DEMONSTRATION STUDY: CONSERVING AN S1/G5/T2 MUSTARD AT A SOUTHCENTRAL MONTANA COAL MINE THROUGH NURSERY PROPAGATION AND TRANSPLANTING[1]

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Abstract. Protected rare plants can hinder mine development if not conserved. Spring Creek Coal Mine (SCCM) in southcentral Montana adopted a proactive conservation/propagation program for an uncommon but not formally protected variety of perennial mustard found in a topsoil-stripping area. The objective is to reestablish a self-sustaining population of *Physaria didymocarpa* (Hook.) A. Gray var. *lanata* A. Nelson, woolly twinpod, in reclamation and elsewhere within the permit area. This recognized variety is rated S1 in Montana (at risk, imperiled); the G5 (common, secure) global designation refers to generic *Physaria didymocarpa* (common twinpod), whereas T2 (less imperiled than S1, it occurs also in WY) refers to the trinomial (var. *lanata*). In the wild, fruits aren't produced every year and empty capsules are common, hence prospects for collecting seed appeared dim. Fifty mature plants were collected from the nexus of the population, transplanted, and used for tissue culturing (cloning) and later for seed collection. Between 500 and 1,000 plants annually were transplanted into the mine permit area beginning in fall 2008. In addition to the problems inherent to a stenotopic functional annual of very limited competitive ability, these limitations have manifested in the transplant program:

- Windblown dust accumulation in the foliage inhibited plant survival. The epithet "lanate" refers to long, tangled, woolly hairs.
- The fresh scoria into which transplants were planted in a few months became a dense sward of 5 dm tall kochia (*Bassia scoparia*) with a scattered twinpod understory. Kochia was more successful in capturing water, nutrients, and light.
- Disturbed or placed scoria subsequently becomes a magnet for yellow sweetclover (*Melilotus officinalis*), a tall nitrogen-fixing legume and copious seeder that overtopped and apparently competed with twinpod, the growth and survival of which did not appear to be assisted by increased mineral N, if present.
- Herbivory from ungulates and insects.

Spring transplanting is now performed into both mined and unmined areas. While transplants survive, a self-sustaining population is not yet assured. Further transplanting and adaptive practices continue at the mine. These lessons may guide others similarly engaged.

Additional Key Words: rare plant conservation, tissue culture propagation, outplanting, habitat, competition.

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Rare Taxon Found During Premine Inventory

During a 1993-1994 vegetation/floral baseline inventory necessary for a mining permit revision at the Spring Creek Coal Mine (SCCM), owned by Cloud Peak Energy in Big Horn County, Montana, one of the authors found an unusual variety of twinpod mustard in a few proximate, mostly similar habitats. Total number of plants within the study area was estimated at slightly more than one thousand.

Dr. Robert Dorn provided positive identification as *Physaria lanata* (A. Nels.) Rydb., which was listed for several regions of Wyoming in *Vascular Plants of Wyoming* (Dorn, 1992). Later, the nomenclature was revised by Reed Rollins (1993) as discussed below.

The site with the most plants was a scoria scarp 50 m high and 150 m long, slope orientation was 255 degrees, and the slope was 65%. The substrate was very channery to very gravelly scoria. The fine-earth fraction consisted of sands, loamy sands, and sandy loams. A smaller number of twinpod plants occurred in association with a nearby sandstone outcrop. Occasional plants occurred on semi-barren shaley soils, but only near the core population on the scarp. Stress adaptation to substrates that limit competitors appears to be more important than competitive ability, which it apparently lacks as it was never seen at densely vegetated sites.

On a continuum from steep, barren, rocky scoria to well-vegetated scoria soil, the modal site for woolly twinpod was intermediate, skeletal scoria soil with about 40% stratified plant cover on slopes of 30-65%. The main associate was bluebunch wheatgrass, Montana's state grass, a very eurytopic (broadly adapted) species occurring throughout the state on a variety of site types in different climates. There is some irony in it cohabiting with uncommon, stenotopic (narrowly adapted) mustard.

