

**Implementation of the
Conservation Strategy for Tahoe Yellow Cress**
(Rorippa subumbellata).

**I. Seed Collection, Assessment of
Reproductive Output and Propagation for
Reintroduction**

prepared for

Tahoe Yellow Cress Technical Advisory Group
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This report replaces an earlier version (April 2002) that did not contain information on propagation or the 2002 seed collections.

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Introduction

Timely implementation of the Conservation Strategy for Tahoe Yellow Cress (Pavlik et al. 2002) requires that propagation materials (seeds) be collected from three ecogeographic quartiles of the lake for propagation under greenhouse conditions. The resulting plants will be used to create experimental populations for testing restoration hypotheses. We have devised a long-term project to enhance population sizes and increase the persistence of TYC at core and priority restoration sites. Over an eight-year period, a total of 18,800 plants could be transferred to nine restoration sites.

During the process of seed collection, we assessed the ability of individuals of this species to produce seeds. Estimates of reproductive output (= seed output per plant) were made by counting the number of fruits on plants of different sizes, estimating the mean number of apparently viable seeds per fruit, and then multiplying these variables together. The data were subsequently summarized by regressing seed output per plant on plant size. Regressions are useful for estimating reproductive output on individuals from natural and experimental populations (Pavlik et al. 1993, Pavlik and Espeland 1998) and for determining reproductive limitations imposed by breeding system, lack of pollinators or other resources or genetic incompatibilities (Pavlik et al. 1993).

Methods

Collection in 2001

Sites and Procedures

TYC seed were collected by Alison Stanton on September 4-7, 2001 at nine locations in the Tahoe Basin (Table 1). The locations were chosen to include each of three ecogeographic quartiles (NW, SW and SE) that support core populations (*sensu* Pavlik et al. 2002) of Tahoe Yellow Cress. Core populations included Blackwood N and S, Tallac, Taylor Creek, Upper Truckee East (= Barton Beach) and Edgewood. Seed collection activities accompanied the annual population census efforts. Cascade is ranked a high priority restoration site in the conservation strategy, while Tahoe Meadows and Lighthouse are medium priority restoration sites.

TYC fruits are small siliques (<5mm long), with a plump, round to oblong shape. Each fruit typically contains 10-50 tiny seeds that turn dark brown at maturity. During the September sampling period, fruit maturity ranged from fully dehisced to immature, fleshy green siliques. Most fruits were still green, but not fleshy, and it appeared that the whitish seeds would mature with an after-ripening period. Darkened, obviously mature seed were found only within dehiscent fruits. We collected both green and dehiscent fruits, but only those lacking obvious insect damage. Fruits were collected from different positions and stems on each plant and sealed in a separate paper envelope for each maternal parent. Envelopes were labeled by site and individual (maternal) plant # and placed within an insulated, ambient temperature cooler for transport.

Collection protocols were designed to maximize the capture of genetic variation within the seed sample. At each site we selected plants with a wide range in size and from as many different microhabitats as possible (e.g. low beach, berms, backbeach depressions). The Center for Plant Conservation (CPC) guidelines (1991) for genetic sampling of endangered plants recommends collecting from 10-50 individuals per population depending on a variety of attributes of the plant species and its habitat. However, the California Department of Fish and Game seed collecting permit for TYC prohibits collecting more than 10% of the total available seed for any population. We therefore established a target to collect ten mature fruits from 30 individual plants in each population. This target was only met at Upper Truckee East, the site with largest TYC population.

The actual number of sampled individuals varied from a low of 3 at Blackwood North, to 28 at Blackwood South (Table 1). Accordingly, the number of fruits collected per plant was increased to a maximum of 30 to compensate for the lower per population sample sizes. More than 2,000 fruits were collected from a total of 177 individuals plant across the nine population locations.

