OREGON DEPARTMENT OF AGRICULTURE

NATIVE PLANT CONSERVATION PROGRAM

Cultivation and Propagation Studies for Large-Flowered Wooly Meadowfoam-Year 2: *Seed Bulking*

Submitted by the
Oregon Department of Agriculture to
U.S. Fish and Wildlife Service, Region One
(OR-EP-2, Segment 21)
April, 2012

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INTRODUCTION

Limnanthes floccosa ssp. *grandiflora*[2] (or large-flowered wooly meadowfoam; Fig. 1) is a short-lived, semi-autogamous annual species of significant conservation concern, known only from the deeper portions of vernal pools in the "Agate Desert" area of Jackson Co., Oregon, with an historic range that may have originally been as large as 130 km² (USFWS 2006). Its current distribution is now smaller (OFP 2011), likely due to development in the Rogue Valley, with plants restricted to only a few remaining pools. The *grandiflora* subspecies is an ORBIC List 1 taxon, with a G4T1/S1 ranking (ORNHIC 2007), indicating it is critically imperiled throughout its range. In addition, *L. floccosa* ssp. *grandiflora* is listed as *endangered* at both the federal and state levels.

Figure 1. Flowers of *Limnanthes floccosa* ssp. *grandiflora.* (Photo credit: M. Carr).

Seeds of this extremely local endemic germinate in late winter to early spring under submerged conditions (Meyers, pers. comm.), plants grow and bloom in April,

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[2] Recent studies have determined that the meadowfoam populations historically known as "*L. floccosa* ssp. *grandiflora*" are best treated as *L. pumila* ssp. *grandiflora* (see Chambers and Meyers 2011), based on a comprehensive molecular phylogenetic evaluation of the genus by Meyers (2010). To remain consistent with the first year report for this project (i.e., McGonigle 2011), the "old" name is retained here, with the caveat that ODA will be using the "new" name in future reports and publications. Since "floccosa" means "wooly," the common name of the species may also need to changed, perhaps to the *large-flowered dwarf meadowfoam*!

and fertile seeds are produced and plants senesce as pools dry by late spring and early summer. Improving our understanding of the reproductive ecology of the species, and ways to use this knowledge to improve restoration and management prospects for remaining populations, has been the impetus for this and earlier work by ODA. Morphological, genetic, and evolutionary studies of *Limnanthes floccosa* have shown it to be a highly polymorphic, taxonomically complex species, with meadowfoam specialists traditionally recognizing five subspecies (Arroyo 1973; Ornduff 1993; Oregon Flora Project 2011; Meyers, Liston, and Meinke 2010, 2012), i.e., *L. floccosa* ssp. *floccosa, L. floccosa* ssp. *californica* Arroyo, *L. floccosa* ssp. *bellingeriana* (Peck) Arroyo, *L. floccosa* ssp. *grandiflora* Arroyo, and *L. floccosa* ssp. *pumila* (Howell) Arroyo. With the exception of *L. floccosa* ssp. *floccosa*, the subspecies of *L. floccosa* are considered rare, or are believed in danger of extinction and formally protected by state or federal authorities (see USFWS 2006; ORNHIC 2007; ODA 2012).

In addition to the intrinsic value of all native plants, the *L. floccosa* complex and related species are of scientific interest, being the subject of many landmark genetic and evolutionary studies (see above references and their bibliographies). Moreover, conserving wild *Limnanthes* populations also has commercial implications, with regard to the unique oil generated by their seeds (Meyers and Liston 2010). Unlike the seed-based oils of other plants, *Limnanthes* seeds produce a very long chain oil with novel physical and chemical characteristics, making it extremely stable at high temperatures and pressures (Miller et al.1964; Isbell 1997). As a result, meadowfoam oil is a valuable alternative for sperm whale oil (Gentry and Miller 1965), previously used as a premium industrial lubricant but no longer available due to the moratorium on cetacean harvests. *Limnanthes* oil has potential as a raw material in many products, including lubricants, cosmetics, waxes, and polymers (Erhan, Kleiman, and Isbell 1993). Research on commercial meadowfoam seed oil production began in the late 1950s, with *L. alba* x *L. floccosa* ssp. *grandiflora* cultivars having been grown on a limited scale for more than 25 years in Oregon. Today, meadowfoam plays an increasingly important role in Willamette Valley agriculture, where it is primarily grown as a rotation crop by grass seed producers (Meyers and Liston 2010).

