REVIEW OF METHODS FROM AGRICULTURAL AND HORTICULTURAL PRACTICE USED IN PROJECTS RELATED TO THE RESTORATION OF NATURAL HABITATS

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Abstract

Review of methods from agricultural and horticultural practice used in projects related to the restoration of natural habitats. Ecological restoration supports the reconstruction of degraded, damaged or destroyed natural habitats. Restoration activities lead to an increased demand for seeds or seedlings of plant species characteristic of different habitats. This creates a demand that cannot be met by the supply of wild plant seeds.

In order to ensure sufficient quantities of seeds for restoration activities, methods typical of agricultural production are increasingly applied. Often, seeds intended for ecological restoration are obtained under natural conditions from natural populations that reflect the genetic diversity of the given species. Obtaining seeds in this way is a labour-intensive and expensive process and does not meet the requirements for large-scale restoration activities required by practice.

In this case, to improve seed production and to reduce costs, different wild seed production systems are integrated that are based on agricultural or horticultural production methods (Pedrini et al. 2020), providing a new intersection between habitat restoration and agriculture. Both the classic selection methods and the methods of modern selection science are applied in the restoration practice. Restoration ecology in its application combines selection, agricultural seed production and plant physiology. Some of the reviewed methods were applied in practice during the implementation of a project related to the restoration of natural habitat 62C0 Ponto-Sarmatian steppes in Bulgaria.*

***Key words:** Ecological restoration, habitats, methods, seed*

INTRODUCTION

Restoring damaged ecosystems is an efficient and cost-effective way for people to address the most pressing challenges that humanity faces today. The biggest global initiative to protect ecosystems was announced in 2019 – it is the "Decade on Ecosystem Restoration" 2021-2030. The Decade on restoration activities is a call for all practitioners, scientists, local communities and other stakeholders to work together to reverse the existing trend of ecosystem degradation and improve ecological integrity for future generations (Valderrábano et al., 2021, UNEP/FAO Factsheet June 2020).

The term "Habitat Restoration" refers to the manipulation of the physical, chemical, or biological characteristics of a site in order to return most of its natural functions (Meine, 2018). According to Meine, this term also includes aspects related to various restoration activities, including: activities that restore the habitat to a condition closest to that in which it was before its degradation; activities that restore one or more of the original functions of the habitat; and activities that remove disturbance or degradation factors until the habitat is fully functional. The publication “International Principles & Standards for the Practice of Ecological

Restoration" (Gann et al., 2019) describes in detail three main approaches to habitat restoration: a) Natural regeneration b) Assisted regeneration c) Reconstruction.

When restoration activities are carried out in natural habitats, plants are the main focus of the work, and the process aims to create plant populations that: establish on the designated sites; reproduce; function normally; and are sustainable. Large scale restoration requires an agronomic approach integrated with restoration ecology to increase native plant resources, propagate and restore population numbers and vitality. On the other hand, the agronomic approach leads, to some extent, to the use of artificial selection and breeding, which allow for modification and interference with the gene pool of wild populations (Erin et al., 2016).

With intensive breeding work, in the near future, ecologically suitable plant material could emerge that has increased stress resistance, competitiveness with invasive and weedy species, and broad ecological plasticity (Jones, 2013). As a result of human activity, ecological challenges are growing and classical restoration is becoming increasingly difficult (Hobbs et al. 2009).

MATERIAL AND METHODS

During the implementation of habitat restoration activities, methods typical for agriculture are used with great success, and various methods from plant breeding, seed production, seed approbation and herbology are easily applied.

The methods used for restoration must be easy to implement, technically feasible, socially acceptable and suitable for use in different places and situations. Agricultural methods, in their majority, meet these requirements.

This study describes key methods inherent to agricultural practice applied in the implementation of restoration activities in the natural habitat 62C0* "Ponto-Sarmatian Steppes" in the Republic of Bulgaria within the project "Restoration of priority natural habitat 62CO* "Ponto-Sarmatian Steppes" in the Kaliakra region". Included in the study are: a method of vegetative propagation of plant species characteristic of the habitat, collecting seeds from wild plants that have a structurally defining function in the habitat, and cutting hay from a representative portion of the habitat and laying it on damaged areas intended for restoration.

