In situ and *ex situ* conservation of the endangered plant species *Artemisia laciniata* (Asteraceae) in Austria

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The aims of the present project were to reinforce the existing population of *Artemisia laciniata* (Asteraceae) in Austria's Neusiedler See – Seewinkel National Park (NP) and to implement *ex situ* conservation measures to support this effort. The plants growing in the slightly saline meadows of the NP represent the last remaining population of this critically endangered species in Europe. In 2015, only 11 individuals were known to exist.

Monitoring of the wild population was conducted, and early observations identified the mowing regime as a critical factor for the species' survival. The mowing management was optimised based on the artificial mowing experiments performed in garden beds at the University of Vienna. The results showed that cutting the plants back to 80 mm in mid-June could lead to about 25 % reduction in the number of plants developing generative stems and a 75 % decrease in the number of generative stems compared to the control group.

The attempt to activate the soil seed bank in an extinct field site was unsuccessful. However, sowing achenes and planting pot plants proved to be effective reinforcement measures. The survival rate of plantings reached up to 95 % after five years, and seedlings from achenes had a 20 % survival rate. However, the success was highly dependent on the location and its microhabitat.

By 2023 the *Artemisia laciniata* population has been stabilised with more than 270 plants on site, and achenes collected over three years are stored at the Millennium Seed Bank. While reintroduction protocols are now in place and the measures appear promising, it is important to continue monitoring to evaluate the long-term success of the plantings, particularly in light of global warming.

Kodym A, Pirker T, Kiehn M, Klaps M, Knickmann B, Schumacher F (2026) *In-situ-* und *Ex-situ-*Erhaltung der gefährdeten Pflanzenart *Artemisia laciniata* (Asteraceae)

Ziel des Projekts war die Stärkung der bestehenden Population von *Artemisia laciniata* (Asteraceae) im österreichischen Nationalpark (NP) Neusiedler See – Seewinkel und die Durchführung von *Ex-situ-*Erhaltungsmaßnahmen zur Unterstützung dieser Bemühungen. Die Pflanzen, die in den leicht salzhaltigen Wiesen der Zitzmannsdorfer Wiesen wachsen, stellen die letzte verbleibende Population dieser stark gefährdeten Art in Europa dar. Im Jahr 2015 waren nur noch 11 Exemplare bekannt.

Es wurde ein Monitoring der Wildpopulationen durchgeführt, und erste Beobachtungen ergaben, dass das Mähregime ein kritischer Faktor für das Überleben der Art ist. Das Mähmanagement wurde optimiert, basierend auf den künstlichen Mähexperimenten in Gartenbeeten an der Universität Wien. Die Ergebnisse zeigten, dass ein Rückschnitt der Pflanzen auf 80 mm Mitte Juni zu einer ca. 25 prozentigen Verringerung der Anzahl der Pflanzen, die Blütenstände entwickeln, und zu einer Verringerung der Anzahl der Blütenstände um 75 % im Vergleich zur Kontrollgruppe führt.

Der Versuch, die Samenbank im Boden an einem erloschenen Standort zu aktivieren, blieb erfolglos. Die Aussaat von Achänen und das Ausbringen von herangezogenen Topfpflanzen erwiesen sich jedoch als wirksame Maßnahmen zur Verbesserung der Populationsgröße. Die Überlebensrate der Anpflanzungen war nach fünf Jahren bis zu 95 %, während die Überlebensrate der Sämlinge aus Achänen bei 20 % lag. Der Erfolg hing jedoch stark vom Standort und seinem Mikrohabitat ab.

Bis 2023 konnte die Population von *Artemisia laciniata* mit mehr als 270 Pflanzen vor Ort stabilisiert werden, und die über drei Jahre gesammelten Achänen sind in der Millennium Seed Bank eingelagert. Obwohl nun die entsprechenden Protokolle vorliegen und die Maßnahmen vielversprechend erscheinen, ist es wichtig, das Monitoring fortzusetzen, um den langfristigen Erfolg der Anpflanzungen zu bewerten, insbesondere im Hinblick auf die Klimaerwärmung.

Keywords: conservation measures, Millennium Seed Bank, monitoring, mowing regime, national park, reinforcement.

Received: 2025 04 28

DOI: https://doi.org/10.25365/azba.162.21

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Introduction

Artemisia laciniata Willd. (Siberian wormwood) is a deciduous semi-rosette perennial with a growth height of 15 to 50 cm (with the generative stems). The generative stems are upright and carry numerous inflorescences. The main distribution area of *A. laciniata* is Central Asia (Fischer & Fally 2006), where it is common (Ellmauer 2005). In this part of the world, it occurs as a frequent companion of fresh, herbaceous larch forests or in aspen and birch forests, but also in meadows and meadow steppes (Jäger 1987).

The last natural occurrence of *A. laciniata* in Europe is found in Austria in the Zitzmannsdorf meadows at the Neusiedler See – Seewinkel National Park (Burgenland) (Kästner & Fischer 2011). The Zitzmannsdorf meadows have been part of the preservation zone of the NP since 1993 and are the largest contiguous meadow area in Burgenland, covering about 400 ha (Naturschutzbund Burgenland 2020). According to Fischer & Fally (2006), the nearest occurrence of *A. laciniata* to Austria is in southern Russia. In Europe, as well as in Austria, the status "critically endangered" applies (Niklfeld & Schratt-Ehrendorfer 1999; European Commission 2011; Schratt-Ehrendorfer et al. 2022). *A. laciniata* is listed as a priority species in Annex II and IV of the Habitats Directive (Council Directive 92/43/EEC 1992).

In the past, a second Austrian population was situated in Lassee (Lower Austria), but it became extinct in the 1960s. German populations in Saxony-Anhalt and Thuringia existed until around 1900 (Jäger 1987). The causes of extinction in Germany and Austria were probably habitat destruction and change, e.g. drainage, but also the collection of herbarium specimens seem to have contributed to the decline (Wendelberger 1959; Jäger 1987).

