# Pathogenicity of Some Select Soilborne Dematiaceous Hyphomycetes on Germinating Seed of *Festuca rubra*

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## ABSTRACT

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The pathogenicity of *Drechslera sorokinia, Curvularia geniculata,* and *Alternaria alternata,* alone or in combination, to germinating seed and emerging seedlings of *Festuca rubra* was evaluated. Seed inoculation and soil and seed infestation methods with *D. sorokiniana* reduced the amount and rate of seedling emergence and increased seedling mortality. *Curvularia geniculata* reduced seedling emergence, but had no effect on rate of emergence or on seedling mortality. Pathogenicity of both organisms generally was greater in autoclaved soil than in nonautoclaved soil. These observations confirmed the pathogenicity of *D. sorokiniana* and established *C. geniculata* as a potentially important pathogen of germinating seed of *F. rubra.* Seedling emergence in soil infested with the combination of *D. sorokiniana* + *C. geniculata* was greater than that in response to either organism alone which suggested competition between the organisms in the soil environment. Seedling emergence from seed infested with the combination of *D. sorokiniana* + *C. geniculata,* however, was below that in response to either organism alone which suggested a synergistic pathogenic interaction between the organisms. *Alternaria alternata* had no consistent effect on seedling emergence or on seedling mortality, and it was not a pathogen. The combination of *A. alternata* with *D. sorokiniana* neither enhanced nor inhibited the pathogenicity of *D. sorokiniana.* The interaction of *C. geniculata* + *A. alternata* on seedling emergence was erratic, but suggestive of potential interactions that could inhibit or enhance the pathogenicity of *C. geniculata.*

*Additional key words: Bipolaris sorokiniana, Helminthosporium sativum.*

The dematiaceous hyphomycetes contain numerous genera such as *Drechslera, Curvularia,* and *Alternaria* which are commonly associated with disease of grasses and contain species that are primary pathogens, weak pathogens, and saprophytes, respectively. *Drechslera sorokiniana* (Sacc.) Subram. & Jain is a primary pathogen that produces leaf spot and seedling, crown, and root rots of numerous grass species (4,7,10,12,13,16). *Curvularia geniculata* (Tr. and Earle) Boed. is a weak primary leaf pathogen (2,5,6), but some isolates are pathogenic to roots and cause preemergence killing of seedlings of some grass species (15,16). *Alternaria alternata* (Fr.) Keissler (= *A. tenuis* Nees) is extremely common on grasses, but is generally classified as a saprophyte (16).

It is common knowledge that *D. sorokiniana, C. geniculata,* and *A. alternata* often occur together on diseased grass tissues. Little is known, however, about how these closely related species interact relative to disease expression. *C. geniculata* and *A. alternata* usually are regarded as secondary invaders of the diseased tissue. Recent studies have shown that the combination of *D. sorokiniana* and *C. geniculata* may reduce disease expression on leaves of *Poa pratensis* at moderate temperatures (20–25 C); at higher temperatures (30 C), however, the combination of organisms increases disease expression (6). It is not uncommon to find *A. alternata* associated with *D. sorokiniana* on diseased grass leaves and on seed of numerous grass species (9,16), but the significance of the association is obscure.

The common association of *D. sorokiniana, C. geniculata,* and *A. alternata* on diseased tissue of grasses and the fact that these organisms are closely related members of the dematiaceous hyphomycetes have resulted in many efforts to establish primary

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pathogenic characteristics of *C. geniculata* and *A. alternata* similar to those of *D. sorokiniana* on leaf tissue of grasses. The conclusions are that *C. geniculata* is a very weak primary leaf pathogen (2,5,6) and that *A. alternata* is a saprophyte (16). Little research, however, has been conducted on the potential pathogenicity of *C. geniculata* and *A. alternata* as soilborne seed and (or) seedling pathogens of grasses. *D. sorokiniana* is well established as a soilborne pathogen (7,16), and *C. geniculata* and *A. alternata* are common soilborne organisms that frequently contaminate the seed of numerous grass species (16).

