. Numbers of macroconidia of *Fusarium solani* f. sp. *phaseoli* adhered to second-order roots of *Vigna radiata* as predicted by the polynomial model for the effects of temperature and hydrogen ion concentration on spore attachment. The response surface was described best by a cubic-by-quadratic polynomial equation expressed as: $(Y + 1)^{0.25} = 460.7 - 46.8(T) + 0.86524(T^2) + 0.00237(T^3) - 398.4(pH) + 124.1(pH^2) - 16.4(pH^3) + 0.78528(pH^4) + 40.5(T{\cdot}pH) - 12.6(T{\cdot}pH^2) + 1.66(T{\cdot}pH^3) - 0.07911(T{\cdot}pH^4) - 0.77297(T^2{\cdot}pH) + 0.24957(T^2{\cdot}pH^2) - 0.03340(T^2{\cdot}pH^3) + 0.00159\ (T^2{\cdot}pH^4) - 0.00127(T^3{\cdot}pH) + 0.00012(T^3{\cdot}pH^2)$ ($R^2 = 0.81$), in which Y represents the predicted value of the number of macroconidia attached per linear centimeter of root, and in which T represents the temperature and pH represents the hydrogen ion concentration of the nutrient solution at the time of root inoculation.

TABLE 4. Effects of temperature and hydrogen ion concentration on germ tube elongation of *Fusarium solani* f. sp. *phaseoli* after spore attachment to second-order roots of *Vigna radiata*

Temperature (C)	Germ tube lengths (μm) at pH				
	3	4	5	6	7
15	0 (0)[z]	17 (3)	20 (5)	27 (2)	21 (8)
20	13 (3)	67 (6)	84 (8)	88 (6)	87 (87)
25	15 (4)	110 (5)	140 (5)	141 (7)	135 (8)
30	1 (1)	81 (8)	101 (16)	104 (15)	104 (13)
35	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[z]Treatment mean of nine replicates (three replicates per treatment in each of three repetitions of the experiment); values in parentheses are half the confidence intervals for each treatment (±95% confidence interval). Data were transformed to square roots and then subjected to two-phase regression analyses for the effects of hydrogen ion concentration within each temperature and orthogonal polynomial contrast analyses for the effects of temperature within each hydrogen ion concentration; table values are presented as detransformed numbers.

Elongation of germ tubes of macroconidia on root surfaces of *V. radiata* was greatest at 25 C and pH 5–7 (Table 4). Macroconidia failed to germinate at 35 C, and at 15 C and pH 3. At pH 3, as compared to higher levels, germination also was suppressed at 20–30 C. Germ tube elongation generally was not affected by H^+ between pH 5 and 7 at 15, 20, 25, or 30 C, but it was affected by temperature within each H^+ tested (Table 4).

The effects of H^+ within each temperature between 15 and 30 C were best described by two-phase regression models (Table 5). The slopes of the first phase regression lines increased as temperature increased from 15–30 C, indicating that the rate of germ tube elongation between pH 3 and 4 increased as temperature increased. The slopes of the second-phase regression lines at each temperature were not different from zero ($P > 0.05$), and the maximum plateau was predicted to occur at 25.6 C. Spline points between the first- and second-phase regression lines of each model

TABLE 5. Parameter estimates for two-phase regression models describing the effects of hydrogen ion concentration within each temperature on germ tube elongation by macroconidia of *Fusarium solani* f. sp. *phaseoli* on root surfaces of *Vigna radiata*

Temperature (C)	Parameter[w]	Estimate[x]	95% Confidence interval[y]
15	μ_1	−12.09	2.47
	β_1	4.03	0.68
	t_1	4.16	0.14
	p_1	4.69	0.77
	R^2	0.81	...[z]
20	μ_2	−10.42	2.47
	β_2	4.65	0.68
	t_2	4.24	0.13
	p_2	9.28	0.82
	R^2	0.95	...
25	μ_3	−15.89	2.47
	β_3	6.61	0.68
	t_3	4.20	0.10
	p_3	11.77	0.90
	R^2	0.98	...
30	μ_4	−26.11	2.47
	β_4	8.77	0.68
	t_4	4.13	0.08
	p_4	10.10	0.99
	R^2	0.95	...
35	μ_5	...	...
	β_5	...	...
	t_5	...	...
	p_5	...	...
	R^2	...	...

