Effect of Separating Giant Foxtail (Setaria faberi) Seeds from Soil Using Potassium Carbonate and Centrifugation on Viability and Germination

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Abstract
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Disciplines
Agricultural Science | Agriculture | Agronomy and Crop Sciences

Comments
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Effect of separating giant foxtail (Setaria faberi) seeds from soil using potassium carbonate and centrifugation on viability and germination

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Changes in weed seedbank composition are often monitored by removing seeds from soil samples. One extraction method accomplishes this by creating a slurry of soil and a concentrated inorganic salt solution. Centrifugation is then used to separate constituents of differing densities. We have found that centrifugation of giant foxtail seeds in 3.2 M potassium carbonate solution as conducted in a centrifugation/flotation extraction method can reduce viability as measured by germination and tetrazolium tests. In one experiment, centrifugation/flotation separation reduced germination of giant foxtail seeds from 94 to 52%. The likely cause of seed damage was the high pH of the potassium carbonate solution in conjunction with the increased hydrostatic pressure due to centrifugation. While centrifugation affected quantitative measures of seed viability, it did not alter qualitative viability estimates using a pressure test.

Nomenclature: Giant foxtail, Setaria faberi Herrm. SETFA.

Key words: Weed seed bank, seed extraction, SETFA.

Estimation of the seed content of a soil sample is required for many vegetation monitoring projects, including treatment effects of weed management systems, decision aid models, and seasonal seedbank population dynamics. This estimation is invariably accomplished by direct germination from the soil sample or physical extraction of the seeds combined with a viability assessment. The relative merit of these methods has been the subject of much discussion (Cardina and Sparrow 1996; Gross 1990; Roberts 1981). The benefits of physical extraction include increased speed of assessment, better control of the germination environment, and recovery of dormant and dead seeds. Quantification of nonviable seed material may potentially provide information about seed fate. However, the presence of nonviable seed material makes hand sorting tedious and requires an arbitrary standard for acceptable damage (Roberts 1981).

Labor is saved in testing viability if the selection process excludes most of the nonviable material. One commonly used method that provides a standard for seed selection is the pressure or forceps test. This procedure involves subjecting a seed to gentle forceps or dissecting needle pressure to determine whether it is firm. This selection process, though based on the nonquantified standard of “firmness,” is often rigorous enough to be used as a nonlaborious approximation of viable seed number (Ambrosio et al. 1997; Ball and Miller 1989; Cardina and Sparrow 1996; Forcella 1992; Roberts and Ricketts 1979; Schreiber 1992). Comparisons of the forceps method with other methods of viability assessment have shown the accuracy of the forceps method (Rothrock et al. 1993).

The procedures used to separate seed material from soil invariably alter the seed environment for some period. This environmental exposure has the potential to affect seeds. At present we can only detect gross changes, such as altered germination behavior or death. Many studies only require estimates of viable seed density. In these instances, showing that a separation method does not affect gross viability is sufficient. Studies attempting to draw significance from germination behavior of extracted seeds, however, are faced with the more difficult task of proving that separation does not alter seed germinability.

Determining that seed separation does not alter viability is usually accomplished by subjecting seeds of known viability to the removal process and documenting changes. Extraction techniques involving concentrated solutions of inorganic salts are often used to separate seeds (Roberts 1981). Such solutions are also known to affect seed viability under certain circumstances (Kropac 1966). Electrolyte solutions of high pH are also known to alter germinability and viability at certain concentrations and exposure times, as Hou and Simpson (1994) showed for wild oat (Avena fatua L.).

In their original methodological work on potassium carbonate centrifugation (PCC) extraction, Buhler and Maxwell (1993) tested the effect of exposing seeds (including giant foxtail) to 3.2 M potassium carbonate. They found that for exposure times of less than 0.5 h, seed germination was unaffected by exposure to the salt solution. Therefore, they concluded that the extraction method did not decrease seed viability. However, the effect of centrifugation in the presence of the salt solution, which more closely approximates the experimental conditions of the extraction process, was not tested. Anomalous results from giant foxtail seeds obtained using this extraction procedure (Dekker et al. 1997) led us to further question the influence of the salt solution on seed viability.

The purpose of this investigation was to document and quantify the potential for viability loss in giant foxtail due to the PCC extraction process, to determine the likely mechanism by which viability was compromised, and to speculate what effect this would have on the estimate of viable seed number.

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Materials and Methods

Seed Extraction from Field Soil

Tests on soil samples collected at the Agronomy and Agricultural Engineering Research Center near Ames, IA, were conducted in December 1996. The soil was Webster clay loam (Typic Haplaquolls), pH 6.8 and 4.5% organic matter. The area had been cropped to soybean [Glycine max (L.) Merr.] the previous growing season and managed to create a heavy infestation of foxtails (Setaria spp.). Samples were collected from four 1-m² plots within a randomized complete block design in a 10- by 34-m area with treatments assigned for repeated measure of the seed bank. Fifteen soil cores (5 cm diam by 10 cm deep) were randomly collected from each plot. Samples were composited by plot and transported to the laboratory for immediate analysis.