The collection of *Physaria didymocarpa* var. *lanata* in the study area may have been a state record. On that basis it was assigned an SU ("possibly in peril, but status uncertain; more information needed") by the Montana Natural Heritage Program (MNHP) in its May 1994 report. Information including an estimate of abundance and map of known occurrences was provided to the MONT herbarium at Montana State University.

A search for woolly twinpod on adjacent lands, while limited by access and ownership, found no additional populations. Prior and subsequent vegetation surveys nearby likewise failed to document more plants. Since it grew to a limited degree on several habitats near the scarp, never those well-stocked with other species, we suspect that limited dispersal contributes to its rarity.

A cautious approach to evaluating the rarity of this taxon is appropriate for two reasons. First, woolly twinpod occurs on some suitable sites in nearby Wyoming. Second, this species likely occurs on sites to which it is suited elsewhere in Big Horn County, awaiting discovery by trained botanists. In Montana, it has been reported also from Rosebud County, home to several coal mines and competent botanical inventories.

Physaria didymocarpa **(Hook.) A. Gray var.** ***lanata*** **A. Nelson, Woolly Twinpod**

Physaria didymocarpa var. *lanata*, woolly twinpod, is yellow-flowered perennial mustard with distinctive subangular basal leaves that form a rosette, the leaves frequently overlapping near the center (imbricate). Stem leaves are often short or lacking toward the center. Under the magnification of a hand lens, leaves and pods are seen to be covered with stellate hairs. In the trinomial, lanate (from L. *lanatus*) refers to a tangle of woolly hairs. Dorn (1992) distinguishes this taxon based on spreading basal-leaf hairs causing a shaggy appearance, the rays appearing as long simple hairs on the most densely hairy leaves.

There are four ovules (later seeds) in each locule (chamber of the ovary, lobe of the pod), hence 8 seeds per fruit. Based upon a small sample, seed weight is about 180,000/pound (400,000/kg), but seed size has proven to be quite variable. Genus *Physaria*, twinpod, derives from the Greek word *physa*, (bellows), which aptly describes the inflated pods that are notched at the tip, while the style persists in the cleft between the two locules (Fig. 1). The epithet *didymocarpa* is Greek for double-fruit. The botanical term didymous refers to twin-like pairs.

Aven Nelson (1904) initially described the plant as *Physaria didymocarpa* var. *lanata*. Rydberg (1912) transferred it to the species rank, *P. lanata* (A. Nels.) Rydb. Rollins (1993) sided with Nelson, listing this taxon as *P. didymocarpa* (Hook.) Gray var. *lanata* A. Nels. Some experts still think that species rank is justified.

Figure 1. Transplanted in fall 2006, this specimen was typical of prolific seed production in late spring 2007. Part of black Smartpots™ is evident, as are volunteer sweetclover seedlings.

Conservation at the Spring Creek Coal Mine

After confirming identification, providing a specimen and information to MONT herbarium, and informing MNHP, the botanical consultant encouraged mine personnel (1993-1994) to consider conserving the local woolly twinpod population. The conservation goal would be to reestablish a self-sustaining population of *Physaria didymocarpa* (Hook.) A. Gray var. *lanata* A. Nelson, woolly twinpod, in reclamation and elsewhere within the permit area. However, mining that pit area (Pit #4) was several years away due to permitting time frames. As a result, the subject lacked urgency. During this interval, the status of woolly twinpod in Montana changed to S1, "imperiled within state borders." This variety is currently rated S1 in Montana (at risk, imperiled); the G5 (common, secure) global designation refers to generic *Physaria didymocarpa* (common twinpod), whereas T2 refers to the trinomial (var. *lanata*). It occurs also in Wyoming and Alberta.

