

Sporulation and Survival of Conidia of *Cephalosporium gramineum* as Influenced by Soil pH, Soil Matric Potential, and Soil Fumigation

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ABSTRACT

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In growth-chamber studies conducted at 5 and 15 C, sporulation of *Cephalosporium gramineum* on artificially colonized oat kernels on soil and survival of conidia in soil were significantly greater at soil pH 4.7 than at 5.7-7.5. Conidial survival also was greater at pH 7.5 than at 5.7-6.7, which produced a significant curvilinear relationship between soil pH and conidial survival. Sporulation of *C. gramineum* on oat kernels increased, whereas conidial survival decreased, as soil matric potential

increased from -0.06 to -0.01 MPa. Survival of conidia always was high in soil at -0.06 MPa regardless of soil pH, but was low and influenced by pH at matric potentials of -0.03 and -0.01 MPa. Pretreatment of moderately acid (pH 5.5) soil with chloropicrin (0.2 ml/kg of moist soil) resulted in large reductions in total population densities of fungi and actinomycetes (but not bacteria) and in a significant increase in conidial survival.

Additional keywords: Cephalosporium stripe, selective medium, *Trichoderma hamatum*, *Trichoderma viride*, winter wheat.

Cephalosporium stripe, incited by the soilborne fungus *Cephalosporium gramineum* Nisikado & Ikata (sporodochial stage = *Hymenula cerealis* Ell. & Ev.), is an important vascular disease of winter wheat (*Triticum aestivum* L.), particularly in the northwestern United States. *C. gramineum* forms no specialized resting structures and survives exclusively as mycelium in plant residue (15). The pathogen invades the vascular system of host plants and is restricted to xylem elements before tissue senescence; hyphae then ramify throughout all portions of infected plants (9). Small ($1-3 \times 2-7 \mu\text{m}$), unicellular conidia are produced in large numbers (up to $1 \times 10^5/\text{g}$ of soil and higher) on infested plant residue near or on the soil surface during winter and spring, as sporulation is favored by cool, wet conditions (5,32). Conidia serve as primary inoculum for host infection. Pathogen ingress apparently is through injured or stressed roots, such as occur in response to soil heaving (5).

The highest incidence and greatest severity of Cephalosporium stripe occurs in acid (pH 4.5-5.5) and wet soils (2,3,18,28). Wheat is only moderately tolerant of acidic soil conditions, particularly cultivars grown in eastern Washington and northern Idaho (19), and probably becomes more susceptible to infection by *C. gramineum* at low soil pH (2). Soil matric potentials in the range of -0.01 MPa (-0.1 bar) also may predispose wheat to infection by *C. gramineum* (2).

Incidence of Cephalosporium stripe is related directly to pathogen inoculum concentration (20,23), and some effects of low soil pH and high soil moisture may be the result of increased survival and sporulation of *C. gramineum*. Bruehl and Lai (8) reported that *C. gramineum* survived better and sporulated more profusely on wheat straw buried in acid than in alkaline soil. A wide-spectrum, antifungal antibiotic, most active at pH 4.0-5.0, was isolated from culture filtrates of the fungus (9). Bruehl et al (10) hypothesized that antibiotic production aids in survival of *C. gramineum* in straw, since wild-type isolates that always produced antibiotic survived longer than isolates that had lost the ability to produce antibiotic.

The amount of sporulation of *C. gramineum* on artificially colonized oat kernels on soil was 50% lower at pH 7.5 than at 4.5-6.5 (26). Similarly, the in vitro growth and sporulation of *C. gramineum* both were greater at pH 4.5-5.5 than at 7.5 (25,26). Love (17) found that conidia of *C. gramineum* survived longest in the coolest (9 C) and most acid (pH 4.5) soil tested but died rapidly at 25 C regardless of soil pH or other factors. Moisture contents of 10-22% in a silt-loam soil had no influence on survival of *C. gramineum* in buried straw (15), but sporulation on straw on soil was most abundant under moist conditions (21,33).

Soil pH and soil moisture each may have direct and indirect effects on *C. gramineum*. Indirect effects could be mediated through changes in the biological, chemical, or physical environment of soil, such as through alterations in microbial activity, nutrient availability, and soil aeration (14). This study was

undertaken to further quantify the effects of soil pH and soil matric potential on sporulation and survival of conidia of *C. gramineum*. The influence of soil treatment with chloropicrin was tested to help distinguish the role of any biologically mediated effects of these factors on *C. gramineum*.

MATERIALS AND METHODS

Selective medium for *C. gramineum*. Preliminary studies (unpublished) with a previously reported selective medium (31) gave unsatisfactory results. A new selective medium, therefore, was developed for use in these studies. Initial tests indicated that a weak basal medium such as half-strength, acidified corn meal agar (0.5 ACMA) (Difco Laboratories, Detroit, MI) was best for isolating *C. gramineum* from soil on dilution plates. Of 41 fungicides that were incorporated into 0.5 ACMA at concentrations of 1–100 mg a.i./L, dicloran, tolclofos-methyl, and triphenyltin hydroxide inhibited the growth of many soil fungi (but not *C. gramineum*) and were tested further. The final medium, acidified to pH 4.0 with 1.25 ml of 25% lactic acid, included 0.5 mg of dicloran, 1.0 mg of tolclofos-methyl, and 0.5 mg of triphenyltin hydroxide per liter. Lactic acid and fungicides were added after cooling the sterile molten medium to 48 C. Germination of conidia on this medium (CGSM) was 95–99%. CGSM was used to quantify population densities of *C. gramineum* in all studies.

