



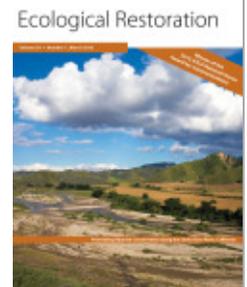
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# Unintentional Selection and Genetic Changes in Native Perennial Grass Populations During Commercial Seed Production <sup>©</sup>

Andrew R. Dyer, Eric E. Knapp and Kevin J. Rice

## ABSTRACT

For habitat restoration, seed of native plant species is frequently transferred over wide geographic areas and planted in environments that differ from the original collection environment. When such collections are grown for seed production in agricultural fields, the genotypes favored under agronomic conditions may differ from those favored at the eventual planting location. The resulting agronomically-produced seed may be poorly matched to intended restoration sites. Populations of two native perennial grasses commonly used in restoration projects in California were grown in typical agronomic conditions for seed production. We compared traits of the plants produced from seed harvested in the first and second years of agronomic production to plants produced from the original seed collections. We found strong evidence for genetic shifts in both species as a result of selection associated with biotic and abiotic factors. A four-population mixture of *Elymus glaucus* produced seed comprised mainly of two populations, primarily due to differential sensitivity to disease. With two populations of *Nassella pulchra*, early and late harvests selected for early and late maturing genotypes, respectively, and a reduction in the variance in phenology within the seed from the early harvest. We found that agronomic techniques for seed production have the potential to cause unintentional genotypic selection and result in shifts away from the genetic composition of the original seed collection. We offer recommendations for seed increase protocols to reduce these outcomes.

**Keywords:** *Elymus glaucus*, genetic shift, habitat matching, *Nassella (Stipa) pulchra*, phenology

## Restoration Recap

- Commercially-produced seed mixes of native species are often used in restoration projects with the assumption that the proportions of the original field-collected source populations are maintained in the harvested seed.
- We grew populations of two species of perennial grasses commonly used in restoration projects and found that seed production differed significantly between populations thereby skewing the proportions of the populations in the harvested seed. In particular, population differences in phenology influenced the ease of mechanical and manual seed collection.
- Plant phenology, susceptibility to disease (rust) and seed harvesting techniques probably all contributed to genetic shifts in the harvested seed in comparison to the parental populations.
- Results suggest that care should be taken when native species are grown in agricultural conditions to ensure the genetic makeup of seed mixes accurately reflects the source populations.

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The limited availability of seed of native species has often resulted in the widespread use of commercially available, but non-native, cultivars for revegetation projects (Millar and Libby, 1991, Montalvo et al. 1997). These revegetation efforts obviously do not “restore” habitats and in many cases may have contributed to weed invasions and erosion problems. Current regulations frequently require

the use of native seeds in revegetation and restoration efforts whenever possible.

Native seed collected from environments similar to the planting site is likely to be the best adapted to local conditions. Several reviews of the concepts and the literature described the importance of local and regional adaptive patterns (Knapp and Rice 1994, 1996, Knapp and Dyer 1998, McKay et al. 2005) and the potential hazards of mixing germplasm sources (Millar and Libby 1991, Hufford and Mazer 2003) to the success of restoration efforts. The need for habitat-specific or climate-specific populations of seed has been demonstrated in failed revegetation efforts at numerous spatial and temporal scales (Kitzmilller 1990, Millar and Libby 1991). An outgrowth of these conceptual and practical considerations has been an emphasis on “local” seed collections for ensuring better germplasm and environment matching (McKay et al. 2005).

Practitioners of ecological restoration are faced with a dilemma in that they wish to use genetically-appropriate germplasm to ensure greater probability of project success, yet the required quantities of site-appropriate plant material may exceed what can be sustainably collected from native populations (Knapp and Rice 1994). This has contributed to the development of a seed production industry devoted to providing the volume of seed needed for large projects. Agricultural production allows a small amount of seed from single native population or a mixture of populations to be increased for use in large planting projects. An important assumption is that the seed produced from such a process is genetically similar to the original seed collection.

When seed are taken from native populations and grown for seed increase in agricultural fields, not only are the growing conditions different, but the genotypes that are favored may be different from those favored at the eventual planting location (Knapp and Rice 1994). In addition, certain aspects of agronomic production such as the relatively uniform field conditions and mechanical harvest can result in a reduction of genetic variability during seed increase. Populations of native species often contain a great deal of genetic variation for traits such as seed ripening and plant stature, as opposed to most food crops which are bred for uniformity, in part to make harvest easier. A mechanical harvester may capture only those seeds from plants of a certain height. Additionally, agricultural plantings of a native species are often harvested only once, which has the potential to favor genotypes that are mature at the time of harvest and not capture the entire range of genotypes of differing phenology found in the original seed collection.

