Preserving Rare and Endangered Calcific Steppe-Dwelling East-European Species ex situ

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Abstract. We have summed up the results of experimental efforts for preserving threatened and endangered calcific steppe-dwelling East-European species in the N V Tsitsin Main Botanical Garden of the Russian Academy of Sciences. The results of experiments carried out since 2009 on the preservation of rare and endangered calcephilic-steppe species of Eastern Europe, which require alternative methods of protection, including in connection with the suppression of their populations in modern reserves of Russia, are presented in the conditions of the RAS GBS. The previous experience and methods of organizing such collections and expositions are analyzed. The main factors that negatively affect the life cycle of species at our point of introduction are identified, among them are inappropriate cultural conditions and competition that determine the duration of the existence of samples, that is, their stability. In this regard, it is proposed to create a system of specialized rockeries that imitate the characteristic natural habitats of various calcephilic communities: outcrops of rocky limestone, outcrops of writing chalk or calcareous soils. The developed approaches made it possible to study ex situ the peculiarities of adaptation of more than 170 rare and stenotopic plant species, mainly difficult in terms of cultivation. As a result of observations during the research period, preliminary results of their cultivation were summed up and the following ratio of species in the groups of introduction resistance was revealed: unstable (8%), weakly resistant (15%), resistant (40%), highly resistant (37%), that is, positive results were obtained for almost 80% of species undergoing a full life cycle in Moscow. The research results can find practical application not only in the creation of such collections in botanical gardens, but also for the restoration of populations of protected species in anthropogenically disturbed natural habitats.

1. Introduction
In the early days of the Botanical Garden of the Russian Academy of Sciences when the collections of vegetation and phytogeographical exhibits were being developed, the special attention was given to steppe plants because this species group is highly diverse in terms of taxonomy, morphology and biology. Another reason is that the vegetation cover of steppes is highly sensitive to human impact. In many steppe regions fields and other types of agricultural land dominate over indigenous communities, resulting in loss of population of some species, especially stenotopic ones.

The theoretical background for these exhibits has been established by the ecological and historical method by M V Kultiasov [1] and ecological and phytocenotic method by N V Trulevich [2]. Meadow steppe and feather-grass steppe exhibits have been created; the cultivation method for these plant communities has been perfected; the efforts for preserving them have proven to be effective [3].
However cultivating calcific plants presented significant difficulties such as competition with habitat-forming species. Due to this, it was decided to create a specialized exhibit of calcific species based on the ecotron as an imitation of a natural habitat [4]. We made our best efforts to recreate the lithological structure and the mechanical texture of the soil typical for chalk exposures.

Despite the consistency and scientific validity of the methods used, they did not become effective, since to a greater extent they determined the theoretical orientation, first of all, to the selection of plants for introduction and were used in a small area, where it was not possible to recreate all the variety of necessary conditions for such a complex in terms of cultivation of a group of plants, therefore, to date, only a few resistant species have been obtained, which are the most environmentally plastic [6]. Adaptation of more demanding stenotopic taxa was unsuccessful, and subsequently the cultivation of such plants was considered inexpedient, and the first expositions of the Cretaceous and limestone regions of Central Russia have not survived to date. A similar experiment was carried out in the botanical garden of Moscow State University during the implementation of a large rock garden project, but even here the exposition was not preserved, while it was noted that the plants of the chalk slopes are as difficult to grow as the arctoalpine species.

For the fruitful development of this direction, it is important not only to take into account the errors of the first experiments in creating such landscape expositions in the largest botanical gardens of Russia: the N V Tsitsin Main Botanical Garden RAS and the botanical garden of Moscow State University, but also to understand what essential points in the organization of the process of plant adaptation were not taken into account earlier.

Therefore, it was decided to create a specialized system of expositions of calciphilous species. Its creation was based on the idea of constructing an ecoton as a model of natural habitat [6]. In this case, the main focus was on reconstructing the lithology and texture of the substrate typical of Cretaceous outcrops.

As part of the program for conserving biodiversity of calcific steppe-dwelling species ex situ, the Botanical Garden of the Russian Academy of Sciences launched in 2009 a special collection [5]. More than 50 acquisition missions were sent to Belgorod, Volgograd, Voronezh, Kursk and Samara regions and to the Republic of Crimea in order to sample seeds. In 2013 the Main Botanical Garden named after N V Tsitsin of the Russian Academy of Sciences and the Zhiguli Natural Reserve named after I I Sprygin signed an agreement on cooperative research of the current state and the genetic conservation ex situ of threatened and endangered flora species dwelling on Zhiguli Upland and subsequent selective repatriation [6].

