

CONSERVING AN S1/G5/T2 MUSTARD AT A SOUTHEAST MONTANA COAL MINE THROUGH NURSERY PROPAGATION AND TRANSPLANTING¹

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Abstract. An uncommon variety of perennial mustard occurred in a topsoil stripping area at a southeast Montana coal mine. Spring Creek Coal Company is attempting to reestablish *Physaria didymocarpa* (Hook.) A. Gray var. *lanata* A. Nelson, woolly twinpod, in reclamation. 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*). It is more of a stenotopic stress-tolerator than a competitor. In the wild, fruits aren't produced every year and empty capsules are common, hence the prospects for collecting seed appeared dim. Fifty mature plants were collected from the area permitted for mining and transplanted into five-gallon Smartpots™ with a mix of native scoria soil, manure-based compost, and commercial mycorrhizal inoculant. One year later, most were magnificent specimens bearing more and bigger fruits than seen in nature. Because seed production is naturally meager, cloning was implemented to propagate seedlings to become nursery-raised transplants. Tissue culture was easily started from seed provided by three of the original transplants. However, transplanting and acclimatizing them to commercial greenhouse conditions presented other problems. Seedling survival was just 15%. Seed collected from the initial transplants, prompted by their prodigious fruit production, yielded about 90% survival. From these two propagation methods, more than one thousand 115-ml (seven-cubic-inch) “stubby cells” were raised in 2008. An experimental population was planted in fall 2008 into suitable mine reclamation. Approximately 800 seedlings will be transplanted in spring 2009, this effort to be repeated in subsequent years. In addition to delicate clone roots/indurate agar, challenges include nursery rot due to the tendency to collect water at the rosette center, providing good root/soil contact when transplanting into appropriate coarse substrate, and possible herbivory. Flexibility and adaption are key elements of the revegetation program. Some lessons may guide others similarly engaged.

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

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Rare Taxon Found During Premine Inventory

During a 1993-1994 vegetation/floral baseline inventory prior to applying for a mining permit revision at the Spring Creek Coal Mine (SCCM), owned by Rio Tinto Energy America (RTEA) in Big Horn County, Montana, a plant ecologist 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 as discussed below.

The site with the most plants was a scoria scarp 50 m high and 150 m long, azimuth = 255 degrees, slope = 65 percent. 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 on a nearby sandstone outcrop with petroglyphs. Occasional plants occurred on semibarren 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 on well-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 30-55%. 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, turned up 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 dispersal is a major limitation.

A cautious approach to evaluating the rarity of this taxon is appropriate for two reasons. First, woolly twinpod occurs on 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 a yellow-flowered perennial mustard with distinctive subangular basal leaves that form a rosette, the leaves frequently overlapping. Stem leaves are often lacking. Under the magnification of a hand lens, leaves and pods are seen to be covered with stellate hairs. In the trinomial, *lanata* (from *L. latanus*) 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 eight seeds per fruit. Based upon a small sample, seed weight is about 180,000/pound (400,000/kg). Genus *Physaria*, twinpod, derives from the Greek word *physis*, (bellows), which aptly describes the inflated pods that are notched at the tip (Fig. 1). The specific epithet *didymocarpa* is Greek for double-fruit. The botanical term didymous refers to a twin-like pair. In the cleft between the two locules, the style persists.

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.

Conservation at the Spring Creek Mine

After confirming identification, providing a specimen and information to MONT herbarium, and informing MNHP, the botanical consultant urged the mine, then owned by NERCO, to consider conserving the local woolly twinpod population.