An unintentional genetic shift during seed increase may thus reduce genetic variation for adaptations to conditions existing at the original seed collection site. Such a loss of genetic variation during seed increase may also reduce a population’s ability to respond to changing conditions

and limit its capacity for future adaptive change (Jump et al. 2008). Although the need for such genetic variation is potentially of great evolutionary importance, maintaining within-population genetic variation during the seed-increase process can be difficult in agronomic practice. In particular, restoration practitioners must consider the complications arising from climate change and how new selective challenges from human-induced environmental change may affect the long-term success of their restoration projects. If native populations contain adequate genetic variation for adapting to climate change, it becomes essential for the seed increase process to capture that variation. While restoration efforts attempt to match plant germplasm to appropriate site conditions, climate change presents a challenge wherein the site conditions could change over time creating a “moving adaptive target” (Rice and Emery 2003). As a result, restoration practitioners must maintain sufficient within-population genetic variation in anticipation of shifts in the environmental conditions faced by the restored populations (e.g., Jump et al. 2008).

Because of the considerations mentioned above, the seed production industry is faced with a complicated set of criteria for increasing seeds of native species with the goal of providing large quantities of seed adapted to specific environmental conditions and containing levels of genetic variation resembling the diversity of the seed source populations. Population mixtures may be one means of countering issues related to the loss of genetic variation and site-appropriate seed collections (Millar and Libby 1991, Lesica and Allendorf 1999). Given that a single population of a species is a restricted sample of the available species variability, mixing several populations from a region presupposes that the larger sample will contain greater range of available genetic variation and the probability will be higher for at least some genotypes in the mixture to be a genetic match to the restoration site (Knapp and Rice 2011). Mixtures of populations may, in principle, be appropriate for use across a wider range of habitat conditions than a single population, if locally superior genotypes successfully “sort themselves out” over time (Burton and Burton 2002). A largely untested assumption of this approach is that the growth, survival, and seed output of every genotype in the mixture will be the same following seed increase (Burton and Burton 2002).

To test for genetic changes when native seed mixes are grown and harvested under agronomic conditions, we conducted an experiment using four northern California populations of blue wildrye (*Elymus glaucus*) and two populations of purple needlegrass (*Nassella pulchra*). Both species exhibit strong ecotype formation and demonstrable genetic differences over relatively short distances (Knapp and Rice 1996, 1998, 2011, Rice and Knapp 2008). Population mixtures of each species were grown at a commercial seed propagation farm under standard production conditions and followed for two years.

We predicted that standard agronomic conditions and practices would result in unintentional selection that differentially affects the survival, growth, and reproduction of genotypes from the source populations within the experimental mixtures. Additionally, we sought to identify specific management practices, as well as unexpected environmental events, that contribute to genetic shifts in trait means and variances during seed increase.

## Methods

### Study species

We chose two perennial native grasses with contrasting breeding systems because differences in spatial patterns of genetic structure can be affected by not only selection but also gene flow and genetic drift that shaped the parental populations. Purple needlegrass is believed to be a predominantly outcrossing species (Knapp and Rice 1998), while blue wildrye is a strongly selfing species (Jensen et al. 1990). The amount of genetic structuring at the landscape scale can be relatively low in outcrossing species because of the mixing effects of gene flow, while self-pollinating species are more likely to maintain high levels of localized genetic differentiation (Heywood 1991). In addition to the breeding system differences, we focused on these two species because they are among the most widely planted native grass species in California.

### Blue Wildrye

In the spring and summer of 1997, seeds were collected from at least 50 widely-spaced plants growing in natural populations from a range of environments in Northern California. These were:

1. Coleman Valley (10 km from the coast, near Bodega Bay, Sonoma County, elev. 240 m; N38°24'08", W122°00'20");
2. Winters (local population near Winters, Yolo County, elev. 60 m; N38°32'24", W122°00'25");
3. Sierra Foothill (UC Research and Extension Center, Yuba County, elev. 450 m; N39°15'4", W121°18'47"); and
4. Sierra High (Washington Road, Nevada County, elev. 1350 m; N39°19'04", W120°48'36").

Greenhouse grown seedlings (264 per population) were randomly arranged and planted in an agricultural seed production field near Winters, Yolo County, California (elev. 56 m; N38°37'18", W121°59'23"). Annual precipitation data were collected from the Brooks, CA weather station (Western Regional Climate Center). The mean annual precipitation was 45.5 cm (1981–2010) and during the experiment was 55.58 cm in the first growing season and 31.45 cm in the second.

Transplanting into cultivated rows, with individuals spaced approximately one meter apart, was done in late fall of 1997. The rows were arranged in four blocks, each containing an equal number of plants from the four different populations. Each plant was labeled so it could be followed during the course of the experiment. Prior to harvest the following spring (i.e., end of the growing season for the first year of the experiment), data were collected on several variables to assess individual plant health and fitness in the agricultural environment. Many plants became infected with a rust (*Puccinia* sp.) and rust incidence was scored in April 1998, by the density of lesions on the leaves (0 = no rust to 4 = fully covered) on a random subset of plants. Flowering culms were counted on each plant and seeds per culm were counted on a subset of culms per population in May 1998. Seed production and survival of individual plants in the agricultural field was determined again in late spring of 1999, at the end of second growing season.

