Influence of pH and Matric Potential on Sporulation of *Cephalosporium gramineum*

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ABSTRACT

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Sporulation by *Cephalosporium gramineum* on mineral salts agar (MSA) containing phosphate or citrate-phosphate buffer, respectively, was 10-fold and 10^4-fold greater at pH 4.5–5.5 than at pH 6.5–7.5, regardless of temperature (MSA-phosphate buffer only) or fungal isolate. Sporulation on MSA with phosphate buffer was greatest at 20 and least at 5 C, which corresponds to the temperatures for greatest and least hyphal growth, respectively, on this medium. Sporulation on oat kernels artificially colonized by *C. gramineum* or on naturally colonized wheat straw, on or buried 2 cm below the soil surface, was twofold to threefold greater at soil pH 4.5–5.5 than at 6.5–7.5. In contrast to the effect of temperature in vitro, sporulation by *C. gramineum* on oat kernels on soil was 28-fold to 50-fold greater at 5 than at 15 C. Sporulation on oat kernels or straw on soil increased from twofold to 10^3-fold as soil matric potential decreased from −0.001 to −0.07 MPa. Greater sporulation of *C. gramineum* at low soil pH may partially explain why Cephalosporium stripe is more severe in acid soils (pH 4.5–5.5) than in soils of higher pH. However, the influence of soil matric potential on sporulation observed in this study was not consistent with the increase in Cephalosporium stripe at high soil moisture contents.

Additional keywords: inoculum production, *Triticum aestivum*, vascular wilt

Cephalosporium gramineum Nis. & Ika. (syn. *Hymenula cerealis* Ellis & Everh.) causes a vascular wilt of winter wheat (*Triticum aestivum* L. em. Thell.), other cereals, and grasses. It is generally believed that *C. gramineum* infects plants during the winter and spring through wounds in roots created by soil freezing and heaving (2,4,11,13,14,17). However, infection of winter and spring wheat by *C. gramineum* in the absence of root wounding induced by frozen soil has been demonstrated (2). In greenhouse studies where frozen soil was not a factor, the incidence of Cephalosporium stripe increased from near 0 to 100% infected stems as soil pH decreased from 7.5 to 4.5 (10). These results have been confirmed, and an interaction between soil pH and matric potential has been demonstrated (1). Both the incidence and severity of Cephalosporium stripe increased as soil pH decreased from 7.5 to 4.5 and as soil matric potential increased from −1.0 to −0.1 bar (−0.1 to −0.01 MPa).

From field observations and previous studies, other researchers (3,4,17) have emphasized the importance of high soil moisture for the development of Cephalosporium stripe. Greater disease at high soil moisture content is attributed to increased host susceptibility (17), increased sporulation and dissemination of the pathogen (4), and increased root wounding as a result of more freezing and heaving (4). Conversely, less disease in drier soils is attributed to a reduction in inoculum density resulting from adsorption of conidia to soil particles (17).

In the absence of soil freezing, a definitive explanation for greater Cephalosporium stripe in soils with low pH and high soil moisture is lacking. Soil pH and moisture may directly influence host susceptibility, pathogen virulence, or both, or may indirectly influence the pathogen-suscept interaction by altering the soil microbiota (7,8), availability of micronutrients (18), or some other component of the soil system. Evidence for both direct and indirect effects of pH on *C. gramineum* exists. For example, growth of *C. gramineum* in vitro is greatest when pH is < 5.5 and least when near 7.5 (15). Survival of *C. gramineum* in infested straw is greatest in soils with pH values of 3.9–5.5, which is attributed to reduced competition as a result of increased antibiotic production by *C. gramineum* (6). Sporulation by *C. gramineum* on straw recovered from soil after burial for 11 mo is also greatest from pH 3.9–5.5 (6).

Increased inoculum density resulting from increased sporulation by *C. gramineum* at low pH values may explain the increase in Cephalosporium stripe in acid soil (pH 4.5–5.5). Some physical and chemical factors that influence sporulation of *C. gramineum* in vitro have been studied (12), but little is known about the

influence of pH on sporulation in vitro, or how soil pH and soil moisture content affect sporulation on artificially or naturally colonized substrates. The purpose of our study was to determine the influence of substrate pH on sporulation by *C. gramineum* in vitro and the effects of soil pH and matric potential on sporulation on artificially and naturally colonized substrates. A preliminary report of this work has been published (16).

