The Willamette Valley Seed Increase Program

Developing genetically diverse germplasm using an ecoregion approach

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ABSTRACT

The goal of the Institute for Applied Ecology's Willamette Valley Seed Increase Program is to develop a supply of ecologically appropriate, genetically diverse native plant material for restoration of prairie ecosystems in the Willamette Valley. In creating restoration germplasm we seek to maximize genetic diversity while simultaneously protecting genetic integrity of extant native populations. In the absence of genetic data to guide appropriate movement of native seeds, we are testing the use of an ecoregion approach using a variety of research techniques. We collected seeds, defined preliminary seed transfer zones, and planted seed increase fields for each of 21 historically widespread, common species. We captured spatial and temporal genetic diversity by sampling from many populations per species over a 3-y period. Seed zone boundaries for each species were drawn at the scale of the ecoregion or smaller, depending on life history characteristics and potential for adverse genetic effects of translocation. To minimize loss of diversity through domestication selection, we planted increase fields using a novel design, the Diversity Enhancement Block. Seedlots from populations with different phenology or from different areas within the ecoregion were planted in separate adjacent blocks. This design allows harvest of each block separately as seeds mature, while still permitting plants from different regions of the valley to cross-pollinate and to produce crop seeds with maximum genetic diversity. All of our production fields have been entered into the Oregon Seed Certification Service Pre-Variety Germplasm program. We are looking for partners to participate in a buyer's cooperative.

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native plant material development, prairies, restoration genetics, seed transfer zones, domestication selection, Diversity Enhancement Block design

NOMENCLATURE

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Successful habitat restoration includes sufficient species diversity to create plant communities representative of the original habitat, resilient to environmental fluctuations, and capable of supporting a diverse assemblage of wildlife (Bradshaw 1987; Ehrenfeld 2001; Meninger and Palmer 2006). Within those species, the genetic quality of germplasm can be equally important in achieving success (Falk and others 2006). Restoration germplasm should be both locally adapted and genetically diverse (McKay and others 2005).

The use of locally adapted germplasm improves the chances of establishment and persistence on restoration sites (Gustafson and others 2005), while protecting genetic integrity of indigenous plant populations by preventing swamping of ecologically inappropriate genes (Lesica and Allendorf 1999;

Asclepias speciosa Torr. (Asclepiadaceae) in the Willamette Valley, Oregon. Photo by Matt Blakeley-Smith

Hufford and Mazer 2003; McKay and others 2005). Carefully targeted seed collection can also conserve genetic diversity of extant native plants.

Meanwhile, genetic diversity is required to ensure the evolutionary potential and long-term sustainability of restored populations through maximizing their adaptive capability (Newman and Pilson 1997; Moritz 1999; McKay and others 2005). There is a potential cost to developing germplasm from sources that are too local (Havens 1998), particularly for species that were historically widespread but have now been artificially fragmented into small, isolated populations. Such source populations may have experienced a loss of genetic diversity through genetic bottlenecks or a reduction in fitness due to inbreeding depression (Ellstrand and Elam 1993; Keller and Waller 2002). Previously connected populations that have been artificially isolated may require the re-establishment of gene flow to restore genetic diversity to prior levels (Newman and Tallmon 2001; Vergeer and others 2004).

Therefore, creating high-quality restoration germplasm requires a balance between maximizing genetic diversity by sampling from wide-ranging source populations while limiting the scope of collections to sources that are locally adapted. This balance can be achieved by using seed transfer zones. Seed transfer zones are geographic areas where adaptive genetic variation among populations is similar to patterns of variation in environmental factors (Johnson and others 2004), allowing safe collection and transfer of seeds with reduced risk of adverse genetic or ecological effects (Hufford and Mazer 2003).

Unfortunately, seed transfer zones have not been delineated for many native plants (Hufford and Mazer 2003) because of unavailability of genetic and ecological data and (or) the lack of time and resources to conduct the research (McKay and others 2005). Even when seed transfer guidelines have been established, acquiring high-quality native seeds for restoration can be difficult. Collecting seeds from the wild is cost-prohibitive and can negatively impact wild plant populations (Smith and others 2007). Moreover, assuming that growers accept the financial risks of producing native germplasm for a limited market (Smith and others 2007), purchasing those seeds requires accepting a level of risk due to the difficulty of verifying source locations and the possibility that commercially produced seeds have reduced genetic diversity (Knapp and Rice 1994; Rogers 2004).

The Willamette Valley Seed Increase Program, a partnership of the Institute for Applied Ecology's (IAE) Native Seed Network, the US Fish and Wildlife Service, and the USDA Natural Resources Conservation Service, has been wild-collecting seeds and cultivating native prairie species in western Oregon to develop a supply of ecologically appropriate, genetically diverse native seeds for the region. Our goals are: 1) to produce germplasm that is broadly applicable throughout the region; 2) to increase the availability and decrease costs of seeds for a diverse suite of native prairie species; and 3) to foster the local native seed industry by working with local seed producers.

MOTIVATION: PRAIRIE RESTORATION IN THE WILLAMETTE VALLEY

The Willamette Valley was historically a wide expanse of wetland and upland prairies, oak savannas, and oak woodlands (Habeck 1961; Johannessen and others 1971). Agriculture, fire suppression, land development, and other land-use changes have, however, reduced Willamette Valley prairies to less than 1% of their pre-European settlement extent (Wilson 1998; Floberg and others 2004), making them one of the most endangered habitats in North America (Noss and others 1995).

Fortunately, much prairie restoration has commenced over the last decade through collaboration among federal and state agencies, city and county governments, conservation organizations, and private landowners. Until recently, the emphasis of prairie restoration was establishment of native grasses, often resulting in development of monocultures of tufted hairgrass (*Deschampsia cespitosa* (L.) P. Beauv. [Poaceae]). While accomplishing the goal of establishing cover of native species, this does not result in the desired prairie plant community (Palmer and others 1997). Creation of functioning prairie habitat requires reintroduction of biological diversity to provide habitat structure, nectar sources, and other resources for wildlife (Howe 1994).