Soil treatments. A soil-mix containing 90% Thatuna silt loam, 5% vermiculite (Terra-lite 3, W. R. Grace & Co., Cambridge, MA), and 5% washed river sand (w/w) was used in all studies; characteristics of the Thatuna silt-loam soil have been described (2). For soil pH studies, the soil-mix was adjusted to pH values (measured in 0.01 M CaCl₂ [22]) of 4.5–7.5 with either H₂SO₄ or Ca(OH)₂ (2). For studies with different matric potentials, the soil-mix was gravimetrically adjusted to moisture contents (oven-dry basis, 105 C) of 20, 32, and 45%, which corresponded to –0.06, –0.03, and –0.01 MPa, respectively, based on a moisture-release curve generated with pressure plates. Except when matric potential was the experimental variable, all studies were conducted with soil adjusted to –0.01 MPa. For fumigation, 10-kg quantities of soil (15% moisture content) were treated with chloropicrin (0.2 ml/kg of soil) for 24 hr at 20–25 C in tightly sealed polyethylene bags and then aerated thoroughly before use.

Soil was added to either 10- or 15-cm-diameter plastic pots, with 450 or 1,150 g of oven-dry soil per pot, respectively. Pots were filled with soil and placed in controlled-temperature growth chambers under fluorescent light at 100–200 $\mu\text{E m}^{-2} \text{sec}^{-1}$. Soil was watered initially to saturation (i.e., 60% moisture content) and then was allowed to dry to the desired moisture content. Thereafter, soil was maintained gravimetrically within 1–4% of the desired moisture content by watering 2–3 times per week.

In some experiments, winter wheat cultivars Stephens or Nugaines were used; four pregerminated wheat seeds were sown 1 cm deep in each pot. After sowing, pots were maintained at 15 C with 12 hr of light per day for 3 wk, or until seedlings were in the four- to five-leaf stage. Chamber temperature then was decreased to 5 C with 8 hr of light per day for 6 wk, followed by 15 C with 12 hr of light per day for another 3 wk. Other experiments were conducted in nonplanted soil with the same regime, except that chambers were maintained at 5 C once the temperature was decreased (from 15 to 5 C) at 3 wk.

Survival in soil. A virulent culture of *C. gramineum* (isolate CG85-4, originally isolated from soil taken from a winter wheat field in the Pullman, WA, area) was used to produce conidial inoculum. The fungus was cultured for 3 days at 20–23 C in 25 ml of freshly made potato-dextrose broth (200 g of potato and 20 g of dextrose per liter) in 250-ml Erlenmeyer flasks on a rotary shaker (85 rpm). Fungal mycelium was removed by filtering the liquid culture through cheesecloth. Conidia then were pelleted by low-speed centrifugation and resuspended in sterile deionized water. Concentrations of germinable conidia in the final suspensions were quantified by dilution-plating on CGSM.

Conidial suspensions were applied as drenches to the surface

of soil in pots 1–3 days after the chamber temperature was decreased to 5 C. Drenches consisted of either 40 ml (10-cm pots) or 100 ml (15-cm pots) of appropriately diluted spore suspension containing between 2×10^6 and 3×10^7 germinable conidia per milliliter. Initial concentrations of conidia in soil (ranging from 1×10^5 to 3×10^6 per gram) were calculated on the basis of oven-dry weight of soil per pot. Soils were sampled for *C. gramineum* 6–84 days after drenching with conidia.

Sporulation on soil. Oat kernels (2.0 g per 10-cm-diameter pot) artificially colonized by *C. gramineum* (11) were placed on the surface of soil in pots 1–3 days after the chamber temperature was decreased to 5 C. The air-dried oat kernels were presoaked in water for 10 min and then pressed into the soil surface to ensure good contact. Oat kernels and soil were incubated for 15 days and then both were sampled for conidia of *C. gramineum*.

Soil assays. The dilution-plate technique was used to immediately assay soil in each pot for *C. gramineum* on CGSM. Soil dilutions (dry weight basis) of 2×10^{-4} to 3×10^{-3} were used, depending on expected population densities. Three to six Petri dishes were prepared per soil sample and were incubated for 10–12 days at 15 C before the characteristic colonies of *C. gramineum* were counted. The mean number of colony-forming units (cfu) of *C. gramineum* per gram of oven-dry soil then was determined for soil in each pot with the equation: cfu = total number of colonies counted/(number of Petri plates \times soil dilution). A 50-g subsample of soil from each pot also was air-dried and measured for pH in 0.01 M CaCl₂.

Population densities of total aerobic bacteria, total actinomycetes, total fungi, and *Trichoderma* spp. in soil were determined. Bacteria and actinomycetes were enumerated on soil extract and caseinate agars (29), respectively, whereas total fungi and *Trichoderma* spp. were enumerated on Martin's rose bengal agar (pH 5.5) containing 200 mg of streptomycin sulfate and 2.5 g of oxgall per liter (27).

Survival in water. Twenty milliliters of a freshly prepared conidial suspension of *C. gramineum* (made in sterile deionized water and containing 1.2×10^8 germinable spores per milliliter) was placed in a sterile plastic container and stored at 5 C in the dark. After specified durations in storage, which ranged from 0–86 days, the conidial suspension was shaken and a 0.1-ml sample was diluted 10-fold in sterile water. Small samples (0.1–0.5 ml) of the diluted suspension were spread on CGSM in petri dishes. Conidial germination was determined by direct microscopic observation after incubation for 48 hr at 15 C. Percentage of germinable conidia was based on a minimum of 600 conidia for each sample period.

