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# INTEGRATED CONSERVATION OF THE RARE AND ENDANGERED THERMOPHILOUS SPECIES *CYPERUS POLYSTACHYUS*: INVESTIGATION ON RECRUITMENT STRATEGIES AND SHORT-TERM ASSESSMENT OF TRANSLOCATION

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ABSTRACT - *Cyperus polystachyus* Rottb. is a threatened species occurring in Europe only in two sites located in wetland habitat in mediterranean Southern Italy. According to the IUCN red list for Italy (1997), the species is listed as critically endangered (CR). This study investigated recruitment and habitat limitations referred to the population located south of Rome, in the Tor Caldara Regional Reserve. The site is an isolated island of vegetation. Natural expansion of the species is highly limited by the absence of "wet" sites in the surroundings.
Importantly the study also endorsed an "in wild translocation program", which aimed to decrease the risk of species extinction linked to demographic stochasticity, extreme climatic events or mismanagement.
Seed germination, seed and site limitations, and germplasm translocation were investigated. Seed viability and seed germination were tested at the Rome Botanical Garden; results showed that the species has high recruitment potential, especially in thermophilous conditions. Germplasm (seeds) was translocated in a vegetation remnant within the Protected Area of Decima Malafede overseen by RomaNatura, a Regional Agency for the management of PAs, which, together with the National Environment Research Institute (ISPRA) was one of the partners in this research. The site limitation and translocation studies were undertaken in a glade with a seasonal pond. Seeds were sown and seedlings planted within an experimental framework, protected by a metal cage to prevent grazing. The new site is within the natural dispersion range of the species in an area with similar ecological and biogeographic conditions. It is surrounded by thermophilous oak woodland and cultivated fields which exclude germplasm escape. Results showed that, due to the germination behaviour, the establishment is limited by the absence of microsites suitable for recruitment. Competition with perennial species and delayed germination are the main obstacles for recruitment, especially during prolonged drought episodes under climatic changes. Short-term assessment of the translocation process was undertaken. Values of new seed set, seed viability and germination were similar to those present in the original site. Translocation was, consequently, proved successful until this first stage. Outcomes will be useful both for the conservation of the species and for the production of an Italian handbook for *ex situ* conservation of threatened species. It is hoped that monitoring, ongoing care and evaluation will continue to be carried out also in the future.

KEY WORDS - *CYPERUS POLYSTACHYUS,* DECIMA MALAFEDE, GERMPLASM STORAGE, INTEGRATED CONSERVATION, TRANSLOCATION.

## Introduction

Sedges mostly occur in wetland habitats, which are, nowadays more than ever, threatened by anthropogenic activity such as land clearing, increased use of fertilizer in agriculture, water table exploitation and climatic changes. In Europe, some species/taxon may even be in danger of extinction (Rich, 1999). Their disappearance is an indicator of

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damage to the ecosystem, which is often irreversible.

*Cyperus polystachyus* is a rare and endangered species that occurs in Europe only in two locations in Italy: on Ischia, in the Campania Region, and in Tor Caldara (TC), in the Lazio Region (Tenore, 1811-38; Moraldo *et al.*, 1987-88, Conti *et al.*, 2005). Both locations are distinguished by the presence of sulphur waters. According to Conti *et al.* (1997) the species is within the CR (critically endangered) National IUCN Red List category, facing an extremely high risk of extinction in the wild. The original home range of the species is unknown as the species was identified for the first time in Europe in 1802. Effective wetland drainage (bonifiche) since the late 1700's, in Central-Southern Italy, likely reduced and/or disjuncted the original range reducing the extent of occurrence of the taxa. For example, marshlands once spread throughout the mainland between Ischia and Tor Caldara.

In TC the species was identified only in 1987. It was suggested (Moraldo *et al.*, 1987-88) that seeds from Ischia reached the site, in the past, thanks to migrating birds. At present, in TC the species is now found in two main stations, all with water content at field capacity. The population, however, experienced a decrease in recruitment in 2008 due to water caption, which favoured summer pond desiccation near the more prolific station.

