











YOUNG VOICES AND VISIONS FOR THE
UN DECADE OF RESTORATION

RESEARCH ARTICLE

Native species can reduce the establishment of invasive alien species if sown in high density and using competitive species

Edina Csákvári^{1,2,3,4} , Nóra Sáradi^{1,2,3} , Boglárka Berki⁵ , Anikó Csecserits^{1,2} ,
Anna Cseperke Csonka⁵ , Bruna Paolinelli Reis^{2,6} , Katalin Török^{1,2} , Orsolya Valkó^{1,7} ,
Márton Vörös^{2,5} , Melinda Halassy^{1,2} 

Invasion of alien species is one of the main drivers of land degradation threatening both natural and managed ecosystems. Ecological restoration is crucial in controlling invasion to improve biotic resistance and avoid further land degradation. We investigated the possibility of controlling the establishment of invasive alien species (IAS) by native seed addition. We tested if trait similarity or increased propagule pressure of native species results in the suppression of IAS at the early stage of development. We set up a sowing experiment with three widespread IAS in Hungary of different life forms and functional groups (*Asclepias syriaca*, *Conyza canadensis*, *Tragus racemosus*) and four Pannonic sand grassland species (*Festuca vaginata*, *Galium verum*, *Gypsophila paniculata*, *Saponaria officinalis*). We found no significant differences in germination ability and seedling emergence between native species and IAS, despite the differences in thousand-seed weight. Using univariate general linear models, we found that the seedling establishment of IAS can be reduced by adding native species at high densities but also depending on the species identity. Instead of species of similar traits, the seeding of a competitor perennial grass of sand grasslands (*F. vaginata*) reduced the seedling emergence of all studied IAS the most. Our results confirm that IAS can be effectively controlled by native seed addition in the early establishment stage, especially applying higher densities and competitive species. We conclude that invasion-resistant restoration can be achieved by the combination of several factors, including high-density sowing of native species that match IAS in the early stage of development.

Key words: germination capacity, grassland restoration, invasion-resistant restoration, seed-based restoration, seedling establishment, thousand-seed weight

Implications for Practice

- To increase biotic resistance and avoid primary or secondary invasion, species-specific native seeding can be an effective strategy in ecological restoration.
- The establishment of invasive alien species can be suppressed by using high-seed density of native species to match invasive propagule pressure.
- Invasion-resistant seed mixtures can be created by adding competitive species, for example, dominant perennial grasses and species with early life traits (germination and seedling establishment) similar to IAS.
- Targeting multiple factors simultaneously (e.g. propagule pressure and trait similarity) can increase community resistance to invasion, but further research is needed on the factors to be included at a species-specific level.

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¹National Laboratory for Health Security, Institute of Ecology and Botany, Centre for Ecological Research, Karolina út 29, 1113 Budapest, Hungary

²Centre for Ecological Research, Institute of Ecology and Botany, Alkotmány u. 2-4, 2163 Vácrátót, Hungary

³Environmental Sciences Doctoral School, Hungarian University of Agriculture and Life Sciences, Páter Károly u. 1, 2100 Gödöllő, Hungary

⁴Address correspondence to E. Csákvári, email csakvari.edina@ecolres.hu

⁵Eötvös Loránd University, Doctoral School of Biology, Department of Plant Systematics, Ecology and Theoretical Biology, Pázmány Péter sétány 1/C, 1117 Budapest, Hungary

⁶Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, C2, Piso 5, 1749-016 Lisbon, Portugal

⁷Centre for Ecological Research, Institute of Ecology and Botany, "Lendület" Seed Ecology Research Group, Alkotmány u. 2-4, 2163 Vácrátót, Hungary

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Introduction

Invasive alien species (IAS) are among the major drivers of human-driven biodiversity loss globally (Jaureguiberry et al. 2022). The problem is well illustrated by the fact that the economic cost of damages caused by IAS is estimated for at least US\$1.288 trillion over the past 50 years globally (Zenni et al. 2021). Ecological restoration is increasingly recognized as a relevant tool to combat land degradation (Aronson et al. 2020) and directly controlling IAS is often part of restoration projects (Weidlich et al. 2020). However, restoration activities generally also imply disturbance, and restored habitats with insufficient vegetation cover are prone to invasion (Hobbs & Richardson 2010), including secondary invasion following the suppression of a dominant invader (Pearson et al. 2016). There is an urgent need to develop innovative, effective, and proactive strategies that help improve the biotic resistance of restored communities limiting the establishment and further spread of invasive species, particularly in newly disturbed areas and after the removal of invasive species to avoid secondary invasion (Guo et al. 2018).

