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Effect of Spent Mushroom Substrate on Seed Germination of Cool-season Turfgrasses

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Abstract. **Spent mushroom substrate (SMS) is used by the turf industry in the northeastern United States for soil improvement. When tilled into soil at high rates, some turfgrass managers claim that SMS inhibits turf seed germination. The authors' objectives were 1) to determine whether fresh SMS inhibits turf seed germination and, if so, which species are most adversely affected; 2) to evaluate whether any inhibition incited by SMS is the result of osmotic effects or toxicity of compounds in SMS extracts; 3) to determine whether any negative effect of SMS on germination can be eliminated by leaching the SMS-amended soil before seeding; and 4) to assess the performance of SMS on seedling emergence in the field. Germination of nine turfgrass species was evaluated in mixtures made from fresh SMS (electrical conductivity of saturated paste extract = 11.9 dS·m^{-1}) and a loamy sand soil. Germination inhibition resulting from SMS was most pronounced in the following order: Colonial bentgrass (*Agrostis capillaris* L.) > sheep fescue [*Festuca ovina* L. ssp. *hirtula* (Hackel ex Travis) Wilkinson] > Kentucky bluegrass (*Poa pratensis* L.) > hard fescue [*Festuca trachyphylla* (Hackel) Krajina] > creeping bentgrass (*Agrostis stolonifera* L.) > chewings fescue [*Festuca rubra* L. sp. *commutata* (Thuill.) Nyman] = strong creeping red fescue (*Festuca rubra* L. ssp. *rubra* Gaud.) > slender creeping red fescue [*Festuca rubra* L. sp. *litoralis* (Meyer) Auquier] > perennial ryegrass (*Lolium perenne* L.). SMS had a stronger negative effect on germination rates than on final germination percentages. Germination of perennial ryegrass and Kentucky bluegrass on blotter paper moistened with SMS extracts or polyethylene glycol of equivalent osmotic potentials showed that the inhibition was primarily the result of osmotic effects. In an experiment with a 50% soil/50% SMS (v/v) mixture, Kentucky bluegrass germinated better in pots that had been watered with 133% or 167% of the evaporation rate for 10 days prior to seeding than in unleached pots. Although the negative effect of SMS on seed germination was not confirmed in a field study in which ECe values never exceeded 4.1 dS·m^{-1}, the authors conclude that incorporation of high rates of SMS represents a potential problem for turfgrass establishment.**

Spent mushroom substrate (SMS) is the organic material remaining after a crop of mushrooms has been harvested. The ingredients may vary among mushroom farms, but typically mushrooms are grown in a composted mixture of horse-bedded straw, hay, poultry manure, ground corn cobs, cottonseed hulls, brewer's grain, cottonseed meal, cocoa bean hulls, and gypsum (Beyer, 1999). After harvest, the SMS is pasteurized with steam to kill insects, pathogens, and mushroom remnants. The substrate may be sold immediately after removal from the mushroom houses ('fresh SMS') or it may be placed in windrows and further composted for at least one winter ('weathered SMS') (Beyer, 1999).

In the northeastern United States, SMS is an important source of organic material for the turf industry. SMS improved soil physical properties when tilled into a clay loam subsoil before turfgrass establishment (Landschoot et al., 1993) and resulted in increased water retention, lower bulk density, improved turf cover, and decreased surface hardness when applied as topdressing to an athletic field during a 3-year period (McNitt et al., 2004). When used for soil improvement, fresh SMS is frequently applied to the soil surface in a 2 to 5-cm layer and incorporated to a depth of 10 to 15 cm. Alternatively, SMS may be blended with soil at a 50% soil : 50% SMS volume ratio and used as a topsoil.

Some turf managers have reported that soil amended with fresh SMS may result in a delay or inhibition of turf seed germination. Such phytotoxic effects may be attributed to total salinity (Carrow and Duncan, 1998; Landschoot et al., 1993) or ammonia concentrations (Adriano et al., 1973; Bennett and Adams, 1970), but heavy metals, phenolic compounds, and fatty acids have also been linked to inhibition of germination (Hoekstra et al., 2002; Keeling et al., 1994; Marambe et al., 1993).

The effects of salinity on plant growth are commonly divided into osmotic effects (physiological drought), ion toxicities, or ion imbalances causing nutritional disorders (Carrow and Duncan, 1998). The most prevalent toxic ions are Na^+ and Cl^-, although negative effects of B, HCO^-, and OH^- have also been reported. Based on reported threshold electrical conductivity of saturated past extract (ECe) values [i.e., the ECe value at which shoot growth starts to decline below that of a nonsaline condition (Carrow and Duncan, 1998)], the cool-season turfgrasses have been ranked for tolerance to salts in the order of tall fescue (*Festuca arundinacea* Schreb.) > perennial ryegrass > fine fescues > creeping bentgrass > Kentucky bluegrass > rough bluegrass (*Poa trivialis* L.) > colonial bentgrass (Turgeon, 2005). Although not included in Turgeon's listing, annual bluegrass (*Poa annua* L.) is commonly regarded as very salt sensitive (Carrow and Duncan, 1998), whereas annual ryegrass (*Lolium multiflorum* Lam.) has a higher salt tolerance than perennial ryegrass and tall fescue (Sun et al., 2000; Yu and Shen, 2004). Significant cultivar differences in salt tolerance during germination were found in rough bluegrass (Camberato and Martin, 2004), Kentucky bluegrass (Horst and Taylor, 1983; Torello and Symington, 1984), and bentgrasses (McCarty and Dudeck, 1993). Horst and Dunning (1989) suggested that a salt concentration with an EC of 23.5 dS·m^{-1} should be used to screen genotypes of perennial ryegrass for improved germination; however, Dudeck and Peacock (1985) found no difference in salt tolerance among six cultivars of perennial ryegrass. Germination and seedling development are generally the most salt-sensitive stages during plant ontogeny, and rankings of cultivars and species for salt tolerance during these stages are often different from those of mature turf (Marcar, 1987; Marcum, 2001; Sanda, 1978).