At seed maturity in mid-June, 1998, seed was mechanically harvested, with whole plants swathed with a cutting implement mounted on a tractor and culms spread onto large plastic tarps for drying. After five days of drying on tarps, culms were vigorously shaken and the bulked seed collected for each block. (Hand shaking was done rather than mechanical separation due to the small amount of harvested material.)

Extent of genetic shifts was evaluated by planting a sample of the harvested agronomic field grown seed, along with seed from the original populations, and comparing electrophoretic markers and morphological traits among seed sources. Genotypes for electrophoretic markers were identified within a sample of harvested seed and as well as a random sample of seed from each of the four populations of the originally planted mixture, using methods described in Knapp and Rice (1996). The morphological trait evaluation of these same seed collections was conducted using seedling transplants established in the greenhouse in containers (Steuwe and Sons, Corvallis, Oregon) in the summer of 1998, several months after seed harvest. In November 1998, while still in the greenhouse, seedlings were scored for leaf length, leaf width, leaf shape (length/width), tiller number, and plant height. Seedlings were planted into the agricultural field at Winters, California in mid-December, 1998. In the late spring of 1999, approximately six months after planting in the agricultural field, phenology, culm number, rust incidence, and final plant height were scored. Phenology was scored using an ordinal scale: 0 = no inflorescences present, leaves entirely basal; 1 = inflorescence present but awns not emerging from sheath; 2 = tips of awns emerging from sheath; 3 = seeds emerging from sheath; and 4 = inflorescence fully elongated and all branches visible.

Data were analyzed using the SAS general linear models procedure with block and population source as main effects. Linear orthogonal contrasts allowed comparison of

**Table 1.** Estimates of seed production by four populations of *Elymus glaucus* planted into four replicate blocks in an agricultural field at Winters, CA. Culms were counted in each of two years; all plants were living at the time of the first count. Seeds per culm were counted on a subset of culms in year 1 and the same number of seeds per culm was assumed in year 2 ± SE. Numbers within columns followed by different letters are significantly different at  $p < 0.05$ .

Population	Year 1				Year 2			
	Seeds/culm	Culms/plant	Seeds/plant (estimate)	% of total (estimate)	Survival (%)	Culms/plant	Seeds/plant (estimate)	% of total (estimate)
Coleman Valley	97.4 ± 3.7 <sup>a</sup>	38.1 ± 1.4 <sup>c</sup>	3711	39	95.7 ± 2.1 <sup>c</sup>	79.8 ± 4.4 <sup>b</sup>	7438	40
Winters (local)	129.1 ± 5.0 <sup>b</sup>	23.5 ± 1.4 <sup>b</sup>	3034	32	97.7 ± 1.6 <sup>c</sup>	69.7 ± 5.6 <sup>b</sup>	8791	48
Sierra Foothill	88.5 ± 3.3 <sup>a</sup>	19.7 ± 0.9 <sup>b</sup>	1743	19	58.5 ± 5.5 <sup>b</sup>	30.6 ± 3.0 <sup>a</sup>	1584	9
Sierra High	98.5 ± 8.2 <sup>a</sup>	9.7 ± 1.2 <sup>a</sup>	955	10	22.7 ± 4.5 <sup>a</sup>	27.5 ± 5.6 <sup>a</sup>	615	3
<b>F (df)</b>	<b>17.94 (3,166)</b>	<b>88.10 (3,232)</b>			<b>93.80 (3,345)</b>	<b>25.78 (3,171)</b>		
<b>p</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>			<b>&lt; 0.001</b>	<b>&lt; 0.001</b>		

mean responses between the original seed collections and populations produced from seed mechanically harvested from the agronomic plantings.

### Purple Needlegrass

In the spring of 1997, seed were collected from a minimum of 50 widely spaced plants within natural populations growing at:

1. Winters (local), Yolo County, CA; Elev. 60 m; N38°38'13", W122°01'42"; and
2. Santa Rosa Plateau, Riverside County, CA.; Elev. 600 m; N33°32'36", W117°16'11".

Greenhouse grown seedlings (n = 432 per population) were randomly mixed and planted in four blocks in an agricultural field near Winters, California. Planting time and method was the same as for the blue wildrye experiment described previously. In late spring, 1998, after one growing season and prior to the first mechanical seed harvest, status (alive or dead) and number of flowering culms were counted on each plant and number of seeds per culm were counted on a subset of culms per population. Plant status, seed production, and mortality were evaluated again at the end of the second growing season, in late spring of 1999.