PROJECT GOALS

Through the **first year of funding for this study** (under OR-EP-2, Seg. 20), we visited all accessible sites currently supporting populations of *Limnanthes floccosa* ssp. *grandiflora*, collected seed samples for greenhouse work (Fig. 2), observed pollinators during field trips, and made assessments of preferred habitat, hydrological relationships, and distribution in the vernal pool environment—see McGonigle (2011) for results and discussion. Seeds from sampled populations were brought back to greenhouse and growth room facilities used by ODA at Oregon State University (OSU), and a series of plants were grown for each wild population. We used plants from these grow-outs (completed in 2009 and 2010) to:

- evaluate the breeding system of *L. floccosa* ssp. *grandiflora* in the insect-free greenhouse environment, comparing our data with observations of earlier workers (Arroyo, 1973);
- contrast the seed production rates from the different geographic races within the Rogue

Valley to determine if any potential productivity differences exist between natural populations;

- determine reasonable and cost-effective methods for large-scale cultivation in the greenhouse, for farmed seed production;
- assess potential differences between plants grown from wild-collected (and potentially outcrossed seed) with inbred seed collected from greenhouse plants, to look for inbreeding depression; and
- evaluate the best methodology for long-term storage of greenhouse-produced seed.

Figure 2. Collecting *Limnanthes* seed in the Rogue Valley, Jackson Co., Oregon. (Photo credit: J. McGonigle)

Results and discussion of the above topics have been presented in McGonigle (2011).

The **second funding year of the project** (covered through OR-EP-2, Seg. 21) focused on **seed bulking**, i.e., growing large numbers of plants under cultivation for seed harvest, in this case following the protocols discussed in McGonigle (2011). This was identified in the *Section 6 project statement* accompanying the FY2011 federal assistance agreement as the primary objective of the Seg. 21 work. The use of bulked seed to establish new populations of rare or endangered species, or to augment existing (but waning) populations, is recognized as an important tool for restoration ecologists. Assuming cost-effectiveness and biological/ ecological feasibility, using cultivated seed to reestablish native annuals, such as *L. floccosa* ssp. *grandiflora*, can be a useful approach (Kramer and Havens, 2009).

Our goal here was to produce **at least 60,000** *Limnanthes floccosa* ssp. *grandiflora* seeds in a single grow-out—the results of this effort are presented and discussed in this report. The 60,000 seeds we hoped to produce would be destined for subsequent augmentation work (via sowing) on lands managed by one or more partner agencies or groups (see below). As with most rare plants, information on the reproductive ecology and seed biology of *L. floccosa* ssp. *grandiflora* is critical to the development of (1) management plans for remaining populations, and (2) protocols to be used in any re-introduction or related recovery projects for the subspecies. Site selection and outplanting timing would be based on information gathered in the first year of this project, as well as through consultations with partner agencies and meadowfoam experts (notably Dr. Stephen Meyers at OSU and TNC staff in Medford).

Outcomes adjustment. In the original project proposal for the second year of this work (under Seg. 21), the focus was clearly on seed bulking through greenhouse grow-outs, as discussed above. However, we also planned to initiate a small-scale, preliminary re-introduction effort during this phase of the work, using seed previously acquired in 2008, focusing on vernal pool habitat owned and managed by TNC, BLM, or possibly the Oregon Dept. of Transportation (ODOT) or other agencies. The primary goal here was to get partner "buy-in" of the overall project, by (1) identifying and discussing with them management goals for ssp. *grandiflora* (and how land managers might participate), and (2) selecting potential sites and negotiating access for some initial field work after 2010.

The longer-term objective, of course, was to establish contacts and initiate pre-planning for a broader re-introduction or augmentation program after 2012, in part making use of the bulked seed expected to be produced through the Seg. 21 funding, as well as additional seed that could potentially be produced in the future (using the protocols established here). However, the second year for the current project (under Seg. 21) was funded at just under 90% of our budget request, which required some adjustments. To offset the reduced federal funds, we elected to postpone the test augmentation work until late 2012 or early 2013 (assuming appropriate support would be available at that time, federal or otherwise), considering this was a minor part of the overall Seg. 21 project plan. However, we did move forward in discussion in 2010 with BLM, TNC, Oregon State Parks (OPRD), ODFW, and ODOT, in hopes of identifying potential sites for meadowfoam population augmentation. The goal was to pursue this further in 2012 or 2013. If other or better options presented themselves sooner, we hoped to go ahead with some preliminary seed sowing at one or more selected sites this winter (2012-13), depending on staff and site availability.