In cases in which the inhibitory effect of SMS is incited by total salinity or specific ions, a logical remedy would be to leach the amended soil before turfgrass establishment. Although this can be achieved by flooding, it is usually more efficient to apply water through an irrigation system at 12- or 24-h intervals for a certain number of days before seeding (Carrow and Duncan, 1998). The leaching requirement depends on the quality of the irrigation water and is usually given as a surplus to the daily evapotranspiration (ET) rate. Carrow and Duncan (1998) presented evidence that an additional 5% to 33% above ET replacement will usually be sufficient to maintain an acceptable salinity level;

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however, none of these examples were related to an establishment situation, let alone organic amendments causing the high salinity level.

The objectives of this research were 1) to determine whether fresh SMS inhibits cool-season turf seed germination under laboratory conditions and, if so, which species are most adversely affected; 2) to evaluate whether any inhibition incited by SMS is the result of osmotic effects or toxicity of compounds in SMS extracts; 3) to determine whether any negative effect of SMS on germination can be eliminated by leaching the SMS-amended soil before seeding; and 4) to assess the performance of SMS on seedling emergence in the field.

Materials and Methods

Turfgrass seed. Seed of colonial bentgrass 'Bardot' and 'Nor', creeping bentgrass (*Agrostis stolonifera* L.) 'Penn A-4' and 'Nordlys', chewings fescue [*Festuca rubra* L. sp. *commutata* (Thuill.) Nyman] 'Koket' and 'Wilma', slender creeping red fescue [*Festuca rubra* L. sp. *litoralis* (Meyer) Auquier] 'Barcrown' and 'Louisa', strong creeping red fescue (*Festuca rubra* L. ssp. *rubra* Gaud.) 'Leik' and 'Pernille', hard fescue [*Festuca trachyphylla* (Hackel) Krajina] 'Pintor' and 'Ridu', sheep fescue [*Festuca ovina* L. ssp. *hirtula* (Hackel ex Travis) Wilkinson] 'Lillian' and 'Quatro', Kentucky bluegrass 'Limousine' and 'Conni', and perennial ryegrass (*Lolium perenne* L.) 'Bargold' and 'Caddy' were kindly provided by the seed companies Felleskjøpet Øst Vest (Holstad, Norway) and Seedsuperstore.com.

Spent mushroom substrate and soil used in pot experiments. Fresh, pasteurized SMS was obtained from the Pennsylvania State University (PSU) mushroom facility in Oct. 2005 on the same day it came out of the building. The substrate was made from horse-bedded straw [74% of dry weight (DW)], switchgrass straw (14% of DW), ground corn hulls (4% of DW), dried poultry manure (4% of DW), and gypsum (4% of DW); the water content of the mixture had been adjusted to 72%. The substrate was used to produce one crop of mushrooms and, immediately after harvest, the SMS was removed from the house. The SMS was allowed to dry for 1 to 2 weeks in an unheated greenhouse before grinding in a hammer mill with a 2-mm sieve. Chemical and physical analyses of the SMS are given in Table 1. A loamy sand (85.3% sand, 7.3% silt, 7.4% clay) with a pH of 7.3, 97 mg·kg^{-1} P and 230 mg·kg^{-1} K (Mehlich 3 extracts) was used in the control treatments and for soil/SMS mixtures.

Expt. 1: Effect of SMS on germination of nine turfgrass species. Four mixtures of ground SMS and soil were mixed in a concrete mixer. The four treatments were 1) 100% SMS, 2) 50% soil/50% SMS (v/v) = 79% soil/21% SMS (w/w), 3) 75% soil/25% SMS (v/v) = 93% soil/7% SMS (w/w), and 4) 100% soil (control). EC values of each mixture were measured both in saturated paste extracts (ECe values) (USDA, 1954), in a dilute 1 soil : 5 water (w/w) extract as used for analyses of compost by the Agricultural Analytical Services Laboratory at PSU, and in a 1 soil : 2 water (v/v) extract as used by some laboratories for analysis of saline soils. EC values were determined both before and after the 25-d germination period (Table 2). Samples collected after the germination period were pooled from the 2-cm surface layer in five random pots for each SMS treatment.

Table 1. Chemical and physical analyses of fresh SMS conducted by the PSU Agricultural Analytical Services Laboratory.[z]

	Mean[y]	Range
pH	6.5	6.4–6.5
Dry matter (%)	89	86–94
On a dry matter basis		
Organic matter (%)	68	67–68
Total N (%)	1.6	1.4–1.7
Total C (%)	35	34 to 36
C:N ratio	22	22–24
Organic N (%)	1.5	1.4–1.7
Ammonium-N (mg·kg^{-1})	169	152–185
P (%)	0.4	0.4 to 0.5
K (%)	1.9	1.9 to 1.9
Ca (%)	5.9	—
Mg (%)	0.9	—
S (%)	1.5	—
Na (mg·kg^{-1})	1930	—
Al (mg·kg^{-1})	2824	—
Fe (mg·kg^{-1})	8033	—
Mn (mg·kg^{-1})	1027	—
Cu (mg·kg^{-1})	49	—
Zn (mg·kg^{-1})	139	—

[z] Methods used for analyzing fresh SMS can be accessed at www.aasl.psu.edu/methods.htm

[y] pH, dry matter, organic matter, total N, total C, C:N ratio, ammonium-N, P, and K were analyzed in three replicate samples; the other elements, only in one sample.

