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Seed germination trials of the invasive plant *Crassula helmsii* from nature restoration sites in Belgium

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Running title

Seed germination in *Crassula helmsii*

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Abstract

Background and aims – *Crassula helmsii* is a highly invasive aquatic plant in Europe, subject to international policy measures (EU Regulation 1143/2014). While its vegetative reproduction is well-documented, the role of sexual reproduction and seed ecology remains poorly understood. This study aimed to determine the germination capacity of *C. helmsii* seeds and revisit its potential for dormancy and seed bank formation.

Material and methods – We conducted *in vitro* germination trials using plant material collected from ten populations in Belgium. Following cold storage (winter conditions), seeds were sown on agar, and germination was tracked. In a comparative trial, flowers from the same populations were sown on a soil substrate, with germination estimated based on the number of seeds per flower – the only method previously used to study the species.

Key results – *C. helmsii* populations exhibited high germination rates, with an estimated overall mean of 68.5%. When excluding seeds that proved non-viable, germination was nearly complete (95.7%). The flower-based trial yielded lower germination (13.8%), consistent with earlier results. This lower emergence is likely real, not an artifact, and suggests environmental constraints that are relevant under more realistic germination conditions.

Conclusion – Our study demonstrates that *C. helmsii* populations in Belgium produce numerous viable seeds that germinate readily. Considering additional evidence from the Crassulaceae family, we hypothesize that *C. helmsii* is self-compatible, positively photoblastic, exhibits physiological dormancy broken by chilling, and is capable of forming persistent seed banks. The species can thus combine asexual (clonal growth and dispersal) and sexual strategies (seed dispersal, dormancy, and seed bank formation). These insights have important implications for the management of this invasive species.

Keywords

Crassulaceae, biological invasions, Australian swamp stonecrop, New Zealand pygmyweed, Regulation 1143/2014, species of Union concern, *Tillaea*

INTRODUCTION

Crassula helmsii (Kirk) Cockayne is a small succulent plant species of the Crassulaceae family, characterized by an amphibious growth habit (Smith and Buckley 2020). On land, it forms low, dense carpets, while in shallow water, it produces short upright shoots. In deeper water, it develops long trailing stems. The terrestrial form in particular can flower profusely, bearing many small, white to pale pink flowers. *C. helmsii* is native to Australia and New Zealand but has become widely established in Europe, especially in the Atlantic biogeographic region (Van der Loop et al. 2024). Here, it thrives on mineral soils in areas with strongly fluctuating water levels, often in habitats of conservation concern (e.g. dune slacks, oligotrophic waters, wet heaths and natural lakes; Van Kleef et al. 2024). Consequently, *C. helmsii* is a much-feared guest in nature restoration projects, as it often appears rapidly after topsoil removal, impeding the optimal development of target plant communities (Brouwer et al. 2017). The species is therefore considered one of the most troublesome aquatic invasive plants in Europe, and this has led to its inclusion on the list of invasive alien species of Union concern under European law (Regulation No. 1143/2014; Van der Loop et al. 2024). This legal status requires authorities and site managers to prevent the species' spread and minimize its ecological impacts as effectively as possible.

C. helmsii exhibits a strong capacity for vegetative reproduction, both over short distances (clonal growth) and longer ranges (dispersal). Bok et al. (2025) demonstrated that the meristematic tissues of the stem apex and axillary buds readily develop into new plants. Shoot tips (which easily detach) or stem fragments (even with a single node) thus act as propagules, which can be dispersed by water, animals, humans, or machinery (Dawson and Warman 1987, Denys et al. 2014, Coughlan et al. 2022).

In theory, the species also has the capacity for generative reproduction, provided that flowering leads to recruitment from seeds. Seeds may then open additional dispersal pathways, enabling the colonization of new sites while also escaping pathogens or herbivores that target vegetative tissues (Howe and Smallwood 1982). Furthermore, seed dormancy may allow recruitment to be timed to favorable conditions (dispersal through time), and, if maintained over extended periods, can lead to the formation of seed banks (Bakker et al. 1996, Baskin and Baskin 2014). Except when produced apomictically, seeds are the product of sexual reproduction. They may therefore also contribute to genetic diversity among individuals and, consequently, to the adaptive potential of populations (Fenner and Thompson 2005). While seed formation has been confirmed for *C. helmsii* populations from Belgium, The Netherlands, France and the United Kingdom (D'hondt et al. 2016), our understanding of its ecological relevance – including the seeds' germination capacity and dormancy traits– remains limited.

The seeds' small size, rounded shape and fragility make them relatively hard to extract from flowers and handle in trials, which is why flowers have been used in previous work of ours (Denys et al. 2014, D'hondt et al. 2016). In these experiments, wilted flowers presumed to contain mature seeds were sown

on a germination bed, and seedling emergence was tracked. However, estimates of germination using this approach have limited precision, as the number of seeds must be estimated from separate measurements of the number of seeds per flower. Crucially, it also does not allow for germination to be observed directly, as seeds remain enclosed by floral tissues and the substrate (in the case of e.g. a sand bed). Though the only credible interpretation for any young plant emerging in such trials is for it to be a seedling (given that a flower cut by the pedicel at this stage no longer contains meristematic tissues), more rigorous trials with individual seeds are needed to allow for more accurate observations (Smith and Buckley 2020).