MATERIALS AND METHODS

Media and fungal isolates. Mineral salts agar (MSA) was adjusted from pH 4.5 to 7.5, at intervals of approximately 1 pH unit, with either phosphate buffer (K_2HPO_4) (PB) or citrate-phosphate buffer (Na_2HPO_4 + citric acid monohydrate)(CB) (15). pH after solidification of the agar was measured with a flat-surface combination electrode. Actual pH values were usually within 0.1 pH unit of the target value and were used in statistical analyses and figures. Three pathogenic isolates of *C. gramineum* (Cg85-4, Cg84-16, and Cg84-30) (15) were used in all studies unless otherwise indicated. Inoculum was prepared by culturing the fungus in unbuffered potato dextrose broth on a rotary shaker (100 rpm) for 2–5 days at 20 C. Conidia were harvested by centrifuging at 2,000 g for 10 min, then washed three times in sterile deionized water. The concentration of conidia was adjusted to 1×10^6 conidia per milliliter with the aid of a hemacytometer.

Sporulation in vitro. Sporulation in vitro was evaluated with techniques similar to those described previously (12). Drops (25 μl) of a conidial suspension were spotted on MSA-PB and MSA-CB (adjusted to pH values of 4.5, 5.6, 6.7, and 7.6 and 4.5, 5.4, 6.6, and 7.0, respectively) in plastic petri dishes. Inoculated dishes were incubated in covered polyethylene boxes in the dark at 15 C. Sporulation was evaluated 7 and 14 days after spotting on MSA-PB and MSA-CB, respectively, by removing four individual colonies with a cork borer and placing them in 3 ml of sterile distilled water. After standing for 5 min, samples were agitated for 15 sec with a vortex mixer, sonicated for 5 sec in a Bransonic B-32 ultrasonic cleaner (Branson Cleaning Equipment Co., Shelton, CT), and agitated for another 15 sec. Each sample was serially diluted, and 0.1 ml subsamples were spread on acidified corn meal agar (aCMA: 2 ml 25% lactic acid per liter) and incubated for 7–10 days at 20 C, when the number of colonies of *C. gramineum* was counted. This experiment was repeated once, with four replicates in each experiment.

The effect of temperature on sporulation was evaluated on MSA-PB at pH 4.5, with isolate Cg84-30, after incubation for 1 or 3 wk at 5, 10, 15, or 20 C. Potential interactions between pH (4.7, 5.6, 6.6, and 7.5) and temperature (5–20 C) were also studied on MSA-PB. All combinations of pH and temperature were tested with a factorial arrangement of treatments. Inoculation and incubation of test media, sampling times, and quantification of sporulation were as described above. Experiments on temperature only had three replicates, whereas those on pH × temperature interaction had four. These experiments were repeated once.

Soil mix and inocula. All experiments were conducted in 500-ml polypropylene jars having screw caps. Each jar contained approximately 200 g oven-dry equivalent of a soil mix containing Thatuna silt loam, vermiculite, and quartz sand (16 mesh) (90:5:5, w/w/w)(1). Soil pH was adjusted to values from 4.5 to 7.5 at intervals of approximately 1 pH unit with reagent grade calcium hydroxide or hydrochloric acid (1).

Soil matric potential was adjusted gravimetrically by comparison with a soil moisture release curve and, unless indicated otherwise, was maintained at approximately −0.06 MPa. In all experiments, soil in jars was first wetted to near saturation (approximately 50% gravimetric moisture) with sterile deionized water, covered with cheesecloth, left to dry to the desired moisture content, and then recapped. Final gravimetric moisture content was determined at the end of each experiment.

Oat kernels artificially colonized by *C. gramineum* (1) or mature wheat straw from plants with symptoms of Cephalosporium stripe were used to infest soil. Oat kernels and straw pieces were soaked in deionized water for 4–8 hr, surface disinfested in NaOCl, rinsed in sterile deionized water, blotted on a paper towel, and then placed on the soil surface or buried. Oat kernels or straw incubated on the soil surface were gently pressed into the soil to ensure complete contact. Buried inocula were covered, without packing, with about 2 cm of soil that had been removed from the surface of the soil in jars after moisture equilibration. After inoculation, jar caps were replaced loosely enough to reduce desiccation but tightly enough to prevent anaerobic conditions. The air space above the soil surface and the cap was approximately 5 cm.

Sporulation in soil: Colonized oat kernels. The effects of soil pH and position of colonized oat kernels were studied at 10 C. About 15 oat kernels were removed from each jar after incubation in the dark for 3 wk. Care was taken to avoid disturbing soil adhering to the oat kernels during removal. Oat kernels were placed in 10 ml of sterile deionized water in 60-ml polypropylene bottles and shaken for 10 min on a wrist-action shaker. The wash water was diluted serially and 0.1 ml spread on a medium semi-selective for *C. gramineum* (19). Petri dishes were incubated for 10 days at 15 C before the number of colonies were counted. Washed oat kernels were oven-dried (105 C) and weighed to determine the amount of colonized tissue. Sporulation was expressed as the number of colony-forming units per gram (cfu/g) of oven-dried oat kernel. This experiment was repeated once; there were three replicates in each experiment.