It has been suggested that rapid establishment of a competitive native vegetation cover can reduce the invasion and spread of alien species (Hess et al. 2019, 2022 and citations within) mainly through niche pre-emption and resource acquisition resulting in increased biotic resistance (Levine et al. 2004; Yannelli 2021). Native seed addition is an effective tool for restoring degraded areas (Török et al. 2011; Kövendi-Jakó et al. 2019) and also in post-invasion restoration (Bucharova & Krahulec 2020). However, restoration measures rarely enable communities to totally resist invasion in the long term, rather they control the abundance and further spread of invasive species (Levine et al. 2004). The question arises, what to seed and how to increase biotic resistance? Funk et al. (2008, 2016) proposed assembling communities relying on trait similarity based on the limiting similarity hypothesis (Hess et al. 2020). Incorporating species functionally similar to known high-risk invaders in seed mixtures may result in restored communities that better resist invasions (Young et al. 2009; Drenovsky & James 2010; Cleland et al. 2013; Larson et al. 2013; Laughlin 2014). Yannelli et al. (2018) suggest that instead of trait similarity, propagule pressure can be more important in shaping biotic resistance. Higher sowing density of native species to match propagule pressure of invasive species increases the chances of native establishment and the formation of a dense native cover that increases biotic resistance (D'Antonio et al. 2001; DiVittorio et al. 2007; Schantz et al. 2015; Yannelli et al. 2018). These mechanisms do not act separately, and their success is dependent also on the life form or functional group of invader species, the native species involved and environmental conditions.

Early traits, such as high germination rate, fast germination or great germination plasticity, are particularly important to the success of invasive species (Colautti et al. 2006; Wainwright & Cleland 2013; Gioria & Pyšek 2017; Gioria et al. 2018) and should be also considered in ecological restoration (Yannelli et al. 2017). Although much knowledge has been accumulated about the general comparison of plant characteristics of native and invasive species (Pyšek & Richardson 2008), little is known about how early resource limitation influence the invasion

success of non-native species during early establishment phases (Yu et al. 2020). Even less is known about the possible suppressive impact of the combination of trait similarity and higher propagule pressure of native species in relation to IAS (Yannelli et al. 2017, 2018).

The aim of the present study is to test the impact of trait similarity and native propagule pressure on the germination of invasive species. European dry grasslands are very vulnerable to neophyte invasion (Axmanová et al. 2021), therefore, it is crucial to develop effective methods to prevent and control their invasion (Hobbs & Humphries 1995). We used sand grasslands (6260 Pannonic sand steppes in the EU Habitats Directive, ŠeffEROVÁ StanOVÁ et al. 2008) as model systems, which still cover large areas within Hungary and can regenerate well on abandoned croplands (Csecserits et al. 2011; Csákvári et al. 2021), but the sandy regions and abandoned croplands in Hungary are also prone to invasion (Török et al. 2003; Botta-Dukát 2008; Csecserits et al. 2011). We included three widespread invasive species that represent different life forms and functional groups: horseweed (*Coryza canadensis* [L.] Cronquist; annual forb), common milkweed (*Asclepias syriaca* L.; perennial forb), and stalked bur grass (*Tragus racemosus* [L.] All.; annual grass). We have set up a laboratory germination experiment and a pot experiment to answer the following questions: (1) Is the germination capacity and seedling establishment of invasive species higher than that of native species? (2) Is the seedling establishment of invasive species less successful when grown together with native species? (3) Can trait similarity or propagule pressure be used to decrease the seedling establishment of invasive species? We hypothesized the following: (i) similar traits of selected native and IAS result in similar germination capacity and seedling establishment; (ii) competition reduces seedling establishment of IAS when sown together with native species, and (iii) propagule pressure suppresses seedling establishment of IAS more than trait similarity because it results in earlier resource limitation.

