Propagation of Endangered Species: Variable Germination of Pink Sandverbena from Pacific Coast Beaches

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Pink sandverbena (*Abronia umbellata* ssp. *breviflora*) is an endangered plant of Pacific Coast beaches. Restoration efforts have focused on the development of seed germination and field propagation techniques. Germination in the laboratory was highest when seeds were removed from the fruit and received alternating temperatures (20°C/30 C) and photoperiods. Benefits from cold stratification differed strongly from site to site and year to year. Field-sowing of seeds is also a viable propagation method.

INTRODUCTION

Reintroduction may be essential for endangered plant conservation, especially for species with few remaining wild populations. Descriptions of effective procedures for rare plant propagation and establishment in the field are crucial for advancing the practice of species re-establishment (Falk et al., 1996).

Many sandverbenas (*Abronia* spp., Nyctaginaceae) are rare and prone to extinction, a characteristic that led Wilson (1972) to call them "disappearing species." Pink sandverbena (*A. umbellata* ssp. *breviflora*) is indigenous to the Pacific Coast of North America from British Columbia to northern California. Due to the invasion and subsequent stabilization of foredune systems by European beachgrass (*Ammophila arenaria*) (Wiedemann, 1984) and disturbance by off-road vehicles, pink sandverbena is now restricted to three wild populations on the southern Oregon Coast and perhaps a dozen in California. It is listed by the state of Oregon as endangered and it is considered a Species of Concern by the U.S. Fish and Wildlife Service. The primary goal of this report is to document germination techniques for pink sandverbena under laboratory and field conditions.

MATERIALS AND METHODS

Pink sandverbena is a low-growing, herbaceous annual plant with a central taproot (Kaye et al., 1999). Each flower produces a single-seeded fruit (achene) with 3 to 5 broad wings that presumably promote dispersal. Germination experiments were performed at Oregon State University's Seed Lab and at two field sites on the Oregon Coast. Field sites included dredged sand placed on the beach at Port Orford on the southern Oregon coast and a natural beach at Greggs Creek, about 20 miles south of Port Orford. Seeds for germination tests came from naturally occurring populations at Port Orford, Harris Beach, and Gearhart, Oregon, as well as a population at Coos Bay that was restored with seeds originally collected at Port Orford.

I conducted a series of germination tests to develop an effective protocol for producing large numbers of seedlings. Prior to germination tests, viability of seeds collected in 1991 and 1992 from Port Orford were analyzed with tetrazolium chloride; viability in these tests was nearly 100%.

In the first experiment, seeds from Port Orford received alternating temperatures and photoperiods (20°C, 16 h dark/30°C, 8 h flourescent light) as recommended by Chirco and Turner (1986). I compared germination of seeds with the following treatments: whole fruits on moist sand, clipped fruits on moist sand, dry-stored seeds on moist germination paper, dry-stored seeds on paper soaked with 0.2% potassium nitrate solution (KNO_3, see Copeland and McDonald [1995]), cold-stratified seeds on moist paper, stratified seeds on paper with KNO_3, dry-stored seeds on moist sand, and dry-stored seeds on sand with KNO_3 solution. Each treatment was replicated 6 times, with 40 seeds per replicate in the first six treatments and 25 seeds per replicate in the last two. Germination, defined as 5 mm growth of the radical, was recorded after 2 weeks. I used ANOVA to test for a treatment effect and Tukey's multiple range test to compare means.

To compare germination of seeds from different natural populations, a second experiment used seeds collected from Harris Beach, Port Orford, and Gearhart, Oregon, in 1993. These seeds were removed from the fruit and subjected to alternating temperatures and photoperiods (20°C, 16 h dark/30°C, as above). Each population was replicated 6 times, with 40 seeds per replicate.