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

But mining that pit area was years away; it wasn't yet permitted, so the issue lacked urgency. Mine personnel and ownership changed and it was not a regulatory issue, the status of the mustard was not highlighted during the transition to current mine ownership by RTEA. During this period, 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

In summer 2006, the woolly twinpod issue suddenly resurfaced while reviewing baseline information. While no law or mining regulation specifically protects S1 species, and mining through the woolly twinpod area had been approved, RTEA was sympathetic to conserving the little mustard. As a result, a voluntary plan for conservation was devised and initiated in

October. At that time, a dragline was nibbling away at the scoria scarp near the twinpod population (Fig. 2). The first phase of the plan called for transplanting at least 50 plants to a suitable location nearby. The second phase involved tissue-culture propagation because the prospect of collecting enough seed from the native population seemed unlikely. 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 even considered.

In October 2006 when the dragline was not operating, 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 (perhaps 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 nonmycorrhizal, 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 just as it was during transplanting as described below.)

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.



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



Figure 3. Flags indicate prospective transplants, but substrate here was too coarse to keep roots anchored in soil.

The same day, Smartpots™ were transported to a pair of undisturbed 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 Smartpot™ 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.

While a few plants went missing, the remaining plants did not show signs of grazing. The transplanted twinpods had become magnificent specimens by spring 2007, probably abetted by twice the normal amount of May precipitation. 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). In late July, three fine specimens were taken to SMK Plant Tissue Culture Micropropagation in Billings, Montana.

The 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 how long-lived this mustard is.

However, a greenhouse incident suggests that herbivory is rare. Overwinter, many nearby plants were grazed/browsed 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!

Propagation

A rare plant that in nature is a sparse, erratic seed producer is a candidate for cloning. Propagation was successful, but some problems in establishing nursery seedling ensued. SMK Plant Tissue Culture Micropropagation is presenting on this subject at this symposium.

The initial plan was to raise 2,000 seedlings in “stubby cells” Cone-Tainer™, a cylindrical pot that measures 3.8 cm in diameter by 14 cm deep with a 115 milliliter volume. Survival of tissue-culture clones was approximately 15 percent, which didn’t provide the desired quantity of plants. The seed collected from the initial transplants saved the day with about 95 percent

survival at the nursery. From clones and mainly seed, Westscape Nursery near Bozeman, Montana, grew about 1,000 plants in 2007-2008. Nursery customers frequently wanted to purchase woolly twinpod as an attractive landscaping forb.

Initiating and growing 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 dried by time and liquid extraction by roots. Combined with the exceptionally fine roots of woolly twinpod, removal was difficult and injury common. This was a prime cause of transplant mortality, according to the nursery. An intermediary step 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 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 roadmix, a coarse substrate physically similar to scoria available in Bozeman.

Outplanting

The goal is to transplant woolly twinpod into scoria mine reclamation in spring 2009. However, a trial run of just 50 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 20 cm 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 5.7 deciliter (15 gallons) of water. Starting at the site, it took about 1.5 hours for two workers to plant the 50 seedlings, which suggests 120 man-hours for the remaining thousand plants.

Outlook

The conservation efforts directed at woolly twinpod at SCCM are consistent with the biodiversity policies and environmental stewardship standards of RTEA. The plan is to continue propagating a similar number of seedlings annually for the next few years, transplanting them to different units of suitable habitat in reclamation. These reclamation areas will be monitored as

part of the routine vegetation monitoring required for bond release. No doubt there is more to learn about nursery practice and establishing and sustaining them in contrived habitat.

We encouraged Westscape Nursery to sell woolly twinpod to interested landscapers. There is something gratifying in knowing that an S1 taxon is growing around upscale houses in the Bozeman area.

Lessons

The first lesson is that transplants may develop into vigorous specimens rarely seen in their natural habitat. If time allows, attempt increasing plants 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 in situ would be fruitless.

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 those populations. While we were dissuaded by cost and logistic considerations, we believe that the best course would be to collect plants as we did when initially transplanting, harvesting seed the following year assuming they blossomed prolifically as they did at the Spring Creek Mine.

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, irrigation concentrates water at the center of the basal rosette, 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 a rare mustard to a hardy grass found in contaminated mine waste, and vice versa. This applies to all phases of conservation from transplanting to propagation to nursery practice to outplanting.

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