[w]Parameter estimates were significant at $P \leq 0.05$; μ = y-intercept, β = slope of the first-phase regression line, t = spline point (pH at which the plateau was reached) for the first- and second-phase regression lines, p = estimated plateau, and R^2 = correlation coefficient.
[x]Parameter estimates are presented as generated from square-root transformed data.
[y]Half-width confidence interval (estimate, ± confidence interval).
[z]Parameter was not estimable.

TABLE 6. Quadratic regression models describing the effects of temperature within each hydrogen ion concentration on germ tube elongation by macroconidia of *Fusarium solani* f. sp. *phaseoli* on root surfaces of *Vigna radiata*

pH	Model[z]	R^2
3	$-11.06 + 1.209(T) - 0.0252(T^2)$	0.61
4	$-36.67 + 3.892(T) - 0.08004(T^2)$	0.95
5	$-42.04 + 4.441(T) - 0.09132(T^2)$	0.94
6	$-39.17 + 4.247(T) - 0.08808(T^2)$	0.94
7	$-41.69 + 4.411(T) - 0.09072(T^2)$	0.93

[z]Models and polynomial terms significant at $P \leq 0.05$; T = temperature. Polynomial terms are presented as generated from square-root transformed data.

were between pH 4.13 and 4.24. Macroconidia failed to germinate at 35 C. Effects of temperature within each H^+ were best described by quadratic polynomial equations (Table 6).

In the germ tube elongation experiment, the numbers of macroconidia were dramatically lower on roots 6 h after inoculation when incubated at 35 C or pH 7 than on roots incubated at lower temperatures or H^+. The numbers of macroconidia observed on roots incubated for 6 h at 35 C or pH 7 were consistent with the numbers of macroconidia observed on second-order roots in the experiment on the effects of temperature and H^+ on spore attachment (Table 3).

Germ tubes were distorted and variable in shape in treatments at pH 3 and 20–30 C. Under these conditions, germ tubes generally emerged from lateral walls of terminal, foot, or intercalary cells; these results were consistent with in vitro and spore attachment experiments in which macroconidia were incubated at pH 3.

Effects of inoculum density and temperature on root disease. Disease symptoms in *V. radiata* infected with *F. s. phaseoli* in hydroponic nutrient solutions include root necrosis, stunting of foliar and stem tissues, and plant wilt (30). The severity of root and shoot symptoms on *V. radiata* caused by *F. s. phaseoli* was influenced by temperature and inoculum density. At 20 C and inoculum densities of 100–3,000 propagules per milliliter, necrotic flecks were observed on all root orders 72–96 h after inoculation. Necrotic flecks coalesced into large necrotic lesions by 7 days, and the roots were moribund by 10–14 days after inoculation. Stunting of inoculated plants was observed between 5 and 7 days after inoculation at 500–3,000 propagules per milliliter and between 10 and 14 days after inoculation at 100 propagules per milliliter. At 25 C and inoculum densities of 500–3,000 propagules per milliliter, root and shoot symptoms were less severe, and took longer to develop, than symptoms of plants inoculated at 20 C and inoculum densities of 500–3,000 propagules per milliliter. At 25 C and 100 propagules per milliliter and at 30 C and 100–3,000 propagules per milliliter, root symptoms generally were lacking on inoculated plants. However, a few necrotic flecks were observed on second-order roots near crowns when plants were inoculated at 25 C and 100 propagules per milliliter and at 30 C and inoculum densities of 500–3,000 propagules per milliliter.