One subsample of soil from each plot was processed according to the protocol of Buhler and Maxwell (1993), and another was wet-sieved over a 1-mm mesh (#18) screen. The latter was accomplished by rinsing the soil with distilled water. Residues from both extraction methods were then dried for 16 h at 35 °C in small mesh screens, cleaned with a seed blower and sorted under a ×10 dissecting microscope. Seeds were subsequently placed into 5-cm diam glass petri dishes (20 seeds/dish) on a single disk of germination paper1 wetted with 2 ml distilled water and sealed with plastic film. Germination conditions were 26 °C under constant fluorescent light at 150 µmol m⁻² s⁻¹. Germination, defined as radicle extension of 5 mm, was recorded after 10 d.

Effect of Potassium Carbonate and Centrifugation on Seed Behavior

The seeds used in these experiments were from two collections: one highly germinable and one highly dormant. Both lots were collected from the Iowa State University Curtis Farm in Ames, IA, in fall 1991 and 1996, respectively. They were stored at 4 °C until use. The first experiment was conducted on the highly germinable seeds in December of 1996. Seeds were randomly divided into sets of 60 and treatments replicated three times. The controls were subjected to the previously described germination test without salt exposure or centrifugation. Treatments included seeds exposed to the potassium carbonate solution without centrifugation; seeds centrifuged in distilled water at 9,450 × g (10,000 rpm); seeds centrifuged in 3.2 M potassium carbonate solution at 9,450 × g; and seeds centrifuged in potassium carbonate solution at 9,450 × g with 100 g of giant-foxtail-free Webster clay loam soil. The treatment solutions were placed in 250 ml centrifugation bottles with 75 ml of either 3.2 M potassium carbonate solution or distilled water. The bottles were then shaken for 3 min on an orbital shaker according to Buhler and Maxwell (1993) before centrifugation. After treatment, seeds were rinsed with distilled water and allowed to dry at ambient laboratory conditions for 24 h. Germination was then tested as described above.

A second experiment was conducted on both the highly germinable and dormant seed lots in June 1997. The purpose was to determine if the harmful effects of the extraction process decreased with decreasing centrifugation force and if the germinability state of the seeds affected the response.

Table 1. Germination of giant foxtail seed from the field seed bank after using two extraction methods.

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Seeds tested</th>
<th>Germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium carbonate centrifugation</td>
<td>160</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Wet sieving</td>
<td>320</td>
<td>40 ± 2</td>
</tr>
</tbody>
</table>

The experiment was conducted in triplicate, each experimental unit consisting of 60 randomly chosen seeds from each seed lot. Controls consisted of seeds that were placed in centrifugation bottles with 75 ml 3.2 M potassium carbonate, shaken for 3 min on the orbital shaker, and rinsed after another 10 min exposure. The remaining treatment units were centrifuged at 95, 2,360, or 9,450 × g (1,000, 5,000, and 10,000 rpm, respectively) for 10 min.

Following centrifugation, seeds were rinsed with distilled water and allowed to dry at ambient laboratory conditions for 24 h before being subjected to a germination test. Germination tests were carried out in 5-cm diam petri dishes lined with a disk of germination paper that had been soaked in water for 5 to 10 min and pressed lightly between two absorbent towels for approximately 2 sec. Twenty seeds were placed in each petri dish and the dishes were sealed with plastic film and placed in a germination cabinet with 20/30 C, 12/12 h coincident thermal and light (150 µmol m⁻² s⁻¹) cycle for 10 d.

Seeds were considered germinated upon radicle emergence. Seeds that did not germinate were classified as decayed if they did not pass a forceps pressure test at the end of the germination period. Remaining seeds were split and placed in a 0.1% tetrazolium (TZ) solution in the dark at room temperature for 24 h. Seeds that did not stain at all were deemed to be dead. Seeds that stained were classified TZ-viable or TZ-nonviable by the “evaluation group B: large seeded grasses” standard (Association of Official Seed Analysts 1970). The determination of whether a seed is TZ-viable or nonviable is based on a visual standard of staining patterns. Total viability was calculated as the sum of germinable and TZ-viable fractions and is hereafter referred to as percent viability.

Data were subjected to analyses of variance and means were compared by paired t tests and presented as the mean and standard error of three 60-seed replicates.

Results and Discussion

The PCC extraction process decreased the germinability of giant foxtail seeds obtained from field soil compared with wet sieving (Table 1). Hand-washed seeds germinated 40%, while the PCC samples germinated 14%. This result was in qualitative agreement with preliminary investigations that had shown an even larger reduction in germination. It might be argued that the two methods extract a different (biased) sample from the soil. However, visual inspection of the soil following the extraction showed no evidence of remaining seeds.

Exposure to potassium carbonate without centrifugation did not affect germination of the germinable giant foxtail seeds (Figure 1) compared with controls, which was consistent with the results of Buhler and Maxwell (1993). Addi-
tionally, centrifugation in distilled water reduced germination relative to potassium carbonate controls (P = 0.001), but not dry controls (P = 0.054). The PCC methods without soil caused a decrease in germinability from 94 to 33%. The addition of 100 g of soil, as would be the case in a PCC extraction, mitigated some of this effect, but germinability was still reduced to 52%.