The natural habitat itself includes diverse petrophytic calciphilous communities belonging to the *Pimpinello-Thymion zigoidii* alliance, the order *Festucetalia valesiacae* and the class *Festuco-Brometea* (Kavrakova et al.2009). The habitat is important for the conservation of rare and endangered plants, some of which dominate the composition of steppe cenoses. This natural habitat has a limited distribution and is subject to constant negative anthropogenic influences. Within the territory of the Republic of Bulgaria, the natural habitat 62C0* "Ponto-Sarmatian steppes" is divided into 2 subtypes (Кавръкова, и кол. 2009): A) Western Pontic Wormwood steppes) and B) Western Pontic feather grass steppes, whose characteristics can be seen in Table 1.

From the table we can see that the typical species for subtype A) are 14, for subtype B) are 13, their total number is 27 and they belong to 16 families (*Brassicaceae, Asteraceae, Fabaceae, Amaranthaceae, Linaceae, Lamiaceae, Ephedraceae, Lamiaceae, Plumbaginaceae, Poaceae, Caryophyllaceae, Convolvulaceae, Cistaceae, Rosaceae, Paeoniaceae, Iridaceae*). The plant species listed in **Table 1** have different development cycles, they include both annual species (e.g. *Avena eriantha*) and perennial species (e.g. *Thymus zygioides*), all reproduce by seed, some of them have potential for vegetative reproduction, which has been preliminarily demonstrated.

The determination of the plant species was carried out with “Key to the native and foreign vascular plants in Bulgaria” (Stoyanov, 2021) and “Flora of the Republic of Bulgaria” (Yordanov, 1979).

Table 1. Plant diversity and subtypes of natural habitat 62C0* "Ponto-Sarmatian steppes" in the Republic of Bulgaria

Type	A) Western Pontic Wormwood steppes	B) Western Pontic feather grass steppes
Association	*Alysso caliacrae-Artemisietum lerchianae*	*Paeonio tenuifoliae – Koelerietum brevis*
Distribution	- limited - on steep slopes; landslide terraces above the sea; - on Miocene (Sarmatian) limestones and clays; - only in the area between the village of Kranevo and the town of Kavarna;	- leveled terrain with soil cover; - on limestone rocks, (carbonate chernozems or renzins), on Sarmatian limestones; - along the edge of the Dobrudja Plateau;
Typical species	*Alyssum caliacrae; Artemisia lerchiana; Agropyron cristatum subsp. brandzae; Aster oleifolius; Astragalus glaucus; Astragalus spruneri; Jurinea stoechadifolia; Kochia prostrata; Linum austriacum; Thymus zygioides; Ephedra distachya; Nepeta parviflora; Matthiola odoratissima; Goniolimon besserianum;*	*Achillea clypeolata; Koeleria brevis; Artemisia pedemontana; Cerastium bulgaricum; Chamaecytisus jankae; Convolvulus cantabrica; Helianthemum salicifolium; Potentilla bornmuelleri; Stipa lessingiana; Stipa ucrainica; Paeonia tenuifolia; Iris pumila; Avena eriantha;*

DISCUSSION

Reproduction is the ability of all living organisms to reproduce. In plant species, it occurs by seed and by vegetative means and can be carried out under both natural (outdoor) and controlled laboratory conditions.

The methods used are divided into two groups: those applied outdoors and those applied under laboratory conditions.Outdoor methods include: vegetative propagation, seed collection from wild populations, various seed production methods, hay cutting methods, determining soil seed bank content, mulching, soil transfer (from other locations). Laboratory methods include seed quality testing under laboratory conditions and micropropagation under *in vitro* conditions.

Out of the listed methods, typical for agricultural production and horticulture in real conditions of implementation of restoration activities in Ponto-Sarmatian steppes, all listed methods were included, without micropropagation under *in vitro* conditions. Three key methods are included in this review: vegetative propagation, seed collection and hay cutting and laying.

1.Vegetative propagation

Vegetative propagation of plants is a method of great scientific and practical application. This method is based on the ability of plants for complete regeneration of an individual from its organs and from parts of its organs (Sendor et Estrada, 2007). This ability of plant organisms has been traditionally used in crop production, viticulture, fruit growing, floriculture, horticulture, forestry, etc.

