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Research Article

Long-term seed survival of common ragweed (*Ambrosia artemisiifolia* L.) after burial

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Abstract

Ambrosia artemisiifolia is a serious threat to human health and agricultural yield. Due to its annual growth form management should focus on the prevention of seed production in the long run. The long-term survival of ragweed seeds depends on the implementation of viable seeds to the persistent soil seed bank. In a field study, we tried to find out how long this species must be surveyed/managed to reach the goal of complete eradication after burial of seeds into mineral soil. We tested for the influence of different seed sources (origin), different soil depths of burial, different experimental sites in Middle Europe (labs), and duration of burial on the viability of seeds by germination test plus TTC-test. In our study, seed origin had a highly significant influence on the seed survival. In all the 10 years of the experiment, seeds sampled from a rural stand in Austria showed significantly lower viability rates than seeds from Hungary. The Hungarian seeds from arable fields had viability rates of up to 90% even after 10 years' burial. Burial depth (7 cm/25 cm) had no significant influence on the viability rates but we detected a serious influence of the experimental sites which can be caused either by the burial site conditions (differences in soil and climate) or by different implementation of the manuals for germination tests and colouration test using 2,3,5-triphenyltetrazolium chloride. The decline of viability within the 10-year period differed by seed origin, but was generally faster in the first few years but relatively low in the following years. Due to the fact that we found 30 to 90% viable seeds after 10 years burial there is substantial evidence that soil perturbation (digging animals, ploughing) should be avoided for even more than ten years in habitats that are highly infested with ragweed.

Key words: Control measures, dormancy, germination, invasive species, soil seed bank, weed management

Introduction

Common ragweed (*Ambrosia artemisiifolia* L.) is considered one of the most dangerous invasive alien weed species in Europe (Smith et al. 2013; Essl et al. 2015). First of all, its pollen causes allergic diseases in humans, inducing enormous costs due to resultant necessary medical care and sick-leaves (Schindler et al. 2015; Schaffner et al. 2020). Furthermore, ragweed causes substantial yield losses in several crops (Pimentel et al. 2005; Soliman et al. 2010; Novák et al. 2022), and alters the competitive balance in rare weed communities (Pál 2004) and some species rich grasslands (Karrer et al. 2011). Many projects were initiated by national and EU authorities that aimed at developing measures against ragweed (Buttenschøn et al. 2009; Karrer et al. 2011; Bullock et al. 2012; Söltner et al. 2016a; Müller-Schärer et al. 2018). A leaf feeding beetle (*Ophraella communa* Lesage) from the native region of ragweed got established in some parts of Europe (Bonini et al. 2015), something that might help greatly to overcome the problems caused by this dangerous weed (Augustinus et al. 2020; Schaffner et al. 2020; Keszthelyi et al. 2023).

The invasiveness of common ragweed was documented for Europe but also for other continents, i.e., Asia (Watanabe et al. 2002; Qin et al. 2014) and Australia (Parsons and Cuthbertson 2001). Both mechanical and chemical measures are able to reduce the aboveground populations of ragweed (Kazinczi et al. 2008; Buttenschøn et al. 2009; Söltner et al. 2016a). Nevertheless, in the case of long-time persisting populations ragweed recovers easily from the soil seed bank because the seeds can survive in the soil for up to 40 years. Toole and Brown (1946) documented that at least a few seeds were able to germinate after this long period of subterranean dormancy. Considering that one single individual of common ragweed is able to produce up to 94.000 seeds (Kazinczi et al. 2008) and these seeds are commonly installed in deeper soil horizons by ploughing, ragweed develops to a nasty weed in many crops, i. e., summer crops like maize, sugar beet, sunflower, and soybean (Kazinczi and Novák 2014; Karrer 2014).

In agroecosystems the average seed production is around 4000 achenes (Kazinczi et al. 2008) for one plant individual. The higher the plant biomass, the higher the seed yield (Lommen et al. 2018). The infestation of the arable soil weed seed bank with ragweed seeds is very high in Hungary and Austria (Kazinczi et al. 2008; Karrer et al. 2011) and was even enhanced during the last decade (Kazinczi and Pál-Fám 2018), ensuring long-term ragweed infestation in the cultivated areas.

Long-term experiments showed that the survival rates of weed seeds under field conditions were best in deeper soil layers. Toole and Brown (1946) found 6% of ragweed seeds still germinable after burial for 39 years at a depth of 22 cm. At least 21% and 57% of seeds buried in the soil at 8 cm and 22 cm, respectively, germinated 30 years after the start of the experiment.

However, ragweed seeds deposited on the soil surface under field conditions were found viable for 4 only years (Beres 2003; Kazinczi et al. 2011). Kazinczi et al. (2011) reported viability rates of 18% when ragweed seeds were stored under dry conditions at room temperature (20 °C) after 5 years. Intraspecific differences regarding germination characteristics of common ragweed are also known (Kazinczi et al. 2006; Onen et al. 2020). After 7, 6 and 4 years of dry storage, ragweed seed viability was 15, 45, and 72%, respectively (Kazinczi et al. 2011). After three years of storage, viability rates of ragweed seeds varied between 62 and 90%, depending on the origin of the tested populations (Kazinczi and Kerepesi 2016).

