Dixon Prairie, Chicago Botanic Garden. Photo by Robin Carlson, Chicago Botanic Garden

Evaluating seed viability in prairie forbs: a test of three methods

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ABSTRACT

A fundamental aspect of establishing native plant species in restorations is ensuring that the seed used is viable. We test whether seed viability estimates for wild-collected seed of 4 forb species native to prairie habitat differ when using 3 methods: 1) germination, 2) tetrazolium, and 3) X-ray. Study species include *Eryngium yuccifolium* Michx. (button eryngo [Apiaceae]), *Lespedeza capitata* Michx. (roundhead lespedeza [Fabaceae]), *Liatris aspera* Michx. (rough prairie blazing star [Asteraceae]), and *Ratibida columnifera* (Nutt.) Woot. & Standl. (upright prairie coneflower [Asteracae]); multiple populations of each species are used to compare seed collected in the same year from different populations. Additionally, we test whether seed pretreatment (gibberellic acid or scarification) could improve germination estimates of viability by overcoming seed dormancy. Results show that viability estimates do not significantly differ by testing method for 3 of our 4 study species, with the exception being *E. yuccifolium*. We suspect that *E. yuccifolium* may have yielded different results for each viability testing method because its pretreatment was not enough to break dormancy, among other factors. Pretreatment yielded significantly higher viability estimates in *L. capitata* and in one population of *L. aspera* and *E. yuccifolium* in the germination study. These results confirm that restoration practitioners should calculate seeding rates based on viability measures on a per-species and per-accession basis. For many species, the method used to calculate viability can be determined by available equipment and expertise, but for species with unknown dormancy requirements, or those that may lose viability when stored, multiple methods may be needed.

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grassland, tetrazolium, germination, X-ray, seed quality, Apiaceae, Fabaceae, Asteraceae

NOMENCLATURE

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The prairie ecosystem, once the dominant landscape of the midwestern US, has now been reduced to 1% of its original land area (Robison and others 2013). To counteract this extreme habitat loss, prairie restorations are occurring across the Midwest, each with a unique set of management practices that affect the diversity and structure of the restored community (Ehrenreich and Aikman 1963; Larson and others 2011). Prairie restorations generally have lower species richness than do remnants (Sluis 2002) and are particularly bereft of native forbs (Dickson and Busby 2009). Forbs play an important role in providing ecosystem services, like supporting pollinator communities and fixing nitrogen (Chapin and others 2000; Isbell and others 2011), and research is ongoing on how to ensure they are a component of restored habitat (for example, research on forb seeding rates; Peters and Schottler 2010; Carter and Blair 2012).

Low forb diversity in restored prairies may be attributable to a variety of factors, including seed limitation as well as poor germination and establishment (Dickson and Busby 2009; Hillhouse and Zedler 2011). While most factors that limit germination and establishment are often out of the control of practitioners, incorporating information on seed viability in restoration planning can lower the risk that seed limitation and poor germination might occur. Accurate seed viability estimates for all seedlots used in a restoration allows seeding rates to be adjusted for each lot to ensure the desired amount of viable seed is used for each species. While viability estimates are often available for purchased seedlots, viability testing for wild-collected seed often falls to restoration managers. Unfortunately, seed viability of wild-collected native forb species can vary greatly among species, populations, and collection years. These differences may be caused by environmental factors at the collection site (for example, drought; Fenner 1991), genetic factors in the source population (for example, inbreeding depression; Menges 1991), or seed collection conditions (Elias and others 2006). Though knowing seed viability is clearly an important component of achieving desired restoration outcomes, many methods of estimating seed viability can be used, and the ideal testing method may vary by species and project.

Three primary methods exist for formally testing seed viability: 1) germination, 2) tetrazolium, and 3) X-ray. Each method has its own strengths and weaknesses (Table 1). Germination tests estimate the potential for a seedlot to produce plants under favorable conditions (AOSA 2012). This test may undercount viable seed if the moisture, light, and temperature conditions are not sufficient to overcome seed dormancy (Gosling 2003). These factors can be a particular challenge for native species, as dormancy-breaking requirements are often unknown, and in some cases dormant seed may take months or years to germinate even under optimum conditions (Baskin and Baskin 2001). Tetrazolium (TZ) tests stain respiring tissues in the seed (Baskin and Baskin 2001; Miller and Peters 2010), which are then dissected to identify living seeds. TZ tests require species-specific protocols that involve some training to implement and interpret. Finally, X-ray equipment uses low levels of radiation to identify seeds that are "filled," or contain

TABLE 1

Comparison of the strengths and weaknesses of 3 different methods commonly used to assess seed viability: germination, tetrazolium, and X-ray.

