Research Practical Germination rate and seedling height of threatened plant species in Switzerland under different irrigation treatments



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Abstract

Only little is known about germination requirements of threatened plant species in Switzerland. As rare plant species are especially prone to local extinction due to climate change, it is important to develop efficient conservation strategies. Ex-situ conservation allows species to be transplanted outside of their natural habitats to prevent further extinction. In order to establish themselves in a given habitat, species must be able to germinate under specific environmental conditions. As a result, the conditions in which a species germinates have an impact on the seedlings' establishment and survival and thus may contribute to species rarity. To test the effect of three different irrigation treatments on germination rate and seedling height of threatened plant species in Switzerland, a blocked split-plot experiment was conducted. Only Tephroseris helinitis showed a significant effect in average germination rate under different irrigation treatments and we thus suggest keeping T. helinitis under moist conditions to obtain high germination success. None of the species showed a significant response to different irrigation quantities on seedling height. There was no clear pattern between water requirements for germination and a species' Landoldt indicator value. However, we need more information on specific germination needs of threatened plant species in order to counteract local extinction of these species. Therefore, multispecies studies are needed to examine abiotic conditions favourable for seed germination to develop germination protocols for individual threatened species.

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1 Introduction

Due to global change, biodiversity decreases on regional, local and global scale (Finderup Nielsen et al. 2019; Dirzo and Raven, 2003). Especially threatened species are prone to extinction (Kempel et al. 2020). Thus, it is important to conserve these species to counteract further biodiversity loss.

Ex-situ conservation offers the possibility to transplant species outside their natural habitats and attempts to prevent further losses. Furthermore, *ex-situ* conservation also provides the opportunity to support wild populations and habitats (Maunder and Byers 2005) and thus acts as a back-up and augmentation for small populations which may otherwise be lost (Li and Pritchard, 2009). *Ex-situ* strategies can therefore support the conservation of species within habitats where they were once present (Schoen and Brown, 2001). *Ex-situ* conservation strategies begin with the germinating of seeds. Unfortunately, information about the cultivation of threatened plant species and their germination is scarce. Multispecies studies, identifying abiotic conditions under which many different species can germinate, reduce the amount of tries required to discover germination protocols for individual threatened species.

Plants are restricted in their environmental range by abiotic and biotic factors. Theses factors define the environmental space where species are present in nature, which is known as the realized niche of a species (Landolt et al. 2010; Born and Linder, 2018). The fundamental niche describes the space, in which a species is able to persist based on its physiological competence (Born and Linder, 2018). While the realized niche of a species can be determined by observational studies, the fundamental niche must be studied by conducting experiments (Born and Linder, 2018). Information on the realized niche of plant species in Switzerland are provided by Landolt et al. (2010). The environmental conditions are divided into six different indicators for climatic and soil factors (Landolt et al. 2010). While these indicator values inform us about the realized niche of a species, there are no standardized information on a species

fundamental niche and how much Landolt indicator values inform us on the germination requirements of a species. They can thus give a guideline about how to cultivate species *ex situ*, but it does not show the full range of abiotic conditions allowing the germination of a species.

While it is important to know how abiotic factors influence the germination of threatened plant species *ex situ*, species must be able to germinate under specific environmental conditions in order to establish in a given habitat. Thus, the environmental conditions under which a species germinates influences the establishment and survival of a species (Donohue et al. 2010). According to the niche-breath hypothesis, rare plant species have a narrow realized niche breadth, meaning that they are restricted in their environmental range within which they can maintain viable populations (Vincent et al. 2020 a). Based on their narrow geographic distribution, they are said to be more vulnerable to environmental changes (Kempel et al. 2020) and climate change will, as a result, affect their growth, survival (Vincent et al. 2020 a) and probably the germination of threatened plant species more than widespread ones (Vincent at al. 2020 b). However, as plant species are expected to shift their ranges within their fundamental niche to cope with environmental changes (Vincent et al. 2020 b, Born and Linder, 2018), information about their specific requirements for germination gives a more complete picture on how a species might react in different climate change scenarios and therefor allow to inform better on conservation actions.