Methods

Study Species

We selected three IAS representing different life forms and functional groups that are of concern in the Pannonian region (Botta-Dukát & Balogh 2008) and at a broader scale (Axmanová et al. 2021; Follak et al. 2021). Horseweed (*Coryza canadensis*) native to Central and North America is one of the most widespread invasive annual forbs in Hungary that threatens primarily agriculture and vineyards, but can develop dominant stands also in sandy areas in abandoned fields and can invade primary grasslands along roadsides. Common milkweed (*Asclepias syriaca*) is a perennial clonal forb from the lowlands of the eastern part of North America causing the most serious problems to agriculture and nature conservation in the sandy regions of Hungary (Csiszár 2012). Once established it can stall the regeneration of abandoned fields, but it can also spread into semi-natural grasslands in case of soil disturbance (Kelemen et al. 2016; Csecserits et al. 2021). Stalked bur grass (*Tragus racemosus*) is a C4 annual grass native to the

subtropical and Mediterranean parts of South Africa. In Hungary, it occurs mainly in sandy areas with exposed soil, like abandoned arable land, vineyards, orchards, or forest renovations (Csizsár 2012).

Three native species were selected to match IAS based on multi-trait similarity following the methodology proposed by de Bello et al. (2021). Native species pool was derived from a regional study in the Kiskunság region, Central Hungary (Cseceserits et al. 2011). We involved all traits with available data for 214 native sand grassland species in the analysis (in total 12 traits; Table S1). Trait data were measured locally (Lhotsky et al. 2016) or originated from the Pannonian Database of Plant Traits (PADAPT, <https://padapt.eu/>) and TRY database (Kattge et al. 2011). Multi-trait dissimilarity of species was calculated using the “gawdis” function of the “gawdis” package (de Bello 2021), where the connected traits (flowering min and flowering length, generative height min and max) were grouped. In the case of life form fuzzy coding was used. Since generally no single species can match the invasive species, we selected from the first 30 native species with the best multi-trait similarity to each IAS. The final choice was determined based on the fidelity of species to sand grasslands (Cseceserits et al. 2011), environmental requirements, and seed availability. The final list of matching native species included Lady’s bedstraw (*Galium verum* L.), Common baby’s-breath (*Gypsophila paniculata* L.), and Wild Sweet William (*Saponaria officinalis* L.). Additional to species selected based on trait similarity, we included a native competitor, perennial grass *Festuca vaginata* Waldst. & Kit. ex Willd. and a seed mixture containing all four native species in a ratio 1:1:1:1. Multi-trait similarity of the selected native species to IAS is presented in Table S2. Nomenclature follows Király (2009).

Generative diaspores of all IAS were collected from natural populations along roadsides or on former arable lands in the Kiskunság National Park. *T. racemosus* was collected in the Pészéradasi rétek protected area on 1 September 2021. *C. canadensis* and *A. syriaca* were collected in the Fülöpháza Sand Dunes protected area on 17 September 2021. The seeds of *A. syriaca* were separated from the tufts of silky hairs. *C. canadensis* and *T. racemosus* were cleaned using sieves of different mesh sizes (up to 0.32 mm). In the case of *C. canadensis* achene remained attached with the pappus. *T. racemosus* was cleaned to get caryopsis without any appendices. All native diaspores were purchased from a local seed producer. All diaspores were dry-stored after cleaning until further processing. Names of the morphological units follow Bojnanský and Fargašová (2007). In the following, the term seed is used for all generative diaspores.

Thousand-Seed Weight Measurement

Five seed sorts of 100 seeds for each species were counted to measure thousand-seed weights (TSW). Only intact and solid seeds were included. The air-dry weights of seeds were measured with 0.0001 g accuracy using a Sartorius BL1205 type analytical balance. The results were averaged for each taxon. The TSW was expressed in grams of 1,000 seeds (g).