Data collected for each plant include: GPS location, the number of fruits collected, an estimate of the total number of fruits on the plant, two measures of size, and notes on the microhabitat (Appendix A). Plant size measurements included the length of the longest and the shortest radius of the basal rosette. Attempts were made to take a third measure of size that might correspond to fecundity (e.g. number of stems), but this was abandoned because an accurate stem count would have been prohibitively time consuming. Fruit count was estimated on each plant by rounding the numbers of fruits on each stem to the nearest 5. Stems were visually examined and assigned a fruit number (usually from 5-20), and all stem counts added successively. On several extremely large plants at Upper Truckee East, this method was applied to a proportion of the plant e.g. 10% and then scaled up to give a total fruit estimate. Fruit production varied from 4 to over 45,000 per plant (rosette, the apparent individual).

A hand drawn map was developed for each population showing access routes, distance to the lakeshore, and a diagrammatic sketch of sampled plant locations. Additional notes were made about site access, habitat condition, and an estimate of the total area occupied by the population (raw datasheets are included in the Appendix A).

Processing and Calculation of Seed Output

Envelopes were brought in the lab and kept at ambient temperatures (15 - 28 C) and humidities (25-60%) for a month (September-October 2001). During that time, the green fruits dried (without molding) and began to dehisce.

Fruits from three core populations (Blackwood South, Taylor Creek and Upper Truckee East) within three lake quartiles (II, III, and IV, respectively) were used to determine the mean number of seeds per fruit. A fruit from each individual ($n = 28, 27, \text{ or } 30$, respectively) was randomly selected

and dissected. All seeds were sorted into two categories (regular and irregular) and counted. The average number of seeds per fruit (regular or irregular) was computed by site.

Calculation of seed output per plant within these same core populations was done for each individual plant ($n = 28, 27, \text{ or } 30$, respectively) by multiplying the number of fruits/plant with the estimate of mean number of regular seeds/fruit for the population as a whole. This product was regressed against canopy area for each individual plant ($\text{area} = \pi (r_1 + r_2/2)^2$ where r_1 and r_2 are the measurements of largest and smallest canopy radius made in the field). Attempts were made to fit linear, logarithmic, power, and exponential curve fits to the data. In general, the linear fit had the highest regression coefficients and was selected for analysis of all datasets.

Preliminary Germination Trials

Regular seeds were counted into lots of 25 each, placed on filter paper disks within sterile petri dishes and given one of several treatments. Treatments included 1) stratification at 4 C for one month, 2) no stratification, 3) exposure to constant temperature of 7 C, 12 C and 17 C, and 4) burial with either sand or potting mix. Another set received similar treatment without exposure to the filter paper disks. Dishes were watered and kept in near darkness for more than 10 days of observation.

Collection in 2002

Although every effort was taken to ensure uniform seed collection, any differences in greenhouse seedling establishment that may emerge between the sites or between individuals within a site is probably a function of the quality of seed collected rather than any intrinsic genetic factor (i.e. seed from some individuals was underdeveloped). Therefore, we did not find it necessary to record or measure phenotypic details about individual maternal plants during the 2002 seed collection.

Our 2001 collection criteria that focused on green undamaged seed may have lead to the harvest of some underdeveloped seed lots. In 2002, we expanded our criteria to include open, dehiscing fruits and obviously darkened, mature fruits. We still kept the collection quantity to 10% or less of the total seed production.

Alison Stanton collected seed from 11 sites on September 3-4, 2002 using the 2001 procedures described above. Currently (December 2002), the seed has not been cleaned, sorted, and counted, but the quantity is sufficient for another full-scale propagation effort in 2003. The seed is being stored at room temperature and humidity in dry manila envelopes at Mills College.

Nursery Propagation

Propagation protocols were developed in cooperation with three separate nursery facilities to maximize yield of founding plants while minimizing artificial selection and *ex situ* loss of genetic variation. The object is to raise hardy, rather than productive, founders that will survive transplanting. A subsample of founders will be held over to the next year for aging in anticipation of an age-structured outplanting.