Creeping red fescue (*Festuca rubra* L.) is a fine-textured species adapted to turf culture in cool humid regions, is somewhat more shade- and abuse-tolerant, and is more susceptible to *D. sorokiniana* leaf spot (1,3,5,14) and root rot (17) than *P. pratensis*. *Curvularia geniculata* causes leaf-tip dieback (2) and is associated with leaf and seedling blighting, and seed rot of *F. rubra* (8). This organism also is often isolated from the basal portion of the leaf sheath of diseased *F. rubra* from plots at the Horticulture Research Station, Ames, IA. *Alternaria alternata* occasionally is found on the seed bracts of *F. rubra* and on dying and dead tissues. The fact that these three organisms occur alone and in combination on numerous species of the Gramineae, including *F. rubra,* suggested that the potential pathogenicity of each organism alone and in combination should be investigated. Research has been initiated to determine the effects of *D. sorokiniana, C. geniculata,* and *A. alternata* on the establishment and persistence of *F. rubra* as a turf cover. The results presented here are those of the first of these studies which were designed to evaluate the pathogenicity of these organisms singly and in combination on the seed germination and seedling establishment of *F. rubra* in soil.

## MATERIALS AND METHODS

*D. sorokiniana, C. geniculata,* and *A. alternata* were grown on 20 ml of 1.0% Czapek Dox Broth (10 g/L) in 3.0% Bacto-agar (wt/vol) in 15 × 150 mm sterile plastic petri dishes under continuous cool-white fluorescent light (75–80 $\mu E/m^2/sec$) at 22 ± 2 C. Conidia of the organisms used for seed inoculation, seed infestation, and soil infestation, respectively, were collected from 15- to 20-day-old cultures in distilled water. The suspensions were filtered through a 90-$\mu$m (pore-size) 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). Combinations of the conidia of the respective organisms were prepared by mixing equal volumes of individual organism suspensions.

*F. rubra* L. 'Dawson' was used for all studies. Seeds were surface sterilized in a 10% Clorox solution (0.53% sodium hypochlorite) for 10 min under vacuum, rinsed 20 times in distilled water, and air dried before they were inoculated with conidia, surface-infested with conidia of the respective organisms, or planted in soil infested with conidia. Laboratory germination tests (22–24C) were conducted on filter paper with surface-sterilized seed to determine percent germination and the potential presence of *D. sorokiniana, C. geniculata,* or *A. alternata* on or within the seed. Five seeds were planted in an autoclaved (2 hr, 120 C) and in a nonautoclaved loam-sand-peat (2:1:1, v/v) mixture in each of 20 compartmentalized plastic flats (6 × 4 × 5 cm) for each seed inoculation, seed infestation, and soil infestation treatment (100 seeds per treatment); each treatment was replicated twice. All studies were conducted in a greenhouse (23–30 C) under natural light. Results were analyzed as factorials and Duncan's multiple range test was applied to means to determine significant interactions.

The study consisted of 18 soil infestation, seed inoculation, and seed infestation treatments with conidia of the respective organisms and their combinations in nonautoclaved and in autoclaved soil. Controls were established in autoclaved and nonautoclaved soil for each treatment. Soil infestation treatments were applied by placing 10 ml of condidial suspensions of *D. sorokiniana* (D), *C. geniculata* (C), *A. alternata* (A), and all possible pairings of the respective organisms (D+C, D+A, and C+A), to the surface of autoclaved and nonautoclaved soil in the compartmentalized flats 1-day before the seeds were planted.

Seed inoculation treatments were applied by placing seed in 200 ml of the appropriate conidial suspension (D, C, A, D+C, D+A, or C+A) in a 500-ml vacuum flask under partial vacuum (faucet aspirator) for 15 min. One drop of Tween-80 was added to the suspension to reduce surface tension and provide better entry of conidia under seed bracts as air was removed. Conidia and seed were kept in suspension by means of a magnetic stirrer. Seed was collected on Whatman No. 1 filter paper in a Büchner funnel and immediately planted in autoclaved and nonautoclaved soil.

Seed infestation treatments were carried out by vacuum inoculating seed with the appropriate conidia (D, C, A, D+C, D+A, or C+A), collecting the seed on filter paper as previously described, and then incubating the seed in 50-ml Pyrex culture tubes with ventilating plastic caps under 75–80 $\mu E/m^2/sec$ of continuous fluorescent light at 22 C ± 2 for 4 days. This treatment provided a period for infestation of the seed bracts by the respective organisms. After incubation, the seeds were planted in autoclaved and in nonautoclaved soil.