Germination Capacity Measurement

We tested the germination capacity of the species in a germination chamber. Fifty unsterilized seeds were placed on standard filter paper in 9-cm diameter sterilized Petri dishes in 10 replicates for each species ($N = 500$ seeds in total per species). For species known to have dormancy (*A. syriaca*, *T. racemosus*, and *S. officinalis*), seeds were stratified at $5 \pm 2^\circ\text{C}$ for 3 weeks prior germination (25 October 2021–15 November 2021). The filter paper was moistened by 5 mL distilled water and Petri dishes were sealed with parafilm to retard loss of water during the stratification. After the stratification period, all other samples were moistened by 5 mL distilled water and put in a germination chamber (PHCbi Plant Growth Chamber MLR-352) on 15 November 2021 and kept under alternating light and temperature conditions (8 h light under 3 LS 25°C and 16 h dark 10°C). Petri dishes were checked every 2–3 days and the filter paper moistened if necessary. Germinated seeds were counted every week for 7 weeks until 3 January 2022. Seeds were considered to be germinated when the radicle or plumule protruded ≥ 2 mm. Germinated seeds were removed from the Petri dishes. In the end, the maximum germination percentage (%) was determined to express germination capacity.

Seedling Establishment in Pot Experiment









We set up a pot experiment to test the impact of native species and their propagule pressure on the seedling establishment of IAS in a factorial design (Table 1). We used 20 cm \times 20 cm \times 23 cm (5.7 L) pots, filled with a 2:1 mixture of potting soil and sand. In the basic treatment, we placed 12 seeds of native species and 12 seeds of IAS in each pot in a regular design to avoid overlaps on 5 October 2021. In case of higher propagule pressure for native species, we used 60 seeds. All seeds were put on the soil surface, except for *A. syriaca* that is known to require burial (Yenish et al. 1996). The seeds of *A. syriaca* were pressed 1 cm deep in the soil and covered with sand. All pots were placed in an unheated foil tent in seven blocks corresponding to the seven replicates per treatment and rows within blocks were permuted according to native species. All pots were kept under natural light and temperature conditions and were watered regularly. We monitored hourly temperature and humidity data by EasyLog EL-USB-2 data logger (Table S3). We counted emerging seedlings for 9 weeks from 20 October 2021 to 15 December 2021, then additionally once in March and April 2022 (Fig. 1). In the end, the maximum germination percentage (%) was determined to express seedling emergence (%) of species.

Data Analysis

Paired samples *t*-tests were used to calculate significant differences between germination capacity and seedling emergence of native and IAS.

Two-way analysis of variance (univariate general linear model) with Tukey’s honest post hoc significance test was used to find the dependence of the seedling emergence of IAS on treatment (native species and mix), propagule pressure and their interaction. We built three separate models for the three studied IAS: *A. syriaca*, *C. canadensis*, and *T. racemosus*. In all models,

Table 1. Experimental design in the pot experiment including the number of seeds for native species (green numbers)/number of seeds of IAS (red numbers).

		Invasive Alien Species			
		X	<i>Asclepias syriaca</i> 	<i>Conyza canadensis</i> 	<i>Tragus racemosus</i> 
Native species	Propagule pressure				
X	None	0/0	0/12	0/12	0/12
<i>Festuca vaginata</i> 	Low pressure	12/0	12/12	12/12	12/12
	High pressure	60/0	60/12	60/12	60/12
<i>Galium verum</i> 	Low pressure	12/0	12/12	12/12	12/12
	High pressure	60/0	60/12	60/12	60/12
<i>Gypsophila paniculata</i> 	Low pressure	12/0	12/12	12/12	12/12
	High pressure	60/0	60/12	60/12	60/12
<i>Saponaria officinalis</i> 	Low pressure	12/0	12/12	12/12	12/12
	High pressure	60/0	60/12	60/12	60/12
1:1:1:1 Mix 	Low pressure	12/0	12/12	12/12	12/12
	High pressure	60/0	60/12	60/12	60/12

the dependent variable was the seedling emergence of invasive species, and fixed factors were treatment with six levels (control, seeded together with *F. vaginata*, *G. verum*, *G. paniculata*, *S. officinalis*, and mix) and propagule pressure with three levels (0, 12, or 60 seeds). In each case, model residuals were checked for normality (Shapiro–Wilk test) and homogeneity of variances (Levene test). Statistical analyses were carried out with the R statistical software (R Core Team 2019).

