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Rabbit ears gilia (*Ipomopsis aggregata* (Pursh) V.E. Grant ssp. *weberi* V.E. Grant & Wilken [Polemoniaceae]) is part of seed increase efforts at the USDA Forest Service Coeur d'Alene Nursery.

Seed increase protocol for rabbit ears gilia (*Ipomopsis aggregata* ssp. *weberi*) and yellow Indian paintbrush (*Castilleja flava* var. *flava*)

Nathan Robertson and Emily Overton Rhoades

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ABSTRACT

The United States Department of Agriculture Forest Service Coeur d'Alene Nursery seed increase program incorporates Regional Forest Service seed transfer protocols to ensure genetically appropriate seed sources are used for revegetation efforts. We have developed seed increase protocols for Region 2 US Forest Service Sensitive Species *Ipomopsis aggregata* (Pursh) V.E. Grant ssp. *weberi* V.E. Grant & Wilken (rabbit ears gilia [Polemoniaceae]) and hemiparasitic *Castilleja flava* S. Watson var. *flava* (yellow Indian paintbrush [Orobanchaceae]). Both species exhibit life history traits such as biennial habit, obligate outcrossing pollination strategy, and indeterminate capsule ripening or hemiparasitism, requiring development of efficient and less expensive seed increase methods.

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KEY WORDS

seed source, seed collection, increase efficiency, biennial, outcrossing, hemiparasite, Orobanchaceae, Polemoniaceae

NOMENCLATURE

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SEED INCREASE OF RABBIT EARS GILIA (*IPOMOPSIS AGGREGATA* (PURSH) V.E. GRANT SSP. *WEBERI* V.E. GRANT & WILKEN [POLEMONIACEAE])

The US Department of Agriculture Forest Service Coeur d'Alene Nursery (CDA) seed increase program includes range-restricted and USFS Sensitive Species in support of using genetically appropriate seed sources for revegetation work (Plant Conservation Alliance 2021). We are currently growing rabbit ears gilia, a Region 2 USFS Sensitive Species restricted to higher elevation open woodlands of south-central Wyoming and north-central Colorado. Rabbit ears gilia is a monocarpic, obligate-outcrossing, biennial that can be successfully grown for 2-plus years under cultivation in the greenhouse for seed increase.

More broadly known as scarlet gilia, this species is an important nectar source for nesting hummingbirds (Trochilidae) and is pollinated by native bees and bumblebees (Apidae). In sagebrush ecosystems and open woodlands of the Great Basin, scarlet gilia is also forage for nesting hens and chicks of Greater sage-grouse (*Centrocercus urophasianus* Bon. [Phasidae]). Seven recognized subspecies of scarlet gilia exist in western North America. *Ipomopsis aggregata* ssp. *aggregata* is the more common and is a scarlet-flowered subspecies found throughout the species range. In the Central Rocky Mountains, rabbit ears gilia (*I. aggregata* (Pursh) ssp. *weberi*) is a rare subtaxa restricted to only one site in south-central Wyoming and a few sites in north-central Colorado.

Rabbit ears gilia is a rare subtaxa of scarlet gilia and is found primarily in Colorado and Wyoming.

SEED PROCESSING

Initial rabbit ears gilia seed sourcing was from the Medicine Bow-Routt National Forest, Colorado, near Dumont Lake. Original wildland collection efforts were focused on representing a broad spectrum of individuals within the population so as to capture and maintain as much genetic diversity as possible.

To remove seeds from capsules, we processed hand-collected raw material through a Westrup LA-H brush machine with a size 10 wire mesh mantle and stiff bristled brushes. Following brushing, the seed material was processed through a Clipper Office Tester seed cleaner using a single, size #6 round hole screen. Finally, seed-bearing material was passed through a Hoffman continuous air stream separator at a setting of 20.0. If any large chaff remained in the seedlot, it was passed over the clipper a second time.

Using this method, seed can be easily cleaned to 95% or higher purity and 95% filled seeds. Following cleaning, we air-dried seed under ambient room temperature conditions to 30 to 35% RH at 20 °C (68 °F) as measured with a Rotronic HygroPalm AW1 (Rotronic Instrument Corp, Hauppauge, New York). Once dried to this moisture content, we sealed seed in 3 mil poly bags and placed them in long-term freezer storage at –15 °C (5 °F).