Statistical analyses. In survival studies, population densities of *C. gramineum* were converted to percent recovery based on the number of germinable conidia initially added to soil. Values for population densities of other soil microorganisms were transformed to log₁₀ (cfu/g of oven-dry soil). In all experiments, pots were placed on bench tops in growth chambers in completely randomized designs, with three to eight replicates (i.e., pots) per treatment or combination of factorially arranged treatments. All data were subjected to analyses of variance (ANOVA), and the effects of soil pH and soil matric potential were partitioned into linear and quadratic sums of squares (16). All studies were repeated and analyzed separately to confirm the observed trends in the data. Some experiments were resubjected to combined ANOVAs (with the separate experiments included as blocks in the analyses) for purposes of data presentation; data from these studies always showed similar trends and are reported here as the mean of two experiments.

RESULTS

Survival of conidia of *C. gramineum* in soil. Influence of soil pH. Soil pH changed very little (less than 0.2 pH units) from the initial values over the course of the experiments; the value presented for each treatment represents the mean soil pH, which was obtained by averaging measurements across all sample dates. Recovery of conidia from soil planted with cultivar Stephens

initially was high (74–83% after 10 days), with no significant differences among soil pH values (Fig. 1). Fewer germinable conidia were found after 29, 43, and 63 days at all soil pH values, but the percentages of recovery were higher at pH 4.7 than at 5.9, 6.7, and 7.5 (Fig. 1). Quadratic ($P < 0.001$) (i.e., curvilinear) relationships occurred between soil pH and percent recovery of conidia on the latter three sample dates, owing to higher recovery rates not only at pH 4.7 but also as pH increased from intermediate values (5.9 and 6.7) to 7.5 (Fig. 1). Curvilinear relationships between soil pH and survival of conidia also occurred in nonplanted soil after 35, 55, and 84 days (Fig. 2).

Conidial survival (in soil in 15-cm-diameter pots) was examined over time at soil depths of 0–3, 3–6, and 6–12 cm. Initial population densities (at 10 days) of conidia of *C. gramineum* generally were

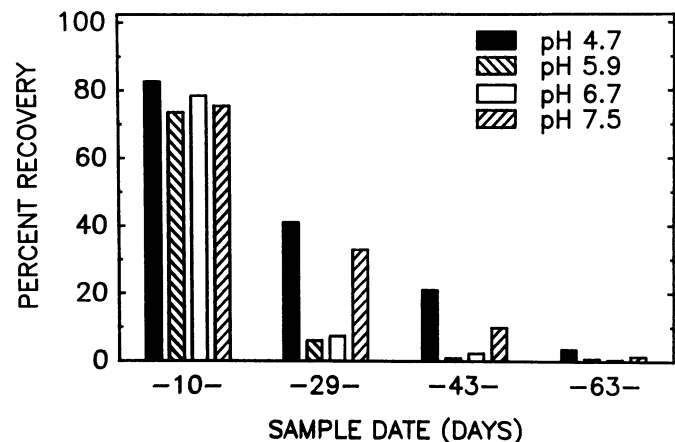


Fig. 1. Effect of soil pH on survival of conidia of *Cephalosporium gramineum* in nonfumigated soil. Soil was planted with cultivar Stephens and maintained at -0.01 MPa. Growth-chamber temperatures were maintained at 5 C from days 0–43, and 15 C from days 44–63. Each bar represents the mean of four replicate pots with five Petri dishes per replicate. Separate ANOVAs were made for each sample date. No significant ($P = 0.05$) relationship was found between soil pH and percent recovery of conidia after 10 days. Quadratic regressions of percent recovery on soil pH after 29, 43, and 63 days were described, respectively, by the equations: $Y = 643.1 - 205.2X + 16.47X^2$ ($P < 0.001$), $Y = 302.4 - 94.60X + 7.41X^2$ ($P < 0.001$), and $Y = 43.09 - 13.14X + 1.01X^2$ ($P < 0.001$).

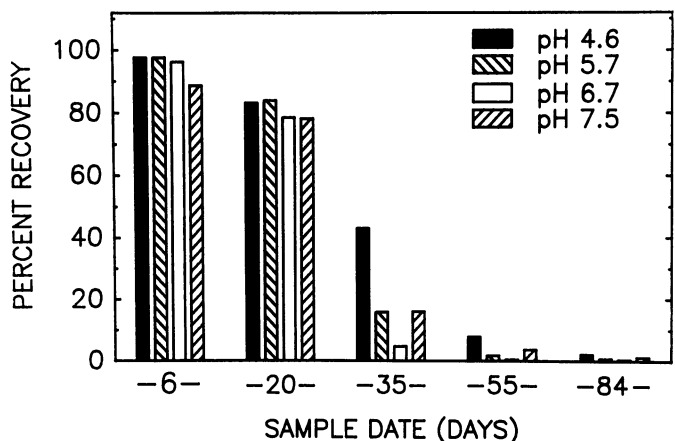


Fig. 2. Effect of soil pH on survival of conidia of *Cephalosporium gramineum* in nonfumigated, nonplanted soil. Soil was maintained at -0.01 MPa and 5 C. Each bar represents the mean of three replicate pots with six Petri dishes per replicate. Separate ANOVAs were made for each sample date. No significant ($P = 0.05$) relationships existed between soil pH and percent recovery of conidia after 6 and 20 days. Quadratic regressions of percent recovery on soil pH after 35, 55, and 84 days were described, respectively, by the equations: $Y = 453.6 - 137.8X + 10.63X^2$ ($P < 0.001$), $Y = 108.6 - 34.44X + 2.74X^2$ ($P < 0.001$), and $Y = 26.53 - 8.34X + 0.665X^2$ ($P < 0.001$).

