Feasibility of out-planting small populations of spreading globeflower *Trollius laxus* in a forested fen in central New York, USA

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SUMMARY

Where sufficient seeds of a rare plant species are available, out-planting small populations may be an effective conservation practice, given certain rare species persist naturally as small populations, sometimes within metapopulations. I investigated the feasibility of out-planting small populations of the rare and declining fen plant spreading globeflower *Trollius laxus*, which is easily grown in greenhouse and garden settings. In 2004 greenhouse-raised seedlings were planted at 14 plots (n = 10 plants per plot) located within a protected area where a well-studied metapopoulation of *T. laxus* already occurred. Suitable plots were identified using a GIS-based, macroscale habitat model; seven were under canopy gaps and seven were under intact canopy. The populations were monitored one, two, three and eight years after out-planting. Two plots that were lost less than one year after out-planting were not included in subsequent monitoring and analysis. I compared survival between gap and non-gap populations and quantified the vigour of the surviving plants over time. Overall, survival was very poor (only 10 of the original 120 transplants survived to year eight), but surviving plants were vigorous, showing increases in size and flower production. Plant survival to year three was significantly greater under canopy gaps than intact canopy. These results suggest that out-planting *T. laxus* at new sites may be difficult, that success will be greater under canopy gaps than intact canopy, and that out-planted populations may need regular supplementation with new transplants in order to be viable over the long-term.

BACKGROUND

Increasingly, restoration and translocation are used to bolster declining populations of rare species. Rare and declining plant species are vulnerable to extinction, making it problematic to acquire large numbers of seeds for out-planting projects. At the same time, some rare species persist in small populations that may be distributed within a larger metapopulation structure (e.g. Freckleton & Watkinson 2002, Alexander *et al.* 2012). The population dynamics of these species suggest that out-planting proximate small populations may be effective in some cases.

When choosing plots for out-planting, it is convenient to use just a few macroscale (i.e. regional or site-level) characteristics, particularly those that are readily available for use in a geographic information system (GIS). Using a GIS for plot selection improves efficiency because it reduces the need for time-consuming field work in which mesoscale (i.e. plot-level) and microscale (i.e. plant-level) factors are measured. However, many plants respond to mesoscale and microscale environmental conditions, so the use of macroscale characteristics to choose sites for out-planting may be ineffective.

Spreading globeflower *Trollius laxus* (sometimes called *T. laxus* ssp. *laxus*) is a spring-blooming perennial that is considered critically imperiled (S1, the most critically at-risk of the state rankings, which range from S1-S5) in three of the five northeastern US states in which it occurs (Natureserve 2014). *T. laxus* was considered a candidate for listing under the US Federal Endangered Species Act (Mitchell & Sheviak 1981). Its stems (5-52 cm tall) emerge from a central basal area, each stem supporting one palmately-divided leaf, creating a clumped form (Parfitt 1997). The 2.5-5 cm yellow, bowl-shaped, bisexual flowers, which occur singly on peduncles that are extensions of the stems (Mitchell & Dean 1982, Parfitt 1997), are believed to be weakly self-compatible. *T. laxus* occurs in minerotrophic (calcareous) wetlands (Parfitt 1997), reaching its greatest population densities in non-forested rich fens (Bliss 1985). In forested fens, *T. laxus* is often patchily distributed (Paine 1865, Bliss 1985). Quite easy to propagate and grow in home and horticultural gardens (Jones 2001), *T. laxus* populations vary from small (up to several dozen plants) to large (thousands of plants) at natural locations in the wild. At the protected area discussed in this study, *T. laxus* often occurs in small natural subpopulations of 5–30 plants (Scanga 2014). Some small populations of *T. laxus* have shown long-term persistence and seem to be genetically related (Zielinkski 1993), indicating that they may function as a metapopulation (Jones 2001).

The first objective of this study was to develop a replicable method for propagating *T. laxus* seedlings. The second objective was to evaluate the utility of a macroscale GIS habitat model as a tool for choosing plots for out-planting. Because *T. laxus* population vigour seems to be linked to the presence of moderately sized gaps in forested fens (Scanga & Leopold 2012, Scanga 2014), the out-planting plots were located under two comparative conditions, gap or intact canopy. The final objective was to determine the feasibility of out-planting proximate small populations (n = 10 plants).