The Austrian population grows in a weakly saline meadow (Fischer et al. 2008; Kästner & Fischer 2011) where the reduced competitive pressure due to salinity may have allowed the species to persist until today. The mowing regime of the meadows plays a key role in conservation efforts. As *A. laciniata* flowers from August to October (Hegi 1929; Jäger 1987; Fischer et al. 2008; Kästner & Fischer 2011), mowing must take place before the plants develop generative stems. In late autumn and winter, the meadows are too wet for bringing in mowing machinery. The window for mowing is further constrained by the fact that the meadows are an Important Bird Area (IBA) and protected as breeding grounds in springtime.

Over the previous years, protocols for the *in vitro* propagation and cryopreservation of *A. laciniata* have been developed (Kodym et al. 2018) and a population genetics study was conducted by the Research Group Plant Ecological Genomics of the Department of Botany and Biodiversity Research at the University of Vienna (Ovidiu Paun, unpublished data). This study showed that the population on the Zitzmannsdorf meadows, despite the low number of individuals, has a low inbreeding factor and high genetic diversity.

The aims of the present project were to maintain and reinforce the wild population of *A. laciniata* and to implement *ex situ* conservation measures. Since 2015 various conservation efforts have been carried out, with the main activities outlined in this paper: (a) monitoring of the natural population, (b) population expansion by sowing achenes and by planting, (c) attempts to activate the soil seed bank, (d) establishment of a mowing management, (e) expanding the living collection, and (f) storing seeds in the Millennium Seed Bank.

Materials & Methods

Monitoring of wild population

In 2015, at the onset of this project, only eleven individuals of *A. laciniat*a were known at the original site, which covered an approximate area of 200 m². These plants are thus referred to as the "original stand", as opposed to the new stand which was discovered only in 2018 (Fig. 1). All plants of the wild stands were labelled with numbered galvanised steel screws in the ground, in order to allow relocating with a metal detector. This was essential to quickly find and clearly identify individual plants in the field. The individual plants were additionally mapped using a GPS (SEPTENTRIO "Altus NR2") with a position accuracy of less than 20 mm horizontally as well as vertically. New plant discoveries in following years have been marked and recorded in the same way. During regular monitoring, a GPS device (GARMIN Oregon 600) with positional accuracy ± 3 m was used to locate the sites.

The meadow surrounding the *Artemisia* plants was excluded from the farmer's mowing activities, which happened once a year around June. However, the vegetation around the plants were cut with different tools including brush cutters, lawn mowers, grass clippers and a Stanley knife.

Vegetation surveys of the original stand, the new stand, the 8 Z plots, the *in vitro* 2018 site and the seed bank mound were carried out in 2017 or 2022. (Fig. 1) (Arge Vegetationsökologie und Landschaftsplanung, AVL, 1060 Vienna, Austria). The species nomenclature was based on Fischer et al. (2008) and the vegetation units on Mucina et al. (1993). The weather data for the nearest monitoring station in Podersdorf am See was retrieved from GeoSphere, Austria (https://data.hub.geosphere.at).

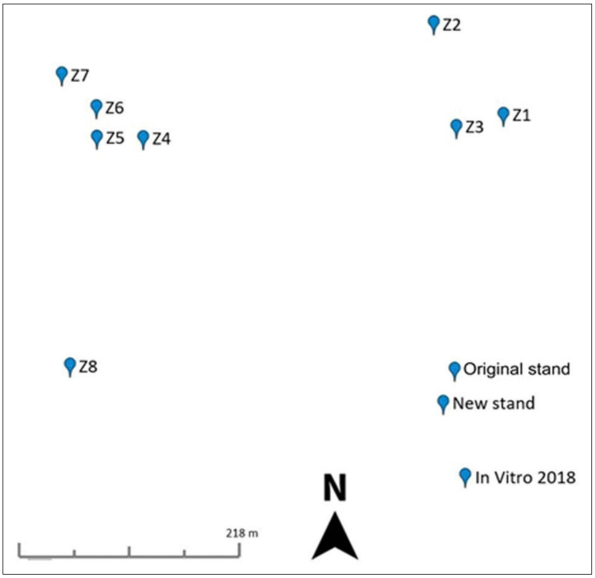

Fig. 1: Location map in the Zitzmannsdorfer Meadows, showing the wild populations of *Artemisia laciniata* (original stand known before 2018 and new stand dicovered in 2018) and additional reinforcement stands (Z1-Z8 and the *in vitro* site) | **Abb. 1:** Standortkarte in den Zitzmannsdorfer Wiesen mit den Wildpopulationen von *Artemisia laciniata* (ursprünglicher Bestand, bekannt vor 2018 und neuer Bestand, entdeckt 2018) und zusätzlichen Verstärkungsbeständen (Z1-Z8, sowie der *In-vitro*-Standort)

Expansion of the population by means of sowing

Sowing into the original stand

Sowing of achenes was carried out on February 1, 2018 in a one square meter plot only a few meters away from the original stand. The vegetation of the plot was removed and the area refilled with fresh soil from nearby mole hills. Fruit stalks of the original stand from 2017 were crushed between fingers and spread out evenly. The material was pressed down and the area lightly covered with grass clippings for protection against drift and to keep the moisture. Later, wild carrot seedlings and upcoming thistles were removed as part of the maintenance.

A second sowing took place on December 18, 2020 with crumbled seed heads from the 2020 harvest. Three narrow strips were set up (100 cm × 20 cm) to reduce exposure to environmental elements.

Spring sowing into the new Z plots

Eight plots (6 × 6 m) were established in the larger area of the Zitzmannsdorf meadows (referred to as Z1-Z8, Fig. 1). A short hollow metal rod painted in red was hammered in at the east bottom corner of the plot, which can be located with a metal detector (BOSCH digital locator "Truvo"). The corner point was mapped using two different GPS devices (GARMIN Oregon 600 and SEPTENTRIO). The sites were selected based on criteria of vegetation and soil moisture similarity with the original population and considering the presence of mole hills. Sowing took place on March 5, 2020. The achenes (five each) were sown into five molehills from previous years, as the sites were mostly free of other plants. Each sowing point was marked with a coloured wooden skewer, sticking out about 20 mm above ground. A map was also drawn.

Winter sowing into the new Z plots

On December 18, 2020, 10 achenes were sown at five sites in each of the eight Z plots. The vegetation was removed and the uneven ground filled with molehill soil before the sowing took place. A total of 400 achenes were used. The sites were marked as described in the spring sowing.