Specific ions were determined in saturated paste extracts before the start of the experiment (Table 2). Cations were determined by inductively coupled plasma emission spectroscopy as specified by the USDA and U.S. Composting Council (2002). Anions were determined using a Dionex 500 ion chromatograph (Dionex, Sunnyvale, Calif.) with a GP40 gradient pump and a combination of Dionex AG11 guard column (75 × 4 mm) and Dionex AS11 analytical column (250 × 4 mm) with sodium hydroxide eluant (USA EPA, 1994).

In Oct. 2005, pots (10 cm high and 10 cm in diameter) were filled with the four soil/SMS mixtures, and samples comprised of 100 seeds were distributed on the surface. After seeding, 3 mm of the respective mixtures were added to cover the seeds. Pots were placed in trays that had been filled with water to a height of 12 mm 1 day before seeding, and trays were replenished with water every day during the germination period. To avoid leachate from pots filled with one soil/SMS mixture to contaminate pots filled with a different mixture, the pots were arranged according to a split–split plot design with three blocks. The trays containing the four different soil/SMS mixtures served as main plots, turfgrass species as subplots, and turfgrass cultivars as sub-subplots. Germinated seeds were counted and removed every second day from day 5 until day 25 after seeding, as soon as the coleoptile appeared. The average daily mean, minimum, and maximum temperatures during the germination period were 18.6 °C, 13.0 °C, and 21.1 °C respectively.

Germination in the various treatments was described by the final germination percentage ($FGP = \Sigma n$, where n is the number of seeds that germinated at each counting) and germination rate [$GR = \Sigma\ (n/D)$, where D is the number of days to germination] (Maguire, 1962).

Statistical analyses were accomplished using the 9.02 release of the SAS procedure PROC ANOVA (SAS Institute, Cary, N.C.). FGP values were arcsine-transformed to ensure homogenous variances and normality. Because initial analyses performed on means within subplots indicated that the soil/SMS mixture × species interaction was significant for both FGP and GR (Table 3), subsequent analyses were performed independently for each species. These analyses did not indicate soil/SMS mixture × cultivar interactions except in perennial ryegrass and Kentucky bluegrass; hence, only mean values across cultivars are presented in this paper and the effects of SMS treatments within species identified by Duncan multiple comparison tests at $P < 0.05$. Probably because of poor-quality seed, perennial ryegrass 'Caddy' and Kentucky bluegrass 'Conni' had poor FGP (<70) even in the control treatment, and these cultivars were therefore excluded from the analyses, leaving perennial ryegrass and Kentucky bluegrass to be represented only by 'Bargold' and 'Limousine', respectively.

Based on mean GRs for each subplot, a relative SMS tolerance value (R-SMS-T) was calculated for each species in each block as

$$\text{R-SMS-T} = 100 \times \frac{\textit{mean GR in pots with } 25\%,\ 50\% \textit{ or } 100\% \textit{ SMS}}{\textit{GR in control pots}}$$

The R-SMS-T values were subjected to analyses of variance after arcsine transformation.

Expt. 2: Effect of equivalent osmotic potentials created by SMS extract or polyethylene glycol on germination of perennial ryegrass and Kentucky bluegrass. In Dec. 2005, a new saturated paste extract was made based on the same SMS used in expt. 1. This extract showed an ECe value of 10.9 dS·m^{-1}, corresponding to an osmotic potential of $\psi_o = -0.39$ MPa (USDA, 1954). Some of the extract was diluted with one or two parts distilled water to provide ECe values of 7.3 dS·m^{-1} ($\psi_o = -0.26$ MPa) and 3.6 dS·m^{-1} ($\psi_o = -0.13$ MPa) respectively.

To determine whether the inhibition of germination resulting from SMS was a true osmotic effect or the result of inhibitory compounds in the SMS extract, solutions of

Table 2. EC values, cations, and anions in soil/SMS treatments used in Expt. 1.

	100% SMS	50% soil/50% SMS (v/v)	75% soil/25% SMS (v/v)	100% soil
Electrical conductivity[z] ($dS \cdot m^{-1}$)				
Before germination period				
Saturated paste extract[y]	11.9	10.4	8.0	3.7
1 soil : 5 water (w/w) extract	11.2	1.9	0.9	0.3
1 soil : 2 water (v/v) extracts	NA[x]	4.2	2.1	0.5
After germination period				
Saturated paste extract (SME)	15.4	18.2	17.6	8.4
1 soil : 5 water (w/w) extract	13.0	3.1	1.9	0.6
1 soil : 2 water (v/v) extracts	NA[x]	6.9	4.3	1.2
Cations and anions in saturated paste extracts made before germination period ($meq \cdot L^{-1}$)				
NH_4^+	2.4	1.8	1.1	0.2
Ca^{2+}	24.0	24.3	24.3	19.0
Mg^{2+}	32.4	25.5	18.5	7.4
K^+	68.7	45.1	28.3	4.7
Na^+	13.5	11.6	10.3	8.1
Cl^-	51.7	36.2	31.8	11.4
NO_3^-	0.1	4.1	8.7	10.4
SO_4^{2-}	78.6	54.9	40.3	6.5
PO_4^{3-}	10.1	3.4	1.3	6.6

[z] Values for the saturated paste and 1:5 extraction methods before initiation of the germination period are means of two samples per soil/SMS mixture. Extracts of ratio 1:2 collected before the initiation of the germination period and all extracts taken after the germination period were made from one sample per soil/SMS treatment.

