

INTEGRATED *IN SITU/EX SITU* PLANT CONSERVATION PRACTICES MANAGED BY  
UNIVERSITY BOTANIC GARDEN OF MODENA

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#### ABSTRACT

The University of Modena's Botanic Garden recently started a series of *in situ/ex situ* integrated conservation projects in cooperation with Parks and other land management institutions. Although these projects only involved a limited number of endangered and/or vulnerable species/populations, some interesting and noteworthy results were achieved.

Activities conducted in the past aimed to increase populations of rare or endangered species rather than true reintroduction of locally extinct *taxa*. They were developed inside the Garden through *in vitro* and *in vivo* growing practices and museum-type educational activities and outside the Garden through partnerships with Parks, nature reserves and other administrative bodies or institutions.

This paper illustrates some of the noteworthy cases undertaken by Modena Botanic Garden staff.

#### KEY WORDS

plant conservation, *ex situ*, *in situ*, Modena Botanic Garden.

#### INTRODUCTION

The Emilia–Romagna Region, where the University of Modena Botanic Garden is located, has been historically characterised by a strong industrial expansion, and significant environmental conservation actions were only introduced in the recent past. There are four University Botanic Gardens in the Region (Bologna, Ferrara, Modena and Parma): they are able to provide Parks and public bodies with the necessary knowledge about ecosystems and plant conservation. There are also several local alpine or thematic Gardens, generally owned and managed by local Institutions or Associations, which can be able to give important support to conservation programs.

The University Botanic Garden of Modena has been involved in plant and ecosystem conservation and management projects for several years. The action lines adopted by the Garden are compliant with the Global Strategy for Plant Conservation and are akin to those adopted by numerous Botanic Garden in the framework of the Working Group for Botanic and Historical Gardens of the Italian Botanic Society (Bedini et al., 1997).

The goals of these projects are: a) to produce vegetational and floristic information on protected areas and promote awareness of them; b) to provide guidelines on plant and habitat conservation and landscape management; c) to co-operate with public and institutional bodies in managing Biotopes; d) to share knowledge with other local institutions; e) to start *ex situ* and *in situ* integrated plant conservation programs; f) to promote public awareness of these projects.

#### CONSERVATION PRACTICES

The Botanic Garden of Modena has implemented various conservation projects through research on ecosystem management and integrated *in situ/ex situ* conservation practices. Some important cases,

regarding the Emilia - Romagna Region in particular are outlined in this paper, with the aim of comparing the various experiences and to characterise their strong and critical points (for each case see extensive papers in Bibliography too).

case 1) *Marsilea quadrifolia* L. in an irrigation and drainage canal near Mortizzuolo (Mirandola, Modena)

*M. quadrifolia* L. is a sporocarpic hydrophytic fern typical of wet environments with variable water



Figure 1. *Marsilea quadrifolia* L. in a ditch near Mirandola (Modena).

levels, such as pools, rice paddies, canals and ditches with slow-moving water (Figure 1).

*M. quadrifolia* is widespread in Europe and Asia up to Manchuria, in Japan and the Mekong delta. It also lives in the Midwest of the U.S.A., where it is probably not native (Bonafede et Al., 2003). The plant was once quite common in the wetlands of central-southern Europe, including the Po valley. Its numerical consistency fell dramatically in many European countries in recent times. Although *Marsilea quadrifolia* L. has been reported in several regions of Italy, all the records date back to the 19th century with the sole exception of a few areas in the plain north of the Po river. The species can therefore be considered in progressive extinction in vast areas, to the point that it has been included in the “Red List of the Plants in Italy” (Conti et al., 1992)

In 1993, we found a population of *M. quadrifolia* L near Mirandola (Province of Modena) in the Dugale Montirone irrigation and drainage canal. We subsequently collected and mapped all the bibliographic records and herbarium specimens data in the Emilia-Romagna Region. We simultaneously investigated the plain territory enclosed by the Via Emilia (Western boundary), the Adriatic coast (Eastern boundary), and the Po river (Northern boundary), using the middle-European floristic grid (6° latitude, 10° longitude), and assuming one square as the mapping unit, in order to draw an up to date distribution map for Emilia-Romagna. These data confirm the plant’s phase of decline in the investigation area (Bonafede et al., 2003). Moreover, at the date of this study, *M. quadrifolia* only grew in limited areas of the Modena lowlands. In September 1996, the Authority responsible for maintaining the irrigation canals and ditches (Consorzio di Bonifica Burana, Leo, Scoltenna e Panaro) needed to excavate and clean the canal for water management reasons (Figure. 2).