Plant fresh weights decreased with increasing inoculum densities at 20 and 25 C but not at 30 C (Fig. 4). At 20 C, the decrease in the fresh weights of inoculated plants was best described by the linear equation $Y = 6.93 - 1.71(\log_{10} ID)$ ($R^2 = 0.67$), in which ID = inoculum density. At 25 C, the decrease in the fresh weights of inoculated plants was best described by a two-phase regression model in which the y-intercept for the first phase was 11.9, the slope of the first phase was not different from zero ($P > 0.05$), the spline point was predicted to occur at an inoculum density of 140 propagules per milliliter of nutrient solution (2.15 $\log_{10} ID$), and the slope of the second phase was -5.79 ($R^2 = 0.53$). At 30 C, the relationship between inoculum density and plant fresh weight was described by the equation $Y = 16.18 - 0.11(\log_{10} \text{ID})$ ($R^2 = 0.002$), in which the slope was not different from zero ($P > 0.05$).

Effects of H^+ on root disease. Fresh weights of inoculated plants were less than those of uninoculated controls when plants were inoculated at pH 4, 5, or 6, but not at pH 7 (Table 7). The effect of H^+ on the severity of disease was greatest at pH 4; fresh weights of plants inoculated at pH 4 were 54–61% less than

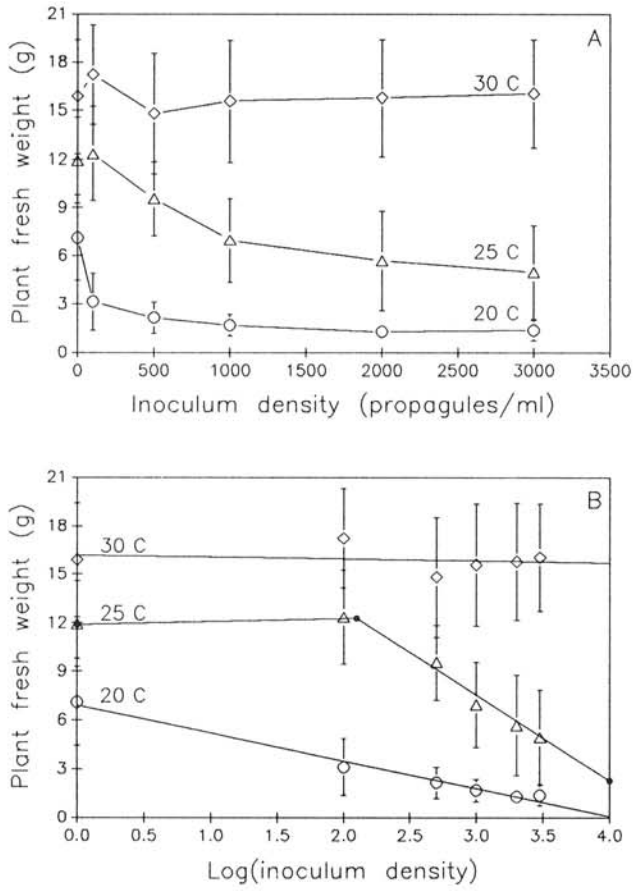

Fig. 4. Effects of inoculum density of *Fusarium solani* f. sp. *phaseoli* and temperature on the severity of disease in *Vigna radiata*. **A,** Plant fresh weights plotted against untransformed inoculum densities. **B,** Linear and two-phase regression lines of plant fresh weights plotted against inoculum densities transformed to $\log_{10}$ (R^2 for 20, 25, and 30 C were 0.67, 0.53, and 0.002, respectively). Bars indicate standard deviations of the means.

TABLE 7. Effects of hydrogen ion concentration at the time of inoculation on disease caused by *Fusarium solani* f. sp. *phaseoli* in *Vigna radiata*

	Plant fresh weight (g)	
pH[y]	Uninoculated	Inoculated
4	10.35 (1.05)[z]	3.81 (1.21)
5	10.54 (0.72)	7.09 (1.60)
6	10.51 (1.71)	7.00 (0.85)
7	9.38 (1.23)	8.64 (1.69)

[y] Hydrogen ion concentrations in separate treatments were maintained for 24 h after plants were inoculated, and then all treatments were adjusted to pH 6.

[z] Treatment mean of nine replicates (three replicates per treatment in each of three repetitions of the experiment); values in parentheses are half the confidence intervals for each treatment (± 95% confidence interval). Untransformed data were subjected to an orthogonal polynomial contrast analysis; table values are presented as detransformed numbers.