Metric	Germination	Tetrazolium (TZ)	X-ray
Equipment expense	Intermediate (incubators)	Intermediate (dissecting microscope)	High (X-ray machine)
Reagent/materials expense	Low (growth medium, containers)	Intermediate (tetrazolium, containers)	None (for digital X-ray machines)
Dedicated time required (person hours)	Low	Intermediate	Low
Length of time required	2 or more wk	2 d	< 5 min
Staff expertise required	Low (intermediate if unknown dormancy)	Intermediate	Low
Type of viability identified	Germinable seed	Respiring seed	Filled seed
Role of dormancy in assessing viability	Important	Not important/estimate not affected	Not important/estimate not affected
Destructive?	No (seedlings can be transplanted)	Yes (seeds destroyed)	No (seeds not damaged)
Noteworthy challenges	Germination conditions must break dormancy, yet dormancy-breaking requirements are unknown for many native species. They may also vary among populations of the same species.	Difficult analysis on very small-seeded species	Difficult analysis on very small-seeded species

a fully formed embryo (Gosling 2003; Terry and others 2003). X-ray analysis can be a cost-effective way to quickly estimate viability for unstudied species, and it is used by numerous conservation organizations, including the Millennium Seed Bank at Royal Botanic Gardens, Kew, and the Dixon National Tallgrass Prairie Seed Bank at Chicago Botanic Garden.

These tests vary in needed expertise, equipment, time commitments, and required funding, and they measure slightly different aspects of viability. It is not clear, however, whether they return similar estimates of seed viability. To our knowledge, such an assessment has not been carried out consistently and rigorously for multiple species and populations. We begin to address this by assessing seed viability using all 3 techniques for multiple populations of 4 native prairie forb species. We expect that X-ray tests will show a higher measured viability than the other 2 tests, with TZ showing the next highest level of measured viability (if some filled seeds contain dead/not respiring embryos) and germination tests showing the lowest measured viability (if some respiring seeds do not germinate).

METHODS

Study Species and Seed Sources

We selected 4 forb species that are relatively common, widespread, and native to prairie ecosystems in the midwestern US: 1) *Lespedeza capitata* Michx. (roundhead lespedeza or roundhead bushclover [Fabaceae]), 2) *Liatris aspera* Michx. (tall blazing star or rough prairie blazing star [Asteraceae]), 3) *Eryngium yuccifolium* Michx. (button eryngo or rattlesnake master [Apiaceae]), and 4) *Ratibida columnifera* Nutt. Woot. & Standl. (upright prairie coneflower or Mexican hat [Asteraceae]). All seeds in this study were collected and stored using the same protocols as those used with the Seeds of Success Program (SOS; BLM 2012). Collections were made in 2011 and banked in the Dixon National Tallgrass Prairie Seed Bank in 2012 (Chicago Botanic Garden, Glencoe, Illinois; Figure 1), with the exception of *E. yuccifolium,* which was collected in 2010 and banked in 2011. SOS protocols are designed to ensure that healthy seed is collected from large populations at peak maturity, and then handled and stored appropriately to maintain long-term viability. Different seedlots for each species were collected at different times, as the timing of seed maturity varied widely across the range of our study species. For all species, cleaned seed was stored in dry (15% relative humidity) conditions for at least 2 wk prior to being banked at –20 °C (–4 °F) (Yates 2009). Seed count and viability estimates (determined by X-ray), as well as herbarium vouchers, for all accessions are available at http://www.scicollections.org. In summer 2013, at least 2 populations of each species from different states (Table 2; Figure 2) were removed from the seedbank, counted, and placed in paper envelopes to thaw at room temperature. Only fully formed seeds (appearing mature, full, and undamaged; Figure 3) were counted and included in the following viability tests (all carried out concurrently in July 2013 at Chicago Botanic Garden).

Viability Test: Germination with and without Pretreatment

To determine percent viability for each species and population, 8 replicates of 25 seeds for each accession were plated on 1.5% distilled water agar (Mondoni and others 2012) using a 5 × 5 printed grid to ensure even spacing of seeds. Immediately prior to plating, seed was bleached for 30 s in a 0.25% sodium chlorite solution and then soaked in a DI water rinse for 1 min. This step was to minimize contamination by superficial fungal and bacteria (Clauss and Venable 2000). Plates were sealed with Parafilm to ensure that the agar did not dry out.

