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Germination ecology of *Rorippa subumbellata* (Tahoe yellow cress), an endangered, endemic species of Lake Tahoe

M. INGOLIA[1,2], T.P. YOUNG[1] AND E.G. SUTTER[3]

[1] Department of Plant Sciences, University of California, One Shields Avenue, Davis, CA 95616 USA (E-mail: meingolia@ucdavis.edu)
[2] Present address: UC Davis Arboretum, One Shields Avenue, University of California, Davis, CA 95616 USA
[3] Deceased

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Summary

We studied the germination ecology of *Rorippa subumbellata* Rollins (Tahoe Yellow Cress, Brassicaceae), an endangered perennial plant endemic to the shores of Lake Tahoe (California and Nevada, USA). The effects of light, temperature, cold stratification, dry storage, and dispersal by hydrochory were tested in both greenhouse and growth chamber experiments. Seeds from four collection years (2001, 2002, 2003, and 2004) were either cold stratified for 14, 30, 60, or 90 days or not stratified and incubated in three alternating day/night temperature regimes (13/-1, 18/4, and 24/10°C) for thirty days. Hydrochory and light experiments were carried out in a cool greenhouse (mean temperature 21°C). Temperature was the most important factor for germination, and germination reached 60-80% when germinated at 24°C. Cold stratification at 5°C for more than a week decreased germination. Seeds required light to germinate. Seeds did survive flotation, but with decreased germination. Seeds dry stored for four years or less germinated nearly as readily as freshly harvested seeds, and germination in the older seed lots exhibited less sensitivity to lower temperatures.

Introduction

Rorippa subumbellata Rollins (Tahoe Yellow Cress, Brassicaceae), is an endangered plant endemic to the shores of Lake Tahoe (California and Nevada, USA) in the Sierra Nevada mountain range. *Rorippa subumbellata* is found exclusively on the beaches of Lake Tahoe and it is the only known lakeside endemic in the Sierra Nevada mountain range (Pavlik *et al.*, 2002). It has been defined as a federal candidate for listing by the U.S. Fish and Wildlife Service since 1975 (Greenwald, 2004), and California and Nevada have classified the species as endangered since 1982.

Rorippa subumbellata is a decumbent herbaceous perennial characterized by deeply divided alternate leaves and a terminal umbel-like inflorescence of small yellow flowers (Hickman, 1993). It reproduces both by seed and vegetatively by horizontal underground stems. Individuals of *R. subumbellata* flower continuously from May to October (Pavlik *et al.,* 2002), and the months of May to September comprise the growing season and average about 7.5 cm of precipitation. Fruit and seed development persist throughout the growing season, with growth and reproduction curtailed by either inundation or the onset of winter (Pavlik *et al.*, 2002). Seeds are small (± 1 mm) and buoyant. Small plants are often seen in late spring and early summer, but the specific month(s) in which the seeds actually germinate have not been determined (Pavlik *et al.*, 2002). Populations of *R. subumbellata* are mainly restricted to sandy beaches and dunes on the lake's margin, and these high elevation microsites are often characterized by extreme light intensity and longwave radiation that results in large inputs from reflective or radiating surfaces such as the sand surface (Pavlik *et al.*, 2002).

Two previous germination trials failed to germinate any seeds of *R. subumbellata*. In the first trial, seeds were either cold stratified for eight weeks at 5°C or not stratified and then placed in two separate growth chambers that were maintained at either a constant 20°C or an alternating 10/20°C temperature regime with an 8/16 hour light/dark cycle (E. Guerrant, pers. comm.). In the second trial, seeds were either cold stratified for 30 days or not stratified, then germinated at constant temperatures of 4, 7, 12, and 17°C for ten days of incubation (Pavlik *et al.*, 2002). Subsequent propagation attempts at three nurseries yielded enough plants for restoration out-planting; however, propagation methods were inconsistent and required large amounts of seeds due to low germination (Pavlik *et al.*, 2003). The specific objective of this study was to characterize aspects of the germination ecology of *R. subumbellata* through greenhouse and growth chamber experiments, controlling for storage time, light regime, seed flotation, temperature, stratification, and collection source, in the hopes of maximizing future germination attempts for restoration.