Total seedling emergence, rate of seedling emergence, and seedling mortality were recorded for each treatment. Seedling counts were initiated with emergence of the first seedling and recorded for 25 days after planting. The number of emerged seedlings 25 days after planting was recorded as total emergence. Rate of seedling emergence was expressed by the coefficient of velocity of emergence (CVE) (11). Seedling mortality ratings were determined by counting the number of dead seedlings after 25 days. Isolations from all dead or dying seedlings were made to determine the presence of the organisms applied in the various treatments.

## RESULTS

**Comparisons of treatment effects within each soil mix on seedling emergence.** The various organisms tested singly and in combination reduced seedling emergence of *F. rubra*. All treatments with D, except seed inoculation, reduced emergence in nonautoclaved soil (Fig. 1A). In autoclaved soil, all D treatments reduced emergence (Fig. 1B). Seed inoculation and infestation with C reduced emergence in nonautoclaved soil (Fig. 1A) and all treatments with C reduced emergence in autoclaved soil (Fig. 1B). Soil infestation with A reduced emergence in nonautoclaved soil (Fig. 1A); other inoculation methods with A in either nonautoclaved or autoclaved soil had no effect on emergence.

Seed inoculation and infestation with D+C reduced emergence in both nonautoclaved (Fig. 1A) and autoclaved (Fig. 1B) soil. Inoculum combination D+A reduced emergence in all inoculation treatments in nonautoclaved soil (Fig. 1A) and with soil infestation in autoclaved soil (Fig. 1B). The combination C+A reduced emergence only with seed infestation in both nonautoclaved (Fig. 1A) and autoclaved (Fig. 1B) soil.

Laboratory germination tests of surface sterilized seed failed to yield D, C, or A and the percentage germination (82%) did not differ from that of the controls in soil (75–82%) (Fig. 1A and 1B).

**Comparisons of treatment effects between each soil mix on seedling emergence.** Most inoculation treatments resulting in significant reductions in seedling emergence failed to show significant differences between nonautoclaved and autoclaved soils; ie, seed infestations with D, C, D+C, D+A, and C+A, seed inoculations with D+C, and soil infestation with D+A (Fig. 1A and 1B). Reductions in seedling emergence in nonautoclaved soil occurred in response to D- and A-infested soil and D+A-inoculated seed (Fig. 1A); these reductions in emergence either were greater than that in autoclaved soil (D) or did not reduce emergence in the autoclaved soil (A, D+A) (Fig. 1A and 1B). Conversely, autoclaved soil favored reductions in seedling emergence in response to soil infestation with C and seed inoculation with D and C (Fig. 1B); nonautoclaved soil infested with C- and D-inoculated seed germinated in the nonautoclaved soil did not reduce emergence (Fig. 1A).

**Rate of seedling emergence.** The CVE of seedlings was reduced significantly in nonautoclaved and in autoclaved soil infested with

D (Table 1). Infestation of autoclaved soil with C and with D+C, also reduced CVE. The CVE of seed in autoclaved soil infested with A was significantly greater than that of the control. Seed inoculated with D+A reduced CVE in nonautoclaved soil, but other inoculations of seeds planted in nonautoclaved and autoclaved soil had no effect on CVE (Table 1). Seed infested with D+C and germinated in nonautoclaved and in autoclaved soil reduced CVE (Table 1). Seed infested with D or with A reduced the CVE in nonautoclaved soil, and seed infested with C or with C+A showed an increase in CVE in autoclaved soil.

**Seedling mortality and organism reisolation.** Seedling mortality in response to D and D+A increased from seed infestation to seed inoculation to soil infestation in autoclaved soil (Table 1). Seedling mortality was increased in nonautoclaved soil by D- and D+A-infested seed and by A- and C+A-infested soil (Table 1). Seedling mortalilty in nonautoclaved soil in response to D was greater with seed infestation than with soil infestation or seed inoculation. The various inoculation methods in nonautoclaved and autoclaved soil with C and D+C had no effect on seedling mortality. Seedling mortality generally remained unchanged or increased in response to all inoculation treatments from nonautoclaved to autoclaved soil.