Restoration has been hampered in the region by limited availability of diverse native seeds, particularly seeds from flowering herbaceous plants (forbs) (Sinclair and others 2006). Prior to establishment of the Willamette Valley Seed Increase Program in 2005, the few native species available for purchase were expensive and derived only from areas near Portland, Eugene, and Marion County (Figure 1). For many species, seeds from a single source were the only germplasm available for use on restoration sites. Whether germplasm from these disjunct sources had sufficient genetic diversity or the adaptive capability for successful use in restoration region-wide was unknown.

Established seed transfer zones were not available for most native Willamette Valley prairie species. Many potential seed

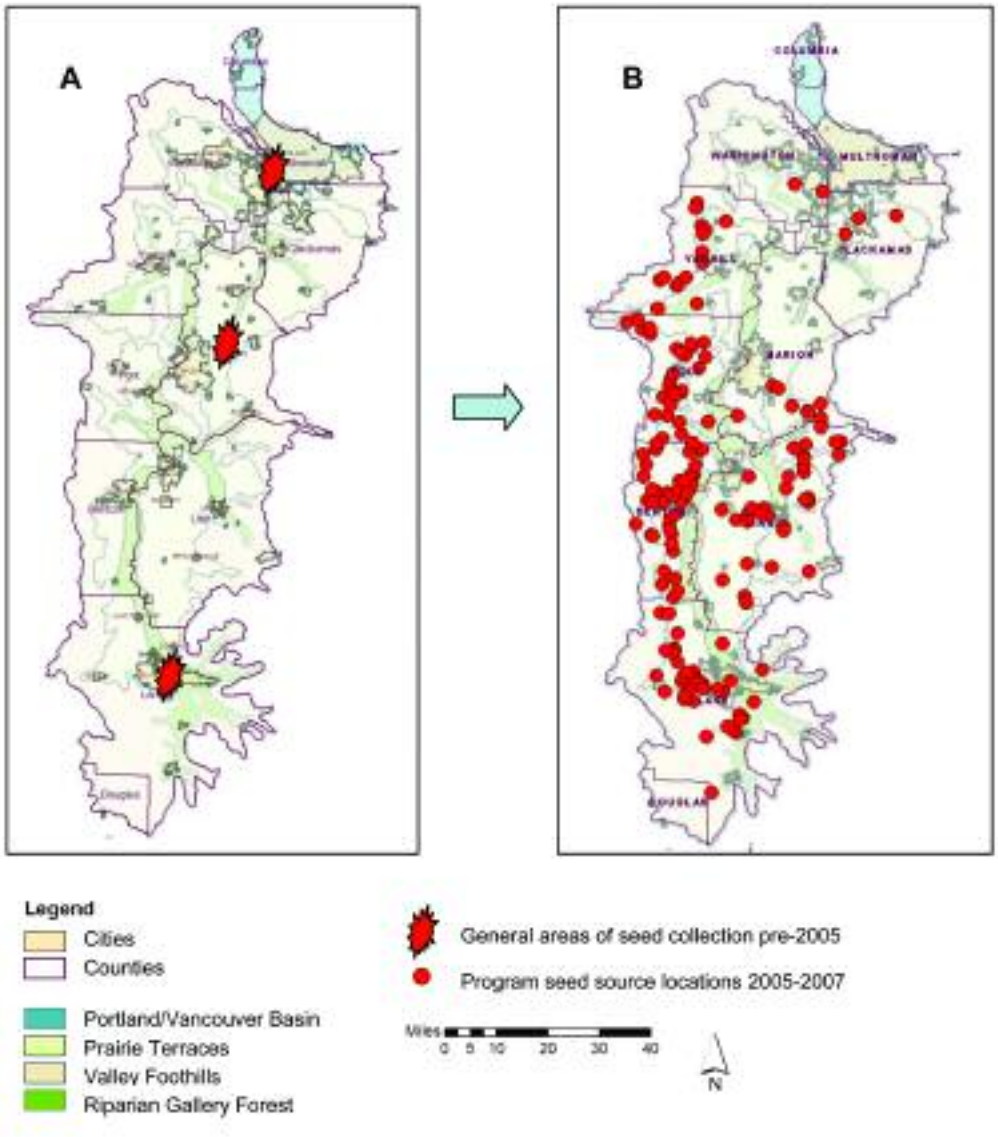

Figure 1. Prior to the establishment of the Willamette Valley Seed Increase Program, source locations for native prairie species were limited to 3 disjunct areas within the valley (A). In 3 y the program has increased the number of documented seed source locations to 207 sites spread throughout the ecoregion (B).

buyers, skeptical of the source identity and low genetic diversity of seeds harvested from large-scale production fields, were resorting to collecting and increasing their own seeds (Sinclair and others 2006). Local seed producers were hesitant to cultivate native species because of an unpredictable market and lack of data on species biology, germination requirements, and efficient harvesting techniques.

Here we describe how the Willamette Valley Seed Increase Program has met the challenges of: 1) selecting target species for collection and increase; 2) maximizing genetic diversity; 3) developing seed transfer zones; 4) maintaining diversity in production fields; 5) creating products that buyers can trust; and 6) creating a sustainable market for native seeds.