Also facing conservation threats is the population in Ischia, where a survey conducted by the authors in the summer of 2008 recorded the absence of the species in several sites described by Merola in 1957 and the development of new touristic infrastructures near the thermal springs within the area of occupancy of the taxa.

The species is listed as present in other parts of the world such as Northern Africa, Asia and Oceania (Moraldo *et al.*, 1987-88) but, to our knowledge, a genetic or morphological comparison was never made among the different taxa. In particular, Merola (1957) considered the species a possible termophilous relict in Europe and a cosmopolite in the other locations.

Protection and conservation of *Cyperus polystachyus* germplasm is both of national interest, for the preservation of the country's floristic heritage, and of international interest as the conservation of endangered flora is within the objective of the GSPC (Global Strategy for Plant Conservation) adopted from the CBD (Convention on Biological Diversity) of which Italy is a member State.

The success of plants establishment, both in natural and altered habitats, is determined by the interaction of many complex factors, of which the most important is the potential for regeneration from vegetative parts and from seeds. While most of the sedges are perennials, *Cyperus polystachyus* in TC displays an obligate seeder regeneration strategy, therophyte, whereas on Ischia it has a perennial habit with specimen surviving the unfavourable winter season as an hemicriptophyte with buds at or just above the soil surface protected by stem leaves.

Complex storage techniques, such as cryostorage (Merrit *et al.*, 2005) or ultra dry storage in sealed glass tubes (Pérez-García *et al.*, 2008), can guarantee that seed viability and germination values remain almost constant; but in normal storage in dry conditions (common in most of the "seed bank" facilities), seed viability and germination values decrease over time.

In particular, in the case of *Cyperus polystachyus* Fabrini *et al.* (submitted) showed that viability (cut test) and germination values in growth cabinet (30°/15° with a 12 hours photoperiod) of post harvest seeds were significantly higher than the values of seeds stored for 4 years in constant dry room conditions (Figs. 1 & 2). Also, a greenhouse experiment on seed permanence in the soil seed bank showed that the species displays a transient behaviour with most of the seeds germinating the first year after seed set.

These experiments showed the not complete reliability of *ex situ* conservation, in ordinary conditions, and the fact that the species, which in TC has an annual behaviour, is also vulnerable also to sporadic events.

*Cyperus polystachyus* in the original sites grows near small ponds and/or springs, always in open habitat. The species usually disperses prolific quantities of viable seeds in the proximity of the mother plant and germination occurs preferably at high temperatures (Merola, 1957; Fabrini *et al.*, in prep). *Cyperus polystachyus*, as confirmed by the Ellenberg ecogramme (Fig. 3), is a hygrophilous, heliofilous and thermophilous species (Pignatti *et al.*, 2005).

These features give the species only a small time window (from late spring to early summer) for germination. Climatic changes, including a prolonged cold and wet season or an extended drought, may reduce the viability of the annual populations of TC, leading to local extirpation. In addition the species suffers from competition with other native species, which display a perennial habit or earlier germination and tend to increase their presence from early spring.

The TC region was originally a wide area of marshlands. From the 1930's wetland drainage for land development limited the presence of wet habitats exclusively to the area of the TC Regional Reserve. The Reserve is now completely surrounded by human settlements and crop fields (plus sea on the western side) preventing the species from expanding outside the site. In all, there is a clear absence of nearby "stepping stones" or ecological corridors.

For all these reasons a partnership among the Germplasm Bank of the Rome Botanical Garden "La Sapienza University", the National Environment Research Institute (ISPRA) and the Regional Authority for management of APs in the Rome area (RomaNatura), supported by volunteer researchers, launched a project to conserve the germplasm of this species.