The operation, as it was planned and described, would have led to the certain extinction of *M. quadrifolia* in what was the only well ascertained point at that time, and where ecological studies were



Figure 2. Digger carrying out excavation work in a ditch.

undertaken. We devised an alternative programme in agreement with the Land Reclamation Consortium. This initially contemplated remodelling the right bank restricted to the area above the mean water level of the canal in the sector where *M. quadrifolia* was present. This operation was performed in Spring, 1997. Starting in July 1997 - using the same sectors - flora surveys were performed through transects along the Montirone canal, with the aim of comparing the bank that underwent the work with the untreated left bank, in order to collect data useful for defining the ecological needs of the species.

Cluster Analysis produced a dendrogram that identified two main groups of floristic relevés:

- a first group performed on the right bank, subject to remodelling in spring 1997 (characterised by *Phragmites australis* (Cav.) Trin. and *Marsilea quadrifolia* near the water and by *Agropyron repens* (L.) Beauv., in part replaced by *Cynodon dactylon* (L.) Pers. in late summer, and *Potentilla reptans* L. on the bank (Two main groups of samples were distinguished on the right bank according to their distance from the water).

- a second group taken from the left bank, which was not remodelled and which had only undergone two cuts per year for the past 6 to 7 years (this group of relevés was characterised by *Carex riparia* Curtis, *Potentilla reptans* L. and *Calystegia sepium* (L.) R. Br.; the difference according to the distance from the water line was less evident). *Marsilea quadrifolia* was more widespread and with greater numerical consistency in the relevés on or near the water line (87.5% of the samplings were positive for *Marsilea*), however the most interesting fact in this context was that 62.5% of the relevés positive for *Marsilea* were located on the right bank, subject to remodelling. This demonstrated the pioneer capabilities of the plant, although the first “pioneers” evidently originated either from the opposite bank (the one we protected from management practices) or from rhizomes fortuitously escaping the bank remodelling. It should be stressed that we observed a substantial increasing of in *Marsilea* located downstream where the current had transported rhizomes.

The studies were completed with chorological analysis in the sites characterised by the presence of *Marsilea*, which highlighted the dominance of the wide-distributed chorotypes (about 50% of the total) in particular cosmopolitan and subcosmopolitan species (belonging often to the antropocore chorotype), characteristics of environmental situations that had been strongly and continuously reworked by man. This is not surprising, given the location of the small ditch, which is in direct contact with farmland subject to intensive farming and bearing a rich infesting flora.

Additionally, some preliminary analysis was performed on mud and waters of the collecting station of the Dugale Montirone canal. This analysis revealed a overall framework that does not differ greatly from those in other canals of the Modena plains and it was in agreement with the plant species that had been observed. Overall, no significant differences were found, although these data require more in depth evaluation. We noted that the plant presence was more abundant where the ditch widened or curved and where the water flow was reduced. Other details on these results are reported in Bonafede et al. (2003).

In brief, we concluded that the plant populations in the station involved in the intervention remained to a great extent unchanged: this is consistent with the results obtained from the surveys performed (in 1997 and 1998) in the same collection stations before and after remodelling of the one of the banks. This comparison confirms the validity of the indications we gave to the management Authorities on the excavation and cleaning of the canal in 1997.

Regarding the conservation activities carried out directly in the Botanical Garden of Modena, *M. quadrifolia* was initially cultivated in a ditch constructed in 1993. It was designed by one of the authors of this paper, based on a plan by the late Prof. Daria Bertolani Marchetti. The ditch (about fifty meters long and with a depth of about 30 cm) was set up as an example of a rural biotope in danger of extinction, with the aim of promoting awareness among visitors to the Botanical Garden on the issues of conservation of vulnerable or threatened plants of local interest (Figure 3).