Initial Transplanting. Mining in Pit #4 started in 2001, and the pit progressed in close proximity to the population of the wooly twinpod in 2005. In summer 2006, the idea of conserving the woolly twinpod resurfaced while reviewing baseline information. While no law or mining regulation specifically protects S1 species, and mining through the woolly twinpod area had been permitted, SCCM was sympathetic to conserving the little mustard. As a result, a voluntary plan for conservation was devised and initiated in October 2006. At that time, a dragline was progressing towards the scoria scarp near the main twinpod population (Fig. 2). The first phase of the plan called for transplanting at least 50 plants to a suitable location nearby. Phase two involved tissue-culture propagation. Because seed production is naturally meager (fruits aren't produced every year and empty capsules are common), obtaining a useful amount of seed from 50 transplants wasn't identified as an option.

Figure 2. Main slope on which woolly twinpod occurred, although mostly higher on the slope where other plants were less dense.

In October 2006 a crew of three began transplanting. First, a small, flat staging area was dug by shovel into the twinpod slope. Here "potting soil" was concocted by combining scoria soil/subsoil with manure-base, commercial compost (roughly five percent by volume) and some of RTI's AM 120® "basin and high plains suite" at the rate recommended for a nursery medium. This product consisted of 50 percent *Glomus intraradices* from Utah, 25 percent of the same from Arizona, and 25 percent *G. etunicatum* from Nebraska. While mustards are reputedly non-mycorrhizal, we reasoned that a perennial such as *Physaria* might associate with a common *Glomus*, and inoculating the soil could hardly hurt.

The best donor site was where the mustards occurred among sparse bluebunch wheatgrass plants (Fig. 2). Here the slope was less steep, making digging from above possible so body weight could be used to push the shovel in, and the soil had some cohesion. Coarse scoria (Fig. 3) was hard to dig and fell off the roots. Only plants spaced at least several inches from bunchgrasses were collected. If closer, separating the intertwined roots resulted in bare-root transplants. For that reason, more densely vegetated spots were avoided. (Ironically, maintaining root integrity was a problem when deflasking clones, but in the opposite way; tiny roots couldn't be separated from the agar medium.)

Figure 3. Flags indicate prospective transplants, but substrate here was too coarse to keep roots anchored in soil. Note the lack of plant competition.

Meanwhile, five-gallon High-Caliper Smartpots™ were prepared with the custom "potting soil" described above. Transplants were dug with a shovel and transported with soil intact on the shovel face to Smartpots™, which were filled within five centimeters of the top with the potting mixture. The potting soil was lightly compacted to reduce voids. The filled Smartpots™ were carried to a trailer and watered.

The same day, Smartpots™ were transported to a pair of undisturbed scoria sites within the permit area not scheduled for mining. Previously, divots of suitable size had been dug with a backhoe for easy placement and future identification of the Smartpots™ locations. One substrate was similar to the donor site, but the other was somewhat more productive and had too much cheatgrass (*Bromus tectorum*), which by the following spring had invaded some Smartpots™. Channers (flat rocks) were placed around plants to deter volunteers from colonizing. The plants were again watered. The final step was to spray a two-part, rain-resistant, mint/casein, anti-browsing chemical on the plants. Subsequently, they were on their own.

The transplanted twinpods had become magnificent specimens by spring 2007, probably abetted by twice the normal amount of May precipitation, but perhaps some element of the potting soil (e.g., AM inoculation) contributed. The poorest of them equaled the finest specimens ever seen at the donor site, now gone, whereas the best were such robust, oversexed specimens that it hardly seemed credible that they might be imperiled (Fig. 1). While a few plants went missing, the remaining plants did not show signs of herbivory. In late July 2007, three fine specimens were taken to SMK Plant Tissue Culture Micro-propagation in Billings, Montana.

Transplants remaining at the mine had so many large fruits that we harvested seed. This was fortuitous because the seed later proved critical for nursery propagation. By the following summer, most transplants were missing, which raises questions about possible herbivory and the life span of the mustard. (In important respects, it can be considered a functional annual.)