The primary goal was the *in situ* conservation of species and habitat, complemented by *ex situ* conservation in the Rome Botanical Garden and an experimental translocation of species germplasm. The *ex situ* establishment in the wild of a new population was considered vital for the conservation of *Cyperus polystachyus* because its life cycle, distribution, and recruitment features make the species vulnerable to local extirpation and consequent extinction in Europe from factors such as localised catastrophic or anthropogenic events, demographic stochasticity, or a sequence of unfavourable seasons in the current climatic change regime. According to Thuiller *et al.* (2005), the risks of extinction for European plants may be high, even in moderate climate change scenarios.

Flora translocation is the deliberate transfer of plant germplasm from one area to another for the purpose of conservation (Vallee *et al.*, 2004). In our case, translocation concerned the attempt to establish new populations in an area with appropriate ecological and management characteristics, within the potential natural dispersal range of the species. According to Hallingbäck *et al.*, (2000; in Società Botanica Italiana, 2008), in the absence of detailed information on dispersal capacity of a seeder species, 50 km is considered the minimum distance between populations to avoid biological isolation. Therefore this distance may be considered the maximum dispersal range of an obligate seeder population.

Compared to conservation in seed bank facilities, new population establishment would have the advantage of having a new seed set from acclimatized plants each year. Germplasm translocation tools, methods and experimental procedures were also acquired by the following handbook and guidelines: Akeroyd & Wyse Jackson (1995), IUCN (1987; 1998; 2002); Vallee *et al.* (2004), Guerrant *et al.* (2004), Rossi *et al.* 2005.

As biological knowledge of the species is incomplete, translocation was carried out in an experimental framework designed to clarify both biological and management characteristics and the needs of the species (Gordon, 1994; 1996). The framework included a contingency plan to remove the translocated propagules, in case they threatened the habitat or other species.

This research also sought to combine information from a related study on the recruitment biology of the species (Fabrini *et al.*, in prep).

AIM

This research aims, through an integrated conservation strategy approach, to improve knowledge about the recruitment biology of *Cyperus polystachyus*. This should enable the survival of the species through conservation measures both *in situ* and *ex situ*. The study also endorsed an "in wild translocation program", to promote a self-sustaining population and therefore delist the conservation status of the species ("blue shift").

A further aim was to contribute to the development of appropriate translocation techniques for endangered flora and detailed protocols for Italian guidelines for *ex situ* conservation of threatened species and assessment of translocation success.

## MATERIALS AND METHODS

THE SITES

The original site of the TC Regional Reserve (41°.29' 22,38" N; 12° 35' 39,41" W) is approximately 40 km south of Rome. *Cyperus polystachyus* grows near small ponds and water springs in assemblage with the following species: *Juncus articulatus* L., *Osmunda regalis* L., *Lythrum salicaria* L., *Agrostis canina* L.. The original germplasm was collected as mature seeds. A harvest was carried out from more than 50 specimens, randomly chosen to limit the genetic homogeneity of the new station (Royal Botanic Gardens Kew, 2005). Successively the seeds were sown (in pastorised nursery potting mixture) and stored in the Rome Botanical Gardens.

The translocation site (41° 41' 24,69" N; 12° 28' 6,60" W), located less than 25 km from the source site, is a glade with a seasonal pond located within the Protected Area of Decima Malafede (DM) -see

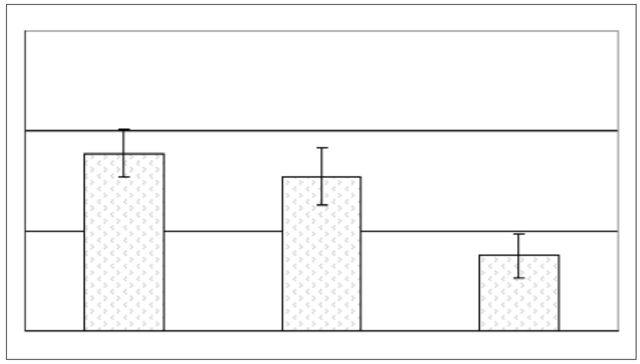

Fig. 1 - Mean percentages (±1 S.E.) of viable seed (cut test).

