Nitrogen Effects on the Pathogenicity of *Drechslera sorokiniana* and *Curvularia geniculata* on Germinating Seed of *Festuca rubra*

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ABSTRACT

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The effects of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ on the pathogenicity of *Drechslera sorokiniana* and *Curvularia geniculata*, singly and in combination on germinating seed of *Festuca rubra* were evaluated. Both nitrogen sources at 10^{-2}M reduced the rate of seedling emergence and total emergence from uninoculated seed in autoclaved and nonautoclaved soil. Total seedling emergence from uninoculated seed in nonautoclaved soil was less than that in autoclaved soil in response to both nitrogen sources at 10^{-2} M. These responses were suggestive of direct nitrogen toxicity to the germinating seed in autoclaved soil and of stimulation of unknown biotic factors in nonautoclaved soil that, when combined with direct nitrogen toxicity, additively reduced total emergence. A concentration of 10^{-3} M of both nitrogen sources generally stimulated rate of seedling emergence and had no effect on total seedling emergence from uninoculated seed. The lowest concentration (10^{-4}M) of the nitrogen sources tested showed mixed effects on rate of seedling emergence and total emergence from uninoculated seed in autoclaved and nonautoclaved soil. The highest concentration of both nitrogen sources enhanced the pathogenicity of *D. sorokiniana* and *C. geniculata*, inoculated on seed both alone and in combination. Rate of seedling emergence was slowed by *D. sorokiniana* and the combination of *D. sorokiniana* + *C. geniculata*, and each pathogen and their combination also reduced total seedling emergence. The combination of the pathogens, together with the highest concentration of either nitrogen source, produced the most severe reduction in total seedling emergence. These results suggest a combination of direct toxicity to germinating seed and an enhancement of the pathogenicity of *D. sorokiniana* and *C. geniculata* induced by nitrogen-containing compounds.

Festuca rubra L. (creeping red fescue) is a fine-textured species adapted to turf culture and is susceptible to *Drechslera sorokiniana* (Sacc.) Subram. & Jain which induces a leaf spot (3,5,8,16), seed and(or) seedling rot (8,12), and root rot (17). *Festuca rubra* also is susceptible to leaf-tip dieback (4) and seed and(or) seedling rot (9,12) caused by *Curvularia geniculata* (Tr. and Earle) Boed. Recent studies have shown that *C. geniculata* potentially is a more severe pathogen of emerging seedlings of *F. rubra* than is *D. sorokiniana* and that, under some circumstances, the combination of the pathogens may additively reduce seedling emergence below that caused by either pathogen alone (12).

Nitrogen fertilization often increases the severity of diseases caused by *D. sorokiniana* and other *Drechslera* spp. on perennial grasses (1,7,13,15). The potential interactions of nitrogen fertilization and the pathogenicity of *C. geniculata* are unknown. That *D. sorokiniana* and *C. geniculata*, both alone and in combination, are potentially severe pathogens of germinating seed of *F. rubra* (12) and that nitrogen may enhance the pathogenicity of *D. sorokiniana* suggests that the practice of applying nitrogen fertilizer to seedbeds before seeding could stimulate these pathogens and reduce the seedling stand of *F. rubra*. The research presented was initiated to determine the influence of nitrogen fertilization on the pathogenicity of *D. sorokiniana* and *C. geniculata*, alone and in combination, on germinating seed of *F. rubra*.

MATERIALS AND METHODS

D. sorokiniana and *C. geniculata* were grown on 20 ml of 1.0% Czapek Dox broth (10 g/L) in 3.0% Bacto-agar (w/v) in 15×100-mm sterile plastic petri dishes under continuous cool-white fluorescent light (75–80 μEin/m^2/sec) at 22 ± 2 C. Conidia were collected from 15- to 20-day-old cultures in distilled water. The suspensions were filtered through a 90-μm sieve to remove mycelial fragments and adjusted to 500 (±25) conidia per milliliter of distilled water with an automatic particle counter (High Accuracy Products Corp., Montclair, CA 91763). The combination of *D. sorokiniana* + *C. geniculata* was prepared by mixing equal volumes of the individual organism suspensions.