In two additional experiments, the effects of soil (pH 5.6) matric potential (−0.007, −0.015, and −0.061 MPa) and position of oat kernels at 10 C and of soil (pH 5.5) matric potential (−0.005, −0.01, and −0.065 MPa) with buried oat kernels at 5, 10, or 15 C were evaluated. Oat kernels were removed after incubation in the dark for 2 and 6 wk, respectively. There were four replicates in each experiment.

In two final experiments conducted with buried oat kernels, the effects of soil pH and soil matric potential were studied at 10 C in one experiment (2-wk incubation), and at 5, 10, and 15 C in the second experiment (6-wk incubation). Soil pH was adjusted to 4.7, 6.0, and 7.1 and 4.5, 5.6, 6.4, and 7.5 in the first and second experiments, respectively. Soil matric potential was adjusted to −0.006, −0.015, and −0.065 MPa and −0.001, −0.01, and −0.063 MPa in the first and second experiments, respectively. There were three replicates in each experiment.

Sporulation in soil: Naturally colonized straw. Two experiments were conducted with naturally colonized mature wheat straw to determine if the effects of soil pH, soil matric potential, and straw position on sporulation were similar to those on colonized oat kernels. Soil pH was adjusted to 4.7, 6.0, and 7.1 and 4.8, 6.4, and 7.7 in the first and second experiments, respectively. Soil matric potential was adjusted to −0.006, −0.015, and −0.065 MPa and −0.005, −0.01, and −0.07 MPa in the first and second experiments, respectively. Straw was recovered after incubation in the dark for 2 or 6 wk at 10 C, respectively. Sporulation in all experiments was evaluated as described above for oat kernels. There were three replicates in each experiment.

Statistical analyses. All experiments were arranged as randomized complete block designs. The total number of cfu from in vitro studies was transformed to $\log_{10}$ cfu, whereas sporulation data from oat kernels and straw were transformed to $\log_{10}$ cfu/g of inoculum before analysis of variance. The significance of response curves for pH or soil matric potential was determined by partitioning the sums of squares for pH into linear, quadratic, and cubic components (9). Orthogonal polynomials for equally spaced treatments were used to analyze significant trends for temperature responses.

RESULTS

Sporulation in vitro. Phosphate buffer (PB) and citrate-phosphate buffer (CB) affect growth differently (15) and, therefore, were treated as separate experiments. For both buffers, pH and isolate had significant effects ($P < 0.01$) on sporulation, and there were significant ($P < 0.01$) interactions between pH and isolate. All three isolates exhibited significant ($P < 0.01$) cubic responses to pH on both buffers. Because the interactions between

isolate and pH resulted from differences in the magnitude of the response to pH as opposed to qualitative (directional) differences, the mean values for pH across isolates are presented. Typically, sporulation decreased from a maximum near pH 4.5–5.5 to a minimum of about pH 6.5, followed by a slight increase as pH approached 7.5 (Fig. 1).

The effect of temperature on sporulation was the same in both experiments, therefore, data for only one experiment are presented. Both temperature and length of incubation had significant ($P < 0.01$) effects on sporulation on MSA-PB at pH 5.5, and there was a significant ($P < 0.01$) interaction between these factors. Sporulation at 5, 10, 15, and 20 C after 1 and 3 wk was 5.4, 7.8, 7.7, and 8.3 log cfu and 8.0, 8.5, 8.5, and 8.6 log cfu, respectively. The response to temperature was significantly ($P < 0.01$) cubic after both 1 and 3 wk incubation.

Results of the initial pH × temperature interaction study were similar to those of the single-effect studies, with pH, temperature, and isolate all having significant effects ($P < 0.01$) on sporulation. At 7 days, sporulation was greatest at pH 4.5–5.5 at 20 C and least near pH 6.5 at 5 C (Fig. 2); the response to pH was significantly ($P < 0.05$) cubic at all temperatures. In addition, there were significant ($P < 0.01$) two- and three-way interactions among pH, temperature, and isolate. Interactions involving isolates resulted largely from differences in magnitude of response to pH and temperature, rather than qualitative differences among isolates. In the follow-up pH × temperature interaction study with a single isolate, both pH and temperature had significant effects ($P < 0.01$) on sporulation; the temperature × pH interaction was not significant. The pH response curve was significantly ($P < 0.01$) cubic, with sporulation decreasing from a high of 6.4 log cfu at pH 4.4 to a minimum of 5.2 log cfu at pH 6.3, then increasing to 5.8 log cfu at pH 7.3. The temperature response curve was significantly ($P < 0.01$) quadratic, with sporulation increasing from 3.5 to 5.9, 6.9, and 6.7 log cfu at 5, 10, 15, and 20 C, respectively.