The original source collection for this seed increase effort was 4 y old prior to being sown. Seeds were soaked for 24 h in a cold running water bath prior to cold, moist stratification in a temperature-controlled cooler. For the stratification period, seeds were air-dried until the seed surfaces were no longer shiny with moisture, then placed into a fine mesh bag that was inserted into an unsealed, 2 mil poly bag. Seeds were stratified at 1.6 °C (35 °F) for 60 d. Every 2 wk during stratification, seeds were rinsed with cold running water, dipped into a mild (3%) hydrogen peroxide bath for 1 min to remove surface pathogens, air-dried until the surfaces were no longer shiny with moisture, and then returned to the stratification cooler. After the 60-d stratification period, we sowed seeds into containers in a temperature-controlled greenhouse.

Germination was mostly complete during the first 14 d; however, seedlings continued to emerge for up to 4 wk after sowing. First-year germination of this seed source was estimated at 35%, although some seeds remained dormant and

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germinated in the second year. Seedling establishment was slow, and seedlings required cool, dry conditions with good airflow to prevent infection with damping-off pathogens.

SEEDLING PRODUCTION AND SEED INCREASE

Seedlings were grown in a double-wall, polycarbonate, hardwall greenhouse with an evaporative cooling system. Environmental controls included humidistat-regulated overhead fog system and a forced air heating system under greenhouse benches. Greenhouse plants were irrigated and fertilized with a traveling overhead irrigation boom system.

Seeds were hand-sown into deep propagation trays (1.2 m (l) × 0.45 m (w) × 10 cm (d) [4 ft × 1.5 ft × 4 in]) filled with a growing medium of 3:1 (v:v) sphagnum peat moss and aged, finely screened Douglas-fir (*Pseudotsuga menzeisii* (Mirb.) Franco [Pinaceae]) bark, amended with approximately 44 kg/m^3 (75 lb/yd^3) of slow-release nitrogen fertilizer (39N:0P2O5:0K2O). During seed germination and active growth, greenhouse temperatures were maintained from 18 to 24 °C (65–75 °F). Germinated seedlings were lightly misted during early morning to ensure foliage dried by midday. Careful attention to irrigation frequency was required because this species is adapted to dry, well-aerated soils and produces a slender, unbranched taproot.

Seedlings were fertilized by an overhead boom system. During active growth, we used Peters Professional Conifer Grower fertilizer (20N:7P2O5:19K2O) at the rate of 200 ppm once per week. Irrigation frequency and fertilization were gradually reduced during late summer so that seedlings could harden for 6 to 8 wk in late summer and fall. During reduced fertilization and irrigation frequency in the hardening phase, we used Peters Professional Conifer Finisher fertilizer (4N:25P2O5:35K2O) at the rate of 40 ppm applied every 2 wk. Plants are usually fully dormant by late October. Plants were overwintered in the greenhouse and kept at temperatures just above freezing for 4 to 5 mo.

Greenhouse growing temperatures were resumed in March. We placed plants outdoors in April or early May after the last average frost date. Most 1-y-old seedlings flower during the second year from late April to June.

Nursery seedlings were observed being pollinated at the CDA nursery by bumblebees (*Bombus* species [Apidae]) and especially by long-tongued bee flies (Anthophoridae), in addition to other, unidentified pollinators. Rabbit ears gilia is a biennial with an indeterminate inflorescence. Flowering and capsule maturation occur over a period of several months. Dry

Cultivated rabbit ears gilia in containers at USDA FS CDA Nursery, showing flowering color and morphology. Plants shown are in their second (reproductive) year of growth.

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Rabbit ears gilia seed cleaned to 90–95% purity and fill prior to freezer storage.

capsules have explosive dehiscence and must be hand-collected just as capsules turn brown. Each capsule yields an average of 5 to 8 seeds.