Isolations of test fungi from seedlings killed in nonautoclaved soil infested with A and C+A (Table 1) were not consistent, but those from seedlings killed in autoclaved soil infested with D and D+A (Table 1) were 67 and 100% *D. sorokiniana,* respectively. Reisolations from seed inoculation and infestations with D and D+A from seedlings killed in nonautoclaved and in autoclaved soil yielded 100% *D. sorokiniana* from the D inoculations and infestations and 67% *D. sorokiniana* from the D+A inoculations and infestations.

## DISCUSSION

**Pathogenicity of individual organisms.** Test organisms D, C, and A were selected for study of their comparative pathogenicity to germinating seed of *F. rubra* because they are closely related species generally recognized to be a strong primary pathogen (1,3,5,14,17), a weak primary pathogen (2,8), and a saprophyte (16), respectively, on *F. rubra.* The primary pathogenicity of D on germinating seed of *F. rubra* (7) was confirmed by reductions in seedling emergence in response to most inoculation methods in both nonautoclaved and autoclaved soil (Fig. 1A and 1B). The ability of D to reduce seedling emergence in nonautoclaved soil (Fig. 1A) suggests that it can function efficiently as a pathogen in soil under the most competitive circumstances. This is further illustrated by the low CVE of seedlings in nonautoclaved D infested soil (Table 1). It seems, however, that for D to successfully reduce seedling emergence in nonautoclaved soil, it must be established in the soil (soil infestation) or on the seed (seed infestation) (Fig. 1A, Table 1). Bringing conidia and seed together (seed inoculation) had no effect on seedling mortality in nonautoclaved soil (Table 1), or on CVE in nonautoclaved or autoclaved soil (Table 1). It also is clear that D is less capable of killing emerged seedlings in nonautoclaved soil than in autoclaved soil (Table 1); this probably reflects competition by other microorganisms in nonautoclaved soil.

