# Effect of seed treatment on germination and emergence of 3 warm-season grasses

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#### Abstract

Seed dormancy can hinder stand establishment and delay progress of genetic and plant improvement studies. Our objective was to evaluate the effect of 2 chemical seed treatments on germination and on emergence and survival of 3 warm-season grasses. Freshly harvested seed of kleingrass (Panicum coloratum L.) weeping lovegrass [Eragrostis curvula (Schrad.) Nees] and wilman lovegrass (Eragrostis superba Peyr.) were treated with concentrated H<sub>2</sub>SO<sub>4</sub> or a 2-chloroethanol sodium hypochlorite (CHL) solution and then germinated on blotters or planted in a commercial peat-vermiculite mix. The 3 grasses responded differently to acid and CHL treatment. Acid treatment increased germination of all 3 species but did not increase the emergence and/or 2 week establishment of weeping or wilman lovegrass. The difference between germination and emergence appeared related to chemical injury from the acid treatment that adversly impacted seedling growth and development. Weeping lovegrass responded to CHL treatment with increased germination and emergence. Wilman lovegrass did not respond well to either chemical.

## Key Words: weeping lovegrass, wilman lovegrass, kleingrass, seed dormancy, establishment, sulfuric acid, 2 chloroethanol

Seed dormancy is widespread in warm-season grasses and can contribute to establishment and stand persistence when a reserve of dormant seed preserves the opportunity for plant establishment over time (Voigt et al. 1987). Excessive levels of seed dormancy, however, can hinder successful stand establishment when rapid germination of large numbers of seed is desired. Post-harvest seed dormancy can also hinder genetic research by increasing time required for selection cycles and delaying evaluation studies.

Acid treatment, to reduce levels of seed dormancy, has been used in both legumes (Townsend and Miklas 1987) and grasses (Fulbright 1987, Jones 1990). We needed to produce seedlings of wilman lovegrass (*Eragrostis superba* Peyr.), for progeny evaluation of winter hardiness, from seed lots that had been harvested about 90 days previously. Preliminary germination tests indicated high levels of post-harvest dormancy. We found that scarification with concentrated sulfuric acid increased germination of dormant wilman lovegrass seed to near-maximum levels. We observed, however, that following transplanting from petri dishes to flats containing commercial potting mix, most of the seedlings failed to develop beyond the 1 or 2 leaf stage and then died. Because of its excellent establishment characteristics (Tischler and Voigt 1983a, 1983b) wilman lovegrass usually transplanted with close to 100% survival.

We hypothesized that our failure to obtain adequate numbers of wilman lovegrass seedlings was caused by the acid treatment that had promoted germination. Our objectives were to evaluate the effects of acid treatment duration on germination and emergence of wilman and weeping lovegrass [*Eragrostis curvula* (Schrad.) Nees] and to reevaluate our earlier work with kleingrass (*Panicum coloratum* L.) (Tischler and Young 1983). We also wanted to evaluate chloroethanol, an alternative chemical treatment for breaking seed dormancy (Burton 1969).

### **Materials and Methods**

Seed of 'Selection 75' kleingrass, 'Ermelo' weeping lovegrass, and 'Palar' wilman lovegrass were harvested by hand at Temple, Tex. during late May to mid June 1993. Inflorescences of weeping lovegrass containing mature seed were cut from the plants and stored in paper sacks to air-dry. They were processed by hammermilling, hand screening, and air-column separation to obtain clean caryopses. Kleingrass seed were collected by bending inflorescences over a beaker and tapping them against its side or by rubbing inflorescences between the fingers. Air-column separation was used to clean samples. Wilman lovegrass seed were obtained by hand stripping ripe spikelets from the inflorescences and processing the seed as described for weeping lovegrass. Seed were stored at about 25° C and 40% relative humidity until used.

### **Germination Studies**

Six replicates of 100 seed each were placed on moistened germination blotters in plastic petri dishes and germinated at  $35/25^{\circ}$  C (12 hours thermo- and photoperiod) using a randomized complete block design. Photosynthetic photon flux density at the surface of the petri dishes was about 5 µmol m<sup>-2</sup> s<sup>-1</sup>. Germination, defined as appearance of both a shoot and a root, was counted at 3, 6, 10, and 14 days after the start of the trials. Seed that did not germinate were assumed to be dormant.

