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Effects of habitat management on newly found populations of the endangered weed, *Mummenhoffia alliacea* (Brassicaceae)

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Abstract

Garlic pennycress (*Mummenhoffia alliacea*) is a rare weed of the Brassicaceae family, protected in Hungary. In 2021, three new sites of the species were discovered. In addition to these previously unknown occurrences, a detailed study of a roadside occurrence known for a longer time but not yet published was carried out. Main aim of our study was to find out how habitat management practices, such as mowing and winter-deicing effects seed mass and germination. Seeds collected earlier had significantly lower mass than those from later collections, suggesting that late-spring mowing would be optimal for the reproduction of *M. alliacea*. The type of mowing (chopping the stem into small pieces or cutting the stem at one point at few centimetres above ground) had no significant effect on seed germination but did have a significant effect on the seed mass. Seed mass deriving from whole individuals was significantly higher than seed mass of chopped individuals, suggesting, that traditional mowing methods are better than the use of modern lawnmowers for the survival of the species. The highest NaCl (used for winter-deicing) concentration where germination was observed was 0.75 m/m% (mass percentage), which is comparable with strongly saline soils in nature. The newly discovered sites were all disturbed, one of which was located on roadsides (paved and dirt roads) and two on embankments, suggesting that anthropogenic habitats could be optimal for the species, but the long-term survival depends on the management of these habitats.

KEYWORDS

germination, mowing, river embankment, roadside, salt tolerance, *Thlaspi alliaceum*

1 | INTRODUCTION

Over the past century, the proportion of land under agricultural use has been increased significantly. The extensive agriculture being replaced by intensive farming, leading to major changes in the agro-ecosystem, resulting in the impoverishment of native weed flora and a drastic loss of diversity (Albrecht et al., 2016; Pinke & Pál, 2005). Modern agriculture is one of the most important driver of biodiversity loss (Reichholf, 1989, Dudley & Alexander, 2017) The main features of intensive agriculture are mechanisation, chemical inputs and monoculture farming, with new seed cleaning techniques, and the use of fertilisers and herbicides in large quantities (Pinke, 2020; Storkey et al., 2012). Some of the native weed species are now only found in extensively farmed areas (Bátori, Kiss, et al., 2020; Kovács-Hostyánszki et al., 2011; Pinke & Gunton, 2014; Tölgyesi et al., 2022). Extensive arable systems are characterised by a high level of biodiversity, especially when mixed with semi-natural grasslands and forests (Márkus, 1994). Pinke et al. (2011) reported in their study that 149 species of Hungarian weed flora are at risk, including the Atlanto-Mediterranean garlic pennycress (*M. alliacea* (L.) Esmailbegi & Al-Shehbaz syn. *Thlaspi alliaceum*).

Members of the genus *Thlaspi* with 75 species are known worldwide, are annual to perennial weeds (Koch & Al-Shehbaz, 2004). They are characterised with tap root, mostly self-pollination or pollination by various insects. Several species of the genus occur in Hungary, of which *T. arvense* and *T. perfoliatum* are the most common and are considered to be widespread weed species also in the distribution area of *M. alliacea*. *Mummenhoffia alliacea* is a rare, protected weed in Hungary. Pál (2005) describes the species as critically endangered, and Pinke et al. (2011) classified the species to the vulnerable category of the Red list of the vascular flora of Hungary (Király, 2007). A few occurrences have been described from north-eastern, western and south-western Hungary in previous decades (Csiky, 2005; Károlyi et al., 1972; Pinke et al., 2005), but no new occurrences have been recorded from new sites in the last 20 years. Its occurrences in Hungary have been found in fields, vineyards and along dirt roads (Csiky, 2005; Károlyi et al., 1972). According to the Vascular plants of Hungary online database (Bartha et al., 2022), until 2021, a total of 32 populations have been documented from 16 floristic quadrats.