Before applying vegetative propagation, it is important to carry out botanical species selection. Plants that are donors of cuttings must correspond to the specific species, be collected from different populations and from different locations, which ensures genetic diversity. It is not necessary to purposely search for certain traits in order not to point the selection in a particular direction. No direct information on vegetative reproduction of typical habitat plant species listed in Table 1 was found, yet this study provided a good start to initiate such a study. The next step before proceeding to vegetative propagation consists of preparing an indicative list of typical perennial species occurring in the 62C0* "Ponto-Sarmatian steppes" habitat to be subjected to vegetative propagation. The list includes 8 species from 3 families described in **Table 2**.

Table 2. Typical species, from the habitat "Ponto-Sarmatian steppes", to study their potential for vegetative propagation.

Family	Latin name
Asteraceae	*Artemisia lerchiana*
	Artemisia pedemontana
	Aster olefolius
	Jurinea stoechadifolia
Fabaceae	*Astragalus glaucus*
	Astragalus spruneri
	Chamaecytisus jankae
Lamiaceae	*Thymus zygoides*

Of the 8 characteristic species listed in Table 2, 4 have been successfully propagated, including *Artemisia lerchiana, Jurinea stoechadifolia, Astragalus glaucus* and *Thymus zygoides,* and research in this field is ongoing. All the rooted plants were reintroduced in micro experimental sites and in the field intended for the implementation of restoration activities.

The application of the vegetative propagation method does not require the use of special expensive equipment or difficult to implement techniques. The advantage of vegetative propagation is the production of a large number of living rooted plants ready for reintroduction into damaged habitats. The plants obtained by vegetative propagation are identical copies of the original parent plants. In order to maintain genetic diversity during vegetative propagation, it is necessary to have at least 50 donor plants from different populations in the habitat. (Albrecht and Maschinski, 2012). Depending on the maturity of the plants, 3 types of cuttings are used in practice: mature, semi-mature and green (Stoltz et Strang 2004, Landis et all. 2014). Rooting can be done in open areas, in growing containers and in greenhouses, on different substrates, including substrate collected from the habitat. For the purpose of the restoration activities in the case of the "Ponto-Sarmatian Steppes", we mainly work with mature and green cuttings of the plant species described in Table 2. Successful rooting of cuttings was obtained for species representing all three families on substrate taken from the habitat. All the plants obtained after rooting were successfully reintroduced on micro plots and on site where active restoration work is taking place. The process of reintroducing plants into damaged habitats combines the theory and practice of horticulture, ecology and evolution (Guerrant 1996, Maschinski et Albrecht, 2017). In Figure 1, cuttings of habitat-specific plants can be seen, which were subsequently rooted, planted and adapted under *ex situ* conditions in a damaged area intended for restoration.

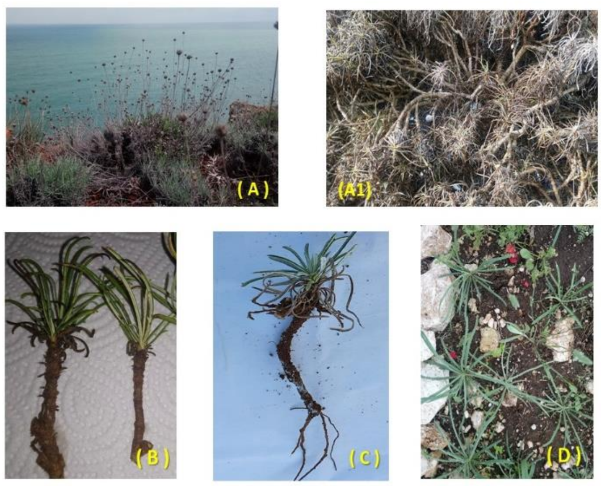

Figure 1. *Jurinea stoechadifolia*: A) et A1) plant in nature, (B) cutting, (C) rooted cutting, (D) adapted plant.

2.Seed collection

The scientific approach applied in restoration activities is increasingly multidisciplinary, a consequence of which is the massive transfer of agricultural technologies based on work with seeds. In applied habitat restoration, the difference between working with agricultural seeds and wild seeds is that in the case of working with wild seeds (including collecting them): no targeted breeding work has to be conducted (Jones, 2009); the physical and physiological requirements of the seeds of each species used must be taken into account, and an appropriate or similar methodology for testing them must be pursued, (Edrisi and Abhilash, 2021). In general, these are plants for which little laboratory and practical information has been collected. Seeds play a critical role in the ecology of *ex situ* conservation and restoration (Miller et al., 2017; Kildisheva et al., 2020).

