Factors Affecting Seedling Emergence of Sorghum for Short-Season Areas

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ABSTRACT

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The potential for extending the range of sorghum (*Sorghum bicolor*) into southern Alberta depends on improving the percentage of seedling emergence. Studies were done to examine the effect of rooting medium and ambient temperature on percentage of emergence, to determine the part played by soilborne and seedborne pathogens in reducing emergence, and to identify pathogens that might be important in southern Alberta. Increased impedance in the rooting medium decreased percentage of emergence as did low temperatures, which increased the pathogenicity of seedborne and soilborne fungi. *Penicillium oxalicum*, *Aspergillus* spp., and a *Rhizopus* sp. were prevalent in seed lots produced in southern Alberta and Texas, but poor seed viability and stunting of the roots of coleoptiles could be attributed only partially to seedborne fungi. Two *Fusarium* spp., *F. oxysporum* and *F. tricinctum*, isolated from Alberta soils were pathogenic to sorghum at low temperatures. Chilling injury per se was not the cause of reduced emergence, and stresses caused by low temperatures or heavy soils enhanced the pathogenicity of soilborne and seedborne pathogens.

Grain sorghum (*Sorghum bicolor* (L.) Moench) is considered to have potential as a new crop in southern Alberta, but poor seedling emergence has discouraged its promotion and development (12). Initially, poor emergence was attributed to chilling injury (12,15), but field studies demonstrating that seed treatment with fungicides increased emergence (5,6) suggested that seedling blight was responsible. Deterioration of sorghum seed by fungal infection and other causes has been described as "grain weathering" and "field deterioration" (8,16). Leukel and Martin (11) concluded from greenhouse tests that seed rots and seedling blights in sorghum were caused primarily by seedborne *Fusarium moniliforme* Sheldon and *Rhizopus nigricans* Ehr. and by soilborne *Pythium* spp., *Penicillium oxalicum* Currie & Thom, and *F. culmorum* (W.G. Sm.) Sacc. Pathogenicity of these fungi was more severe at soil temperatures of 15–20 than at 25 C (11).

The objectives of our study were 1) to measure the influence of low soil temperatures and different rooting media on seedling emergence and survival, 2) to identify prevalent seedborne and soilborne organisms associated with diseased seeds and seedlings, and 3) to assess the ability of selected soilborne *Fusarium* spp. to reduce emergence of disease-free seed lots in autoclaved soil at low temperatures.

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MATERIALS AND METHODS

Seed of Pride brand P145, a commercial single-cross hybrid produced in Texas in 1981, and four open-pollinated populations (Lethbridge 1–4) produced at the Lethbridge Research Station in 1980 were chosen to study emergence and to isolate soilborne pathogens. The Lethbridge populations were harvested at about 25% moisture and spread on a floor at room temperature to dry to about 10% moisture. All seed lots were stored in a seed-storage room.

Effect of temperature and rooting medium on emergence. Emergence of the five seed lots from three rooting media was studied in two model E-7 controlled-environment cabinets (Controlled Environments Ltd., Winnipeg, Man.) set to a 12-hr photoperiod. The rooting media were Cornell mix (1), sand, and soil (Typic Haploboroll silty clay loam) from the sorghum plot area at the Lethbridge Research Station. The three rooting media were either not autoclaved or autoclaved at 121 C and 15 psi for 6 hr. Light was provided from a combination of wide-spectrum Gro-Lux and incandes-

cent lamps at a 3.3:1 ratio of input wattage and photosynthetic photon flux density of 323 μmol sec $^{-1}$m $^{-2}$, which was measured 1 m below the lamps with an LI-188B integrating photon photometer (LI-COR Inc., Lincoln, NE).

The experimental design was a split plot with controlled-environment chambers as main plots, the three rooting media (autoclaved and not autoclaved) in wooden flats as six subplots, and the five sorghum genotypes as sub-subplots. There were two replicates. The two temperature treatments in the controlled-environment cabinets were 12-hr day/night air temperatures of 30/20 and 15/5 C maintained at ±0.3 C and monitored periodically with shaded thermocouples connected to a Version 5 model Polycorder (Omnidata Inc., Logan, UT) set to record temperatures every 10 min over a 24-hr period. Numbers of emerged seedlings were recorded daily for 4 wk.