1. SPECIES SELECTION

Selection of species appropriate to the site is one of the most important steps in restoration planning (Rogers and Montalvo 2004). Our first task was to gather a committee of local restoration biologists, botanists, and seed producers to identify target species. The committee selected 21 upland and wetland prairie species with potential to provide a foundation for development of species-rich plant communities (Table 1; Figure 2). Selection criteria included that plants be: 1) common and historically widespread; 2) easy to collect and grow in agricultural production fields;

TABLE 1

Target species of the Willamette Valley Seed Increase Program and common-garden studies

Common name	Scientific name	Common-garden studies[z]
FORBS		
common yarrow[1]	*Achillea millefolium* L. [Asteraceae]	
showy milkweed[2]	*Asclepias speciosa* Torr. [Asclepiadaceae]	
denseflower willowherb[3]*	*Epilobium densiflorum* (Lindl.) Hoch & P.H. Raven [Onagraceae]	X
woolly sunflower[4]	*Eriophyllum lanatum* (Pursh) Forbes [Asteraceae]	X
Puget Sound gumweed[5]	*Grindelia integrifolia* DC. [Asteraceae]	X
barestem biscuitroot[6]	*Lomatium nudicaule* (Pursh) J.M. Coult. & Rose [Apiaceae]	X
bigleaf lupine[7]	*Lupinus polyphyllus* Lindl. [Fabaceae]	X
Spanish clover[8]*	*Lotus unifoliolatus* (Hook.) Benth. [Fabaceae]	
slender cinquefoil[9]	*Potentilla gracilis* Douglas ex Hook. [Rosaceae]	X
lanceleaf selfheal[10]	*Prunella vulgaris* L. ssp. *lanceolata* (W. Bartram) Hultén [Lamiaceae]	X
western buttercup[11]	*Ranunculus occidentalis* Nutt. [Ranunculaceae]	
straightbeak buttercup[12]	*Ranunculus orthorhynchus* Hook. [Ranunculaceae]	
Oregon saxifrage[13]	*Saxifraga oregana* Howell [Saxifragaceae]	X
meadow checkermallow[14]	*Sidalcea campestris* Greene [Malvaceae]	X
rose checkermallow[15]	*Sidalcea malviflora* (DC.) A. Gray ex Benth. ssp. *virgata* (Howell) C.L. Hitchc. [Malvaceae]	X
Hall's aster[16]	*Symphyotrichum hallii* (A. Gray) G.L. Nesom [Asteraceae]	X
GRAMINOIDS		
California oatgrass[17]	*Danthonia californica* Bol. [Poaceae]	X
tufted hairgrass	*Deschampsia cespitosa* (L.) P. Beauv. [Poaceae]	
dense sedge[18]	*Carex densa* (L.H. Bailey) L.H. Bailey [Cyperaceae]	
one-sided sedge[19]	*Carex unilateralis* Mack. [Cyperaceae]	
poverty rush[20]	*Juncus tenuis* Willd. [Juncaceae]	

Notes: Species with an asterisk are annuals. Superscripts indicate the photo number in Figure 2.
[z] Native Willamette Valley species included in common-garden studies conducted by the Native Seed Network in partnership with the NRCS Corvallis Plant Materials Center.

Figure 2. Target species in the Willamette Valley Seed Increase Program. Numbers correspond to species in Table 1. Photos by Saundra Miles (3, 5, 8, 9, 11, 13, 15, 16, 17, 18, 19, 20), Lynda Boyer (4, 14), Ben Legler (7, 10), Tom Kaye (1, 6, 12), and Matt Blakeley-Smith (2)

and 3) known to readily establish on restoration sites. We targeted flowering herbaceous plants, particularly those that are important nectar sources for local butterflies, because of their scarcity on the market. We included several graminoids because of their utility in restoration and the need for increased genetic diversity in commercially available local germplasm.

2. MAXIMIZING GENETIC DIVERSITY

Our program has made an unprecedented valley-wide effort to locate, map, and collect from as many source populations as possible for each target species. We attempted to capture spatial and temporal genetic diversity by sampling across a geographic and topographic range over several years. In 3 y we collected from 207 sites (see Figure 1), averaging 38 sites per species. This allowed us to collect sufficient volumes of stock seeds for establishment of production fields without placing undue collection pressure on a small number of populations (Falk 1991; Guerrant and others 2004).

At each site, we sampled from as many plants as possible across the entire extent of the population to capture within-population diversity and potential adaptations to microhabitat variation. We collected arbitrarily from plants of varying appearance, vigor, and fecundity to avoid inadvertent selection for only those genotypes that prospered under existing environmental conditions. We minimized impacts on source populations by visiting sites no more than 2 consecutive years and collecting no more than 50% of available seeds from perennial species and 25% from annuals. Our protocol follows guidelines of the West Eugene Wetlands Program (City of Eugene 2008) and should not negatively affect populations of perennial plants (Menges and others 2004).

Because population sizes differed from site to site, so did the amount of seeds available for collection. When combining accessions from several populations into a seedlot for cultivation, we balanced the contribution from each population so that seed mixes were not overwhelmed by the largest populations. For example, in many cases we had small, medium, and large volumes of seeds from different accessions. We then included all available seeds from small accessions in the seedlot, but limited the amount of seeds from medium and large accessions to a standard, balanced amount.