F. rubra 'Dawson' was used in all studies. Treatments were established to determine the effects of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$, and the effects of *D. sorokiniana* (D), *C. geniculata* (C), and *D. sorokiniana* + *C. geniculata* (D+C), with and without $(NH_4)_2SO_4$ and $Ca(NO_3)_2$, on rate of and total seedling emergence. All seed was surface-sterilized in a 10% Clorox solution for 10 min under vacuum, rinsed 20 times in distilled water, and air-dried before planting and(or) inoculation. Five seeds were planted in an autoclaved or nonautoclaved 2:1:1 loam-sand-peat soil mix in each of 20 compartments (6×4×5 cm) of compartmentalized plastic flats per treatment (= 100 seeds per treatment). $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ treatments (10 ml of 10^{-4}, 10^{-3}, or 10^{-2}M solution per compartment) were applied to the soil mix 2 days before and 2 days after planting. All of the treatments described above were conducted in the greenhouse (23–30 C) under natural light and replicated twice. Laboratory germination tests (22–24 C) were conducted on filter paper with surface-sterilized seed to determine percent germination and potential presence of *D. sorokiniana* and(or) *C. geniculata* on or within seed.

The methodology of soil infestation, seed inoculation, and seed infestation treatments with D, C, and D + C is described in a previous study (12). The various inoculation treatments applied to surface-sterilized seed were conducted and replicated as previously described, but all evaluations of pathogenicity of D, C, and D + C, with and without $(NH_4)_2SO_4$ or $Ca(NO_3)_2$, were conducted only in the nonautoclaved soil mix.

The results of the soil infestation, seed inoculation, and seed infestation treatments were analyzed separately and collectively (pooled) for each organism and their combination, with and without the application of the nitrogen sources. Total seedling emergence and the rate of seedling emergence were recorded for each treatment. Seedling counts were initiated with emergence of

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the first seedling and recorded through 25 days after planting. The number of emerged seedlings 25 days after planting was recorded as total seedling emergence. The rate of seedling emergence was expressed by the ratio coefficient of velocity of emergence (CVE) (11). All data were analyzed according to a 4 organism (including the control) × 2 soil conditions × 3 nitrogen forms (including the control) × 3 molarities factorial design.

RESULTS

Nitrogen and seedling emergence. The application of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ to soil in which seed of *F. rubra* is germinating has both direct and indirect effects on total seedling emergence. The percentage of emerging seedlings from control seed germinated on filter paper in the laboratory was 82% and did not differ from that of seed germinated in autoclaved and nonautoclaved soil without the application of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ (Figs. 1A and B). The application of $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ at 10^{-2}M to the autoclaved soil mix reduced seedling emergence (Fig. 1A); applications of either nitrogen source at 10^{-4} and 10^{-3}M to the autoclaved soil mix had no effect on seedling emergence. The application of $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ at 10^{-4} and 10^{-2}M to the nonautoclaved soil mix reduced seedling emergence below that of the nonautoclaved control (Fig. 1B) and below that of the same treatments in autoclaved soil (Figs. 1A and B). Applications of either nitrogen source at 10^{-3}M to the nonautoclaved soil mix had no effect on seedling emergence (Fig. 1B).

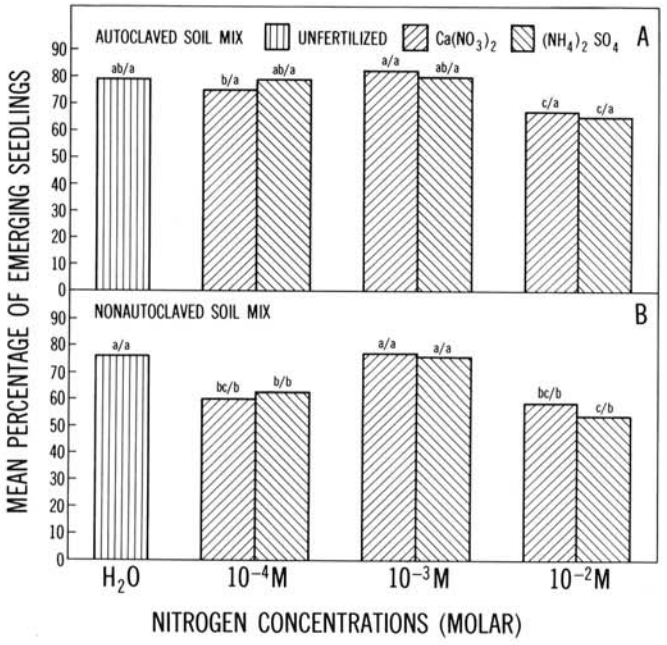

Fig. 1. Mean percentage of emerging seedlings of *Festuca rubra* seeded in **A,** autoclaved and **B,** nonautoclaved soil mixes that received various concentrations of $Ca(NO_3)_2$ and $(NH_4)_2SO_4$. Means among concentrations of the respective nitrogen sources within autoclaved or nonautoclaved soil mixes (across a/), and means between autoclaved and nonautoclaved soil mixes within specific concentrations of the respective nitrogen sources (down, /a) followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