A third experiment was conducted in 1997 and 1999 to re-examine seed dormancy and effects of stratification. In 1997, six replicates of 20 seeds each from Port Orford (1996) were stored dry while an identical set of replicates were cold-stratified on moist pads at 4°C for 8 weeks. I used a *t*-test to compare mean percentage

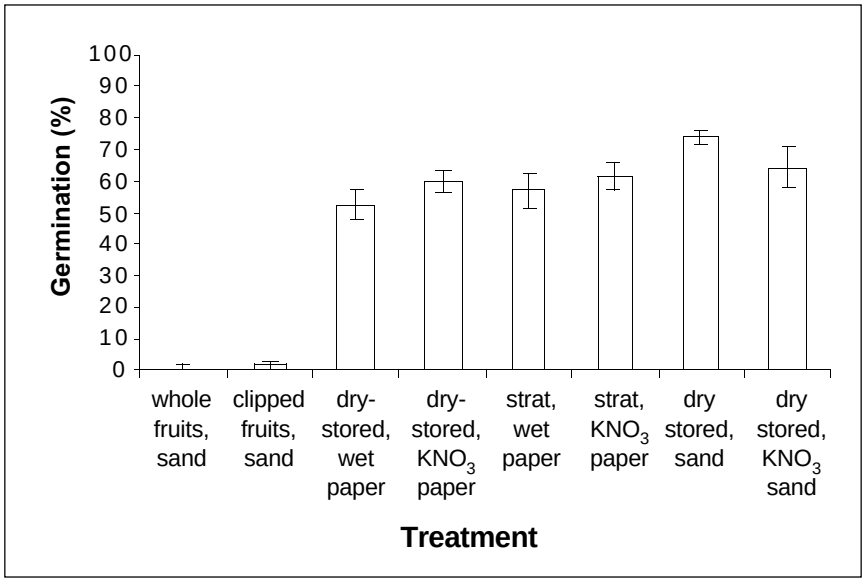

Figure 1. Germination of sandverbena seeds in various treatments (error bars represent ±1 SE). See text for explanation of treatments.

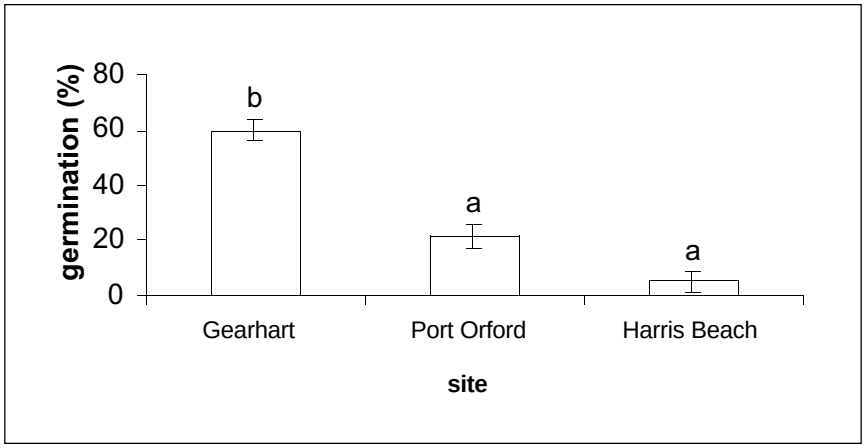

Figure 2. Germination of seeds collected from three sites. Bars with the same letter are not significantly different.

germination. In 1999, groups of 150 seeds from Port Orford (1997) and Coos Bay (1998) were chilled at 4°C for 0, 1, 2, 3, 4 , 6, and 8 weeks, then placed at alternating temperatures (20°C/30°C) to document minimum stratification periods.

Field experiments were performed in a fourth experiment. In November 1993, sandverbena fruits were sown on the sand surface, buried 3 cm, and buried 10 cm. In all of these treatments, 50seeds were placed in each plot. Each treatment was replicated 16 times in a randomized block design, duplicated at Port Orford and Greggs Creek. Seedlings occurring in these plots were counted in June 1993 to determine percentage seedling recruitment for each seeding treatment. For more details of field methods, see Kaye et al. (1999).

RESULTS

In Experiment 1, treating seeds prior to placing them in alternating temperatures had a significant effect on germination (df=7, F=34.73, P<0.0001). Specifically, removing the fruit from the seed increased germination several-fold: only 0.8% and 1.7% of seeds with fruit husks left intact or clipped germinated, while those with the fruit husks removed ranged in germination from 52% to 74% (Fig. 1). Differences among the other treatments were not significant. Experiment 2, however, suggests that germination of seeds collected the following year differed among populations (Fig. 2). Although seeds from Gearhart had relatively high germination (60%), those from Port Orford and Harris Beach had significantly (P<0.0001, one-way ANOVA) lower rates (5% to 22%). Seed germinability and/or dormancy clearly differed from site to site and year to year.