TABLE 2

Seed source information for all 4 study species, including scientific and common name, as well as each source population location (county and state) and collection date.

Scientific name	Common name	Population location (County, State)	Latitude	Longitude	Elevation (m)	Collection date
Eryngium yuccifolium	Rattlesnake master	Shawnee, Kansas	38.953N	95.699W	321	6 Oct 2010
		Crawford, Missouri	38.181N	91.203W	311	24 Sep 2010
Lespedeza capitata	Roundhead bushclover	Franklin, Missouri	38.266N	90.111W	259	6 Oct 2010
		Iowa, Wisconsin	43.243N	89.733W	231	22 Sep 2010
Liatris aspera	Rough prairie blazing star	Mason, Illinois	40.151N	89.846W	207	7 Nov 2011
		Phelps, Missouri	38.019N	91.574W	325	25 Oct 2011
Ratibida columnifera	Upright prairie coneflower	Jefferson, Colorado	39.767N	105.222W	1808	3 Aug 2011
		Chase, Kansas	38.409N	96.503W	397	10 Aug 2011
		Johnson, Wyoming	44.103N	106.541W	1354	9 Aug 2011

Note: 1 m = 3.3 ft.

Figure 1. Dixon National Tallgrass Seed Bank. Photo by Robin Carlson, Chicago Botanic Garden

As our species are known to have either physical or physiological seed dormancy, we also applied treatments designed to break dormancy for all of our study species and populations. For this, an additional 8 replicates of 25 seeds per accession were pretreated following recommendations in the literature prior to preparing and plating them in Petri plates as for the non-pretreated seed, described above. For *L. capitata*, which is known to have physical seed dormancy, the pretreatment was scarification (recommended for germination of legumes in Smith and others 2010). Scarification consisted of rubbing seed with fine-grained sandpaper for approximately 1 min with only slight pressure to break the seedcoat. The remaining 3 species (*E. yuccifolium*, *L. aspera,* and *R. columnifera*) were reported to have physiological dormancy, requiring moist cold stratification for many weeks to break dormancy. Because of time constraints, we applied gibberellic acid (GA_3) treatments instead of moist cold stratification, as GA_3 is known to stimulate germination of seeds with physiological dormancy (Baskin and Baskin 2001). For this, seed was soaked in 500 ppm GA_3 overnight (Watkinson and Pill 1998; Çetinbaş and Koyuncu 2006; Miller and Peters 2010).

Following plating, all pretreated and non-pretreated seed was placed in an incubator (Percival Scientific, Model I-36LLVL) set to 12-h photoperiods and day/night temperatures of 25/15 °C (77/59 °F), based on optimal germination conditions for our study species (Cole and others 1974; Baskin and Baskin 2001). The location of each Petri plate was randomized in separate plastic tubs for each species. Germination checks were performed twice weekly for 28 d, with germination recorded when radicle emergence (> 1 mm) was visually confirmed. Tubs were rotated within the incubators twice each week. Percent germination was calculated for each Petri plate and used as the percent viability estimate in all analyses (however, only percent viability calculated for *pretreated* seeds was used to compare testing methods). We did not distinguish if any viable but dormant seed remained in the Petri plate with further tests, so this calculation of viability may underestimate true viability. In the replicates for which pretreatment was applied, however, this occurrence was expected to be minimal.

Viability Tests: Tetrazolium

Tetrazolium (TZ) tests were conducted according to the recommendations in the AOSA Tetrazolium Handbook (Miller and Peters 2010). For this, 8 sets of 25 seeds were soaked in 500 ppm GA_3 for 24 h for all species and populations. Seed was then cut and soaked in 1% tetrazolium chloride in Petri plates for 24 h at 30 °C (86 °F). Last, the seedcoat was removed and the seed was visually inspected for viability under a dissecting microscope. A seed was considered viable when the embryo was stained red and matched the description for a viable seed in the Tetrazolium Handbook (Miller and Peters 2010). Percentage of appropriately stained embryos was calculated for each set of seed and used as the percent viability estimate in all analyses to compare testing methods.