[y] The amounts of water required for saturation of the 100% SMS, 50% soil/50% SMS, 75% soil/25% SMS, and 100% soil treatments were 343%, 62%, 37%, and 22% of their respective dry weights.

[x] Not applicable; all water was absorbed by the SMS.

Table 3. Analyses of variance of FGP (arcsine-transformed values) and GR, expt. 1.

		Arcsine (FGP)		Germination rate	
	df	Mean square	*P* value[z]	Mean square	*P* value[z]
Block	2	0.099	NS	3.79	NS
Soil/SMS mixture	3	1.717	0.0001	223.84	0.0001
Error A (block × soil/SMS mixture)	6	0.025	—	2.01	—
Species	8	0.286	0.0001	33.77	0.0001
Soil/SMS mixture × species	24	0.027	0.0272	2.37	0.0032
Pooled error B (block × species + block × soil/SMS mixture × species)	64	0.015	—	0.98	—

[z] In this and the following tables, exact probability values are given for $P < 0.05$; otherwise, NS (nonsignificant) is indicated.

polyethylene glycol (PEG 6000; EMD Chemicals, Gibbstown, N.J.) with the same osmotic potential as in the SMS dilution treatments were prepared based on the equation

$$\psi_o = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T$$

where C is the concentration of PEG 6000 in grams per kilogram H_2O and T is the temperature in degrees Celsius (Michel and Kaufmann, 1973).

Expt. 1 showed that Kentucky bluegrass and perennial ryegrass represented extremes in response to SMS; thus, these were the only species included in expt. 2. Blotter papers were placed in Petri dishes and moistened with 5-mL distilled water (control) or the respective concentrations of SMS or PEG solutions before seeding samples of 100 seeds per dish. The Petri dishes were sealed with parafilm to avoid evaporation and were placed in an incubator set to a regime of 8 h light at 25 °C and 16 h darkness at 15 °C (International Seed Testing Association, 1996). Germinated seeds were counted and removed every day from day 4 until day 14 (both species) and every second day from day 15 until day 21 (Kentucky bluegrass only). A seed was considered germinated as soon as the coleoptile was visible. FGPs and GRs were calculated and subjected to analysis of variance. Orthogonal contrasts were calculated to compare germination in SMS extract versus PEG 6000, the effect of concentration of SMS or PEG 6000 (linear and quadratic effects), and their interactions.

Expt. 3: Germination of Kentucky bluegrass after leaching of 50% soil/50% SMS (v/v) mixture. A preliminary pot experiment showed that the ECe values of the 2-cm surface layer increased temporarily during the first week after incorporation of SMS unless daily watering exceeded the equivalent of 125% of daily evaporation. Hence, in Feb. and Mar. 2006 we conducted an experiment comparing 1) unleached 50% soil/50% SMS mixture with 50% soil/50% SMS mixtures that had been watered daily with 2) the equivalent of the daily evaporation, 3) 133% of evaporation, and 4) 167% of evaporation, for a 10-d period before seeding. During the leaching period, distilled water was added according to the daily weight of four random pots (average daily evaporation, 4.0 mm). After 5 and 10 d of leaching, samples for saturated paste extracts were pooled from the surface 2 cm from additional pots that were not seeded. Samples were also taken at the end of the experiment, 25 d after seeding.

The experiment was performed with Kentucky bluegrass 'Limousine'. During the germination period, pots were misted lightly three times a day to avoid drying of the soil surface. Germinated seeds were counted upon emergence and removed every day from day 6 to day 15 after seeding, and susequently every second day. The experimental design was a randomized complete block with four replicates for the treatments in the germination study and two replicates for soil analyses. FGPs and GRs were calculated and analyzed as described earlier.

Expt. 4: Effect of SMS on Kentucky bluegrass seedling emergence in the field. In early May 2006, the same SMS as used in the pot experiments was applied at two rates in a field experiment on a Hagerstown silt loam (fine, mesic Typic Hapludalfs) at the Joseph Valentine Turfgrass Research Center, University Park, Penn. The soil had a pH of 7.0, and P and K values (Mehlich 3 extracts) of 180 $mg \cdot kg^{-1}$and 264 $mg \cdot kg^{-1}$ respectively. The experimental design was a Latin square with plot size of 1 × 1 m, three replicates, and the following three treatments: 1) control, no SMS; 2) 280 m^3 (30,000 kg DW) SMS ha^{-1}; and 3) 560 m^3 (60,000 kg DW) SMS ha^{-1}.

In treatments 2 and 3, the SMS was tilled manually into the top 10 cm soil layer. After incorporation, and again after 6 d and 12 d, soil samples were collected from each plot and ECe values were determined (USDA, 1954). Kentucky bluegrass 'Limousine' was seeded at a rate of 1.0 kg $(100\ m^2)^{-1}$ just after the SMS had been incorporated. Starter fertilizer, 19–25–5 (The Scotts Company, Marysville, Ohio) was applied to the surface of all plots at a rate of 0.5 kg N $(100\ m^2)^{-1}$ before covering the plots with a geotextile cover. The experiment received 70 mm as irrigation and rainfall for 12 d after seeding. On day 12 (i.e., before any fertilizer effect of SMS on seedling growth was expected to have occurred), the geotextile cover was removed and seedling emergence was assessed on a visual scale from 0 to 9, where 9 is the best emergence. A 10 × 10-cm grid was placed over each plot and the number of seedlings was counted in eight random quadrates corresponding to a total area of 800 cm^2/plot.