The rate of seedling emergence from autoclaved and nonautoclaved soil not fertilized with $(NH_4)_2$ or $Ca(NO_3)_2$ did not differ (Table 1). The application of $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ at 10^{-2}M slowed the rate of seedling emergence in autoclaved and nonautoclaved soil. Both nitrogen sources slowed seedling emergence at 10^{-4}M in nonautoclaved soil, but had no effect on emergence in autoclaved soil. Rate of seedling emergence was increased by both nitrogen sources at 10^{-3}M in autoclaved and nonautoclaved soil.

Pathogen-nitrogen interactions and seedling emergence. Inoculation of *F. rubra* seed by the soil infestation, seed inoculation, and seed infestation treatments, in nonautoclaved soil, and in combination with the various concentrations of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ showed few significant differences and no consistent trends in total seedling emergence or in rate of seedling emergence. The pooled values of all three inoculation treatments, however, showed significant interactions between the pathogens, nitrogen concentrations, total seedling emergence, and rate of seedling emergence. Therefore, all subsequent results are presented on the basis of the pooled treatments' effect.

Total seedling emergence was reduced by D, C, and D + C below that of uninoculated, unfertilized controls independent of the application of either nitrogen sources, and the reduction in emergence in response C and D + C was significantly below that induced by D (Figs. 2A–D). The addition of $Ca(NO_3)_2$ at 10^{-4}, 10^{-3} and 10^{-2}M to D-inoculated seed (Fig. 2B) and 10^{-2}M to C- and D + C-inoculated seed (Figs. 2C and D) reduced emergence below that of their respective unfertilized controls. The combination of D + C with $(NH_4)_2SO_4$ at 10^{-4}M and 10^{-2}M (Fig. 2D) and the D and C inoculations with $(NH_4)_2SO_4$ at 10^{-2} (Figs. 2B and C) also reduced emergence below that of their respective unfertilized controls.

The reduction in seedling emergence was most consistent with $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ concentrations at 10^{-2}M with D, C, and D+C; i.e., the reductions in emergence were consistently below the D, C, and D+C unfertilized controls and below the uninoculated controls that received either nitrogen source at the 10^{-2}M concentration (Figs. 2A–D). The effects of the nitrogen sources at the 10^{-4} and 10^{-3} M concentration in combination with D, C, and D+C was more complex; eg, $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ at 10^{-3}M did not reduce emergence below that of the respective D, C, or D+C controls (Figs. 2B – D), but all organism combinations with $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ at 10^{-3}M reduced emergence below that of the uninoculated control that received the 10^{-3}M concentration (Figs. 2A–D). At the 10^{-4}M concentrations of either nitrogen source, this relationship occurred only with $Ca(NO_3)_2$ and D+C (Figs. 2A and D).

The rate of seedling emergence from seed inoculated with D, C, and D+C was slowed by D and D+C (Table 2). The addition of $(NH_4)_2SO_4$ or $Ca(NO_3)_2$, at concentrations of 10^{-4} or 10^{-2}M, to seed inoculated with D, C, or D+C reduced the rate of seedling emergence below that of their respective unfertilized controls; the same seedling emergence reductions occurred with the uninoculated seed subjected to these concentrations (Table 2). The combination of D+C and either nitrogen source at a concentration of 10^{-4} and 10^{-2}M consistently slowed the rate of seedling emergence below that of the uninoculated controls that received the same nitrogen concentrations. The application of $(NH_4)_2SO_4$ or

TABLE 1. Rate of seedling emergence (CVE[y]) of *Festuca rubra* seeded in autoclaved and nonautoclaved soil in response to applications of $Ca(NO_3)_2$ and $(NH_4)_2SO_4$

Soil mix condition	Unfertilized	Nitrogen source and concentration					
		$Ca(NO_3)_2$			$(NH_4)_2SO_4$		
		10^{-4}M	10^{-3}M	10^{-2}M	10^{-4}M	10^{-3}M	10^{-2}M
Autoclaved	16.6 c/a[z]	16.0 c/a	21.2 a/a	11.6 d/a	15.4 c/a	19.1 b/a	10.7 d/a
Nonautoclaved	17.5 b/a	14.5 c/a	19.4 a/b	12.7 d/a	14.5 c/a	18.7 ab/a	11.9 d/a

[y] CVE = coefficient of velocity of emergence (11). The larger the value, the faster is the rate of emergence.