We seized the opportunity presented by a nature restoration project involving *C. helmsii* management to set up a more methodological study. We conducted a new round of *in vitro* germination trials using the previous method (hereafter referred to as 'germination from flowers'), while also expanding the experiments to include seed-level germination tests ('germination from seeds'). The results of these trials enable us to make more accurate statements about germination propensity, the potential for dormancy and seed bank formation, and the general importance of sexual reproduction in this invasive species.

MATERIAL AND METHODS

Populations

Material was collected over two consecutive years from ten established populations of *C. helmsii* in northwestern Belgium (Fig. 1, Table 1). All sites were protected as nature reserves and/or have their habitats designated under the EU Habitats Directive (mainly oligotrophic to mesotrophic standing waters). For each population, the species was harvested by hand from a representative location where it formed dense mats and appeared to be flowering.

Germination from flowers (GfF)

Plants were collected in October (November) 2024, and then stored at low temperature (6 °C) for 66-86 days, after which flowers were isolated from the plants using tweezers.

As a substrate, we prepared a 1:2 mix of potting soil and sand, the latter of which was sterilized by heating it for 24h at 120 °C. The substrate was put in plastic trays (16 × 11 × 6 cm). Each tray had a wool felt pad at the bottom, was perforated, and was placed inside a larger tray to facilitate the uptake and drainage of excess water. Twenty flowers were sown per tray and buried very superficially. The trays were then subjected to a 14 h light / 10 h dark regime at 22 °C (± 0.57, standard deviation) and 15 °C (± 0.59), respectively. (Temperature data are based on measurements from a logger. A two-hour transition between day and night has been omitted from the calculations.) The lamps were LED tube lights (42 W, 6500 K). The trays were regularly watered, and their positions changed every four weeks.

The trays were checked for seedlings for 127 days. Seedlings were removed upon detection. When in doubt, a needle was placed next to a developing plant until confirmation. There were four trays (replicates) per population (Table 1). Eight trays with only substrate acted as controls; none of these showed germination except for a single graminoid specimen.

Additionally, 20 flowers from each population were dissected to determine the number of seeds per flower. We denote the mean number of seeds per flower for population n as μ_{Sn}, which was then used to estimate the actual number of seeds sown in the trays (i.e. $20 \times \mu_{Sn}$).

Germination from seeds (GfS)

Plants from each of the populations were sampled in October 2025, and then stored at low temperatures (fridge; 4 °C) for 112-122 days, after which flowers were isolated from the plants.

Seeds were extracted from flowers under a stereoscope and transferred to 9 cm Petri dishes containing pure agar (VWR product code: MERC1.01614.1000). Given prior experience with *C. helmsii* stock indicating that microbial growth (fungi, bacteria) can be rapid and severe, and considering the relatively low number of seeds available, we opted to increase the number of replicates (dishes) and space the seeds adequately apart. Consequently, five seeds were sown per dish. While this number is lower than typical for germination tests (Poschlod et al. 2026), it was sufficiently robust for the intended (simple) analysis. The number of dishes per population are included in Table 1.

The dishes were subjected to a 14 h light / 10 h dark regime at 20 °C (± 0.35) and 15 °C (± 0.81), respectively (logger data, as above). The same lamps as in the previous experiment were used. The positions of dishes were changed at every scoring occasion.

Germination in the petri dishes was scored for a minimum of 65 days. A fraction of the seeds were removed at the earliest scoring times due to sanitary concerns arising from rapid microbial growth. Germinated seeds (seedlings) were removed upon discovery; a seed was considered germinated as soon as distal plant parts (root tip or cotyledons) emerged from the seed coat. Monitoring of the dishes was discontinued if no new germination had been observed in any dish from the same population for the past 14 days.

Each remaining seed at the end of the trial was evaluated under a stereoscope by longitudinal sectioning and assessment of the inner tissue contents. Indeed, non-germinated seeds vary fundamentally (Poschlod et al. 2026): they can either be viable (with an intact embryo, capable of germination but not yet germinated) or non-viable (incapable of germination, whether dead, empty or containing an aborted or malformed embryo).

Analysis

We analyzed the proportion of seeds germinated (odds of germinated vs non-germinated seeds) using a generalized linear model (GLM) with a binomial distribution and logistic link function. Population was included as the sole explanatory variable (fixed effect), as to illustrate variation among the sampled locations. However, as our populations represent a random sample from a broader set of *C. helmsii* populations, and our primary objective is to gain a general understanding of germination rates (rather than, for example, differences among specific populations), we also fitted a generalized linear mixed model (GLMM) with population as a random effect. This allowed us to estimate the overall mean germination probability and the cross-population variation. Population BE07 was excluded from these analyses due to insufficient seed availability (Table 1).