Semi-natural habitats, which facilitate the survival of *M. alliacea* and many other weed species, have also declined in Hungary due to the intensification of agricultural cultivation (Pinke & Pál, 2005). However, it is well-known, that valuable semi-natural vegetation remained in anthropogenic habitats, like river embankments (Bátori, Vojtkó, et al., 2020), cemeteries (Löki et al., 2019) or roadsides (Fekete et al., 2017, 2020) playing an important role in maintaining diversity and provide shelter for many plant species (Hobbs et al., 2009). Furthermore, roadside verges can also act as ecological corridors (Tikka et al., 2001), but this role is mainly known in case of the spread of alien, invasive and salt-tolerant species (Fekete et al., 2020, 2022; Follak et al., 2013; Gelbard & Belnap, 2003). The spread of the latter is due to winter de-icing with NaCl, which causes salt accumulation in the roadside soil. The effects of high salt concentrations on wildlife have been reported in several studies, including contamination of surface water, groundwater, alteration of soil pH, nutrient availability, osmotic stress and the potential for the spread of salt-tolerant plant species (Amrhein et al., 1992; Davison, 1971).

In the spring of 2021, we found several new, previously unknown populations of *M. alliacea* in Hungary, which were found on paved roadsides, along dirt roads near the settlements of Nyékládháza (Süveges, 2022) and Olaszliszka, and on river embankments near the settlements of Gyula and Körmend. Due to the occurrence in new habitats and the endangered status of the species, we considered it important to conduct further research.

The aim of our work was to study the newly found populations of *M. alliacea* in anthropogenic habitats. Therefore, in this study we present (1) a detailed systematic sampling of the Olaszliszka road section and associated byways, (2) a study of the reproductive characteristics of the species, (3) an assessment of the effect of different mowing methods on seed mass and germination success and (4) an evaluation of the effect of salt on seed germination.

2 | MATERIALS AND METHODS

2.1 | Survey of the populations

On 30 April 2021, we conducted the first survey of the population of the Olaszliszka region, which has been known for some years but not yet published, along the main road 37. At that time, we collected green whole plant individuals. On 10 May 2021, we re-visited the Olaszliszka population conducting a systematic survey with sampling points located at every 1 km along a 20-km-long road section. At each stop, we surveyed a 50-m-long transect in a 6-m-wide line on both sides of the road, looking for individuals of *M. alliacea*. Where dirt roads joined the main road, we also surveyed the edge of the dirt roads within 50 m. At each sampling point, we recorded geocoordinates and number of individuals. We randomly selected 20 individuals and recorded the distance from the road and height of the shoots. At points where population size allowed, green whole plant individuals were also collected.

On 30 May 2021 we found a small roadside population near Nyékládháza, in this case we also recorded the geo-coordinates, the number of individuals, the distance of individuals from the road and the height of the shoots. We also surveyed the edge of the road and the roadside branching from it on a 100-m-long stretch. The population consisted of only a few individuals, so we were unable to collect specimens.

At Gyula, the population was found on 19 April 2021 on the embankment of the Fehér-Körös river. Individuals were found in small and large groups for about 9 km along the river in northwestern direction. In this case, we also recorded geo-coordinates, then estimated the number of individuals and collected seeds.

In Körmend, specimens of *M. alliacea* were found on 22 May 2021. on the embankment of the Rába river and in the weedy edge of the Rába bridge. A specimen was also found along the Pinka river at a distance of 3 km from the Rába bridge. We recorded geo-coordinates and numbers of individuals and collected seeds from the population along the Rába.