Materials and methods

Seed harvest and storage

We collected seeds of *R. subumbellata* on 14 August 2004 from five sites (Upper Truckee East Beach, Blackwood Beach [North and South], Tallac Creek Beach, Baldwin Beach, and Edgewood Beach) around Lake Tahoe in California and Nevada. Collection site selection was determined based on the availability of donated seeds collected by the Tahoe Yellow Cress Technical Advisory Group in previous years (2001–2003). The 2001, 2002, and 2003 seeds were collected in the first week of September of each year and placed in manila envelops and/or paper bags (Pavlik *et al.*, 2003, Alison Stanton pers. comm.). The 2004 seed collection was authorized by a California Department of Fish Game permit (permit 04-11-RP) that restricted collection of seeds per population to 5% of available seeds. Seeds were harvested by lightly shaking stems until mature seeds fell into paper bags.

Seeds collected in 2001-2003 were stored at BMP Ecosciences in South Lake Tahoe, CA at room temperatures in paper bags until August 2004. From August 2004 to February 2005, our 2004 collections and donated seeds from previous collection years were stored in paper bags at room temperature at the University of California, Davis until the initiation of the experiments. Although dry storage may not be optimal for some species, seeds stored in this manner often show similar responses to those stored under more "natural" conditions (Baskin and Baskin, 1998).

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Greenhouse germination

In September 2004, greenhouse experiments were initiated to test the effects on *R. subumbellata* seed germination on parameters specific to light regime, collection year, and hydrochory (seed dispersal by flotation). This was a 2 × 3 × 3 × 3 factorial experiment, testing the effects of flotation, light levels, collection site, and collection year. The Baldwin Beach, Upper Truckee East, and Tallac Creek Beach seed sources were used for this experiment. A cool greenhouse at UC Davis was chosen to correspond with spring air temperatures of the Lake Tahoe Basin. Mean air temperature during the experiments was 21°C, typical high temperature was 25°C and the low was 16°C. Stuewe pots (2.5 cm × 12 cm) were filled with UC Soilless mix (25% sand, 33.3% peat, and 41.7% fir bark), and placed in trays containing 200 pots each, with the two outside rows on each side not seeded. Each pot was sown with one seed and topped with ≤ 1 cm of 20-mesh sand (grain size ≤ 0.85 mm). Individual trays were used as replicates, and five replicates were used for each variable in each of the treatment combinations performed. Trays were randomly arranged in the greenhouse. Twenty seeds from each of the three seed sources (Tallac Creek Beach, Upper Truckee East, and Baldwin Beach) were planted, making a total of 60 seeds for each replicate and 300 seeds for each of the 18 combinations of the three treatments described below.

Hydrochory/flotation experiment

In the flotation experiment, 300 seeds were floated for one week and 300 seeds were floated for one month in 40 ml clear plastic snap cap vials at 17°C in a Percival growth chamber with a 14/10 hour light/dark cycle. Each vial contained 20 seeds. After the flotation interval, the numbers of seeds still floating, seeds germinated during flotation, and seeds that sank during the flotation interval were all recorded. Seeds were sown randomly in the replicate trays. Each of the five replicate trays had a total of 120 seeds (60 floated for one week/60 floated for one month). Seeds floated for one week were planted on 28 September 2004, and seeds floated for one month were planted on 28 October 2004.

Light regime experiment

Seeds in the light regime treatments were sown on 25 September 2004 in 100% light, 50% shade, or dark conditions. Three hundred seeds were sown in full light treatments, 300 in 50% shade treatment, and 300 in dark treatments. Each replicate of 60 seeds were sown in a single tray for a total of 15 trays. Dark treatments were covered in aluminium foil, and seeds were not checked for germination until the termination of the experiment after 30 days. The 50% shade treatment was created by covering the target tray with an empty tray supporting a 50% shade cloth (to allow room for seedlings to grow).