For the *GfF* trial, germinated vs non-germinated seeds were equated to the number of observed seedlings vs the estimated number of non-germinated seeds in a tray. Since this latter number relies heavily on the estimated number of seeds per flower (μ_{Sn}), we explored the sensitivity of the estimate to this parameter by refitting the model using variations in μ_{Sn} (0.1–0.9 × μ_{Sn}). Indeed, since not all seemingly healthy seeds are necessarily viable, μ_{Sn} could theoretically be an overestimate (and the germination rate an underestimate).

For the *GfS* trial, germinated vs non-germinated seeds were equated to the germinated seeds (observed directly) vs the seeds sown. As a complement, we repeated the analysis by excluding the seeds that had either been removed early due to sanitary risks, or were identified as non-viable during post-trial evaluation. This ensured this analysis was limited strictly to viable seeds.

Analyses were performed in R (RStudio 2026.04.0), involving, among others, packages *tidyverse* v2.0.0 (Wickham and RStudio 2023), *stats* v.4.6.0, *lme4* v.2.0-1 (Bates et al. 2026) and *emmeans* v2.0.3 (Lenth et al. 2026).

AI statement

Vibe (chat.mistral.ai) was used as an AI tool to polish the draft text, and as an occasional troubleshooter for coding.

RESULTS

Germination from flowers (GfF)

The number of seeds per flower varied strongly, with the majority (60%) having no developed seeds, and about one in four containing a single seed (1 seed: 24%; 2 seeds: 12%; 3 seeds: 4%).

Germination could only be detected at a relatively late stage, when at least the cotyledons were fully developed (Figs. 2, 3). For the ten populations combined, a total of 67 seedlings were recorded across

the 40 trays (800 flowers, equaling an estimated 500 seeds). 90% of the seedlings were recorded within 75 days (Fig. 4).

The fixed-effect model estimated germination rates ranging from 6.8% (±2.7% s.e., standard error) to 43.8% (±12% s.e.; Fig. 5). The random-effect model yielded an overall mean germination probability of 13.8%. Accounting for variation among populations, this model estimates that 95% of *C. helmsii* populations have germination probabilities ranging from 4.8% to 34.1%.

The sensitivity analysis is shown in Fig. 6. The estimate of the overall germination rate proved fairly robust to variations in μ_{Sn}, in that conceivable misestimates of the number of viable seeds per flower (~0.9–0.6 × μ_{Sn}) still yielded low germination rates.

Germination from seeds (GfS)

Germination was clearly observable in the agar plates, following an expected pattern (Figs. 2, 3). During germination, seeds swelled slightly, the radicle emerged from the seed coat, fine root hairs developed, the hypocotyl elongated, the cotyledons unfolded, and the green intensity of the upper plant half increased. In a few cases, the cotyledons were the first organ to rupture the seed coat. The developing seedling either completely detached from the seed coat or the seed coat remained attached to the tip of one of the cotyledons. Notably, the radicle apices consistently exhibited a reddish coloration.

Germination was observed more rapidly than in the previous experiment (90% within 40 days; Fig. 4). Although filamentous fungi rapidly developed, they eventually receded, and germination did not appear to be hindered. Later observations also detected microbial growth with a more mucilaginous habitus, sometimes associated with seeds. However, it remains unclear whether this had a neutral or potentially negative effect on germination.

From the 333 sown seeds for the ten populations combined, 227 seedlings were counted. The fixed-effect model estimated germination rates ranging from 60.0% (±7.7% s.e.) to 86.7% (±8.8% s.e.; Fig. 5). The random-effect model yielded an overall mean germination probability of 68.5%. Accounting for variation among populations, this model estimates that 95% of *C. helmsii* populations have germination probabilities ranging from 57.1% to 78.0%.

46 seeds were promptly removed due to microbial growth (sanitary concerns) and were consequently considered non-viable. Thus, a total of 60 non-germinated seeds remained at the end of the trial for detailed evaluation. The majority of these (50 seeds, 83%) were found to be non-viable (empty, or with incomplete or amorphous tissues). Only 10 seeds (17%) appeared fully intact.

Based on these more conservative data, the fixed-effect model estimated germination rates across populations to range from 88.9% (± 6.0% s.e.) to 100% (± 0%). However, due to the limited number of seeds, high germination rates, and low variation, the model now fitted poorly (residual deviance <<

degrees of freedom). Similarly, attempts to include population as a random effect failed due to singular fits. This only leaves the intercept-only (null) model, which estimated an overall mean germination probability of 95.7%.

DISCUSSION

Results

Our results confirm that a significant proportion of *C. helmsii* flowers in the studied region contain seemingly healthy seeds (estimated at 40%). After simple cold storage, these seeds exhibited high germination rates (estimated at 68.5% for a given population), with subsequent analysis revealing that non-germinating seeds were essentially non-viable. When these were excluded, nearly all seeds successfully germinated (estimated at 95.7%). The small fraction (~5%) that failed to germinate may represent very late germinators, not captured by the experiment (but see below). There is variation among populations, though rates are of the same order of magnitude (and never zero).