The seed crop in the four blocks was mechanically harvested twice (May 22 and June 3, 1998), using a Woodward Flail-Vac seed stripper, a device designed to harvest native grass seed in agricultural production. Because the Flail-Vac does not cut the flowering culms but only strips ripe seed from them, multiple harvests timed for different periods of seed ripeness are possible.

To evaluate the extent of genetic shifts in phenology as a result of selection caused by timing of the mechanical harvest, and whether genetic variation is lost in the process, seeds from both the early harvest and the late harvest along with seeds from the original field collections were planted in containers in the greenhouse in the summer of 1998. Prior to transplanting into the agricultural field, leaf length, leaf width, leaf shape (length/width), and plant

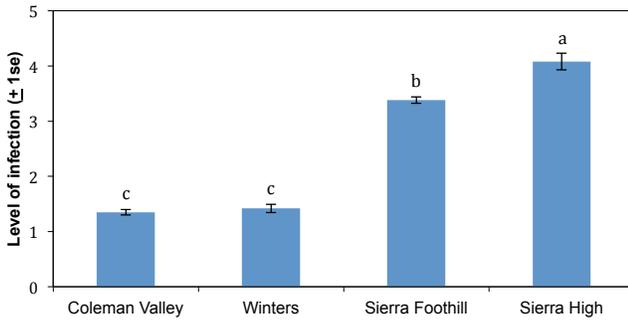
height were measured. Electrophoretic markers could not be used on the purple needlegrass seed collections due to both the complicated autopolyploid inheritance (Knapp and Rice 1998) and lack of markers that clearly differentiated the parental sources. In late spring, 1999, after plants had fully established in the agricultural field at Winters, CA, phenology, total number of culms per plant and final height were measured.

Data were analyzed using the SAS general linear models procedure with block and population as main effects. Linear orthogonal contrasts allowed the mean of the field population to be compared with populations derived from each mechanical harvest. Using the Levene test, variances of the response variables were compared between the mechanically harvested populations and the original seed collections to look for effects of agronomic growth environment and harvest timing on within-population genetic variation.

## Results

### Blue Wildrye

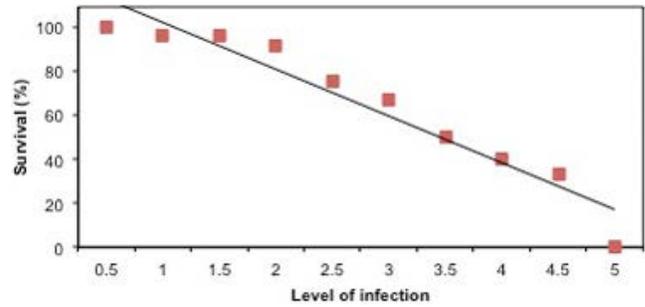
*Genetic shifts within mixtures.* Direct counts of culms and numbers of seeds per culm showed that Coleman Valley and Winters populations produced an estimated 71% of the seeds in the field even though they together comprised only 50% of the initial seed mixture (Table 1). By the end of the second growing season, the Coleman Valley and Winters populations produced an estimated 88% of the total seed with the local Winters population producing about 48% despite representing only 25% of the initial planting (Table 1). In addition, a strong (but not uncommon) wind event three days prior to harvesting may have contributed to even greater genetic shifts. Due to differences among populations in ease of seed shattering and ripeness, a larger proportion of Coleman Valley and Sierra Foothill seed probably fell to the ground in this wind event compared to the Winters and Sierra High populations.



**Figure 1.** Intensity of rust infection (mean  $\pm$  SE) on four field-collected populations of blue wildrye (*Elymus glaucus*) grown under agronomic conditions. The level of infection was assessed using a 6-point scale of 0 = “no rust” to 5 = “leaves fully covered with rust” on a random subset of plants from four planting blocks. Columns with different letters are significantly different at  $p < 0.05$ .

*Differential mortality among populations.* Rust infection in the first growing season was more pronounced on plants from the two Sierra populations than on plants from the coastal and local populations (Figure 1). Mortality was strongly associated with degree of rust infection (Figure 2) and by the beginning of the second growing season most of the plants that were heavily infected in the first year had died (Table 1). The rust infection caused differential mortality within populations as well. The Sierra High population consisted of two distinct phenotypes: some with green leaves and sheaths and some with purplish leaf sheaths. The plants with the purple sheaths appeared to be much more rust resistant than the green plants. These two phenotypes occurred with an approximate proportion of one-third purple and two-thirds green in the original planted population; however by the second growing season, only the purple phenotype remained.

*Overall genetic shifts: comparison of mechanically harvested populations and original seed collections.* Less tightly held seed may have also led to more seed falling to the ground from Coleman Valley and Sierra Foothill plants as a result of the physical process of harvesting. Electrophoretic



**Figure 2.** Percent survival of blue wildrye (*Elymus glaucus*) ( $n = 352$ ) across all populations after the second growing season relative to the intensity of apparent rust infection assessed during the first growing season. Regression;  $r^2 = 0.93$ ,  $p < 0.0001$ .

markers indicated that approximately 86% of the mechanically harvested seed originated from the local Winters population (Table 2).

