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RESCUING CRITICALLY ENDANGERED SPECIES IN BELGIUM - AN AMBITIOUS REINTRODUCTION PROGRAM OF THE BOTANIC GARDEN MEISE

In addition to seed collection, young leaf material has been sampled in each source population (and target population, in case of reinforcement) for genetic analyses.

Plant vigour prior to transplantation was estimated by measuring rosette diameter or leaf length and width (as shown here for Dianthus deltoides*). Differences in plant size were detected between seed source populations (Godefroid* et al.*, 2016).*

Introduction

In Europe semi-natural grassland habitats and their characteristic species are considered as highly threatened (Pedro Silva *et al*., 2008). Southern Belgium is an important area for the conservation of semi-natural grassland types listed in the annexes of the Habitats Directive. There is an urgent need to preserve, restore and manage these few remaining, often degraded, habitat patches. This task includes genetic rescue of populations of critically endangered plant species that, without intervention, would not naturally regenerate because of restricted seed dispersal abilities and the absence of a persistent seed bank in the soil. In the framework of the EU LIFE project 'Herbages' (LIFE11 NAT/BE/001060), the Botanic Garden Meise, as a centre of excellence in *ex situ* conservation and plant propagation, has implemented population translocations into the wild for four critically endangered plant species (*Dianthus deltoides, Helichrysum arenarium, Arnica montana* and *Campanula glomerata*). The aim is to increase the effective size of the remaining populations (by reinforcement) and to restore extinct populations (by reintroduction) in order to improve connectivity in the landscape.

A four-step approach

We first undertook a review of past and existing reintroduction projects worldwide (Godefroid & Vanderborght, 2011). This exercise helped to identify several factors that positively influence plant reintroduction outcomes (Godefroid *et al*., 2011). It also revealed shortcomings of common experimental designs that jeopardize the success of plant reintroductions. With this knowledge in mind, we started to prepare reintroductions using a four-step

After testing several propagation methods and visiting our colleagues of the LIFE project 'Arnikawiesen' in Luxembourg, we have developed an adequate protocol to produce 2,100 healthy plants of Arnica montana *that were transplanted at the age of two months in three populations of 700 individuals each.*

approach (Godefroid *et al*., 2016): 1) the selection and profiling of the target species; 2) the source population selection and seed collection; 3) the development of propagation protocols; and 4) the assessment of plant fitness of the populations used as seed source before planting out.

Species profiling is essential before implementing plant reintroductions

A detailed profile of each species has been produced in order to properly evaluate the species' biotic and abiotic needs, its basic biology and interspecific relationships (Godefroid *et al*., 2016). We compiled information for the following aspects:

- conservation status in Belgium and in the surrounding countries;
- ecological requirements (e.g. habitat type, soil pH and trophic level);
- morphological traits (e.g. life form, plant height, leaf arrangement, root depth);
- reproductive biology (breeding system, dicliny, dichogamy, phenology, and pollination);
- propagation ability (e.g. clonality, lateral spread); seed bank type; establishment strategy; aggregation; persistence; seed dispersal capacity; ploidy; mycorrhizae;
- interactions with predators and herbivores; diseases;
- seed germination requirements;
- cultivation methods (soil requirements, timing);
- possible causes of decline;
- (un)favourable ecological management practices.

A careful selection of potential seed sources

The selection of the potential populations that may serve as sources for seeds was based on the following criteria: 1) a similar habitat to the translocation sites; 2) a size preferably of more than 50 flowering individuals, and 3) a location as close as possible to the translocation sites. For each species, seeds were collected following the recommendations of ENSCONET (2009). Quality assessment of harvested seeds of *A. montana* showed a positive relationship between flowering population size and seed weight and germination rate, the non-germinated seeds corresponding to empty (aborted) seeds (Godefroid *et al*., 2016). Germination rate in *D. deltoides* was positively correlated with population size (Godefroid *et al*., 2016).

These findings show that if local seed sources only consist of small remnant populations, seed quality may not be optimal. Therefore, population size can be a valuable criterion for selecting seed source populations. We recommend to use seeds from the largest possible source populations in order to optimize the quality of the source material used in plant reintroductions. Practitioners should also examine seed quality of source populations before undertaking reintroductions. Seed weight might be used as an estimation of seed quality. This is essential as seed size is known to affect long-lasting differences in fitness among offspring (Halpern, 2005).

Producing quality plant material requires identifying the most appropriate propagation method

Preliminary tests using different kinds of growing medium were conducted in the seed laboratory and the nursery of the botanic garden. They provided valuable information for optimizing plant propagation protocols. The choice of growing medium strongly influenced the germination rate of the target species (Godefroid *et al*., 2016). For some species (e.g. *C. glomerata* and *A. montana*), changing the soil texture (e.g. by adding sand) increased germination rate by a factor of two. Such preliminary tests therefore provide valuable information for not wasting seeds, since reintroductions concern rare species where the seed amount that can be collected is limited. It also helps to know how many seeds need to be sown in order to obtain the required number of plants. The results also show that each species responds differently to a given growing medium, confirming that there is no universal protocol and that comparative trials using different kinds of substrate are necessary to optimise propagation protocols.