Expansion of the population through planting

In vitro-derived seedlings

In May 2018, a new additional large plot (appr. 14 × 14 m) was set up south of the original stand, where pot plants were planted (In Vitro 2018, Fig. 1). The plants were derived from *in vitro* germination. Achenes were harvested from the original stand in October 2017 and cultured *in vitro* in December following the protocol by Kodym et al. (2018). The germination rate was 31%. The rest of the achenes were either contaminated or did not germinate. The *in vitro* plants were acclimatised in the glasshouse. Three weeks before planting in the field, they were brought into a courtyard for hardening off.

On May 23, 2018, 88 seedlings were planted in four transects, each 10 meters long with 22 plants in a zig zag line. The dense vegetation was first removed for each site with a spade, refilled with soil from mole hills and compressed slightly. The distance between the transects was 1 m. All plants were watered after planting.

Beginning of August 2018, the plants were additionally watered due to persistent drought and heat. Over the coming years, the area was mown once a year and monitoring was carried out regularly.

Nursery raised seedlings

Plants from achenes (crop year 2019, original stand) were raised in the Botanical Garden of the University of Vienna, Rennweg, 1030 Vienna. Sowing took place on January 30, 2020. The achenes had been stored at room temperature until sowing. One half of the achenes was germinated frost-free in the cold house (7 °C minimum in winter), the second half in the outdoor bed (cold frame). The cold house plants were moved outdoors in a cold frame after pricking out in April.

The best developed 80 plantlets from both treatments were selected and planted on October 9, 2020 at the eight Z plots. Per plot, five plants each were placed in one- year-old molehills (after one year they are flat and have settled down) and five in newly dug sites. At the new sites, holes were punched out with the help of a hollow spade (LIECO, Austria). Soil from surrounding molehills, outside the plots, was used to fill the holes. The plants were marked with a coloured wooden skewer.

Activation of seed bank

A. laciniata was discovered in the Zitzmannsdorf meadow in the early 1950s by Melzer (1952a, 1952b, 1952c) at the foot of a small mound, which presumably formed the remains of a former gun emplacement of World War II. Plants were last reported at this location in 1959 (Wendelberger 1959). The vegetation on and around the mound was partially removed in November 2017 in three transects approx. 0.5 × 5 m to activate the potential seed bank.

Mowing trials *ex situ*

A "mowing trial" was carried out in raised garden beds at the University of Vienna, Spittelau, 1090 Vienna in order to determine the latest possible mowing time for the future mowing regime on the Zitzmannsdorf meadows that allows plants to fructify. The trial was conducted twice over the span of 2020 and 2021 and included a total of 108 vital plants. Over eight weeks, twelve plants (randomised) were pruned back to 80 mm (= mowing height of the bar mower in the field) with secateurs every week. The eight-week pruning phase lasted from June 12, to July 31.

Subsequently, monitoring was carried out until October 2, 2020/ October 15, 2021. The number of generative stems per plant was recorded every week. In 2020, the height of plants was also measured on a weekly basis. Phenological changes (e.g. appearance of first generative stems, first flowering...) were also documented. At the end of October/beginning of November, the plants with seed heads were checked for the presence of achenes. As soon as one achene was found, this plant was rated as "achenes present".

During the trial, the plants were only watered in particularly dry weather via irrigation hoses at a maximum of twice a week.

Ex situ conservation

Living collection at the botanical garden

In 2006, a living collection was established at the Botanical Garden of the University of Vienna from achenes harvested 2005 from the Zitzmannsdorf meadows. At that time, the living collection comprised 20 seedlings. In the 2014 inventory, eleven genotypes were counted, by 2016 the number had decreased to ten.

The plants were grown in pots with a substrate consisting of one part T6 planting substrate for woody plants and perennials (KRANZINGER, Austria) and one part permeable special substrate (consisting of 10 parts Cocopeat, 10 parts bark humus, 3 parts compost, 3 parts sand, 2 parts lava, 6 parts pumice and 10 parts gravel). Pots were set into coarse sand within a cold frame so that any roots growing through the drainage holes can take up moisture from the surrounding and remain undamaged during repotting. In summer, the plants are watered 1–2 times a week, but generally kept rather dry. They are not fertilised, but weeds are removed regularly.

An inventory of the living collection was carried out in 2020. The living collection was supplemented with plants raised at the Botanical Garden of the University of Vienna to widen the genetic pool in 2022.

Millennium Seed bank

Achenes from the years 2020, 2021 and 2022 were sent to the Millennium Seed Bank for long-term storage.

Results

Monitoring of wild population

In 2018, the number of known individuals in the original stand had risen from 11 to 14, while a "new stand" with eleven plants was discovered on April 19, 2018, just 25 m south of the original stand. The plants were remarkably large and vigorous with at least 20 shoots each (Fig. 2), spreading over an area of approximately 200 m². This part of the meadow had been cut regularly with a tractor trailed mower by the local farmers. Over time, more plants were discovered in both stands, but also two small plants in the old stand disappeared. Thus, a total of 35 individuals (19 original and 16 new stand) were known in September 2023.

Fig. 2: Vigorous *Artemisia laciniata* plants in the new stand discovered in 2018 | **Abb. 2:** Kräftige *Artemisia laciniata* Pflanzen des 2018 entdeckten neuen Standortes

Inspired by discovering another stand, a search for other populations described for the Zitzmannsdorf meadows by Kasy (1961) and Melzer (1955) was carried out. However, the descriptions of the locations were cryptic and no plants could be found.

As part of the regular monitoring of the original and new stand, the generative stems were counted (Tab. 1). In the years 2015 and 2016 only one generative stem was recorded at the original stand.

The number of generative stems per individual and the percentage of plants flowering was highly variable from year to year. The biggest difference though was seen between the

Tab. 1: Overview of the number of generative stems of the original and new stock (2017–2023). The date in brackets indicates the day of the count. The light grey shading indicates which individuals were already known that year. A hyphen means the individual could not be located on the day of observation. | **Tab. 1:** Übersicht über die Anzahl der Blütenstände des originalen und neuen Bestandes (2017–2023). Das Datum in den Klammern ist der Tag der Zählung. Die hellgraue Schattierung zeigt an, welche Individuen in diesem Jahr bereits bekannt waren. Ein Bindestrich bedeutet, dass dieses Individuum am Tag der Beobachtung nicht gefunden wurde.