Results

Thousand-Seed Weight

The invasive species had both the highest (*Asclepias syriaca* 5.1022 g) and the lowest (*Conyza canadensis* 0.034 g) TSW. Among the native species, *Saponaria officinalis* had the highest

(1.7032 g), and *Festuca vaginata* had the lowest TSW (0.3576 g) (Table 2).

Germination Capacity and Seedling Emergence of Native and Invasive Species

The germination capacity under laboratory conditions was the highest for native *S. officinalis* (89.6%), followed by invasive *A. syriaca* (81.8%) and native *Gypsophila paniculata* (75.6%). The lowest germination capacity was found for native *Galium verum* (34.4%). The germination capacity of IAS was not different from native species on average ($t = -1.602$, $df = 29$, $p = 0.120$; Table 3).

In the pot experiment, when seeded separately, *Tragus racemosus* had the highest (59.5%) and *Galiumverum* (21.4%) the lowest percent of emerging seedlings. The emergence of



Figure 1. Seedlings of (A) *Festuca vaginata*, (B) *Galium verum*, (C) *Gypsophila paniculata*, (D) *Saponaria officinalis*, (E) *Asclepias syriaca*, (F) *Conyza canadensis*, (G) *Tragus racemosus*. Photos taken by the Restoration Ecology Research Group.

invasive species was better than native ones on average, but this difference was not significant ($t = -1.791$, $df = 20$, $p = 0.088$; Table 4).

The Impact of Native Species and Propagule Pressure

The univariate general linear model showed that propagule pressure and the interaction of treatment and propagule pressure had a significant impact on the seedling emergence of *A. syriaca* at $p < 0.05$ (Table S4; Fig. 2). Larger propagule pressure of native species resulted in a lower emergence of *A. syriaca*, but this also

depended on the species in question. The highest emergence was found in pots seeded together with the smaller pressure of seeds of *S. officinalis* and significantly lower emergence was found in pots seeded with *F. vaginata*, *G. verum* or Mix with high propagule pressure and in *G. paniculata* pots with low propagule pressure. Significant difference was also found between the *S. officinalis* and Mix treatments, the latter resulting in lower seedling emergence (Table S5).

In the case of *C. canadensis*, only the interaction of treatment and propagule pressure had a significant impact ($p = 0.0187$) on the seedling emergence (Table S4; Fig. 3). According to

Table 2. TSW data with measured morphological units of native and invasive species. TSW was measured in the laboratory based on five samples of 100 seeds per species. Data from HUSEED (Peti et al. 2017), PADAPT (<https://padapt.eu/>), and SID (Royal Botanic Gardens Kew 2018) databases are given for comparison.

Species	Measured TSW (g)			Database TSW (g)		
	Morphological Unit	AVG	SD	HUSEED	PADAPT	SID
<i>Festuca vaginata</i>	Caryopsis	0.3576	0.0188	0.3350	0.647	0.5438
<i>Galium verum</i>	Achene	0.3838	0.0047	0.3128	0.418	0.4957
<i>Gypsophila paniculata</i>	Seed	0.5578	0.0424	0.6597	0.406	0.7570
<i>Saponaria officinalis</i>	Seed	1.7032	0.0386	1.5329	1.600	1.6911
<i>Asclepias syriaca</i>	Seed	5.1022	0.1523	na	5.858	6.2266
<i>Conyza canadensis</i>	Achene with pappus	0.0340	0.0015	na	0.056	0.0672
<i>Tragus racemosus</i>	Caryopsis	0.3580	na	na	0.591	0.4599

Table 3. Germination capacity (%; mean \pm SE) of native and IAS in the laboratory experiment. The mean value and the standard error of the measured data were calculated from the original dataset. No significant difference was found in germination capacity between native and invasive species (paired samples *t*-test, $p < 0.05$). Laboratory germination was tested in Petri dishes under controlled conditions (8 h light under 3 LS 25°C and 16 h dark 10°C) based on 10 samples of 50 seeds per species. Data from HUSEED (Peti et al. 2017) and SID (Royal Botanic Gardens Kew 2018) databases are given for comparison. In case of data from HUSEED and SID databases, we reported mean values and mean \pm SE values, where it was possible.