We collected capsules and seeds and stored them at 18 to 21 °C (65–70 °F) in paper bags. When cleaned to a standard of 95% purity, 95% filled seed, and 30 to 35% RH at 20 °C (68 °F), yield averaged 990,000 seeds/kg (458,100 seeds/lb) for this accession. Under this style of high-intensity greenhouse seed production, yields of 60 to 70 g of pure live seed (PLS) were harvested per square meter of crop growing space. Cleaned seed is used in custom restoration seed mixes and for additional rotations of seed increase at the nursery.

SEED INCREASE OF YELLOW INDIAN PAINTBRUSH (*CASTILLEJA FLAVA* S. WATSON VAR. *FLAVA* [OROBANCHACEAE])

Yellow Indian paintbrush is a western species that ranges from the northern and southern Rocky Mountains to throughout the Great Basin region. All *Castilleja* species are hemiparasitic and are germinated and grown with companion plants. We used white sagebrush (*Artemisia ludoviciana* Nutt. [Asteraceae]) as the companion plants that were directly transplanted with paintbrush seedlings into seed production rows.

YELLOW INDIAN PAINTBRUSH PROPAGATION AND SEED INCREASE

Paintbrush seeds were stratified for 30 d at 1.6 °C (35 °F). To accomplish this, we soaked seeds for 24 h in a cold running water bath prior to cold, moist stratification in a temperature-controlled cooler. Prior to stratification, seeds were air-dried until the seed surfaces were no longer shiny with moisture, then placed into a fine mesh bag that was inserted into an unsealed, 2 mil poly bag. Every 2 wk during stratification, we rinsed seeds with cold running water, dipped them into a mild (3%) hydrogen peroxide bath for 1 min to remove surface pathogens, air-dried them until the surfaces were no longer shiny with moisture, then returned seeds to the stratification cooler. Seeds in stratification were routinely inspected for signs of initial seed dormancy break, as evidenced by hypocotyl emergence.

We air-dried scarified seed until the seed surfaces were no longer shiny with moisture, then seeds were sown with a vibrating hand seeder. If hypocotyl emergence was extensive, seeds were sown by hand while still moist, taking care to preserve hypocotyl integrity where possible. Seeds were sown into containers in a temperature-controlled greenhouse. We sowed stratified seeds of *Castilleja* and unstratified seeds of *Artemisia* into Ray Leach Cone-tainer SC7 cells (Stuewe & Sons, Tangent, Oregon). Based on findings that members of the Asteraceae are good hosts for *Castilleja* species (Love and McCammon 2017), we selected white sagebrush as a host plant. This species is often present in yellow Indian paintbrush habitat, exhibits similar horticultural requirements under cultivation, and maintains a low, spreading habit. Most important, white sagebrush seeds mature during late fall, which allows for uncontaminated

Established seed increase plot of yellow Indian paintbrush (*Castilleja flava* S. Watson var. *flava* [Orobanchaceae]) growing alongside white sagebrush (*Artemisia ludoviciana* Nutt. [Asteraceae]) host plants at the USDA FS CDA Nursery.

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Castilleja seed harvest well before white sagebrush flowers and produces seeds.

The 2 species were sown simultaneously in the greenhouse, with the intention of establishing the host/parasite relationship as quickly as possible and reducing labor costs associated with multiple transplant efforts. Some *Castilleja* seedlings grow slowly in the absence of a host in the same container. Surplus white sagebrush seedlings were hand-transplanted into cells containing only *Castilleja* seedlings when needed. With this method, we obtained approximately 90% container fill rate after transplanting and initial establishment of both seedlings. During active growth, white sagebrush seedlings were pruned to reduce canopy cover and to maintain *Castilleja* seedlings' competitive advantage. Once the seedlings were root tight, they were extracted from cells and outplanted into the nursery seed increase field beds.

Seed increase beds at the CDA nursery consisted primarily of a sandy loam soil with good drainage. Beds were approximately 1.8 m (6 ft) wide and consisted of 5 rows of plants, spaced 30 cm (12 in) apart. Plants within rows were spaced approximately 35 cm (14 in) apart to accommodate growing space for both host and hemiparasitic *Castilleja*.