Results

Expt. 1: Effect of SMS on germination of nine turfgrass species. The EC values of the SMS soil/mixtures varied according to the extraction method (Table 2). The saturated paste method resulted in the highest conductivities across treatments, followed by the 1 soil : 2 water (v/v) method and the 1 soil : 5 water (w/w) method. At the start of the

Table 4. FGP^{zy} and GR^{z} of colonial bentgrass (COL), creeping bentgrass (CRB), chewings fescue (CF), slender creeping red fescue (SlCRF), strong creeping red fescue StCRF, hard fescue (HF), sheep fescue (SF), Kentucky bluegrass (KB), and perennial ryegrass (PR) as affected by soil/SMS treatments.

	COL	CRB	CF	SlCRF	StCRF	HF	SF	KB	PR
FGP									
100% soil (control)	88 a^{x}	85 a	83 a	80 a	85 a	83 a	80 a	76 a	95 a
75% soil/25% SMS	68 b	77 ab	80 ab	75 b	77 a	71 ab	59 b	65 ab	84 a
50% soil/50% SMS	57 b	63 b	71 b	69 c	76 a	56 bc	59 b	54 b	90 a
100% SMS	34 c	34 c	52 c	55 d	54 b	32 c	18 c	9 c	78 a
P value	0.0001	0.0028	0.0042	0.0002	0.0050	0.0237	0.0010	0.0009	NS
GR									
100% soil (control)	12.5 a	11.6 a	10.4 a	10.7 a	11.1 a	10.4 a	9.2 a	8.6 a	13.1 a
75% soil/25% SMS	8.0 ab	9.9 a	9.2 a	9.2 b	9.1 b	8.1 a	5.9 b	6.7ab	11.1 ab
50% soil/50% SMS	5.8 b	6.9 b	7.2 b	7.7 c	8.4 b	5.6 b	5.6 b	4.9 b	10.4 bc
100% SMS	3.1 c	3.4 b	4.9 d	5.9 d	5.3 c	2.8 c	1.4 c	0.7 c	8.2 c
P value	0.0001	0.0017	0.0002	0.0002	0.0009	0.0012	0.0005	0.0009	0.0141

^{z}FGP = Σ n, GR = Σ (n/D), where n is the number of seeds that germinated at each counting and D is the number of days to that counting.

^{y}Analyses of FGP were performed on arcsine-transformed values.

^{x}Means followed by the same letter within columns are not significantly different according the Duncan multiple comparison test at $\alpha = 0.05$.

^{NS}Nonsignificant.

experiment, decreasing soil-to-SMS ratios increased EC values regardless of the extraction method. Because evaporation was replenished from the bottom rather from the top of the pots, the salinity of the top 2-cm layer increased during the germination period. In relative terms, this increase was most pronounced for treatments with a high soil-to-SMS ratio.

The dominating ions in the SMS were SO_4, K, and Cl (Table 2). Ca, Cl, and nitrate made the largest contribution to the ECe value in the control treatment. Compared with other ions, Na levels were low in all treatments. The sodium absorption ratios ranged from 2.2 in the 100% soil and 75% soil/25% SMS treatments to 2.3 in the 50% soil/50% SMS treatment and 2.5 in the 100% SMS treatment (data not shown). Ammonium contributed less than 1% of the total ion concentration in all soil/SMS mixtures.

Analyses of variance revealed significant treatment and species effects for FGP and GR. There were also significant treatment × species interactions for FGP and GR (Table 3). Incorporation of 25% SMS into the loamy sand reduced the FGP of colonial bentgrass and sheep fescue by 20 U or more compared with the controls (Table 4). In the 50% soil/50% SMS treatment, only strong creeping red fescue and perennial ryegrass were not significantly affected compared with the controls. The FGP values in 100% SMS were all significantly lower than those of the controls with the exception of perennial ryegrass. The FGPs of sheep fescue and Kentucky bluegrass were more than 60 U lower than in the controls.

As the amount of SMS increased in soil/SMS treatments, the GR of all species decreased (Table 4). As with the FGP data, colonial bentgrass was the most strongly affected species at 25% or 50% SMS in the mixture, whereas germination of sheep fescue was most retarded in the 100% SMS treatment. With respect to GR, perennial ryegrass was the least affected of all species.

The calculation of R-SMS-T values resulted in the following ranking of the species' ability to germinate in soils amended with high rates of fresh SMS: perennial ryegrass > slender creeping red fescue > chewings fescue = strong creeping red fescue > creeping bentgrass > hard fescue > Kentucky bluegrass > sheep fescue > colonial bentgrass (Fig. 1).

Expt. 2: Effect of equivalent osmotic potential created by SMS extract and polyethylene glycol on germination of perennial ryegrass and Kentucky bluegrass. SMS extract and PEG had no significant effect on the FGP of perennial ryegrass regardless of the osmotic potential (Table 5). In Kentucky bluegrass, FGP decreased linearly with decreasing osmotic potential for both SMS extract and PEG.

Highly significant linear reductions in GR occurred with increasing solute concentrations in both species. When averaged over concentrations, Kentucky bluegrass germinated faster in PEG than in SMS extract, but the opposite was true in perennial ryegrass.