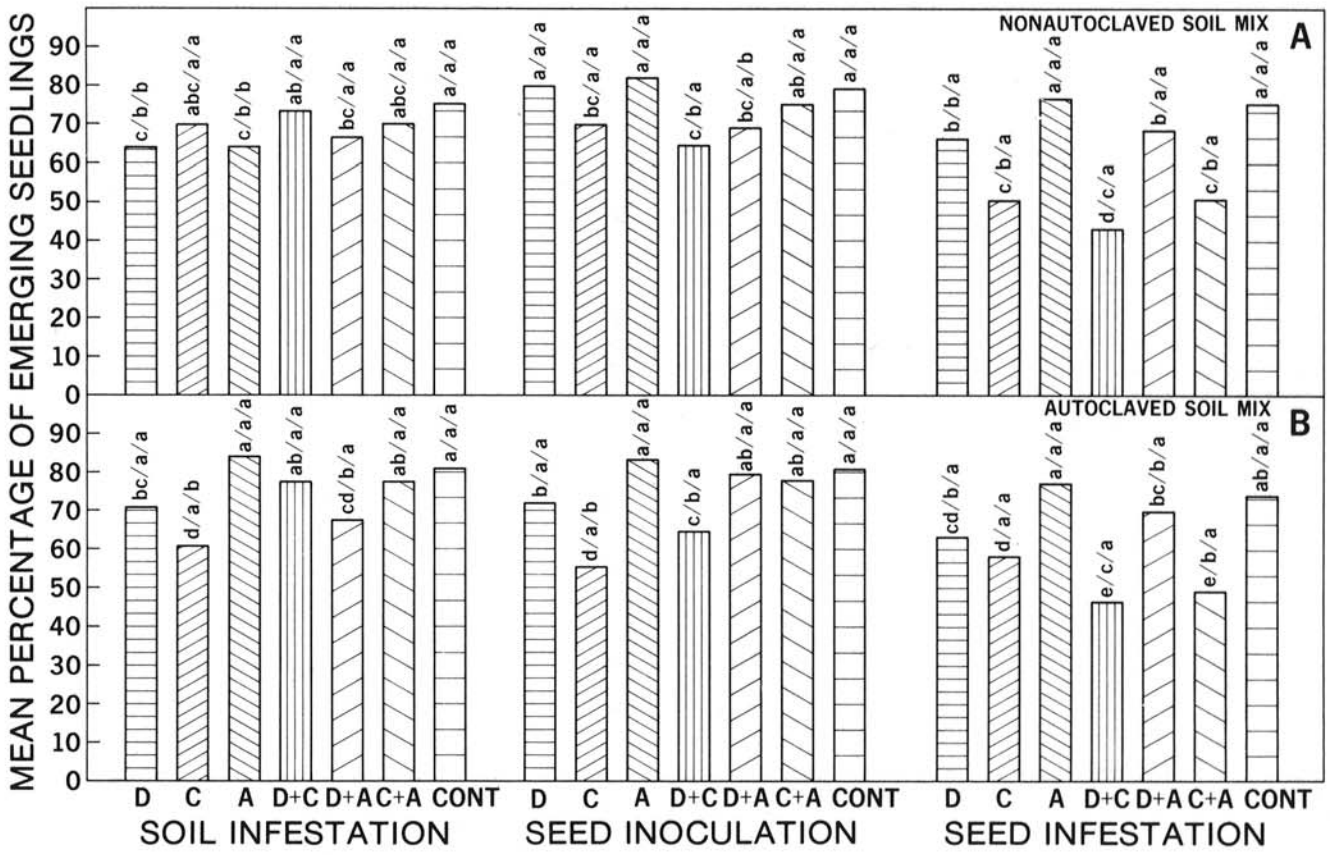


**Fig. 1.** Mean percentage of emerging seedlings of *Festuca rubra* from seed in nonautoclaved (upper section labeled **A**) and in autoclaved (lower section labeled **B**) soil and inoculated with *Drechslera sorokiniana* (D), *Curvularia geniculata* (C), or *Alternaria alternata* (A) and their combinations (D+C, D+A, C+A) by various methods. Seed inoculation was accomplished by planting seed in soil containing conidia of the respect organisms (soil infestation), by combining seed and conidia of the respective organisms (seed inoculation) before planting seed, and by establishing the respective organisms on the seed bracts (seed infestation) by incubating the combination of seed and conidia before planting seed. Means among organism(s) treatments within each inoculation method (across, a//), means for specific organism(s) treatments between inoculation methods (across, /a/), and means for specific organism(s) treatments between nonautoclaved soil (down, //a) followed by the same letter are not significantly different. Duncan's multiple range test ($P = 0.05$).

TABLE 1. The rate of seedling emergence (CVE)[x] and seedling mortality of *Festuca rubra* in response to seed inoculation methods with *Drechslera sorokiniana, Curvularia geniculata,* and *Alternaria alternata* and their various combinations in nonautoclaved and autoclaved soil

| Inoculation method and organism(s)[y] | Nonautoclaved soil mix | | Autoclaved soil mix | |
|---|---|---|---|---|
| | CVE[x] | Seedling Mortality (%) | CVE[x] | Seedling Mortality (%) |
| Soil infestation | | | | |
| D | 12.9 d/b/a[z] | 0.3 b/b/b | 13.2 d/b/a | 3.2 a/a/a |
| C | 13.8 bcd/b/a | 0.5 b/a/a | 13.1 d/b/a | 0.0 b/a/b |
| A | 15.4 ab/b/b | 1.5 a/a/a | 17.5 a/b/a | 0.0 b/a/b |
| D+C | 14.1 abcd/b/a | 0.0 b/b/b | 14.0 cd/b/a | 0.5 b/a/b |
| D+A | 15.6 a/b/a | 0.3 b/b/b | 14.7 bc/b/a | 3.3 a/a/a |
| C+A | 14.5 abc/b/a | 1.7 a/a/a | 14.9 bc/b/a | 0.8 b/a/a |
| Control | 14.5 abc/b/a | 0.0 b/a/a | 15.6 b/b/a | 0.0 b/a/a |
| Seed inoculation | | | | |
| D | 14.0 ab/b/a | 0.5 a/b/c | 13.5 ab/b/a | 2.5 a/a/b |
| C | 14.3 ab/b/a | 0.0 a/a/a | 14.0 ab/b/a | 0.5 b/a/a |
| A | 14.6 a/b/a | 0.3 a/a/a | 12.6 b/c/b | 0.2 b/a/a |
| D+C | 13.8 a/b/a | 0.5 a/ab/a | 12.5 b/c/a | 0.7 b/a/a |
| D+A | 12.8 b/c/a | 0.8 a/b/a | 13.3 ab/b/a | 1.7 a/b/a |
| C+A | 14.5 a/b/a | 1.0 a/a/a | 14.5 a/b/a | 0.5 b/a/a |
| Control | 14.3 a/b/a | 0.0 a/a/a | 13.1 ab/c/a | 0.0 b/a/a |
| Seed infestation | | | | |
| D | 21.0 d/a/a | 2.0 a/a/a | 21.1 c/a/a | 2.0 a/a/a |
| C | 22.6 bc/a/a | 0.5 c/a/a | 23.5 a/a/a | 0.2 c/a/a |
| A | 21.9 cd/a/a | 0.7 bc/ab/a | 21.4 bc/a/a | 0.5 c/a/a |
| D+C | 18.7 e/a/a | 1.7 ab/a/a | 17.0 d/a/b | 0.8 bc/a/a |
| D+A | 25.0 a/a/a | 2.5 a/a/a | 20.8 c/a/b | 1.7 ab/b/a |
| C+A | 24.2 a/a/a | 0.7 bc/a/a | 22.7 ab/a/b | 0.0 c/a/a |
| Control | 23.7 ab/a/a | 0.8 bc/a/a | 21.1 c/a/b | 0.0 c/a/a |