results for site selection- where the following marshland species occur: *Juncus effusus* L., *Alisma plantago-aquatica* L., *Damasonium alisma* Miller, *Ranunculus trichophyllus* Chaix, *Paspalum paspaloides* (Michx.) Scribner. In the translocation site, seeds were sown and seedlings planted in two 75x150 cm plots fenced by wire mesh to prevent herbivores (mainly roe deer and wild boar) and soil was cleared from vegetation. Both seed and seedling were used to different types of "selection" and seed set time. In fact seedling grown in a nursery would have been selected to specific "greenhouse" pressures while direct sowing would have mimicked the natural dispersal process and new seedlings would have been selected more on site natural selection. Seedling founder, however, have a higher growth and establishment rate and shorter time to flowering (Jusaitis *et al.*, 2004).

THE SPECIES

While none investigated the genetic difference of the two European populations (as well, to our knowledge, differences between the European and north African populations) preliminary observation on the winter survival of specimens of the two populations in greenhouse confirmed the different habit, perennial and annual, with only the Ischia specimen surviving the winter. The two populations also have slight morphological differences: the Ischia population features bigger stems and leaf and shorter flower culms originating compact spikelets. These differences suggest that there could be a variation between the two European populations. The species lacks a long-distance seed dispersal mechanism, such as wings or hooks.

Different surveys and experiments were undertaken, both in the Botanical Garden facilities and in the wild, to investigate the recruitment biology of the species and the success rate for translocation.

**Seed reproductive biology (a)**

a1) Seed and microsite limitation: availability of seeds and microsites suitable for germination and seedling survival are two factors that can limit recruitment more than any other.

To investigate these limitations and to quantify the values of seedling emergence a specific designed experiment was undertaken, in spring 2008, in the DM site, nearby, and concurrently, the translocation plots.

Seeds were sown within a confined unit made of cylindrical containers of 10 cm diameter (without tops or bottoms) inserted into the soil protruding just 2 cm.

The seed limitation experiment had two treatments, with 5 and 25 seeds; the site limitation experiment also had two treatments, with vegetation inside the cylinder (to test if the presence of other plants can impact both the emergence and growth of *Cyperus polystachyus* seedlings) and on bare soil (to eliminate the competitive effect of other species). Each cylinder had double sided sticky tape on the internal border to avoid seed predation (the sticky tape was replaced at each survey until germination). In total there were 4 treatments each one replicated 4 times. The experiment assessed both the germination percentage and the seedling survival. The units were watered just for the first months and records were collected every two weeks until the end of the experiment.

**Translocation (b)**

the following investigations were undertaken:

b1) biogeographical study of the areas surronding the TC site to evaluate areas suitable for translocation;

The use of multiple sites for translocations may increase the potential for proliferation of new populations, thus creating a metapopulation capable of reducing the extinction risk; for this reason within the potential natural dispersal range of the species a