2.2 | Study of reproductive traits

From the Olaszliszka population, fruiting specimens were collected randomly and with the collected specimens two kinds of mowing methods were imitated in the laboratory The shoot of 85 randomly selected green individuals were cut into 5 cm long segments and 85 randomly selected individuals were left whole. The first method

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represents when the mower chops the stems of the individuals into pieces of 1–2 cm above the soil surface, and the second method is when the stems are cut 1–2 cm above the soil surface, leaving the stems in one piece. The number of flowers per individual and the number of seeds per fruit, and thus the average individual seed production were estimated from the plants left whole. We counted the number of flowers for 85 individuals and randomly selected five flowers per individual and counted the number of seeds per individual, in the five randomly selected flowers to estimate the seed production. We measured the seed mass of seeds deriving from chopped and whole individuals on an analytical balance to five decimal point accuracy. We weighed 50 seeds together, which we later placed in one Petri dish during germination experiments. Based on the methodology of Török et al. (2016), we estimated the thousand-seed mass of the species. The seeds were then stored in paper bags at room temperature until the start of the germination experiment.

2.3 | Testing the effect of mowing

We tested whether there was a difference in the seed mass and germination of chopped and whole individuals. The germination experiment was carried out on 1.0% agar-agar medium in 20 pieces Petri dishes with 50 seeds per Petri dish. For each treatment (chopped and whole individuals) 500 were included in the germination test. The diameter of the Petri dishes was 8.7 cm. The experiment was carried out at room temperature, under natural light. The seeds were placed on the medium on 07 October 2021 and the number of germinated seeds was recorded daily for 28 days.

We tested for effects of collection date at Olaszliszka on seed mass and germination of the species: (30 April and 10 May 2021). Germination was carried out on 1.0% agar-agar medium in Petri dishes, where 10 $\times$ 50 seeds were germinated from each collection in total 1000 seeds were examined during this experiment.

2.4 | Testing salt tolerance

To test the salt tolerance of the species, seeds from Olaszliszka were germinated at eight different NaCl concentrations. We made eight agar-agar media with different NaCl concentrations. The concentrations were chosen to cover the soil categories defined by the FAO (Abrol et al., 1988). The concentrations in g L^{-1} were 0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0 and 10.5, corresponding to 0, 25.67, 51.33, 77.00, 102.67, 128.34, 154.00 and 179.67 mol/L respectively. At each concentration, four replicates were performed, thus 200 seeds per concentration and 50–50 seeds per Petri dish were germinated. In the experiment of salt tolerance, a total of 1600 seeds were germinated in 32 Petri dishes.

2.5 | Data analyses

All data analyses were performed in the R statistical environment (R version 4.1.3. R Core Team, 2022) using the *lme4* package (Bates, 2010). To test the effect of two types of mowing methods on the species seed mass, a linear mixed model (LMM) was used, with seed mass as the dependent variable and mowing type as the explanatory variable.

The effect of mowing type on germination was also tested using a binomial generalised linear model (GLM), where germination outcome (germinated/non-germinated) was used as the dependent variable, while mowing type as the explanatory variable, and the Petri dish code as a random factor.

To study the difference in the germination rates between seeds collected at different times in Olaszliszka, we used binomial GLM germination outcome (germinated/non-germinated) as the dependent variable, the time of collection as the explanatory variable and the code of the Petri dish as the random factor.

To analyse germinability of seeds at various salt concentrations we used a binomial GLMM, where germination outcome (germinated/non-germinated) of seeds was used as dependent variable and NaCl concentration was the sole explanatory variable, included as a second-degree orthogonal polynomial. All models included the identifier of the Petri dish as a random factor.

3 | RESULTS

3.1 | Survey of new populations of *M. alliacea*

During our field surveys, we found about 34 000 individuals of *M. alliacea* at four different sites (Figure 1). The largest population was found at Olaszliszka.

Number of surveyed individuals along dirt roads in Olaszliszka region was 791 and 1107 at the surveyed 50 m sections of the main road 37 during the 20 systematic stops along the 20 km road section. Based on these, the estimated population size of Olaszliszka was 23 000 individuals (Figure 2). In Gyula the estimated population size was 10 000 individuals. In Körmend 1000 individuals were found and a single specimen along the Pinka River near Körmend. The smallest population was in Nyékládháza, where we found 12 individuals of the species. In Olaszliszka, *M. alliacea* was found 14 times out of 20 systematic stops, which is 70% of the stops. The average shoot height of the species is 53 ± 30 cm (min.: 6 cm, max.: 104), based on our measurements. The average distance from the pavement of the individuals is 260 ± 167 cm (min.: 7 cm, max.: 500 cm).