		Number of generative stems						
	Plant ID	**2017 (10.11.2017)**	**2018 (20.07.2018)**	**2019 (6.11.2019)**	**2020 (30.10.2020)**	**2021 (4.11.2021)**	**2022 (31.10.2022)**	**2023 (16.10.2023)**
Original stand	**1**	7	9	0	2	0	1	0
	2	4	2	0	0	0	3	3
	3	1	3	2	0	0	0	0
	4	0	1	0	0	0	0	0
	5	0	3	0	0	0	0	0
	6	0	0	5	0	0	0	0
	7	-	1	0	-	0	0	0
	8	4	7	9	6	4	4	1
	9	1	1	2	0	0	1	1
	10	0	1	2	0	0	0	1
	11	0	-	-	-	-	-	-
	12	0	-	4	0	2	0	0
	13	2	2	1	0	7	0	0
	14	0	0	-	-	-	-	-
	15		1	0	5	4	5	1
	16		3	0	0	1	3	0
	33				0	0	0	0
	34						0	0
	35						1	0
	36						0	0
	37							1
	Subtotal	**19**	**34**	**25**	**13**	**18**	**18**	**8**

		Number of generative stems						
	Plant ID	**2017 (10.11.2017)**	**2018 (20.07.2018)**	**2019 (6.11.2019)**	**2020 (30.10.2020)**	**2021 (4.11.2021)**	**2022 (31.10.2022)**	**2023 (16.10.2023)**
New stand	**17**		1	0	1	0	0	3
	18		24	6	22	0	11	17
	19		33	17	66	3	22	12
	20		28	12	43	4	40	9
	21		11	25	45	2	14	26
	22		17	0	11	1	1	1
	23		2	20	21	0	2	3
	24		1	0	0	0	0	0
	25		8	0	0	0	1	0
	26		12	11	31	0	0	0
	27		39	2	7	1	0	2
	28			24	60	22	33	43
	29			2	13	1	5	0
	30			3	14	3	4	8
	31				0	0	3	1
	32				2	5	1	6
	Subtotal	-	**176**	**122**	**336**	**42**	**137**	**131**
	In total	-	**210**	**147**	**349**	**60**	**155**	**139**

Tab. 2: Average number of generative stems at the original and new stands for the years 2017–2023. The new stand was only discovered in 2018. | **Tab. 2:** Durchschnittliche Anzahl an Blütenständen des originalen und neuen Bestandes der Jahre 2017–2023. Der neue Bestand wurde erst 2018 entdeckt.

	2017	**2018**	**2019**	**2020**	**2021**	**2022**	**2023**
Original Stand							
% of plants flowering	46	86	50	20	33	39	31
Generative stems per plant	3.2	2.8	3.6	4.3	3.6	2.6	0.4
New Stand							
% of plants flowering	-	100	71	81	56	75	75
Generative stems per plant	-	16	12.2	25.8	4.7	11.4	8.2

original and the new stand. The new stand consisted of large, vigorous plants and produced more generative stems (on average 4.7 to 25.8 per plant) than the original stand (0.4 to 4.3) (Tab. 2). Not all generative stems carried achenes; e.g., in 2020, a quarter of the plants produced none, others however were bearing many fruits.

The vegetation surveys assigned the original stand to a rather dry formation of the *Succiso-Molinietum caeruleae* (Pannonian bluegrass-pipegrass meadow). It shows transitional character to the Pannonic semi-arid grassland of the *Cirsio-Brachypodion pinnati*. The stand could be assigned to FFH-type 6410. Ecologically, it is an alternately moist mowing meadow with changing dominances of *Molinia caerulea* with approx. 22.5 % cover, *Brachypodium pinnatum* (8 %) and *Bromus erectus* (3 %). The area exhibits a slight browsing tendency, indicated by comparatively high cover values of *Peucedanum cervaria* (Korner & Staudinger 2018).

The new stand was also categorised as *Succiso-Molinietum caeruleae* and was dominated by *Molinia caerulea* (10 %), *Brachypodium pinnatum* (8 %) and *Scirpoides holoschoenus* (6 %). *Bromus erectus* was present with 4 %, *Peucedanum cervaria* was not found (Korner & Staudinger 2022).

Expansion of the population by means of sowing

Sowing into the original stand

In April 2018, two months after the sowing, the first *Artemisia* seedlings appeared, but were still difficult to identify. On June 8, the peak of germination was reached with 120 seedlings. It was noticeable that the seedlings germinated best along the edge of the plot, which provided partial cover. The number of plantlets dropped sharply to 29 by October 2019. From the original 120 seedlings, around 20 % seem to have been established as plants after five years. In April 2022 and 2023, 23 plants were counted, of which 12 were clusters. Since *A. laciniata* propagates vegetatively via rhizomes (Jäger 1987), it was very difficult to

Fig. 3: Seedlings in the year 2020 | **Abb. 3:** Sämlinge im Jahr 2020

distinguish between large individual plants or plants with multiple shoots. First flowering occurred two years after sowing. In 2022, a total of 20 generative stems were counted (Fig. 3).

From the 2020 sowing into three strips, a total of 29 seedlings were counted in April 2022 (14/11/4 per strip). By September 2023, 17 plants were present (7/5/5). They carried no generative stems.

Spring sowing into the new Z plots

In contrast to the preliminary sowing experiment from February 2018 near the original stand, sowing achenes across eight new Z plots in mole hills at the beginning of March 2020 was not successful. On April 28 and July 13, 2020, no seedlings of *A. laciniata* could be found. On October 9, 2020, one seedling was recorded (germination rate = 0.5 %). However, it did not survive.

A comparison of the rain data shows that the field conditions in 2018 were more favourable than in 2020 and 2021 (Tab. 3).