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## **Emergence Studies**

Six replicates of 100 seed each were planted in 3 rows (33 or 34 seed per row) at a depth of 1 cm each replicate in a separate 9cm square pot containing commercial peat-vermiculite potting mix. Pots were watered to capacity, allowed to drain, and placed in a randomized complete block design in the same germinator as the petri dishes. Number of seedlings emerged were counted on the same days as the germination counts. Seed that did not produce seedlings were considered either dormant or unable to produce viable seedlings. After 14 days the seedlings with 3 or more leaves were harvested by cutting them off at the media surface. Seedlings with less than 3 leaves were not growing normally and were producing no new leaves, the first 2 leaves are present in the embryo. Such seedlings invariably died. The seedling tops were oven dried and weighed. Experiments were started on 11 June (kleingrass), 18 June (weeping lovegrass), and 2 July (wilman lovegrass) 1993.

### Seed Treatments

Length of acid treatment, based on preliminary studies, was varied with the species. Treatment times were 1, 2, and 4 min; 2, 4, and 8 min; and 5, 10, and 15 min for weeping and wilman lovegrass and kleingrass, respectively. Acid treatments were administered by adding reagent grade concentrated  $H_2SO_4$  (17.8 M) to the seed in a test tube. After the prescribed treatment time, seed were tapped from the test tube into a tea strainer where they were thoroughly rinsed under a stream of cold water. A solution of 1 M sodium bicarbonate was then poured through the seed mass to neutralize any remaining acid. After 1 min, the seed were rinsed again with cold water and spread thinly on dry paper towels to facilitate rapid drying.

The chloroethanol (CHL) treatments were identical for all grasses. Prior to germination the seed were soaked for 1 hour in a solution of 2-chloroethanol and 67 mM (0.525%) sodium hypochlorite in water. Concentrations of CHL were 0.6 M (4%), 1.2 M (8%), or 2.4 M (16%). All seed were air dried prior to use.

Control seed were untreated. In previous studies with kleingrass (Tischler and Young 1983) and preliminary studies with weeping lovegrass, soaking seed in water had little effect on germination. Because of this previous experimentation we saw little need to include a short-term water soak and rinse as an additional seed treatment.

Kotowski's coefficients of velocity (Scott et al. 1984) were calculated as an estimate of speed of germination or emergence.

 $CoV = 100 (\Sigma N_i / \Sigma N_i T_i)$ 

where N is the number of seeds germinated or emerged on day i and T is the number of days from sowing.

Prior to analysis all germination and emergence data were arcsin transformed (Little 1985); however, actual percentages are presented. Data were analyzed by analysis of variance (ANOVA) procedures. As part of the ANOVAs the quantitative variables, concentration (chloroethanol) or time (acid) were divided into single degree of freedom linear and quadratic components in order to determine if response to increasing levels of treatment were linear, curvilinear, or not signifcant (P < 0.05).

## **Results and Discussion**

Kleingrass responded to increasing length of acid treatment with increased total germination percentage, faster coefficients



Fig. 1. Effect of  $H_2SO_4$  treatment on germination, speed of germination (CoV), emergence and seedling weight of Selection 75 kleingrass.

of velocity (CoV) of germination, and increased emergence (Fig. 1). We believe the decreased seedling weight observed with increasing acid treatment of kleingrass was caused at least in part by the crowding of seedlings in the small pots. For the 15 min acid treatment an average of 69 seedlings were growing in about 27 cm of row length. The vigor and health of the seedlings from the 15 min treatment is indicated also by the percentage of emerged seedlings having 3 leaves, 95% compared to 69% for the control. The response across all acid treatments was linear (P < 0.05 from ANOVA).

Increasing concentrations of chloroethanol (CHL) also increased germination and emergence of kleingrass, but the maximum response was obtained with the intermediate 1.2 rather than the maximum 2.4*M* concentration (Fig. 2). The response of CoV of germination to CHL concentration, however, was linear. Seedling weight was not significantly affected by CHL concentration (P < .05). With CHL treatment the maximum seedling emergence was 32%, half that of the best acid treatment.