There are various possibilities to obtain the necessary characteristic /typical/ seeds for restoration activities. These are described by (Dorner J., 2002) and include:

- Classical (manual) collection of seeds typical for the habitat;
- Use of hay collected clear of weedy and invasive species, derived from meadows and pastures having typical habitat characteristics;
- Use of donor soil from the natural habitat or use of soil from digging and construction activities taking place in the same habitat;
- Purchase or production of typical seeds of species forming the habitat.

Classical (manual) collection of seeds

Manual seed collection is an important part of habitat restoration, but it does have its advantages and disadvantages. As an advantage it is noted that manual collection guarantees authenticity and species purity of the seeds, while a disadvantage may be the insufficient time to collect the mature seeds, especially if large quantities are required (Dorner J., 2002), and the high value of the seeds obtained in this way (Knight and Overbeck, 2021). Janet Dorner, in her 2002 publication „An introduction to using native plants in restoration projects“ formulated principles to follow when collecting seeds. In the implementation of the restoration project in the Natural Habitat 62C0* “Ponto-Sarmatian steppes", these principles have been followed, as listed in **Table 3**. **Figure 2** shows manually collected seeds of plant species typical for natural habitat 62C0*: *Linum austriacum; Koeleria brevis; Iris pumila; Avena eriantha;*

Figure 2. Hand-collected seeds of 62C0* plant species typical of the natural habitat: (A) *Avena eriantha; (B) Linum austriacum; (C) Iris pumila; (D) Koeleria brevis*

Based on the plant species typical for the habitat 62C0* "Ponto-Sarmatian steppes", the following list of plants suitable for seed collection was prepared: *Achillea clipeolata, Agropyron cristatum subsp. Brandzae (Agropyron brandzae, Artemisia lerchiana, Artemisia pedemontana, Astragalus glaucus, Astragalus spruneri, Avena eriantha, Chamaecytisus jankae, Convolvulus cantabrica, Galatella villosa (Aster oleifolius), Helianthemum salicifolium, Iris pumila, Koeleria brevis, Linum austriacum, Paeonia tenuifolia, Stipa lessingiana, Thymus zygioides.* Seeds of 17 plant species were collected, including annual and perennial species. In the process, the principles of wild species seed collection were followed, which are described in **Table 3**.

A major disadvantage of working with wild seeds is that for many of them there are no established standardised testing methodologies (germination, germination energy). The failings with regard to wild plant seed handling are to some extent being remedied by The International Seed Testing Association (ISTA), which provides testing in independent laboratories where wild plant seeds can be tested using standardised methodologies.

Table 3. Principles of work in the collection of seeds, from wild species, intended for the implementation of restoration activities (prepared according to Dorner J., 2002)

Principles of work in the collection of seeds from wild species.	**Reasons**
Comparing seed donor location and restoration site: landscape: slope, hydrology, soil, temperature, sunshine, altitude, etc.Vander Mijnsbrugge et al., 2010	Plants are adapted to the conditions, there is a high probability of adaptation to a new place.
Seeds are collected in an area near a planting site.	Native plants adapt more easily to the planting site.
No seeds are collected in highly sensitive and protected areas.	Protection of populations.
Do not collect seeds from or near ornamental plants.	Decorative varieties have no adaptation to the place of planting.
Do not collect seeds from weedy areas.	Prevents mixing with weed seeds.
Dry seeds should be collected on days without rain	Seeds with high humidity lose their vitality quickly, unlike dry seeds.
Only ripe seeds are collected.	Increases germination.
Using paper bags or other "breathable" containers to store dry seeds.	The seeds dry faster and retain their viability longer.
Seeds are collected only from large populations.	Genetic diversity is promoted and inbreeding depression is avoided.
Seeds to be collected from different microhabitats within the habitat.	Genetic diversity is promoted and inbreeding depression is avoided.
Determining the factors that affect the viability of seeds before their collection and processing.	Short-lived seeds should be planted immediately after collection.
Collecting a small number of seeds from many plants rather than many seeds from a small number of plants.	Genetic diversity is supported.
Collecting seeds of different phenotypes of a species: short, tall, multi-seeded, low-seeded, etc.	Genetic diversity is supported.
Leave at least 2/3 of the available seeds.	Protection of natural populations
Conservation of seed donor populations	Protection of natural populations
After collection, the seeds are cleaned and dried.	Dry and clean seeds have higher vitality.