*M. quadrifolia* was abundant for many years over a stretch of about 10 meters along the ditch, inhibited where water flows into a shady area of the ditch. The species initially maintained a floating bearing; since 1998, the plant started to colonize the banks, up to about one-half meter from the water level, assuming an erect bearing in the plants at the centre of the ditch, especially in the sites with slow water flow. The plant never produced sporocarps. It is interesting to note that the banks of the experimental ditch are not subject to cutting, in contrast to the practices carried out in the ditches of the Land Reclamation Authority; periodic weeding is selectively performed manually.

An act of vandalism suffered by Botanic Garden in 2003 destroyed the hydraulic system of the artificial ditch and the resulting prolonged dryness led to the gradual demise of the station. This confirms the difficulties in maintaining *ex situ* living collection of plants for long periods, especially in the case of species connected to particular habitat and when their survival is critically dependent on continued human care (Heywood, 1989).

case 2) *Viola pumila* Chaix in the Novellara and Reggiolo Valley (Province of Reggio Emilia).

*Viola pumila* Chaix (Figure 4) only grows in Italy as small populations in the Po valley, in the Emilia-



Fig 4. *Viola pumila* Chaix

Fig 3. The didactic ditch in the Botanic Garden of Modena, an example of a rural biotope in danger of extinction, were *M. quadrifolia* was cultivated *ex situ* (1998).

Romagna Region, in two areas in the Novellara and Reggiolo Valley (Province of Reggio Emilia) (Pignatti, 1982) and near Mirandola (Province of Modena). Other population in Northern Italy had not confirmed for over 50 years. The reasons of the rarity of this breed can be attributed to land reclaiming for agricultural activities, bank management that causes an increase in ruderal aspects and floristic corruption by the invasion of common and alien plants.

Due to its scarce distribution and to the exiguity of populations, the species is considered rare and in danger of extinction in Italy. A project of integrated *in situ/ex situ* conservation was therefore launched by the Botanic Garden of Modena in co-operation with local Administrations and the Association of Voluntary Ecological Guards of Reggio Emilia (Dallai & Sgarbi, 2005). The municipalities of Novellara and Reggiolo established an “Area di Riequilibrio Ecologico” (Ecological restoration area) between the banks of Bagna, Fiuma, Valletta and Bruciati in order to restore and preserve these decaying marsh areas. A number of *V. pumila* plants were planted in the Area, which allowed us to have seeds to obtain an *ex situ* conservation collection. Seed viability was tested using Tetrazol (Water solution of TTC 1%, 24 hours of incubation at 30 °C). Seed sterilisation/scarification was obtained using NaOCl 1.5% solution + 2 drops of TWEEN 80 for 14 minutes. Seeds were rinsed 3 times in sterile H<sub>2</sub>O and used in *in vivo* and *in vitro* germination.

*In vivo* germination tests: seeds were sown in pots (3 seeds per pot) and placed in closed greenhouse-cases. Greenhouse-cases were stored in a climatic chamber (23 °C and with a 12-hour photoperiod).

*In vitro* germination tests: a synthetic medium, MS 1/2 concentration (Murashige and Skoog, 1962) was used. Sowing was done under a laminar flow hood: 20 seeds were put in each holder (Vitrovent™), which was stored in a climatic chamber (T. 23 °C, Photoperiod 12 hours).

The best results were obtained by *in vitro* germination, with 62.3% mean germination (Figure 5). The plantlets were transplanted onto compost 40 days after germination and were later ready for repotting (Figure 6).



Figure 5. Plantlets of *Viola pumila* 15 days after sowing.



Figure 6. Plantlets of *Viola pumila* 2 months old cultivated in holder after re-potting.

Several hundreds of plants were obtained *in vitro* from seeds collected from the wild and some were subsequently transplanted into the natural sites in the Novellara and Reggiolo Valleys (Province of Reggio Emilia), with the involvement of local Administrations, Schools and the Media. Others were planted in a bed in the Botanic Garden of Modena with the aim of creating a conservation live collection (Figure 7)



Figure 7. *Viola pumila*: *ex situ* collection in the Botanic Garden of Modena.