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ISSN 1758-2067

ACTION

Seed collection and propagation: Approximately 600 seeds were harvested from two spatially distinct subpopulations at Nelson Swamp Unique Area (NSUA, Madison County, New York, USA, 42º53'N, 75º47'W) in July 2003. NSUA is a forested rich fen with a mixed conifer canopy (Scanga & Leopold 2010, Scanga 2014). NSUA was chosen for this study in part because it supports one of the largest remaining *T. laxus* populations, so any negative demographic impacts from collecting seeds were likely to be minimised. In addition, the approximately 5000 plants at NSUA are patchily distributed in over 20 spatially distinct subpopulations across the 850 ha forested wetland, leaving much apparently suitable habitat unoccupied by *T. laxus*.

Propagation methods were based on previously developed protocols (Parsons & Yates 1984, Brumback 1989). Seeds were immediately potted (n = 40 per 13 cm pot) in moistened PRO-MIX "BX" (Premier Horticulture, Quakertown, Pennsylvania). The pots were misted with a 10% bleach solution, covered with plastic and put in a greenhouse without climate control (in the shade, under a bench) for 90 d. The pots were subsequently lightly watered if necessary, then transferred to a dark room at 4 °C for 120 d, at which point some of the seeds had germinated (Figure 1a). All pots were then transferred to a heated greenhouse. Ambient light in this greenhouse was augmented from 08:00 h to 20:00 h with two 1000 W metal halide bulbs. Only about a third (31%) of the seeds germinated into viable seedlings. Seedlings were transplanted as they grew into individual 5 cm pots (Figure 1b), and eventually individual 13 cm pots (Figure 1c), filled with a mix of 2:2:1:1 Earthgro topsoil (The Scotts Company, Marysville, Ohio), *Sphagnum* peat, vermiculite and perlite, as well as 1% by weight crushed limestone.

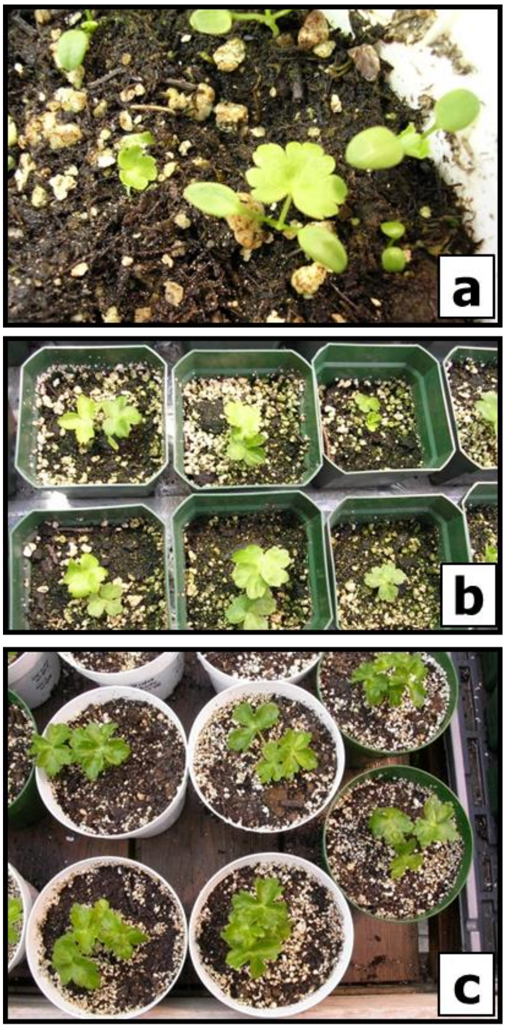

Figure 1. Seedlings of *Trollius laxus* in the greenhouse. Seedlings are shown a) just after seed germination; b) after the first transplant into 5 cm pots; and c) after the final transplant into 13 cm pots.