SEED PRODUCTION PROTOCOLS

Selection of seed donor populations and seed collection. An important goal of the first year of this project was to try and identify which wild populations of *Limnanthes floccosa* ssp. *grandiflora* might be the most appropriate as seed donors for the seed bulking work. And then to maximize the genetic diversity of bulked seed, we planned to include multiple donor populations representing several germplasm lines, with those populations then ranked (based on the data from the first year of the project) according to overall *vigor and fecundity* (USFWS 2006; McGonigle 2011), defined by germination rate, number of flowers per plant, number of seeds produced per flower, and seed weight—see pp. 26-28 in McGonigle (2011) for relative performance data for tested populations. The seed bulking work would then stress the use of propagules from the most vigorous wild populations available.

Upon visiting 30 historic localities for *L. floccosa* ssp. *grandiflora* during 2010 field surveys (see McGonigle 2011), ODA botanists located only 19 sites with extant populations. Many of these were on private land with no access, or were considered too small to serve as seed donors for seed bulking work. After field evaluations were complete, seeds were collected (with permission) from public land or TNC property at five separate sites—see Appendix 2 in McGonigle (2011) for details. These were *Agate Desert Preserve* (TNC), *Denman Wildlife Area* (ODFW), *Jackson Co. School* (Jackson Co.), *Savannah Whetstone Preserve* (TNC), and *Weigh Station* (Jackson Co.).

Seeds from four of the sites (Denman was excluded) were ultimately used for the initial greenhouse cultivation work described in McGonigle (2011), which focused on germination requirements, breeding system, and cultivation protocols. A total of 567 seeds were collected in 2010 from the four sites to grow plants in support of this work, representing a minimal take, considering the sampled populations were estimated to have supported over 100,000 flowering plants. Considerably more seeds would be needed in 2011 for the seed bulking work (see below).

Population comparisons. Data from the first year of the study (McGonigle 2011) indicated that plants from the Jackson Co. School and Agate Desert Preserve study sites were the most vigorous and fecund, perhaps not surprising, considering that these were the largest natural populations located (ca. 50,000 flowering individuals at each in 2010). Accordingly, these study sites were emphasized in the 2011 seed bulking growout, with roughly 70% of the plants grown originating from seeds of these two populations (collected in spring, 2011). In order to include all four study populations (in the interest of genetic diversity in the bulked seed lot), ~30% of the seeds used were from the less vigorous Savannah Whetstone Prairie and Weigh Station study populations—about half of these were wild-collected in 2011, with the rest previously taken from selfed plants that were grown in the greenhouse in 2010 (during the first year of the project).

Germination data reported by McGonigle (2011) showed variability in germination percentages for wild seeds from the different study sites, ranging from the Jackson Co. School site at 47% to the Savannah Whetstone Preserve at just 14%. Collectively, germination in the lab for all sites was well under 50%, regardless of the methods used. When we also considered the potential for seedling losses in the greenhouse due to disease and other factors, we anticipated we would need to start with about 5 seeds for every plant we expected to survive and reproduce in the greenhouse. To reach our goal of at least 60,000 cultivated seeds, we planned on using around 8,000 seeds from the four study sites to grow the plants for the seed bulking work. After collection, these were mechanically cleaned at the OSU Seed Laboratory, to remove the outer husk and chaff (i.e., the calyx) and break up the one-seeded nutlets (which occur in attached clusters of up to 5 per flower).

GREENHOUSE WORK AND SEED BULKING RESULTS

Cultivation. After the study site material had been collected and sorted for cultivation work, we ended up with ca. 8,850 apparently viable seeds available. We used the germination and potting protocols described in McGonigle (2011), staring the first week of August, 2011, and then culled any diseased and obviously unfit seedlings and young plants during the initial two weeks of greenhouse cultivation, leaving **1,624 plants** in individual 4-inch pots (in a standard loamy-mix potting soil). These were grown at ca. 65-75º F, thoroughly watered daily, fertilized every four to six weeks (DynaGro liquid micronutrient fertilizer), and maintained under an artificial 16 hour day length to supplement the ambient natural lighting. The grow-out was terminated in late October.