Sporulation on oat kernels. Both soil pH and inoculum position had significant ($P < 0.02$ and 0.01, respectively) effects on sporulation after 3 wk incubation at 10 C. The overall response to pH was significantly ($P < 0.01$) linear, with sporulation increasing twofold as soil pH decreased from 7.5 to 4.5 (Fig. 3). Sporulation was nearly fourfold greater (8.7 compared with 8.1 log cfu/g, $P < 0.01$) when inoculum was on the soil surface

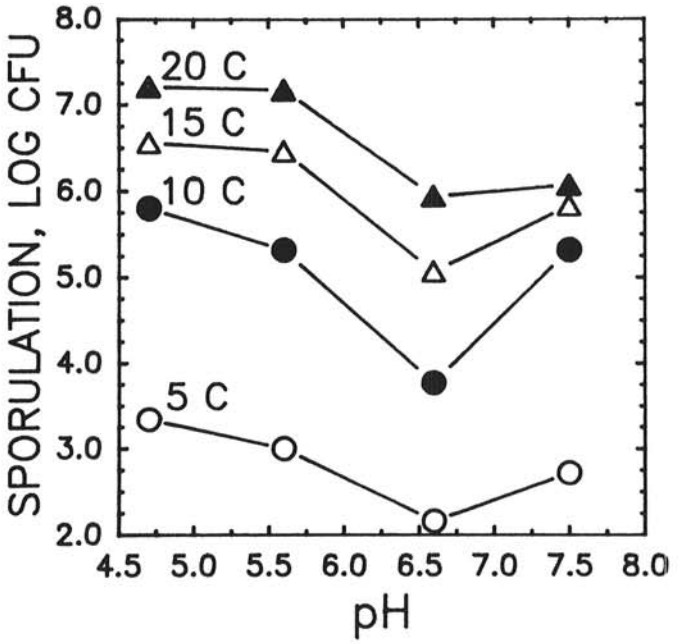

Fig. 2. Influence of temperature and substrate pH on sporulation of *Cephalosporium gramineum* on mineral salts agar containing phosphate buffer after incubation in the dark for 7 days. Each point represents the mean of three isolates and four replicates. Cubic regressions of sporulation ($\log_{10}$ colony-forming units) on substrate pH at 5, 10, 15, and 20 C are described by the equations $Y = -63.5 + 36.0X - 6.3X^2 + 0.4X^3$ ($P < 0.05$), $Y = -146.8 + 81.5X - 14.2X^2 + 0.8X^3$ ($P < 0.05$), $Y = -125.6 + 69.2X - 11.9X^2 + 0.7X^3$ ($P < 0.05$), $Y = -93.1 + 52.0X - 8.8X^2 + 0.5X^3$ ($P < 0.05$), respectively.

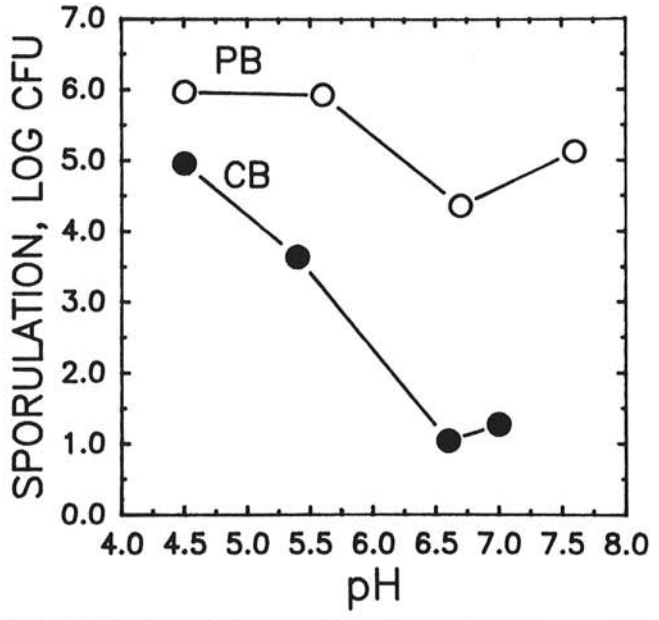

Fig. 1. Effect of substrate pH on sporulation by *Cephalosporium gramineum* in vitro on mineral salts agar with phosphate buffer (PB) or citrate-phosphate buffer (CB) after incubation in the dark for 7 days at 20 C. Each point represents the mean of three isolates and four replicates. Cubic regressions of sporulation ($\log_{10}$ colony-forming units) on substrate pH for PB and CB are described by the equations $Y = -106.4 + 59.5X - 10.2X^2 + 0.6X^3$ ($P < 0.01$), and $Y = -127.9 + 75.2X - 13.8X^2 + 0.8X^3$ ($P < 0.01$), respectively.