2. Materials and Methods

More than 200 samples of seeds and living plants of calcific taxons were collected from the natural habitats for study ex situ. 172 of them (see the Table) are listed as rare and endangered and also included in some regional Endangered Species Lists of the European part of Russia. 25 of them are included in the Federal Endangered Species List (Red Book) [7].

The previous experience of cultivating this group of species had shown that the lithological and edaphic factors had been underestimated. These factors together with the challenging climate conditions of Moscow had usually led to damping off and rotting of most plants which subsequently perished and dropped from the collection [8, 9]. In order to adjust the weather conditions, a system of specialized ecotrons of the rockery type [4] has been created that replicated the natural habitats of calcific plants. The regional features of the calcific ecological and floristic complexes have been recreated in their respective exhibits, e.g. exposures of writing chalk stone and calcareous soils for the Central Russian Upland exhibit and lime rock for Zhiguli Upland and Southern Crimea flora exhibits. Such diversification was the key for cultivating plants in those collections. The Zhiguli exhibit was created by planting seeds on a sheer cliff face made of big limestone blocks. For the plants typically found on the Central Russian Upland an unusually steep elevation was made out of various calcareous soils. This approach to experimental site construction eliminated the risk of overdamping and enabled us to study the adaptation and phenology of plants without this inhibitory factor.
Since sampling was often conducted in protected areas, only few samples of each plant were taken (in some cases one or two), and it affected their resistance assessment. For introductory resistance assessment the scale by N V Trulevich has been used which classifies the species into four groups: non-resistant, semi-resistant, resistant and highly resistant [2].

Non-resistant species do not go through their full yearly cycle of sprout development; their rhythms are disrupted; their vitality worsens every year; they often wither away on early stages of ontogenesis or in the first years after planting. Their life expectancy is less than five years.

Semi-resistant species go through their yearly cycle of sprout development, but not every year; their vitality is weak as compared with the plants belonging to the same species in their natural habitats; the ontogenesis is more often accelerated and less often slow; their life form may significantly change; they do not reproduce on their own. Their life expectancy is 5–10 years.

Resistant species go through their full cycle of sprout development, their vitality is high, their rhythms are stable with slight shift in time as compared to their natural calendar; they are well-adapted to local weather conditions. In terms of productivity and size, the samples perform just as in their natural habitats or better. They preserve their natural life forms; their ontogenesis goes at the natural or slightly accelerated pace. They do not reproduce on their own and do not self-seed, but in most cases they respond well to artificial propagation. Their life expectancy is up to 20 years.

Highly resistant species share all the features of the resistant group and in addition they actively self-reproduce, self-seed, vegetatively regenerate and expand their range. Their life expectancy is more than 20 years.

Latin names of taxa are given according to the International Plant Names Index [13].

3. Results and Discussion

According to the introductory resistance assessment of all the calcific steppe-dwelling species in the collection (table 1), 13 of them were non-resistant (NR), 26 semi-resistant (SR), 69 resistant (R) and 64 highly resistant (HR). It should be noted that referring to some species as highly resistant is only anticipatory because the time span of the experiment is less than 20 years. However other evaluation criteria show the characteristics that may range these species among highly resistant.

Table 1. Introductory resistance of calcific steppe-dwelling species in the Botanical Garden of the Russian Academy of Sciences.