Soilborne and seedborne organisms. Fifty seeds of each seed lot in the surface-sterilized and unsterilized form were placed on moist Whatman No. 3 filter paper in 10-cm-diameter plastic petri dishes (10 seeds per dish) and incubated at 20 C. After 7 days, the number of ungerminated seeds, the shoot and root length, the percentage of the seed and seedlings free of fungi, and the type of fungi associated with germinated and ungerminated seeds were recorded. A seedling was considered stunted if the combined root and shoot measurements were less than 30 mm.

Seedlings and ungerminated kernels from the temperature/rooting medium experiment were excavated after 4 wk. Those showing necrosis or discoloration were surface-sterilized by immersion in 0.5% sodium hypochlorite (NaOCL) for 3 min, rinsed in sterile distilled water, and plated on acidified potato-dextrose agar ($^{+}$HPDA). After 4 and 12 days, plates were examined for fungal growth. Fungal isolates were grown for 7 days, subcultured by hyphal tips, and transferred twice on $^{+}$HPDA and stored on potato-dextrose agar (PDA) slants for later identification.

Pathogenicity tests. The pathogenicity of two cultures of *F. oxysporum* and one of *F. tricinctum* isolated from diseased sorghum seedlings grown at the 15/5 C regime was tested in the same controlled-environment cabinets at the same temperature regimes and lighting conditions used for the previous experiment.

The *Fusarium* isolates were grown on PDA for 2 wk at about 20 C under constant near-UV light. Cultures were homogenized in a Waring Blendor in sterile distilled water for 15 sec and filtered through cheesecloth. A suspension of about 10^6 conidia and mycelial fragments per milliliter for each of the isolates was used as inoculum.

Uniform seed lots of three of the homozygous maturity genotypes, SM80, SM100, and 100M (13), which had been grown in a greenhouse at the Lethbridge Research Station Phytotron, were used in the pathogenicity tests. These seed lots, when plated on $^{+}$HPDA, were free of seedborne fungi and germinated at a rate of 95–100% on moistened filter paper.

Three kernels of each genotype were seeded 3 cm deep in 15-cm clay pots containing autoclaved or nonautoclaved soil or Cornell mix. About 15 ml of inoculum was poured over each seed and allowed to soak in. Each pot was placed on a saucer containing sufficient water to keep the rooting medium moist until the seedlings emerged. The experimental design was a randomized complete block with three replicates. Numbers of emerged seedlings were recorded after 4 wk, and ungerminated seeds and discolored and necrotic seedlings were excavated, washed thoroughly, surface-sterilized, and placed on $^{+}$HPDA for reisolation of pathogenic fungi.

RESULTS AND DISCUSSION

Effect of temperature and rooting medium on emergence. Rooting medium and seed lot had an effect ($P = 0.05$) on seedling emergence at both temperature treatments, but no significant interactions were detected. The overall emergence was 45% for the 30/20 C regime and 35% for the 15/5 C regime. Autoclaving produced a significant increase in emergence only at the low temperature (Table 1). Averaged over the genotypes and rooting media, the benefit from autoclaving at 15/5 C was 14% but the benefit from autoclaving at the high temperature was 7% (Table 1). The difference between P145 seed treated with fungicide at the high and low temperatures was very small. Cornell mix (made of sand, peat, and vermiculite) was porous and offered very little impedance to the sorghum seedling. Conversely, soil had a tendency to become too wet and then dry very hard. Thus, both low temperature and compacted soil appeared to have an additive effect of reducing emergence. These results demonstrated that many factors influence survival of sorghum seedings, and it is not difficult to envisage considerable stand reductions in southern Alberta resulting from cold soils and seedborne and soilborne organisms. These factors were also mentioned by Ross and Webster (15) as being important in Nebraska.