Collection year experiment

Seeds from three collection years (2001, 2003, and 2004) were sown on 16 September 2004. The 2002 seeds were not available for this experiment. Three hundred seeds were sown from each collection year, and each replicate tray contained 180 seeds (60 from each year).

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Seeded trays in all three experiments were watered with a mister everyday. Trays covered with aluminium foil in the light regime experiment ("dark" treatments) were bottom watered by placing the trays in water-filled containers for 10 minutes to insure saturation of the substrate. Germination was monitored regularly (except the "dark" treatments) and experiments were terminated after 30 days. Seedlings were left in place until termination of the experiment. Preliminary tetrazolium viability tests yielded inconclusive results due to small seed size and were therefore not conducted on seeds that did not germinate.

Growth chamber experiment

Although several greenhouse treatments had significant effects (see Results), overall germination percentages were less than desirable for a propagation effort. This prompted the design and initiation of a more in-depth analysis of germination ecology in February 2005. The growth chamber trials consisted of four experiments. The first was 2 × 3 × 4 factorial experiment that simultaneously tested the effects of seed source, germination temperature, and collection year. This experiment was repeated five times. The second was a 2 × 3 × 4 × 4 factorial experiment that simultaneously tested the effects of seed source, germination temperature, collection year, and stratification time. In addition, two similar experiments were carried out on seeds from Edgewood Beach that were collected in 2001. The first tested the effect of temperature regime and was repeated five times. The second was a 4 × 3 factorial experiment that tested the effect of stratification time and temperature. Each of the resultant treatment classes had three replicates. We used 12-50 seeds from each seed source per replicate in each of the four experiments, depending on availability (see table 1). In total, 20,203 seeds were tested in three replicates.

Three Percival growth chambers were utilized and temperature regimes were set to have a 14/10 h light/dark cycle. The temperature regimes were chosen to mimic early spring (13/-1°C), spring (18/4°C), and early summer (24/10°C) day/night air temperatures at Lake Tahoe. Temperatures were chosen using average ambient air temperature data provided from the Western Regional Climate Center from the last 100 years (2001). All chambers remained within 2°C of their setting throughout the experiment.

The two experiments that did not involve stratification were repeated on five different dates: 16 February, 2 March, 16 March, 16 April, and 16 May, 2005. Seeds were sown in 100 mm diameter petri dishes lined with 20 mesh sand and watered with 10 ml of water or until seeds were fully wetted. With the exception of 16 February, each sow date also represents the termination of a stratification interval on a separate set of seeds for the stratification experiments. Prior to stratification these seeds were wetted to insure imbibition and then stratified for 14, 30, 60, or 90 days in a refrigerator at 5°C in the dark. Petri dishes were wrapped in parafilm during stratification to retain moisture. At the end of each stratification time period, parafilm was removed from the petri dishes and seed lots were placed in one of three growth chambers with the different temperature regimes.

Petri dishes were distributed randomly within each chamber. No fungicide was used, and seeds were moistened as needed. Germination was defined as the emergence of the radicle through the seed coat. The number of seeds that had germinated was counted at five day intervals and each experiment concluded after 30 days.

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Table 1. Seed numbers used in each replicate in each of the three temperature regimes of the growth chamber experiments of *Rorippa subumbellata*.