Tab. 3: Monthly mean air temperature and monthly precipitation of the measuring station "Podersdorf" – measuring point no. 7912, Operator: ZAMG (https://data.hub.zamg.ac.at) | **Tab. 3**: Mittlere monatliche Lufttemperatur und monatlicher Niederschlag der Messstation „Podersdorf" – Messstation Nr. 7912, Betreiber: ZAMG (https://data.hub.zamg.ac.at)

	Mean air temperature [°C]			Precipitation [mm]		
	2018	**2020**	**2021**	**2018**	**2020**	**2021**
January	3.2	0.6	1.6	19	7	32
February	-0.4	6.5	2.5	30	8	19
March	3.2	7.0	5.8	40	30	1
April	15.5	12.0	9.0	20	3	21
Sum				**109**	**48**	**73**

Winter sowing into the new Z plots

From the 400 achenes sown mid-December 2020 into freshly dug holes in the Z plots, 94 (23.5 %) had germinated by May 21, 2021. On August 13, 2021, 26 seedlings (6.5 %) had established themselves. However, in July 2022 the number was reduced to 12, and further down to 3 in 2023. The surviving plantlets were still in a three-leave stage.

Expansion of the population through planting

In vitro-derived seedlings

Overall survival of the 88 *in vitro*-derived plantlets (Fig. 4) was 47 % after five years (Tab. 4). The pattern that has been showing over the years, has become more and more prominent:

Fig. 4: Hardened off *in vitro* plants, ready for planting | **Abb. 4:** Abgehärtete *in vitro* Pflanzen, bereit für die Auspflanzung

Tab. 4: Survival rate and number of flowering individuals of the 88 *in vitro*-derived plants (May 2028) | **Tab. 4:** Überlebensrate und Anzahl der blühenden Individuen aus der *in vitro* Kultur (88 Pflanzen, Mai 2018)

Evaluation	plants alive	% survival	flowering
July 2018	88	100	-
Oct. 2018	86	98	3
Oct. 2019	72	82	47
July 2020	64	73	11
Oct. 2021	57	65	23
Sept. 2022	52	59	7
Sept. 2023	47	53	9

survival and vigour depended on the transect they were growing in. The lowest survival rates were recorded in transect 1, the highest in transect 4. The survival rates varied between 18 and 95 %. Plants started flowering in the first year of being planted out. In 2023, generative stems were only found in transect 3 and 4 (Tab. 5).

The vegetation survey identified three distinct vegetation types within the small plot (Korner & Staudinger 2022). In transect 1, both *Euphorbio verrucosae-Caricetum montanae and Filipendulo vulgaris-Brometum* were present. The boundary between the three vegetation types

Tab. 5: Survival rate of the *in vitro* plants and the number of generative stems per transect (September 2023) | **Tab. 5:** Überlebensrate der *in vitro* Pflanzen und Anzahl der Blütenstände pro Transekt (September 2023)

	plants alive	%	generative stems
Transect 1	4	18	0
Transect 2	7	32	0
Transect 3	15	68	3
Transect 4	21	95	7

Tab. 6: Vegetation types of the in vitro 2018 planting site | **Tab. 6**: Vegetationstypen auf der In vitro 2018 Fläche

Vegetation classification	Main species and their cover in %
Euphorbio verrucosae-Caricetum montanae	*Bromus erectus* 58, *Chrysopogon gryllus* 20
Filipendulo vulgaris-Brometum	*B. erectus* 60, *C. gryllus* 5, *Festuca pseudovina* 5
Succiso-Molinietum	*B. erectus* 60, *B. pinnatum* 6

ran diagonally across the plot, with a gradual increase of *Succiso-Molinietum.* Transect 4, which showed the highest survival, was entirely dominated by *Succiso-Molinietum*. The vegetation types and most common species are found in Tab. 6.

Nursery raised seedlings

After sowing achenes in the Botanic Garden of the University of Vienna on January 30, 2020, the first seedling was visible in the cold house on February 6, and over a month later, on March 10, in the outdoor bed. The seedlings from the cold house kept their head start and were pricked out on April 7, those in the outdoor bed on July 14 and August 10.

While the cold house conditions favoured fast germination and development (Fig. 5), the outdoor beds resulted in a higher germination rate. The average germination rate of achenes sown frost-free in the cold house was 47.3 % with a germination time of 1–2 weeks, in the outdoor bed 73.6 % with a germination rate of 5–6 weeks.

Survival in the eight Z plots was promising, although the number decreased steadily over the three years. In October 2023, 48 of 80 plants (60 %) had survived. The survival rate in the new holes (70 %) was better than in the molehills (50 %) (Tab. 7). A total of 40 generative stems was recorded. Various sites showed traces of animal activities from crows, moles, deer and hares. Marking the individual trial sites with environmentally friendly wooden sticks did not persist. Field mowing by the farmer with cutter bars and rakes removed most of them. Therefore, after one season, the locations were marked with bright coloured metal screws in the ground, which can be detected with a hand-held metal detector.

Two vegetation types were present – the *Succiso-Molinietum* and the *Euphorbio verrucos-ae-Caricetum montanae* (Tab. 8). The dominant plant in all plots was *Bromus erectus*, with