	Measured Germination Capacity (%)		Database Germination Capacity (%)		
	AVG	SE	HUSEED	SE	SID
Native species	60.6	3.8	40.8	na	90.0
<i>Festuca vaginata</i>	42.8	3.9	8.0	0.0	91.3
<i>Galium verum</i>	34.4	1.3	66.0	7.2	81.6
<i>Gypsophila paniculata</i>	75.6	2.3	88.3	4.2	92.3
<i>Saponaria officinalis</i>	89.6	1.5	1.0	0.6	94.8
IAS	60.5	4.5	na	na	na
<i>Asclepias syriaca</i>	81.8	1.0	na	na	na
<i>Conyza canadensis</i>	44.0	7.6	na	na	93.1
<i>Tragus racemosus</i>	55.8	7.4	na	na	na

Tukey's post hoc significance test though, no significant difference was found between the paired variables (Table S5).

Both the treatment and the propagule pressure had a significant impact on the seedling emergence of *T. racemosus* at $p < 0.05$, but not their interaction (Table S4; Fig. 4). According to Tukey's post hoc significance test, significant difference was found between the *G. paniculata* and *F. vaginata* treatment, the latter resulting in lower seedling emergence (diff = 25.57, $p = 0.035$, Table S5). Higher propagule pressure also led to lower seedling emergence.

Discussion

Using controlled experiments, we investigated traits related to the early establishment phase (TSW, germination capacity, and seedling establishment) of native and IAS and the early competitive impacts of trait similarity and propagule pressure of native species on IAS establishment to provide guidance for possible seed-based restoration methods. We have found that the selected native species were similar to the studied IAS in

Table 4. Seedling emergence (%; mean \pm SE) of native and IAS in the pot experiment. The mean value and the standard error of the measured data were calculated from the original dataset. No significant difference was found in seedling emergence between native and invasive species (paired samples *t*-test, $p < 0.05$). Seedling establishment was tested in a foil tent in 5.7-L pots on a 2:1 mixture of potting soil and sand with regular watering.

Seedling emergence (%)	Mean	SE
Native species	33.9	3.4
<i>Festuca vaginata</i>	39.3	7.7
<i>Galium verum</i>	21.4	5.1
<i>Gypsophila paniculata</i>	33.3	7.5
<i>Saponaria officinalis</i>	41.7	5.1
IAS	43.3	4.9
<i>Asclepias syriaca</i>	32.1	7.8
<i>Conyza canadensis</i>	38.1	7.9
<i>Tragus racemosus</i>	59.5	6.9

germination capacity and seedling establishment, although different in TSW, and that the seedling establishment of IAS can be reduced by native species when seeded in higher quantities and using competitive species, but the results are species specific.

We measured a lower TSW for almost all species compared to international data (Seed Information Database [SID], Royal Botanic Gardens Kew 2018) and the values presented in the PADAPT (<https://padapt.eu/>), but generally higher than in the Hungarian Seed Database (Peti et al. 2017). Besides slight differences in the methodology, this general tendency probably reflects the aridity of the location of the collections compared to international data (Fenner 1992), and also the aridity of the year of collection compared to national data. In 2021, a 120-year-old Hungarian heat record was broken in Fülöpháza; 40°C were measured in June (OMSZ 2021). The climate affected all species equally, so we assume that it has no impact on the results of the experiment.