Three nurseries received contracts through the USDA Forest Service to propagate TYC: the USDA Forest Service facility in Camino, CA; the USDA Forest Service facility in Washoe Valley, NV; and privately-owned Sierra Valley Farms in Beckwourth, CA. Only the facility at Washoe had previously propagated TYC for restoration efforts. We chose to put the plants at three separate facilities to diffuse the risk of an unsuccessful propagation.

The entire 2001 seed lot was hand-sorted into three equal lots in December 2001 and stored in manila envelopes at room temperature and humidity until it could be delivered to the three nurseries. Each nursery received 177 seed lots, each containing one third of the seed collected from each of the 177 individuals (Table 1). A small portion of seed was retained for laboratory germination tests at Mills College.

Unexpectedly, all efforts failed to germinate seed in the laboratory during fall 2001 to spring 2002 (see results), thereby delaying planting. It did not seem prudent to put the entire seed lot in pots when we could not obtain any germination. However, in May we requested that two nurseries plant out a small number of lots as a pilot propagation. Each nursery subsequently tested slightly different seed storage and propagation methods. Results for each nursery are discussed below.

Results and Discussion

Seed Collection

We estimate that 46,000 seeds were obtained during the 2001 collection from 2,163 fruits of 177 individuals belonging to the nine populations (Table 1, Appendix). A majority of seeds within these fruits developed the same dry, brownish coats and full, angular shapes observed in seeds from dehiscent fruits. Dissection of these regular seeds revealed white, fleshy embryos with filled cotyledons and prominent radicles. Between 1 and 27% of all seeds were irregular (lowest at Blackwood South, highest at Taylor Creek) and probably not viable, appearing small, black and shriveled. Approximately 40,000 regular seeds were repackaged (but still kept in separate envelopes for each individual) and given to Gail Durham (Botanist, USFS) for distribution to three native plant nurseries. At no time were these seeds refrigerated or exposed to high light or humidity.

Table 1. TYC seed collection locations, the number of individual plants sampled, the total number of fruits collected, and mean number of regular or irregular seeds per fruit (\pm SE) for the 2001 lot.

Site	site code	TRPA code	# plants sampled	# fruits collected	mean seeds per fruit (regular)	mean seeds per fruit (irregular)
Blackwood North	BN	4	3	45		
Blackwood South	BS	5	28	317	26.1 \pm 1.5	0.3 \pm 0.2
Cascade	CD	18	24	226		
Edgewood	ED	30	10	70		
Lighthouse	LT	23	11	165		
Tallac	TL	19	21	265		
Taylor Creek	TY	21	27	319	21.7 \pm 3.0	8.0 \pm 2.8
Tahoe Meadows	TM	29	23	325		
Upper Truckee East	UT	25	30	431	30.2 \pm 2.8	7.1 \pm 2.7
Totals			177	2,163		

Seed Output Estimates

Plants sampled in 2001 ranged in canopy area from 78 cm² to more than 350,000 cm², but the majority of observed individuals were generally under 5,000 cm². At Upper Truckee East, it was determined that a single, large clone produced more than 1.4 million seeds (Figure 1), while another plant exceeding 11,000 cm² produced nearly 100,000 seeds. Both of these individuals were found growing in a high beach habitat near the edge of the river floodplain.

Plants were smaller at the Blackwood South and Taylor Creek sites, generally not exceeding 3000 cm² (Figure 2). Seed output per plant, however, was measured in the thousands, with similar slopes of the linear regression.

If the largest (> 5,000 cm²) individuals were excluded from the analysis, the slopes of the regression lines of all three sites were very similar, falling in a range of 3.77 - 3.97 (Figure 3). Despite considerable geographic and ecological distances between sites, the similarity of these relationships indicates few limitations imposed on reproduction by breeding system, pollinator availability, or resources for growth. Wide, site-specific variations in the lines would have suggested an outcross breeding system, dependence on pollinator population size and behavior, and restrictions on canopy and flower production by soil water, temperature, soil nutrients, or other factors. Consequently, data from all three populations (n = 75 plants) can be described by a single line with a high regression coefficient (Figure 4). The equation can be used to predict seed output from canopy area measurements in natural and experimental populations.