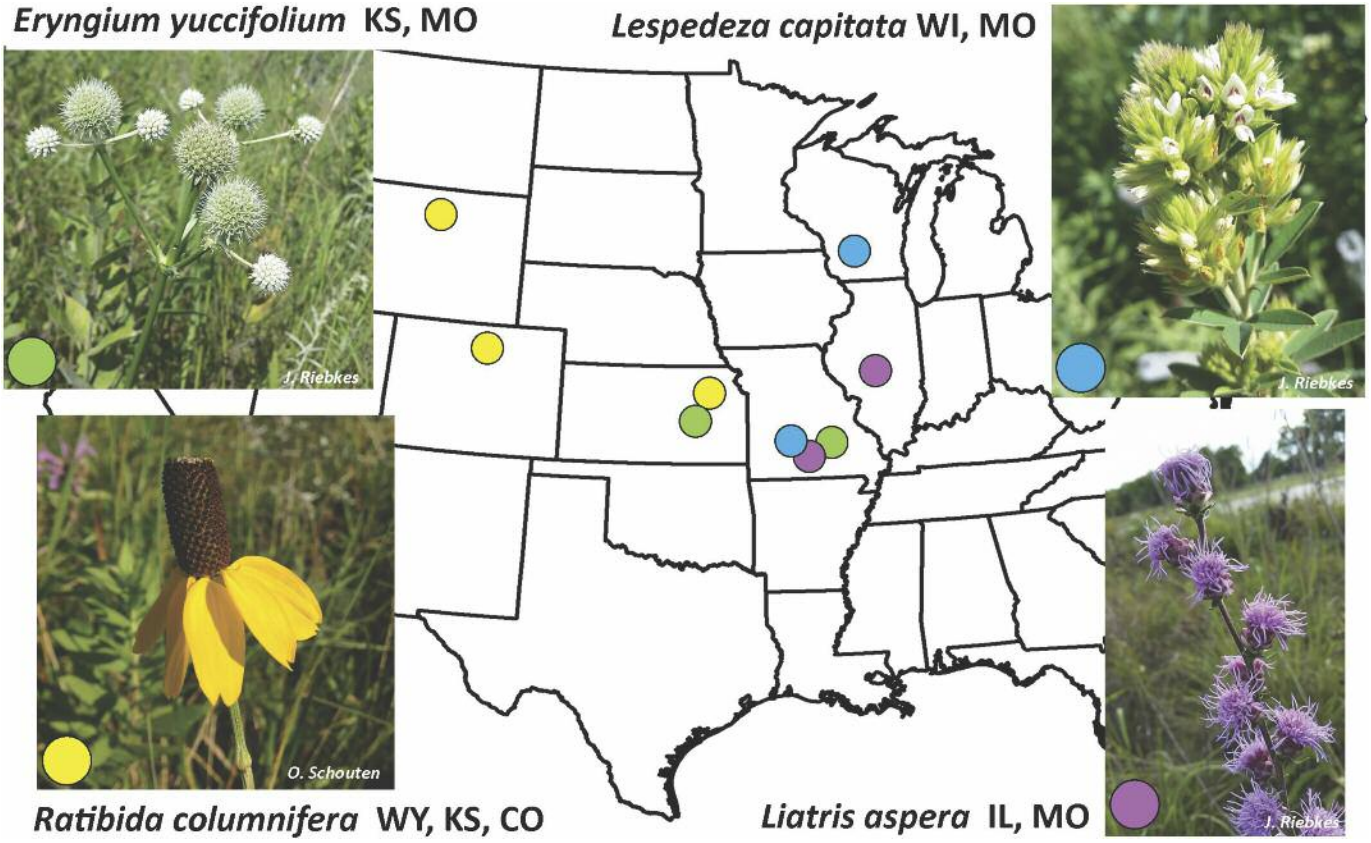

Figure 2. Location of study populations for all 4 study species: *Eryngium yuccifolium* (green circles, 2 populations); *Lespedeza capitata* (blue, 2 populations); *Liatris aspera* (purple, 2 populations); *and Ratibida columnifera* (yellow, 3 populations). See Table 2 for additional collection details. Photos of *Eryngium, Lespedeza,* and *Liatris* by Jessica Riebkes; Photo of *Ratibida* by Olivia Schouten

Figure 3. *Liatris aspera* seeds. Photo by Robin Carlson, Chicago Botanic Garden

Viability Test: X-ray

In order to verify the identification of a fully formed embryo on X-ray images, trial X-rays and cut tests were performed on a small subset of seed for each species. Following this, 8 replicates of 25 seeds each were scanned under optimal conditions to determine whether seed was filled with embryos as an estimate of potential viability. All species and populations were analyzed by digital X-ray (Faxitron, Model MX-W) for 20 s at 18 kV (except *L. capitata*, which was scanned at 22kV to improve image quality). The percentage of all scanned seeds that were filled (the X-ray showed a whole embryo) for each replicate was reported and used in a percent viability estimate in all analyses to compare testing methods.