Experiment 1							Experiment 2						
Source	Repetition Date (2005)	Collection Year				Source	Stratification Time (Days)	Incubation Date	Collection Year			
		2001	2002	2003	2004				2001	2002	2003	2004
Upper Truckee East	16-Feb	10	15	20	50							
	2-Mar	10	17	20	50	Upper Truckee East	14	2-Mar	10	30	20	50
	16-Mar	10	17	20	50		30	16-Mar	10	30	20	50
	16-Apr	10	15	20	50		60	16-Apr	10	30	20	50
	16-May	10	15	20	50		90	16-May	10	30	20	50
Baldwin Beach	16-Feb	12	13	50	50							
	2-Mar	15	13	50	50	Baldwin Beach	14	2-Mar	12	13	50	50
	16-Mar	15	15	50	50		30	16-Mar	12	13	50	50
	16-Apr	15	20	50	50		60	16-Apr	12	13	50	50
	16-May	15	13	50	50		90	16-May	12	13	50	50

Experiment 3			Experiment 4			
Source	Repetition Date (2005)	Collection Year	Source	Stratification Time (Days)	Incubation Date	Collection Year
		2001				2001
Edgewood Beach	16-Feb	20				
	2-Mar	20	Edgewood Beach	14	2-Mar	20
	16-Mar	20		30	16-Mar	20
	16-Apr	20		60	16-Apr	20
	16-May	20		90	16-May	20

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Statistical analysis

Statistical analyses were performed using SAS 9.1 statistical software (SAS, 2005). Logit methods of SAS were used for analysis of the final germination percentage data. Logit regression analysis utilizes the natural log of the odds ratio (Borooah, 2002), and offers a more stable result, in contrast to ANOVA, when some replicates do not germinate.

Results

Greenhouse experiments

Averaged across collection sources, 65-70% of seeds in the hydrochory experiment were still floating in the vials after one or four weeks of flotation. During the flotation interval, 2-4% percent of the seeds germinated on the surface of the water. Once surface germination occurred, seedlings sank to the bottom where their underwater life expectancy was not determined, but none of theses seedlings died before the end of the flotation interval. Only 11% of the seeds that were put in water for one week germinated after sowing, and this was double the germination of seeds floated in water for four weeks (table 4). There was no significant difference between collection sources in the flotation trial (table 2).

Averaged across collection sources, seeds in full light had 22% germination, seeds in 50% shade had 6% germination, and seeds in the dark did not germinate (table 3). The seedlings in the shade trial were distinctly thin and etiolated. The significant interaction of light regime and collection source (table 2) was due to the higher germination percentage (37%) of the Tallac Creek Beach seeds in full and half light (table 3).

Table 2. Logit analysis of *Rorippa subumbellata* greenhouse germination trials.

Effect	DF	Wald Chi-Square	p
Light Regime	2	31.4	< 0.0001
Flotation Interval	1	6.78	0.009
Collection Year	2	112	< 0.0001
Collection Source	2	25.5	< 0.0001
Light Regime * Collection Source	2	23.7	< 0.0001
Flotation Interval * Collection Source	2	1.98	0.4

Table 3. Greenhouse percentage germination of *Rorippa subumbellata* collection sources in 100% light, 50% shade, and dark.

	Final Germination Percentage			
Regime	Blackwood Beach (North and South)	Tallac Creek Beach	Upper Truckee East	Mean Germination
Light	12	37	18	22
50% Shade	2	10	5	6
Dark	0	0	0	0

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Table 4. Greenhouse percentage germination of *Rorippa subumbellata* after one week or one month of flotation.

Float Interval	Source	Percentage Floating After Interval	Percentage Sunk After interval	Percentage Germinated While Floating	Percentage Germination of Floating Seeds	Percentage Germination of Seeds that sank during flotation interval	Total Percentage Germination
1 Week	Blackwood Beach (North and South)	70	28	2	4	1	7
1 Week	Tallac Creek Beach	25	70	5	5	10	20
1 Week	Upper Truckee East	65	31	4	2	1	7
1 Week	Sources Combined	53	43	4	4	4	11
4 Weeks	Blackwood Beach (North and South)	61	39	0	1	0	1
4 Weeks	Tallac Creek Beach	74	21	5	3	0	8
4 Weeks	Upper Truckee East	75	23	2	3	0	5
4 Weeks	Sources Combined	70	28	2	2	0	5

The influence of collection year on germination was averaged across replicates and only tested on one seed source, Upper Truckee East. Seeds collected in 2001 had significantly greater germination (40%) than seeds collected in 2003 (9%) and 2004 (8%) (figure 2).