Soil perturbation by ploughing incorporates weed seeds like those of ragweed to deeper soil horizons where weed seed survival rates increase with burial depth (Froud-Williams et al. 1984). We know the maximum age of buried ragweed seeds from the Durvel burial experiment (Toole and Brown 1946) but there is still a lack of knowledge about the annual decrease of seed viability during the first years of burial. Therefore, a long-time experiment was initiated within the framework of the EU-project HALT Ambrosia (https://ojs.openagrar.de/index.php/JKA/article/view/1792) to test annually the ragweed seed viability buried at different soil depths for a period of one to ten years (Karrer 2016a, Karrer et al. 2016a). The main aim was to find out the annual decrease of seed viability in general but also germinability as major part of the viability.

Materials and methods

Two populations of ragweed seeds were sampled in autumn 2011 from arable fields in Hungary (Kaposvár: 46.368608, 17.851789; 148 m a.s.l.) and from ruderal arable fields in Austria (Hagenbrunn: 48.343333, 16.466278; 178 m a.s.l.). Seeds were air dried and stored at room temperature (± 20 °C).

Field experiments were established in the experimental farm areas of Kaposvár University (Kaposvár: 46.368608, 17.851789; 148 m a.s.l.) and of BOKU University (Groß-Enzersdorf: 48.199417, 16.557611; 154 m a.s.l.). Seeds were buried in winter 2011/2012 at two soil depths (upper layer (5–8 cm), and lower soil layer (25 cm).

In a pre-trial, another seed lot from Austria (Styria, Unterpurkla: 46.731500, 15.901528; 229 m a.s.l.) was sampled in 2010 and buried in the botanical garden of the BOKU University (Vienna: 48.237194, 16.332361; 236 m a.s.l.) in winter 2010/2011 at a soil depth of about 10 cm. This pre-trial gave us some valuable technical and practical experiences for the main trial.

Portions of 50 seeds were enclosed each in polyester mash before burial (Fig. 1, left). For the main trial, we buried 50 bags with 50 seeds each at the two different soil depths in Kaposvár and Groß-Enzersdorf, and for the pre-trial 70 bags with 50 seeds each in Vienna.

The buried seed lots represent spatially independent replicates (Fig. 1).

Excavation of the seeds of the pre-trial (7 bags each year) started in 2012 and ended by 2021. In the main experiment, excavation of 10 bags per year (5 from each layer) ranged from the year 2013 until 2022. Excavated seed bags were transferred to the lab for immediate germination tests.

Seed viability was tested by both labs following the manuals of Karrer et al. (2016a) and Starfinger and Karrer (2016). First step: germinability was tested directly after excavation. Due to the fact that the seeds were buried for one year minimum in the soil under field conditions their need for vernalisation (Willemsen 1975; Bassett and Crompton 1975) was adequately met. Intact seeds from every bag were put into petri dishes on moistened filter paper and placed for 4 weeks into climate chambers running a cycle of 12 hours light at 30 °C and 12 hours darkness at 15 °C (Leiblein-Wild et al. 2014; Karrer et al. 2016a, 2016c). Every second day the number of germinated seeds was counted and removed. A seed was stated “germinated”

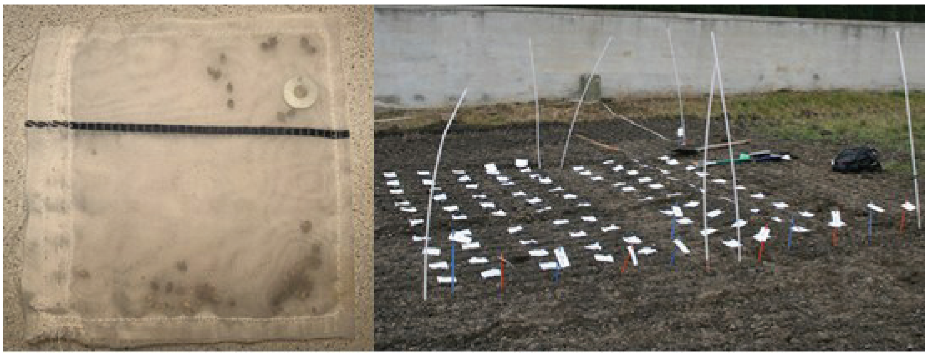

Figure 1. Seed bags (left), prepared for burial (right).