similar at all depths for all soil pH values (Table 1), but fewer ($P < 0.05$) conidia were recovered at the 3–6 and 6–12 cm depths (compared with the 0–3 cm depth) at soil pH 5.9 and 6.7 after 29, 43, and 63 days. Fewer conidia also were recovered at the lower soil depths at both soil pH 4.7 and 7.5, but not until after 63 days (Table 1). Similar results in additional studies confirmed the effects of soil pH and soil depth on conidial survival.

Influence of soil fumigation. Percent recovery of conidia after 34 days at soil pH 4.6 was lower in fumigated (14%) than in nonfumigated (51%) soil (Fig. 3). Conversely, at pH 5.6 and 6.5

TABLE 1. Population densities ($[cfu/g \text{ of soil}] \times 10^3$) of *Cephalosporium gramineum* at three soil depths and four pH values^y

Soil pH	Soil depth (cm)	Sample date (days)			
		10	29	43	63
4.7	0–3	327 a ^x	146 a	97 a	20 a
	3–6	278 a	124 a	60 a	12 b
	6–12	283 a	155 a	68 a	8 b
5.9	0–3	366 a	18 a	9 a	6 a
	3–6	194 b	3 b	1 b	1 b
	6–12	235 ab	3 b	1 b	1 b
6.7	0–3	432 a	70 a	26 a	4 a
	3–6	217 a	6 b	1 b	<1 b
	6–12	218 a	8 b	2 b	<1 b
7.5	0–3	322 a	152 a	62 a	13 a
	3–6	212 b	85 a	21 a	2 b
	6–12	267 ab	129 a	27 a	1 b

^xSoil was planted with cultivar Stephens in 15-cm-diameter pots. Each pot was drenched with 100 ml of a suspension containing 4.1×10^7 viable conidia of *C. gramineum* per milliliter. The initial concentration of conidia in soil was $3.57 \times 10^5/g$. Growth-chamber temperatures were 5 C from days 0 to 43 and 15 C from days 44 to 63. Soil matric potential was -0.01 MPa.

^yEach number represents the mean of four replicate pots with five Petri dishes per replicate. Separate ANOVAs were made for each soil pH and sample date combination. Means followed by the same letter within each pH-sample date group are not significantly ($P < 0.05$) different according to Duncan's multiple range test.

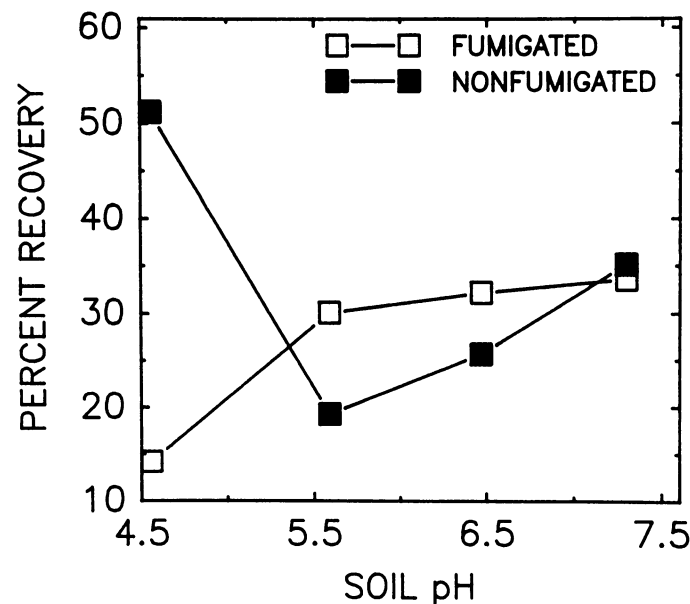


Fig. 3. Effect of soil pH and soil fumigation on survival of conidia of *Cephalosporium gramineum*. Soil (nonplanted) was maintained at -0.01 MPa and 5 C, and was sampled after 34 days. Individual points represent the mean of two experiments, each with three replicate pots and six Petri dishes per replicate. The relationship between percent recovery of conidia and soil pH was linear in fumigated soil ($Y = -12.72 + 6.74X$, $P < 0.05$) and quadratic in nonfumigated soil ($Y = 466.5 - 146.2X + 11.96X^2$, $P < 0.01$).

conidial survival was 56 and 25% higher, respectively, in fumigated than in nonfumigated soil. A significant ($P < 0.01$) interaction was found between soil pH and soil fumigation treatment; the soil pH-survival relationship was curvilinear ($P < 0.01$) in nonfumigated soil and linear ($P < 0.05$) in fumigated soil.

In two other studies conducted with soil that was not "amended" with either H_2SO_4 or $Ca(OH)_2$ (natural pH = 5.5), the percent recovery of conidia after 27–70 days was higher ($P < 0.001$) in fumigated than in nonfumigated soil (Fig. 4). Wheat plants grown in this nonamended soil generally had little effect on survival of conidia regardless of prior fumigation treatment (Fig. 4). Additional studies confirmed the highly favorable effect of soil fumigation on conidial survival in unamended soil adjusted to -0.01 MPa.