Expt. 3: Germination of Kentucky bluegrass after leaching of the 50% soil/50% SMS (v/v) mixture. Daily watering corresponding to the daily evaporation loss did not prevent an increase in the ECe value of the 2-cm surface layer during the 10-d leaching period (Fig. 2). By contrast, ECe values were reduced in pots receiving 133% and 167% of the daily evaporation. During the germination period, ECe values converged in all treatments. The highest leaching rate caused a significant increase in GR compared with unleached pots or pots that had only been replenished with the daily evaporation rate (Table 6). The effect of leaching treatments on FGP was not significant.

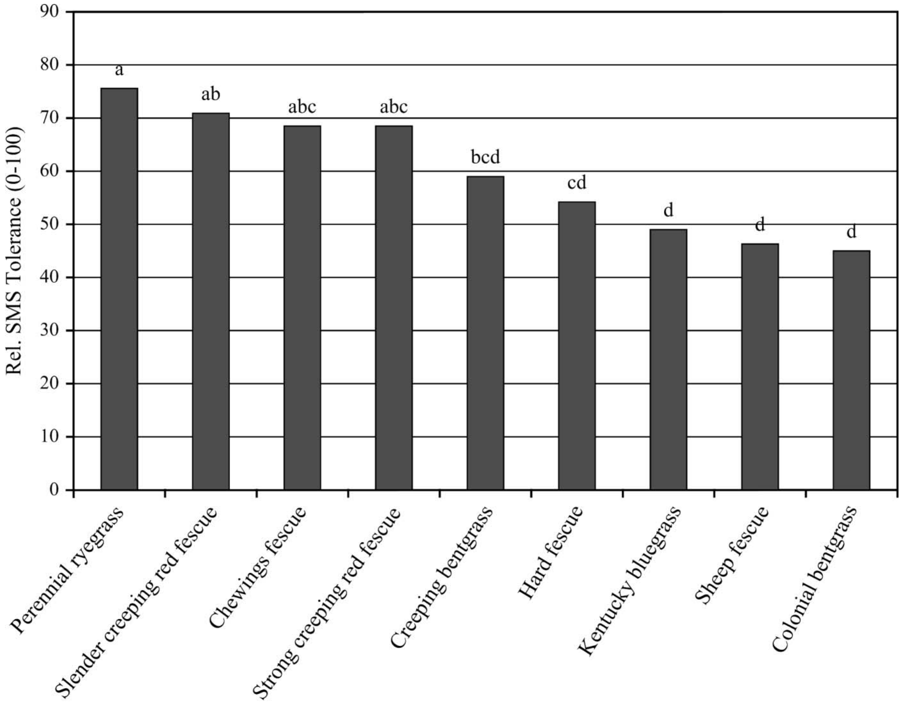

Fig. 1. Relative spent mushroom substrate (SMS) tolerance values calculated as the mean germination rate (GR) in soil/SMS treatments containing 25%, 50%, or 100% SMS relative to the GR in 100% soil. Bars with the same letters are not significantly different according to the Duncan multiple comparison test at $\alpha = 0.05$.

Table 5. FGP and GR as affected by decreasing osmotic potential created by SMS extracts or PEG (expt. 2).

		Perennial ryegrass		Kentucky bluegrass	
		FGP	GR	FGP	GR
Distilled water (control)		93 a[y]	18.8 a	92 a	13.7 a
SMS, ψ_o = –0.13 MPa (3.6 $dS\cdot m^{-1}$)[z]		92 a	16.8 b	88 abc	11.3 c
SMS, ψ_o = –0.26 MPa (7.3 $dS\cdot m^{-1}$)		93 a	15.6 c	85 bcd	9.8 d
SMS, ψ_o = –0.39 MPa (10.9 $dS\cdot m^{-1}$)		91 a	14.2 d	81 d	8.4 e
PEG 6000, ψ_o = –0.13 MPa		88 a	16.1 bc	89 ab	12.7 b
PEG 6000, ψ_o = –0.26 MPa		93 a	15.6 c	84 cd	10.4 d
PEG 6000, ψ_o = –0.39 MPa		91 a	12.8 e	80 d	8.9 e
Contrasts	*df*	*P* value			
Distilled water vs. solutes	1	NS	0.0001	0.0004	0.0001
SMS vs. PEG	1	NS	0.0324	NS	0.0009
Osmotic potential, linear effect	1	NS	0.0001	0.0003	0.0001
Osmotic potential, quadratic effect	1	NS	NS	NS	NS
Type of solute × osmotic potential	2	NS	NS	NS	NS

[z] ψ_o (MPa) = –0.036 × ECe ($dS\cdot m^{-1}$) (USDA, 1954).

[y] Means followed by the same letter within columns are not significantly different according the Duncan multiple comparison test at $\alpha = 0.05$.

[NS] Nonsignificant.