Reproductive yield: 2010 versus 2011 data. The 2011 seed bulking growout (Fig. 3) produced an estimated **70,992** *Limnanthes floccosa* ssp. *grandiflora* seeds. These were distributed (by study population) as follows: *Weigh Station* (12,848 seeds); Savannah Whetstone Preserve (4,012

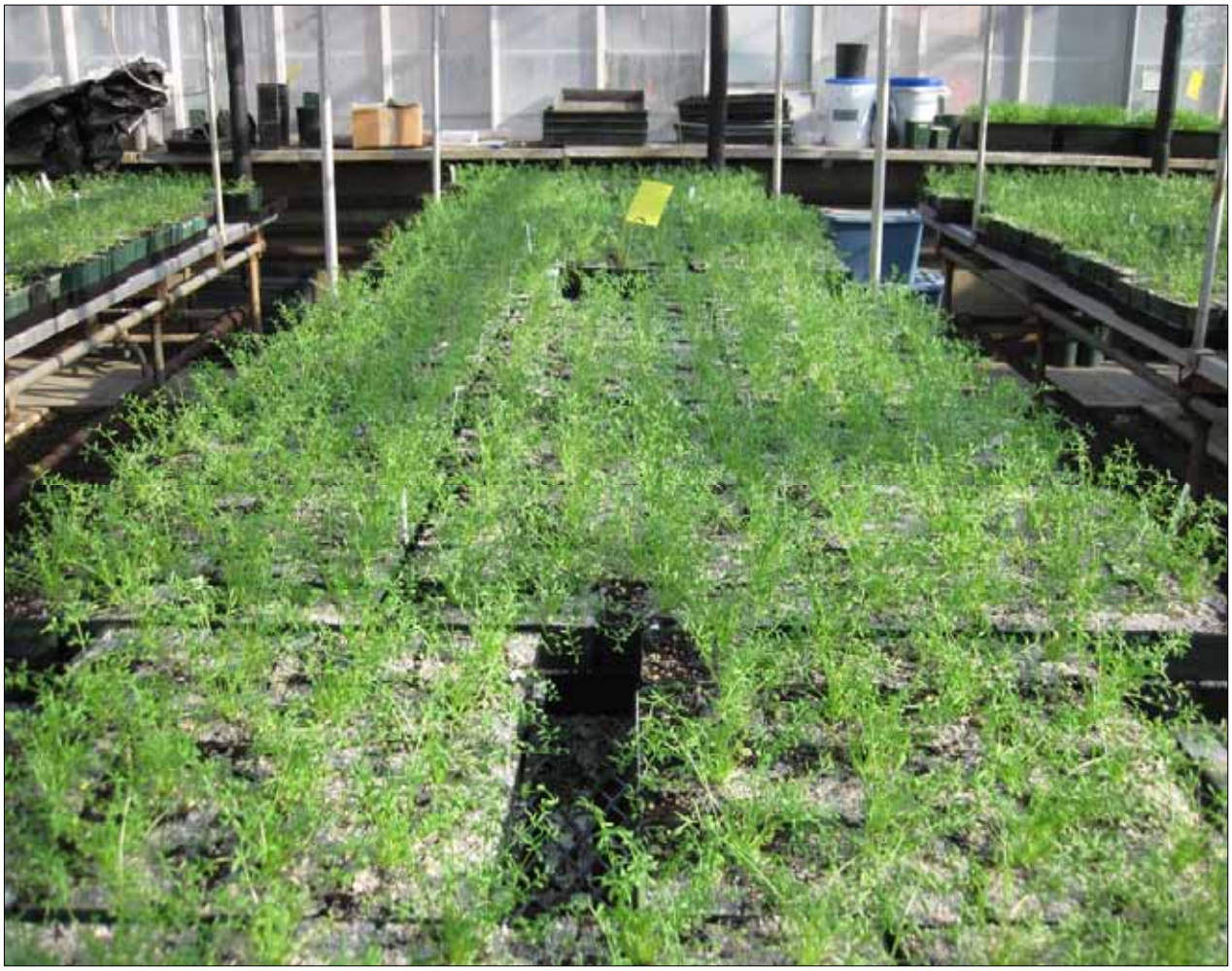

Figure 3. *Limnanthes floccosa* ssp. *grandiflora* plants being grown for seed bulking in OSU greenhouse in Corvallis (September 11, 2011). (Photo credit: ODA staff)