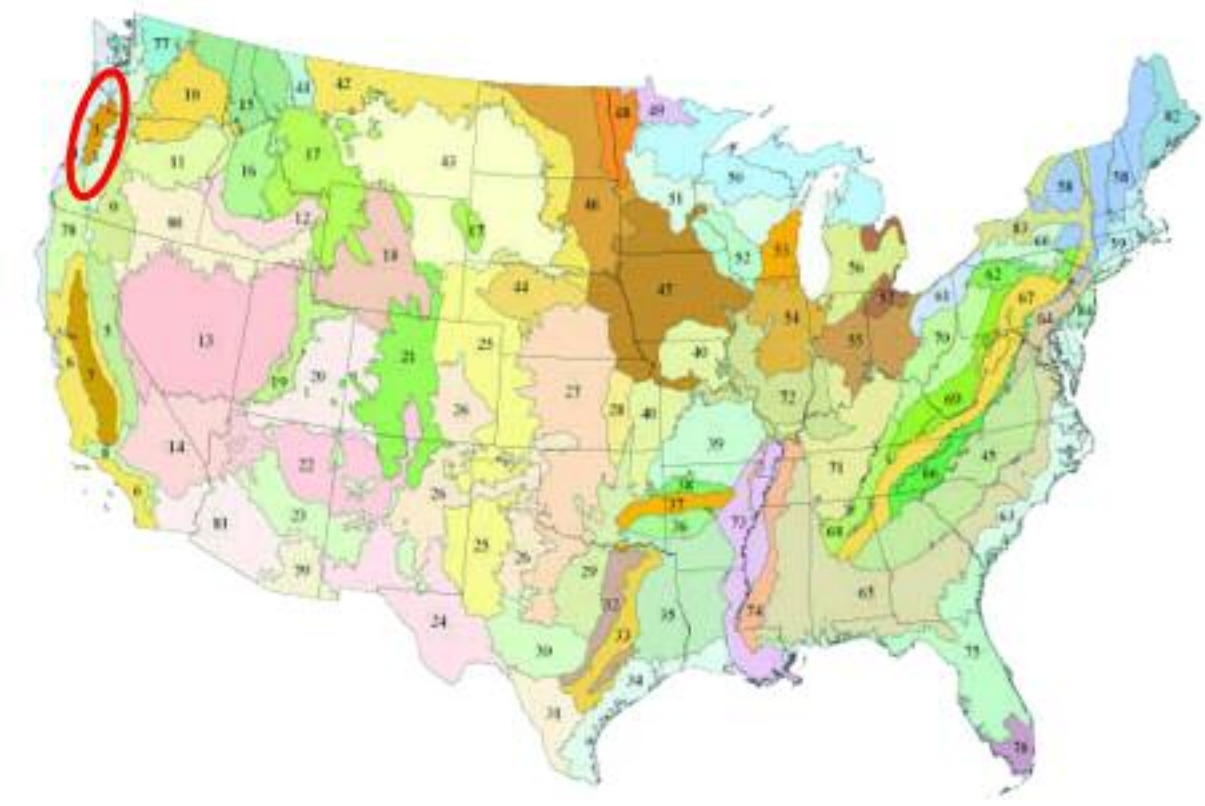

Figure 3. EPA Level III ecoregions of the US (Omernik 1987). The Willamette Valley Ecoregion is circled in red.

3. DEVELOPMENT OF SEED TRANSFER ZONES

Genetic guidelines

Our goal was to develop ecologically appropriate, genetically sound seed movement guidelines for the local native seed industry despite the absence of genecological data. Ecoregions have been applied as a practical starting point for delineating seed transfer zones when genetic data are lacking (MacKay 1993; Jones 2005; Withrow-Robinson and Johnson 2006). Using US Environmental Protection Agency (EPA) Level III ecoregions (Omernik 1987) may be biologically meaningful because their boundaries encompass geographic areas with similar geology, climate, vegetation, soils, wildlife, hydrology, and land-use patterns. Plants within an ecoregion, especially those that were historically widespread and that had high levels of gene flow, will likely share similar adaptations to these relatively uniform environmental variables, although this is not always true (Erickson and others 2004). The Willamette Valley ecoregion is an excellent candidate for a surrogate seed zone because of its moderate size (48 km wide and 161 km long [30 x 100 mi]), little topographic relief (61 to 335 m elevation [200 to 1100 ft], consistent soil pH, and uniform climate (PRISM 2008) (Figure 3). Moreover, the literature and common-garden studies for a variety of species support using the Willamette Valley ecoregion as a seed zone (St Clair and others 2005; Johnson 2008; Wilson and others 2008).

Despite evidence in favor of a single valley-wide seed zone, we were hesitant to universally apply this to all species in our program. Instead, we evaluated this approach on a species-by-species basis. For each species we reviewed literature to determine the likelihood of high historic levels of gene flow as well as evidence of geographic variation, local adaptation, and potential for hybridization, ploidy variation, or taxonomic uncertainty within the Willamette Valley that would necessitate smaller seed zones.

Once we summarized this information for a subset of "case study" species, IAE hosted a workshop to receive input about genetic, ecological, and economic issues on seed transfer guidelines from more than 60 local researchers, restoration practitioners, and native seed producers. The result was clear recommendations for 5 of the 10 case study species evaluated: *Lotus unifoliolatus* (Hook.) Benth. (Fabaceae), *Potentilla gracilis* Douglas ex Hook. (Rosaceae), *Prunella vulgaris* spp. *lanceolata* (Lamiaceae), *Elymus glaucus* Buckley (Poaceae), and Roemer's fescue (*Festuca idahoensis* Elmer ssp. *roemeri* (Pavlik) S. Aiken. (The public may comment on case study species information and workgroup recommenda-