Influence of soil matric potential. At both soil pH 4.5 and 5.9, recovery of conidia after 28 days in soil decreased as matric potential increased from -0.06 to -0.01 MPa (Fig. 5). The relationship between conidial survival and soil matric potential was curvilinear ($P < 0.001$) at both pH values.

Sporulation of *C. gramineum* on oat kernels on soil. Influence of soil pH and soil fumigation. Sporulation was greater ($P < 0.05$) at soil pH 4.7 than at 5.8, 6.6, and 7.3, regardless of whether or not soil had been fumigated (Fig. 6). However, there was a significant ($P < 0.05$) interaction between soil pH and soil fumigation treatment; in nonfumigated soil, sporulation was 48% less at pH 7.3 than at 4.7, but in fumigated soil, it was only

16% less at pH 7.3 than at 4.7. The pH-sporulation relationship was linear ($P < 0.001$) in nonfumigated soil, whereas it was curvilinear ($P < 0.05$) in fumigated soil.

Influence of soil matric potential. The amount of sporulation of *C. gramineum* on oat kernels placed on nonamended soil (pH 5.5) and maintained at 5 C for 15 days was 2.8×10^8 , 6.3×10^8 , and 1.3×10^9 conidia per gram of air-dry oat kernel at matric potentials of -0.06 , -0.03 , and -0.01 MPa, respectively. Each

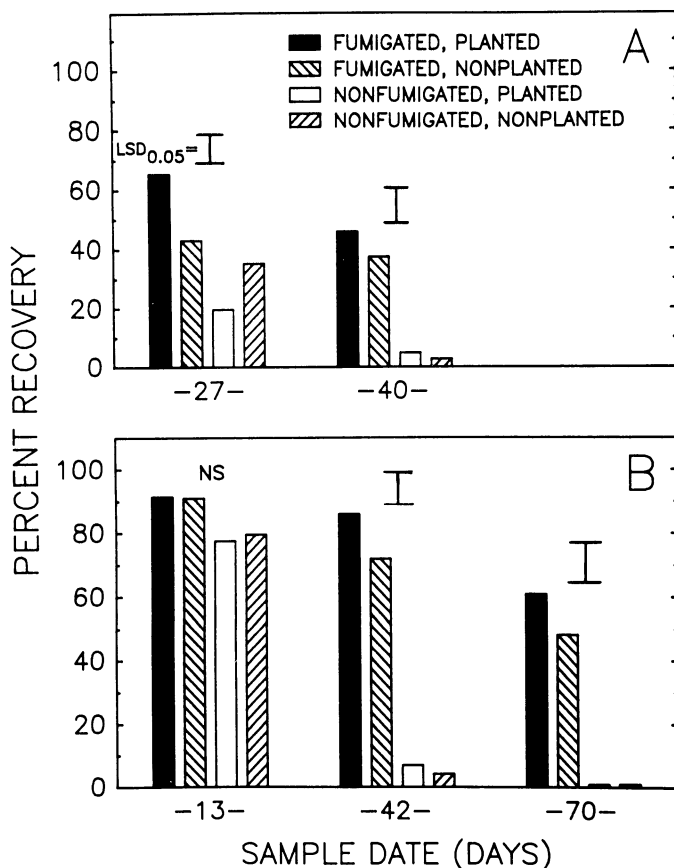


Fig. 4. Effect of soil fumigation and wheat plants on survival of conidia of *Cephalosporium gramineum*. Soil (pH 5.5) was not amended with either H_2SO_4 or $Ca(OH)_2$ and was maintained at -0.01 MPa. A, Experiment 1, conducted with cultivar Stephens (10-cm-diameter pots); growth-chamber temperature was 5 C. B, Experiment 2, conducted with cultivar Nugaines (15-cm-diameter pots); growth-chamber temperatures were 5 C from days 0 to 48, and 15 C from days 49 to 70. Each bar represents the mean of three (experiment 2) or six (experiment 1) replicate pots with five Petri dishes per replicate. Separate ANOVAs were made for each sample date. The vertical line above each group of bars represents Fisher's least significant difference (LSD) ($P = 0.05$) for comparing all treatment means within each sample date; NS = no significant differences.