[x]CVE= coefficient of velocity of emergence (11).

[y]D=*D. sorokiniana,* C= *C. geniculata,* A= *A. alternata,* D+C= *D. sorokiniana* + *C. geniculata,* D+A= *D. sorokiniana* + *A. alternata,* C+A= *C. geniculata* + *A. alternata.*

[z]Means among organism treatments within inoculation methods (down, a/ / ), means for specific organism(s) treatments between inoculation methods (down, /a/ ), and means for specific organism(s) treatments betwen nonautoclaved and autoclaved soil (across, / /a) followed by the same letter are not significantly different. Duncan's multiple range test ($P = 0.05$).

The potential pathogenicity of *C. geniculata* has been researched on grasses (2,5,6), and this organism is recognized as a weak primary leaf pathogen of some grasses (5,6) and as a secondary invader of *D. sorokiniana* (D) leaf lesions (6). The observations of this study, however, establish C as a potentially important primary pathogen of germinating seed of *F. rubra.* Total seedling emergence decreased in response to most inoculation treatments in both nonautoclaved and autoclaved soil, and most reductions in response to C were significantly below those in response to D (Fig. 1A and 1B). Unlike D, the pathogenicity of C is primarily restricted to the seed before or during germination. This is illustrated by the inability of C to affect the CVE, or the mortality of seedlings in nonautoclaved soil (Table 1). It also seems that C must be in close contact with the seed (seed inoculation) or established on the bracts (seed infestation) to reduce seedling emergence in nonautoclaved soil (Fig. 1A); the presence of conidia in soil (soil infestation) did not influence seedling emergence. These observations suggest that future research on species of *Curvularia* should be directed toward investigation of their potential pathogenicity on germinating seed and on the roots of grasses.

No consistent pathogenic characteristics were established for *A. alternata* (A) on germinating seed of *F. rubra.* Some reduction in seedling emergence occurred in nonautoclaved soil infested with A, but effects on CVE or seedling mortality were inconsistent (Table 1).

**Pathogenicity of organism combinations.** D+C reduced seedling emergence from soil infestation to seed inoculation to seed infestation (Fig. 1A and 1B). Seedling emergence in soil infested with D+C generally was greater than that infested with D or C alone. Seedling emergence in response to D+C seed inoculation or infestation generally was lower than that of either organism alone, and the most severe reduction in seedling emergence occurred in response to seed infestation in nonautoclaved and autoclaved soils. These reactions suggest that when both organisms are present in soil (soil infestation) their relationship is competitive and pathogenicity is reduced. Conversely, if conidia of the two organisms are placed on the seed (seed inoculation), or established and growing on the bracts of the seed (seed infestation), their pathogenicities to germinating seeds are synergistic. A similar competitive and synergistic interaction for these organisms has been established on leaves of *Poa pratensis* (6). Where D increased seedling mortalilty the increase in response to the combination of D+C often was significantly lower (Table 1), which suggested competition between the organisms.

The combination of D+A had little effect on the primary pathogenic characteristics of D (Fig. 1A and 1B, Table 1). No competitive or synergistic effects occurred with D+A. Some competition seems to exist with the combination of C+A, however, in that seedling emergence was greater in response to C+A than to C in response to some of the inoculation treatments (Fig. 1A and 1B).

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