seeds); *Jackson Co. School* (24,852 seeds); and *Agate Desert Preserve* (29,280 seeds). Seeds have been processed and are in dry storage at OSU. Output data for the 1,624 plants included an estimated total of 33,118 fruiting calyces (Fig. 4) for the growout, or about *20.4 flowers per plant* (data for all populations lumped). This contrasts with the roughly 23 to 31 flowers per plant (pooled average = 27.7) observed during the more limited 2010 cultivation work (McGonigle 2011). Reasons for the reduction in flower number during the seed bulking work are not entirely clear, as the greenhouse protocols for both years are believed to have been very similar. The primary difference was time of year, with the 2010 plants germinated in early June and grown through late August (flowering largely in July), while the 2011 plants were grown from early August through October or so (with most of these flowering in September).

Although supplemental lighting (high pressure sodium) was used on the 2011 seed bulking plants, to make up for the shortening day length of late summer and early fall, this may not have been enough in the open greenhouse environment to completely offset effects of the natural

photoperiod decline. By mid-July, 2010, climate records show that day length would have been around 15 hours and 10 minutes in Corvallis, while in mid-September, 2011, the time from sunrise to sunset was roughly 12 hours and 30 minutes (a difference of 2 hours and 40 minutes).

Figure 4. Dried calyces (with seeds) from *Limnanthes floccosa* ssp. *grandiflora* plants grown for seed bulking (December 5, 2011). Seeds are in storage at OSU. (Photo credit: ODA staff)

The genus *Limnanthes* is comprised of long-day annuals (Erwin and Warner 2002), i.e., plants that normally initiate reproduction when the natural photoperiod is increasing, generally during the weeks just prior to the summer solstice (OSU 2012). The fact that the seed bulking plants were grown when natural photoperiods were declining, and well past the solstice (in August and September), may have slowed their flowering response despite the added lighting, ultimately resulting in the 20% or so reduction in flowers per plant recorded in 2011 *vs.* 2010.

Conversely, flowers from the 2011 plants were *34% more fecund* than those grown the year before, averaging 2.15 seeds per flower (n=200 calyces measured) under the shorter day length, as opposed to 1.42 seeds per flower (n=63) for plants grown in the early summer of 2010 (McGonigle 2011). The 2011 greenhouse plants likewise produced *more seed per flower* than plants of open-pollinated, wild populations (i.e., 1.94 seeds, n=63; from a mix of plants from the

four study sites), also sampled in 2010. This is a bit more perplexing, since higher seed production per flower and increased flowering would intuitively seem connected, presumably associated with the longer photoperiods that promote reproduction in spring annuals. For example, in *Floerkea proserpinacoides* (a close relative of *Limnanthes*), Smith (1983) demonstrated that seed set per flower was indeed highest during the latter spring, and then significantly diminished as the summer progressed and flowering declined.

However, temporally-related decreases in flower size in relation to resource availability (including photoperiod) are well-known, and have been shown in many plants to improve seed yield through increased autogamy (Cruden 1973). Brock et al. (2009) demonstrated, in the short-lived annual *Arabidopsis thaliana*, that seasonal abiotic variation can influence floral morphology, and that photoperiod was correlated with the length of petals, stamens, and pistils, which changed in relative size for flowers growing in waning day lengths. Meinke (unpublished) has observed this in the greenhouse for several annual, selfing species of *Mimulus* as well. A reduction in flower size over time can lessen the degree of physical separation (known as *herkogamy*) between male and female reproductive parts in flowers, which in self-pollinating plants, such as *Arabidopsis* (or *Limnanthes*), can improve the efficacy of autogamous seed production.