Water is an abiotic factor, which is predicted to be altered by climate change (Born and Linder, 2018). The realized moisture niche of Swiss plant species is indicated by its Landolt indicator value for moisture, ranging from one, indicating dry conditions, to five, representing flooded environments (Landolt et al. 2010). Even though changes in water availability may affect species distributional range (Born and Linder, 2018), only little is known about the effects of changing water conditions on the germination and seedling performance of threatened species

and if for example intermediate watering regimes would work for the germination of species with very different moisture Landolt indicator values.

In this study, we tested the effect of three different irrigation treatments on germination and seedling height of six threatened plant species in Switzerland. The aim of the study was to answer the following questions:

- Does the irrigation quantity affect germination of threatened plant species such as Crepis praemorsa and Tephroseris helinitis?
- 2) How do these different irrigation treatments affect the height of the seedlings of *Crepis praemorsa* and *Tephroseris helinitis*?
- 3) Do the moisture index values explain the performance of the species under the different irrigation treatments?

2 Materials and methods

2.1 Study species and pre-treatments

We included six plant species from five different plant families that are considered threatened in Switzerland. Seeds were collected from natural populations and thus were not available in same quantities for the different species. To create a gradient of species with differing irrigation needs, the species were chosen based on their Landolt indicator values for moisture (*Table 1*).

Table 1: List of study species. Ecological indicator values are stated according to Landolt et al. (2010), the scale ranges from 1 to 5, low values mean that the species grow in habitats with dry conditions and high values that they prefer moist conditions. w means that the species can persist under slightly changing water conditions, w^+ that they can persist under strongly changing conditions. Abbr. IUCN-categories: VU = vulnerable, EN = endangered (infoflora.ch, 2021)

Species	Family	Landoldt value for moisture	IUCN-Category
Bupleurum longifolium L.	Apiaceae	2^{+}	VU
Crepis praemorsa (L.) Walther	Asteraceae	2^+w	VU
Saxifraga granulate L.	Saxifragaceae	2^+w	VU
Trientalis europaea L.	Primulaceae	3^+w^+	VU
Tephroseris helenitis (L.) B. Nord.	Asteraceae	$4\mathrm{w}^+$	EN
Alisma lanceolatum With.	Alismataceae	$5w^+$	EN

In order to compile the experimental design, the seeds were cleaned, counted and weighted. At the end of October 2020, 96 pots (Ø 8cm) were filled with Substrate 167 from RICOTER (30% bark compost, 30% peat substitute, 35% coco-peat and 5% sand). To each pot, eight to 20 seeds from the same species, depending on the quantity of available seeds, were added (*Table 2*). RhizoPlus (Andermatt Biogarten) was diluted in water to a concentration of 0.2%, with which the pots were then watered. The plant strengthening agent contains the soil bacteria *Bacillus amyloliquefaciens*, which benefits the growth of roots and thus increases the intake of nutrients and the growth of the plant (biogarten.ch, 2021). To stratify the seeds, the pots were put into the fridge at 4 °C for almost three months. The seeds were watered once a week from bellow. In mid-January 2021, the pots were taken out of the fridge and the experiment was set up.

Number of seed families	Number of seeds per pot
10	8
6	10
1	10
3	10
3	20
5	20
	10 6 1 3

Table 2: List of study species, number of seed families and number of seeds per pot and species

2.2 Experimental Design

The 96 pots were assigned to three different irrigation treatments ("dry", "medium" and "wet") on the plot level. The "dry" treatment pots were watered each Monday, Wednesday and Friday, in the "medium" treatment they were watered each day. For the "wet" treatment the pots were watered each day for the above and put into pot saucers filled up to 2 cm with water to additionally irrigate them from bellow.

We assigned our treatments according to a blocked split-plot design (*Figure 1*). We had a total of six blocks, each containing one "dry", one "medium" and one "wet" treatment plot. In each of the treatment plots we randomly placed one pot per species (sample function in R, version 4.1.0). An exception was block number six, where we had, due to limitation of seeds for the other species, only pots with seeds from *T. helenitis* and *A. lanceolatum*.