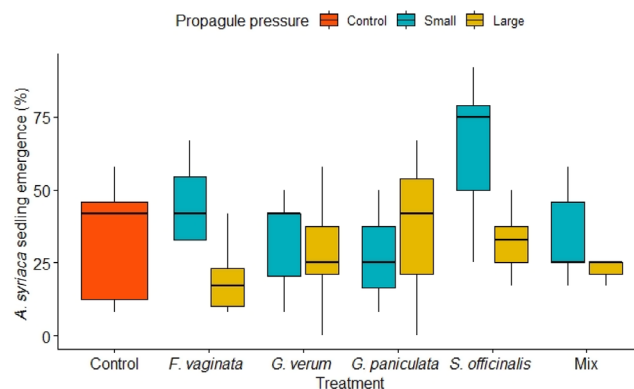


Figure 2. Seedling emergence of *Asclepias syriaca* seeded alone (control) or with native species with large (60 seeds) or small propagule pressure (12 seeds). Vertical axes of box plots show the seedling emergence of species, in percentage. Thick lines are medians, boxes are quartiles, whiskers are non-outlier minimum and maximum.

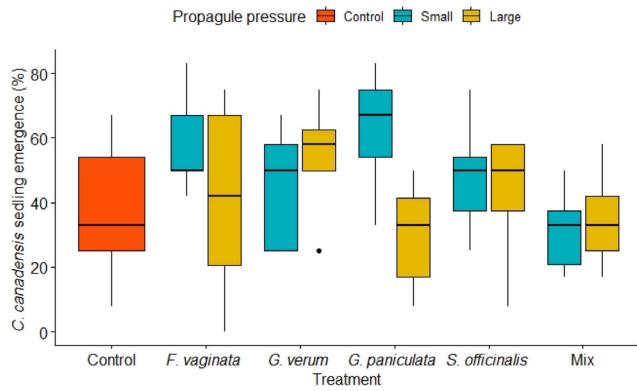


Figure 3. Seedling emergence of *Conyza canadensis* seeded alone (control) or with native species with large (60 seeds) or small propagule pressure (12 seeds). Vertical axes of box plots show the seedling emergence of species, in percentage. Thick lines are medians, boxes are quartiles, whiskers are non-outlier minimum and maximum, dots are outliers.

Our results confirmed the hypothesis that similar traits of selected native and IAS resulted in similar germination capacity and seedling establishment. Germination is a critical step in plant invasion (Gioria & Pyšek 2017), and IAS tend to have higher germination rates than native species (Wainwright & Cleland 2013). Besides selecting native species based on resource-use traits (Funk et al. 2008), restorationists also need to apply species that can match IAS in early traits (Yannelli et al. 2017), such as seed germination traits (Jiménez-Alfaro et al. 2016) to achieve invasion-resistant restoration. Although we did not include seed germination directly when selecting our native species, the multi-trait approach applied resulted in species that fulfilled these criteria.

We also hypothesized that competition reduces seedling establishment of IAS when sown together with native species. This hypothesis was not supported. Seedling establishment in our controls where IAS were sown alone were not significantly different from other treatments. Although both restoration evidence and the literature (Hess et al. 2022) indicate that competition can be used to reduce the abundance of IAS, our data generally did not confirm this claim in the early developmental phase. A possible explanation for this could be that there is no significant competition yet between the species either above or below ground during the early growth period (Yu et al. 2020). Rather, germination and dormancy may be primarily determined by differences in environmental requirements, such as temperature and precipitation (Sax et al. 2007). Another reason could be that competition does exist already at the early phases of development, but the competition is species specific (Gioria & Osborne 2014) and species with higher or lower competition ability were averaged. It is also possible that sown *G. paniculata* and *S. officinalis* have allelopathic effects by saponins that inhibit IAS competition during germination phase (Mahalel 2015), although this was not confirmed in our study, except for *C. canadensis*.

In fact, we found significant differences in the seedling establishment at the species level. The studied three IAS, representing