when the radicula was visible at a length of 2–3 mm. Second step: All seeds that did not germinate (probably because of dormancy) were subjected to a standard viability test of the embryo. For this TTC-test we used 2,3,5-triphenyltetrazolium chloride to induce red colouration of the living cells of the ragweed embryo. The TTC-test procedure followed Starfinger and Karrer (2016) and Hall et al. (2021). The seeds were already in a soaked condition after the germination test and immediately cut with a medical scalpel longitudinally into two halves to check the constitution of the embryo using a microscope. Dead embryos (decomposed or degraded in the sense of Hall et al. 2021) and empty seed coates were classified non-viable before the TTC-test started. Apparently intact embryos were put into 0.5 ml PCR-tubes that were filled with 1%-TTC solution (powder dissolved in demineralised water) and incubated at 30 °C for 24 hours in darkness. Then the embryos were checked for discolouration using a microscope. The discolouration of the embryo was classified to three types (Suppl. material 1: fig. S1): (a) fully stained in red (= viable), (b) partially stained red or orange (intermediate) and (c) not stained at all (dead), following the protocol by Starfinger and Karrer (2016). Intermediate discolouration was always connected with non-coloured radicula. This inactive (= dead) radicula disables the embryo to break through the seed coat for successful germination. Therefore, intermediates were counted as non-viable seeds for statistics (Hall et al. 2021).

Before burial the sampled seeds from 2010 and 2011 were also tested in Vienna for germinability, and viability by TTC-test, after 6 weeks of vernalisation in darkness at 4 °C (n = 100 each seed lot).

In parallel to the burial trial, seeds from the Kaposvár seed lots were stored for 10 years in dry conditions at room temperature and tested for germinability and viability annually following the same procedure as administered to the buried seeds.

Analysis of the data were performed either on the number of germinated seeds, or on the number of TTC-positive seeds, or on the number of viable seeds from both subsequent tests. „Viable" seeds comprise therefore finally the germinated seeds plus TTC-positive seeds from the TTC-test that was applied to the non-germinated seeds. Statistical analysis of germinability and final viability was applied to arcsin-transformed data. GLMM and ANOVA was used to describe differences of viability with respect to the independent factors 'seed origin', 'burial depth', 'burial site' (lab, resp.) and 'year of excavation'. For fine-tuning the results of multiple regression analysis we constructed generalised linear mixed models (GLMM) using R packages lme4 (Bates et al. 2018), MuMin (Bartoň 2018), and AICcmodavg (Mazerolle 2017) aiming to detect the best model (factor combination) that can explain the viability of seeds. Collinearity of the explanatory variables "burial site", "seed origin", "burial depth", and "year of excavation" was tested using R-package corrplot (Wei et al. 2017), and could be precluded. Models were selected by comparing the second order Akaike Information Criterion value (AICc value) corrected for small sample sizes. To identify the most parsimonious model based on the lowest AICc value we computed the AICc differences (ΔAICc) between the different candidate models. As a rough rule Burnham and Anderson (2002) proposed that models for which $\Delta i \geq 2$ receive substantial support as the chance of the smaller AICc value being correct lies at approx. 73%. Group differences considering the year of excavation were post-hoc tested by Tukey HSD. Other groups defined by binomial factors were checked for significant differences of their means at a significancy level of $p < 0.05$ by non-parametric tests (Kruskal-Wallis-Test). The homogeneity of variances was tested with Levene's test. The correlation of germinability and viability-rates was tested by Spearman' Rank Correlation.

Results

As ragweed seeds are known to stay dormant under specific conditions we tested the initial germinability and viability rates of the seed lots before burial. The seeds from Unterpurkla used in the pre-trial in Vienna were germinable at an average of 72% and finally viable (germinated and TTC-positive) at an average of 82%, before burial. Pre-burial-tests of the seed lots from Hagenbrunn and Kaposvár in the lab in Vienna gave 14% and 76% germinability, and 18% and 85% viability, respectively. In general, the seeds started with less than 100% viability due to some dead embryos, which could not be detected from outside the obviously intact seeds.

Pre-trial in Vienna

For the trial at the BOKU-garden in Vienna with seeds from Unterpurkla, excavated seeds started with very low mean viability rates of 43% after the first year of burial (2012). After two years' burial seed viability was measured at 73% which was about the same values as the seeds before burial (2011: 72%). In subsequent years the viability rates dropped to a level of ≥ 40%. Only in the very last year (2021) ragweed seeds showed a marked further viability decrease to 30%. (Fig. 2). The low viability in the year 2012 seems to be accidental.

Main burial experiment

The trial gave results for germination rates as well as for total viability rates (including TTC-positive seeds). Viability rates are generally equal to or higher than the germination rates. Both rates are positively correlated ($R = 0.884$, $p < 0.001$). But there is a difference between the places of burial/analysing labs. The germinability rates were almost as high as the viability rates and reached a perfect linear correlation for the Austrian labs whereas for the Hungarian data the linear regression coefficient was less positive but nevertheless significant (Suppl. material 1: fig. S2).

In the main burial experiment four influential factors on germinability and viability were tested.