Morphological and phenological assessments in the greenhouse and field showed that the source populations differed from each other for all traits assessed, except tiller number, and the mean values from the original seed sources differed from the mean values of the agronomic population for the majority of traits (Table 3). This included the mean rust score for plants grown from the agriculturally produced seed, which was significantly lower (more resistant) than the mean of the original mixture (Table 3).

### Purple Needlegrass

*Genetic shifts within mixtures.* Despite equal numbers at the time of planting, the non-local Santa Rosa population produced an estimated 61% of the total seed in the first season and 58% in the second season, based on counts of culm numbers and seeds per culm (Table 4). The difference in culm production per plant between populations was significant in both years.

*Population differences in mortality.* No mortality was observed in the agricultural seed production environment for plants of either population in both years.

**Table 2.** Isozyme genotype of blue wildrye (*Elymus glaucus*) seeds harvested in 1998 from a mixed planting of four populations in four blocks in an agricultural field at Winters, CA. The Winters population was identifiable by a unique ADH (Alcohol dehydrogenase) genotype. Ranges are given when the exact parental identity was ambiguous. N = number of seeds analyzed. Row totals do not equal N because ambiguous identifications are listed for all possible populations.

	N	Coleman Valley	Winters	Sierra Foothill	Sierra High
Block 1	50	2–6	44	0	0–4
Block 2	43	0–4	35	0–1	0–5
Block 3	56	0–2	49	3	0–2
Block 4	39	0–2	33	1–2	0–3
Totals	188	2–14	161	4–6	0–14
Percent		1.1–7.4%	85.6%	2.1–3.2%	0–7.4%

**Table 3. One-way ANOVA results for the four original blue wildrye (*Elymus glaucus*) sources inter-planted with seed harvested from the same sources after growing for one year in four separate blocks in an agricultural field (blocks showed few differences and means for individual blocks are not shown). Seedlings were scored for several traits in the greenhouse then transplanted into an agricultural field, where additional traits were evaluated ( $\pm$  SE). Leaf shape = length/width. Numbers within columns followed by different letters are significantly different at  $p < 0.05$ . N = number planted for each source; df is variable because all traits were not measured on all plants.**

	N	Phenology	Leaf shape	Culms/plant	Rust score	Height (cm)
<b>Seed source</b>						
Parent 1—Coleman Valley	80	1.86 $\pm$ 0.13 <sup>b</sup>	42.1 $\pm$ 1.1 <sup>c</sup>	25.6 $\pm$ 1.2 <sup>c</sup>	1.41 $\pm$ 0.08 <sup>a</sup>	81.4 $\pm$ 1.0 <sup>b</sup>
Parent 2—Winters (local)	80	2.86 $\pm$ 0.11 <sup>c</sup>	36.1 $\pm$ 1.0 <sup>bc</sup>	17.8 $\pm$ 0.7 <sup>b</sup>	1.91 $\pm$ 0.09 <sup>b</sup>	87.4 $\pm$ 0.9 <sup>c</sup>
Parent 3—Sierra Foothill	80	1.74 $\pm$ 0.12 <sup>b</sup>	35.1 $\pm$ 0.7 <sup>ab</sup>	15.6 $\pm$ 0.7 <sup>b</sup>	2.44 $\pm$ 0.08 <sup>c</sup>	91.4 $\pm$ 1.0 <sup>c</sup>
Parent 4—Sierra High	80	0.84 $\pm$ 0.10 <sup>a</sup>	30.9 $\pm$ 0.9 <sup>a</sup>	7.9 $\pm$ 0.9 <sup>a</sup>	3.34 $\pm$ 0.09 <sup>d</sup>	71.9 $\pm$ 2.6 <sup>a</sup>
<b>F (df)</b>		<b>29.66 (7,511)</b>	<b>10.38 (7,514)</b>	<b>23.75 (7,511)</b>	<b>44.72 (7,511)</b>	<b>18.44 (7,472)</b>
<b>p</b>		<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
Parent mean	320	1.82 (0.07)	36.0 (0.5)	16.2 (0.6)	2.27 (0.06)	83.0 (0.7)
Field harvested seed mean	200	2.43 (0.08)	38.6 (0.7)	19.1 (0.6)	1.76 (0.06)	85.0 (0.6)
<b>Parents vs. field harvested seed</b>						
<b>F (df)</b>		<b>40.76 (1,511)</b>	<b>9.78 (1,514)</b>	<b>14.73 (1,511)</b>	<b>51.01 (1,511)</b>	<b>4.95 (1,472)</b>
<b>p</b>		<b>&lt; 0.001</b>	<b>0.002</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>0.026</b>