Plot selection: Using ArcView 3.3 (Environmental Systems Research Institute 2002), a simple habitat suitability model for *T. laxus* was developed, based on the macroscale physical features of the known *T. laxus* subpopulations at NSUA (Figure 2). The model was limited to open-access GIS layers, including soil survey (1:24,000, United States Department of Agriculture Natural Resources Conservation Service 1998), bedrock geology (1:250,000, New York State Museum / NYS Geological Survey 1999a) and surficial geology (1:250,000, New York State Museum / NYS Geological Survey 1999b). From these GIS layers, polygons were selected in which a *T. laxus* subpopulation was known to occur. By performing sequential intersections of these polygons, potentially suitable *T. laxus* habitat was isolated. Within this habitat, a random point generator extension was used to select randomised locations for 14 plots. The plots ranged from approximately 0.1–2 km from the seed source subpopulations.

Seedling out-planting: After growing the seedlings in the greenhouse for approximately 90 d, they were stratified by size based on the number of stems, and 10 seedlings were randomly selected to be transplanted to each of the 14 plots at NSUA on 15 May 2004. In each of the 14 plots, the average number of stems per plant ranged narrowly from 4.8 to 4.9, indicating that all plots had a similar population structure at the start of the experiment. Seedlings were hardened off outside before taking them to the field.

In the field, the 14 random points were located using a handheld global positioning system unit, and seedlings were planted in either the nearest canopy gap or the nearest non-gap at each point (Figures 2 and 3). I alternated between the gap and non-gap positions so that there were 7 gap plots and 7 non-gap plots in total. Whenever possible, plants were placed at the base of hummocks because a previous study indicated that plants were most vigorous at this microtopographic position (Leimanis 1994). Plants in each plot were marked (Figure 3) and monitored in September 2004, as well as June 2005 (year one), June 2006 (year two), and June 2007 (year three). Of the 14 original plots, 2 were destroyed before the June 2005 monitoring period (one gap plot by beaver-induced flooding of a nearby stream; one non-gap plot by herbivory). Therefore, only the remaining 12 plots were followed from 2005-2007. At each monitoring period, plant survival and stem and flower production were recorded, and each plot was thoroughly examined for new seedlings.

Plant markers were removed in 2007 to meet the restrictions at NSUA, but all plots could still be relocated in June 2012 (year eight) and the number of plants remaining in each plot recorded. Whether survival in the gaps was greater than expected by chance was evaluated using a Pearson's Chi-squared test with Yates' continuity correction in R 2.15.2 (R Core Team 2012). Because the canopy conditions had changed at several plots by June 2012 due to new tree falls, only the 2005-2007 monitoring data were used in statistical analysis.