TABLE 8. Effects of hydrogen ion concentration at the time of inoculation on the numbers of propagules of *Fusarium solani* f. sp. *phaseoli* detected in nutrient solution over time in experiments on disease development in *Vigna radiata*

	Number of propagules (cfu/ml)				
pH	Day 0[y]	Day 1	Day 5	Day 10	Day 14
4	452 (26) a[z]	1 (1) c	2 (1) c	5 (4) c	23 (26) b
5	482 (19) a	6 (2) b	1 (1) c	2 (1) c	6 (4) b
6	525 (33) a	6 (4) b	3 (2) b	3 (2) b	6 (3) b
7	521 (23) a	20 (12) b	17 (10) b	13 (9) b	14 (19) b

[y] Samples on day 0 were taken 30 min after plants were inoculated.

[z] Treatment mean of nine replicates (three replicates per treatment in each of three repetitions of the experiment); values in parentheses are half the confidence intervals for each treatment (± 95% confidence interval). Data were transformed to square roots and then subjected to orthogonal polynomial contrast analyses when testing the effects of hydrogen ion concentration within each day and analyses of variance followed by least-squares mean separation procedures for repeated measures when testing the effects of days within each hydrogen ion concentration; table values are presented as detransformed numbers. Treatments in rows followed by the same letter were not significantly different ($P > 0.05$). Treatments in columns were considered significantly different if the confidence intervals failed to overlap.

those of uninoculated plants. The effect of H^+ on the plant fresh weights of uninoculated plants was described by the linear equation $Y = 11.82 - 0.29(pH)$ ($R^2 = 0.04$) in which the slope was not different from zero ($P > 0.10$). The effect of H^+ on the plant fresh weights of inoculated plants was described best by the linear equation $Y = -1.28 + 1.44(pH)$ ($R^2 = 0.44$). Some variability in the data was observed for the effect of H^+ on the plant fresh weights of inoculated plants between the first repetition ($R^2 = 0.42$) and the second and third repetitions of the experiment ($R^2 = 0.75$ and 0.65, respectively).

When the populations of the pathogen were measured in the nutrient solutions at 30 min after inoculation, fewer propagules (colony-forming units [cfu] per milliliter) were detected in treatments in which plants were inoculated at pH 4 (452 cfu/ml) than in treatments in which plants were inoculated at pH 6 or 7 (525 and 521 cfu/ml, respectively) (Table 8). The numbers of propagules of *F. s. phaseoli* recovered from the nutrient solutions in all treatments decreased rapidly after 24 h and remained low for the remainder of the experiment. A slight increase in the number of propagules per milliliter of nutrient solution was observed between 10 and 14 days after inoculation at pH 4 and 5, but not at pH 6 and 7 ($P \leq 0.05$).

Sporodochia, large, highly branched conidiophores, and macroconidia were observed in greatest abundance on roots inoculated at pH 4. Conidiophores and macroconidia were also observed on roots inoculated at pH 5 or 6, but the numbers of these structures were lower compared to roots inoculated at pH 4. Sporodochia or highly branched conidiophores were not observed on roots treated at pH 7 during inoculation; unbranched monophialides were observed rarely at pH 7 on hyphae present in the rhizosphere.

DISCUSSION

In soils, attachment of phytopathogenic fungi to root surfaces may not be a prerequisite for pathogenesis, because the proximity of infective propagules to host tissues is maintained by the stability of the soil matrix. However, in a hydroponic nutrient solution, nonmotile propagules must interact with root surfaces directly and establish a close association with the host in order to permit adequate time for the germination of the propagule and subsequent infection of the root. Therefore, attachment of nonmotile propagules to root surfaces in nutrient solution may be conceptually similar to spore attachment to aerial plant surfaces. Root pathogens in hydroponic systems that are capable of rapid attachment, germination, and penetration should have a competitive advantage in causing disease.