Figure 5. Flowers of *Limnanthes floccosa* ssp. *grandiflora* are more apt to be pollinated by insects than other selfing subspecies of *L. floccosa*, since flowers are larger and the corolla has an open phase more conducive to occasional cross-pollination. (Photo credit: M. Carr)

So is it better to conduct a seed-bulking growout for *Limnanthes floccosa* ssp. *grandiflora* during spring and early summer (as in 2010), or mid-summer to early fall (as in 2011)? According to the above data, individual plants grown in 2011 produced an average of about 43.9 seeds before senescence (i.e., 2.15 seeds/flower multiplied by 20.4 flowers/plant). Fewer seeds per plant (approximately 39.3; 1.42 seeds/flower times 27.7 flowers/plant) were produced by the 2010 plants, even though they were grown earlier in the year (presumably at a time more conducive to reproduction in a long-day plant). Although the species is self-fertile, *L. floccosa* ssp. *grandiflora* maintains a nominally mixed mating system (Fig. 5) facilitated by floral morphology (Brown and Jain 1979), and it appears that opportunities for outcrossing may be greater earlier in the year, with any plants that germinate late (potentially

subjecting them to greater drought-stress and/or pollinator limitation as the pools dry out) being more apt to self-pollinate.

If the interpretation here is correct, then a roughly 10% greater yield can be expected from ssp. *grandiflora* plants grown under lights later in the year. However, seed yield may very well be increased even further by growing plants outdoors in greenhouse yards (and open to potential pollinators), in which case spring and summer would be the preferred time. Furthermore, evidence from this project suggests the seeds collected from the 2010 plants were heavier, which could suggest greater fertility (though the significance of this is unclear, since both sets of plants were grown indoors in pollinator-free environments). More specific work would need to be done to determine if seeds grown later in the season in fact have lower viability, or if the observed weight difference was due to disease or other factors possibly affecting viability.

OPTIONS FOR A PILOT REINTRODUCTION PROJECT

As discussed earlier, our goal to use *Limnanthes* seeds produced in 2008 in a pilot re-introduction effort in conjunction with the seed bulking grow-out was not realized, due to the FY2011 budget reduction. However, we did engage in discussions in 2010 and 2011 with BLM, TNC, OPRD, ODFW, and ODOT about possible sites for such a pilot project on their lands, with the understanding that we might still try and pursue this over the winter of 2012-13 (probably using non-federal support to supplement the effort). A number of loosely defined options were considered, but without confirmed funding for field work and travel, we were unable to decide on dates and our talks with the various land managers were put on hold.

But then in 2011 another option presented itself, one that included ODOT and a private company. ODA had been working with PacifiCorp (a subsidiary of Pacific Power) on a mitigation project in southern Oregon, which involved the take of *Limnanthes floccosa* ssp. *grandiflora* on land owned by the City of Medford ("take" in this case falls under state law—see ORS 564 and OAR 603). ODA suggested that the loss of the plants on city lands could be compensated for by creating a new (or augmenting an existing) population of ssp. *grandiflora* at an appropriate site in the Rogue Valley, within the historic range of the taxon. ODA entered into an agreement with PacifiCorp that would provide ODA with support for this work (i.e., limited funding to supplement greenhouse and mitigation-related field work). ODOT was simultaneously dealing with a wetland mitigation project in the same area, involving a state-owned site under their management known as the Whetstone Vernal Pool Mitigation-Conservation Bank.

A tentative agreement was reached between ODA, PacifiCorp, and ODOT to use the ODOT site (which historically had a very small *L. floccosa* ssp. *grandiflora* population) for the pilot re-introduction/augmentation effort. This would cover the PacifiCorp mitigation obligation, while at the same time providing an opportunity to meet our goal of a pilot outplanting under OR-EP-2 (Seg. 21). Although ODOT was not required to include the meadowfoam plants in their own mitigation work, they have agreed to do so in the spirit of cooperation, and to enhance the overall quality of the restoration effort at the site.

Finally, due to the time elapsed, we have decided to go ahead and use the fresher seed grown during the 2011 seed bulking work for the joint mitigation/re-introduction project at the Whetstone Vernal Pool Mitigation-Conservation Bank (Fig. 6), as opposed to stored seed from 2008. To offset the take of 87 meadowfoam plants, PacifiCorp and ODA originally determined that a 350 plant minimum population would need to be created (or added as augmentation to an existing population). This figure was based on past work by TNC at one of their preserves, in