| Species | Ra | Species | Ra | Species | Ra |
|---------|----|---------|----|---------|----|
| *Androsace koso-poljanskii* Ovčz. | R | *Centareaea orientalis* L. | HR | *Linum flavum* L. | R |
| *Artemisia hololeuca* Bieb. ex Bess. | R | *Centareaea ruthenica* Lam. | R | *Linum hirsutum* L. | R |
| *Artemisia salsoloides* Willd. | R | *Cephalaria uralensis* (Murr.) Schrad. ex Roem. & Schult., | HR | *Linum nervosum* Waldst. & Kit. | R |
| *Asphodeline taurica* (Pall. ex M. Bieb.) Endl. | NR | *Chamecitisus austriacus* (L.) | HR | *Linum pallasianum* Schult. | SR |
| *Astragalus zingeri* Korsh. | HR | *Clausia aprica* (Seph.) Korn.-Tr. | HR | *Linum perenne* L. | HR |
| *Bulbocodium versicolor* (Ker-Gawler) Spreng. | R | *Cleistogenes squarrosa* (Trin.) Keng Czern., | HR | *Linum ucranicum* L. | R |
| *Cephalanthera rubra* (L.) Rich. | NR | *Clematis integrifolia* L. | SR | *Linum uralense* Juz. | SR |
| *Cotoneaster alaunicus* Golitsin | R | *Clematis lathyrfolia* Bess. ex Reichenb. | SR | *Lithospermum officinale* L. | HR |
| Species | Ra | Species | Ra | Species | Ra |
|---------|----|---------|----|---------|----|
| *Daphne cneorum L.* | HR | Convolvulus lineatus L. | SR | Melampyrum argyrocomum (Fisch. ex Ledeb.) K.- Pol. | NR |
| *Erucastrum cretaceum* Kotov | R | Crambe tataria Sebeok | R | Melica transsilvanica Schur | HR |
| *Genista tanaitica* P. Smirn. | R | Daphne sophia Kalen. | HR | Onosma montana Sm. | SR |
| *Globularia punctata* Lapeyr. | HR | Dianthus acicularis Fisch. ex Ledeb. | HR | Onosma simplicissima L. | SR |
| *Hedysarum grandiflorum* Pall. | R | Dianthus andrzejewskianum (Zapal.) Kulcz. | R | Ornithogalum kochii Parl. | |
| *Hedysarum ucrainicum* Kaschm. | R | Dianthus armeria L. | HR | Ornithogalum ponticum Zahar. | R |
| *Hyssopus cretaceus* Dubjan. | R | Dianthus eugeniae Kleop. | R | Orthanthella lutea (L.) A. Kern. | NR |
| *Iris aphylla* L. | HR | Dianthus pallens Sm. | R | Oites baschkirorum (Janisch.) Holub | |
| *Iris pumila* L. | HR | Dipotaxis cretacea Kotov | R | Oxytropis pilosa (L.) DC | R |
| *Koeleria sclerophylla* P.A. Smirn. | HR | Draba sibirica (Pall.) Thell. | HR | Paronychia capitata (L.) Lam. | NR |
| *Matthiola fragrans* Bunge., | R | Echinops ruthenicus Bieb. | R | Pedicularis kaufmanii Pinzg. | |
| *Paenio tenuifolia* L. | R | Echium russicum J. F. Gmel. | R | Pimpinella tragium Vill. | R |
| *Scrophularia cretacea* Fisch. ex Spreng. | R | Elytrigia pontica Soltok. | HR | Pinus pallasiana D. Don. | SR |
| *Silene cretacea* Fisch. ex Spreng. | SR | Ephedra distachya L. | HR | Plantago maritima L. | SR |
| *Stipa dasyphylla* (Lindem.) Trautv. | HR | Eremogone micradenia (P.A. Smirn.) Ikonn | HR | Polygala cretacea Kotov | R |
| *Stipa pennata* L. | HR | Erysimum canescens Roth. | HR | Polygala sibirica L. | R |
| *Stipa pulcherrima* C. Koch | HR | Euphorbia seguieriana Neck. | R | Prunella grandiflora (L.) Scholl. | HR |
| Aconitum nemorosum L. | R | Galatella angustissima (Tausch) Novopokr. | R | Psathyrostachys juncea (Fisch.) Nevski | HR |
| Adonis vernalis L. | R | Galatella biflora (L.) Ness | HR | Pulsatilla patens (L.) Mill. | R |
| Adonis wolgensis Stev. | R | Galatella dracunculoides (Lam.) Nees | HR | Reseda lutea L. | HR |
| Agropyron cristatum (L.) Beauv. | HR | Galatella linosyris (L.) Reichenb. fil. | HR | Rosa porrectidens Chrshan. & M. Pop. | R |
| Species | Ra | Species | Ra | Species | Ra |
|---------|----|---------|----|---------|----|
| Ajuga chia Schreb. | R | Galatella villosa (L.) Reichenb. | R | Rosa rubiginosa L. | R |
| Ajuga laxmannii (L.) Benth. | R | Galium humifusum M. Bieb. | HR | Salvia nutans L. | R |
| Allium flavescens Bess. | R | Galium verum L. | HR | Salvia verciillata L. | HR |
| Allium globosum M. Bieb. ex Redoute | HR | Genista tinctoria L. | HR | Scabiosa ucranica L. | NR |
| Allium inaequale Janka | SR | Gentiana cruciata L. | R | Schivereckia podolica Besser. Andrz. ex DC. | SR |
| Allium paczoskianum Tuzson | SR | Gypsophila altissima L. | HR | Scorzonera mollis M. Bieb. | NR |
| Allium sphaerocephalon L. | HR | Gypsophila juzepeczukii Ilkon. | HR | Scorcellaria orientalis L. | R |
| Alyssum tortuosum Waldst. & Kit. ex Willd. | R | Haplophyllum suaveolens (DC.) G. Don fil. | SR | Scorcellaria supina L. | HR |
| Anthemis tinctoria L. | HR | Helianthemum canum (L.) Hornem. | HR | Scutellaria radiata (Waldst. & Kit.) M. Bieb. | R |
| Anthericum ramosum L. | R | Helianthemum nummularium (L.) Mill. | HR | Sideritis montana L. | R |
| Artemisia maritima L. | SR | Helianthemum rupifragum A. Kerner | HR | Silene chlorantha (Willd.) Ehrh. | SR |
| Artemisia nutans Willd. | SR | Helianthemum zheguliensie Juz. ex Tzvel. | HR | Silene supina Bieb. | HR |
| Artemisia santonica Web. | R | Helichrysum arenarium (L.) Moench. | R | Stipa capillata L. | HR |
| Artemisia sericea Web. ex Stechm. | R | Helicotrichon desertorum (Less.) Nevski | R | Stipa lessingiana Trin. | SR |
| Asparagus officinalis L. | HR | Hylotelephium maximum (L.) Holub | HR | Stipa tirs Steven | R |
| Asperula petraea V. Krecz. ex Klok. | HR | Hylotelephium zheguliense Tzvel. | HR | Teucrium chamaedrys L. | R |
| Asperula tephrocarpa Czern. ex M. Pop. & Chrshan. | R | Inula ensifolia L. | R | Teucrium polium L. | R |
| Aster amellus L. | HR | Iris arenaria Waldst. & Kit, | SR | Thesium arvense Horvatovszky | SR |
| Astragalus albicaulis DC. | R | Iris halophila Pall. | HR | Thymelaea passerina (L.) Coss. & Germ. | SR |
| Astragalus austriacus Jacq. | SR | Jasminum fruticans L. | HR | Thymus cretaceus Klokov | R |
| Astragalus helmii Fisch. | SR | Juniperus oxycedrus L. | NR | Thymus zheguliensis Kloko et Shost. | R |
As shown in the chart (figure 1), most of the assessed species are resistant (37%) and highly resistant (40%). The proportion of non-resistant and semi-resistant species is low and totals to 23%.