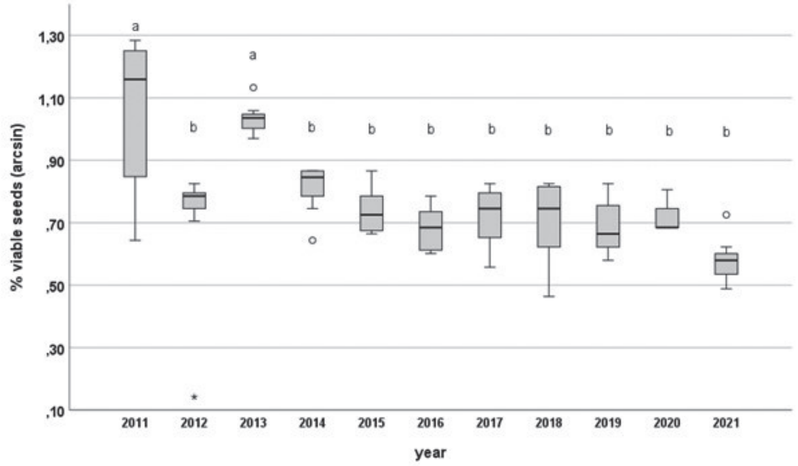

Figure 2. Viability rate variation (box-plots of arcsin-transformed % viable seeds per bag) of ragweed seeds buried at BOKU botanical garden in Vienna from 2012 to 2021. In 2011 the seeds were tested before burial.

The ANOVA with all four factors showed a significant influence of 'seed origin', 'year of excavation', and 'burial site/laboratory' ($p < 0.001$) on the viability rates of buried ragweed seeds (Suppl. material 1: table S1). The factor 'burial depth' had significant effect on viability in the four-factorial analysis but not in the one-factorial ANOVA ($F = 0.674$, $p = 0.350$).

The GLMM analysis started with the calculation of the explanatory power of each stand-alone factor, indicating that seed viability was particularly affected by the factor „seed origin". As null model we used the factor „burial site/laboratory" to test if results are influenced by site specific conditions and/or lab conditions but this could be mainly excluded. However, when calculating the models, it became obvious that seed viability of common ragweed was mainly explained by the interaction of the factors „seed origin", „year of excavation" and „burial site", indicating that there is some influence of site/lab specific conditions. Additive effects of the factors showed only very low AICc values (not shown in Table 1), and can therefore be excluded. Furthermore, in contrast to the four-factorial ANOVA analysis the factor „burial depth" had almost no explanatory power over the results.

Years of excavation significantly affected seed viability rates in general (Fig. 3; overall ANOVA: $F = 4.222$, $p < 0.001$), and specifically, the years 2020, 2021 and 2022 had significant pairwise differences compared to three or more years before at the level of $p < 0.001$.

Table 1. Summary of AICc values used for model selection of dependent variable seed survival rate; number of estimated explanatory parameters and parameter combinations = 8; AICc = Second order Akaike Information Criterion; ΔAICc = difference between AICc to the next most parsimonious model; R^2 = proportion of variance explained by the factors on the (arcsin-transformed) viability rates of buried ragweed seeds.

	Explanatory model	AICc	ΔAICc	R^2
Viability of seeds	Null Model: Burial site (Laboratory)	433.5		
	Seed origin * Year of excavation * Burial site	-206.2	0.0	0.86
	Seed origin * Year	-45.2	161.0	0.81

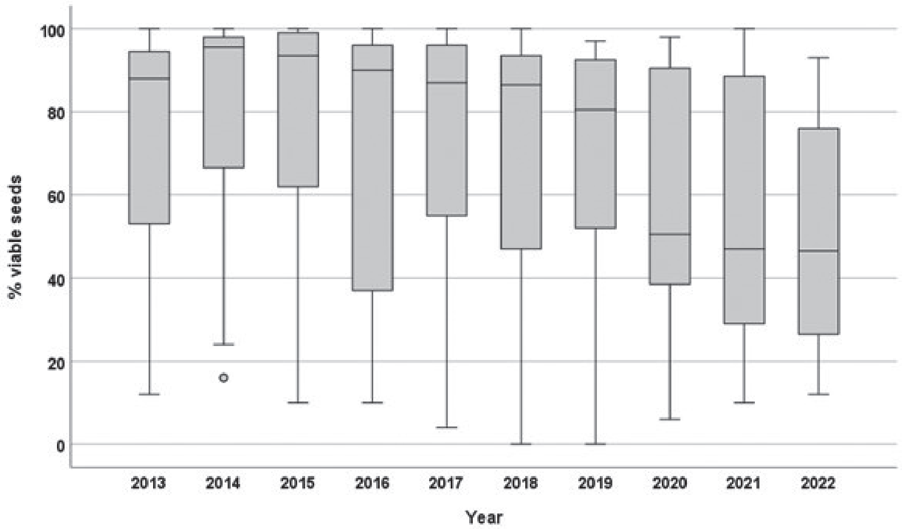

Figure 3. Seed viability rate (box-plots, percentages of viable ragweed seeds per bag) from 2013 to 2022.

In general, seeds originating from Kaposvár/Hungary had significantly higher viability rates than the seeds from Hagenbrunn/Austria (means at 89.45 and 47.92, resp.; Mann-Whitney-U-Test: $p < 0.001$). This significant difference in viability rates was detected for all years (evident from Fig. 4).

Interestingly, the burial site/testing lab showed also significant influence on the viability rates of ragweed seeds (Fig. 5, Suppl. material 1: table S1). In the first 6 years of the experiment the mean viability rates of buried ragweed seeds differed significantly between the burial site/lab. From the 7th year onwards, the viability measures by the different labs (burial sites) were almost identical.