When seeds are subjected to more challenging (realistic) conditions, i.e. germination from floral tissues in an organic/mineral bed, the germination rate dropped to 13.8%. Notably, applying the same analysis *post hoc* to the data from the analogous trial in D'hondt et al. (2016) yielded an estimated rate of 11.3%. The *GfS* trial clearly demonstrated that a proportion of the seeds we initially deemed healthy are, in fact, non-viable (roughly one in three). This suggests that the germination rate from the *GfF* trial is an underestimate. However, the sensitivity analysis revealed that, for a similarly high germination rate as observed in the *GfS* trial, the true number of viable seeds per flower would need to be as low as one-tenth of our observed count. This is implausible, leading us to conclude that the lower germination rate is real and not an artifact. We can thus speculate about processes –pre- or post-germination, lethal or non-lethal– that may cause the lower emergence: e.g., a lack of light for germination (Milberg et al. 2000), or fatal germination at depth (Fenner and Thompson 2005). We suspect that the slower pace observed in Fig. 4 for the *GfF* trial reflects a time lag in the detection of germination, rather than germination itself.

Germination

C. helmsii germinated rapidly under conventional *in vitro* conditions. The observations suggest it to be a positively photoblastic species (i.e. requiring light to germinate). The red radicle tips are likely caused by anthocyanins, which are known in Crassulaceae (Molisch 1928, in Thakur and Nozzolillo 1978; Góraj-Koniarska et al. 2015), although it remains uncertain whether this is a regular or stress-induced phenomenon (e.g., due to intense light). From a methodological perspective, the use of an agar substrate proved effective. The seeds are most easily handled using moistened needle tips. Microbial growth ultimately proved less problematic than initially anticipated. In fact, in the context of our trials, we conducted a small sterilization test using excess seeds from three populations, where seeds were placed

on filter paper with diluted bleach and polysorbate for 15 minutes (Robberechts 2026, unpublished). This treatment had no significant effect on germination success, though visually, the microbial load on the plates appeared reduced. Complete sterilization with bleach (e.g., by submersion) remains practically challenging for these small seeds. Therefore, if sterilization is desired, it is advisable to consider alternative methods, such as heat (Godefroid et al. 2017) or UV treatments (Kamel et al. 2022).

Dormancy

All plant material underwent a period of cold storage representing simplified winter conditions. The observation of near-complete germination thus suggests that *C. helmsii* seeds are either non-dormant or, more likely, physiologically dormant with cold conditions ('chilling') releasing dormancy (Poschlod et al. 2026). Indeed, physiological dormancy is considered the standard type within the Crassulaceae family (Baskin and Baskin 1972, 2014, Martínez-Villegas et al. 2012). It is important to recall that seeds with physiological dormancy –unlike all other dormancy types– can switch back and forth between dormant and non-dormant states in response to environmental conditions (Finch-Savage and Footitt 2017). For example, the acquisition of secondary dormancy has been demonstrated in *Sedum pulchellum* (Crassulaceae), which moreover combines dormant and non-dormant seeds within the same plant (Baskin and Baskin 1977). In fact, acquired secondary dormancy might also have contributed to the lower emergence in the *GfF* trial, and the viable but ungerminated fraction in the *GfS* trial might actually have consisted of dormant seeds (see above).

Seed banks

This transition from dormant to non-dormant also means that seeds with physiological dormancy are capable of forming seed banks. Indeed, among crassulacean species present in Europe, seed bank types are classified as transient in some genera (e.g., *Hylotelephium*, *Phedimus*), but as short- to long-term persistent in others, such as *Sedum* and *Petrosedum* (TRY database; Fitter and Peat 1994, Kleyer et al. 2008, Tavşanoğlu and Pausas 2018, Kattge et al. 2020). For *Crassula*, these sources interpret the results on *C. tillaea* from Levassor et al. (1990) as indicating short-term persistence, whereas its apparent presence in samples from unploughed soil over 40 years might also hint at long-term persistence (i.e., remaining viable for over five years; also see Peco et al. 2003).

Seed and dormancy traits are strongly conserved within families and genera, so the above likely also applies to *C. helmsii* (Willis et al. 2014, Seglias et al. 2018). The emergence of *C. helmsii* from sediment samples in Australian studies has already been interpreted as germination from the seed bank (Nicol et al. 2003, Nicol and Ward 2010). Spontaneous germination in natural populations of *C. helmsii* has not yet been confirmed, and it remains true that distinguishing seedling emergence from emergence via vegetative fragments is extremely difficult. The small size of the seeds and seedlings, combined with the lack of distinguishing features (e.g., cotyledon shape), makes visual confirmation of germination in the field virtually impossible. Nevertheless, there do not appear to be any fundamental barriers to *in situ*

seed formation, dispersal, or seed bank formation, and so we caution that the absence of evidence for germination should not be mistaken for evidence of absence.