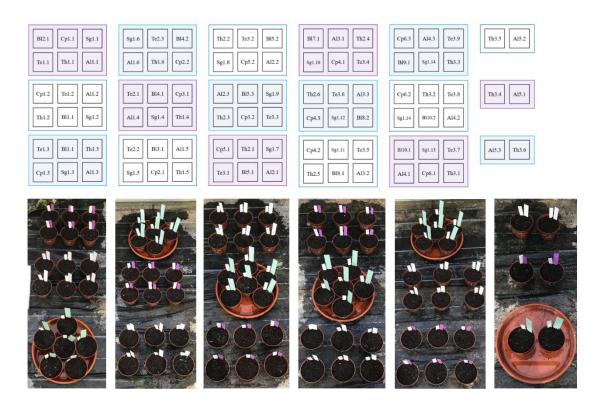


Figure 1: Experimental design (above) and picture at the first day of the experiment (bellow). Abbr.: Bl: Bupleurum longifolium, Cp: Crepis praemorsa, Sg: Saxifraga granulate, Te: Trientalis europaea, Th: Tephroseris helenitis, Al: Alisma lanceolatum. The first number indicates the seed family of the according species and the second the replication of the corresponding seed family. Blue indicates "wet", white "medium" and purple "dry" conditions.

2.3 Measurements

We counted germinated seeds every day to obtain an accurate germination rate per pot. Seeds were considered germinated when the radicle was visible. As seeds were quite small and light, application of seeds to the pots was difficult. In pots Sg1.8 and Cp5.2 more individuals germinated than the highest possible number of seeds (germination rate of 1.3 and 1.2 respectively). For the two pots the germination rate was set to 1 as the maximal possible germination rate was reached. Additionally, we measured the plant height from the soil to the highest point of the largest leaf of the plant for a maximum of three germinated individuals per pot once a week. After three months, some seedlings of *T. helenitis* and *C. praemorsa* were quite big and thus were pricked out in order to prepare them for translocation projects later on. The height of each seedling was measured and the seedlings were planted in separated pots (\emptyset 8cm). As the substrate used at the beginning of the experiment is low nutrient content, seedlings

were planted in Substrate 282 of RICOTER, composed of 10% soil, 25% bark compost, 25% peat substitute, 25% coco peat and 15% white peat. A subset of these seedlings was put back into the according treatments, the rest was regularly watered. We decided to include the pricked seedlings into the statistical analysis to avoid unbalance. As the number of pricked pots differed among the treatments, we had to be careful in interpreting results after day 80, as pricked seedlings had more space and nutrients available. The experiment ended in May 2021 and had a total duration of 100 days.

After we finished the experiment, we pricked out all individuals that were large enough into separate pots (\emptyset 10 cm, Substrate 282) and repotted large individuals (\emptyset 12 cm pots, Substrate 282) for future translocation projects. After finishing the experiment, most individuals of *C*. *praemorsa* started flowering, consequently we collected the seeds for future *ex-situ* propagation projects. Pots in which no seeds germinated were still watered.

2.4 Statistical analysis

We excluded *B. longifolium* from the statistical analysis as the species did not germinate at all. To obtain an overview of the germination of the different species, we plotted their average germination rate within each treatment against time. Even though S. *granulata, T. europaea* and *A. lanceolatum* did germinate during the experiment, they were excluded from the statistical analysis to answer the first two questions as the species did not exceed germination rates over 0.16 within all treatments.

To test the effect of the different treatments on the germination rate and plant height, we only analysed *Crepis praemorsa* and *Tephroseris helinitis*, as the other species did not germinate well enough. For the response variable germination rate we selected the highest quantity of germinated seeds per pot and averaged these within the different treatments and species. For the response variable plant height, we averaged the height of the seedlings within each pot. Afterwards, we analysed the data for both species and response variables separately. For all analysis, we used linear mixed-effects models (*lmer*), requiring the lme4 package in R (Bates et al. 2015). Treatment was included as fixed term and seed family and block as a random factor ((1 | seed.family) and (1 | block) respectively). In the model with germination rate as response variable, the random factor block did not explain any variance (0 %) for either species and we thus excluded the random factor from the final models. For the germination rate, seed family explained 36.6 % of the variance for *Tephroseris helinitis* and no variance for *Crepis praemorsa*. For the response variable plant height of *T. helenitis*, seed family did not explain any variance (0%) and was taken out of the model, but block explained 42.9% of variance. For the plant height of *C. praemorsa* 8 % of the variance for was explained by seeds family and no variance by the different blocks. We thus excluded block from the final model.