Pre-translocation plant fitness as an indicator of genetic diversity

In order to detect possible effects of the seed source population on plant development, we carried out morphometric measures (vegetative plant size) on the produced plants (between 2,000 and 3,000 individuals per target species): rosette diameter for *A. montana, H. arenarium* and *C. glomerata*, and the length and width of the longest leaf for *D. deltoides*. These measurements were performed one month and one week before transplantation (when plants were 4 and 7 weeks old, respectively).

Differences in pre-translocation plant fitness were detected between seed source populations, which might reflect genetic diversity and maternal effects (Godefroid *et al*., 2016). As the translocated plants should retain as much genetic diversity as possible to ensure a high adaptive potential and improve establishment success, multisource reintroductions can be recommended.

More than 9,000 plants reintroduced in 17 populations

For each species, a population of 500 to 700 young individuals was transplanted into three to six sites. Once *in situ* these plants (which are permanently labelled) were precisely mapped to facilitate their long-term monitoring. Transplants were positioned at an equidistance of 30 or 50 cm (depending on the species), and each translocated population consists of a mixture of plants from different origins arranged in order to maximize pollen exchanges between different origins.

Long-term monitoring of the transplants is necessary

Long-term monitoring is necessary because initially high survival rates can be followed by reversals over time (Fahselt, 2007; Hutchings, 2010). A demographic survey (e.g. survival, floral production, reproductive success, and population extension by clonal propagation or seedling recruitment) is recorded yearly in the field. Seeds produced by translocated plants are collected on a subsample and germinated to estimate the offspring's fitness (measure of germination rate, chlorosis, and vegetative growth). Genetic diversity is estimated using molecular markers.

Prior to transplantation, the target areas were 'cleaned' to avoid competition and to maximize the chances of transplant survival. Precise mapping of the permanently labeled individuals was carried out at the time of planting in order to facilitate long-term monitoring.

A total of 2000 plants of Helichrysum arenarium *were transplanted for reinforcement into one site and for reintroduction into three sites, which were 1 km apart in order to promote gene flow between populations. Five months after transplantation, more than 40% of the individuals were already flowering, with a survival rate of 98%.*

Box 1. A country whose flora is among the most endangered in the world

Belgium is one of the most populated countries in the world. As a consequence, about 40% of its flora is endangered and 8% has already disappeared (Delescaille & Saintenoy-Simon, 2006; Van Landuyt *et al*., 2006). The situation is particularly critical for species that are characteristic of very localized and seriously threatened habitats, such as wet depressions on peat soil, oligotrophic ponds or xeric sand calcareous grasslands. Any shrinking of these habitats affects the status of all characteristic species. Barely 9% of the Belgian habitats of European interest are in favourable conservation status; 17% are in inadequate status, 73% are in bad status and 1% are of unknown status (Peeters, 2014). Restoring habitats and endangered species in Belgium is more essential than ever and it has become one of the core businesses of the Botanic Garden Meise.

Encouraging results

The first results are beyond our expectations: over 90% reintroduced individuals survived the transplantation stress, and the flowering rate (> 30% on average) is impressive, sometimes just a few months after transplantation. We have also observed the presence of seedlings, and clonal propagation, indicating a potential for population expansion. Monitoring of reintroduced populations will continue for 10 years as only a long-term monitoring will tell what their future will be in the long run.

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Plant fitness is monitored throughout the year. Special emphasis is put on floral display (number of flowering stalks and of flowers/flower heads per stalk) and on reproductive success, through the sampling of closed ripe fruits or flower heads in order to estimate seed production and quality (aborted and viable seeds).

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Box 2. One of the few LIFE projects involving a botanical garden

The LIFE project 'Herbages' (LIFE11 NAT/BE/001060) started in 2013 in Southern Belgium, coordinated by Natagora, a Belgian nature conservation NGO. The Botanic Garden Meise is one of the partners, together with the Walloon Directorate-General for Agriculture, Natural Resources and Environment. The project runs until 2019 and aims at improving the conservation status of 400 hectares of priority grasslands in 26 Natura 2000 sites. LIFE funds are used to restore 11 grassland types, including six priority habitats, among which xeric sand calcareous grasslands (6120*), semi-natural dry grasslands on calcareous substrates (6210*) and species-rich Nardus grasslands (6230*). The restoration of these habitats involves land purchase, ecological management (deforestation, scraping the top soil, seeding, fencing, and grazing by sheep or goats), and implementation of ecological corridors and stepping-stones. Another important aspect of the project is to prevent the extinction of the habitats' characteristic species, especially those that are most vulnerable to fragmentation, by population reinforcements/ reintroductions after the restoration of their habitat. The project also has a significant socio-economic dimension, by providing work to small local companies (forestry, environmental management, agricultural). Moreover, the restored habitats will subsequently be leased to local farmers who will ensure ecological management of the sites.

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