The species occurred on various types of degraded species-poor grasslands and weedy roadside vegetation. The vegetation of loamy soils near to Olaszliszka were characterised by mesophilic to drought-tolerant grass species (*Arrhenatherum elatius*, *Festuca pseudovina* and *Elymus repens*), but temporarily species of wet grasslands also occurred in the ditches (e.g., *Alopecurus pratensis* or *Ranunculus repens*). Abundance of salt-tolerant weeds (*Plantago major*, *Polygonum aviculare*, *Atriplex tatarica*) increased towards to the roadside. The sites were also characterised by a temporarily high abundance of deciduous crucifers in the April–May season, beside of *Thlaspi perfoliatum* was the most common species, but *Thlaspi arvense* was

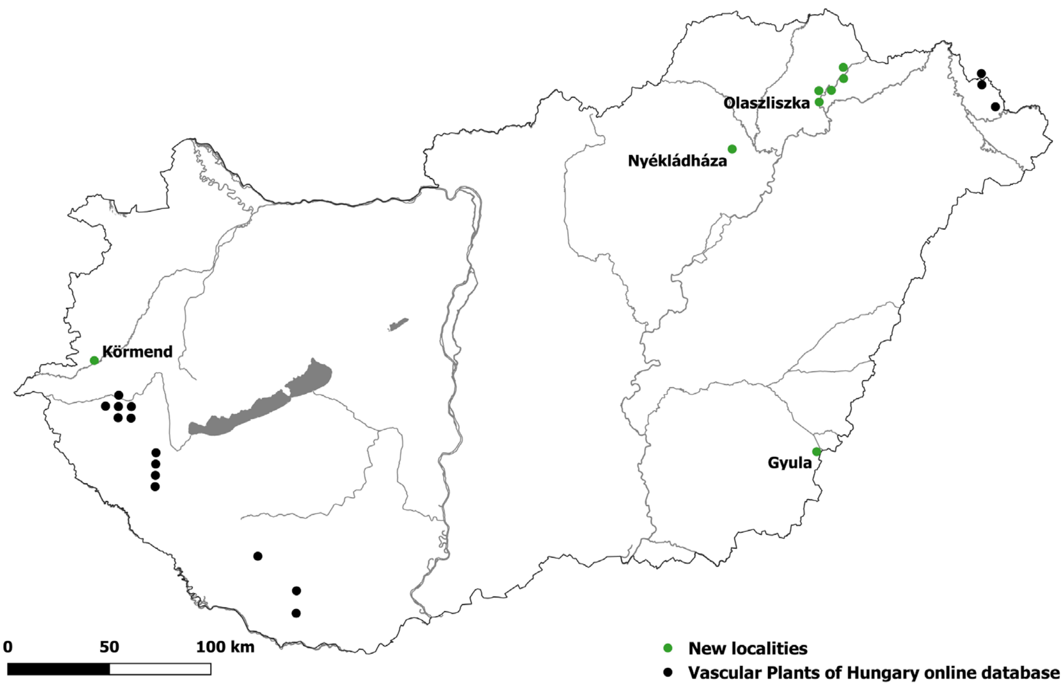

FIGURE 1 Previously known (Bartha et al., 2022) and recently discovered populations of *Mummenhoffia alliacea* in Hungary.

FIGURE 2 Individuals of *Mummenhoffia alliacea* along the road 37 near Olaszliszka (Northeast Hungary).

also found in some places. The sites in Gyula were characterised by weedy loess grassland vegetation formed on loamy soils with a temporary dominance of short-lived weedy dicots. Characteristic perennial graminoids were *Poa angustifolia*, *Carex praecox* and *Festuca rupicola*. Beside of *M. alliacea* many other crucifers were abundant in the springtime including *Calepina irregularis*, *Arabidopsis thaliana*, *Thlaspi arvense*, *Thlaspi perfoliatum* and *Capsella bursa-pastoris*.