Analyses

Percent germination was calculated for each replicate of 25 seeds and used as the response variable for analyses. With this, analysis of variance was used to determine the effects of pretreatment, species, and source population on percent germination. To compare estimates of viability among testing methods, an analysis of variance test was used to determine the effects of test method (excluding germination results for seed that was not pretreated), species, and source population on percent viability. Significant effects and two-way interactions were identified using model simplification, and Tukey's HSD post-hoc tests were used to identify significant differences between groups. All analyses were completed in R version 2.14.0 (R Core Team 2012).

Importance of Pretreatment in Germination Studies

In the overall model, we found interactions between the effects of species and pretreatment ($F = 43.359$, $P < 0.0001$) on percent germination, so we analyzed each species separately. The effect of pretreatment varied by species and source population (Table 3). In 3 of the 4 species, pretreatment improved germination rates in some or all source populations (Figure 4). For example, scarification of *L. capitata* seeds increased germination in both the Missouri and the Wisconsin source populations ($F = 306.46$, $P < 0.0001$).

In *L. aspera,* percent germination of seed pretreated with GA_3 was significantly higher than untreated seed in Missouri ($P = 0.001$), but not in Illinois ($P = 0.906$), and this interaction between pretreatment and population was significant ($F = 6.469$, $P = 0.017$). However, Illinois seeds had higher germination overall than Missouri seeds ($F = 73.690$, $P < 0.0001$).

In *E. yuccifolium,* Kansas seed that received the GA_3 pretreatment had significantly higher germination than did untreated seed ($P < 0.001$), but this was not the case in Missouri ($P = 0.939$). Germination in the Kansas population was significantly higher than in Missouri for the GA_3 treatment ($P < 0.0001$), but not in the untreated seed ($P = 0.584$). This interaction between pretreatment and population was significant ($F = 8.343$, $P = 0.007$).

Germination in *R. columnifera* was not significantly different between untreated and GA_3 pretreated seeds ($F = 3.07$, $P = 0.060$). There was also no significant difference in percent germination between seed from Kansas and Colorado ($P = 0.406$), nor Wyoming and Colorado ($P = 0.471$), but Kansas seed had higher percent germination than Wyoming seed ($P = 0.045$).

TABLE 3

Results of ANOVA tests on the effects of pretreatment (GA_3 or scarification), population, and their interaction on percent germination data for each study species.

Species	Effect	*F*	*P*
Eryngium yuccifolium			
	Pretreatment (GA_3)	**13.70**	**0.0009**
	Population	**22.05**	**< 0.0001**
	Pretreatment*Population	**8.43**	**0.007**
Lespedeza capitata			
	Pretreatment (scarification)	**306.46**	**< 0.0001**
	Population	**25.61**	**< 0.0001**
	Pretreatment*Population	0.0066	0.94
Liatris aspera			
	Pretreatment (GA_3)	**12.23**	**0.0016**
	Population	**73.69**	**< 0.0001**
	Pretreatment*Population	**6.47**	**0.017**
Ratibida columnifera			
	Pretreatment (GA_3)	3.07	0.06
	Population	3.72	0.06
	Pretreatment*Population	0.75	0.48

Note: Significant effects are set in bold.