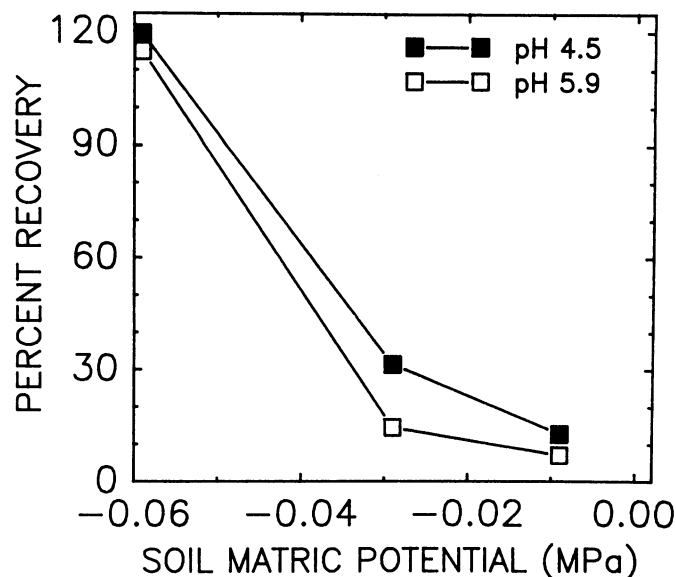


Fig. 5. Effect of soil matric potential on survival of conidia of *Cephalosporium gramineum* at two soil pH values. Soil (nonplanted) was maintained at 5 C and was sampled after 28 days. Pots were covered with polyethylene sheets to reduce evaporation of water from the soil surface. Individual points represent the mean of two experiments, each with eight replicate pots and six Petri dishes per replicate. The relationships between percent recovery of conidia and soil matric potential were quadratic at both soil pH 4.5 and 5.9 and were described, respectively, by the equations: $Y = 15.43 + 665.7X + (4.0 \times 10^4)X^2$ ($P < 0.001$) and $Y = 21.20 + (2.0 \times 10^3)X + (5.9 \times 10^4)X^2$ ($P < 0.001$).

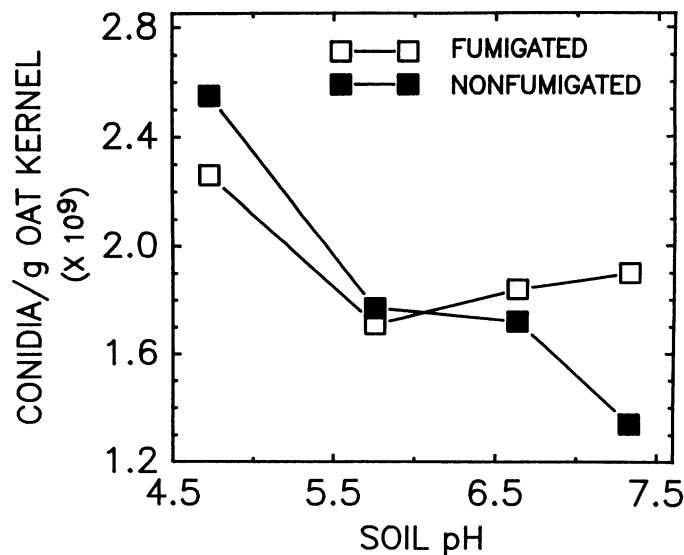


Fig. 6. Effect of soil pH and soil fumigation on sporulation of *Cephalosporium gramineum* on oat kernels on soil. Soil (nonplanted) was maintained at -0.01 MPa and 5 C. Oat kernels were incubated on soil for 15 days before sampling. Individual points represent the mean of two experiments, each with four replicate pots and five Petri dishes per replicate. The relationship between conidial production and soil pH was quadratic in fumigated soil ($Y = 9.94 - 2.60X + 0.206X^2$, $P < 0.05$) and linear in nonfumigated soil ($Y = 4.47 - 0.429X$, $P < 0.001$).

of these numbers represents the mean of two experiments (with similar results), each with eight replicate pots and six Petri dishes per replicate. The relationship between amount of sporulation on oat kernels and soil matric potential was curvilinear ($P < 0.001$). The gravimetric moisture contents (oven-dry basis, 70 C) of oat kernels on the surface of soil at -0.06 , -0.03 , and -0.01 MPa were 89, 146, and 217%, respectively.

Soil microflora. Population densities of bacteria usually increased as soil pH increased (Fig. 7). In general, there were relatively minor differences in numbers of bacteria between fumigated and nonfumigated soil. In contrast, soil fumigation greatly reduced population densities of actinomycetes (Fig. 7); their numbers remained very low at soil pH values of 4.7–6.5 but increased more than 1,000-fold to 5.2×10^5 cfu/g at pH 7.5. Population densities of actinomycetes always were much higher in nonfumigated than in fumigated soil, ranging from 4.0×10^6 to 6.3×10^7 cfu/g, with an eightfold increase in numbers (arithmetic scale) as soil pH increased from 6.5 to 7.3. *Streptomyces* and *Nocardia* spp. were commonly observed on the soil-dilution plates. Soil fumigation also greatly reduced population densities of total fungi and *Trichoderma* spp., except at soil pH 4.7, where fumigated soil was naturally recolonized by *Trichoderma* spp. to very high population densities (Fig. 7). *Trichoderma hamatum* and *T. viride* (13) comprised about 90 and 10%, respectively, of all isolates of *Trichoderma* spp. in fumigated soil, whereas *T. viride* predominated in nonfumigated soil. Neither soil matric potential nor wheat plants had significant effects on population densities of soil microorganisms in the general soil mass (data not shown). Similar results were obtained in repeated studies.

Survival of conidia in sterile water. Percentages of germinable conidia of *C. gramineum* stored in water at 5 C generally paralleled conidial survival in wet soil (i.e., -0.01 MPa) and were 98, 95, 75, 41, 31, and 18% after 0, 2, 8, 22, 37, and 86 days, respectively. A negative correlation ($r = -0.87$, $P < 0.05$) was found between length of storage in water and germinability of conidia. At 86 days, microscopic observation of conidia spread on CGSM indicated that many conidia had lysed and/or their protoplasmic contents were disintegrating. Similar results were obtained in repeated studies.

DISCUSSION

The pH of Thatuna silt loam soil-mix amended with either H_2SO_4 or $Ca(OH)_2$ was very stable over the course (0–84 days) of all experiments. Gravimetric control of soil moisture content prevented the drainage of excess water from pots; thus, problems associated with changes in soil chemical composition (and pH) caused by repeated leaching of soil were avoided. The isolate of *C. gramineum* used here was selected because of its typical growth rate, cultural morphology, and response to pH in vitro (25). This isolate also causes typical symptoms of Cephalosporium stripe on winter wheat plants grown in the greenhouse and is considered by us to be a very representative isolate.