Preliminary Germination Trials

As of February 2002, no 2001 seed had been observed to germinate under any treatment regime. We have examined the seeds, which appeared regular in all respects, and could not find a clear indication of why they did not germinate. Ferreira (1987) apparently had no difficulty in getting all of her summer-collected seeds to produce seedlings. Similarly, efforts to propagate plants to support the 1986 reintroduction effort had no problems with germination of summer-collected seed (Widowski 1987). Perhaps the green fruits we collected required more ripening, but we still should have seen germination of seeds derived from dehiscent fruits (about 1/9th of the entire sample).

Alternatively, our fall-collected seed could have different dormancy characteristics than summer seed, requiring treatments that have not yet been tested (e.g. prolonged stratification).

Much to our surprise and relief, when planted by the nurseries in May 2002, germination and establishment rates were as high as 86% (see below). The discrepancy between laboratory and nursery results most likely reflects a strong dormancy that was either overcome by longer after-ripening or exposure to organic soil under greenhouse conditions.

Nursery Propagation

Sierra Valley Farms

Seed was stored for 90 days at 32 F in a moist environment. This method of cold stratification is often required to obtain germination in plants that experience a freeze at some point in the year. Although we had still not obtained germination in the laboratory (a shorter cold stratification had been tested), total of 1638 supercells were planted with seed lots from 7 of the 9 sites on May 21, 2002 (the Cascade and Blackwood South lots were retained for later use).

The supercells were packed with organic soil-less mix and topped with a one-inch layer of Lake Tahoe beach sand (collected from Baldwin Beach). A variable number of seeds (?) were planted on the soil surface and top-dressed with vermiculite. The cells were placed under fine mist with bottom heat of 78 F. Several weeks after planting when the propagules began to establish, the supercells were moved off the bottom heat source. The plants are currently in a greenhouse receiving a daily mist of ionized water without any fertilizer.

As of October 2002, a total of 1123 plants had become established. This is a 69% establishment rate. Initial germination was not monitored, so the actual germination rate may have been higher. A variable number of replicates were planted of each lot (3-28) and establishment of the replicates varied from 0-100%. In the seed sorting process we noted that several seed lots appeared to be poorly developed. This variability in initial seed quality makes it difficult to determine differences in greenhouse establishment rates between the sites. However, further analysis may reveal trends. Table 2 shows the total number of established plants from each lot.

During the September 2002 visit, a moderate proportion of the plants were flowering and producing fruit. The seed were allowed to drop onto the greenhouse floor. It is unlikely that the second-generation seed will self sow and cross contaminate the seed sources.

USFS Camino

All seed was stored in the freezer until planting. Since we had still not obtained germination in the laboratory prior to May 2002, we requested that the nursery plant out several seed lots as a pilot propagation. Five lots from Blackwood South were planted on May 24, 2002. Supercells were filled with a peat moss organic medium and three seeds were sown on the soil surface of each container and top-dressed with a thin layer of Lake Tahoe beach sand (collected at Baldwin Beach). The cells were placed outside and misted daily with ionized water (no fertilizer).

A final planting of the remaining seed lots occurred on July 10, 2002. The plants were checked on July 22, almost two months after sowing. A total of 44 plants had established in the 61 cells planted in May. This represents a 72% establishment rate. Three seed lots had establishment rates of 80 –86 %, but one had only 31%. During that evaluation, the July planting was beginning to germinate but it was too soon to evaluate germination percentages.

All plants were checked again on October 21, 2002. A total of 909 plants were counted as living out of a total of 1790 planted cells. The plants were still very small, no plant was over two inches in diameter, and many were slightly chlorotic. It appeared that very little growth had occurred in the previous three months, perhaps due to high heat stress or over watering. Small roots were observed growing out of the bottom of the cells, but it is unclear whether these plants will survive through to the spring.