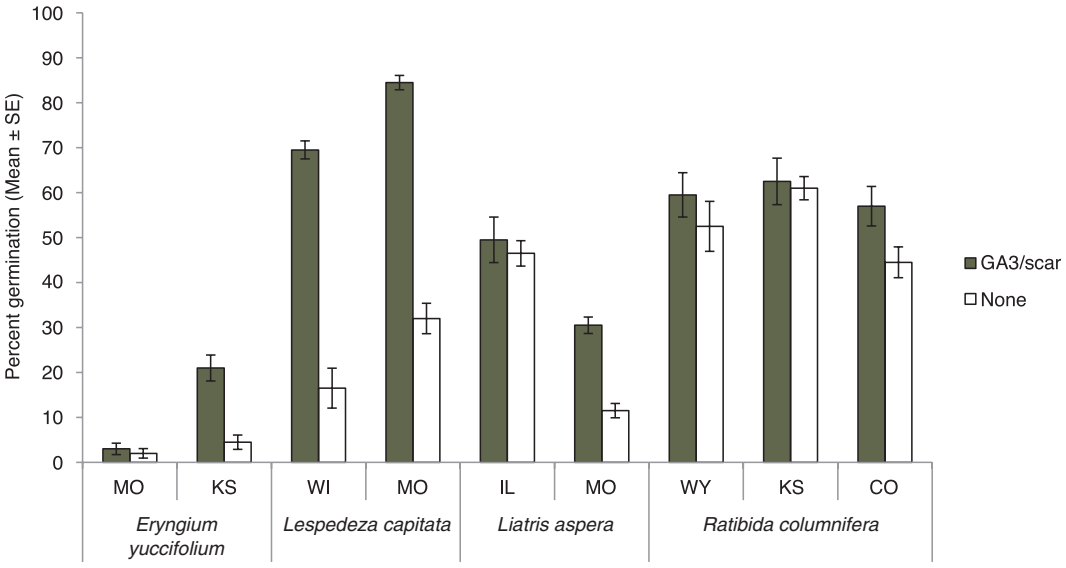

Figure 4. Percent germination (mean ± SE) of 4 prairie forbs with and without pretreatment (scarification for *L. capitata* and GA_3 for all other species) for each species and study population.

Comparison of Testing Methods

We analyzed the effect of testing method on viability for each species separately, because we found significant interactions between species and testing method (F = 17.264, P < 0.0001) in the overall model. Measured viability did not differ with testing method for 3 species (Table 4; Figure 5): *R. columnifera* (F = 1.648, P = 0.200), *L. capitata* (F = 2.727, P = 0.076), or *L. aspera* (F = 0.394, P = 0.676). In *E. yuccifolium*, however, a significant difference occurred in measured viability between the testing methods (P < 0.0001). Germination (with pretreatment) differed from both X-ray (P < 0.0001) and TZ (P < 0.0001), but TZ and X-ray results were not significantly different from one another (P = 0.090).

Population-Level Differences in Viability

In 3 out of 4 species, a significant difference in viability occurred among source populations regardless of testing method used (Table 4; Figure 5). In *L. capitata*, a significant difference occurred between source populations (F = 31.52, P < 0.0001), with Missouri viability higher than Wisconsin. All testing methods also showed a significant difference between populations in *L. aspera* (F = 24.90, P < 0.0001), with Illinois seed having higher viability than Missouri seed and in *E. yuccifolium*, with Kansas seed viability significantly higher than Missouri (P < 0.0001). When comparing all 3 testing methods for *R. columnifera*, however, no significant difference in viability was observed between the 3 populations (F = 2.29, P = 0.11).

TABLE 4

Results of ANOVA tests on the effects of test method (germination, TZ, and X-ray), population, and their interaction on square root-transformed percent viability data for each study species.

Species	Effect	*F*	*P*
Eryngium yuccifolium			
	Test method	**101.91**	**< 0.0001**
	Population	**57.61**	**< 0.0001**
	Test method*Population	0.76	0.47
Lespedeza capitata			
	Test method	2.73	0.08
	Population	**31.52**	**< 0.0001**
	Test method*Population	3.05	0.06
Liatris aspera			
	Test method	0.39	0.68
	Population	**24.90**	**< 0.0001**
	Test method*Population	0.62	0.54
Ratibida columnifera			
	Test method	1.65	0.20
	Population	2.29	0.11
	Test method*Population	0.44	0.78

Note: Significant effects are set in bold.