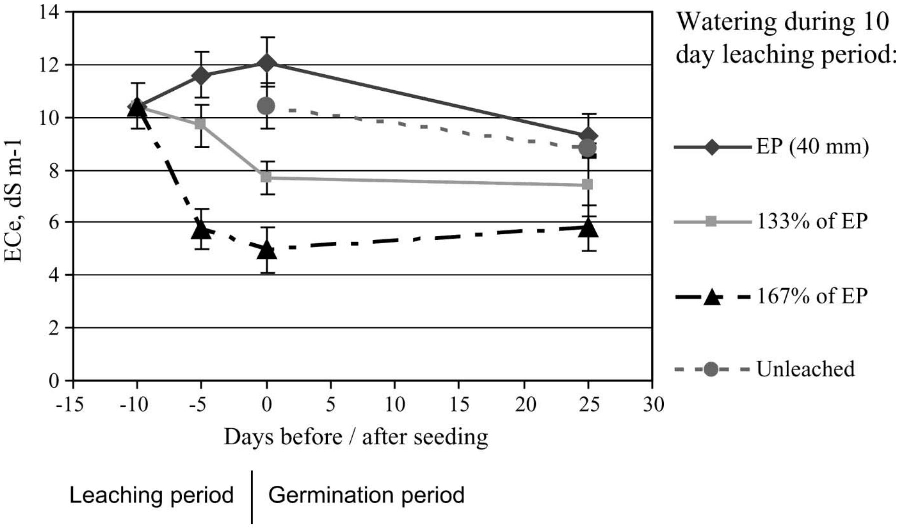

Fig. 2. Electrical conductivity in saturated paste extracts (ECe) from a 50% soil/50% spent mushroom substrate mixture during the leaching and germination periods; ±1 SE is indicated (n = 3 for samples taken at days –5 and 0; n = 3 for samples taken at day –10 and 25). EP, daily evaporation.

Table 6. FGP and GR of Kentucky bluegrass in a mixture of 50% soil/50% SMS (v/v) as affected by increasing daily leaching rates during the 10-d period before seeding.

Leaching treatment	FGP	GR
Unleached	61 a[z]	6.1 bc
EP (40 mm)	51 a	4.9 c
133% of EP	72 a	8.0 ab
167% of EP	70 a	8.2 a
P value	NS	0.0115

[z] Means followed by the same letter within columns are not significantly different according the Duncan multiple comparison test at $\alpha = 0.05$.

EP, replacement of evaporation only.

[NS] Nonsignificant.

Expt. 4: Effect of SMS on Kentucky bluegrass seedling emergence in the field. Incorporation of 2.8 m^3 (100 m^2)$^{-1}$ and 5.6 m^3 (100 m^2)$^{-1}$ increased the ECe value at the time of seeding from 0.5 to 2.9 $dS\cdot m^{-1}$ and 4.1 $dS\cdot m^{-1}$ respectively (Fig. 3). During the following 6 d, the ECe value decreased in plots amended with SMS, but increased in control plots; the latter was probably the result of the application of starter fertilizer after the first soil samples had been collected. The ECe value in all plots decreased from 6 to 12 d after seeding; to some extent this might be the result of a 28-mm rainfall event on day 8 after seeding. Neither visual assessment of seedling emergence nor seedling counts revealed any significant effect of SMS amendment on the germination of Kentucky bluegrass under field conditions (Table 7).

Discussion

The SMS from the PSU mushroom facility used in these experiments had lower ECe values than fresh SMS sampled from some other mushroom farms. We have analyzed samples of fresh SMS products with ECe values ranging from 11.2 to 22 $dS\cdot m^{-1}$. The chemical composition of the SMS from the PSU mushroom facility was mostly within the normal values for fresh SMS, as reported by Beyer (1999). Although the concentrations of aluminum and especially Fe in the SMS were relatively high, it is doubtful that this would have any negative impact on germination in a primarily organic medium with a pH of 6.5.

The results of expt. 1 demonstrated that SMS and mixtures of soil and SMS have the potential to inhibit germination of several cool-season turfgrass species. Based on the fact that the R-SMS-T values of the grass species tested were mostly consistent with the salinity tolerances established by Carrow and Duncan (1998) and Turgeon (2005), we conclude that that the inhibition was primarily the result of salts.

Ammonium toxicity may be one reason for poor germination and seedling growth in soils amended with high rates of manure-based composts, but its effect is often confounded with that of high salt concentrations. O'Brien and Barker (1996) incorporated up to 2300 $mg\cdot N\cdot kg^{-1}$ DW as either $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ to ammonium-depleted municipal solid waste and leaf composts with initial pH values of 7.1 and 7.2 respectively. Germination of perennial ryegrass was more retarded after application of $Ca(NO_3)_2$ than after application of $(NH_4)_2SO_4$, and this was attributed to a stronger and more lasting effect of $Ca(NO_3)_2$ than of $(NH_4)_2SO_4$ on EC. In a recent study conducted at the PSU, Stehouwer (pers. comm., Feb. 2006) found that the concentrations of NH_4-N ranged from 5 $mg\cdot kg^{-1}$ DW in an SMS to 5023 $mg\cdot kg^{-1}$ DW in a biosolid compost; however, despite the higher concentration of ammonium, germination of perennial ryegrass was faster with the use of a biosolid compost. The SMS used in our study was slightly acidic and with an NH_4-N concentration of 169 $mg\cdot kg^{-1}$ DW, which is in the middle of the range (60–240 $mg\cdot kg^{-1}$ DW) as reported by Beyer (1999) for fresh SMS. Such a low level is unlikely to have any toxic effect on turfgrass germination.

The physiologically inactive polymer PEG 6000 was included in expt. 2 in an attempt to distinguish between osmotic effects and toxicity of ions. The results indicate that the inhibitory effect of salinity on germination, incited by SMS, was primarily the result of osmotic effects. However, results indicated a slight, but significant, effect of SMS extract compared with PEG on the GR of Kentucky bluegrass, whereas the opposite appeared to be the case in perennial ryegrass. These results are in general agreement with the literature review by Ungar (1978), who summarized that specific ion effects are common in salt-sensitive species, but inconclusive in salt-tolerant species.