Sexual reproduction

Seed formation also raises questions about the pollination ecology of *C. helmsii*. It is unlikely that seeds are formed apomictically (without pollination), but self-pollination (autogamy) is known within the genus (Thiede and Eggli 2007). The flowers appear to be entomophilous (Thiede and Eggli 2007), and in the species' native range, visits by Syrphidae (hoverflies) have been documented (Diaz 2012). No pollinators have been reported in Great Britain (Smith and Buckley 2020), nor in Belgium. Given the high density of flowering stems (D'hondt et al. 2016), geitonogamy is most likely, and our results thus suggest that *C. helmsii* is a self-compatible species. The population-level implications remain to be determined, but as Green and Noakes (1995) demonstrated in animals, even a small component of sexual reproduction can be highly advantageous in an otherwise clonal life cycle.

Management

Recruitment via seeds has varied implications for management, which is significantly complicated. Prevention must account for the potential presence of seeds, whether targeting pathways (e.g., as stowaways in the water plant trade; Van Den Neucker and Scheers 2022) or in the field (e.g., through the use of filters, Bok et al. 2025). Field interventions aimed at plant removal must be executed with high precision to eliminate any potential seed bank while also ensuring biosecurity (Van Der Loop et al. 2022). Approaches that accept the presence of *C. helmsii* but seek to suppress its dominance through biotic interactions appear more cost-effective in this regard; e.g., by introducing plant competitors (Van Der Loop et al. 2023, Gaudichet et al. 2026), or specialized herbivores (Varia et al. 2022). However, even here, it must be considered that *C. helmsii* possesses a life stage that can at least temporarily evade stressors.

Perspectives

Plant species from aquatic environments often exhibit a narrow syndrome that combines asexual (clonal) and sexual traits, which partly explains their frequently large ranges (Santamaría 2002). The findings of this study suggest that established *C. helmsii* populations in Western Europe also display this combination, as viable, easily germinable, and likely physiologically dormant seeds are readily found. Further research is needed to unravel the exact role of generative versus vegetative reproduction in (meta)populations of this highly invasive species. Potential avenues could include field experiments or small-scale population genetic studies (ramets vs genets; e.g., Kaljund et al. 2013, Holt et al. 2020, Loh et al. 2020).

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Tables

Table 1. Overview of the sampled *Crassula helmsii* populations used for germination testing.

			germination from flowers			germination from seeds		
pop.	**lat**	**long**	**sampling**	**# trays**	**# flowers (total)**	**sampling**	**# dishes**	**# seeds (total)**
BE01	51,3505	3,3286	11/10/2024	4	80	27/10/2025	8	40
BE02	51,2541	4,2605	3/10/2024	4	80	28/10/2025	7	33
BE03	51,2483	2,9881	4/11/2024	4	80	27/10/2025	3	15
BE04	51,1241	3,1867	13/10/2024	4	80	27/10/2025	8	41
BE05	51,1103	3,2891	14/10/2024	4	80	21/10/2025	8	38
BE06	51,1093	3,2872	14/10/2024	4	80	21/10/2025	10	43
BE07	51,0849	3,3246	14/10/2024	4	80	21/10/2025	1	2
BE08	51,0808	3,3425	14/10/2024	4	80	21/10/2025	8	41
BE09	51,0771	3,3433	14/10/2024	4	80	21/10/2025	8	40
BE10	51,0754	3,3455	14/10/2024	4	80	21/10/2025	8	40