These results are in general agreement with those of Tischler and Young (1983). The germination and emergence data both support the conclusion, that acid treatment is an effective method of breaking kleingrass seed dormancy and that CHL is less effective than acid. On a population basis, our data do not support the conclusion that acid treatment adversely affects germination, emergence, or initial seedling growth of Selection 75 kleingrass. Other populations of kleingrass might respond differently (Tischler and Young 1983).

If evaluation of acid treatment had been based on germination and CoV of germination we could have concluded that acid treat-



Fig. 2. Effect of chloroethanol treatment on germination, speed of germination (CoV), emergence and seedling weight of Selection 75 kleingrass.



Weeping lovegrass germination and emergence responded well to low concentrations of CHL treatment, reaching a maximum germination and emergence of 99% (Fig. 4). The highest concentration, however, resulted in a marked decrease in performance. In contrast to kleingrass, seedling weight was increased slightly by the lower concentrations of CHL treatment (P < 0.05).

In contrast to acid treatment, more than 99% of all CHL- treatment seedlings survived until the end of the study, and 90 to 99% of these had 3 leaves. The CHL treatment was very effective in maximizing germination and emergence of the apomictic Ermelo weeping lovegrass genotype. We also found a 0.6 *M* rate very effective for breaking dormancy of other genotypes of *E. curvula* and of *E. lehmanniana* Nees (Voigt, et al., 1996).

Dormancy of fresh seed of wilman lovegrass was very high, with only 2% of untreated seed germinating or emerging (Fig. 5). Acid treatments of 2 or 4 min provided large increases in germination, with a maximum germination of 76%. An 8 min treatment, however, resulted in essentially no germination. Although emergence was increased from 2 min of acid treatment few of those seedlings survived. After 14 days the control treatment had 2% of seed resulting in seedlings and the 2 min acid treatment



Fig. 3. Effect of  $H_2SO_4$  treatment on germination, speed of germination (CoV), emergence and seedling weight of Ermelo weeping lovegrass.



Fig. 4. Effect of chloroethanol treatment on germination, speed of germination (CoV), emergence and seedling weight of Ermelo weeping lovegrass.





Fig. 5. Effect of  $H_2SO_4$  treatment on germination, speed of germination (CoV), emergence and seedling weight of Palar wilman lovegrass.

only 3% (not shown). The weight of the seedlings from the acid treatment was also clearly inferior to that of the control (Fig. 5).

Germination increased to a maximum of 28% at the highest concentration of CHL, but speed of germination and emergence was higher at the lower CHL concentrations (Fig. 6). Despite a wide range in mean performance an effect of CHL treatment on seedling weight was not detected (P < 0.05).

Neither acid or CHL treatments were very effective in producing seedlings from dormant seed of wilman lovegrass. The relatively high viability of the wilman lovegrass seed was demonstrated by the acid treatment, but the damage from this treatment precluded survival of most of the embryos that germinated. The CHL treatment increased the germination and emergence of wilman lovegrass, but the majority of the dormant seed failed to germinate.

## Conclusions

The 3 warm-season grasses responded differently to  $H_2SO_4$ and chloroethanol (CHL) dormancy-breaking seed treatments. Acid treatment increased germination to high levels in all species but adversely affected emergence in weeping and wilman lovegrass. Because there was no indication of disease, e.g., damping off, in either these studies or in the preliminary observations on death loss following acid treatment of wilman lovegrass, we believe the adverse effect of acid-treatment are not from increased incidence of disease as reported for Indian ricegrass (Plummer and Frischknecht 1952, McDonald 1976), but from

Fig. 6. Effect of chloroethanol treatment on germination, speed of germination (CoV), emergence and seedling weight of Palar wilman lovegrass.

direct damage to the seed. Acid treatment in Indian ricegrass resulted in delayed protein synthesis, reduced protein levels and increased leaching of electrolytes while still promoting germination (McDonald and Khan 1983).

These experiments also demonstrate that germination studies of dormancy breaking treatments employing acids may not be predictive of seedling emergence or survival. Similarly, Plummer and Frischknecht (1952) found that the length of acid treatment required to produced maximum emergence of Indian ricegrass from soil were shorter than those required for maximum germination.

Human toxicity of CHL, also known as ethylene chlorohydrin, (Specher et al. 1960) limits its use to laboratory environments where human exposure can be controlled.

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