To test whether Landolt moisture index values may indicate the germination potential of the species under different irrigation treatments, we included all species into the analysis except *B. longifolium*. Again, we used a linear mixed-effects model, containing treatment, indicator value and their interaction as fixed terms and seed family and block as random terms. We set both, germination rate and seedling height as response variables in two different models. For the germination rate, seed family explained 12.3% of variance and the different blocks 17.5%. 48.7% of variance for seedling height was explained by the different seed families and only 1.1% by blocks.

Before running the different models, we checked the data according to Zuur et al. (2009) to ensure that they fulfil the model requirements.

For all statistical analyses, we used R version 4.1.0 (R Core Team, 2021)). We used likelihoodratio tests to determine non-significant terms by comparing the models with and without the respective terms. To analyse the mean differences in germination rate and seedling height under the different explanatory variables we used ANOVA post hoc tests (TukeyHSD and LSD). Both Tukey's 'Honest Significant Difference' method (TukeyHSD) and least significant difference test (LSD) indicated the same differences in significance levels between the different means of the used response variables.

3 Results

3.1 Germination rate and seedling height over time

B. longifolium did not germinate during the experiment. Only few seeds of A. lanceolatum (1.6% over all treatments) germinated and thus the germination rate could not be investigated. Both, S. granulate (5%) and T. europaea (9.8%) germinated under all three treatments but showed lower germination rates (Figure 2). Even though T. europaea showed low germination under dry treatment, height could not be measured as no leaves were existent. Over all treatments, C. praemorsa and T. helinitis germinated best. Germination rate was lowest under dry conditions for both species. C. praemorsa showed increased germination rate during the first 25 days under dry conditions. Afterwards, only small changes in average germination rates could be observed. A similar pattern was observed for T. helinitis but slightly less seeds germinated in total. Around day 37, average germination rate of T. helinitis slightly decreased under these dry conditions. Under medium irrigation conditions both species performed better than under dry conditions. The average germination rate of C. praemorsa increased until day 75 from where the average germination rate stayed relatively constant. In the first 37 days, the average germination rate of T. helinits rapidly increased up to ca. 30%. Around day 60, the overall germination rate decreased. After day 75, the average germination rate of C. praemorsa under wet conditions exceeded the germination rate under medium conditions. For T. helinitis, average germination rates under medium and wet irrigation conditions were relatively similar. Seedling height of both species was variable under each of the irrigation treatments.

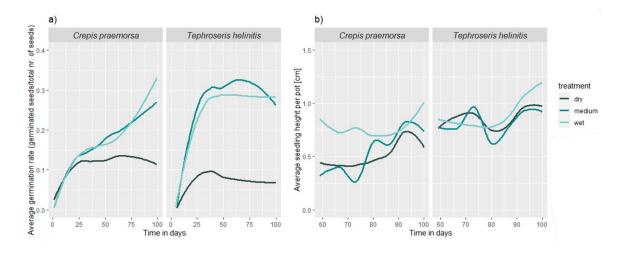


Figure 2: Germination rate and seedling growth over time. a) Average germination rate of Crepis praemorsa and Tephroseris helinitis b) Average seedling height of Crepis praemorsa and Tephroseris helinitis. Dark lines indicate germination rate or seedling height under dry conditions, turquoise represents medium and light blue wet conditions.

Overall, only few seedlings of *S. granulata* and *T. europaea* germinated during the experiment (*Figure 3*). *S. granulata* showed highest germination rates under medium conditions, however only less than 10 % of all seeds germinated under these conditions. *T. europaea* showed slightly higher germination rates, with highest germination under wet conditions reaching up to an average of 15 % of germinated seeds per pot. Seedlings were largest under wet conditions but still smaller than most of the seedlings of *C. praemorsa* and *T. helinitis*.

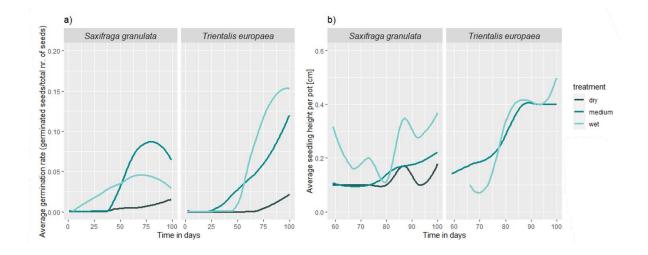


Figure 3: Germination rate and seedling growth over time. a) Average germination rate of Saxifraga granulate and Trientalis europaea b) Average seedling height of Saxifraga granulate and Trientalis europeaea. Dark lines indicate germination rate or seedling height under dry conditions, turquoise represents medium and light blue wet conditions.