USFS Washoe Valley

Seed was stored at room temperatures in paper bags until planting. On June 25, a variable number of seed were sown on the surface of supercells packed with an organic soil-less potting mix containing vermiculite. Anywhere from 0-10 seedlings emerged in each pot. A total of 179 plants established from the June plantings. Initial germination was not monitored, but the establishment rate was very

high, over 95% of the cells contained established plants in September 2002. Only 3% of established plants were flowering during the September evaluation.

A final planting of the remaining seed lots occurred in early September 2002 when a total of 1231 additional cells were planted. Seven replicates were planted of most of the 177 seed lots. Prior plantings had depleted the seed supply of a few lots, but extra individuals from seed-rich lots were planted to make up the difference. As of October, a total of 510 additional seedlings had established, bringing the total number of plants up to 689 of a total of 1410 cells that were planted (49% establishment rate). However, most of the 510 cells contained two seedlings each. These seedlings are being split into two separate pots, but it will take some time before we can determine the success of this transplanting effort.

Table 2. Number of established TYC plants from the 2001 lot by collection site and nursery facility as of Fall 2002.

	USFS Camino	Sierra Valley Farms	USFS Washoe Valley
Blackwood North	22	22	5
Blackwood South	145	not planted	75
Cascade	103	not planted	64
Edgewood	63	34	37
Lighthouse	55	101	23
Tallac	97	123	38
Taylor Creek	138	280	107
Tahoe Meadows	145	262	70
Upper Truckee East	141	301	91
Total Established	909	1123	689
Total Planted	1790	1638	1410

Conclusions

Large numbers of TYC seed are relatively easy to collect for propagation purposes. Average and above-average sized plants produce thousands, if not millions, of seeds in a single season, and these are readily gathered, sorted and stored. Regressions of seed output on simple measures of plant size were best fit with a linear model and had highly significant regression coefficients. The lack of site-specific variations in the regressions indicated that few limitations were imposed on TYC reproduction by breeding system, pollinator availability, or resources for growth. These results will make estimation of seed output by experimental populations relatively easy to evaluate.

Preliminary germination trials of the 2001 seed lot in the laboratory found a strong dormancy that could not be overcome during the first five months after collection. Subsequent planting under nursery conditions three months later indicated that germination could be as high as 86%, but could also vary widely. An after-ripening requirement may prevent early germination of TYC *in situ*, preventing seedling mortality during harsh winter and early spring conditions.

Three nurseries planted a total of 4,838 cells with TYC seed. More than one seedling germinated and established in some cells, but these have been counted as only one plant for our purposes. If all living plants survive to the spring, we will have 2,721 plants available for outplanting. However, one third of the plants may not survive, but we may be able to offset this loss by splitting apart individual seedlings at the Washoe Valley facility, giving a net loss of 400 plants from the current total.

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Figure 1. Seed output (number of regular seeds per plant) as a function of canopy size (projected area) for apparent individuals (isolated rosettes) of *Rorippa subumbellata* (Tahoe Yellow Cress) at the Upper Truckee East population, September 2001.

Figure 2. Seed output (number of regular seeds per plant) as a function of canopy size (projected area) for apparent individuals (isolated rosettes) of *Rorippa subumbellata* (Tahoe Yellow Cress) at the Blackwood South (dark points and line) and Taylor Creek (light points and line) populations, September 2001.

Figure 3. Seed output (number of regular seeds per plant) as a function of canopy size (projected area) for apparent individuals (isolated rosettes) of *Rorippa subumbellata* (Tahoe Yellow Cress) at the Blackwood South, Taylor Creek, and Upper Truckee East populations, September 2001. Plants larger than 4,000 cm² were excluded from this analysis.

Figure 4. Seed output (number of regular seeds per plant) as a function of canopy size (projected area) for apparent individuals (isolated rosettes) of *Rorippa subumbellata* (Tahoe Yellow Cress) at the Blackwood South, Taylor Creek, and Upper Truckee East populations, September 2001. Plants larger than 4,000 cm² were excluded from this analysis and all points (n = 75) have been fitted to a single line.

APPENDIX

Field Datasheets