Two wk after transplanting, we fertilized seedlings with ammonium phosphate (16-20-0 N:P:K) granular fertilizer, broadcast applied at 620 kg/ha (300 lb/ac) to promote establishment. Seed increase beds were irrigated using sprinkler irrigation as needed throughout the growing season to relieve drought stress (typically 3 to 4 times/mo). We controlled weeds through a post-planting application of commercial pre-emergent, as well as hand-weeding as needed.

Plants often flowered and produced seeds during the same year in which they were transplanted. Seed capsules retain seeds after they begin to split open. We hand-harvested dried capsules and placed them into paper bags for short-term storage in a dry warehouse environment at 18 to 21 °C (65–70 °F). Initially, seed-bearing material was brushed on a Westrup LA-H brush machine with a size 14 wire mesh mantle to remove seeds from capsules. Next, it was screened to remove large material using an Office Clipper Tester seed cleaner fitted with a single, size #6 round hole screen.

During final cleaning, seed material was passed through a Hoffman continuous air stream separator. Seed is typically small, but not very uniform in size. Filled seed varies in size and weight, so the air stream separator setting ranges from 9.5 to 11.5, which creates fractions of varying weights and seed sizes. These fractions are hand-screened through brass sieves with various sized wire mesh bottoms to screen out remaining chaff material. Individual fractions are not tested for germination rates, but a small sample of each is X-rayed with a Kubtec XPERT 80L-X laboratory X-ray cabinet (Kubtec, Stratford, Connecticut).

Brushed (Westrup LA-H brush machine) yellow Indian paintbrush seed-bearing material ready to be passed through an air column separator (background) to separate chaff from seed.

We counted filled seeds to determine ratio of hollow:filled seeds. Size fractions were passed through the air stream separator at increasing rates of airflow until X-ray samples confirm 90 to 95% filled seed. Resulting fractions were recombined, and using these methods, seed purity and fill of 90%-plus was achieved. Following cleaning, seed was air-dried under ambient room temperature conditions to 30 to 35% RH at 20 °C (68 °F) as measured with a Rotronic HygroPalm AW1. Once dried to this moisture content, we sealed seed in 3 mil poly bags and placed them in long-term freezer storage at –15 °C (5 °F). Prior to storage, we counted and weighed 100 seeds, and the seed number to weight ratio was used to estimate seeds per lb. For this accession, we determined seed increase efforts yielded approximately 9,900,000 seeds/kg (4,500,000 seeds/lb). Extrapolated over the field plot size (102 m^3 for this increase effort), average yields of 1.2 grams PLS/m^3 annually can be expected.

SUMMARY

Both rabbit ears gilia and yellow Indian paintbrush express life cycle traits that make seed increase efforts expensive and labor intensive. Little information regarding culture and seed increase of either species is available for comparison, although we can make some estimations and insights based on these seed increase procedures. A biennial with indeterminate inflorescence

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Cleaned and packaged seed harvested from yellow Indian paintbrush increase plot at the USDA FS CDA Nursery, showing variation in seed size.

development and expulsive seed ripening, rabbit ears gilia seed increase efforts are labor intensive and short-lived regardless of cultural methodology. Although sufficient for very small-scale wildland restoration or seeding efforts, the yields seen in this trial, and the associated labor costs, are likely prohibitive at a larger scale. We suggest that additional work should be conducted with this species using a direct-sow method into highly sterilized field beds, thus avoiding the labor and expense involved in greenhouse culture. Although harvests would still be limited to a single year given the biennial nature of gilia, ease of harvest may be facilitated in the field with a combine or other mechanical harvesting technique, and a magnitude of scale may be achieved that results in higher cost-benefit for seed increase for this species.

Yellow Indian paintbrush also expresses traits that complicate seed increase efficiency, including hemiparasitism and indeterminate seed ripening. Despite these obstacles, we feel efficient methods for multiyear culture and harvest could be further developed, and seed production per m^3 potentially increased beyond that seen in this trial. Paintbrush plants in our plot declined in number and reproductive ability after only 2 y, indicating either a cultural issue or a possible mismatch with the host species, white sagebrush. We suspect the latter,

Pairing yellow Indian paintbrush with white sagebrush as the host plant may be a mismatch given the proliferation of the host and the decline of paintbrush over time.

as visual assessment confirmed crowding and proliferation of white sagebrush in conjunction with paintbrush decline. A low-growing, non-rhizomatous species such as *Penstemon* (Schmidel) (Scrophulariaceae) or *Eriogonum* (Michx.) (Polygonaceae) may be better suited as a host for yellow Indian paintbrush in future increase efforts, although host seed size and ripening phenology should be considered.