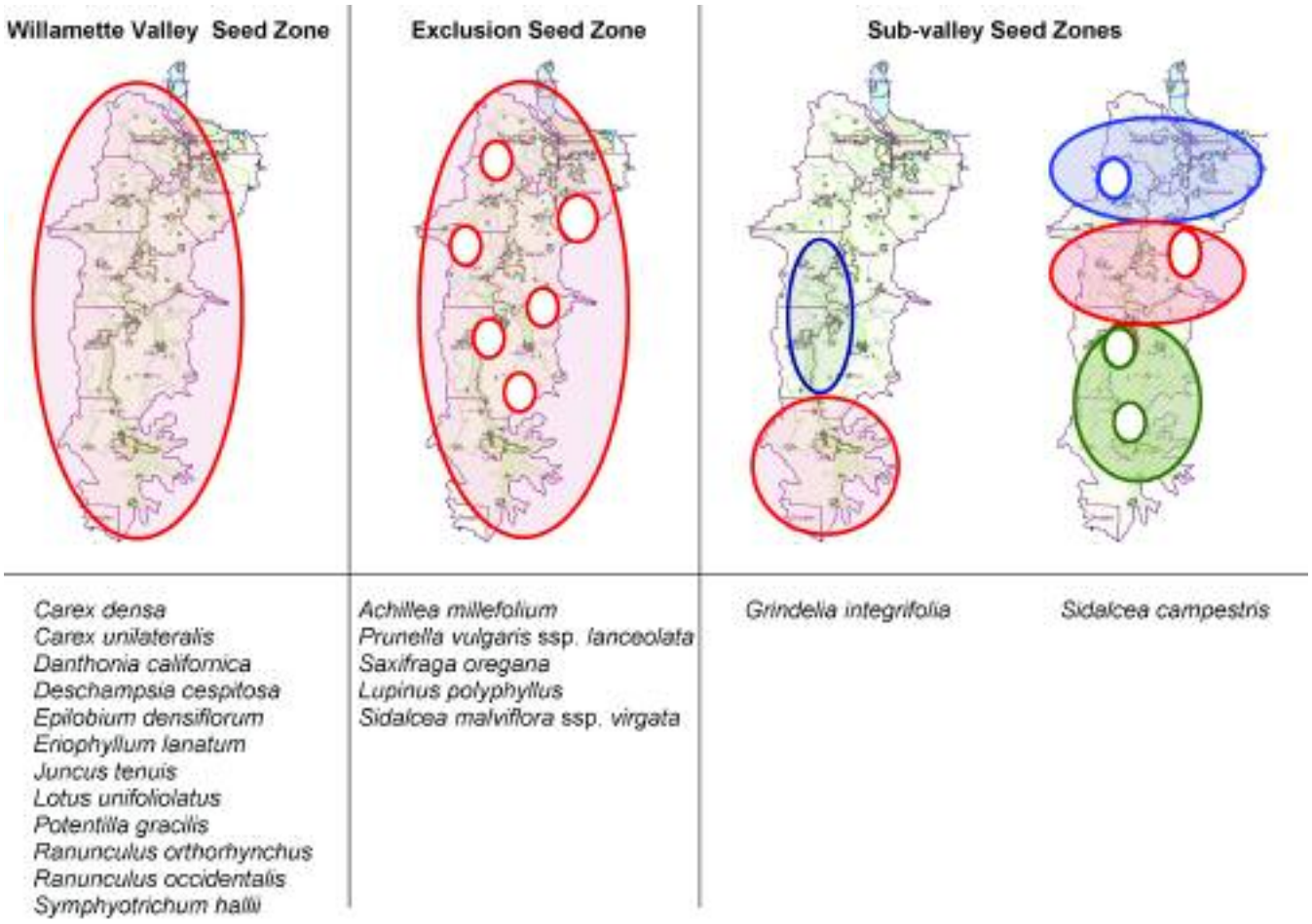

Figure 4. Seed transfer zones applied to target species in the Willamette Valley Seed Increase Program. Size and location of seed zones (hatched areas) and excluded populations (open circles) are schematic and do not represent precise geographic locations.

tions on the Native Seed Network website [Native Seed Network 2006].) Based on this work, we assigned each target species to one of 3 possible seed transfer strategies.

Willamette Valley Seed Zone

This seed transfer zone encompasses the entire ecoregion; seeds may be collected and transferred throughout the valley. This zone is appropriate for the sedges, grasses, and many of the forbs in our program (Figure 4).

Exclusion Seed Zone

This zone is also valley wide but excludes some populations of 5 species (described below) because of concerns regarding very local genetic variants or hybridization with nonnative subtaxa (Figure 4). In the absence of genetic data within the ecoregion, we usually rely on phenotypic variation for exclusion.

We excluded roadside populations of *Achillea millefolium* L. (Asteraceae) to avoid introducing nonnative varieties, which we feel would be more likely along transportation routes, even though no evidence yet exists that nonnative tetraploids are present in the Willamette Valley (Tyrl 1975). We excluded populations of *Prunella vulgaris* L. ssp. *lanceolata* (W. Bartram) Hultèn (Lamiaceae) if individuals exhibited leaf morphology suggesting hybridization with the introduced Eurasian subspecies *vulgaris*. Similarly, we excluded *Saxifraga oregana* Howell (Saxifragaceae) populations if leaf morphology and plant stature suggested hybridization with the native congener, wholeleaf saxifrage (*Saxifraga integrifolia* Hook. [Saxifragaceae]).

For *Lupinus polyphyllus* Lindl. (Fabaceae) we excluded populations from 2 sites because every plant had white flowers, rather than the predominantly blue. Flower color in this lupine is diverse, ranging from bright blue to gray and whitish brown, and this variability is apparent at the within-plant, within-

population, and between-population levels. Although white- and blue-flowered plants are not considered taxonomically distinct (Hitchcock and Cronquist 1973), we excluded white populations until more is understood about the origin and adaptive significance of flower color.

Similarly, we excluded populations of *Sidalcea malviflora* (DC.) A. Gray ex Benth. ssp. *virgata* (Howell) C.L. Hitchc. (Malvaceae) that are white-flowered, rather than the typical pink-flower, and populations of the "giant" form, rather than the more common shorter, less robust plants, until the genetic basis and adaptive significance of these morphologies can be assessed. We freely collected from the shorter, pink-flowered populations because, once widely distributed throughout the Willamette Valley, this species has been artificially fragmented into small isolated populations (Gisler 2007) that we suspect will benefit from restored gene flow.