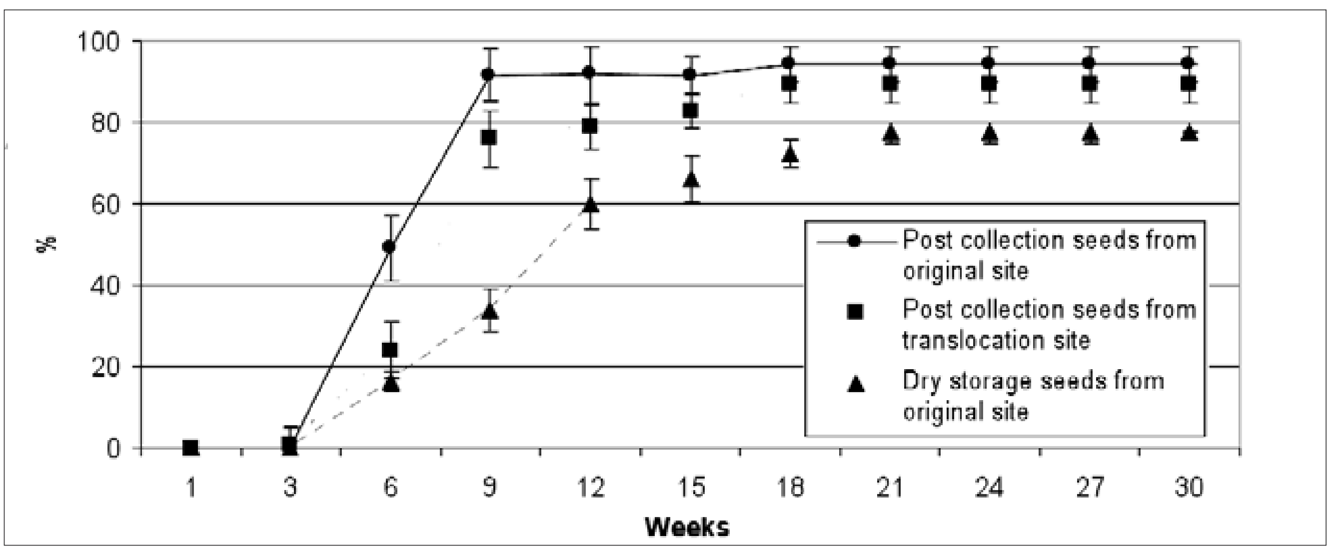


Fig. 2 - Time courses of seed germination from 1–30 weeks. Mean percentages (±1 S.E.) of seed germination in petri dish. Germination results were not adjusted to account for non-viable seeds. smoked; ● =post harvest original site ;■ = post harvest translocated site; Δ=four years dry storage original site.

biogeographic study was undertaken to individuate sites with suitable ecological (b1.1) and specific biological/management introduction features (b1.2).

In particular new sites needed to be:

b1.1) near ponds or in the presence of a spring; in open habitat; with warm climate; in absence of aggressive species; in an habitat within the same sintaxa at order level;

b1.2) within the potential natural dispersal range of the species; within the same phytoclimatic unit; similar edaphic and climatic conditions; within the same water catchment; and to avoid possible, but unlikely, weedy tendency the sites needed to be an "enclosure" surrounded by a specific biological barrier that excluded escapes (Crosti *et al.*, 2009). Lastly sites needed to be within a managed protected area with low impact of tourism/recreation with the authority of the PA willing to be a partner in the conservation project.

b2) Germplasm translocation

The germplasm translocation, which started in June 2008, employed both seed sowing and seedling planting. Self-sustaining should be assessed over a long-term period (at least 10 years or three generations as from the IUCN (2006) criteria for evaluating population reduction) and while abundance and extent of the translocation can be assessed over a short time span, resilience and persistence must be tested over longer periods (Pavlik, 1996). However, this information is often difficult to obtain because the response of vegetation to conservation management is most likely to occur beyond the average time of research projects or funding (Crosti *et al.*, 2007). In this paper we assess the short-term self-sustainability of the translocated population and we considered the first stage of the translocation successful if:

b2.1) seed viability (cut test, 3 replicates 50 seeds) and germination (growth cabinet 30°/15° 12 hour photoperiod, 4 replicates 30 seeds) of the first generation seed set occurred in similar values compared to the original sites;

b2.2) germination of first generation seedlings occurred; second generation seed set produced viable seeds.