Fig 8. *Senecio paludosus* L.

case 3) *Senecio paludosus* L. in the in the Novellara e Reggiolo Valley (Province of Reggio Emilia)

Another practice in *ex situ/in situ* conservation was carried out to save some populations of *Senecio paludosus* L. found in the same area of the Po Valley as described above (*V. pumila* case).

*S. paludosus* (Figure 8) is an helophyte perennial species that grows in oligotrophic marshland. This species has a wide distribution type chorology (Euro-Siberian) but it is almost extinct everywhere on account of land reclamation and farm pollution.

The seeds used for this project were picked by Ecological Voluntary Guards of Reggio Emilia in 1995 and stored in dark conditions at 4 °C. TTC vitality tests (see above) gave results worse than *V. pumila* (mean: 14.65%). Germination tests were run on lots of 10 achenes put in a Petri dish on three layers of filter paper soaked in distilled water. The Petri dishes were putted in Vitrovent™ (adding more distilled water) and kept in a climatic cell (T. 23 °C, Photoperiod 12 hours). After 5 days, we observed the first seed germination; a total of 20% of seeds germinated. Better results were recorded in germination tests carried out on a synthetic culture substrate MS 1/2 concentration (Murashige and Skoog, 1962), in sterile conditions (achenes sterilisation/scarification was obtained using an NaOCl 1.5% solution + 2 drops of TWEEN 80 for 14 minutes). The achenes were put on a sterile medium in Petri dishes and were kept in a climatic cell (T. 23 °C, Photoperiod 12 hours). A pre – chill treatment was tested on two lots of achenes by keeping them in dark conditions and at 4° C (Ellis et al., 1985). Test results gave a mean germination of 26% to 53% in the different lots and maximum percentages were obtained in one of the lots subject to pre –chilling treatment. Another lot of 80 achenes directly sowed onto potting compost gave mean germination of 36.25%, however there was a significant death rate (10.34%) after repotting. Further tests are needed to fine-tune a rational germination protocol for this species.

Germinated seeds at the Botanic Garden - either *in vivo* and *in vitro* – gave a large stock of plantlets that were used *ex situ* (to increase conservation living collection) and *in situ* (for restoration in natural sites). Some plants were introduced into the “Ecological restoration area” between the banks of Bagna, Fiuma, Valletta and Bruciati (Figure 9). Here a marsh area was reconstructed some years ago and has been fenced in and controlled. This zone can be considered a good example of a “nursery field” in a natural area and could play a very important role in the cultivation of plants at risk of extinction in the local landscape.

Some plants were planted in the “nursery field” and other lots were planted in two sites near to the banks of a ditch. One year later (July 2007), they had survived in one site only. However, we observed that these plants have a good offshoot ability after bank mowing. The station is now periodically monitored.

case 4) European orchids from seeds: various projects

The Red List of rare and endangered wild flora includes the whole family of Orchidaceae, particularly the terrestrial orchids. Unfortunately, these species do not germinate with conventional propagation techniques, and the development of wild orchid seedlings depends closely on infection by a mycorrhizal fungus. (Johansen & Rasmussen, 1992).

An interesting project has been carried out since 1996 in the Botanical Garden of Modena, concerning the *ex situ* propagation of Italian terrestrial orchids by *in vitro* asymbiotic seed germination (Sgarbi, 2002). These methodologies were applied to obtain *ex situ* collections of plants (Figure 10) that can be used to re-establish the orchid population in wild sites. Many plants, including *Serapias*, *Orchis* and *Ophrys* species,

obtained by *in vitro* culture techniques, have so far been cultivated *in vitro*. Some of these specimens could be reintroduced in natural protected areas in the same sites where seeds were collected and where the survival rate of the plants will be recorded. The seed treatments and the culture conditions applied resulted in high germination rates, particularly when sowing immature seeds. The main problem is currently transplanting the seedlings from *in vitro* nutrient media to compost in the pots.

The perspective is the use of this know how for germinating and growing other orchid species and the project also aims to involve the public and schools in the conservation of these endangered species and their habitats (Sgarbi et al., 2000, 2001, 2004).



Figure 9. Planting of *Senecio paludosus* in an ecological restoration area into Novellara and Reggiolo Valley.