Sub-Valley Seed Zone

For *Grindelia integrifolia* DC. (Asteraceae) and *Sidalcea campestris* Greene (Malvaceae), populations within the Willamette Valley are divided into 2 or more distinct seed zones (Figure 4). We assigned 2 seed zones (South and Mid-valley) for *Grindelia* because of concerns over possible hybridization with the weedy species Idaho gumweed (*Grindelia nana* Nutt. var. *nana* [Asteraceae]), which is native east of the Cascades but introduced into the Willamette Valley (Chambers 1998). Although Idaho gumweed is found throughout the valley, in northern counties it is thought to be locally naturalized in pastures and along roadsides while *G. integrifolia* remains in remnant low-lying wet prairie. Unfor-tunately, it has been suggested that all *Grindelia* populations south of Finley National Wildlife Refuge in Benton County may be hybrids (Severns and Villegas 2005). Therefore, we excluded roadside populations from both zones because they are more likely to include hybrids, and we will only outplant seeds harvested from the South Zone cultivation fields on restoration sites south of Benton County, never on remnant wet prairie sites harboring extant gumweed stands. Despite the complications presented by potential hybridization, we included *Grindelia* in the seed increase program because it is a dominant component of wet prairies and a critically important nectar source for a variety of endemic insects, including the newly rediscovered great copper butterfly (*Lycaena xanthoides* Boisduval [Lepidoptera: Lycaenidae]) (Severns and others 2006).

Sidalcea campestris, an uncommon endemic to the Willamette Valley and candidate for threatened status at the state level (Gisler 2004), has 4 reputed variants within the region. These include the typical form, an early-flowering form, and 2 morphological variants—the "Yamhill" form and the "Lebanon" form (Alverson 2006)—leading to concerns that these represent genetically distinct types with unknown geographic boundaries (Native Seed Network 2006).

In collaboration with Oregon State University scientists, we are researching the geographic distribution of morphometric and genetic variation in *S. campestris*. Preliminary data do not reveal any geographic pattern of grouping among populations (Lambert 2008), but do provide evidence of hybridization with *Sidalcea nelsoniana* Piper (Malvaceae) as well as some other unusual genotypes (Liston 2006; Lambert 2008). Northern populations contained the most "pure" *Sidalcea campestris*, presumably because congeners with *S. campestris* are absent (Gisler 2003). Therefore, we delineated 3 seed zones: North, Mid-valley, and South (Figure 4). Because of the higher likelihood of hybrids in the south, the South Zone collections were excluded from cultivation until further data indicate which populations can be safely included. To be conservative, we excluded populations that contained the atypical "Yamhill" and "Lebanon" variants within the Mid-valley Zone, even though DNA analysis suggests these may be pure *S. campestris*. We also excluded Benton County collections from the Mid-valley seedlot because some populations occur near congeners and may be vulnerable to hybridization. We hope to conduct further DNA analysis of populations in the South Zone and the Benton County area to determine whether we can include populations from these regions in our production fields. We will evaluate restoration sites for their proximity to extant populations and sow cultivated seeds only on those sites where the risk of undesirable gene flow is low.

Testing the ecoregion approach

In partnership with the NRCS Corvallis Plant Materials Center (PMC), we are conducting common-garden studies for 12 species to support the refinement of preliminary seed movement guidelines (Table 1; Figure 5). For each species, we are measuring a suite of phenological and morphological traits in progeny from 10 to 30 populations grown in a common environment to determine if differences among populations have a genetic basis. If genetic differences revealed among populations follow geographic patterns within the valley, smaller seed zones within the ecoregion may be necessary. Seed transfer zones for future grow-out could then be refined before our germplasm is released to the public.

Figure 5. Common-garden study underway for lance self-heal (*Prunella vulgaris*) at the NRCS Corvallis Plant Materials Center.
Photo by Amy Young

4. MAINTAINING DIVERSITY IN PRODUCTION FIELDS

Seed Increase in Agricultural Production Fields

We have initiated cultivation of our wild-collected seeds, planting a total of 2.8 ha (7 ac) of increase fields with 4 experienced Willamette Valley native seed producers and the NRCS Corvallis PMC. From the 17.7 kg (39 lb) of stock seed sown, we expect to harvest 1225 kg (2700 lb) of seeds during a 3-y period.

The Diversity Enhancement Block Design

Domestication selection can result in the loss of genetic diversity through inadvertent exertion of uniform selection pressures (Rogers 2004). When genetically diverse seeds, collected from many sites with a wide range of environmental conditions, are cultivated in one location, they are subjected to uniform conditions such as hydrology, soil type, weed competition, and farming practices. This can result in genetic shifts through selection for only those genotypes that succeed under a particular set of agronomic conditions (Knapp and Rice 1994). Additionally, when seeds from all populations are grown in a single field, competitively superior populations have the potential to overtake the field and dominate the genetics of the resulting harvest. Last, potential variation in the timing of plant maturation could mean that early- or late-ripening plants (or entire populations) would be excluded during a single, brief harvest period. This could result in low seed yields as well as a loss of genetic diversity in the harvested crop. Variation in phenology within a single field was likely to occur in our program because wild-collected seed sown in individual planting beds originated from diverse mixes of widespread populations.

We implemented 3 strategies to minimize the loss of genetic variation through domestication selection. First, we maximized germination success by providing wild seeds with any known treatments required to break dormancy, such as cold stratification or seed scarification. For species that were difficult to germinate or when little was known about germination requirements, we sowed seeds in individual containers in greenhouses and established production fields with transplants rather than direct-seeding. This prevented loss of genetic diversity through selection for successful germination under farming conditions.

Second, we avoided risking all wild-collected genetics in one opportunity by growing the same seedlot at 2 separate farms whenever possible. This subjected our diverse seed mixes to at least 2 sets of environmental conditions and farming techniques. Presumably some genetic variation will be lost at each farm. If selection pressures differ between farms, however, the harvested crops will contain different complements of the gene pool so that some of the original diversity is restored when the harvests are combined. The risk of complete crop failure is also reduced because it is less likely that both farms would experience adverse growing conditions.

