

Seed Germination and Pollination Requirements of Holy Ghost *Ipomopsis sancti-spiritus*

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Abstract: Holy Ghost *Ipomopsis* is an endangered species known only from a single location in northern New Mexico. To examine its cultivation requirements and breeding system, I conducted seed germination and pollination studies. Results indicated that maximum germination occurred after at least 1 month of cold stratification. Fruit set occurred in response to application of both self and outcrossed pollen, indicating that the species has a flexible breeding system. Fruit set success from outcross pollen varied with pollen donor. This evidence and the observation that anthers often lack pollen may be responses to small population size.

Introduction

For many rare plants, recommended conservation strategies include maintaining an ex situ collection of the species and determining autecological factors necessary for growth and reproduction (USFWS 1989, 1991, 1992). To accomplish this, botanical gardens in the consortium known as the Center for Plant Conservation have collected seeds or cuttings of rare species from the wild to house "captive" populations in their gardens. The captive populations are a resource for studying growth and reproduction requirements, which are essential for the management of the species in captivity and can aid conservation efforts in the wild.

One of the species for which ex situ and biological studies have been recommended is Holy Ghost *Ipomopsis sancti-spiritus* Wilken & Fletcher, housed at The Arboretum at Flagstaff and part of the National Collection of the Center for Plant Conservation. The species is known only from a 1.3 mile stretch along a road through mixed conifer forests of Holy Ghost Canyon in northern New Mexico, Santa Fe National Forest. *Ipomopsis sancti-spiritus* population size is estimated to be between 1250 and 2500 individuals (Sivinski 1991). It was listed as endangered in 1994 (USFWS 1994). Because the majority of the population occurs along the cut-and-fill slopes of the road, it is hypothesized that management practices, which eliminated natural disturbances such as fire and erosion, have shifted the dependence of the species to man-made disturbances for suitable habitat (Sivinski 1991). Like other members of the genus, the species requires well-drained soils and is not very competitive (Maschinski and Whitham 1989, Wilken 1996). The extremely narrow geographic distribution of the species and its lack of competitive ability make it extremely vulnerable to activities related to road maintenance and/or

activities suppressing moderate disturbance (Sivinski 1991).

Little was known about the reproduction and cultivation requirements of the species. Yet, this information is essential to conserve the species ex situ and to carry out the conservation recommendation to introduce the species to new locations (Sivinski 1991). Here I report on seed germination and pollination requirements and suggest avenues for future research.

Methods

To determine the germination requirements of the species, seeds were exposed to six different conditions: (1) direct exposure to moisture on a mist bench in a greenhouse facility with ambient temperatures of 60–80°F without any special treatment; (2) scarification with concentrated sulfuric acid for 1 min., rinsing, and placement on a mist bench; (3) exposure to variable day/night temperatures of 55°/35°F in a growth chamber; (4) exposure to variable day/night temperatures of 76°/38°F in a growth chamber; (5) stratification at 42°F for 4 weeks; (6) stratification at 42°F for 6 weeks; and (7) stratification at 42°F for 8 weeks. Seeds from individual fruits were kept separate for germination trials so that parental origin could be tracked. In addition, the total number of seeds in 55 fruits was counted to determine average seed set. The total number of seeds used for each treatment varied between 60 and 117 in 24 to 38 replicates per treatment. Germination was checked weekly for 7 weeks after the treatment was imposed. Differences in the percentage of seeds that germinated were analyzed using analysis of variance.

To determine whether *Ipomopsis sancti-spiritus* is self-compatible or self-incompatible, 10 plants growing at The Arboretum at Flagstaff were

bagged to eliminate any avian or insect contact with flowers. Within each plant, test flowers were haphazardly selected and emasculated before pollination treatment; five flowers received pollen directly from the anthers of another plant, five flowers received self pollen, and five flowers received no pollen. Fruit set was then quantified at the end of 4 weeks.

Results

The mean number of seeds/fruit was 4.1 in the 55 fruits measured. The percentage of seeds that germinated varied according to treatment ($F = 5.08$, $p < 0.0001$, Figure 1) with the highest percentages occurring in the 4-week and 8-week stratification treatments, 73 percent and 77 percent, respectively. Surprisingly, the 6-week stratification treatment was not intermediate between the 4-week and 8-week treatments. Scarification had the lowest percentage (36%) of seeds germinating. These findings suggest that most germination in the wild would occur in the summer or fall when soils are moist and temperatures warm to $>60^{\circ}\text{F}$ following the winter stratification period.

Seedlings that arose from the germination studies were transplanted to standard potting mix

(2 peat, 3 sand, 4 perlite, 4 composted bark) and then into Flagstaff basalt cinder soils. They survived well on the grounds of The Arboretum at Flagstaff. Thirty-two of 215 or 15 percent of transplants died.

Pollination trials indicated that *Ipomopsis sancti-spiritus* could set fruit when flowers received either self or outcrossed pollen. None of the flowers that received no pollen set fruit. The percentage of successful fruit set with selfed vs. outcrossed pollen varied across individuals and with pollen donor, such that 57 percent of selfing trials successfully set fruit, whereas outcrossed pollen trials had between 9.5 percent and 77 percent successful fruit set, depending on the pollen donor. Finding viable pollen for the experiment was sometimes problematic, because a small, unmeasured percentage of anthers appeared empty of pollen, being completely white instead of blue in color. Blue anthers contained pollen, whereas white ones did not.