Given the results of the spore attachment experiment and on pathogenicity tests (30), it was hypothesized that the effects of H^+ on disease would depend upon the timing of the H^+ treatments. When the H^+ was adjusted after plant inoculation, the severity of disease caused by *F. s. phaseoli* in *V. radiata* was suppressed at pH 4 (30). Furthermore, when pH was maintained at 4 after inoculation, production of secondary inoculum was suppressed at 20 C, a temperature normally conducive to the production of secondary macroconidia. In the current study, when the H^+ was adjusted to pH 4, 5, 6, or 7 during plant inoculation, pH 4 enhanced the severity of disease and increased the production of secondary inoculum at 25 C. In a previous study, few secondary macroconidia were produced at 25 C and pH 4–7 (30). Thus, both a high level of disease caused by *F. s. phaseoli* in *V. radiata* and a high level of spore attachment by the pathogen to roots of *V. radiata* occurred when H^+ was maintained at pH 4 during plant inoculations.

The effects of H^+ on spore attachment by *F. s. phaseoli* to roots were different from the effects of H^+ on germ tube elongation. Both in vitro and in situ experiments support the conclusion that H^+ between pH 5 and 7 had little or no effect on the growth of the fungus. In contrast, spore attachment was greatest at pH 4 and decreased by nearly two orders of magnitude at pH 7. Given the results of the current study and those from a parallel study (30), we propose that the effects of H^+ of the nutrient solution after spore attachment on disease severity are primarily due to the influence of H^+ on host resistance and not due to the influence of H^+ on spore attachment or fungal growth.

For a second series of experiments, it was hypothesized that the effects of temperature on the severity of root disease would depend on the effects of temperature on spore attachment. The numbers of spores attached to roots were similar among plants inoculated at 20–30 C at each H^+ tested between pH 4 and 7. In contrast, both in vitro and in situ experiments support the conclusion that the effects of temperature on the growth of the fungus were best described by quadratic polynomials with an optimum temperature for growth predicted at 25 C. However, results from the inoculum density experiment confirm previous reports that *F. s. phaseoli* causes more disease at cooler (20 C) than warmer (25–30 C) temperatures (30,31). Therefore, we propose that the effects of temperature between 20 and 30 C on the severity of disease caused by *F. s. phaseoli* in *V. radiata* are primarily due to the effects of temperature on host resistance and not to the effects of temperature on spore attachment or fungal growth. Temperatures greater than 30 C may interfere with spore attachment to roots and the subsequent development of disease, but those above 30 C suppress the growth of uninoculated plants of *V. radiata* (30) and, thus, may not be practical for suppressing spore attachment in commercial hydroponic systems. Additional research is required to elucidate the effects of temperature and H^+ on host resistance.

Results of the current study bring into question the root-dip technique used to inoculate plants with *Fusarium* spp. Hydrogen ion concentrations of the spore suspensions usually are not reported in root-dip procedures (1,18,22,25). Although the technique is effective, high densities of spores, often between 10^5 and 10^7 propagules per milliliter, appear to be required for induction of moderate or severe root disease (1,18,22,25). In contrast, a density of 500 propagules per milliliter was adequate in the current study to produce severe root disease at 20 C and moderate root disease at 25 C. If the H^+ are not similar among studies of related pathosystems, then differences in levels of disease may be due to the effects of H^+ on spore attachment and not to biological qualities of the hosts or pathogens. The effects of H^+ on spore attachment in solutions used for root-dip procedures for *Fusarium* spp. should be better characterized and reported.

Physical methods for controlling root diseases in hydroponic systems include ultraviolet radiation, ozonation, ultrafiltration, and thermal heating systems (13,34). However, limitations in efficacy, phytotoxicity, applicability, and energy requirements may reduce the utility of these physical control methods in some hydroponic systems. In contrast, control of some root diseases in hydroponic systems might be possible by inhibiting spore attachment to roots by altering the H^+ of the nutrient solution. If proven effective for other root pathogens, manipulation of H^+ in nutrient solutions may prove to be an inexpensive root disease management tool for hydroponic systems.