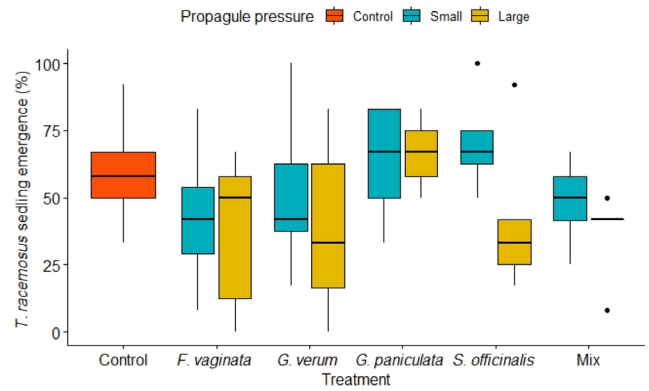


Figure 4. Seedling emergence of *Tragus racemosus* seeded alone (control) or with native species with large (60 seeds) or small propagule pressure (12 seeds). Vertical axes of box plots show the seedling emergence of species, in percentage. Thick lines are medians, boxes are quartiles, whiskers are non-outlier minimum and maximum, dots are outliers.

different life forms and functional groups, behaved differently when sown together with native species. The identity of sown native species had an impact on the seedling emergence of all IAS, at least in interaction with propagule pressure. Species identity had a direct significant impact on the studied annual grass (*T. racemosus*), whereas in the case of the forb species, perennial *A. syriaca* and annual *C. canadensis*, it had an impact in interaction with propagule pressure. Generally, co-seeding with perennial grass *F. vaginata* that is considered a competitor species of sand grasslands (Borhidi 1995) decreased the seedling emergence compared to some other treatments. Perennial forb *G. verum* and the seed mixture of all native species also had an impact on *A. syriaca*, but only in high density. Contrary to our expectations, trait similarity did not negatively affect seedling emergence, rather the presence of a competitor species and in one case a diverse seed mixture led to the suppression of IAS at the early developmental phase. Resistance to invasion was found to be realized through high plant density, biomass, vegetation cover or productivity of native species (Smith et al. 2004; Lulow 2006; Rinella et al. 2007) or species diversity (Levine & D'Antonio 1999; Levine 2000). Our results are in line with the finding that dominant native species, in particular perennial grasses, can effectively suppress IAS, as their extensive root system can well utilize available resources and result in a dense vegetative cover (Whitson & Koch 1998).

In general, larger propagule pressure of native species reduced the seedling establishment of IAS in our study. High propagule pressure of invasive species is a primary determinant of habitat invasibility in comparison with other controlling factors (Von Holle & Simberloff 2005). Taking advantage of this, if we can ensure greater propagule pressure by increasing the amount of native species, the competition can be tilted in favor of native species (D'Antonio et al. 2001; DiVittorio et al. 2007; Schantz et al. 2015; Yannelli et al. 2018). Although competition during the early growth period is generally negligible compared to other factors (Yu et al. 2020), high amounts of seedlings crowded together in the limited space of a pot

environment can result in early resource limitation (Funk et al. 2008) and thus the control of seedling emergence. Similarly to the findings of Yannelli et al. (2017, 2018), our results indicate that increased seed density can be an effective tool in IAS suppression already at the early developmental stages.

Species-specific and propagule pressure differences amplified each other's effects for two of the three studied IAS. Several studies show that targeting multiple factors simultaneously can increase invasion resistance, for example, high propagule pressure of native species applied together with high richness (Carter & Blair 2012), species of fast vegetative development (Yannelli et al. 2018), appropriate seed size (Yannelli et al. 2017) or priority (Schantz et al. 2015); native species of high biomass together with priority (Yannelli et al. 2020) or adequate functional groups (Byun & Lee 2017); priority of native species and limiting similarity (Byun et al. 2020). We suggest integrating more than two factors in searching for possibilities to increase biotic resistance. In a next step, we will study three factors, namely trait similarity, propagule pressure and priority.

Plant invasion causes major problems worldwide, which poses a severe threat to biological diversity. There is a need for close cooperation between ecologists, nature conservation experts and decision-makers to respond to the problem. Our results contribute to the development of a more comprehensive and integrated approach to invasive plant management, and provide a new scientific basis for the seed-based prevention and control of plant invasion in the Pannonian region and a better understanding of early competition between native and invasive species.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. List of functional traits for native sand grassland species used in the analysis.

Table S2. Results of multi-trait similarity of native and invasive species.

Table S3. Results of monitored monthly temperature and humidity data.

Table S4. Results of the univariate general linear model.

Table S5. Results of Tukey's post hoc significance test.

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