Our third strategy was to plant fields with a novel planting design we call the Diversity Enhancement Block (DEB). In the DEB design, a single field contains seeds from throughout the seed transfer zone, but two or more accessions are planted in separate side-by-side blocks (Figure 6). Separate blocks may represent different geographic areas within the zone, different habitats, or a difference in phenology among source populations in the wild. If blocks differ in maturation rates in the agricultural setting, they can be harvested separately at the optimal harvest time for each. After harvest, seeds from both blocks are combined into a seed-zone-wide diversity mix.

This approach minimizes the loss of diversity from plants that mature before or after harvest time and allows the farmer to maximize crop yield. It also prevents competitively dominant populations from overtaking entire fields, allowing for greater representation of the full complement of populations sampled. Placing the separate blocks side-by-side allows outcrossing between plants from many source populations, theoretically producing crop seeds with maximum genetic diversity (Burton and Burton 2002).

During the first year of production we observed marked differences in phenology and general appearance among

blocks within the same field for some species. *Eriophyllum lanatum* (Pursh) Forbes (Asteraceae) was a particularly striking example: the North Block had noticeably more silvery-lanate pubescence, began flowering earlier, had lighter yellow flowers and lighter-colored seed pods than the South Block (Figure 6). These differences in adjacent blocks originating from different regions of the valley suggest the possibility of genetic differences with a large-scale geographic pattern.

Epilobium densiflorum (Lindl.) Hoch and P.H. Raven (Onagraceae) and *Ranunculus orthorhynchus* Hook. (Ranunculaceae), on the other hand, showed no differences among blocks in either appearance or maturation time. This was surprising for *R. orthorhynchus* because phenological differences among wild populations formed the basis of the DEB design, with seeds from early- and late-ripening populations planted in separate blocks. The blocks' similarity in maturation rate when planted in the same field suggests phenological differences among wild populations may be environmentally based rather than genetically determined for this species.

Continual cultivation can amplify the effects of domestication selection as the uniform selection pressures for conformance to agronomic techniques have more time to take effect. We are implementing 2 strategies to prevent the genetics of our cultivated germplasm from drifting too far from the genetics of wild populations over time. First, we plan to maintain fields in production for a limited number of generations. Second, we plan to supplement the genetics of wild-collected seeds on a regular basis.

The peer-reviewed literature provides little guidance for the optimal frequency of refreshing genetics during cultivation of wild germplasm (Smith and others 2007). Furthermore, although these supplemental collection efforts will be much smaller than the initial effort, additional collections could negatively impact wild populations. Therefore, we do not propose a standard protocol for all target species in the program. Instead, the frequency of genetic supplementation will depend on each species' life history and the population sizes available for sampling from the wild (Guerrant and others 2004).

5. CREATING A PRODUCT BUYERS CAN TRUST

All diversity germplasm production fields in the program are entered in the Pre-Variety Germplasm seed certification program of the Oregon Seed Certification Service (OSCS 2007).