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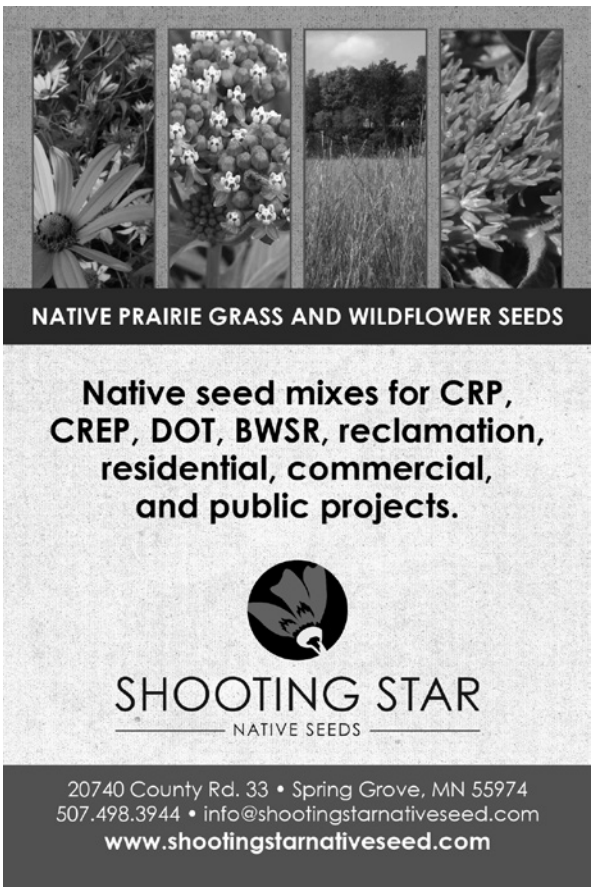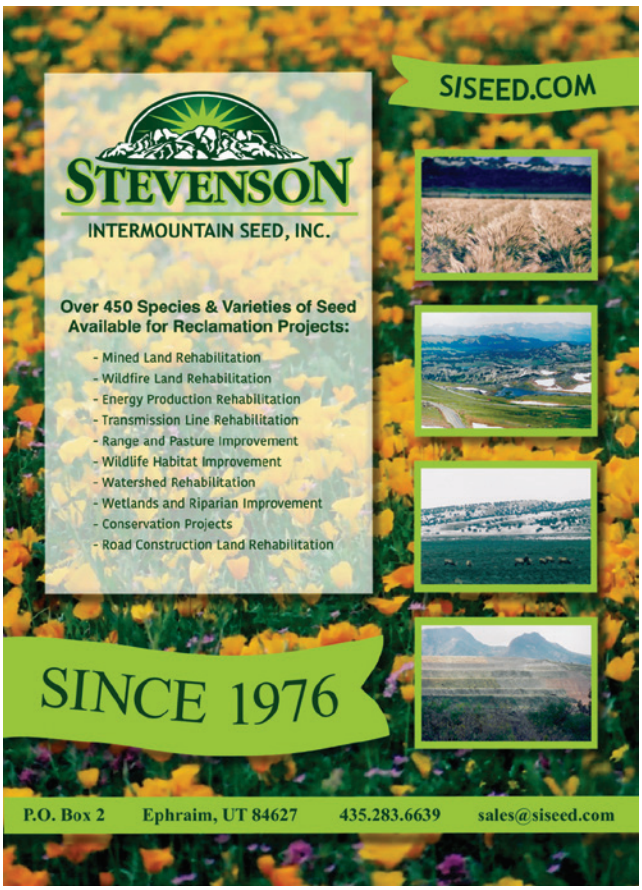
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