[z] Means within autoclaved or nonautoclaved soil mixes and among nitrogen forms and concentrations (across, a/), and means within nitrogen sources and concentrations and between autoclaved and nonautoclaved soil (down, /a) followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

$Ca(NO_3)_2$ at a concentration of 10^{-3}M, to D-, C-, or D+C inoculated seed, or uninoculated seed either had no effect on the rate of seedling emergence or stimulated the rate of emergence (Table 2).

DISCUSSION

Reduction in total seedling emergence by D, C, and D+C (Figs. 2A–D) confirms previous observations on the individual and collective pathogenicity of these organisms on germinating seed of *F. rubra* (12). No significant trends were established from the individual soil infestation, seed inoculation, and seed infestation treatments in combination with either nitrogen source. The pooling of the data from the three inoculation methods, however, established significant trends and functions for nitrogen fertilization in the host-pathogen(s) interaction. The rationale for pooling is based on the assumption that the individual inoculation treatments used in this study would not occur independently of each other in nature. Therefore, the results obtained by pooling may be more representative of the potential interaction of the pathogens and germinating seed of *F. rubra* than that observed for individual inoculation treatments.

There seem to be two distinct effects that occur with either nitrogen source in the host-pathogen(s) interaction. (i) Either nitrogen source may be directly toxic to the germinating seed of *F. rubra*. The toxic effect of the nitrogen sources at 10^{-2}M seems to be compounded by the probable nitrogen stimulation of unknown biotic factors in nonautoclaved soil that further reduce the rate of seedling emergence (Table 1) and total seedling emergence (Figs. 1A and B). (ii) Either nitrogen source may enhance the pathogenicity of D, C, and D+C to germinating seed. Direct enhancement of pathogenicity of D, C, and D+C with either nitrogen source at the lower concentrations is minimal (Figs. 2B–D). The highest concentration (10^{-2}M) of either nitrogen source, however, clearly enhances the pathogenicity of D, C, and D+C to germinating seed of *F. rubra* (Figs. 2A – D). The reduction in seedling emergence induced by D+C with either nitrogen source is significantly lower than that of D and C alone (Figs. 2B–D) and represents the treatment responsible for the most severe reduction in total seedling emergence. Previous observations have shown that the D+C combination has an additive pathogenic effect that reduces seedling emergence more than that of either organism alone (12). The present observations suggest that the additive pathogenic capabilities of D+C may be further enhanced by the ability of the pathogens to exploit the direct toxic effects (Figs. 1A and B) of highest concentration (10^{-2}M) of either nitrogen source to germinating seed of *F. rubra*.

The observations of this study may have practical implications. The highest concentration (10^{-2}M) of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ utilized in this study is about equal to 43 kg N per hectare (0.9 lb N per 1,000 ft^2). This concentration is on the lower end of recommended preplanting nitrogen application rates for seedbeds of temperate grasses (2). Also, both D and C are common soilborne organisms. Hence, there is some potential for the interactions observed to occur in the field. The implications also may extend beyond seed germination and seedling establishment. The inability