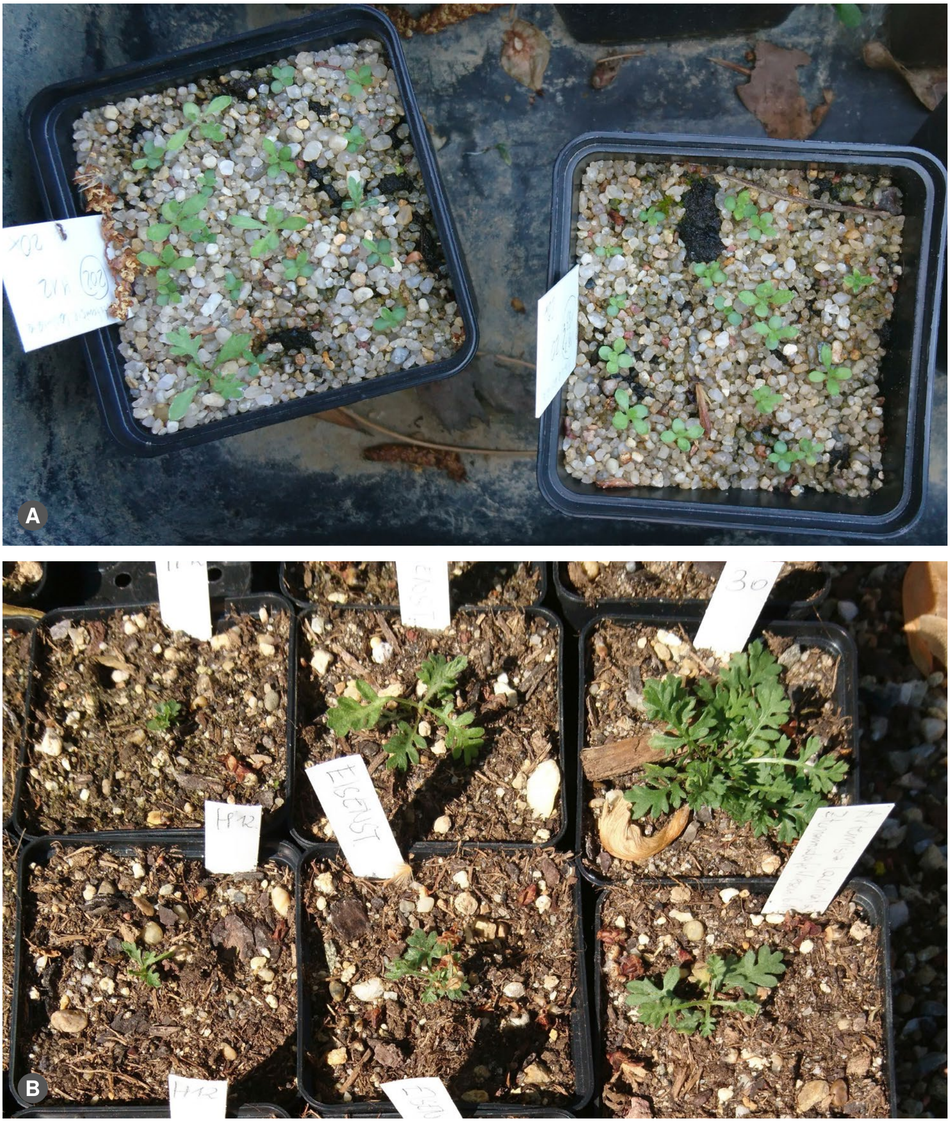

Fig. 5: Comparison of germination in the cold house and the outdoor bed. A: seedlings from the outdoor bed; B: pricked plants from the cold house (both photographed on 28.4.2020) | **Abb. 5:** Vergleich der Keimung im Kalthaus und Außenbeet. A: Sämlinge des Außenbeetes; B: pikierte Pflanzen des Kalthauses (beide Fotos aufgenommen am 28.4.2020)

a cover ranging from 30 % to 70 % (Korner & Staudinger 2022). The accompanying plants were grouped into dry, mesic and wetland species. No correlations could be made when trying to link survival of *A. laciniata* seedlings with the vegetation class or indicator species or their percentage cover. The obtained results were further influenced by animal disturbance and undifferentiated mowing.

Tab. 7: Survival percentages of the 80 cultivated plants in mole hills and new holes (planted Oct. 2020) | **Tab. 7:** Prozentuale Überlebensraten der 80 vorgezogenen Pflanzen in Maulwurfshügeln und neu gegrabenen Löchern (gepflanzt im Okt. 2020)

	molehills	new holes	total
Oct. 2020	95.0	97.5	96.3
May 2021	90.0	97.5	93.8
Aug. 2021	80.0	97.5	88.8
July 2022	67.5	82.5	75.0
Oct. 2023	50.0	70.0	60.0

Tab. 8: Comparison of survival percentages between new holes and molehills in the eight plots linked with the vegetation survey data (July 2022) | **Tab. 8:** Vergleich der prozentualen Überlebensraten zwischen neu gegrabenen Löchern und Maulwurfshügeln in den acht Standorten in Verbindung mit den Daten der Vegetationsaufnahme (Juli 2022)

Plot	Vegetation type	Main accompanying species (with > 5 %)	Cover % dry:wetland plants	Survival %		
				new holes	molehills	total
Z1	*Succiso-Molinietum*	*Bromus erectus* 60, *Festuca pseudovina* 6	67:7	60	100	80
Z2	*Succiso-Molinietum*	*B. erectus* 40, *F. pseudovina* 10, *Colchicum automnale* 6	67:1	100	40	70
Z3	*Euphorbio verrucosae-Caricetum montanae*	*B. erectus* 70	83:4	100	100	100
Z4	*Euphorbio verrucosae-Caricetum montanae*	*B. erectus* 47, *Briza media* 6	82:3	80	60	70
Z5	*Euphorbio verrucosae-Caricetum montanae*	*B. erectus* 30, *Chrysopogon gryllus* 22, *Brachypodium pinnatum* 8	80:9	60	60	60
Z6	*Euphorbio verrucosae-Caricetum montanae*	*B. erectus* 40, *F. pseudovina* 10, *Centaurea scabiosa* 6	69:7	100	60	80
Z7	*Euphorbio verrucosae-Caricetum montanae*	*B. erectus* 66, *C. scabiosa* 6	84:1	60	40	50
Z8	*Succiso-Molinietum*	*B. erectus* 64	72:13	100	80	90

Activation of seed bank

The seed bank trial at the site was started in November 2017. Throughout 2018 many seedlings emerged, especially of thistle species, but none of *A. laciniata*. At the last monitoring in July 2020, the area was partly overgrown again and no seedlings or rosettes of *A. laciniata* could be found.

The vegetation survey could not assign a clear vegetation unit to the quite heterogeneous area. It corresponds most closely to the *Filipendulo vulgaris-Arrhenatheretum*. *Arrhenatherum* *elatius* covered approx. 25 %, a clonal stand of *Carex riparia* and numerous disturbance indicators were recorded.

Mowing trials *ex situ*

In 2020 and 2021 artificial mowing experiments were carried out in the garden beds of the University of Vienna (Fig. 6). Height measurements were conducted during 2020. The average height of the rosette plants in the control group (no mowing) increased continuously until August 7 due to the growth of the generative stems, and reached the maximum at 556 mm. The plants cut back during weeks 1–3 reached an average height of over 300 mm, while those cut back in weeks 4–6 grew over 200 mm. However, plants cut back in weeks 7 and 8 reached less than 200 mm. This was reflected in the height of the generative stems, which were shorter in the treatments than in the control group.