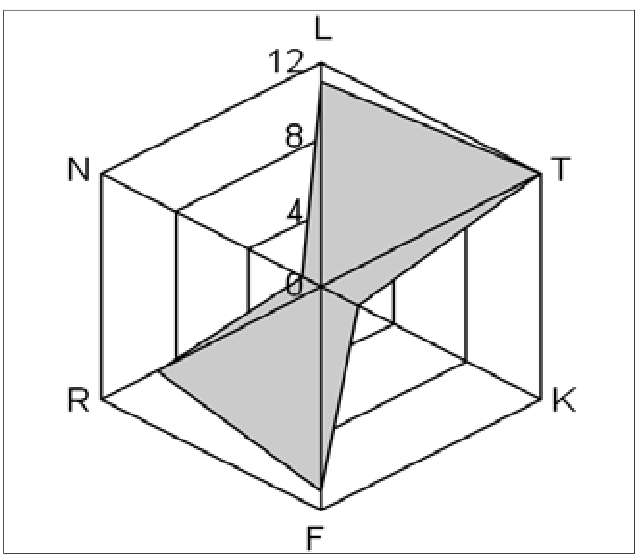

Fig. 3 - Ellenberg ecogramme with the ecological requirements of *Cyperus polystachyus*; L light, T temperature , K continentality, F soil moisture, R pH, N nutrients.

## RESULTS

Seed reproductive biology

a1) The experiment lasted 17 weeks and across all the treatment only three seedlings survived (Fig. 4).

Higher germination percentage values occurred for all treatments during the fifth week after the first emergences. In presence of other vegetation within the experimental unit, the germination value was 10.5% ±2.8, while on bare soil the value was 36% ± 5. Treatments with 5 seeds were not successful, with all the germinant missing by the 13th week (in the treatment with vegetation, germinants were missing at the 9th week scoring). At the end of the experiment only the treatment on bare soil and 25 seeds had 3.0 % ± 1.6 (0.75 ± 0.48 seedlings) survival.

Translocation

b1) following the biogeographical investigation five different sites were considered suitable according to the ecological and introduction/management fea-