3.2 Irrigation treatments and germination rate

The different irrigation treatments did not affect the average maximal germination rate of *C*. praemorsa (p = 0.2218), but it did influence the average maximal germination rate of *T*. helinitis (p = 0.0004) with the germination rate being significantly lower under dry conditions compared with the germination rate under medium and wet conditions. Under medium and wet conditions, the germination rate was approximately the same (*Figure 4*).

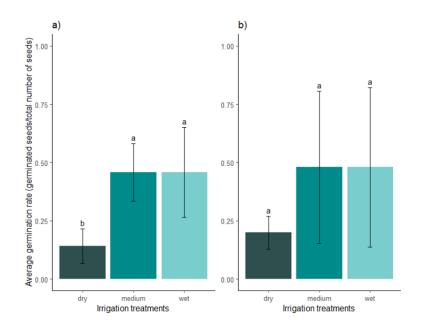


Figure 4: Average germination rate under different irrigation treatments a) Tephroseris helenitis, b) Crepis praemorsa. Letters above the error bars; different letters indicate statistical significance between the germination rate under the three different irrigation treatments.

3.3 Irrigation treatments and height of seedlings

Seedling height varied a lot within and between treatments and for both *T. helinitis* and *C. praemorsa*, irrigation treatments did not have any significant effects on seedling height (*Figure 5*).

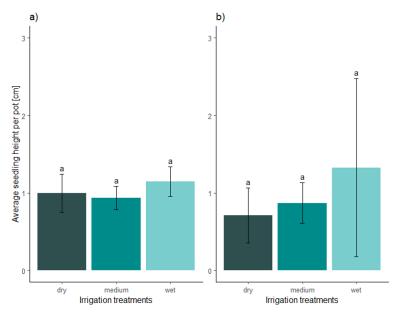


Figure 5: Average seedling height under different irrigation treatments a) Tephroseris helenitis, b) Crepis praemorsa. Letters above the error bars; different letters indicate statistical significance between seedling height under the different irrigation treatments.

3.4 Moisture indicator values and plant performance

When analysing, all five species together, they germinated significantly better under medium and wet conditions than under dry conditions. Further, species with a high Landolt indicator value (submerged) germinated worst compared with the other Landolt indicator values. However, as there was only one species with an indicator value of 5 (*A. lanceolatum*) the low germination rate could also relate to the species itself rather than its indicator value. The interaction between the irrigation treatment and the Landolt indicator value showed no significant effect on the germination rate. Neither the irrigation treatment, nor the Landolt indicator value or their interaction influenced the seedling height (*Table 2*). Germination rate of species with an indicator value of two under medium conditions was significantly higher than the rate of all other species under every other treatment. All species germinated worst under dry conditions irrespective of their indicator value. Except from species with an indicator value of two, all species germinated best under wet conditions (*Figure 6*).

Table 2: Results of the linear mixed effects model testing for effects of treatment, Landolt indicator value and their interaction on germination rate of five threatened plant species. Significances are presented in the following way: p-value: 0.001: ***; 0.001-0.01: **; 0.001-0.05: *; 0.05 - 0.1: .; >0.1: ns.

	Germinati	Height
Fixed terms	p-Value	p-Value
Treatment	0.025172*	0.1563 ns.
Indicator value	0.001487 **	0.6733 ns.
Treatment x Indicator value	0.203671 ns.	0.4785 ns.
Random terms	Variance	Variance
Seed family	0.0077 (12.3 %)	0.1295 (48.7 %)
Block	0.0109 (17.5 %)	0.0029 (1.1 %)

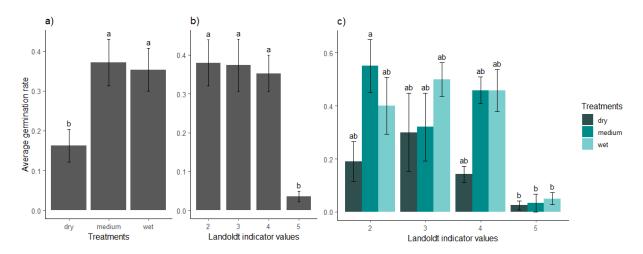


Figure 6: Effects of different irrigation treatments and Landolt indicator values on germination. a) Average germination rate under different irrigation treatments b) Average germination rate of species with different Landolt indicator values for moisture c) Average germination rate under different irrigation treatments along species with different Landolt indicator values. Letters above the error bars; different letters indicate statistical significance