Discussion

Several pieces of evidence from these preliminary studies suggest that *I. sancti-spiritus* has characteristics symptomatic of the ills of small

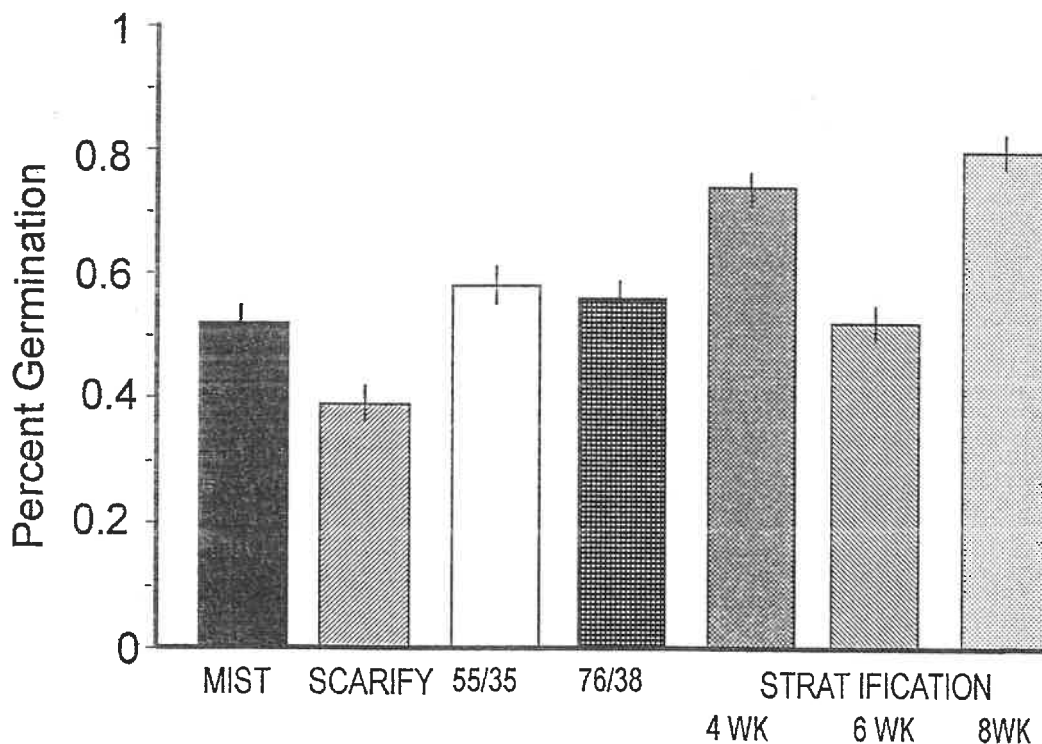


Figure 1. Percentage of seeds germinating with various treatments.

population size. With a mean of 4 seeds/fruit and 10–12 ovules per locule (Wilken and Fletcher 1988), its seed/ovule ratio is approximately 0.38. This value is equal to or below levels seen in other outcrossing species (i.e., 0.37, 0.45, 0.48) and is dramatically lower than the values of 0.92, 0.95, 0.91 measured in inbreeding species (Weins et al. 1987). Apparently, *I. sancti-spiritus* is not receiving adequate pollen at Holy Ghost Canyon to optimize seed set. Studies with the congener *I. aggregata* (Pursh) V. Grant indicated that seed set could be enhanced with pollen supplements, suggesting that the species was pollen limited (Hainsworth et al. 1985). Because the number of flowering *I. sancti-spiritus* may be less than 500 individuals in a season with clusters of <50 plants in a 30-m stretch of habitat (Maschinski, personal observation), pollinator visitation may be very low.

The results of the pollination studies suggest the narrowly distributed *I. sancti-spiritus* is apparently capable of some selfing, but is also outcrossing. In comparison, its more widespread congener, *I. arizonica*, is obligately outcrossing. It should be noted, however, that neither viability of seeds produced from selfed vs. outcrossed pollen nor survivability of offspring produced by the two methods was measured. For now it is possible to state that *Ipomopsis sancti-spiritus* apparently has a flexible pollination system, which may be an adaptation to small population size.

These findings also indicated that outcrossing success varied with pollen donor. Due to the requirement that two individuals must differ by at least one allele at the incompatibility locus (S-locus; de Nettancourt 1977) in order for a self-incompatible species to successfully set fruit, the probability of incompatibility increases in small populations (Byers and Meagher 1992, Byers 1995). Lloyd (1965) and Wyatt (1983) suggested that self-compatibility may evolve from self-incompatibility due to pressures imposed by small population size, such as small numbers of pollinators or a lack of compatible pollen. As Byers (1995) found with the rare plant *Eupatorium resinsum*, low seed set in wild populations of *I. sancti-spiritus* is most likely influenced by insufficient quantities of pollen and incompatible pollen. As population size becomes smaller, the proportion of incompatible mates increases. Further, the observation that anthers were empty of pollen suggests that the species may be experiencing inbreeding depression and subsequent reductions in gamete production.

These preliminary studies have provided a

good foundation for future studies. Among the questions that will need to be addressed are whether selfed or outcrossed progeny have equal fitness, what percentage of pollen is viable, and what the incompatibility ratio is in the population. These are essential pieces of information needed to unravel the breeding system and to determine what measures need to be taken for the conservation of the species.

Acknowledgments

Special thanks to Meg Heim for her help with the germination studies and to Kenric Kesler for his help with the pollination studies. This work was supported by a U.S. Fish and Wildlife Service contract to The Arboretum at Flagstaff.

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General Technical
Report RM - GTR-283

Southwestern Rare and Endangered Plants: Proceedings of the Second Conference

September 11-14, 1995
Flagstaff, Arizona