Seedborne and soilborne organisms. In germination tests on filter paper, the seed lots had a high proportion of ungerminated seeds, ranging between 20 and 64% (Table 2). Surface-sterilization of seed had no effect in reducing the percentage

Table 1. Percentage of emergence of five sorghum lines grown in three autoclaved and nonautoclaved rooting media at 30/20 and 15/5 C

	Observations (no.)	Emergence (%)	
		30/20 C	15/5 C
Rooting medium			
Cornell mix	20	54 a[z]	41 a
Lethbridge loam	20	44 ab	38 ab
Sand	20	39 b	26 b
Cultivars			
Pride P145	12	66 a	63 a
Lethbridge 1	12	51 b	38 b
Lethbridge 2	12	39 c	27 c
Lethbridge 3	12	35 c	24 c
Lethbridge 4	12	36 c	24 c
Soil treatment			
Nonautoclaved	30	42 a	27 b
Autoclaved	30	49 a	41 a

[z] Means within columns and within treatments followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 2. Percentage of nongermination, stunted seedlings, and incidence of seedborne fungi in surface-sterilized and unsterilized seed of five sorghum lines incubated on moistened filter paper for 7 days at 20 C

	Ungerminated seeds				Stunted seedlings[x]			
	Percentage		Percentage with seedborne fungi		Percentage		Percentage with seedborne fungi	
Cultivars	US[y]	S	US	S	US	S	US	S
Pride P145	20 b[z]	12 b	84 a	86 b	17 a	20 bc	80 a	20 a
Lethbridge 1	41 ab	50 a	100 a	64 ab	18 a	30 ab	83 a	12 ab
Lethbridge 2	30 b	32 a	94 a	68 ab	26 a	34 a	100 a	0 c
Lethbridge 3	40 ab	45 a	90 a	78 ab	30 a	25 abc	100 a	0 c
Lethbridge 4	64 a	52 a	100 a	64 a	20 a	12 c	100 a	0 c

[x] Seedlings with a combined root and shoot measurement of less than 30 mm after 7 days at 20 C were considered stunted.

[y] S = surface-sterilized for 3 min in sodium hypochloride; US = unsterilized.

[z] Means within columns followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

of ungerminated seed but did reduce the incidence of seedborne fungi in both germinated and ungerminated sorghum seed. Excluding the P145 seed lot, 100% of the seeds that produced healthy seedlings were contaminated with seedborne fungi in the unsterilized seed lots, whereas only 2–20% of healthy seedlings in surface-sterilized seed lots were contaminated. In the P145 seed lot, which was treated with captan, seedborne fungal contamination in healthy seedlings was 0% in both the surface-sterilized and unsterilized treatments. Fungal contaminants of healthy and ungerminated seed in the unsterilized treatments were primarily *P. oxalicum*, *Aspergillus* spp., and a *Rhizopus* sp. Bacterial ooze was also apparent on the seed surface of ungerminated seed in the surface-sterilized treatments.

The seedborne fungi prevalent in the sorghum seed lots produced in Lethbridge in 1982 probably developed during the postharvest drying process and may have been important in reducing the viability of seed lots. However, the presence of the same fungi on both the ungerminated and healthy seedlings suggested that much of this contamination was superficial. Fredericksen et al (7) reported that infection and colonization of the endosperm and embryo by *F. moniliforme* is characteristic of grain mold development in susceptible sorghum lines. Internal colonization of the seed by these fungi may be necessary before they can adversely affect seed germination. Seedborne *P. oxalicum* and species of *Aspergillus*, *Rhizoctonia*, *Rhizopus*, and *Helminthosporium* have been reported to reduce seed viability and seedling vigor, especially at low soil temperatures (11). Conversely, seedborne *Alternaria* spp. have been associated with seed lots with high germination (3). The poor germination in this study could not have been due entirely to seedborne fungi because surface-sterilization of ungerminated seed reduced the incidence of fungi without increasing the germination (Table 2).

The incidence of stunted seedlings after the 7-day incubation period (Table 2) was similar among the five seed lots, ranging between 12 and 34%. Often, the distal portions of the root and coleoptile were discolored and necrotic. The frequency of stunted seedlings differed among the surface-sterilized seed lots but not among the unsterilized seed lots. Most of the fungal contamination on stunted seedlings appeared superficial because surface-sterilization did not reduce the percentage of stunted seedlings, but it did reduce the incidence of fungi isolated from the affected seedlings (Table 2). Again, a bacterial ooze was often apparent on the seed and on the surface of stunted seedlings. The stunting and discoloration of the roots and coleoptiles were believed to be due to a seedborne bacterium detected in many of the isolations. The bacterial species in question has been tentatively identified as *Pseudomonas syringae* (D. A. Gaudet, *unpublished*). Studies are under way to identify the role of seedborne bacteria in reducing seed viability and vigor.