4 Discussion

4.1 Why some species germinated better and faster than others

During the experiment, not all species germinated equally well. There are several factors affecting the germination success of a seed. For some species, germination is restricted to specific germination seasons, meaning that they germinate only during a specific time of the year (Baskin and Baskin 2014). Thus, the time span of our experiment may not have encompassed the germination season of *B. longifolium* for example. Simulating in situ germination triggers by applying artificial stratification treatments showed positive effects on germination percentage of threatened species and their time needed for germination (Hoyle et al. 2015, Vincent at al. 2020 b). Thus, germination is often postponed until a period of cold, wet conditions that break seed dormancy (Hoyle et al. 2015). Another explanation for these differences may be that some of the species' seeds were dormant. Even if the environmental requirements for germination were ideal, the seeds may not have germinated because the conditions were not favourable for seedling establishment and thus the seeds did not germinate (Baskin and Baskin 2014). Furthermore, germination is affected by the age of the seeds (Baskin and Baskin 2014). The seeds used in the experiment were collected from June to September 2020 and were potted in late October. Thus, some of the seeds were stored up to four months. According to Baskin and Baskin (2014), germination experiments should be started only seven to ten days after collecting the seeds, to avoid modifications in the seed's germination responses during dry storage. However, Godefroid et al. (2010) showed that germination percentages of threatened species were still high (59%) for seeds stored between 1 and 26 years in seed banks. As the university of Bern does not provide such a facility, our seeds were not stored in a seed bank. Furthermore, germination percentage and/or rate of many species differ between seeds of a species which are collected in different locations or at different times in the same location, even though they are subjected to identical dormancy-breaking conditions. Among other factors, this variation is driven by genetic differences and the environment of the mother plant during seed maturation (Baskin and Baskin, 2014). Thus, the time of seed collection and the availability of different seed families may have affected our results, as Heinicke et al. (2016) showed variable germination success among populations and Baskin and Baskin (2014) indicated that differences in germination speed was found for seeds from different seed families. However, the seeds for each species were all collected at the same time and at the same location within a radius of 10 m. Seed families used in our experiment may have germinated better or worse than other families which were not included in the experiment. Especially low germination rates of *S. granulate* may have been affected by the seed family, as only one was included. However, seed family explained between 0 to 48.7% of variance in germination rate and seedling height within our models.

Even though we need more information on germination requirements of certain species to determine the causes of differences in germination success, these results might give us some indications on what we should test to obtain more profound knowledge on the subject. Germination is not only affected by water availability but also by other factors such as effect of temperature, substrate, light and potting depth of the seeds (Baskin and Baskin, 2014; Nelson and Larson, 1984). Thus, further experiments should be conducted, testing for effects of these factors on germination of threatened plant species. While *T. helinitis* and *C. praemorsa* germinated well under the given conditions, they were not favourable for germination of other species. Thus, the germination of the species may have been restricted by environmental factors such as season and stratification as well as by seed age and the genetic makeup of the species (Baskin and Baskin, 2014).

4.2 Germination, seedling height and ecological niche

The germination rate of *T. helinits* was affected by irrigation quantity. The Asteraceae species *T. helinits* is mostly found in wet habitats (infoflora.ch), which is also indicated by its Landolt indicator value for moisture. Within our experiment, *T. helinitis* germinated significantly better under medium and wet than under dry conditions. These results are thus in good agreement with the available information on the species realised niche. However, results from this experiment must be interpreted with caution, as the sample size was quite small. A nationwide survey of rare and threatened plant species in Switzerland showed that especially taxa from wet, moist and flooded habitats are prone to extinction. Water pollution and flow modification are likely to be the main causes of decreased abundance of such species (Kempel et al. 2020). The findings of Kempel et al. (2020) thus highlight the importance for *ex situ* conservation of species in such habitats, as they are especially threatened.