When seed viability rates were compared with respect to year of excavation and burial depth no significant difference was found ($F = 1.478$; $p = 0.155$, Fig. 6).

But when the data are presented in groups by seed origin and burial site (Fig. 7), viability rates differ significantly between all compared groups at $p < 0.001$ (Kruskal-Wallis-Test, two-tailed). Along the years of excavation both seed lots decreased in viability rates in both places of burial, but the Hagenbrunn seeds lost viability

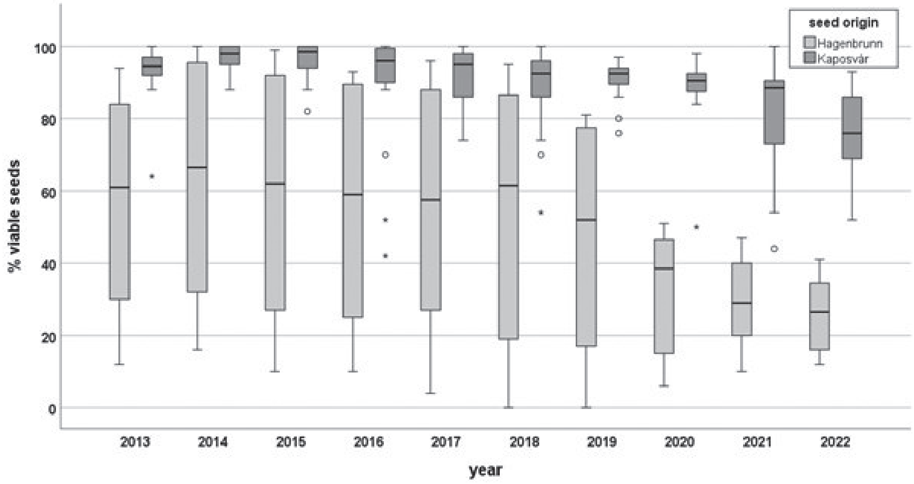

Figure 4. Seed viability rate (box-plots, percentages of viable ragweed seeds per bag) from 2013 to 2022 with respect to seed origin.

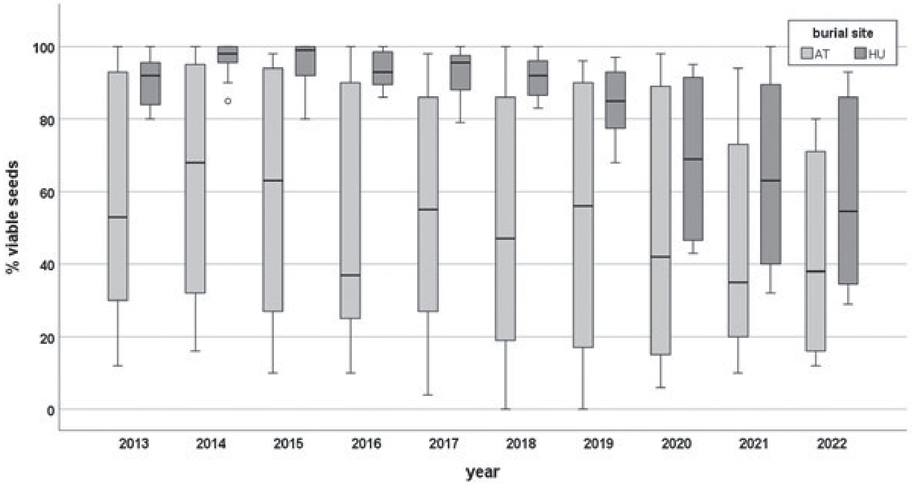

Figure 5. Seed viability rate (box-plots, percentages of viable ragweed seeds per bag) from 2013 to 2022 with respect to burial site (AT = Groß-Enzersdorf in Austria, HU = Kaposvár in Hungary).

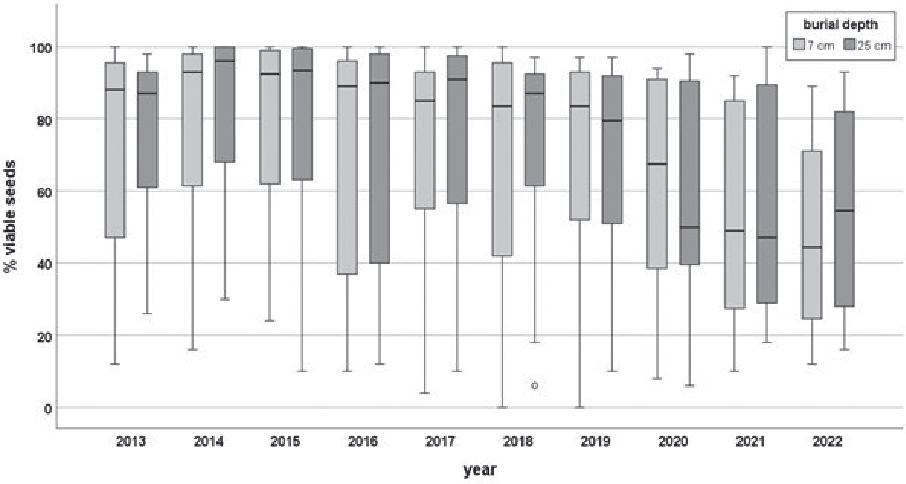

Figure 6. Seed viability rate (box-plots, percentages of viable ragweed seeds per bag) from 2013 to 2022 with respect to burial depth of seeds.