However, a greenhouse incident suggests that herbivory is rare. Over winter, many nearby plants were to various degrees consumed by rabbits, voles, and ground squirrels. In March, a single ground squirrel bit off a chunk of one of the larger woolly twinpods being used for seed propagation. An odor reminiscent of skunk cabbage permeated the greenhouse, and no further herbivory ensued! But deer or antelope pull pedestal led plants and insects sometimes defoliate (shown later).

Propagation

A rare plant that in nature is a sparse, erratic seed producer and doesn't spread vegetatively is a candidate for cloning. Propagation was successful, but some problems in establishing nursery seedlings ensued.

The initial plan was to raise 2,000 seedlings in "stubby cell" Cone-Tainers™, cylindrical pots measuring 3.8 cm in diameter by 14 cm deep with a 115 mL volume. Survival of tissue-culture clones was approximately 15%, which didn't provide the desired quantity of plants. The seed collected from the initial transplants saved the day with about 95% survival at the nursery. From clones and mainly seed, Westscape Nursery near Bozeman, Montana, started about 1,000 plants in 2007-2008.

Starting plants from seed was half as expensive as deflasking, rooting, and transplanting tissue-culture clones into stubby cells. Deflasking involves removing the agar base containing the clones from each flask and soaking them in warm water to loosen the agar so the roots can be removed intact. Inherently indurate agar was further dried by time and liquid extraction by roots. Combined with the exceptionally fine roots of woolly twinpod, removal was difficult and injury common. Cloned seedlings were delicate (Fig. 4), and transplant mortality at the nursery was high. An intermediary step was used by transferring the seedlings to softer agar that could easily be soaked away, might be justified for this and similar delicate species.

By April 2007, the number of surviving clones had been reduced from 2,000 to about 500. Those clones having some semblance of roots and normal shoot formation survived the hardening off process due to the ability to pull water up through the roots. Those lacking that ability rotted before root formation occurred. Cuttings from survivors fortified total quantity. When the seedlings showed some vigor, they were transplanted into stubby cells with a combination of potting soils and road-mix, the latter a coarse substrate that is physically similar to scoria.

Figure 4. Delicate clone in a cup.

Outplanting A trial run of 48 plants was planted in a small patch of pit-run scoria overlying spoil in fall 2008. From this we learned to baby the plants, excavating a conical hole about 2 dm deep in the scoria rather than planting in the usual hoedad manner. In backfilling the unamended scoria, care was taken to place some soil fines next to the roots as the coarser material would leave many voids. The transplants were modestly watered with a total of 57 L (15 gal) of water. Watering-in plants is good practice not only because it provides soil water but because it settles dirt around the roots and helps fills voids.

Subsequently, these quantities were transplanted at SCCM, for which the Office of Surface Mining (OSM) bestowed a 2009 National *Excelling in Surface Coal Mining* award in recognition of the mine's conservation efforts. Table 1 below shows the chronology of transplanting at SCCM.

Table 1. Chronology of Transplanting at SCCM.

Date	Activity	Figure
October 2008	48 plants in PAR 1H Scoria	none
April 2009	905 plants in PAR 7A Scoria knobs (East and West knobs)	5-10
2010	Plants held at nursery; seed collected from PAR 7A plants	none
Spring 2011	About 350 planted in older reclamation with another 70 planted on a native scoria hillside just east of the Neco Road (limited use road)	11
2012	Plants not ready for transplanting; held at nursery	None
2013	370 stubby cells planted with various amendments	None

Lessons

These are the general lessons learned from conservation-through-transplanting of woolly twinpod:

1. Initial transplants may develop into vigorous specimens rarely seen in their natural habitat. If possible, rescue some of the original plants and then attempt to increase them through seed collection before resorting to cloning. A rather small number of vigorous plants, 50 in this case, provided enough seed for several thousand seedlings at less expense than tissue culturing, despite the fact that in situ collecting would have been unproductive. The initial transplants prospered in the transplant medium without competition.
2. To spread the risk of local failure, transplant a portion of each year's plants into appropriate reclamation substrates (scoria in this case) and the rest into promising native areas, in this instance scoria with limited competition and open spots (Fig. 11).
3. Transplant in spring as food for herbivores become abundant, not fall, the beginning of scarcity.
4. Maintain a seed-collection program at the nursery.
5. Don't plant into fresh scoria where a kochia thicket is likely to develop. Woolly twinpod is not adapted to fierce competition or being overtopped by tall foliage. Wait a year or two until the kochia subsides. In the meantime, broadcast a mix featuring mainly light-seeded, fibrous-rooted grasses. The goal is not to pack the site with plants but to suppress weeds with some open spots between plants for (one hopes) twinpod seedlings.

6. Due to lack of competitive ability, a few granules of fertilizer down the hole is better than a fertilizer "teabag" a few inches away as the fertilizer can just encourage a nearby volunteer with great seedling vigor.
7. Sweetclover invasion is always possible on scoria. We don't know how to minimize the risk of towering, tap-rooted competitors that leaves the ground covered with seed.
8. Place transplants in "pods," rather concentrated small areas within larger scoria laydown areas.
9. Watering-in not only provides initial soil saturation, but it helps fill voids near roots.
10. Placing pin flags next to at least 20 transplants per area allows survival counts the next year (Fig. 11).

Figure 5. PAR 7A scoria transplant area. Topsoil laydown was ongoing during transplanting.

Figure 6. Stubby cells were packed with fine roots. Note longitudinal groove to prevent circling and coarse scoria substrate.

Figure 7. Trays of stubby cells, some flowering, await transplanting at PAR 7A April 2009.

Figure 8. Collecting woolly twinpod fruits at PAR 7A amidst kochia, June 2010.

Figure 9. PAR 7A in 2010, copious fruits, but spent parent transplanted spring 2009 suggests a functional annual or one that relies on regeneration from seed every other year.

Figure 10. Clustered regeneration follows proximate seed dispersal. (Spring 2013, 16 plants are visible here at one of the PAR 7A planting locations).

Figure 11. At a native transplant site, pin flags allow survivor counts. Of spring 2011 transplants at native and reclaimed sites, two-thirds survived by summer 2012 in both areas.

Since the original 50 plants prospered so vigorously with compost and mycorrhizal inoculant, we experimented in the 2013 transplanting using compost, a water-retaining polymer, slow-release fertilizer, and a commercial AM product in several combinations.

The efforts associated with woolly twinpod conservation center on a single population, albeit one presumably fine-tuned to local conditions. Still, there may be negative consequences of limited genotypic variation. At one time, we considered collecting plants or seed from other known populations, assuming it could be done without harming them. We were dissuaded by cost and logistic considerations. But in general, we believe that the best course for conserving similar species is to preserve part of the local population for propagation, harvesting seed the following year assuming that they blossom prolifically as they did at SCCM.

Upon being told that we "babied" woolly twinpod when outplanting, Westscape Nursery reported that the same term described their experience with seedlings. In addition to deflasking difficulties, aerial irrigation concentrates water at the center of the basal rosette with its imbricate leaves, causing rot. A well-drained potting soil is critical. This brings up an important distinction in conserving rare taxon. They are likely narrow habitat or niche specialists with a reproductive bottleneck. In propagating them, one does well to consider inherent plant characteristics as well as placing them into Phil Grime's (1979) classification of plants as ruderals, competitors, or stress tolerators. What works for a stress-tolerant competitor, even a stenotopic one, does not extrapolate to rare taxa. One should exercise the greatest circumspection when generalizing from successful revegetation species to rare taxa. This applies to all phases of conservation from transplanting to propagation to nursery practice to outplanting.

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