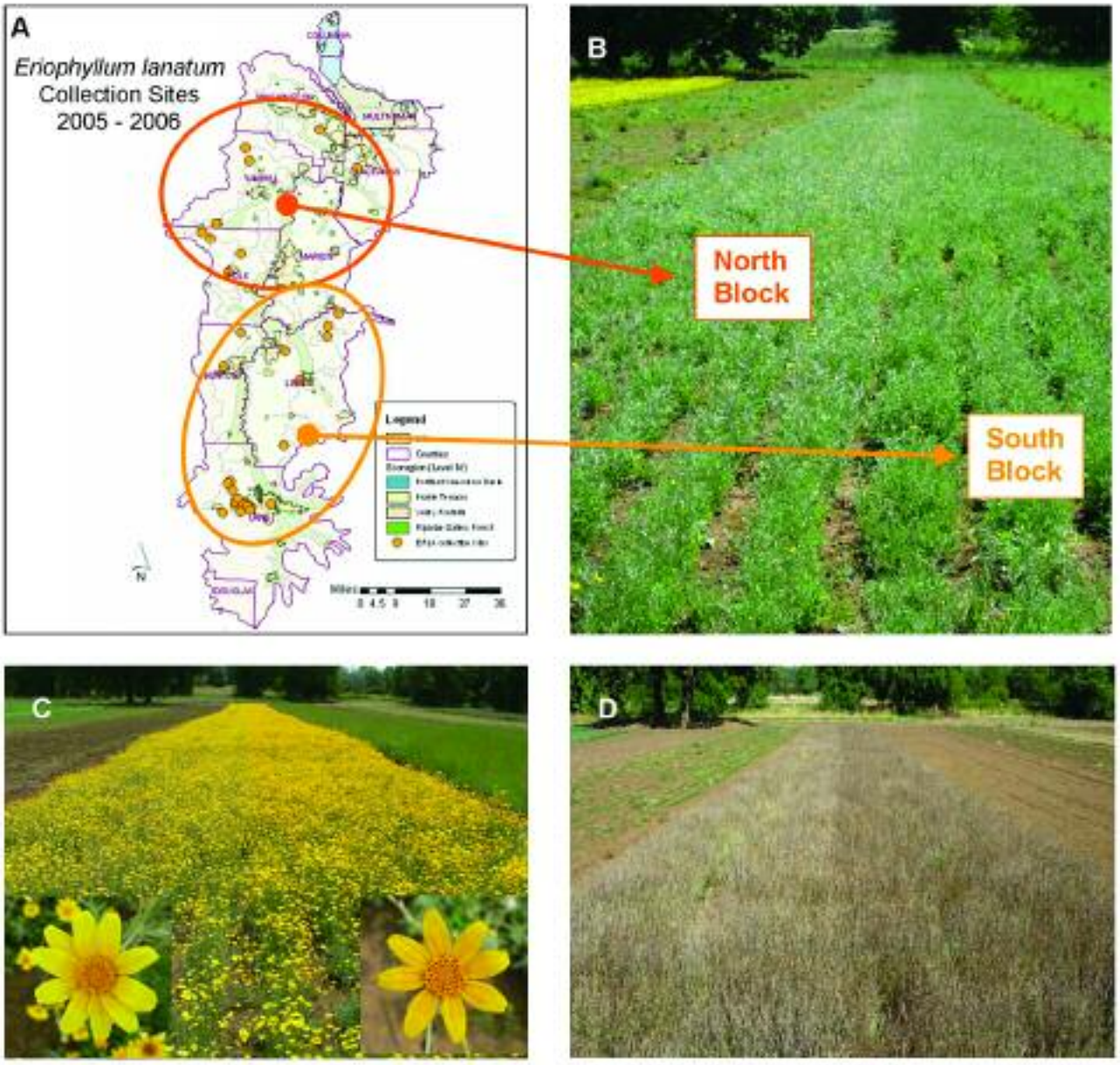

Figure 6. Woolly sunflower (*Eriophyllum lanatum*) field planted with the Diversity Enhancement Block design. Collection sites 2005–2006 (A). In the fall of 2006, seedlots from northern sources and southern sources were planted in adjacent blocks within the same field. Photo taken May 2007. (B) Photo taken June 2007 (C). Photo taken July 2007 (D). Photos by Lynda Boyer, Kimiora Ward, and Amy Young

The Diversity Enhancement Block planting design has been approved and incorporated into the accepted practices recognized by OSCS. Seeds harvested from production fields under the Native Seed Network Willamette Valley Diversity Germplasm name will therefore have third-party verification of source location and identification, and will be subjected to rigorous standards and testing to ensure purity and to quantify viability.

Very few native seed increase programs have taken steps to certify their seeds. Passing certification requires verification of species identification at seed collection sites, demonstration of a clean field history, and the implementation of field isolation distances to prevent introduction of inappropriate genes. Fields must pass a seedling and a pre-harvest inspection by the OSCS. After harvest, seeds must be cleaned in a certified warehouse and tested at the OSCS seed laboratory for purity, viability, and presence of weeds.

Restorationists can be confident of the source and quality of seeds that are purchased with a certification tag attached. The difficulty in verifying the source location of seeds collected without regulatory standards is not only a problem for restoration practitioners seeking to purchase ecologically appropriate seeds, it also deters cultivation of local ecotypes by commercial seed producers (Smith and others 2007). This makes the additional expense and coordination required for certification well worth the effort.

6. CREATING A SUSTAINABLE MARKET FOR NATIVE SEEDS

We have made a significant investment to create high-quality germplasm that is genetically diverse and ecologically appropriate for use in prairie restoration throughout the Willamette Valley. We were successful because of collaboration with our partners, including the US Fish and Wildlife Service, the USDA Natural Resources Conservation Service, and more recently, the Oregon Department of Transportation (ODOT).

Because our partners pooled financial resources and coordinated projected restoration seed needs, the resulting economy of scale allowed us to plant a large acreage of production fields that should yield high returns. The US Fish and Wildlife Service and IAE will outplant these crops on approximately 486 ha (1200 ac) of restoration sites on private lands participating in the NRCS Wetland Reserve Enhancement Program (WREP), as well as on a mitigation site managed by ODOT.

The majority of our production fields are currently grown through contracts with local seed producers. To make this program sustainable and pay for the costs of maintaining fields in the future, we are developing a Native Seed Buyers Cooperative. We hope to increase the number of participating organizations so that we can further combine our production efforts, plant larger fields, and reduce the cost of native seeds. Participants of the buyer's cooperative who conduct prairie restoration using certified Native Seed Network Willamette Valley Diversity Germplasm can have increased confidence they are using high-quality seeds that are source-identified, regionally adapted, and genetically diverse.

Pooling the needs of many seed buyers has the added benefit of allowing communication of future needs to local seed producers in bulk, making the market more predictable and cultivation of native species less risky. The demand for genetically diverse, source-identified, certified seeds among federal restoration practitioners is on the rise (Smith 2008), and land management agencies may soon require that mitigation and restoration projects use certified seeds in order to receive funding. As the market for native species continues to expand, local seed producers may be motivated to grow native plants on speculation instead of by contract. This increase program could then potentially continue to operate independently by private seed producers.

SUMMARY AND CONCLUSIONS

Germplasm developed by the Willamette Valley Seed Increase Program is unique because great effort has been made to: 1) capture and maintain genetic diversity through every step of germplasm development from seed collection to field production and harvest; 2) protect the genetic integrity of locally adapted native populations by developing appropriate seed movement guidelines; and 3) verify the source and quality of our seeds by means of certification through the Oregon Seed Certification Service PreVariety Germplasm Program.

Like many other restoration practitioners, we were faced with budget and timing constraints that meant we could not conduct research to delineate genetically based seed zones before we began seed collection and established production fields (McKay and others 2005). However, we plan to use the first few harvests from our production fields on highly degraded sites in which prairie habitat is being re-established from agricultural grass seed fields through the USDA NRCS Wetland Enhancement Restoration Program. Initiating new populations on highly degraded sites is one condition where it may be advisable to mix germplasm

from many sources (Lesica and Allendorf 1999), thereby maximizing genetic diversity so that restored populations can adapt to the novel environment or the presence of competitive introduced species.

This set of circumstances has provided us the opportunity to make unprecedented strides in developing regional germplasm for a suite of native species within the time frame and financial constraints of a typical "results oriented" restoration funding scheme. The fact that our first harvests will be sown on highly degraded sites affords us extra time to simultaneously acquire funding and opportunities to conduct the genetic research needed to establish sound seed movement guidelines. We expect that much of this research will be completed and seed zones re-evaluated before germplasm is released to the public and potentially sown on less degraded sites that might be more sensitive to the effects of genetic provenance.

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