The first generative stem in the control group appeared on June 19 with the proportion of flowering plants steadily increasing until July 3, when 11 out of 12 plants had generative stems. The flowering period in the control group lasted from August 21 to October 2. For plants cut back during weeks 1 to 6, flowering began one week later, on August 27, while those cut back in weeks 7 and 8 started flowering two weeks later, on September 4. Despite the delayed start, all generative stems eventually bloomed, regardless of when they were produced.

In 2021, the flowering period shifted forwards by about one week as compared to 2020 (Tab. 9). The majority of the plants (10 out of 12) in the control group already had generative

Fig. 6: Plants of the control group and experimental groups in one of the garden beds of the mowing experiment | **Abb. 6:** Pflanzen der Kontrollgruppe und Versuchsgruppen in einem der Gartenbeete des Mähversuches

Tab. 9: Comparison of flowering in the control group in 2020 and 2021 | **Tab. 9:** Vergleich der Blüte in der Kontrollgruppe im Jahr 2020 und 2021

	2020	**2021**
First generative stem	19. June	before 11. June
Flowering period	21. Aug. – 2. Oct.	27. Aug. – 7. Oct.
Main flowering period	27. Aug. – 18. Sept.	3. Sept. – 10. Sept.
First flowers fading	18. Sept.	10. Sept.

Tab. 10: Differences in flower production between the control group and the eight experimental groups (12 plants per group, gs = generative stems) | **Tab. 10:** Unterschiede in der Blühproduktivität zwischen der Kontrollgruppe und den acht Versuchsgruppen (12 Pflanzen pro Gruppe, gs = generative stems = Blütenstände)

		2020			**2021**		
		Plants with generative stems		**No of gs**	**Plants with generative stems**		**No of gs**
	Control	**11**	**92 %**	**80**	**10**	**83 %**	**145**
Cut back	Week 1 (11.6.21)	8	67 %	18	10	83 %	108
	Week 2 (18.6.21)	8	67 %	18	9	75 %	80
	Week 3 (25.6.21)	6	50 %	19	8	67 %	33
	Week 4 (02.7.21)	6	50 %	13	9	75 %	30
	Week 5 (09.7.21)	5	42 %	8	9	75 %	35
	Week 6 (16.7.21)	5	42 %	9	6	50 %	14
	Week 7 (23.7.21)	2	17 %	3	5	42 %	10
	Week 8 (30.7.21)	2	17 %	7	6	50 %	10

stems at the start of the mowing trials on June 11. Over time, the number of generative stems increased, while the number of plants with generative stems remained the same. The flowering period of the control group extended from August 27 to October 7. For the plants that were cut back during weeks 1 to 2, the flowering time was similar to the control group. For weeks 3 to 8, the start of flowering was one week later, on September 3. Again, all generative stems came into flower.

The number of plants with generative stems and the total number of generative stems until mid-September, the end of the main flowering season, are presented (Tab. 10). In 2020, 92 % of the plants in the control group developed generative stems. The later the "mowing" took place, the fewer plants produced generative stems. Weeks 1 and 2, weeks 3 and 4, weeks 5 and 6 and weeks 7 and 8 gave similar results. The number of generative stems produced depended on the time of cutting. Cutting in mid-June had a major impact, resulting in 27 % fewer plants with generative stems and 78 % fewer generative stems compared to the control group. In 2021, a similar overall trend was observed, although the harm was less.

Achenes in the control group were produced in 10 out of the 11 flowering plants (91 %) in 2020. In the experimental group, 36 out of 42 flowering plants (86 %) had produced achenes. In 2021, achenes were found in 8 out of 10 flowering plants (80 %) in the control group, and in 54 out of 62 flowering plants (87 %) in the experimental groups.

***Ex situ* conservation**

Living collection botanical garden

In the 2020 inventory, the ten genotypes from 2016 were still present, in some cases with multiple copies, as they had been propagated by division. Twelve new genotypes, derived from propagation of field-collected seed material, were added. The collection now comprises 22 genotypes.

Seed bank Millenium Seed Bank

Achenes from the years 2020, 2021 and 2022 are stored under the accession numbers 33806, 33807 and 33808 in the Millennium Seed Bank.

Discussion

In 2015, the population of *Artemisia laciniata* in the Zitzmannsdorfer meadows consisted of just eleven known plants. As of October 2023, the known population has grown to 273 individuals and is no longer confined to a small, vulnerable area. Of these 273 plants, 35 belong to the wild population, while 238 have been planted (195) or sown (43), keeping in mind that this paper here is limited to the key propagation activities.

In the initial phase, achenes could only be harvested from the original stand, which were weak and had very low production. This scarcity of seeds led to early attempts of applying *in vitro* culture. However, the discovery of the new stand with vigorous plants marked a turning point, offering new opportunities for conservation and propagation efforts. The sighting of a new stand in April 2018 highlights how easily *Artemisia laciniata*, as a rosette plant, can be overlooked in the meadow. The best time to spot plants is in spring (April), when the rosettes have sprouted, displaying a silvery hue, and other vegetation is not yet fully developed. Despite being in close proximity to the known stand, these individuals, some of them quite large, had remained unnoticed by excursion leaders, botanists and rangers. Attempts to activate the seed bank of a known extinct site were unsuccessful. Efforts to locate the populations described in old references for the Zitzmannsdorf meadows (Melzer 1955; Kasy 1961) were equally unsuccessful due to vague descriptions and the vast areas they referenced. This underlines the importance of providing GPS data to the National Park and using markers such as screws to ensure that future generations can accurately identify and distinguish wild populations and planting sites.

Artemisia laciniata requires small open spaces free from competing vegetation for successful germination and establishment, conditions that today are largely absent in the dense meadows. Small-scale soil disturbances created by mole activity provide openings for colonization, in particular for weak light competitors. Mole hills are very common in our experimental area, but they did not provide suitable conditions for *A. laciniata.* To expand the wild population, two methods were employed: sowing of achenes and planting seedlings. Eight additional plots were established across the meadows to ensure that the plants are no longer confined to small, vulnerable areas. These plots were grouped together to facilitate pollen exchange. It became apparent that germination success is highly dependent on the micro habitat and soil moisture. The sowing within the 1 m^2 open area revealed that the achenes preferably germinated at the edges, where they benefited from the protection of some surrounding vegetation, such as grasses, while still being in relatively open conditions. Freshly dug small holes in the meadow also provided some protection from the weather and from animals and provided a micro climate suitable for germination. In contrast, achenes sown on old molehills with loose soil were highly exposed, leading to fast drying out of open soil and disturbance by animals like moles, crows, deer and hares. As no seedlings were observed on the molehills, it appears that these open conditions prevented successful germination and/or early stage establishment of plants. The same pattern was also observed with plantings: plants in freshly prepared sites had a higher survival rate than those on old molehills.