About half of the non-resistant species are Scrophulariaceae and xerophytic species of other families.

The semi-resistant group mainly consists of perennial xerophytes. Shrubs and sub-shrubs constitute about 20% of the resistant group; perennial rhizome plants share the same proportion.

1/3 of the highly resistant group is roughly equally shared between sub-shrubs and Poaceae family.

Figure 1. Proportions of calcific steppe-dwelling species by their introductory resistance.
has shown better survivability and further development of samples, e.g. this method for preservation ex situ has proven effective for over 90% of rare plants of Zhiguli. On the other side, using soft calcareous soils led to less resistance (85%) of species that grow on such soils in their natural habitats.

4. Conclusion

The manmade ecotron imitating limestone and chalk exposures of the European part of Russia enables us to dramatically increase the biodiversity of rare calcific and steppe-dwelling species cultivated in the N V Tsitsin Main Botanical Garden of the Russian Academy of Sciences and to map possible ways to restore the populations of threatened taxons. This novel, unconventional and effective approach to cultivation of rare calcific steppe-dwelling taxa introduced in the Botanical Garden have proven effective for almost 80% plants, which offers good prospects for further study of resistance of maladaptive species.

The ways of restoring the number of endangered taxa in some reserves are outlined. Such a practical result may be the repatriation of some of the few in the I I Sprygin Zhiguli State Reserve species: Astragalus zingeri Korsh., Dianthus acicularis Fisch. ex Ledeb., Helianthemum zheguliense Juz. ex N.N. Tzvelev, Iris pumila L., Koeleria sclerophylla P.A. Smirn. and others successfully propagated in the culture of N V Tsitsin Main Botanical Garden.

Particularly remarkable results of cultivation were obtained in a number of representatives of the Red Book of the Russian Federation, among which the vast majority are obligate calcephiles. Previously, long-term preservation of such plants in culture was not possible, since the methods of cultivation used did not allow achieving their stability.

In general, thanks to non-standard and effective methods of cultivation of rare calcephilic-steppe taxa, first used under N V Tsitsin Main Botanical Garden conditions, the experience of their conservation has become successful for almost 80% of plants, which indicates the prospects for further studies of the resistance of other species difficult in terms of adaptation and have not yet been tested in similar experiments.

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