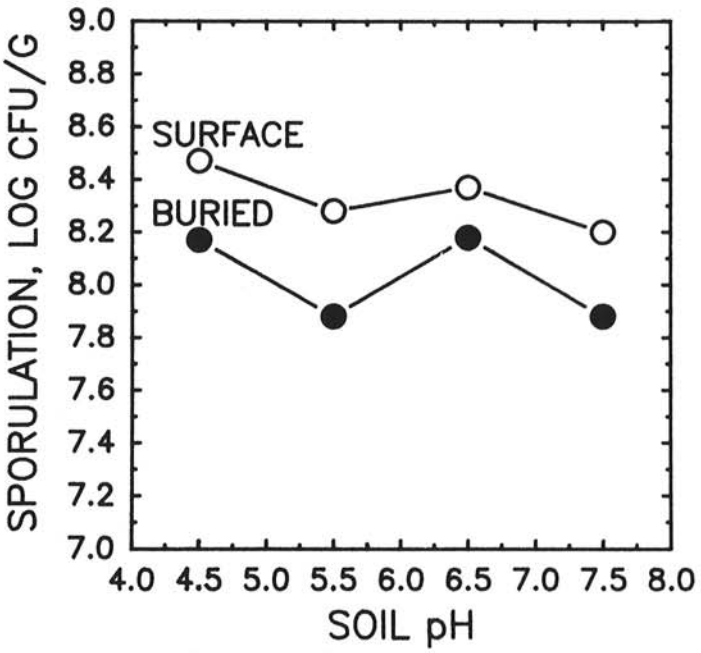

Fig. 3. Effect of inoculum position and soil pH on sporulation of *Cephalosporium gramineum* on colonized oat kernels. Oat kernels placed on or buried 2 cm below the soil surface and incubated in the dark for 3 wk at 10 C. Soil matric potential was adjusted to −0.07 MPa. Each point represents the mean of three replicates. The soil pH × inoculum position interaction was not significant, and, therefore, the linear regression of sporulation ($\log_{10}$ colony-forming units) on soil pH combined over inoculum position is described by the equation $Y = 8.8 - 0.1X$ ($P < 0.01$).

than when buried. The pH × inoculum position interaction was not significant.

Neither soil matric potential nor inoculum position had a significant ($P < 0.05$) effect on sporulation when oat kernels were sampled after incubation for 2 wk at 10 C, even though sporulation was twofold greater at −0.06 than at −0.007 MPa and when oat kernels were on the soil surface than when buried. In the follow-up experiment incubated for 6 wk, the response to matric potential was significantly quadratic ($P > 0.01$), with sporulation increasing 10^3-fold as soil matric potential decreased from −0.005 to −0.07 MPa (Fig. 4). Sporulation was 50-fold greater at 5 than at 15 C (8.3 versus 6.6 log cfu/g, $P < 0.01$); the data for 10 C were lost because of an incubator failure. The matric potential × temperature interaction was not significant ($P < 0.05$).

In two experiments to examine the interaction of soil matric potential and soil pH (2-wk incubation at 10 C), soil matric potential always had a significant ($P < 0.01$) effect on sporulation, whereas the effect of soil pH was significant ($P < 0.05$) only in one experiment. The response to matric potential was quadratic, with sporulation increasing 2.5-fold (from 7.6 to 8.0 log cfu/g) as matric potential decreased from −0.006 to −0.07 MPa. The soil pH × soil matric potential interaction was not significant.

In a similar experiment where oat kernels were recovered after incubation for 6 wk, matric potential, pH, and temperature all had significant ($P < 0.01$) effects on sporulation. On average, sporulation was 28-fold greater at 5 than at 15 C, threefold greater at pH 4.5 than at 7.5, and 100-fold greater at −0.06 than at −0.01 MPa. There were significant two-way interactions between these factors, however. The pH × matric potential interaction resulted from significant ($P < 0.01$) quadratic responses to pH at both −0.06 and −0.01 MPa but a nonsignificant ($P < 0.05$) response at −0.001 MPa (Fig. 5A). The pH × temperature interaction resulted from significantly linear ($P < 0.01$), quadratic ($P < 0.05$), and cubic ($P < 0.01$) response curves at 5, 10, and 15 C, respectively (Fig. 5B). The matric potential × temperature interaction resulted from quantitative differences in the quadratic relationship of sporulation to matric potential at 5, 10, and 15 C (Fig. 6).