The sites in Körmend were characterised by degraded mesophilous grasslands formed on sand, loam and gravel river sediments characterised by *Poa angustifolia*, *Arrhenatherum elatius*, *Dactylis glomerata* and some places with *Alopecurus pratensis*. The species occurred on heavily degraded places where the perennial vegetation was removed by ditch reconstruction works. On these newly formed surfaces the species appeared together with annual weeds like *Thlaspi arvense*, *Capsella bursa-pastoris*, *Lamium amplexicaule*, *Papaver dubium*, *Chenopodium album*, *Bromus arvensis* and various short-lived *Veronica* species (most frequent were *V. persica*, *V. polita* and *V. arvensis*).

The average shoot height of the species is 60 cm, based on our measurements. The average distance from the pavement of the individuals is 253 cm.

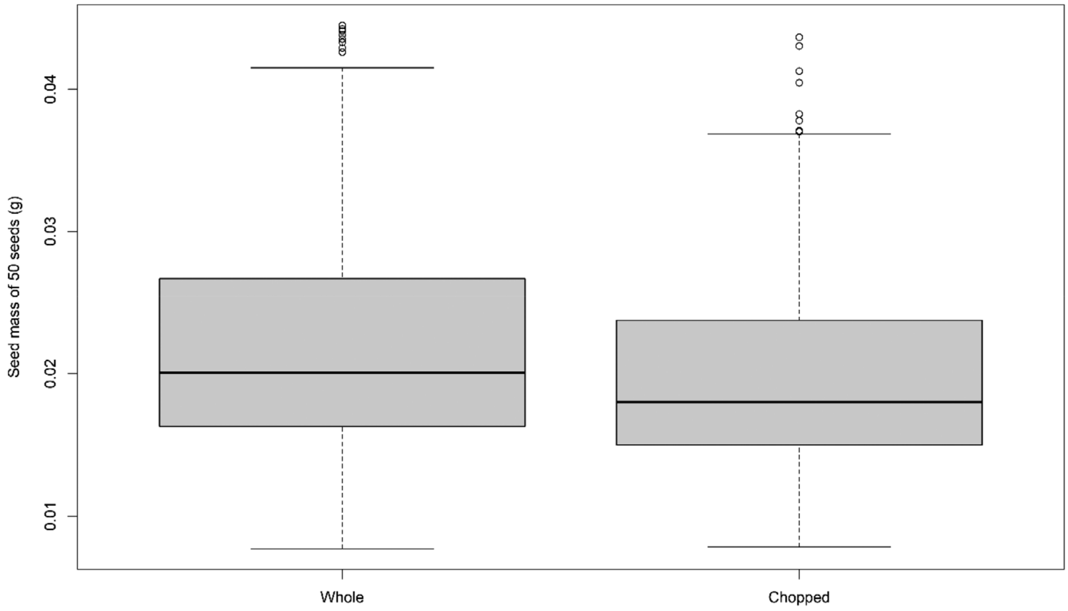

FIGURE 3 Seed mass of *Mummenhoffia alliacea* as a function of mowing type.

TABLE 1 GLMM showing the effect of collection time on seed mass collected at Olaszliszka.

	Estimate	SE	*t* value	*p* value
Intercept	0.01	0.001	11.78	<0.001
10 May	0.01	0.001	5.79	<0.001

3.2 | Study of reproductive characteristics

Mummenhoffia alliacea has a white, loose raceme inflorescence with an average of 123 flowers per individual. The small, four-petalled flowers produce a 6–7 mm silicle fruit with an average of eight seeds, so that the species can produce an average of 984 seeds per individual. The thousand-seed mass of the species is 0.42838 g as measured.

3.3 | Testing the effect of mowing

When testing the effect of mowing type on seed mass, we found that seeds from chopped individuals had significantly lower mass than seeds from whole (mowed) individuals (LMM, $p > 0.01$, Figure 3).