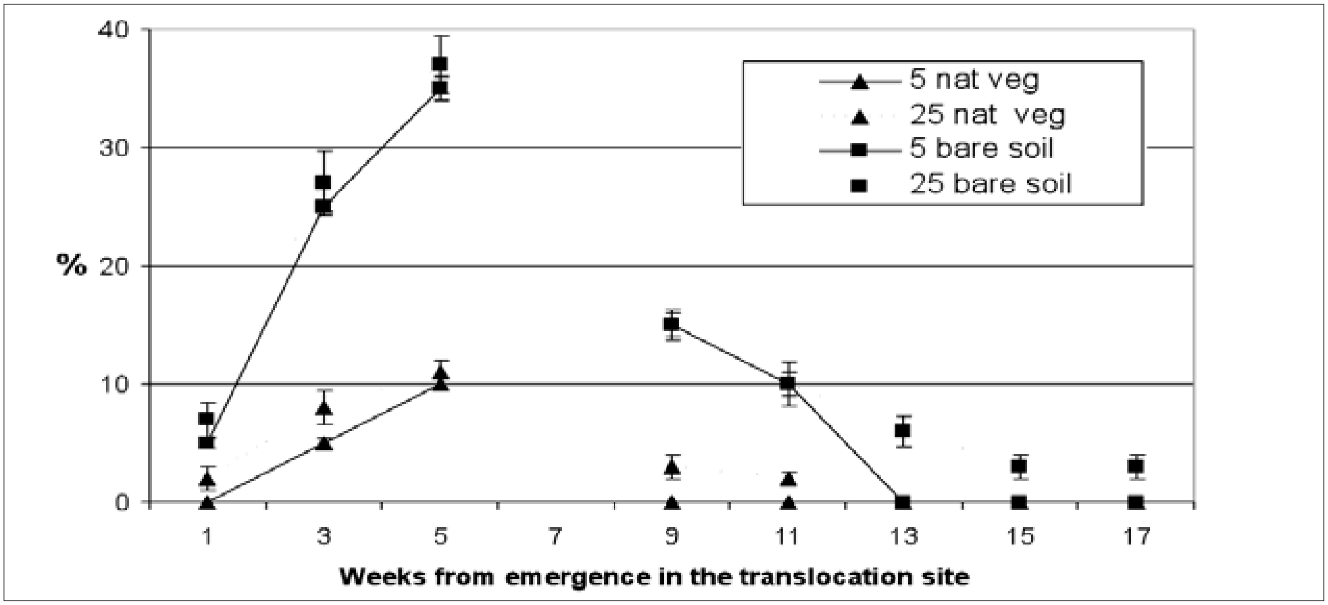


Fig. 4 - Time courses from seedling emergence 1-17 week. Mean percentages (±1 S.E.) of translocated seeds in experimental trail. Solid line 5 seeds, dotted line 25 seeds; ■= with native vegetation, Δ= on bare soil. No record was undertaken at week 7.

tures: Litorale Romano and Castel Porziano State Reserves, Laurentino Acquacetosa and Decima Malafede (DM) Regional Reserves, Foglino woodland City Reserve.

Due to the scarce research funds and to the experimental nature of the project the translocation program was limited only to one site and DM was chosen in particular because it provided a greater biological barrier against germplasm escape (the site is surrounded by a "double buffer" constituted by an oak woodland and crop fields). In addition the Regional Authority that manages the area, RomaNatura, decided to join the conservation project.

Germplasm translocation (b2)

b2.1) viability and germination tests between first year generation seeds harvested from DM with fresh seeds harvested from the original site of TC showed similar percentage values (viability = 85.4 ± 2.9 *vs* 87.7 ± 2.33; germination 89.6 ± 5 *vs* 94.2 ± 4.2) (Figs. 1 & 2);

b2.2) in June 2009 in the DM translocation site germinants of *Cyperus polystachyus* ($1^{st}$ generation seedlings) were present and by September 2009 plants were naturally dispersing the second generation of viable seeds. It should be noted, however, that the number of mature plants in September 2009 was much lower compared to the number of fertile plants in September 2008. The new population did not show any weedy behaviour in the new site.

## CONCLUSION

This study confirmed the importance of an integrated conservation approach which combined in the same project germplasm conservation in a seed bank, research on the seed biology, field studies, translocation and the expertise of a number of public bodies involved in biodiversity conservation.

*Cyperus polystachyus* suffers various threats within its area of occupancy, while loss of wetland habitats (including ecological corridors) and its lack of any long-distance dispersal mechanism limit the species expansion. For natural recruitment, greater numbers of seeds need to reach new sites suitable for germination without the presence of competing species. To germinate, the species needs an extremely specific niche (moist soil and warm temperature), and being a late germination small annual herbaceous is more vulnerable when competing with other perennial species.

The first stage of the translocation proved successful over the 2nd generation seed set and continuing the conservation effort would likely ensure the self-sustainability of the new population.

Within a translocation framework, it is important to be able to link back to the original source of germplasm collection. For this reason beside the publication of this paper, the project is listed within the Italian national inventory of plant reintroduction of the SBI-Italian Botanical Society (Rossi & Bonomi, 2007) and in the global plant reintroductions survey conducted by the National Botanic Garden of Belgium.

Due to the annual behaviour of the species, the sustainability of the population is highly dependent on climate conditions (precipitation and temperature), so only a multi-site translocation program and long-term management could ensure the survival in the wild of this *ex situ* population.

The partnership among the Rome Botanical Gardens, the National Environmental Research Institute (ISPRA), RomaNatura and the volunteer researchers allowed the achievement of the project, until the first stage, at no additional cost beyond personnel and materials. Successful introduction of endangered species, however, requires continuous ongoing care and management of population status and dynamics and only a long-term assessment will be able to evaluate the success of the translocation. At present there is no agreement or available funds to continue the monitoring, evaluation and ongoing management, so the translocation planting may have to be abandoned at this stage. There is the hope, from all researchers involved, that one of the former partners or a new body may support this conservation project.

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