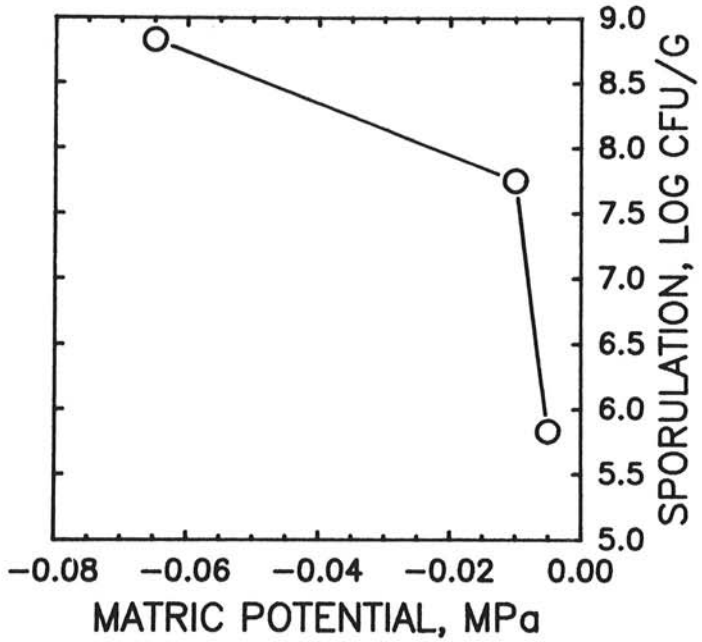

Fig. 4. Effect of soil matric potential on sporulation of *Cephalosporium gramineum* on colonized oat kernels buried 2 cm below the soil surface in plastic jars incubated in the dark for 6 wk at 10 C. Each point represents the mean of three replicates and two temperatures. The quadratic regression of sporulation ($\log_{10}$ colony-forming units) on soil matric potential is described by the equation $Y = 3.6 - 475.1X - 6072.7X^2$ ($P < 0.01$).

Sporulation on naturally colonized straw on soil. Sporulation increased 1.3-fold as matric potential decreased from −0.007 to −0.06 MPa and when straw was on the soil surface (as compared with buried straw); however, neither of these differences was significant ($P < 0.05$).

In two subsequent experiments, both soil pH and soil matric potential had significant ($P < 0.05$ and 0.01, respectively) effects on sporulation. In one experiment (incubated for 2 wk at 10 C), sporulation increased 2.5-fold (from 7.0 to 7.4 log cfu/g) as pH decreased from 7.1 to 4.7, and 1.8-fold (from 7.1 to 7.3 log cfu/g) as matric potential decreased from −0.006 to −0.07 MPa. The response curves were significantly ($P < 0.05$) linear for both pH and matric potential. In the second experiment (incubated for 6 wk at 10 C), sporulation increased fivefold (from 6.9 to 7.6 log cfu/g) as pH decreased from 7.7 to 4.8, and 200-fold