Figures

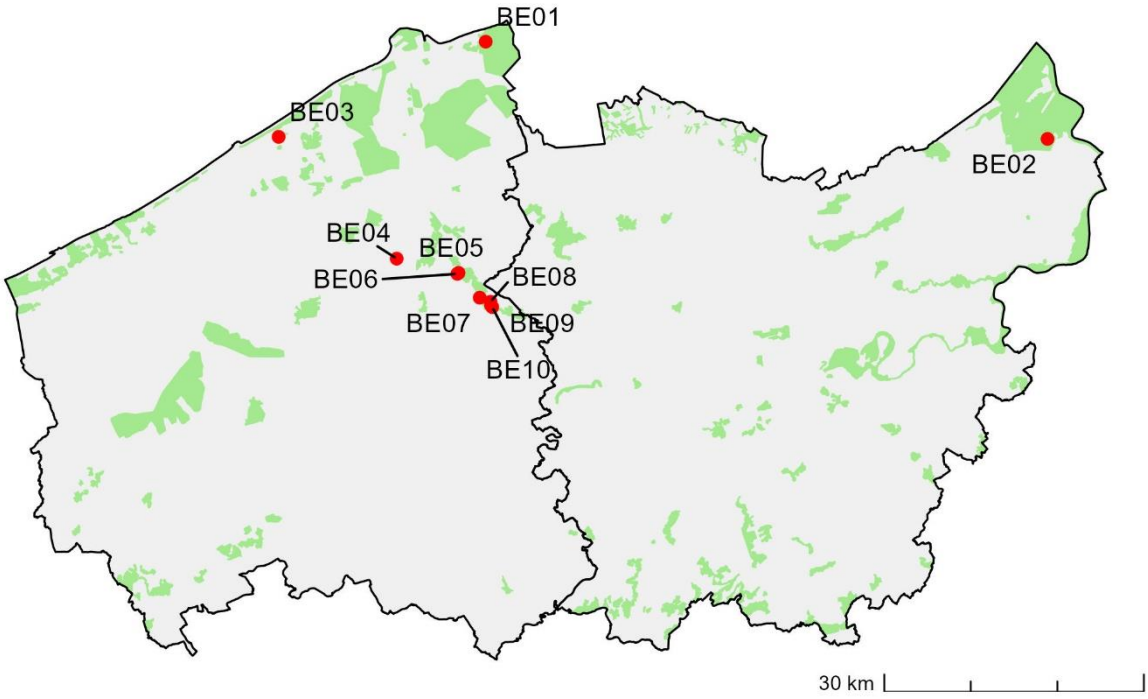