Tests performed in 1997 with seed collected in 1996 at Port Orford showed that stratification significantly improved germination from an average of 32.5% to 80.8% (df=10, t=2.23, P<0.0001). This is inconsistent with results from Experiment 1, in which stratification had no effect. Tests in 1999 with Port Orford (1997 collection) and Coos Bay (1998) seeds also showed a positive effect of stratification. Initial dormancy rates differed among the two populations (despite their genetic similarity). In this test, germination reached about 90% after 2 weeks of 4°C stratification (Fig. 3).

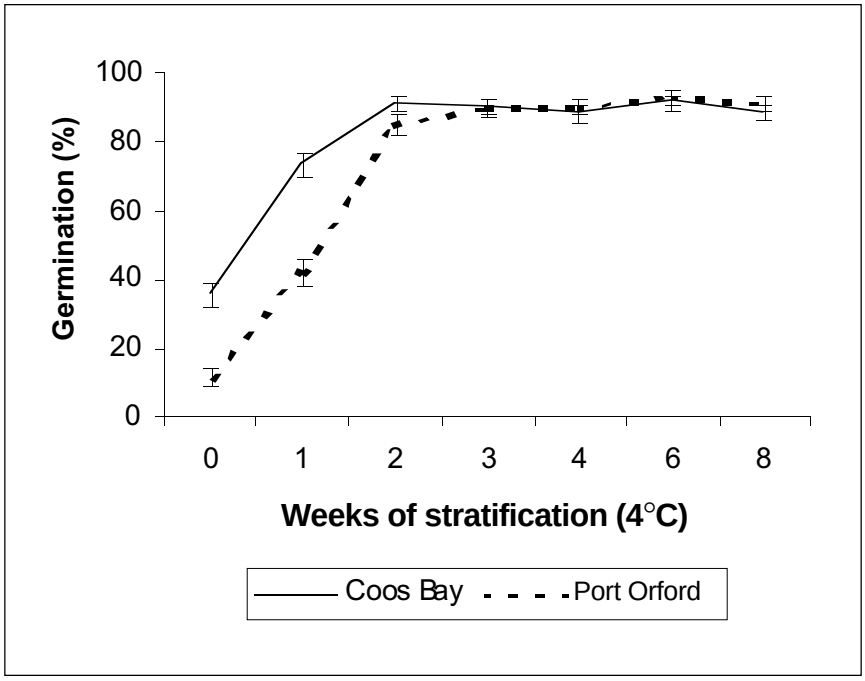

Figure 3. Effect of stratification period on germination of seeds from two populations. Error bars represent ±1 standard error.

Sowing seeds (in fruits) directly in plots in November was an effective method of producing seedlings the following June (Fig. 2). Seed burial at 3-cm produced the best results, with an average of 37.5% emergence at Port Orford and 25.9% at Greggs Creek. Seedling establishment from fruits buried 10 cm was significantly lower than shallow-buried or surface-sown seeds at Port Orford (df=2, F=22.3, P 0.0001) and Greggs Creek (df=2, F=8.02, P=0.002).

DISCUSSION

Seed germination in the laboratory was greatest when the seed was removed from the fruit. Once fruits were removed, germination of seeds collected in 1992 was not affected by stratification, exposure to KNO_3, or substrate (germination pad or sand), all of which yielded about 50% to 70% germination in alternating temperatures and photoperiods. However, stratification substantially improved germination of seeds collected from Port Orford in 1996 and 1997, and from Coos Bay in 1998. Apparently, germination requirements for this species differ from site to site and year to year. One possible explanation is annual variation in climate during seed maturation and while fruits are on the ground, prior to collection. Germination methods for this species may need to be revised periodically to propagate plants from different years and different locations. Fortunately, 2 weeks stratification at 4°C results in high germination rates, regardless of collection site and initial dormancy.

Sowing single-seeded fruits directly into appropriate habitat resulted in short-term plant establishment on dredged material and a natural beach. Sowing seeds or transplanting greenhouse-grown individuals on beaches may be effective methods for re-introducing pink sandverbena. Deep-burial of seeds should be avoided, however, because buried seeds may remain dormant or fail to emerge.

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