These studies were conducted at 5 and 15 C to approximate temperatures that occur in the field during winter and spring, when sporulation of *C. gramineum* on infested plant residue normally occurs. The longer that conidia survive in soil during this time, the greater the likelihood of their eventual contact with and infection of roots of susceptible host plants. Our results show that survival of conidia in soil is affected by pH. At a soil matric potential of -0.01 MPa, the pH-survival relationship always was curvilinear when soil was sampled after 29–84 days, with the lowest and highest recoveries occurring, respectively, at intermediate (pH 5.7 and 6.7) and extreme (pH 4.7 and 7.5) soil reactions. Curiously, the in vitro germination of spores of *Fusarium oxysporum* f. sp. *lycopersici* (another vascular pathogen favored by acid soil) was reported (30) to exhibit a similar double maxima at pH 4.5 and 7.0 and minimal germination at pH 5.0–5.5. Although maximal conidial survival of *C. gramineum* occurred at both pH 4.7 and 7.5 in our studies, survival always was greater at pH 4.7 than at any other soil pH tested. Antibiotic production, reported to

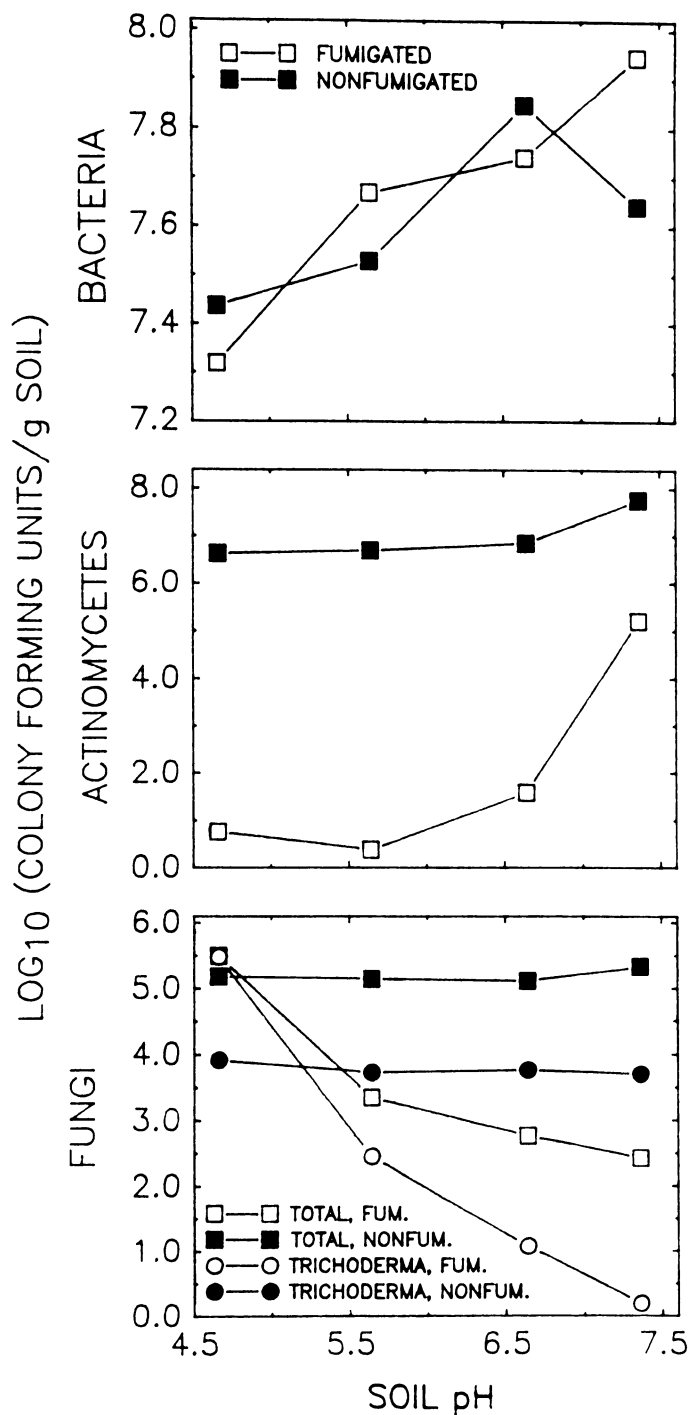


Fig. 7. Effect of soil pH and soil fumigation on population densities of total aerobic bacteria, total actinomycetes, total fungi, and *Trichoderma* spp. Soil (nonplanted) was maintained at -0.01 MPa and 5 C for 9–38 days before sampling. Individual points represent the mean of four sample periods, each with three replicate pots and three Petri dishes per replicate. Separate ANOVAs were made for population densities of each soil microorganism. For actinomycetes, separate analyses were further made for fumigated and nonfumigated soil (due to the inequality of error mean squares). The relationships between population densities of soil microorganisms and soil pH were described by the equations: $Y = 6.37 + 0.214X$ (total bacteria, fumigated soil, $P < 0.001$), $Y = 4.35 + 1.00X - 0.074X^2$ (total bacteria, nonfumigated soil, $P < 0.001$), $Y = 40.89 - 15.01X + 1.38X^2$ (total actinomycetes, fumigated soil, $P < 0.001$), $Y = 14.92 - 3.13X + 0.292X^2$ (total actinomycetes, nonfumigated soil, $P < 0.001$), $Y = 33.28 - 8.56X + 0.553X^2$ (*Trichoderma* spp., fumigated soil, $P < 0.001$), $Y = 5.63 - 0.563X + 0.041X^2$ (*Trichoderma* spp., nonfumigated soil, $P < 0.001$); regressions of total fungi on soil pH were not significant ($P = 0.05$) with either fumigated or nonfumigated soil.

favor survival of *C. gramineum* in a food base such as straw, likely would not play a significant role with individual conidia in soil (6); so pH must affect survival of this pathogen in other ways besides an antibiotic-related mechanism.