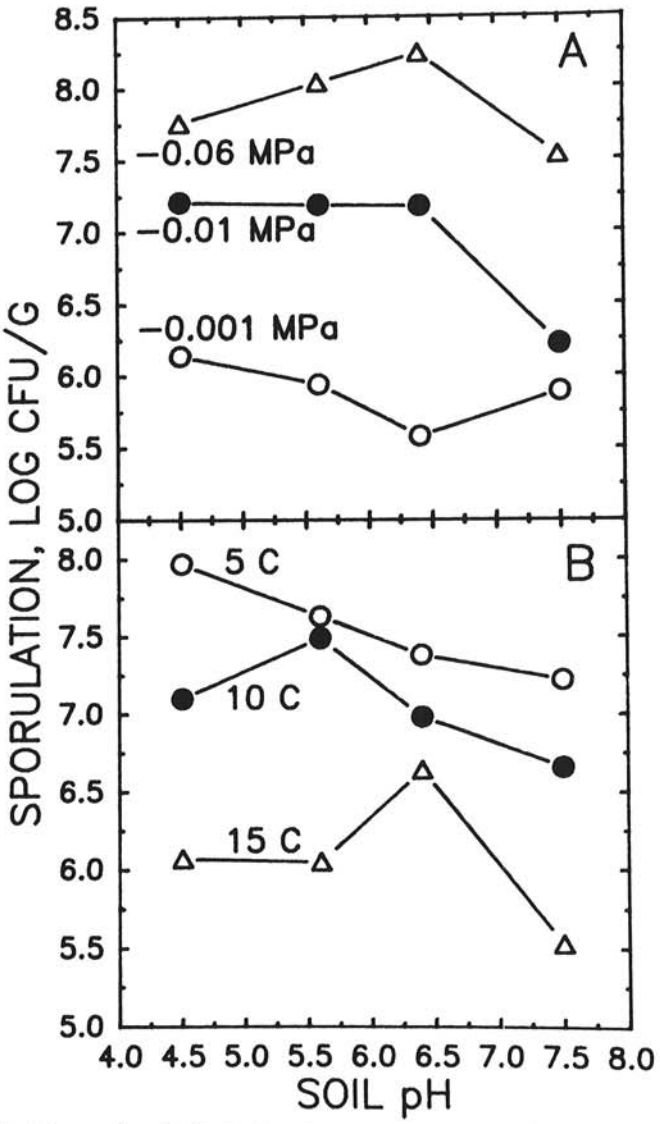

Fig. 5. Interaction of soil pH with soil matric potential (A) and temperature (B) on sporulation of *Cephalosporium gramineum* on colonized oat kernels buried 2 cm below the soil surface in plastic jars incubated in the dark for 6 wk. Each point represents the mean of three replicates and three temperatures (**A**) or three soil matric potentials (**B**). For **A**, the pH response at −0.001 MPa was not significant ($P < 0.05$), but quadratic regressions of sporulation ($\log_{10}$ colony-forming units) on soil pH at −0.06 and −0.01 MPa are described by the equations $Y = -0.02 + 2.8X - 0.2X^2$ ($P < 0.01$) and $Y = 1.0 + 2.4X - 0.2X^2$ ($P < 0.01$), respectively. For **B**, linear, quadratic, and cubic regressions of sporulation ($\log_{10}$ colony-forming units) on soil pH at 5, 10, and 15 C are described by the equations $Y = 8.1 - 0.3X$ ($P < 0.01$), $Y = 1.0 + 1.9X - 0.2X^2$ ($P < 0.05$), and $Y = 67.1 - 33.4X + 5.9X^2 - 0.3X^3$ ($P < 0.01$), respectively.

(from 6.1 to 8.4 log cfu/g) as matric potential decreased from −0.005 to −0.07 MPa. However, there was a soil pH × matric potential interaction resulting from significant ($P < 0.01$) quadratic responses to pH at both −0.005 and −0.01 MPa but a nonsignificant ($P < 0.05$) response to pH at −0.07 MPa.

DISCUSSION

The pH values, temperatures, and soil matric potentials used in this study were chosen because they represent the range of conditions that occur in areas of eastern Washington where Cephalosporium stripe frequently occurs, and because they have been used in greenhouse studies on Cephalosporium stripe (1,10,19,20). The purpose for including three fungal isolates in this study was to evaluate the sporulation response of *C. gramineum* to pH, temperature, and buffer, not to differentiate isolates. Although the isolates did differ significantly and there were significant interactions between isolate and pH or temperature, the biological significance of these differences with respect to disease development is questionable, since they were small relative to the effects of pH and temperature and resulted from quantitative rather than qualitative (directional) differences.

Sporulation of *C. gramineum* on oat kernels and wheat straw (placed on the soil surface or buried in soil to simulate more natural conditions) was twofold to threefold greater when soil pH was 4.5–5.5 then when it was 6.5–7.5 (Figs. 3 and 5). Thus, we agree with Specht and Murray (19), who found that sporulation on colonized oat kernels on the soil surface in pots incubated in a lighted growth chamber was nearly twofold greater when soil pH was 4.7 than when it was 7.3.

The response of *C. gramineum* to pH also was influenced by temperature, soil matric potential, and length of incubation. For example, sporulation increased twofold and threefold in experiments incubated for 2 and 6 wk, respectively, as pH decreased from 7.5 to 4.5. It is likely that both sporulation and survival of conidia at different soil pH values are factors that influence the measurement of conidia on oat kernels and straw in experiments with long (more than 2-wk) incubation times, because Specht and Murray (19) have shown that survival of conidia incubated in soil longer than 3 wk is greater at pH 4.7 than at pH 5.7–7.5.

