Seed viability studies for recovery of *Plagiobothrys hirtus* (Boraginaceae), the rough popcornflower

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**Study Objectives:**

I. To collect seed from a series of populations producing a genetically diverse seed lot suitable for creating transplants for reintroduction.

II. To test collected seeds in the Oregon State University greenhouses to evaluate the seed viability of maternal lines and populations.

III. To identify new publicly owned sites suitable for introduction.

**Abstract:** *Plagiobothrys hirtus* is Oregon’s top priority for endangered plant species recovery, and the wetlands of Douglas County provide the only habitat for this plant in the world. The goal is to establish this species from endangered to threatened, and in order to do that, existing populations must be protected, and new populations must be created. Genetic diversity plays a major role in the long-term viability of most endangered species, as their populations are small, however, little is known about the genetic diversity in populations of *P. hirtus*. Seed viability studies are one way to quantify genetic diversity, as germination is one of the many traits that can be observed indicating variation between individuals. Observing a wide range in traits among maternal lines indicates that there is a wide range in genetic variation within the population. We conducted these studies at the OSU greenhouses to examine variation in germination of maternal lines in four populations.

Unexpected findings indicate that *P. hirtus* expresses dormancy, and that this dormancy is most effectively broken with cold stratification. Total germination was highest (and had the highest range) from maternal lines with the largest populations, and the highest assumed genetic diversity. These observations are consistent with our predictions. Seed management protocols were developed using these findings, providing information needed to create stable populations moving toward delisting this species from endangered to threatened.

**Materials and Methods:** Seed viability

Mature seeds from 25 maternal lines were collected from each of the four sites. Previously, a report investigating the sexual reproduction of *P. hirtus* after-oiling (loss of dormant state usually due to drying) was suspected (Amsberry and Meinke 2001). To help achieve after-oiling through water loss, seeds were dried at 25°C (77°F) in the seed dryer for 24 hours. Ten seeds per maternal line were placed on filter paper moistened with distilled water. The petri dishes were monitored in the OSU greenhouse, receiving natural light and distilled water at 65°F (see Figure 3). Unexpectedly, seven days passed with no germination events, though previous studies involving germination trials with *P. hirtus* observed that most seeds finish germinating within a week of beginning the germination trial (Amsberry and Meinke 2004). Because no germination events were observed, we hypothesized that even with the after-oiling treatment of a 24-hour drying dormancy was still present. To test this hypothesis, we applied three treatments for breaking dormancy.

The three treatments applied to attempt to break dormancy were solutions of gibberellic acid (GA3), potassium nitrate (KNO3), and cold stratification. The temperature for cold stratification was reached by averaging the daily temperatures in Roseburg during the onset of the rainy season, as the observed time for natural *P. hirtus* germination (OBA 2008).

1. GA = 0.25%-maternal lines 1-5 in all populations
2. KNO3=0.25%-maternal lines 1-10 in all populations
3. Cold stratification (2°C) maternal lines 11-15 in all populations
4. DI water (no change)-maternal lines 16-20 in all populations

The seeds were submerged in their respective solutions for 24 hours, transferred to new petri dishes and were watered with DI water for the duration of the experiment. During this time, seeds were monitored daily for germination events, with any developing seedlings transplanted to pots filled with SB 40 Sunshine Growers potting soil.

**Discussion:**

Treating the seeds with cold stratification achieved enough germination (Figure 4) to evaluate variations in germination patterns between maternal lines and populations (Figure 6). Most likely due to the small sample size, not all populations had statistically different total germinations from each other, however, trends indicate that the populations with the highest assumed genetic diversity also had the highest total germination. North Star, as the largest extant population, had a significantly higher total germination than West Gate, which could potentially have less genetic variability due to a small number of founders used for its creation (Table 1, Figure 6).

This trend was highlighted again with the reproductive capacities of each population (the average number of seeds produced per plant) as the total germination for each population. The larger populations had the greater reproductive capacities as well as the highest range in number of seeds germinating (Table 1). The range in total number of seeds germinated achieved between maternal lines suggests their level of genetic diversity, implying that populations may range from most to least genetically diverse in the following order: North Star, South Side Swale, Popcorn Swale, and West Gate. These findings will guide seed selection in the future, emphasizing populations likely to have high genetic diversity when planning for future seed collection, germination, and transplanting.

**Introduction:**

The federally endangered rough popcornflower, *Plagiobothrys hirtus* is a perennial wetland plant found only in Douglas County, Oregon (Figure 1). Threatened with extinction by habitat loss and competition with exotic species, *P. hirtus* is only known at about a dozen remaining sites (Amsberry and Meinke 2006). After overwintering submerged in water, this facultatively aquatic species germinates at 19°C (66°F) (see Figure 3). The flowers need insects to achieve pollination, reaching up to 70 cm in height with white and yellow five-petaled flowers (Figure 5). The flowers need insects to achieve pollination, reaching up to 70 cm in height with white and yellow five-petaled flowers (Figure 5). The flowers need insects to achieve pollination, reaching up to 70 cm in height with white and yellow five-petaled flowers (Figure 5).

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**Site Selection:**

To identify new publicly owned sites for introduction, we began by searching the North Bank Habitat Management Area (NBHMA), which already supports two successful introduced populations of *P. hirtus*, created from 1998-2003 (Amsberry, Amberg, and Meinke 2005). Soil maps were reviewed, sites were visited and then rated according to a habitat suitability analysis developed at Oregon State University (Table 1). Three of the best sites at the NBHMA were then selected as potential sites for outplanting.

BLM maps of potential habitat were developed in collaboration with BLM staff, based on slope and preferred soil type. The layering of these two habitat features provided us with an initial map to begin surveying to vehicle. The best possible sites to survey are located within lime green areas (Figure 7). Intermediate outplanting sites are shaded blue, and the less promising sites are shaded in orange.

**Literature Cited:**


**Figure 1.** Map of seed collection sites. Yellow dots represent each site. Site names are outlined in red.

**Figure 2.** Map of Oregon and surrounding states. *Plagiobothrys hirtus* is shaded in orange.

**Figure 3.** Map of Oregon and surrounding states. *Plagiobothrys hirtus* is shaded in orange.

**Figure 4.** Cold stratification of *P. hirtus*. Flowers prior to being placed on seed paper.

**Figure 5.** Flowers (Figure 5). The flowers need insects to achieve pollination, reaching up to 70 cm in height with white and yellow five-petaled flowers (Figure 5).

**Figure 6.** Total germination of each maternal line across all populations. Population 1 (South Side Swale), population 2 (North Star), population 3 (West Gate), and population 4 (Popcorn Swale). n=5 for all populations. Relevance calculated based on significance using an ANOVA (*) (P ≤ 0.05). Main effects that were not significant are denoted as an “x” (P > 0.05). Overall, these results indicate that populations with higher assumed genetic diversity have a higher germination rate per maternal line.

**Figure 7.** Map of Oregon and surrounding states. *Plagiobothrys hirtus* is shaded in orange.