When averaged across the five variables in the flotation and light experiments, seeds from Tallac Creek Beach had the greatest germination, averaging 15%, while Upper Truckee East averaged 7%, and Blackwood Beach (North and South) averaged 4%.

Growth chamber experiment

Averaged over temperature regime, repetition date, and collection year, Edgewood Beach seeds exhibited 9% germination, Baldwin Beach 34%, and Upper Truckee East Beach to 28% (Logit: df = 1, Wald Chi-Square = 16.8, p = 0.0002). The Edgewood collection source was only available for the 2001 collection year, and because this could skew

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subsequent results, these data were not used for other analyses of the experiment. There was no significant difference between the other two seed sources (p = 0.91) and collection source was not included as a factor in the remaining analyses of the experiment.

Germination percentages declined with cooler temperature regimes. When averaged across all other treatment variables, the 24/10°C regime had 71% germination, and two of the five incubation dates had germination over 80%. In contrast, the 18/4°C regime averaged 18% germination, and the 13/-1°C regime averaged 7% germination (Logit: df = 2, Wald Chi-Square = 565, p < .0001).

Seeds of *R. subumbellata* that were cold stratified for any period of time germinated less than those not stratified (figure 1 and figure 2; Logit: df = 1, Wald Chi-Square = 110, p < 0.0001). When averaged across all other treatment variables, 31% of non-stratified seeds germinated compared to only 19% of stratified seeds. As stratification time increased, germination percentage significantly decreased, but only in the optimal temperature regime (figure 1).

There was a significant interaction between temperature regime and collection year (Logit: df = 6, Wald Chi-Square = 230, p < 0.0001). There were also significant differences among collection years (Logit: df = 3, Wald Chi-Square = 129, p < 0.0001). In the 24/10°C temperature regime, germination varied from 65-76%, and germination increased slightly with more recent collection year. In contrast, in the cooler temperature regimes, germination steadily decreased with more recent collection year (figure 2).

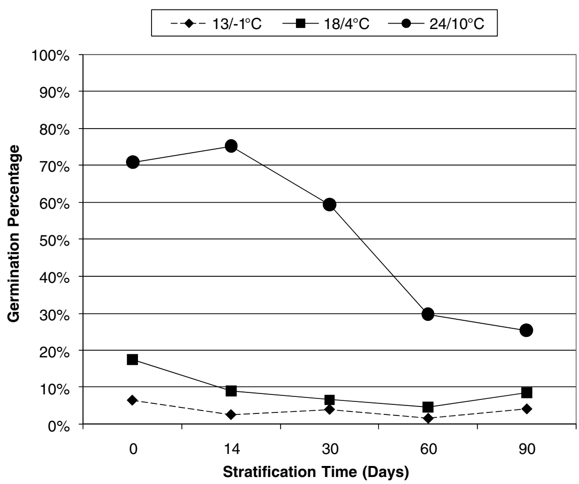

Figure 1. Mean germination percentage of *Rorippa subumbellata* seeds after varying lengths of stratification time in three alternating temperature regimes. Germination percentages were averaged across two seed sources (Upper Truckee East and Baldwin Beach) and four collection years (2001, 2002, 2003 and 2004).

Discussion

Interpretative comparison of greenhouse trials versus lab trials requires recognition of the differences between the experiments. Greenhouse trials were carried out in pots (versus petri dishes), and they were conducted at a single average temperature regime