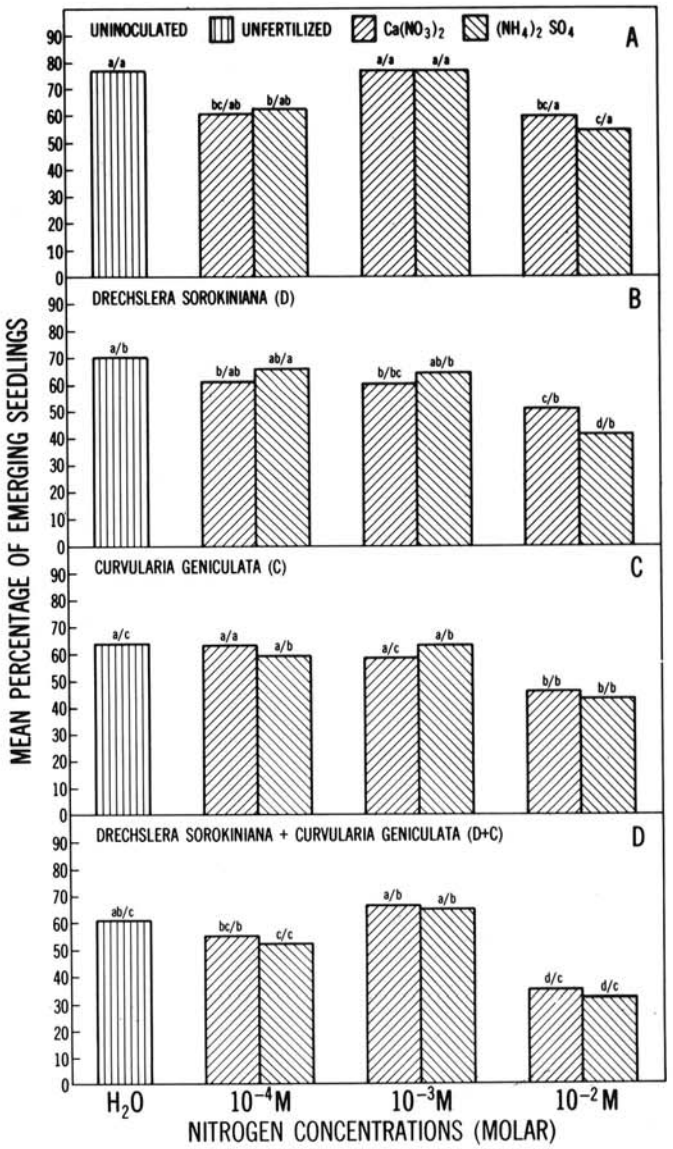

Fig. 2. Mean percentage of emerging seedlings of *Festuca rubra* seeded in nonautoclaved soil receiving various concentrations of $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ and either **A,** uninoculated or **B,** inoculated with *Drechslera sorokiniana,* **C,** *Curvularia geniculata*, or **D,** the combination of the organisms. Emergence of seedlings from inoculated seeds represents the pooled inoculation effect of inoculation methods, inducing soil infestation, seed inoculation, and seed infestation. Section A of the histogram is the uninoculated fertilized control. Means among concentrations of the respective nitrogen sources and within the specific organism(s) used for inoculation (across, a/), and means between the specific organism(s) used for inoculation and within specific concentrations of the respective nitrogen sources (down, /a) followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 2. Rate of seedling emergence (CVE[w]) of *Festuca rubra* seeded in nonautoclaved soil in response to inoculation with *Drechslera sorokiniana* and *Curvularia geniculata* singly and in combination, with and without the applications of $Ca(NO_3)_2$ and $(NH_4)_2SO_4$[x] to the soil

		Nitrogen source and concentration					
		$Ca(NO_3)_2$			$(NH_4)_2SO_4$		
Organism(s)[y]	Unfertilized	10^{-4}M	10^{-3}M	10^{-2}M	10^{-4}M	10^{-3}M	10^{-2}M
Uninoculated	17.5 b/a[z]	14.5 c/a	19.4 a/a	12.7 d/a	14.5 c/a	18.7 ab/ab	11.9 d/a
D	16.0 b/b	13.8 c/ab	19.6 a/a	8.7 d/b	14.4 c/a	19.6 a/a	8.3 d/b
C	16.9 a/ab	14.3 b/a	17.0 a/b	11.9 c/a	14.6 b/a	17.0 a/c	11.5 c/a
D+C	15.5 b/b	12.6 c/b	17.8 a/b	9.9 d/b	12.9 c/b	18.0 a/bc	9.6 d/b

[w] CVE = coefficient of velocity of emergence (11). The larger the value, the faster is the rate of emergence.

[x] Data presented in the table represents the pooled values of soil infestation, seed inoculation, and seed infestation treatments.

[y] D = *Drechslera sorokiniana*; C = *Curvularia geniculata*; and D+C = *D. sorokiniana* + *C. geniculata.*

[z] Means within organism treatments and among nitrogen forms and concentrations (across, a/), and means between organism treatments and within specific nitrogen forms and concentrations (down, /a) followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

of *F. rubra* to persist in mixtures with *Poa pratensis* has long been attributed to the inability of *F. rubra* to withstand the intense management practices applied to *P. pratensis;* ie, high levels of nitrogen fertilization, heavy irrigation, and intense mowing (6,10,14). The susceptibility of germinating seed of *F. rubra* to D, C, and D+C observed in this and previous studies (12) and the potential enhancement of the pathogenicity of these pathogens by nitrogen suggests that the inability of *F. rubra* to persist with *P. pratensis* may be closely related to pathogenic factors that are enhanced by intense cultural practices.

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