Increasing centrifugation force in the presence of potassium carbonate progressively reduced germination of giant foxtail seeds from the germinable lot (Table 2). At 9,450 × g, the force used in the PCC process, centrifugation resulted in approximately 25% dead seeds. A further 1% decayed during the germination test, 12% were rendered TZ-non-viable, and 22% became TZ-Only. Only 40% of the seeds remained germinable following the 9,450 × g treatment compared with 93% for noncentrifuged controls. Total viable seeds decreased from 94% for the control to 62% following centrifugation at 9,450 × g. These deleterious effects diminished progressively as the centrifugation force was decreased (Table 2). At 95 × g, seeds were not affected.

The viability status of dormant giant foxtail seed was also changed when centrifuged with potassium carbonate (Table 2). After the 9,450 × g treatment, 23% of the seeds were dead compared with no dead seeds at lower centrifugation forces. TZ-viable seed decreases from 92% without centrifugation to 66% following centrifugation at 9,450 × g. Percent germination of the dormant seeds was not affected by centrifugation. In contrast to the germinable seeds, the dormant seeds were not affected by centrifugation at 2,360 × g or less.

The total percent viability (germinated plus TZ-viable) did not differ between germinable and dormant seeds (P < 0.05) at each centrifugation dose (Table 2). In addition, the general response to PCC extraction was similar between the field soil and the controlled experiments. This consistency suggests that the deleterious effects are probably not due to some peculiarity of the seed lots used.

A 3.2 M potassium carbonate solution results in a pH (12.6), potassium ion concentration (6.4 M), osmotic potential, and carbon dioxide levels very high relative to anything seeds would normally experience. Undoubtedly, the durable hull of the foxtail seeds could mitigate some of these effects for short exposure times. As originally reported (Buhler and Maxwell 1993), exposure to the potassium carbonate solution was tolerated for up to 0.5 h without a decrease in germination if the seeds were not centrifuged.

Centrifugation, however, probably increases the contact between sensitive seed tissues and the hostile medium by increasing the hydrostatic pressure surrounding the seeds and thus accelerating imbibition. This is supported by the fact that deleterious effects diminished as the force decreased. The very reason that centrifugation is used is that it shortens the seed exposure time to the caustic salt solutions relative to previous techniques (Buhler and Maxwell 1993; Malone 1967). Apparently, the beneficial effects of decreased exposure time are negated by centrifugation.

The magnitude of the centrifugation effect in soil sample processing was decreased by the presence of soil. It is likely that the soil buffers some of the caustic properties of the solution. Supporting this notion, the pH of the supernatant in soil processed samples was 11.4 after centrifugation compared with 12.6 for the original flotation solution.

It is of practical importance, however, that no seeds affected by the centrifugation treatments lost their "apparent viability" under the forceps pressure test. For workers concerned only with giant foxtail viable seed densities and not the behavior of extracted seeds, the use of the easily conducted pressure test avoids the artifacts of the PCC process.

The assessment of seed density, viability, or germinability by extraction of seeds from soil samples is complicated by many potential sources of artificial variability. These include seed recovery efficacy of the extraction procedure, potential seed damage during extraction, the criteria for acceptable seed damage, and viability determination procedures. These artificial sources are often hard to detect because the natural variability among samples may be quite large. Where the effects consistently bias samples, such determinations are more easily accomplished. In this paper we have documented how the PCC method of extraction can influence viability estimates. The effects are quite large and probably restrict those using this method for giant foxtail seeds to a pressure-based determination of seed viability. Additional research is needed.

<table>
<thead>
<tr>
<th>Table 2. Effect of centrifugation force in the presence of potassium carbonate solution on germination and viability of germinable and dormant giant foxtail seed.</th>
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</thead>
<tbody>
<tr>
<td>Centrifuge force</td>
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<tr>
<td>g</td>
</tr>
<tr>
<td>Germinable seed</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>95</td>
</tr>
<tr>
<td>2,360</td>
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<tr>
<td>9,450</td>
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<tr>
<td>Dormant seed</td>
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<tr>
<td>0</td>
</tr>
<tr>
<td>95</td>
</tr>
<tr>
<td>2,360</td>
</tr>
<tr>
<td>9,450</td>
</tr>
</tbody>
</table>

a Decayed seeds did not pass a forceps pressure test. Dead seeds were intact but did not stain with TZ. TZ-viable - nonviable seeds were differentiated using the visual standard of Association of Official Seed Analysts (1970).

b Means in the same column within a seed lot followed by the same letter do not differ based on paired t tests at P < 0.05.

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search is needed to determine how this extraction method affects seeds of other species.

**Sources of Materials**

1 Anchor Paper, 480 Broadway Street, St. Paul, MN 55103.
2 IEC model B-22M centrifuge with a fixed angle rotor #877, International Equipment Co., 300 Second Avenue, Needham Heights, MA 02194.

**Acknowledgments**

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**Literature Cited**


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