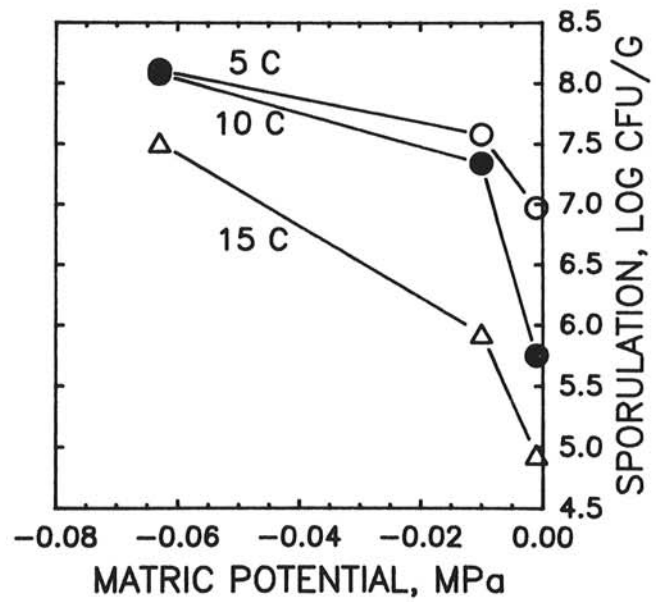

Fig. 6. Effect of soil matric potential and temperature on sporulation of *Cephalosporium gramineum* on colonized oat kernels buried 2 cm below the soil surface in plastic jars and incubated for 6 wk in the dark. Each point represents the mean of three replicates and four soil pH values. Quadratic regressions of sporulation ($\log_{10}$ colony-forming units) on soil matric potential at 5, 10, and 15 C are described by the equations $Y = 6.9 - 78.0X - 931.9X^2$ ($P < 0.01$), $Y = 5.5 - 205.5X - 2624.3X^2$ ($P < 0.01$), and $Y = 4.8 - 124.2X - 1293.4X^2$ ($P < 0.01$), respectively.

Previous workers (1,12) reported that the optimum temperature for sporulation of *C. gramineum* in vitro was below that for hyphal growth, however, we found greatest sporulation in vitro at 20 C, which is also the optimum for hyphal growth on MSA-PB (15). Sporulation on MSA-PB (pH 5.5) after 1 wk was 700-fold greater at 20 than at 5 C. The temperature response diminished with longer incubation such that sporulation after 3 wk was only fourfold greater at 20 than at 5 C. It was apparent that sporulation per unit area was greater and hyphal growth was less at lower temperatures (5 C) than at higher temperatures (20 C), where hyphal growth was abundant. This apparent discrepancy among studies may be attributable to differences among test isolates, culture media, incubation conditions, or the methods used to evaluate sporulation.

In contrast to the effect of temperature in vitro, sporulation on oat kernels or straw in soil was greatest at 5 and least at 15 C, regardless of soil pH or soil matric potential. The contrasting results between studies in vitro and those in soil suggest that a biological factor(s) that inhibits sporulation of *C. gramineum* is present in soil at higher temperatures, although reduced oxygen content due to limited diffusion, especially at high soil moisture contents, may also have affected sporulation. Specht and Murray (19) have shown that fumigation of soil with chloropicrin increases survival of conidia of *C. gramineum* and also alters the pH response for sporulation. The low-temperature optimum for sporulation by *C. gramineum* on soil may partially explain the apparent low-temperature requirement for disease development. *C. gramineum* may have become adapted to a psychrophilic existence as a result of decreased biological competition, which enables it to sporulate more profusely at lower temperatures.

Our findings of increased sporulation on oat kernels and straw with decreasing soil matric potential (Fig. 4) are in contrast to previous work (19), in which sporulation decreased about twofold as matric potential decreased from −0.01 to −0.07 MPa. Differences between these studies may be related to different incubation conditions. In our study, oat kernels and straw were incubated in plastic jars in the dark, whereas in the previous study (19) oat kernels were incubated in open pots in a lighted growth chamber. Under the conditions of our study, with loosely capped jars, the moisture content of the air above the soil was probably in equilibrium, or nearly so, with that of the soil. In the previous study, with open pots (19), the moisture content of the air above the soil would have been much less than in our studies. How these factors may interact to alter the response to soil matric potential is not known.

The sporulation response of *C. gramineum* to soil pH and matric potential was similar whether colonized oat kernels or naturally colonized straw was tested. Oat kernels have been used as inoculum in greenhouse and field studies on Cephalosporium stripe in relation to soil pH and matric potential, and it appears that the use of this type of inoculum is satisfactory for determining the significance of these factors in disease development under natural conditions.

The decreased sporulation of *C. gramineum* at high soil matric potential observed in this study does not explain why Cephalosporium stripe increases in wet soil. Of great significance, however, is that *C. gramineum* sporulated prolifically even at very high soil matric potentials (−0.001 MPa). Sporulation under these conditions is significant, because high soil matric potentials and low temperatures are present during the winter and spring in field soils in eastern Washington, especially when frozen subsurface layers exist.

Increased sporulation and subsequent inoculum density at low soil pH may partially explain the observed increase in Cephalosporium stripe at low soil pH (4.5) (1,10). However, Specht and Murray (20), using a growth chamber-greenhouse system (1) to study the disease, showed that the relationship between inoculum density and disease incidence is logarithmic. Because the increases in sporulation at low soil pH observed in our study were only twofold to threefold, even with long

incubations, increased inoculum density at low soil pH does not fully account for the pH response of Cephalosporium stripe. It is apparent, therefore, that soil pH also has other effects that result in increased disease at low soil pH. Anderegg and Murray (1) suggested that low soil pH may increase host susceptibility to infection because of greater leakage of pathogen-stimulatory root exudates under acid soil conditions.

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