*Overall genetic shifts: comparison of mechanically harvested populations and original seed collections.* Of the seven traits evaluated, three (phenology, leaf shape, and plant height in the greenhouse) differed significantly between the two parental populations (Table 5). Mechanically harvested seed from the agricultural field differed from the mean of the parental mixture for two of the three traits. Only greenhouse plant height showed a significant difference between the two harvesting times, but both phenology and leaf shape were nearly significant (Table 5). In the first season after sowing, plants from the Winters population tended to flower earlier than plants from the non-local Santa Rosa Plateau population (mean phenology score of 3.01 and 2.10, respectively). Flowering phenology of plants from the first (early) mechanical seed harvest was 2.89, whereas the phenology score of the plants produced from second (late) mechanical seed harvest was 2.71 (Table 5), suggesting the early mechanical harvest collected seed with a greater proportion of early-flowering genotypes most prevalent in the Santa Rosa Plateau population, compared to seed collected in the later harvest.

Variance in phenology of plants produced from the original seed collections was compared to the variance in phenology of plants produced from each of the two subsequent mechanical seed harvests. Within-population variance in phenology was significantly reduced in the first harvest (Levene test;  $F_{1,530} = 5.93$ ;  $p = 0.015$ ) compared to the variance of the plants grown from the original seed collections. Variance in phenology in the second harvest was not significantly lower (Levene test;  $F_{1,530} = 0.60$ ;  $p = 0.439$ ) than variance in phenology in the original collection. Variance in phenology when the data from both harvests were combined did not differ statistically from the variance of the original two-population mixture (Levene test;  $F_{1,530} = 3.24$ ;  $p = 0.073$ ).

## Discussion

The large-scale production of native seeds using agronomic production techniques presents unique problems for the use and genetic management of locally adapted populations (Espeland et al., in press). The task of collecting large quantities of seed from native populations in the field for use in restoration projects is a difficult but direct way of assuring that collected seed are from locally adapted genotypes. Unfortunately, the process is labor intensive, time consuming, costly, and often does not yield the necessary quantities of seed for large restoration projects.

An economically reasonable alternative is to collect a comprehensive, random subsample of genotypes from the field population and increase the quantity of seed using well-established agronomic techniques. However, our results suggest that even with seed production taking place within the climatic region of the populations, growing conditions that differ from those of the natural habitat may favor genetic shifts toward a subset of the genotypes within the original seed collection. One potential consequence of such shifts is that the resulting seed stock may no longer be as well-adapted to the environmental stresses of the targeted restoration site (Rice 1995). However, the significance of the loss of within- or among-population genetic variation on the adaptive potential of mass-produced seed remains untested.

In this study, we found significant and rapid shifts in the apparent genetic composition of multi-population seed mixtures of one native perennial grass species in high resource and low stress growing conditions and significant but smaller shifts in a second species. Changes in genetic composition in a four-population seed mixture of blue wildrye were dramatic and related to differential population-level mortality, differential seed production

**Table 4.** Estimates of seed production by two populations of *Nassella pulchra* planted into four replicate blocks in an agricultural field at Winters, CA. Culms were counted on 60 plants per population in year one and 40 plants per population in year two; all plants were living at the time of both counts. Seeds per culm were counted on a subset of culms in year 1 and the same number of seeds per culm was assumed in year 2 ( $\pm$  SE).

Population	Year 1				Year 2		
	Seeds/culm	Culms/plant	Seeds/plant	% of total	Culms/plant	Seeds/plant	% of total
Winters (local)	56.9 $\pm$ 1.6	11.6 $\pm$ 1.1	660	39	59.6 $\pm$ 5.8	3391	42
Santa Rosa Pl.	47.7 $\pm$ 5.4	22.0 $\pm$ 1.2	1049	61	100.2 $\pm$ 5.9	4780	58
<b>F (df)</b>	<b>4.74 (1,99)</b>	<b>39.82 (1,115)</b>			<b>24.88 (1,75)</b>		
	<b>0.032</b>	<b>&lt;0.001</b>			<b>&lt;0.001</b>		

among populations, and tightness with which seeds were held in the inflorescence. Plant mortality in the first growing season, despite the benign growing conditions, was a strong and unanticipated influence on genetic composition of the harvested seed. The populations also differed in seed production and phenology of seed maturation, which came into play during mechanical seed harvest. In addition, after only a single growing season, selection imposed by abiotic, biotic, and management factors had significantly altered population genetic variation relative to the genetic constitution of the initial seed collection.