Figure 6. An example of a potential site for *Limnanthes* re-introduction work at ODOT's Whetstone Vernal Pool Mitigation-Conservation Bank in Jackson Co., Oregon. (Photo credit: ODA staff)

which they showed that roughly 50 seeds needed to be sown for every reproductive plant recruited on-site from re-introduction efforts. We realize there is bound to be considerable variation in such results from year to year and site to site. However, we felt that this was a conservative enough estimate, and we went with the TNC data in lieu of more complete information. So with this in mind,17,500 seeds would be required to create a wild population of 350 plants. However, with the availability of the 2011 bulked seed, we have the opportunity to potentially create a larger population, and we plan to go ahead with this. The estimated 70,992 seeds resulting from the 2011 seed bulking, following the 2% rule of thumb suggested by the TNC data, could be expected to create a wild population of just over 1,400 plants. A larger population (1,400 *vs.* 350)

obviously faces better odds, in terms of successful reproduction and longer-term recruitment over successive years, and the ODOT mitigation site offers the space for this attempt. A draft scope of work (details to be finalized between ODA, PacifiCorp, and ODOT) for the re-introduction work was developed for a created population of 350 plants (see the outline below, which has been modified from the original working draft to factor in the 1,400 plant target number). The date for seed sowing has yet to be determined, but probably will occur well after rains have started and the ODOT vernal pools have become inundated (i.e., January or February).

***Mitigation site information*.** The mitigation site is located in Township 36S, Range 2W, Section 26 of the Willamette Meridian, in Jackson Co., Oregon. PacifiCorp proposes to establish a minimum 350-plant meadowfoam population (we plan to increase this target to 1,400 plants) at ODOT's Mitigation Bank to offset the effects of the loss of a meadowfoam population (87 plants) at PacifiCorp's project site (on City of Medford land). Seed will be sown in unoccupied areas within the ODOT Mitigation Bank site, with the objective to establish a population equal to or greater than 350 plants (now 1,400 plants).

***Site preparation and sowing work*:**

- Planning work with ODA, ODOT, USFWS, and any other necessary regulators or partners will be completed, and necessary permits (if needed) secured.
- Meetings with ODOT (and ODA) to review a map of potential planting areas available for the proposed work will be held; to be followed up with one or more field visits.
- ODOT will be provided with a general seeding plan for approval, including lists of materials and equipment involved. No soil amendments or mulch shall be used.
- The specific vernal pool wetland area to be seeded will be identified, with ODOT and ODA input (areas not known to have a current meadowfoam population will be used):
    - The selected vernal pool wetland area(s) shall be mechanically treated for noxious and invasive weeds, or otherwise appropriately prepared for seed sowing, and then maintained to prevent invasive plants from re-encroaching into seeded areas (without the use of pesticides).
    - Prep work for the sites selected for seeding will include raking away excessive thatch and litter, as appropriate.
    - Surface boundaries will be laid out for sowing at preferred density (for a target of 24 plants/m^2); sowing area of ca. 58 m^2 needed if target is 1,400 plants.
- Seeds will be sown at roughly 1,200 seeds/m^2 (~70,000 seeds total for 1,400 plants) (*Rationale*: A pilot sowing project for ssp. *grandiflora* by the TNC in 2009-2011 resulted in a recruitment rate of 2%, following a 5% initial establishment/germination rate from sowing. Note, however that the reduced second year precipitation and inundation resulted in lower than normal abundance in most wild patches of the species, so the 2% estimate may be considered a conservative estimate.)

***Proposed monitoring methods and sampling protocols*:**

- Before or immediately after sowing, measurable success criteria will be established (in consultation with ODA and USFWS), focusing on the number of reproductive plants appearing in the establishment area by a certain year, their reproductive vigor, etc.

- The established population will be monitored during peak blooming (typically in April) in the first, third, and fifth years following the initial sowing. Descriptive reports will be prepared following each monitoring visit, and submitted to Oregon Department of Agriculture, U.S. Fish and Wildlife Service, and ODOT.
- If the established population does not exceed an estimated 1,500 plants, a complete census will be conducted to gather survival data, employing a method to physically grid the patches to ensure comprehensive and non-duplicative counts. However, if the population exceeds 1,500 plants, it may be appropriate to use temporary transects within in the planted vernal pool, to then estimate overall population size via a suitable quadrat-based sampling method.
- The reproductive effort of established population will be assessed via subsampling; data on numbers of flowers and seeds produced will be collected each sampling year.
- A series of permanent photo points will be established for each planting area.
- Any re-encroaching weedy species within the meadowfoam establishment sites will be evaluated and treated, as needed.
- If by the 3rd year the monitoring success criteria are unmet, it will be necessary to replant and then survey in the 5th year.

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