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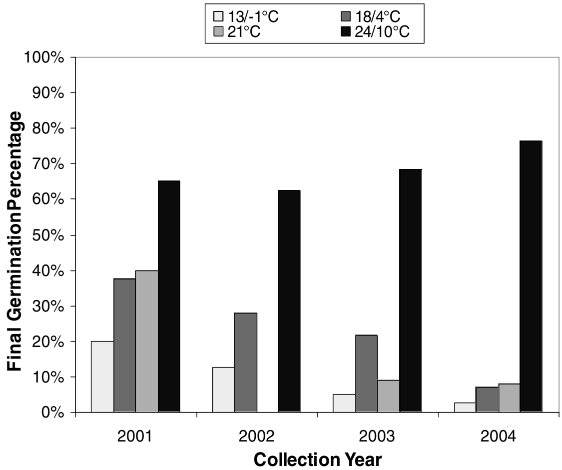

Figure 2. *Rorippa subumbellata* germination percentages of non-stratified seeds by collection year in the greenhouse and growth chamber experiments. Growth chamber results are averaged across the two seed sources (Upper Truckee East and Baldwin Beach) and five repetition dates. Growth chamber experiments are split up by the day/night alternating temperature. Greenhouse temperature is depicted as an average temperature over a 24-hour cycle and greenhouse collection year experiments were conducted solely on the Upper Truckee East seed source. 2002 seeds were not available for the greenhouse experiments.

(21°C), instead of three temperature regimes. Germination percentages in the greenhouse experiment were lower than those in the growth chamber experiment, but not particularly low relative to their cooler germination temperatures (figure 2). In addition, the <1 cm top covering of sand may have inhibited germination in *R. subumbellata* seeds (see "Light regime," below).

Light regime

As with seeds of the cogeners *R. islandica* (Matsuo *et al.*, 1984), *R. nasturtium-aquaticum* (Biddington and Ling, 1983), and *R. palustris* (Klimesova *et al.,* 2004), *R. subumbellata* seeds possess a distinct requirement for light to germinate. Baskin and Baskin (1998) note that many species found in seasonally flooded habitats have light requirements for germination to ensure emergence in moderately shallow and/or clear water so that seedlings can access light for photosynthesis and growth. A light requirement for *R. subumbellata* seeds gives this species the same advantage.

Klimesova *et al.* (2004) suggested that the light requirement for *R. palustris* ensures germination in a less competitive environment. Our results indicated that emergence in densely colonized habitats would be deterred both by the high light requirement, and because shaded soil will be cooler, another deterrent to *R. subumbellata* germination. The preservation of open habitat is therefore likely a necessity to insure success of the species. The sensitivity of *R. subumbellata* to light and the high germination percentages in the appropriate temperature regime indicate that the species may have an ability to support a seed bank. Further investigation of seed bank dynamics for *R. subumbellata* may prove important for the conservation effort.

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Flotation

The flotation experiment tested the ability of seeds to germinate while floating on water and to survive water dispersal, a common dispersal method among wetland habitat species. *Rorippa palustris* seeds have water-repellent surfaces and an ability to float at least one month during water dispersal (Klimesova *et al.*, 2004) Seeds of *R. subumbellata* exhibited an ability to float for at least one month, but there was a reduction in germination percentage after flotation. Although floated seeds had low germination percentages, and some seeds germinated before the termination of one- and four-week flotation intervals, some seeds remained viable, and perhaps sufficiently so to be ecologically viable as colonizers. It would also be valuable to investigate the longevity of germinated seedlings after submersion. The ability to remain alive while underwater and then colonize open, moist beach habitat after the spring melt could be central to survival, given the annual flooding found in some of the core *R. subumbellata* sites around the Lake Tahoe. Overall, flotation dispersal offers a way to increase colonization and as the lake margins recede during the progression of the growing season, seeds and/or seedlings are abandoned onto a moist, open substrate, optimal for establishment. Confirmation of this adaptation is evident from the documented observations of *R. subumbellata* seedlings in the "bathtub ring" of debris left on beach shores as water levels recede during the growing season (Pavlik *et al.*, 2002). It is probable that both seed and seedling dispersal via flotation are abilities that are crucial for survival and lake-side colonization in the metapopulation. However flotation does not come without a cost, since flotation inhibits germination.