3.Using hay

Hay collected from a representative sample of a habitat offers a **"bouquet"** of seeds in its composition. It does not have an exact ratio between the seeds of the different plant species included in its composition (Galatowitsch et van der Valko, 1994). Hay collected without weed and invasive species admixtures, obtained from meadows and pastures, has the typical characteristics of a given habitat.

The technology of working with hay as a seed source permits cutting from representative parts of the habitat at a time when the seeds are in the waxy maturity phase and can complete their maturation after cutting. Like seed, hay is also valued by (Knight et Overbeck, 2021), but its cost is not as high. Its low cost compared to other seed sourcing options is the reason it is widely used in habitat restoration projects. The use of hay as a seed donor has its advantages and disadvantages, as well as principles for cutting and collecting hay that are highlighted in **Table 4**.

Table 4. Good practices, advantages and disadvantages of hay, to be used as a seed source in habitat restoration projects

Good practices	**Reasons**
Determining suitable places to collect hay.	Sites must correspond to a specific habitat and characteristic species composition.
The hay is mowed and harvested free of weeds and invasive species.	Invasive and weedy species create problems at the restoration site.
Hay is harvested when the seeds of the habitat-specific species are ripe but not dispersed.	Difficulties in logistics due to the large geographical distance between the donor and recipient sites of hay.
Hay is collected in small areas at different times, for a variety of seeds of different characteristic types.	The seeds of different species mature at different times.
When mowing, leave 30% unmowed area of the donor sites to maintain the habitat.	Donor sites should maintain an optimal species composition.
Making phenological observations related to the flowering and ripening of typical species, which is a guarantee that the hay is harvested at the optimal time.	Phenological observations provide information on the phases that plants go through
Advantages of using hay	**Disadvantages of using hay**
Harvesting hay provides a "bouquet" of seeds of plant species.	Hay takes up large volumes, or must be baled at appropriate humidity
Hay provides an abundance of seeds from typical plant species with different genotypes.	Some of the plant species may be missed due to very late or early flowering and seed formation.
Once collected, the hay can be transported and spread over the site prepared for restoration.	Some external factors (extreme rainfall, extreme temperatures, lack of pollinators) can cause not enough seeds in the hay.
Hay is suitable both for large-scale restoration	Different time of maturation of the seeds of the typical

projects on large areas and for restoration of smaller areas.	species
It has a lower cost compared to other recovery options.	

Within the project for restoration of natural habitat 62C0* "Ponto-Sarmatian steppes" the method of hay cutting from a representative part of the natural habitat was used. In the hay cut from a representative section of the habitat, typical species were found, such as: *Artemisia lerchiana; Agropyron cristatum subsp. brandzae; Aster oleifolius; Astragalus glaucus; Linum austriacum; Achillea clypeolata; Koeleria brevis; Artemisia pedemontana; Chamaecytisus jankae; Convolvulus cantabrica; Potentilla bornmuelleri; Stipa lessingiana; Iris pumila; Avena eriantha;*

Figure 3. Laying of dried hay from a representative part of the habitat on an area intended for restoration.

Cutting was carried out over an area of 24ha, the hay collected was used to lay over a 1.4ha restoration area. Cutting was carried out in several stages in different months of the year and in 2 consecutive years in order to saturate the site with seeds of typical species, and to cover as many typical species of the habitat as possible. Cutting was adapted to the maturation periods of the different species. Part of the work carried out can be seen in **Figure 3**.

CONCLUSIONS

A general conclusion that could be made from this review of the agricultural methods used in implementing restoration activities in natural habitats is that a multidisciplinary approach and integration between agriculture and ecological restoration is possible. The genesis of the methods used is irrelevant, as long as they work well, are relatively inexpensive and are easy to apply to a variety of habitat restoration cases. Each of the methods listed has its place in the practice of a wide range of restoration activities, having its own positives and negatives.

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