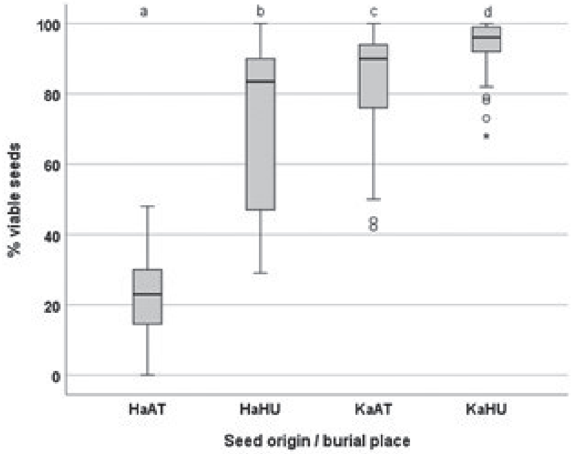

Figure 7. Seed viability rate (box-plots, percentages of viable ragweed seeds per bag) with respect to seed origin (Ha=Hagenbrunn/Ka=Kaposvár) and burial site (AT=Austria/HU=Hungary); letters correspond to significant group differences in means.

from the first years onwards with a very low starting value when buried in Austria, whereas the same seed lot buried in Hungary start their loss of viability from a far higher level (Fig. 8, Suppl. material 1: fig. S3, table S3); only in the last three years, their viability dropped almost to the level of Hagenbrunn seeds buried in Austria. The Kaposvár seeds lost barely no viability when buried in Hungary (> 95%). Only in the last year (2022) viability dropped to a mean of less than 90%. When the same seed lots were buried in Austria we found more than 90% viability in the first three years only. The overall means for the Kaposvár seeds dropped slowly but not significantly to just above 90% viability until 2020. In 2021 and 2022, the mean viability of Kaposvár seeds buried in Austria dropped to 89 and 83%.

Discussion

Seeds of common ragweed are known to stay dormant when the conditions (i.e., burial in deeper soil horizons, long dry periods, missing stratification by several

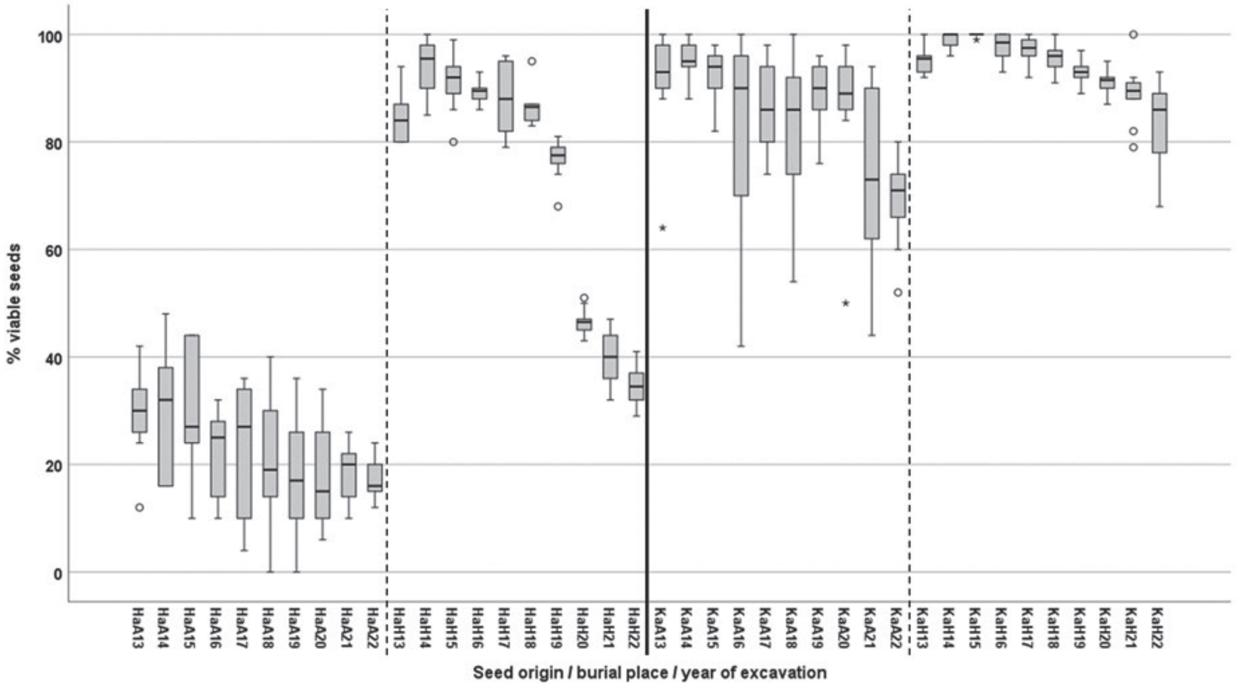

Figure 8. Seed viability rate (box-plots, percentages of viable ragweed seeds per bag) from 2013 to 2022 with respect to factor combinations of seed origin (Hagenbrunn/Kaposvár) and burial site (**A** = Austria / **H** = Hungary).