Collection source

Differing germination percentages among seed sources have been noted in numerous ecological studies (Lord, 1994, Hamze and Jolls, 2000, Dhanai *et al.*, 2003, Severns, 2003), and both of our experiments revealed significant variation among sites in terms of germination percentages of *R. subumbellata*. It is not known whether the germination differences among these collection sites can be attributed to genetic, environmental, or seed developmental differences. Genetic analyses done on *R. subumbellata* have revealed little genetic variation (Saich and Hipkins, 2000), so it is likely that the variation reflects differences in seed quality, due to environmental differences during seed development.

Temperature regime

In general, temperature is the single most important factor triggering changes in dormancy states in seeds (Baskin and Baskin, 1998). Conditional dormancy dependence with temperature was exhibited by *R. palustris*, which germinated at 80-100% in a 25/10°C (day/night) regime, but only 20% in a 15/10°C temperature regime (Klimesova *et al.*, 2004). Seeds of *R. subumbellata* also exhibited a conditional dormancy broken at high temperatures, with increases in incubation temperature dramatically correlating with increased germination percentages. The 24/10°C temperature regime corresponds to air temperatures in the Lake Tahoe Basin in mid-June to July. However, the lower heat capacity of sand in conjunction with intense solar heat absorption at high elevations makes the sand surface temperatures reach similar and higher temperatures earlier in the growing season, when *R. subumbellata* appears to be germinating in nature.

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Moisture content of the sand affects the substrate's ability to warm, and this likely has implications for germinating *R. subumbellata* seeds. Due to a lack of summer precipitation, wet sand is found closer to water's edge during the growing season, perhaps making the temperature requirement act as a safeguard against inundation. Likewise, the sand is more likely to be close to saturation and therefore cooler, at the start of the growing season when there is still a chance of a late spring frost, and the warm temperature requirement would be another safeguard against earlier, precarious germination.

Stratification trial
Cold stratification, the storage of moist seed at a characteristic winter temperature (usually between 0 to 10°C), often breaks non-deep physiological dormancy in seeds (Baskin and Baskin, 1998). Conversely, a pattern of dormancy induced by cold stratification has been confirmed in a limited number of perennial species (Baskin and Baskin, 1998). The decrease in germination percentages of *R. subumbellata* seeds as stratification time increased may represent conditional dormancy and the designed incubation conditions to which seeds were subjected after stratification were not sufficient for dormancy break of the stratified seeds. In practice, using non-stratified seeds is the most productive way to germinate *R. subumbellata* seeds.

Collection year
It is common that as seeds increase in age, percent germination and speed of germination decrease (Liu and Spira, 2001). The reduced vigour of two year old *R. nasturtium-aquaticum* seeds produced lower germination percentages and slower overall germination (Biddington and Ling, 1983). *Rorippa subumbellata* seeds tended to follow the same pattern in our experiments, with older seeds having less overall germination. However, the effects of seed age were relatively small, especially at the (optimal) higher germination temperatures. Older seeds were more likely to germinate than younger seeds in the cooler temperature regimes, and at the lowest temperature regime older seeds germinated to the highest germination percentages in every instance (figure 2). The ability of older seeds to germinate at cooler temperatures may be indicative of the seeds losing dormancy requirements with age. This could be a strategy to give seed a last opportunity before all viability is lost.

Conclusion

Restoration recommendations
To insure success of the species, seed storage is a must considering the small habitat range of *R. subumbellata* and an apparently unavoidable increase in construction and development in the Tahoe Basin. Due to the prolific seed production of *R. subumbellata*, it may be possible to store large amounts of seed for later germination. Although better storage conditions than those utilized in this experiment may increase the longevity of seeds in storage, even less than ideal seed storage conditions were sufficient for high percentages of germination of seeds stored up to five years, when germinated at warm temperatures with ample moisture and no stratification. Propagation efforts should take

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into consideration that seeds of *R. subumbellata* are likely to germinate best in the field through top sowing seeds in full sun conditions. Out-planting in open habitats with a low probability of intense competition should also increase survivorship of transplants.

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