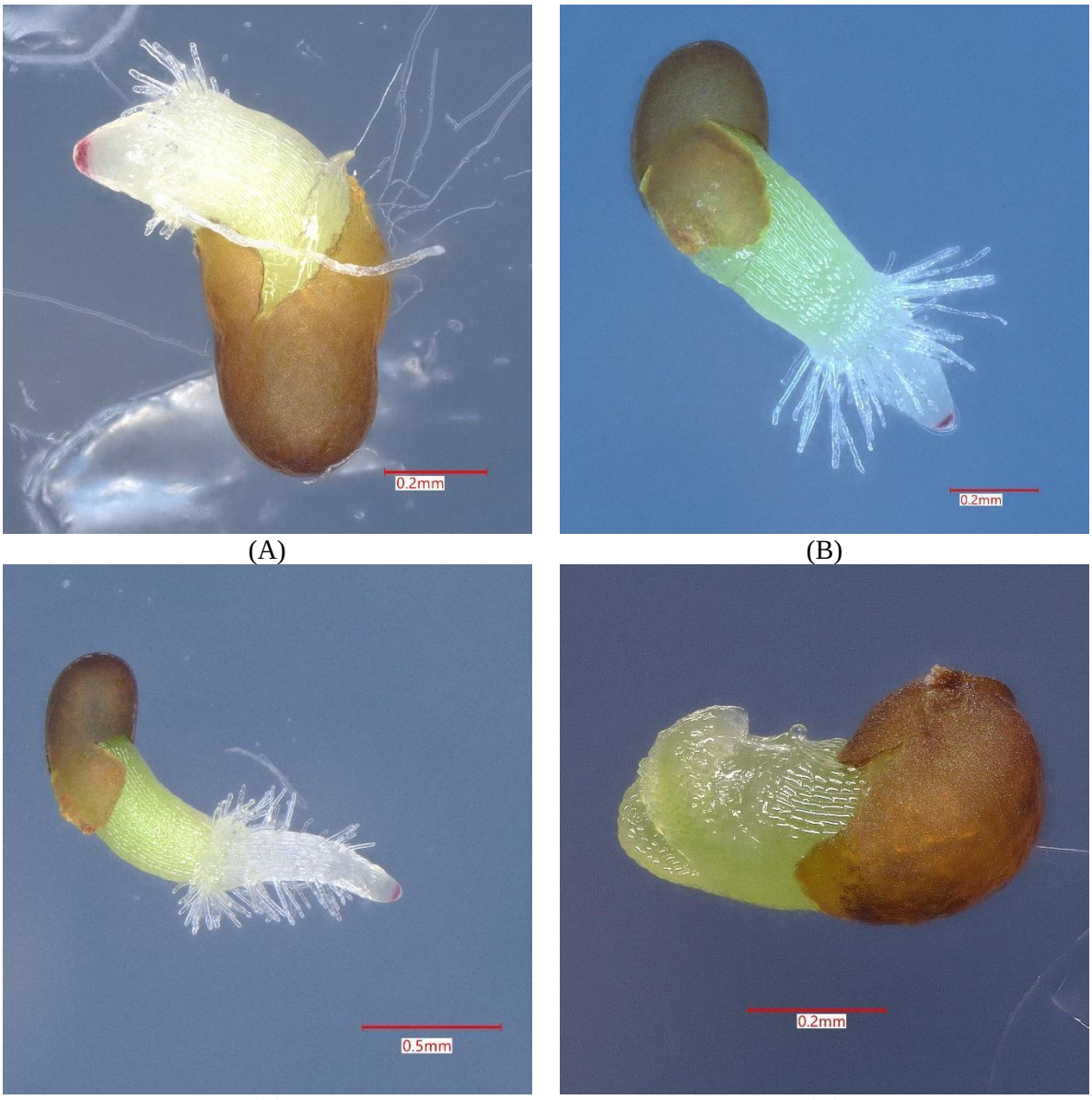
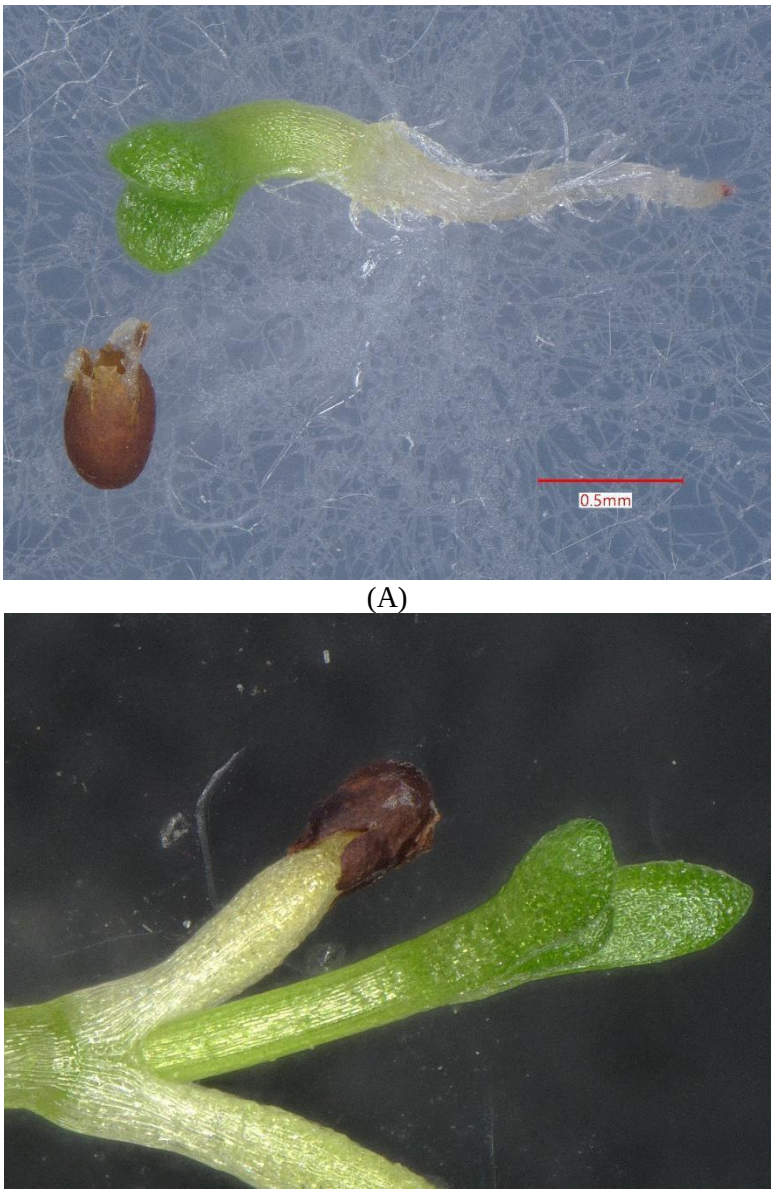