weeks of low temperature after seed ripening) do not allow germination (Dickerson 1968; Willemsen 1975; Bazzaz 1979). In earlier experiments, Dickerson (1968), Kazinczi et al. (2008), and Farooq et al. (2019) found reduced germinability when seeds were buried at six to eight centimeters soil depth. It can be expected that those seeds that are buried in even deeper soil horizons are not stimulated to germinate and stay in enforced dormancy over years. But long-time burial cannot hold the viability of buried seeds of any kind of weeds at high levels. In the case of common ragweed, Duvel started a burial experiment of several weedy species in 1902 to determine seed longevity under natural conditions. Maximum age for survival was 39 years (Toole and Brown 1946) for seeds that were buried in depths of 56 cm (germination rate at 6%) and 106 cm (22%). In our experiment we tested germinability and final viability of ragweed seeds buried in two different soil depths. In general, germinability and overall viability rates differed slightly but not significantly. Only in some years and exclusively at the Hungarian burial site, the seeds buried in deeper soil showed higher germinability and viability rates than those from shallower soil depths (Suppl. material 1: table S4).

Viability rates comprised of germinated seeds and subsequent TTC-test on the non-germinated seeds. Therefore, germination rates were somehow lower than rates of viability, but the burial and test conditions in Hungary differed obviously to those in Austria. This confirmed the fact that all germinating seeds are viable but not all viable seeds germinate – due to seed dormancy – similar to the majority of any weed seeds (Baskin and Baskin 1980, 1998).

Our experimental design did not allow to clarify the role of potential factors that may cause some of the differences in the viability results. Climatic conditions during burial (Suppl. material 1: table S5) might have some influence but we found no interpretable differences. Some influence in the viability results may be caused by differences in the seed manipulations during the experiment (conditions of excavation and transportation to the lab) or by differences in the interpretation of colouration of seeds by the TTC-test. As long as human interfaces are involved in the interpretation of TTC-colouration of seeds, this may cause images under discussion (Zhao et al. 2010; Busso et al. 2015; Hall et al. 2021). In fact, Austrian TTC-positive seeds were few and did not raise the total viability rates this much compared to Hungary. Possibly, the Hungarian lab classified less intensive stained seeds as not viable in some cases.

Viability values of the pre-trial in Vienna indicated a significant decrease in the first 3 years from 72% to about 40%. In subsequent years there was no further loss of viability except for the last year (30%). This result runs contrary to the main experiment results (Fig. 8) where specifically the seeds originating from Kaposvár started at very high viability rates of more than 95%. Even after 10 years, the viability rates of buried seeds originating from Kaposvàr were reduced only to 85% in the Hungarian lab, and to 70% in the Austrian lab. The seeds originating from Hagenbrunn in Austria started in the Hungarian lab also with relatively high viability rates of around 90% and dropped drastically only in the last three years to 35%. On the other hand, the same seed lots buried and analyzed in the Austrian lab started in the first year with only 30% viability and dropped slowly to less than 20%. Burial depth had no significant influence on these interesting differences.

Several factors may influence the germinability and viability in burial experiments like ours: When seeds are sampled in the field seed sizes and ripening stage may vary to some extent. The conditions of storage or transportation of weed seeds may have an influence on their viability (Moravcová et al. 2006; Karrer 2016b, Kazinczi and Kerepesi 2016; Starfinger and Söltner 2016), i. e. when the seeds differ already in size, weight or ripening stage (Karrer et al. 2012; Karrer 2016c; Söltner et al. 2016b). Furthermore, the soil conditions (dynamics and range of soil temperature and moisture) at the place of burial might have an influence on seed survival (Farooq et al. 2019; Nikolić et al. 2020).