Sowing worked well near the original and new stand, but not in the more distant sites. Generally, planting out had a much higher success rate than direct sowing of achenes, although this method is more costly and time-consuming. Germination and cultivation of *A. laciniata* was very effective under protected nursery conditions, when seed were sown at the end of January, both in the cold house and in outdoor beds. This supports Kasy's (1978) observation that *A. laciniata* can be easily propagated from achenes.

A comparison of the field performance of *in vitro*-derived plants and nursery-raised plants revealed that their survival rates were identical after one summer, both at 98 %. However, a suitable location appears to be a highly critical factor just like in seed sowing. A noticeable gradient in survival was observed in the *in vitro*-derived plants, likely influenced by varying soil conditions affecting plant development. Salinity in particular may play a significant role, prompting plans for future soil sampling. Although the vegetation cover varied across the transects, no correlation was found with successful establishment of plants.

Implementing an appropriate mowing regime is crucial for the long-term conservation of *A. laciniata*. The positive impact of mowing on the plant's vegetative growth became apparent, when a new stand was discovered in 2018. These plants, which were included in the farmer's regular summertime mowing, grew large and vigorously. In contrast, the original stand, which had not been regularly mown for an extended period to protect achene production, consisted of weak plants. The difference in vitality of the plants was also reflected in reproduction, with the new stand to produce the majority of achenes. Consequently, a regular mowing regime has been introduced in recent years for the original stand, but the effects are yet to be seen.

The mowing trial conducted in the garden of the University of Vienna showed that summer mowing significantly reduced the number of flower heads. The trial was conducted to develop an effective mowing regime for the Zitzmannsdorfer meadows without incurring long travel distances and harming natural seed production. The flowering period in 2020 and 2021, from mid-August to early October, closely aligned with the flowering period from August to October reported in the literature (Kästner & Fischer 2011). In both experimental years, the phenology of the garden plants mirrored that of the wild population. The experiment clearly demonstrated that mowing should not occur too late, as it would lead to a reduction in the number of plants with generative stems, the total number of generative stems and their length. Nevertheless, plants with flower heads, in both control and experimental groups, produced ripe achenes by late October to early November. Additionally, *A. laciniata* was found to tolerate cutting back to ground level, quickly sprouting back afterward.

The optimal mowing period for *A. laciniata* is between November and March/April. However, autumn and winter mowing is only possibly in a small time window, due to wet soil conditions, making it difficult to bring in mowing equipment. On the other hand, mowing in spring and early summer is also not feasible, as the Zitzmannsdorfer meadows are designated as an Important Bird Area (IPA) (Dvorak 1995). For example, the meadows are a nesting site for the Eurasian curlew (*Numenius arquata*) (Dvorak et al. 2017), a species vulnerable in Europe and endangered in Austria. The curlew's breeding season extends from April to June (Ulmer et al. 2012). Notably, the breeding population in the Zitzmannsdorf meadows tripled between 1991 and 2011, partly contributed to the practice of late mowing (Dvorak et al. 2011).

Hand mowing, which was partially utilized during the project, is too costly for the long-term maintenance of the whole area, but may be possible when limited to the wild stands and selected plots. To simplify the mowing regime, it may be advantageous to completely omit mowing in individual strips for a year and alternate this practice annually. This approach would allow the plants to reach full fructification every other year and would also be beneficial for other plants with late seed set, and for animals, like over-wintering insects, eggs and larvae (Unterweger et al. 2018). The risk of a hay ball being stored on the plot or the tracks of the tractor running across remains a big concern.

Since 2006, a living collection of *A. laciniata* has been maintained in the Botanical Garden of the University of Vienna, safeguarding a significant portion of the genetic variation of the original population (unpublished data from the Research Group Plant Ecological Genomics, Ovidiu Paun, University of Vienna). Nevertheless, the species cannot be considered fully secured and there are plans to further expand the *ex situ* collection in the near future in order to encompass the entire genetic spectrum. Additionally, storing achenes in the Millennium Seed Bank from the wild population was on the agenda to minimise the risk of genetic loss. This is particularly important because it is known that species can adapt to cultivation conditions, which might result in the loss of adaptations necessary for survival in its natural habitat (Ensslin & Godefroid 2019).

Despite all the progress made, it is crucial to closely monitor the wild population and its reinforcement plantings in the coming years, incorporating observations into the ongoing adjustment of conservation measures. Natural spontaneous germination has not yet been observed, but would be essential for sustainable conservation. Additionally, climate change poses a significant threat to the species' vitality due to rising temperatures and increased drought. As a result, assisted migration to wetter locations may need to be considered to ensure the long-term survival of the species.

Acknowledgements

We thank our colleagues from the Botanic Garden of the University of Vienna (Alena Binder, Heather Libby, David Prehsler and Franz Tod) for their help with raising plants and sharing their knowledge. Special thanks go to Ovidiu Paun and his working group at the University of Vienna for providing insights on the genetics of *A. laciniata*.

Thanks go to Hans Lehner, ranger at the National park, who has immensely pushed the project by discovering the new stand of *A. laciniata*. We are also grateful to Michael Kuttner for GPS tracking our plants and Erwin Köllner, Viktoria Werner and Thomas Zechmeister from the Biological Station Illmitz for their support and insights. For critical and constructive comments of the manuscript, thanks to Matthias Kropf and Karin Tremetsberger.

This research was supported by a) the LE Project A4/LA.LE4-10380-16-2020 with support from the federal government, the states, and the European Union, b) the Landscape conservation fund in the frame of the Burgenland's species and habitat protection program and c) the FWF Elise Richter Project V 351-B22. The propagation of the *in vitro* plants and the vegetation surveys in 2017 were financed by the Biological Station Illmitz.

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