Seeds of whole plants showed a germination rate of 16.8%, while seeds of the chopped plants germinated at a rate of 10.4%. This corresponded to a germination of 84 out of 500 seeds for whole plants and 52 out of 500 seeds for the chopped plants. Although seeds germinated at a lower rate in the chopped individuals, it was shown that mowing type had no significant effect (GLMM, $p = 0.637$) on the germination rate of *M. alliacea* seeds.

We also found that seeds collected in May were characterised with higher seed mass than those collected in April (Table 1, Figure 4).

A total of 86 germs were observed when examining the germination of seeds collected at different times, which represented a germination rate of 8.6% for the entire experiment (Table 2).

Based on our results, it seems that the germination rate of seeds collected in May highly increases compared to seeds collected in April (Table 3).

3.4 | Testing salt tolerance

During the experiment a total of 113 seeds germinated out of 1600 tested. The highest germination rate was 27.5%, which was observed at 0.15% NaCl concentration, and no germination was observed at the two highest concentrations (Table 4). Germinability decreased significantly with increasing substrate NaCl concentration as shown by GLMM (Table 5).

4 | DISCUSSION

In our study, we surveyed three new populations of *M. alliacea* and one known but undocumented population. Of these, two populations were found along roadsides and two on river embankments. Based on the literature, the populations we studied were the first to be found on paved roadsides in Hungary, and no records of the species occurring in river embankment in the past were found. We have shown that there was a significant difference in the mass of seeds collected at different times. Significant differences were also found in the seed mass of individuals mowed by different methods, but the type of mowing had no significant effect on seed germination. Furthermore, the remarkable salt tolerance of the species was also highlighted.

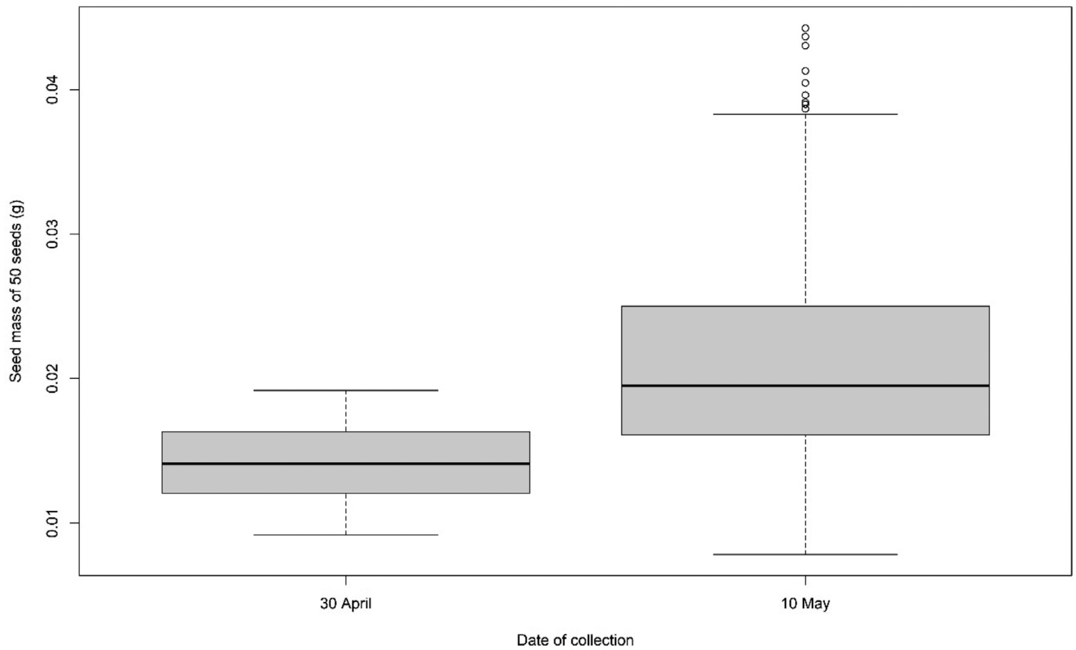

FIGURE 4 Differences between seed masses collected at different times at Olaszliszka.