F. oxysporum and *F. tricinctum* predominated in isolations on †HPDA from ungerminated seeds and unemerged seedlings in nonautoclaved sand and soil treatments, especially those grown at the 15/5 C regime, suggesting a role for these fungi as soilborne pathogens of sorghum seedlings. A *Trichoderma* sp. predominated in all isolates from unsterilized Cornell mix.

Pathogenicity tests. The *F. tricinctum* strain and the two *F. oxysporum* strains isolated from nonautoclaved sand and soil in the first experiment significantly reduced emergence of disease-free sorghum seed at the low-temperature regime (Table 3). These results confirmed that the capacity of soilborne fungi to infect sorghum seedlings was enhanced at low temperatures and supported the recommendation that sorghum not be seeded until soil temperatures approach 20 C (15). The 15/5 C regime was similar to the mean day/night air temperatures of 17/4 C and only slightly lower than the mean day/night soil temperatures of 16/11 C recorded at 5-cm depth in Lethbridge during May.

The three isolates differed in their ability to reduce seedling emergence, with *F. tricinctum* causing the largest reduction. There were no differences among the three cultivars, SM80, SM100, and 100M, in the amount of preemergence rot caused by the fungi. As in the first experiment, a 10% reduction in emergence for 100M was observed in soil compared with Cornell mix, but SM80 and SM100 experienced greater difficulty in soil than in the Cornell mix, with 29 and 23% reductions, respectively.

Isolations obtained from ungerminated seeds and from seedlings showing root and coleoptile necrosis and decay yielded the *Fusarium* spp. added to the soil, indicating that the inoculants caused the reduced emergence in autoclaved soils.

Low-temperature effects in promoting damage by *P. oxalicum*, *F. moniliforme*, *F. culmorum*, and *Aspergillus* spp. have been reported for sorghum in Kansas (11). *F. oxysporum* and *F. tricinctum* were considered only weak pathogens of forage legumes and grasses (2,10), although they were more pathogenic on plants subjected to temperature or nutritional stress (4,10). These two *Fusarium* spp. have not been reported as pathogenic to sorghum seeds and seedlings although *F. oxysporum* has been reported to be pathogenic to maize (2). Reed et al (14) reported that *F. oxysporum* and other *Fusarium* spp. were frequently associated with sorghum roots early in the growing season in Nebraska, whereas *F. tricinctum* was more frequently associated with sorghum roots later in the growing season.

The difficulties encountered with stand establishment of sorghum have prevented commercial production in southern Alberta. Even weakly pathogenic soil fungi appear to have the potential to seriously reduce sorghum emergence. Chilling injury induced by low soil temperature probably does not reduce emergence as previously thought (12,15). Seed damage caused by harvesting and by insects may also increase disease problems by facilitating the access of fungi to the endosperm and embryo (9). The stunting of the root or coleoptile observed in this study could have an effect of delaying emergence, thereby predisposing the seedlings to seedborne and soilborne disease organisms. It is apparent that under field conditions in southern Alberta, where emergence is restricted by low soil temperatures or soil impedance, adapted cultivars will require a greater capacity to resist infection by soilborne disease organisms than that which traditional sorghum cultivars offer. The potential for extending the range of sorghum into southern Alberta depends on the potential for developing genotypes that have greater resistance to both seedborne and soilborne diseases and for developing management practices that reduce the stresses that may provide a relative advantage to the pathogens.

Table 3. Combined percentage of emergence of three sorghum cultivars grown in two autoclaved rooting media at 30/20 and 15/5 C after inoculation with *Fusarium oxysporum* and *F. tricinctum*

	Observations (no.)	Emergence (%)	
		30/20 C	15/5 C
Rooting medium			
Cornell mix	72	94 a[z]	70 a
Lethbridge loam	72	78 b	40 b
Isolate			
Check	18	90 a	82 a
F. oxysporum (No. 1)	18	93 a	46 bc
F. oxysporum (No. 2)	18	79 a	57 b
F. tricinctum	18	82 a	35 c

[z] Means within columns and within treatments followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

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