Significant differences in seed yield between sources and a strong influence of phenology on harvestable seed also existed within the two-population seed mixture of purple needlegrass. Population differences in survival were non-existent (all plants survived); genetic shifts were caused primarily by differences in seed production and the timing of seed maturity relative to harvest. The timing of mechanical seed harvest produced an apparent shift in flowering phenology, with the early harvest producing earlier-flowering

phenotypes, and the late harvest producing seed tending to be later flowering. While the phenology difference was not quite statistically significant ( $p = 0.054$ ), the three traits that showed the largest affect of harvest timing all shifted more strongly in the direction of the earlier flowering Santa Rosa Plateau population with the first harvest, providing stronger evidence for flowering time in relation to harvest being the cause of the genetic shift. Importantly, dissimilar harvesting techniques between the two species and chance weather events, such as high wind, very likely contributed to the differences observed and such considerations are likely to be common influences on phenological and genetic variation under all field situations. In addition to genetic shifts in mean flowering time, there was also a significant reduction in within-population variance for flowering between the early mechanical harvest and the original seed collection. However, the range of genetic variation from the original seed collection was apparently captured when the data from early and late mechanical harvests were combined.

**Table 5.** One-way ANOVA results and mean trait values ( $\pm$  SE) of *Nassella pulchra* original populations (source) and seed grown from two mechanical harvests of the same populations in four separate blocks in an agricultural field (blocks showed few differences and means for individual blocks are not shown). All seed (parents and progeny) were germinated in the greenhouse and later randomly planted into an agricultural field with data collected in both locations. Numbers in columns followed by different letters are significantly different at  $p < 0.05$ . N = number planted for each source; df is variable because all traits were not measured on all plants.

	N	Phenology score	Leaf shape	Height (cm)	Culms/plant
<b>Seed source</b>					
Parent 1—Winters (local)	80	2.10 $\pm$ 0.13 <sup>a</sup>	34.8 $\pm$ 1.1 <sup>b</sup>	28.6 $\pm$ 1.2 <sup>a</sup>	12.4 $\pm$ 0.9
Parent 2—Santa Rosa Pl.	80	3.01 $\pm$ 0.10 <sup>b</sup>	30.0 $\pm$ 0.6 <sup>a</sup>	46.2 $\pm$ 1.3 <sup>b</sup>	12.7 $\pm$ 0.7
<b>F (df)</b>		<b>30.74 (1,529)</b>	<b>19.69 (1,517)</b>	<b>57.05 (1,529)</b>	<b>0.06 (1,528)</b>
<b>p</b>		<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>0.809</b>
<b>Parents vs. field harvested seed</b>					
Parent mean	160	2.62 $\pm$ 0.09	32.1 $\pm$ 0.6	38.7 $\pm$ 1.2	12.5 $\pm$ 0.5
Harvest mean	400	2.80 $\pm$ 0.05	31.8 $\pm$ 0.3	42.2 $\pm$ 0.7	12.7 $\pm$ 0.3
<b>F (df)</b>		<b>6.60 (1,529)</b>	<b>1.04 (1,517)</b>	<b>12.90 (1,529)</b>	<b>0.06 (1,528)</b>
<b>p</b>		<b>0.010</b>	<b>0.308</b>	<b>&lt; 0.001</b>	<b>0.818</b>
<b>Harvest 1 vs. harvest 2</b>					
Field harvest 1 (early)	200	2.89 $\pm$ 0.17 <sup>b</sup>	31.2 $\pm$ 0.4 <sup>ab</sup>	44.3 $\pm$ 1.0 <sup>c</sup>	12.8 $\pm$ 1.4
Field harvest 2 (late)	200	2.71 $\pm$ 0.09 <sup>b</sup>	32.4 $\pm$ 0.4 <sup>b</sup>	40.3 $\pm$ 1.1 <sup>b</sup>	12.5 $\pm$ 0.4
<b>F (df)</b>		<b>3.60 (1,529)</b>	<b>3.73 (1,517)</b>	<b>8.74 (1,529)</b>	<b>0.16 (1,528)</b>
<b>p</b>		<b>0.054</b>	<b>0.054</b>	<b>0.003</b>	<b>0.688</b>

The importance of genetic shifts to the goals of restoration ecology will vary with the species involved and the amount of time since the agronomic populations were established. For example, in this study, the four blue wild-rye populations showed a very strong shift toward dominance by two populations within a single year while both purple needlegrass populations remained reasonably well represented in seed from both early and late mechanical harvests. Significant genetic shifts, particularly in blue wildrye, could be compounded over time depending on the agronomic techniques and if the same fields are used repeatedly for seed increase collections. Several methods could be used to prevent such an outcome including frequent replanting with wild-collected seed, greater attention to harvesting protocols that avoid over-sampling certain populations, and careful observation of the health of different populations within the plant mixtures. Given that natural selection is an inevitable and predictable outcome in any environment, any agronomic planting of wild seed for commercial seed increase is likely to have a “useful lifespan” of only a few years (Darris 2005).