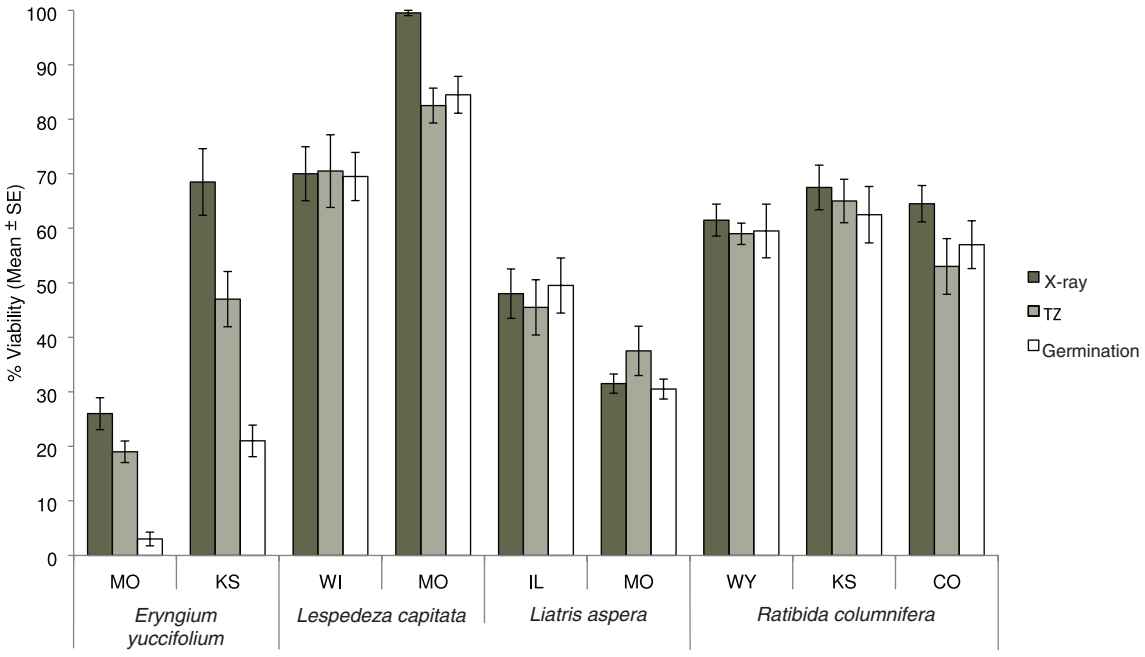

Figure 5. Seed viability (mean ± SE) as determined by X-ray, TZ tests, and germination (shown with pretreatment only) for each study species and population.

DISCUSSION

Consistency of Viability Measurements among Testing Methods

All 3 methods used to estimate seed viability (germination with pretreatment, TZ, and X-ray) returned viability estimates that were not significantly different from one another in 3 out of 4 study species (the exception being *E. yuccifolium*; Figure 5, Table 4). And even in *E. yuccifolium,* TZ and X-ray tests provided similar estimates of viability. This finding suggests that, at least for orthodox seed stored in cold and dry conditions for 2 to 3 y or less, no single viability testing method is best, and individuals can generally select the viability testing method that is most appropriate for their situation (time, expertise, and equipment; Table 1).

In the species that proved the exception (*E. yuccifolium*), the germination test led to significantly lower viability estimates than both TZ and X-ray tests. Almost no *E. yuccifolium* seed germinated without a GA_3 pretreatment, and even following pretreatment, germination was never more than 25% (Figure 5). It is not clear if this was because our pretreatment was not successful in sufficiently breaking dormancy, or if seeds that appeared viable in the other 2 tests were actually no longer germinable. The seed used for all species was collected and stored following the same Seeds of Success protocols, but site-specific differences at collection sites (for example, drought or genetic issues) may have negatively affected the viability of *E. yuccifolium* seedlots, so seed that appeared full on X-ray analysis and had respiring tissue with TZ were actually incapable of germination. Production of underdeveloped embryos is common in the Apiaceae family, which may be difficult to distinguish with X-ray or TZ tests (Baskin and Baskin 2001; Goodman and others 2006.). This may help explain why, even though they appeared to contain a respiring embryo, few *E. yuccifolium* seeds actually germinated in this study.

It is also possible that at least some germinable *E. yuccifolium* seed remained dormant at the end of our study despite the GA_3 pretreatment. Recent research has shown that *E. yuccifolium*, like most species in the Apiaceae family, produces seed that exhibits deep physiological dormancy (Necajeva and Ievinsh 2013). *Eryngium yuccifolium* typically requires 12 wk of moist, cold stratification for optimal germination, and even then germination percentages are low (below 50%; Greene and Curtis 1950; Smith and others 2010). While the GA_3 pretreatment we used has overcome dormancy in some *Eryngium* species (Necajeva and Ievinsh 2013), the concentration of GA_3 in our pretreatment may not have been sufficient. A higher concentration of GA_3, or a combined pretreatment/cold stratification method, may be a more successful strategy to break dormancy of *E. yuccifolium* seed.