The mechanism that mediates spore attachment of *F. s. phaseoli* to roots of *V. radiata* is not known, but given the results from the current study and from parallel studies (28,30), several mechanisms of spore attachment should be considered. Mechanisms described for the attachment of other fungi to plant surfaces include hydrophobic interactions (10,12), secreted fungal adhesives (12,21), lectins (11,12,20,24), adsorptive processes (4), surface charge phenomena, and protein- or glycoprotein-mediated interactions (4,23,24). However, hydrophobic interactions probably did not mediate spore attachment of *F. s. phaseoli*, because macroconidia failed to attach readily to the hydrophobic surfaces on hypocotyls of *V. radiata*. Furthermore, although secretions of adhesive materials have been reported for *Fusarium* spp. (21,28), the secretion of spore mucilage by macroconidia of *F. s. phaseoli* was not correlated in time with spore attachment to roots of *V. radiata* (28); high numbers of macroconidia attached to plant roots within 5 min of inoculation, but spore mucilage was not observed before 1 h.

Lectins have been described in roots and hypocotyls of *V. radiata* (11,16), reported on root hairs of other legumes (5), and

implicated as functional components in fungal spore attachment to plant surfaces (12,20). Furthermore, macroconidia of *F. s. phaseoli* can be agglutinated by the lectin concanavalin A (Con A) (3,17). However, seven sugar haptens of plant lectins, including the hapten for Con A, failed to prevent attachment of *F. s. phaseoli* macroconidia to roots of *V. radiata* (28). Therefore, chemical binding phenomena other than lectins may have been involved in spore attachment of *F. s. phaseoli* to roots of *V. radiata*.

The suppression of spore attachment by *F. s. phaseoli* at high temperature (35 C) and low acidity (pH 7) in the current study supports the hypothesis that the binding properties of the host–fungus interaction may involve proteins or protein moieties in larger macromolecules. Extremes of temperature and H^+ can change the stoichiometries of proteins, alter the surface charges of macromolecules, or induce denaturation of proteinaceous compounds.

Adsorption of fungal spores to plant surfaces might be involved in attachment of macroconidia of *F. s. phaseoli*. Adsorption can be suppressed or reversed by altering the H^+ of the medium (4) or be inhibited when ions involved in cation bridges are rendered unavailable in neutral or alkaline environments (2). In the current study, extremes of pH near 3 or 7 inhibited macroconidium attachment to root surfaces. In addition, macroconidia that bound to root surfaces when plants were inoculated at 25 C and pH 5 in the germ tube elongation experiment detached from roots when plants were transferred to 35 C or pH 7. Presumably, equal numbers of macroconidia attached to second-order roots during plant inoculations, and, thus, the reductions in the numbers of macroconidia observed at 35 C or pH 7 represented a loss of macroconidia from the rhizoplane of *V. radiata* during the 6-h postattachment period.

A neutral or slightly alkaline environment may also interfere with spore attachment by reducing the availability of micronutrients in the infection court. Effective suppression of disease caused by *F. oxysporum* f. sp. *lycopersici* on tomato by the addition of lime to soils has been demonstrated (14,15); micronutrient deficiencies created by alkaline conditions were suggested as the mechanism of disease suppression (14). At H^+ greater than pH 6.5, Fe^{3+} and Ca^{2+} ion species begin to precipitate from nutrient solution (2). Calcium has been implicated as a required cofactor in the adsorption of *F. moniliforme* to root mucilage of *Zea mays* (9), and iron may be essential for spore germination by *Fusarium* spp. in the rhizosphere (6,27).

Additional research is required to characterize the mechanism of spore attachment by *F. s. phaseoli* to roots of *V. radiata*. Results from the current study are consistent with the adsorptive and protein-mediated mechanisms, but the possible effects of neutral or slightly alkaline H^+ on nutrient availability should also be considered. In addition, caution should be exercised in interpreting results from studies on the effects of temperature and H^+ on the severity of plant diseases in hydroponic solutions if concomitant studies on spore attachment to roots are not available. Future research should consider the possibility that separate mechanisms at different temperatures or H^+ might be involved in spore attachment of *F. s. phaseoli* to roots of *V. radiata*. Although a single mechanism of spore attachment may be operating, Nicholson and Epstein (24) concluded that there is no evidence in the literature to dispel the possibility that several binding mechanisms are concomitantly involved in spore attachment.

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