In all species examined in our experiments, salinity had a greater influence on the GR than on the FGP. Similar results have been reported by Camberato and Martin (2004), Marcar (1987), and Sun et al. (2000). Germination rate is an expression of seed vigor (i.e., a seed's ability to germinate under nonoptimal conditions, such as reduced water availability) (International Seed Testing Association, 1996). Larsen et al. (2004) found that seed of Kentucky bluegrass had a higher base water potential for imbibition

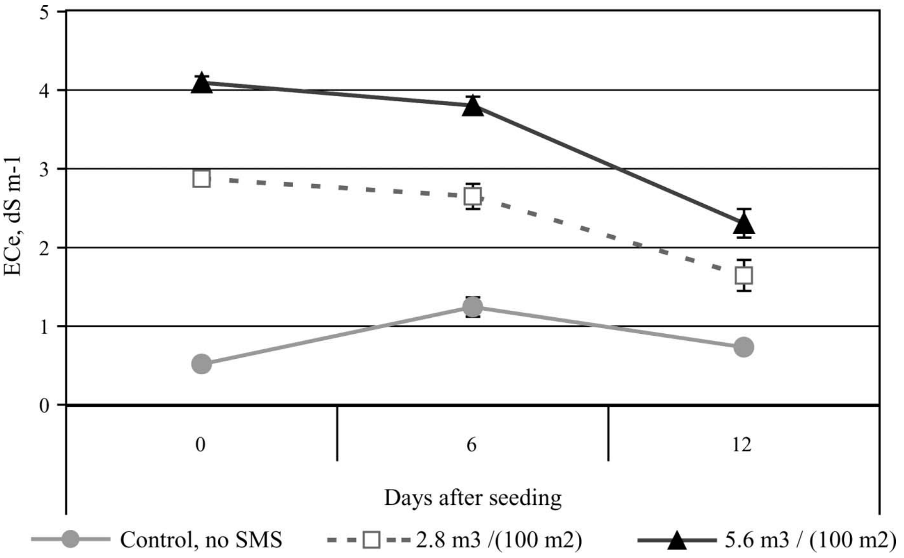

Fig. 3. Electrical conductivity in saturated paste extracts (ECe) after incorporation of various rates of fresh spent mushroom substrate (SMS) in a field experiment; ±1 SE is indicated (n = 3).

Table 7. Visual seedling emergence (range, 1–9; where 9 = best emergence) and seedling counts 12 d after seeding on plots amended with increasing rates of SMS.

SMS rate	Visual seedling emergence	Seedlings m^{-2}
Control, no SMS	8.3 a^z	2517 a
2.8 m^3 (100 m^2)$^{-1}$	7.7 a	1892 a
5.6 m^3 (100 m^2)$^{-1}$	8.7 a	2196 a
P value	NS	NS

^{z}Means followed by the same letter within columns are not significantly different according the Duncan multiple comparison test at α = 0.05.

^{NS}Nonsignificant.

than seed of perennial ryegrass and red fescue. Lowering the soil water potential from 0 to –0.8 MPa increased the time to 50% germination by 34 d, 10 d, and 6 d in Kentucky bluegrass, perennial ryegrass, and red fescue respectively. The reason why GR was less affected by salinity in perennial ryegrass than in red fescue in expt. 1 may be that specific ion toxicity occurred only in red fescue. Sanda (1978) found perennial ryegrass to be more salt tolerant than red fescue during germination, but the opposite was true for mature plants of these two species.

Expt. 3 showed that germination inhibition resulting from additions of SMS to soil can be ameliorated by leaching the amended soil before seeding. However, the experiment was not designed to determine the exact leaching requirement of the 50% soil/50% SMS (v/v) mixture. The fact that both FGP and GR were similar in treatments receiving 133% and 167% of evaporation might indicate that the lower salt concentration resulting from a higher irrigation volume was countered by adverse effects resulting from lack of oxygen. Along this line, Roberts et al. (2001) reported that excessive leaching before seeding reduced the germination of creeping bentgrass in a biosolid waste medium.

During the course of expt.1, replenishment of water from the bottom rather than from the top of the pots caused a gradual increase in the salinity of the germination layer. This was probably more detrimental to slowly germinating species such as Kentucky bluegrass and sheep fescue than to a quickly germinating species such as perennial ryegrass. It is noteworthy that the highest accumulation of salts did not occur in the 100% SMS treatment, but in mixtures of soil and SMS. Under field conditions, soils with a low cation exchange capacity but a high capillary capacity will probably be most susceptible to salt accumulation in the germination layer after incorporation of SMS.

Unlike the soil used in the pot experiments, the soil used for the field experiment (expt. 4) had a very low salt concentration before incorporation of SMS. Even with the highest rate of SMS, the ECe of the amended soil never exceeded 4.1 dS·m^{-1}, which is comparable with the lowest ECe value used in expt. 2. With such a low salinity level, it is not surprising that seedling emergence of Kentucky bluegrass was unaffected.

Although there was no significant effect of SMS on seedling emergence in the field experiment, this research has, as a whole, shown that high rates of fresh SMS used for soil amendment represents a potential problem for turf seed germination under certain conditions. The risk for delayed or inhibited germination will be greatest when high amounts of SMS are blended with soil and used as topsoil, or when high amounts of SMS are tilled into soils that already have a potential salinity problem. In such situations, SMS rates should be low, and perennial ryegrass should be used instead of salt-sensitive species such as colonial bentgrass, sheep fescue, and Kentucky bluegrass. Because there is considerable variation in salinity levels of SMS from various mushroom farms, EC values should always be determined before using SMS for soil amendment of turfgrass areas.

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