TABLE 2 Germination of seeds from two different collection dates.

Collection place	Collection time	Total number of seeds	Number of seeds germinated	Germination rate
Olaszliszka	30 April 2021	500	2	0.4%
Olaszliszka	10 May 2021	500	84	16.8%

TABLE 3 GLMM showing the relationship between germination (rate) and the date of collection of seeds.

	Estimate	SE	*t* value	*p* value
Intercept	−6.491	1.054	−6.159	<0.001
10 May	4.003	1.133	3.534	<0.001

4.1 | Assessment of the occurrence of the species

In Hungary, *M. alliacea* has previously been described mainly from arable fields (Csiky, 2005; Károlyi et al., 1972), but our results showed that paved roadsides and river embankments can be suitable habitats for the species. Károlyi and Pócs (1968) stated, that it is likely that there may be more populations of the species in Hungary than previously thought due to the mild sub-Atlantic-Sub-Mediterranean climate of south-western Hungary, that provides favourable conditions. However, according to Pinke et al. (2006) the distribution of *M. alliacea* in Hungary is not primarily determined by climate but rather by soil type. Our study further confirms this, since the recently discovered populations are all found on loamy, compact soils (Pásztor et al., 2018).

Literature showed that the species is able to adapt to a wide range of habitats under anthropogenic influence. Populations are known from very different disturbed habitat types in several countries. In Germany *M. alliacea* has been found in gardens and courtyards (Zange, 1995) and in Austria along a cycle and hiking path (Hofbauer, 2005). In the United States, it is considered as an alien species from Europe (Thompson et al., 2013). A few specimens of the species were first discovered in several states in the mid-20th century and since then it has become widespread in several northern states (Al-Shehbaz, 2010; Block & Rhoads, 1998; Lamont & Young, 2006; Thieret & Baird, 1985; Thompson et al., 2013). In the United States, it occurs in fields, pastures and roadsides (mainly along highways) (Steury, 2000), and in recent decades due to its rapid spread it has become an invasive species (Cusick, 2015).

In Hungary, the propagules of the species can easily be transported from former cultivation sites to the paved roadside by machines working in the fields. From there they can be transported by motor vehicles, as the mud attached to the vehicles can contain large amounts of propagules (Clifford, 1959). Airflow generated by vehicles (Ross, 1986) and mowing machines can also help the dispersal of seeds into the road network (Vitalos & Karrer, 2009). However, several factors make it difficult for some species to survive along roads, such as trampling by vehicles, heavy metal pollution or high salinity of the soil. In winter, very high levels of salt are deposited on roads, but concentrations decrease rapidly with the distance from the road (Zehetner et al., 2009). Heavy metal contamination along roads is also significant (Huber et al., 2016), and like salt, heavy metal concentrations also decrease rapidly away from the road (Pagotto et al., 2001).

TABLE 4 Germination rates on different NaCl media.

NaCl concentration	0.0%	0.15	0.30	0.45	0.60	0.75	0.90	1.05
Germination rate	13.5%	27%	11%	1.5%	2.5%	0.5%	0%	0%

TABLE 5 Effect of NaCl concentration on germination (both linear and non-linear).

	Estimate	SE	t value	*p* value
Intercept	−4.93	0.40	−12.24	<0.001
NaCl concentration (linear term)	−119.11	13.70	−8.69	<0.001
NaCl concentration (non-linear term)	−37.11	11.01	−3.37	<0.001

In addition to trampling, increased salinity and high heavy metal concentrations may also play a role in the zonation of different species along roads. Several hyperaccumulator species belong to the genus *Thlaspi* and although *M. alliacea* is not classified as a hyperaccumulator, it is able to accumulate higher amounts of heavy metals (Reeves, 1988).