The favorable effect of high soil acidity on longevity of conidia was negated by fumigation with chloropicrin, i.e., survival actually was lowest in fumigated soil at pH 4.7, where very high population densities (3.0×10^5 cfu/g) of *Trichoderma* spp. occurred. *Trichoderma* spp. are well-known antagonists of many soilborne plant pathogens and often multiply to high populations in soil after disinfection treatments (24). Postfumigation recolonization by *Trichoderma* spp. did not occur to such high levels in moderately acid (pH 5.5–6.5) soil. The greater survival of conidia that occurred following fumigation of moderately acid soil was associated with large reductions in population densities of total actinomycetes and total fungi, suggesting that possibly these microorganisms (or specific species representing these groups of microorganisms) somehow adversely affected *C. gramineum* in nonfumigated soil. Soil fumigation did not greatly influence numbers of total bacteria, and bacterial activity was not directly related to survival of conidia of *C. gramineum*. Bruehl et al (7) also were unable to demonstrate a detrimental effect of bacteria on *C. gramineum*. Our methods did not measure qualitative changes in the species composition of bacteria that may have affected survival of conidia. Populations of *Pseudomonas* spp., which are often implicated as important antagonists of plant pathogens (12), may have been reduced by soil fumigation, but this was not determined.

Soil matric potential had a major effect on conidial longevity, with greatest survival occurring in the driest (–0.06 MPa) soil tested; recovery of conidia after 28 days at this matric potential was more than 100% regardless of soil pH. Recovery of more conidia than initially introduced can probably be explained on the basis that not all conidia were completely separated when added to soil, in spite of vigorous shaking of final suspensions. This was confirmed by direct microscopic observations of conidial suspensions. Possibly, those conidia initially joined together (about 10–20% of all conidia) separated into individual colony-forming units during incubation in soil.

Soil matric potentials of –0.06 to –0.01 MPa had no influence on population densities of soil microorganisms, and no microbial-related effects of soil water on survival of conidia of *C. gramineum* could be demonstrated directly. Poor survival of conidia at –0.03 and –0.01 MPa (compared with very high survival at –0.06 MPa) probably was due partly to the physical environment associated with very wet soil. Diffusion of oxygen and other gases becomes progressively more restricted as soil matric potential increases (4,14). Very low oxygen concentrations in the wetter soils, perhaps accentuated by a higher rate of microbial activity in nonfumigated soil, may have caused the rapid death of conidia. Neither soil oxygen levels nor microbial respiration (CO₂ evolution) were measured in these studies, but we did find other indirect evidence to support the possible role of oxygen in conidial longevity, including: population densities of *C. gramineum* decreased more at lower soil depths (3–6 and 6–12 cm) than near the soil surface (0–3 cm), where oxygen would be most available (14); and conidia survived poorly when stored in sterile water at 5 C (e.g., 41% germinable conidia after 22 days), which probably was due to oxygen starvation because the solubility of oxygen and its rate of diffusion in water are very low (14,23).

Low soil pH (4.5–4.7) seems to partly offset the otherwise adverse effects of very wet, nonfumigated soil on conidial longevity. Because low soil pH limits biological activity (1,14), this also may have indirectly favored survival of conidia in wet soil (–0.03 and –0.01 MPa) by decreasing microbial respiration and thus leaving more oxygen for *C. gramineum*. However, further experimentation would be required to conclusively determine whether or not soil oxygen concentration has a direct effect on conidial survival. Soil pH probably exerts important direct effects on survival of conidia of *C. gramineum* in soil as well, since low pH favors both the growth and sporulation of *C. gramineum* under in vitro conditions (25,26).

Sporulation of *C. gramineum* on oat kernels was lower at soil pH 5.8–7.3 than at 4.7. The lower amount of sporulation at pH 7.3 (the highest pH tested) was more pronounced in nonfumigated than in fumigated soil. The occurrence of very high population densities of actinomycetes (6.3×10^7 cfu/g) was the only microbiological change associated with the greatly reduced sporulation of *C. gramineum* in nonfumigated soil at high pH. Conversely, the very high population densities of *Trichoderma* spp. that occurred in fumigated soil at pH 4.7 did not have as adverse an effect on sporulation (Fig. 6).

The greater sporulation of *C. gramineum* and the increased longevity of conidia in soil at low pH probably are important factors in the epidemiology of *Cephalosporium* stripe. Another epidemiological factor that should favor disease is the increased sporulation, but not survival of conidia, of *C. gramineum* in wet soil. The greatest levels of pathogen sporulation probably occur near or on the soil surface, where highly aerobic conditions exist. Abundant soil water also would increase the development of *Cephalosporium* stripe (in spite of decreased conidial longevity) by favoring passive movement of conidia to infection sites at root surfaces.

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