Obviously, the burial of ragweed seeds at seven or more centimetres of soil depth is deep enough to stop initiation of germination. We found only a slightly higher (but not significant viability of seeds buried deeper into the soil at 25 cm. This is in line with the results of Toole and Brown (1946) who found also higher survival rates of ragweed seeds buried at about one metre in the soil. Benvenuti et al. (2001) found that typical annual weeds of arable fields did not germinate in depths of ≥ 6 cm. This seems to hold also for our annual weed species. Other weeds like *Echinochloa crus-galli* showed also sensibility of age and storage conditions of seeds. Moravcová et al. (2022) found that storage of barnyard grass at room temperature ended in lower germination rates and the loss of germinability within 2 years whereas seeds buried for 8 years in 20 cm soil depth showed germination rates of 40–60%. In the soil the seeds experienced several dormancy/non-dormancy periods without germination and could stay germinable for longer time. We tested also the germinability and viability of seeds of the different origins stored either at room temperature (Hungary) or at 4 °C in darkness (Austria) and found that the Kaposvár seeds stored at room temperature lost viability continuously (2013: 96%, 2014: 96%, 2015: 80%. 2016: 47%, 2017: 23%, 2018: 13%, and 2019 onwards: 0%). The Hagenbrunn seeds stored in darkness and at 4 °C in Vienna were measured with lower and quickly decreasing viability levels; i. e., 2013: 54%, 2014: 14%, 2015: 12%, 2016 onwards: 0%. Obviously, the 'seed quality' of Kaposvàr seeds was far better than those provided from Hagenbrunn/Austria. This is astonishing as the average seed weight of the Hagenbrunn seeds was significantly higher than in the Kaposvár seeds: means (n = 200, each) 5.37, sd ± 1.89 mg and 3.59 ± 1.29 mg, respectively. The seeds for the pre-trial (from Unterpurkla) were measured with 4.01 ± 1.66 mg. In our experiments, the smaller the seeds, the better the germination performance and viability. Similar results were found in the tree *Copaifera langsdorffii*, where smaller seeds germinated quicker than big ones and invested more to the root system (Souza and Fagundes 2014). Following these authors, this behaviour can be interpreted as adaptation to the colonisation of transient habitats and early successional stages like arable fields (Baskin and Baskin 1998). Kazinczi and Kerepesi (2016) showed in earlier experiments that seed age and origin greatly influenced seed viability.

Our results confirmed the fact that under field conditions ragweed seeds can remain viable for a long time, especially in the deeper soil layers, so its seeds can enrich the persistent soil seed bank in habitats with regular soil perturbance as in arable fields. Seeds that are deposited on the soil surface or beneath shallow litter layers undergo the dormancy/non-dormancy environmental influences in temperate regions every year. They are prone to germinate easily due to nice water and light supply after having experienced break of dormancy until early spring. In such populations the soil seed bank is lower in numbers of viable seeds (Karrer et al. 2011).

In our experiment it became evident that the exactness of sticking to the experimental protocols is essential to gain comparable data. Starfinger et al. (2012), Karrer et al. (2016b) and Hall et al. (2021) used ragweed germination and viability testing data to show the great influence of lab conditions and discipline, circumstances of sampling and storage of seeds and environmental conditions of burial sites, as well as the rules for interpretation of primary viability results (colouration of seeds in TTC-tests).

So from the point of integrated weed management in arable fields we suggest to prefer preventive control procedures, primarily to prevent the flowering and seed production of ragweed (Karrer et al. 2011; Kazinczi and Novák 2014; Karrer 2016d). This represents a long- term control strategy in terms of reducing ragweed populations.

To conclude, we found significant differences in the viability of ragweed seeds with respect to seed origin which was interestingly negatively correlated to seed weight. Effects of burial site/lab conditions were also significantly different over the whole duration of the experiment, but towards the end (after 8 years) the differences collapsed. Our tested soil depths had no significant influence on viability, indicating that burial at ±7 cm fulfils the need of ragweed seeds for continuation of innate dormancy. The loss of viability with ageing of buried seeds was expected although this effect was less prominent in the Hungarian seed origin. Hungarian seed populations from Kaposvár experienced a far longer period, by about 70 years, of successful invasion and establishment of common ragweed compared to the Austrian population with about 10 years. This might have promoted the development of a segetal weed population with prolonged buried seed survival rates adapted to the local agricultural regimes in Hungary whereas the ruderal population in Austria was far younger and still not adapted so much to burial processes.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualisation, resources, project administration and supervision: GKAR, GKAZ. Formal analysis: GKAR, GKAZ, RH, JP. Investigation: GKAR, FL, NW, RH, BK, JP, IJ. Methodology: GKAR, GKAZ. Writing – original draft: GKAR, GKAZ. Writing – review and editing: GKAR, GKAZ, RH.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary data

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Data type: pdf

Explanation note: **figure S1.** Discolouration and viability stage after TTC-test of common ragweed seeds; **figure S2.** Linear correlation of germinability and total viability of ragweed seeds buried in Austria and in Hungary; **figure S3.** Synoptic boxplots of viability rates of ragweed seeds with respect to seed origin, burial site/lab, year of excavation, and grouped by burial depth; **table S1.** ANOVA results about the influence of the factors seed origin, year of excavation, place of burial/lab, and burial depth on the viability rates of buried ragweed seeds; **table S2.** ANOVA results about the influence of the factors seed origin, year of excavation, place of burial/lab, and burial depth on the germination rates of buried ragweed seeds; **table S3.** Viability rates of ragweed seed lots originating from Hagenbrunn or Kaposvár, buried in Austria or Hungary and excavated from 2013 to 2022; **table S4.** Differences of means of ragweed seed germination and viability rate in the burial experiment performed in Hungary; **table S5.** Climatic variables in the 10 years of the experiment at the burial sites Kaposvár and Groß-Enzersdorf.

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