In their study, Pysek and Prach (1994) highlighted the role of linear facilities in the spread of species. In addition to roads, river embankments can also be considered as linear facilities, which are mowed twice a year in Hungary (Bátori, Vojtkó, et al., 2020). In total, river embankments cover 150 000 ha in the country. These areas are mainly used for mowing, although grazing used to be practised, but was banned due to flood protection concerns (Sallai et al., 2011). Bátori et al. (2016) reported that river embankments provide habitat for a number of vascular plant species that disappeared from the surrounding landscape or are rare, and therefore play an important role in maintaining landscape diversity. Along the Fehér-Körös river, we found small to large clusters of individuals along 9 km of embankment, so it is possible that mowing vehicles and machinery may have played a role in seed dispersal in this case.

4.2 | Reproductive traits and germination experiments

It was estimated that the species can produce almost 1000 seeds per individual, while Matthies (1990) reports that a single individual of *T. arvense* can produce up to 2500 seeds based on his measurements. The thousand-seed mass of *M. alliacea* is 542 mg according to Török et al. (2016) and 428 mg according to our own measurements. The seeds of the chopped individuals had a significantly lower seed mass, than of the whole ones. This is probably due to less nutrients available for the formation of fruits from the smaller shoot pieces than from the whole shoots. It has been observed previously in other species that mowing decreases seed mass (Rampton, 1969). A significant difference was also found between the seed mass of seeds collected at two different times, with seeds collected earlier having significantly lower mass than those collected later, as they had less time to develop.

Our germination experiment was a good way to find out the lowest salt concentration at which the species would definitely not be able to survive. According to the FAO (Abrol et al., 1988), soils with salt concentrations between 0.60% and 0.75% are already classified as strongly saline, where few species can survive. These concentrations were the highest salinity soils on which germination was still observed in our study. In a previous study, we tested the salt tolerance of several roadside halophyte plant species and the maximum salt tolerance of *Podospermum canum* coincided with the salt tolerance of *M. alliacea* (Fekete et al., 2022). According to Fekete et al. (2018), the average salinity of roadside soil in Hungary is 0.14–0.15 m/m% at a distance of 1 m from the pavement and decreases with distance from the road. We can therefore see that the salt tolerance of *M. alliacea* is remarkable.

Mowing type had no significant effect on seed germination, contrary to our previous hypothesis. We have seen that the germination of seeds collected in the second half of May has increased fifty-fold compared to seeds collected in April. Therefore, mowing in May or early June may be optimal for the conservation of the species.

In recent decades, intensive agricultural cultivation has led to the decline of several weed species previously common in Hungary, such as *Adonis flammea*, *Vaccaria hispanica*, *Misopates orontium*, *Vicia lutea* and *Ranunculus lateriflorus*, which are similar to *M. alliacea* (Bauer & Barna, 1999; Csiky, 2004; Farkas, 1999; Frank et al., 1998). The decline of these species is also being attributed to the spread of neophyte and nitrophilous weed species such as *Ambrosia artemisiifolia*, *Amaranthus retroflexus*, *A. chlorostachys*, *Chenopodium album*, *Datura stramonium*, *Consolida orientalis*, *Galium aparine*, *Sorghum halepense*, *Cirsium arvense* and *Solanum nigrum* (Pinke et al., 2011; Pinke & Pál, 2005). The impoverishment of weed flora due to intensive agricultural cultivation is a common trend in other countries too (Fried et al., 2009; van Elsen, 2000). Native weeds are pioneer species of secondary habitats (Bunting, 1960) and play an important role in maintaining biodiversity, since they facilitate the maintenance of pollinators and provide habitat and food sources for many other organisms, including species suitable for human consumption, medicinal plants and fodder. They are also useful from an agricultural point of view, as they protect the soil from erosion, reduce soil nitrate oversaturation and create a favourable microclimate for soil microorganisms (Pinke & Pál, 2005).

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/wre.12584.

DATA AVAILABILITY STATEMENT

All sample data used in the analyses are intended to be available from figshare.

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