ISSN 1758-2067

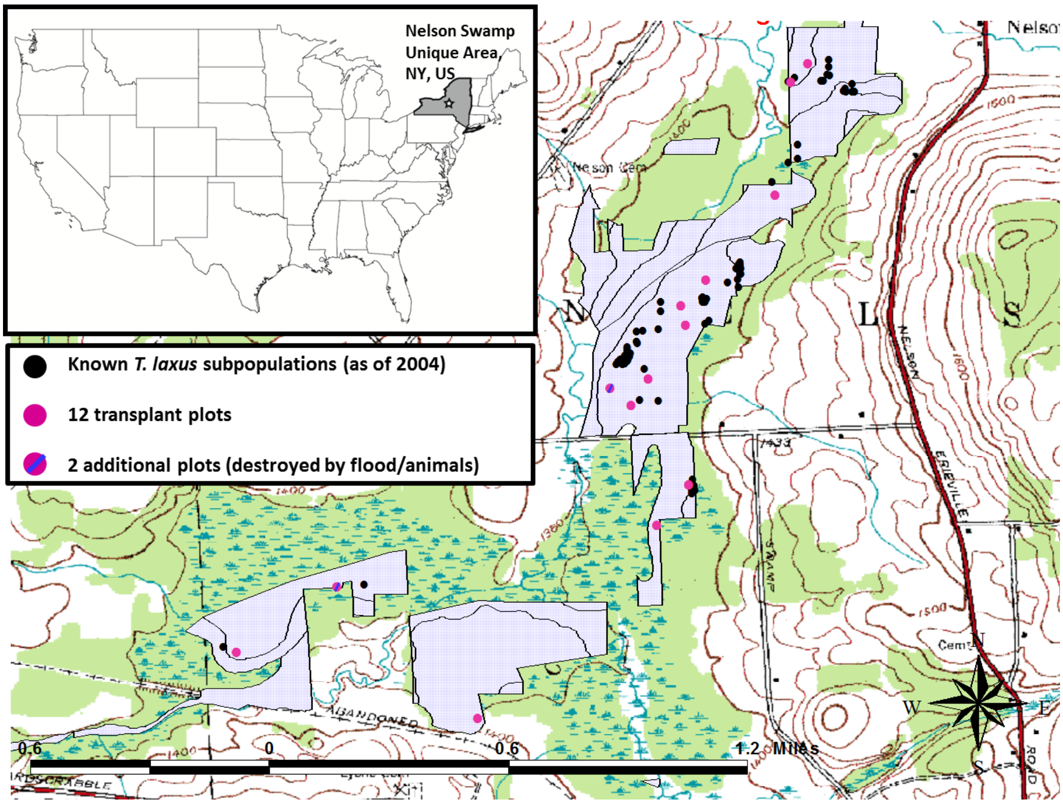

Figure 2. Potentially suitable habitat (pale shaded area on the topographical map) for *T. laxus* within the protected Nelson Swamp Unique Area, and locations of naturally occurring *T. laxus* subpopulations as well as the out-planting plots used in this study.

CONSEQUENCES

Overall, survival of transplants was poor (Figure 4). Although 76 seedlings survived at least one year, only 29 of the original 120 out-planted seedlings survived three years (to 2007). These survivors occurred in 8 of the 12 plots (n = 6 gap plots; n = 2 non-gap plots). Even fewer survivors (n = 10 plants in 6 different plots) were found in the 2012 survey.

Plant survival to year three was significantly greater under canopy gaps than non-gaps (n = 25 plants in canopy gaps; n = 4 plants in non-gaps; $\chi^2 = 18.2$, $p < 0.001$). It is noteworthy that all gap plots had at least one surviving plant by year three, and that 5 of the 6 gap plots had at least one survivor by year eight.

The 29 plants that survived to year three (2007) were healthy. They maintained their year one increase in size (number of stems) and produced flowers (Figure 5) and fruits (data not shown). However, only two new seedling recruits were found over the course of the study in all plots, suggesting that seed germination, seedling establishment or both germination and establishment were very low.

DISCUSSION

Although *T. laxus* grows easily in gardens and greenhouses, and occurs in small populations in the wild, its survival in the out-planted small populations was very low, even at a fen where a very vigorous patchy population with many small *T. laxus* subpopulations already exists. The GIS-based macroscale environmental conditions at all 12 plots should have been appropriate for *T. laxus*, so these results suggest that it is not sufficient to use macroscale variables alone when choosing sites for out-planting.

Most of the plants (75% under canopy gaps; 52% under intact canopy) were still alive one year after out-planting in 2005. This observation indicates that the transplanting procedure itself was not a major cause of plant death. Instead, most plants seemed to gradually die off over time, suggesting that certain aspects of the mesoscale or microscale environment were inappropriate. Plot-level (mesoscale) light

Figure 3. Out-planting *T. laxus* seedlings at Nelson Swamp Unique Area: a) marked seedling after out-planting; b) 10 marked seedlings in a plot.

ISSN 1758-2067

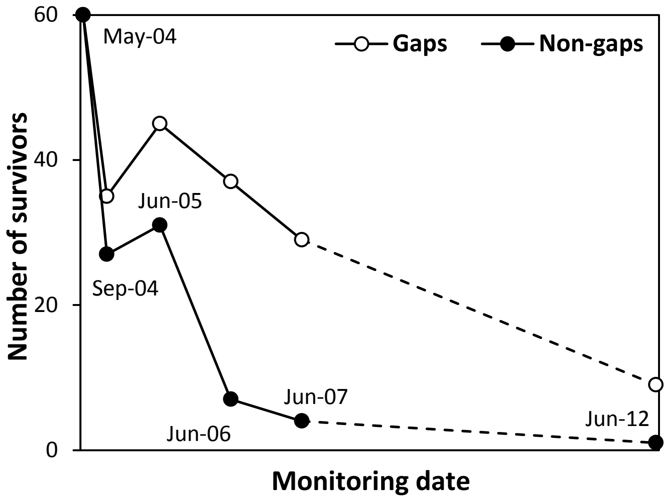

Figure 4. Survival of 120 *T. laxus* seedlings in six canopy gaps and six non-gaps from 2004–2007, and in a 2012 follow-up visit to the site.