Figure 10. Orchids plants grown *in vitro* by Laboratory of Micro-propagation and *in vitro* Culture of the Botanic Garden of Modena

case 5) Conservation of boreal and alpine species in the area of Mount Falterona (Tuscan - Emilian Apennine)

Significance activities of *ex situ/in situ* conservation is actually carried out by Botanic Garden of Modena in co-operation with the National Forestry Service in the Area of Mount Falterona (National Casentine Forest Park, Mount Falterona, Campigna), in order to increase the local endangered populations of boreal and alpine species whose southern distribution border is in this area. The goal of these projects – which are still in an early stage - is to strengthen natural populations by producing plantlets by seed in the Botanic Garden with the prospect of introducing them in their original places. The species considered so far are: *Hieracium villosum* L., *Saxifraga oppositifolia* L., *S. paniculata* Miller, *S. moschata* Wulfen, *Rynchosinapis cheirantos* (Vill.) Dandy (Sgarbi et al., 2007). In this phase, protocols for *in vitro* germination and cultivation had been studied. The National Forestry Territorial Office for Biodiversity – Pratovecchio (Arezzo) provided the Botanic Garden with several lots of seeds and the Botanic Garden now has access to plantlets for strengthening operations. This point is dealt with in detail in another paper in this issue.

#### DISCUSSION AND CONCLUSIONS

The cases illustrated underline a number of strong and critical points and can be classified into two different types: *in situ* interventions/ecosystem management and *ex situ* conservation practices.



Case 1 (*Marsilea* project) belongs substantially to the first type: greatest problems in these cases are related to harmonising conservation projects with social/economic requirements and anthropic activities. The experiences on the “Marsilea project” provided the Botanic Garden of Modena with a starting point in this sense and they confirmed the possibility – and the necessity – of undertaking a non-conflictual policy between Institutions serving different purposes. Although certain errors were committed, the experience has provided an important basis for cooperation with the Authority responsible for maintaining the irrigation canals and ditches in the local territory. These good relations with Land Reclamation Authority “Consortium of Burana, Leo, Scoltenna and Panaro Reclamation” allowed important results: 1) for many years, the Botanic Garden led monitoring on many hydro-hygrophilous species in a lot of local canals and ditches, even with the help of means and staff of the Consortium; 2) during this time, we had the chance to organise meetings with engineers/technicians and workers of the Consortium to put forward biodiversity conservation problems and to show pictures of threatened/rare hydro-hygrophilous species; 3) in the most recent period, the Consortium proposed the Botanic Garden a partnership on an experimental project of vegetation management, which is still in progress and that has involved over 100 km of banks (Dallai et al., 2006); 4) every year. During public cultural activities, the Botanic Garden and Consortium underline their partnerships in conservation projects through thematic exhibitions and other initiatives (Dallai & Garbari, 1998).

The other cases, more recent than the *Marsilea* project, regard the production of plantlets – generally by seed - in the Botanic Garden with a view to introducing them into the origin locations. For many years, Botanic Garden of Modena has played an active role in specialised micro-propagation activities, that agree to obtain a high percentage of germination, to cultivate a large lot of plants in small places and to obtain a scalar production that is very helpful to reintroduction programs, as is well known. However, significant difficulties often emerge during the outside acclimatisation phases and when plants are transferred into potting compost. In a Botanic Garden, holding a few specimens of a species in cultivation induced the known problems (Heywood, 1989) and the plantlets destined for restoration in nature would not be kept *ex situ* for long periods of time. For these reasons, our experiences confirm the strategic function – where possible - of “nursery field” near the site designated for introduction, where plants can live in semi-natural conditions (see cases 2, 3).

Although the Botanic Garden of Modena is focuses primarily on conservation projects involving regional flora, difficulties on *ex situ* cultivation persist with regard to species suited to particular and vulnerable habitats, such as aquatic ones. Furthermore, tested protocols for germination and growth for many wild species do not exist. It is important to stress that Botanic Garden projects should address four different aims: knowledge of the *in situ* floristic/ vegetation situation and threat reasons; study of *ex situ* germination and cultivation protocols; attempts to improve relations with Institutions and Administrative bodies involved in countryside management (this could constitute a critical point for many Botanic Gardens) and focusing attention on education programs for the public and schools and involving them in practical activities.

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