To our knowledge, the potential for the large differences between field and farm environments to cause genetic shifts during the “seed increase” process has not been investigated for populations of native species used in restoration projects. However, agronomic research indicates that genetic shifts during seed production may be quite common in cultivars of crop species. For example, a cultivar of rye (*Secale cereale*), when grown for several generations in different regional environments, was no longer adapted to the original environment for which it was intended (Hoskinson and Qualset 1967). The “Balbo rye” cultivar was released in Tennessee but was later grown as far west as Colorado and as far north as Michigan and seeds produced in those differing environments were always sold as “Balbo rye”. After many years, when the seeds from the different environments were planted together in a common garden in Tennessee, striking differences were found in growth habit, plant height, and heading date, indicating that selection had altered the genetic composition of the original population (Hoskinson and Qualset 1967). Similarly, Fu et al. (2005) examined accessions of hard red spring wheat (*Triticum aestivum*) and found significant progressive loss of allelic variation over six growing periods dating back 100 years.

Other examples exist of genetic shifts in crop cultivar seed production occurring over very short periods of time. Standford et al. (1960) found that a single generation of increase of “Pilgrim” Ladino clover in different environments altered the genetic composition of the seed because of genotype by environment interactions in both male and female fitness components, producing genetic shifts for early flowering and for flowering intensity. The effect was greatest in the first year but continued in succeeding years (Standford et al. 1962). Fernandez et al. (2005) studied genetic shifts in two cultivars of slender wheatgrass (*Elymus*

*trachycaulus*), an outcrossing perennial grass commonly used in revegetation and reclamation projects. After two generations of cultivation, seed collection, and resowing, 92% of the original AFLP variation remained although each generation lost significant variation compared to the original seed collection. In a similar study on the same species, Fu et al. (2004) used an 11 population composite seed collection, which greatly reduced the loss of AFLP variation.

Our results indicate that populations of native species, when removed from their home site and grown in an agronomic environment to produce seeds, may experience significant selection pressures that may alter genotype frequencies in potentially unpredictable ways. The resultant population(s) may be less suited to the restoration site and higher mortality and lower adaptability may be a result, although this possibility remains untested.

## Recommendations

One goal of the restoration project manager is to avoid unnecessary costs associated with using germplasm that is poorly matched to the restoration site. If seeds are inadvertently mismatched to the restoration site as a result of genetic shifts during seed increase, restorationists are then faced with more costly alternatives. Below, we summarize several methods that can be used to avoid or minimize genetic shifts associated with seed increase:

1. Grow locally-collected seed in an environment as similar to the native environment as possible. For example, when increasing seed, coastal and inland populations or low and high elevation populations should be grown in settings as similar as possible to the selective environment they are adapted to. From the evidence in the current study, one important consideration may be pathogen resistance in populations from spatially proximate or similar environments.
2. When populations are mixed for the purposes of seed increase, select populations from a relatively narrow range of natural environments and then adhere to suggestion #1. The likelihood of generating a representative mix of genotypes in the seed population will be increased by using a mixture of genotypes that are all reasonably well-adapted to the growing conditions.
3. Do not rely on long-lived plants for continuous seed production. Replant the population frequently using original field-collected seed, not farm-produced seed. Perennial plants growing in agronomic conditions may very quickly “sort themselves out” with some genotypes surviving and others dying. The resulting population will reflect the subset of genotypes that are favored by conditions in the farm environment rather than the natural population, and the genetic make-up of seeds produced will be skewed toward those genotypes.

4. A knowledge of the breeding system of the species may help reduce genetic loss and achieve better habitat matching. For example, highly selfing species are not expected to generate new genotypic combinations as readily, and may be better able to maintain adaptive gene combinations. In contrast, outcrossing species may generate within and among population genetic combinations that facilitate more rapid habitat matching. While this may be a useful characteristic in the restoration site, it could present problems when the new genetic combinations are produced in an agricultural environment that is very different from the intended restoration environment.
5. Where possible, conduct multiple seed harvests to capture the range of phenotypic (and therefore genotypic) variation. When collecting seeds from seed increase fields, harvest at different times of the season, use different seed collection techniques, and combine the seed collected from the different harvests. Because loss of some genotypic variation during the seed increase process is likely, a seed collection protocol that samples the range of variation is a necessity.
6. Identify and anticipate sources of selection during the seed increase process and how both biotic (e.g., disease) and abiotic (e.g., severe weather) selective agents might influence differential seed production by individual plants. Field-to-field variation may create significant differences in the selection regime within the same population. Chance weather events can affect genotypes differently depending on phenological stage and result in greatly narrowed genetic variation in the collected seed.

It is unlikely that any approach, regardless of the care taken, will result in a seed population that completely mimics the original population. However, by recognizing the importance of unintentional selection and the potential for rapid genetic shifts in seed increase operations and instituting the protections listed above, we believe that the goal of maintaining well-adapted and genetically diverse populations of native species can be attained. Minimizing genetic shifts and maintaining genetic variation will help assure the adaptive potential of a restored population for future selective challenges and increase the probability of long-term restoration success.

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