Figure 1. Locations of the sampled populations (western Belgium). Black: province borders; green: Natura2000 areas.



548 **Figure 2.** Early germination stages in *Crassula helmsii* (pictures from different seeds). **A.** Radicle
549 protrusion through the ruptured testa. **B.** Hypocotyl emergence. **C.** Hypocotyl elongation with further
550 root hair development. **D.** Inverted germination (cotyledon-first emergence).



(A)

(B)

Figure 3. Late germination stages in *Crassula helmsii*. **A.** Seedling with fully emerged cotyledons. **B.** Seedling with first true leaf pair, retaining testa remnants on a cotyledon.

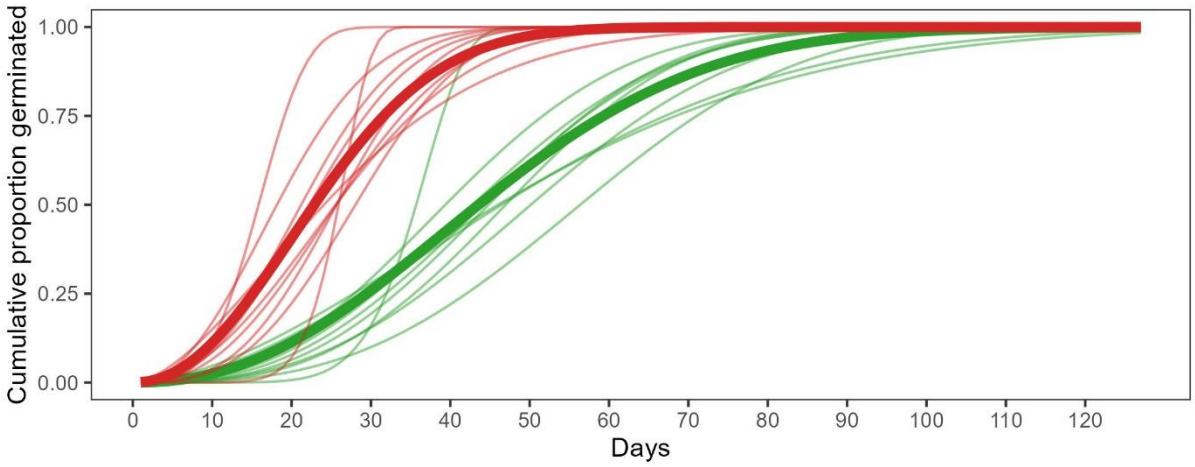


Figure 4. The timing of germination, shown as fitted Weibull distributions. Green: germination from flowers. Red: germination from seeds. Thick lines: populations taken together. Thin lines: single populations (n = 10).

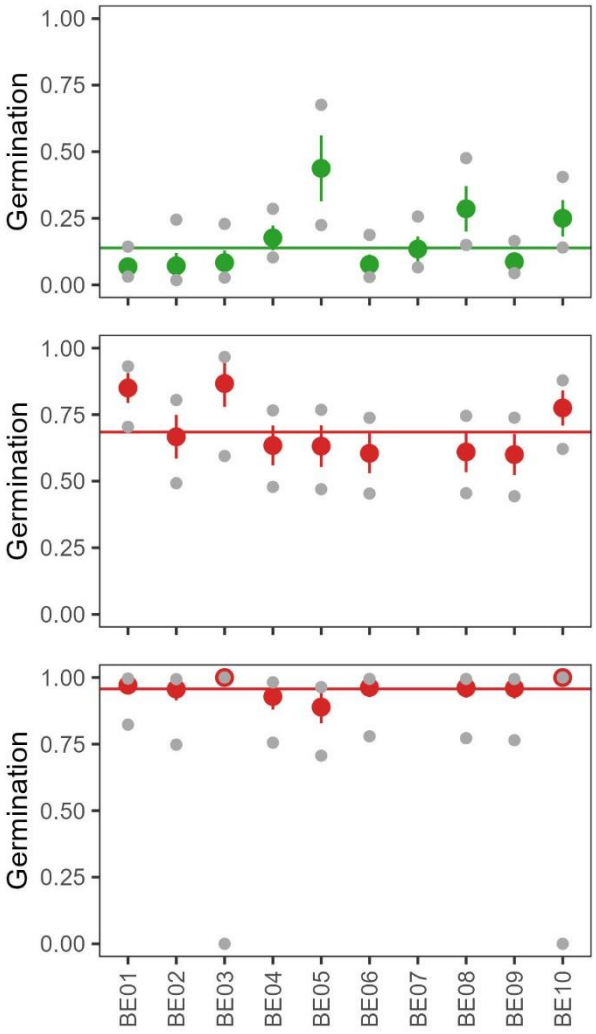


Figure 5. Estimates of germination. Upper panel: germination from flowers. Middle panel: germination from seeds. Lower panel: germination from seeds, non-viable seeds excluded. Vertical bars represent standard error; grey points indicate the 95% confidence level. Population means and variation were derived from a fixed-factor model. The horizontal line shows the overall estimate from the random-factor model.

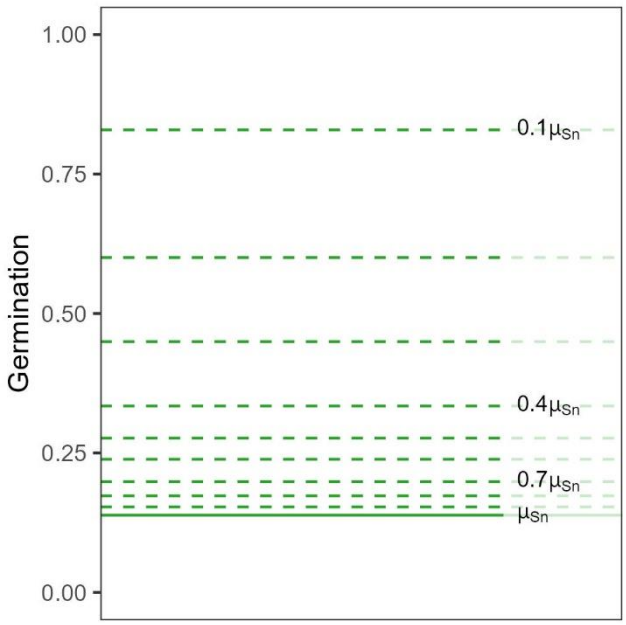


Figure 6. General estimate of the germination rate from the random-effect model when the number of seeds per flower (μ_{Sn}) is artificially reduced (sensitivity test). The solid line shows the actual results. The dashed lines represent reduced values of 0.9–0.1 × μ_{Sn} (bottom to top).