These results demonstrate a limitation of using germination studies to assess viability. If dormant but viable seed does not have the right conditions to break dormancy and germinate, it will appear nonviable (Gosling 2003). When using germination as a viability test, it is important to test the seed using conditions known to break any potential dormancy. For species in which specific dormancy-breaking conditions are not known (often the case for understudied native species), it may be necessary to use multiple pretreatments and to conduct trials over multiple months to ensure dormancy is broken and germination results indicate true viability. Finally, these results suggest that multiple approaches to assessing viability may be useful to determine viability in species whose dormancy is poorly understood or difficult to break.

This study used seed that had been stored in optimal conditions (15% relative humidity, –20 °C [–4 °F] in a seedbank) for 2 to 3 y, demonstrating that banked native seeds for orthodox prairie species can remain viable and germinable, at least over the time frame of this study (and see Turner and others 2013). We would expect similar results for tests run on fresh, recently harvested seed (unless the species requires dry after-ripening to break dormancy mechanisms; Baskin and Baskin 2001). For seed that is older, recalcitrant, or that has not been stored under appropriate conditions, the estimated viability using X-ray, TZ, and germination is predicted to become increasingly disparate, with germination percentage showing the lowest viability (Bewley and others 2013).

Among-Population Differences in Viability

We found significant differences among populations regardless of testing method used for 3 of our 4 study species (the exception being *R. columnifera;* Table 4), illustrating the importance of testing the viability of wild-collected seed prior to use in restoration seed mixes. For each study species, the seed from different populations was collected in the same year, minimizing potential differences between populations caused by storage length. Other factors that we could not measure, however, such as local biotic and abiotic conditions (precipitation, temperature, pests or pathogen presence and abundance), may have been different at each population and therefore influenced viability in unknown ways. For example, differences in estimated viability between populations of the same species have been found to be attributable to local drought conditions (Dornbos and others 1989) or to be a result of local adaptation to climatic differences (influencing dormancy and response to pretreatment in germination tests as in Naylor and Jana 1976). Additionally, genetic differences between populations (including the inbreeding depression that may affect seed germinability; Menges 1991) may have also affected our seed viability estimates. In future studies, repeating this experiment to compare seed from additional populations, or multiple collection years from the same populations, would allow us to better understand why and when viability may differ across the range of a species.

Among-Population Differences in Germination and Pretreatment Response

All of our species exhibited some level of seed dormancy, as pretreated seed (with either GA_3 or scarification) germinated at higher rates in all species and populations (Table 3; Figure 4). These patterns were significant in *E. yuccifolium* and one population each of *L. aspera* and *L. capitata*. The results are consistent with the literature, which report increased germination following treatment with gibberellins for many prairie species, including the species from this study (Watkinson and Pill 1998; Baskin and Baskin 2001; Çetinbaş and Koyuncu 2006; Madeiras and others 2007). As in many legume species, scarification increased germination in *L. capitata* (Baskin and Baskin 2001; Voigt 2013).

In *L. aspera* and *E. yuccifolium*, pretreatment affected germination response in a variety of ways among different populations. For example, in *L. aspera,* pretreatment improved germination in the Missouri, but not the Illinois, population. This response is because Illinois seed had little to no dormancy (both untreated and pretreated germinated to a similar high degree), while the Missouri seed had dormancy that was broken by GA_3 (untreated seed had low germination relative to pretreated seed). Similarly, in *E. yuccifolium*, GA_3 pretreatment significantly increased germination in the Kansas, but not the Missouri, population. This may reflect true differences in the viability of each population (with Kansas having more living, germinable seed than Missouri has), or it may be a reflection of different degrees of seed dormancy in each population. Among-population differences in response to GA_3 have been found in other species (for example, *Onopordum acanthium* L. [Scotch cottonthistle; Asteraceae]; Qaderi and Cavers 2000), and it has been speculated that populations with lower responses to GA_3 may produce seed that persists for longer periods of time in the seedbank.

CONCLUSION

The use of X-ray, tetrazolium, and germination tests on multiple populations of 4 wild-collected forb species native to prairie habitat provided similar estimates of viability. These results are encouraging, as they suggest that practitioners can use the testing method best suited to their individual resources. For species that produce seed with challenging or unknown dormancy-breaking requirements, however, germination tests will likely underestimate true seed viability. Additional research on seed germination is needed for these species. Furthermore, within-species population-level differences in viability and germination underscore the importance of conducting viability tests on wild-collected seed as an early step in restoration to ensure seeding rates are appropriately adjusted. Knowledge about seed viability can inform management for conservation and restoration actions to improve outcomes for native plants.

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