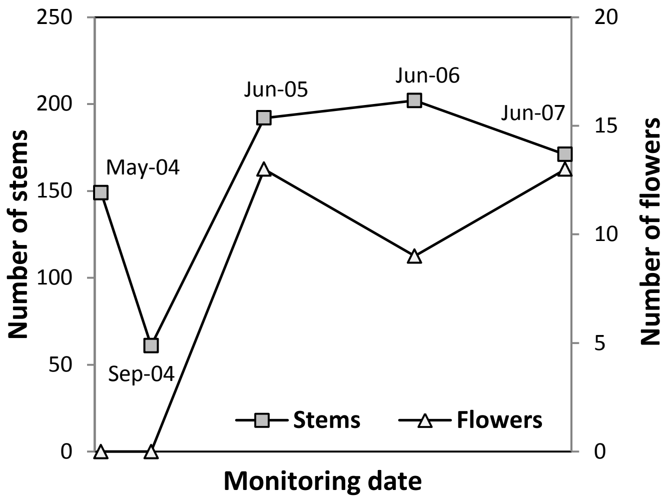

Figure 5. The number of stems and flowers produced by 29 plants that survived to 2007, recorded between 2004 and 2007.

levels are clearly important: survival was greater under canopy gaps than intact canopy, a result that supports previous studies that indicate that *T. laxus* has the greatest vigour under intermediate levels of light in forested fens (e.g. Scanga 2014).

Although mesoscale light levels are important, microscale factors most likely explain why the surviving seedlings were scattered among multiple plots. In fens, relatively small differences in microtopography (hummocks and hollows) can result in marked differences in microclimatic factors, including soil temperature, water levels, snow depth and groundwater chemistry (Raney *et al.* 2014), and calcareous soil substrates are known to be important for *T. laxus* persistence (e.g. Parfitt 1997). Sunlight can also vary greatly at very small spatial scales in forested ecosystems (Canham *et al.* 1990, Lieffers *et al.* 1999, Scanga & Leopold 2010). Such differences in microenvironment can have important effects on plant community composition in fens (Raney *et al.* 2014), and likely impacted the survival of individual *T. laxus* transplants.

It would be logistically simpler to out-plant fewer, larger subpopulations rather than more, smaller subpopulations. However, a large subpopulation approach would greatly reduce the spatial distribution of the out-planted populations, making them more vulnerable to catastrophic disturbances like the stream flooding that eliminated all plants at one of the plots at NSUA. In this study, a small subpopulation approach ultimately resulted in survivors that were widely distributed across the field site (at six different locations by 2012). Given that *T. laxus* plants may live for almost four decades (Scanga 2009), the 10 survivors may produce many more propagules before they die.

The results of this study indicate that out-planting seedlings of *T. laxus* may be unproductive, even at sites where it already occurs. To improve plant survival, it will be important to consider mesoscale conditions like canopy cover; microscale conditions are also likely to be very important. Regular supplementation of out-planted populations with new transplants may be needed. Such supplementation can overcome the effects of temporal variability in environmental conditions, e.g., due to unusual weather conditions. Using the propagation methods outlined in this study, it should be simple to raise new seedlings for future out-planting projects.

ACKNOWLEDGEMENTS

I thank G. Owens (New York State Department of Environmental Conservation), L. Goldmann, C. Schirmer, D. Kurtz, and A. Blowers for assistance with fieldwork, and W. Cullina (New England Wild Flower Society) for valuable advice on *T. laxus* propagation. I also thank D. Leopold for his support and guidance. Funding was provided by Utica College and the US Environmental Protection Agency (EPA) under the Science to Achieve Results Graduate Fellowship Program. EPA has not officially endorsed this publication and the views expressed herein may not reflect the views of the EPA.

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ISSN 1758-2067