The different irrigation treatments showed no significant effect on plant height at all. Due to the high Landolt indicator value for moisture of *T. helinitis*, it was expected that the seedling would perform best under rather wet or moist conditions. Even though water availability has been shown to be a major factor influencing seedling survival (Nelson and Larson, 1984), *T. helinitis* seemed to be no longer affected by soil moisture. Literature indicates that *T. helinitis* can persist under changing water availabilities (Landolt et al. 2010) which may explain these results. However, there are other factors affecting seedling performance. Soil properties as well as the aerial environment (for example temperature, water supply etc.) can have high impacts on seedling growth and development.

Interestingly, *C. praemorsa* showed no statistically significant responses to the different irrigation treatments. Born and Linder (2018) showed that species performed best under irrigation treatments similar to the conditions within their habitat. Thus, species from dry habitats such as *C. praemorsa* are expected to perform best under rather dry conditions, which

is not in agreement with our results. As seeds seem to germinate not only under conditions favourable for germination but also for seedling establishment (Baskin and Baskin 2014), this might suggest that *C. praemorsa* would be able to persist under wetter conditions but is somehow restricted to more unfavourable environments. Thus, our findings suggest that the fundamental niche of *C. praemorsa* is larger than its realised niche, as irrigation quantity did not have significant effects on its germination. The germination of *C. praemorsa* showed higher variation within the different treatments than *T. helinitis*, which could explain non-significant differences between the irrigation treatments. Additionally, the sample size of *C. praemorsa* was smaller than the one for *T. helinitis*, reducing the statistical power of the model, which may have led to non-significant results. However, experimental replication was limited as only few seeds were available for the experiment.

C. praemorsa is often found in rather dry environments and thus we would have expected that the plant species performs best under dry or medium conditions. Species which are found in dry habitats are expected to have deep and complex rooting systems in order to absorb water from the dry soils (Born and Linder, 2018). As the rooting system of a seedling is not fully developed, they may not receive enough water and thus perform worse than expected under these dry conditions. Thus, it would have been interesting to record seedling heigh over a longer period of time.

T. helinitis germinated best under medium and wet conditions, which may suggest that the threatened species will struggle with dryer conditions under the ongoing climate change. As *T. helinitis* showed relatively high germination rates (up to 50%) under medium and wet irrigation, we suggest keeping the species under moist conditions in order to obtain high germination success, as germination may only occur when habitats of *T. helinitis* are moist, e.g. due to seasonal fluctuations in water availability (Baskin and Baskin, 2014). Climate change is expected to induce dryer conditions (Cook et al. 2018), which probably will have serious

consequences for the threatened species. Our results showed that germination and seedling height were not statistically affected by different irrigation treatments. Thus, *ex situ* multiplication of *C. praemorsa* is not restricted by moisture availability. Nevertheless, the results suggest that the fundamental hydrological niche of *C. praemorsa* is larger than its realised niche and thus may be able to shift its range within a larger fundamental hydrological niche under climate change. Nevertheless, we do not know how the species performs under the different conditions as a full-grown plant and how other factors such as temperature will affect the species performance.

5 Conclusion and outlook

Our study showed that *T. helinits* and *C. praemorsa* germinated well under the given conditions and thus were fostered. We suggest keeping *T. helinitis* under moist conditions to obtain high germination success. *C. praemorsa* started to flower after the experiment and seeds for future resettlement project could be collected. The other species showed relatively low germination rates. In order to improve the germination of these species and thus provide *ex situ* augmentation, we need to test the effect of temperature, substrate, light and potting depth of the seeds on seed germination and seedling establishment (Baskin and Baskin, 2014; Nelson and Larson, 1984). To do so, multispecies studies are needed to examine abiotic conditions favourable for seed germination of different species. These studies offer the possibility to develop germination protocols for individual threatened species to reduce deficient resettlement attempts. As we only studied germination rate and seedling height of these species, we do not have any information on the species performance under the different treatments when they are full-grown. Thus, long-term experiments on these matters should be conducted. With these information, specific conservation strategies and resettlement projects for the different species can be evaluated. In terms of climate change, our results suggest that *T. helinits* may be negatively affected by dryer conditions. As *C. praemorsa* showed no differences in germination rate or hight under the different treatments, climate change may have less consequences in terms of satisfying its water requirements. We did not find a consistent relationship between Landolt indicator values and species germination success under different irrigation treatments, which may indicate that some species can germinate under a wider range than expected based on their realised niche. Due to the low germination rates of the other species, we cannot conclude how climate change will affect their germination and performance and thus further experiments are needed.

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