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Evaluation of the viability of old seeds of several important agricultural weeds

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SUMMARY

Persistent seed banks are equally important in agriculture and invasion biology. While seed vitality persistence exemplifies an eternal uphill battle for weed control in agriculture, it signals a potential invasiveness of species in invasion biology. Considering yield losses caused by *Amaranthus retroflexus*, *Abutilon theophrasti*, *Chenopodium album* and *Datura stramonium* in agriculture, and the importance of *Ambrosia trifida* as an emerging invader in Europe, the objective of this study was to test the viability and longevity of the aged seeds of these economically important weeds. Three seed viability/longevity tests were conducted: the crush test, germination test in Petri dishes, and 3,5-triphenyltetrazolium chloride (TTC) test. The results revealed a significant variation in germination potential among the tested species. The highest vitality was observed in 7-year-old seeds of *A. retroflexus* (41.67 %), followed by 16-year-old *A. theophrasti* seeds (17.78 %), 13-year-old *C. album* seeds (15.00 %) and 17-year-old *D. stramonium* seeds (7.5 %). Furthermore, a remarkable seed longevity was documented in the tested species (with the exception of *A. trifida*), preserving their germination potential for over half a century. Seed germination was recorded in 49-year-old seeds of *D. stramonium*, 53-year-old seeds of *A. retroflexus*, 58-year-old seeds of *A. theophrasti* and 59-year-old seeds of *C. album*, in strong evidence of the persistence of these weed species' seeds in the environment.

Keywords: germination, seed longevity, seed persistence, viable seeds, weed seeds

INTRODUCTION

Amaranthus retroflexus, Abutilon theophrasti, Chenopodium album and *Datura stramonium* are considered to be among the world's worst weed species (Costea et al., 2004; McDonald et al., 2004; Ziska, 2013; Sarabi et al., 2013; Bajwa et al., 2019), while *Ambrosia trifida* has recently been detected in Serbia, where it currently invades agricultural areas (Vrbničanin, 2015) and has the potential to cause health problems (Ghosh et al., 1991; Savić et al., 2019). In general, all of these weed species cause significant losses in agriculture. In maize

and soybean crops, *A. trifida* can cause yield losses of over 50% at densities of only 1 plant m^{-2} (Baysinger & Sims, 1991; Webster et al., 1994; Harrison et al., 2001). Studies on sugar beet yield losses caused by *A. retroflexus* have shown 12% yield reduction at densities of 4000 plants per ha, and 31% at 15000 plants per ha (Stebbing et al., 2000). In general, weeds compete with crops for light, nutrients and humidity. A well-planned management of commercial seed production and storage, and successful weed control, require that weed seed viability/longevity be determined. Additionally, data on seed longevity and seed bank are important indicators of the invasiveness potential of studied species (Daehler et al., 2004).

Viability is the percentage of viable seeds in the seed bank which have the potential to germinate. Genetic factors are the key determinants of seed persistence (Bekker et al., 2003), enabling weed seeds to remain viable for long periods of time in the soil, where seed dormancy is also an important trait. However, Conn and Farris (1987) showed that there is no significant relationship between initial seed dormancy and seed longevity. Seed dormancy is cycling, ensuring the survival of a weed, making it one of the most important adaptive traits in plants (Gonzáles-Alday et al., 2009). Viable seeds are often remarkably similar to nonviable seeds visually, which often leads to erroneous conclusions, and therefore makes seed viability/longevity testing a requirement in estimating the number of viable seeds in seed banks.

Numerous tests enable seed longevity/viability verification: germination test directly from soil samples (Bekker et al., 1998); controlled ageing test (CAT) (Newton et al., 2009); standard germination test (International Seed Testing Association, 1985); 3,5-triphenyltetrazolium chloride test (TTC, International Seed Testing Association, 1985), and seed crush test (Sawma & Mohler, 2002). In general, each test has its specific advantages and disadvantages. Germination seed testing directly from soil is optimal as it does not require seed extraction from soil, unlike Petri dish tests. However, its downside is that it can take up to two years to complete, until all seeds have germinated. Meanwhile, Petri dish tests offer a more rapid and time-efficient procedure due to the flexibility of using variable temperature conditions. Of course, it is important to note that a portion of seeds can degrade or show high level of dormancy, in both germination tests (Roberts & Dawkins, 1967; Roberts & Feast, 1973). The crush test requires seeds to be removed from soil, germinated and then crushed. Its advantage is that the entire seed sample can be used directly, with those seeds that are brittle or discolored instantly being classified as nonviable (Warnes & Anderson, 1984; Wilson, 1985; Wilson & Lawson, 1992). The TTC test is considered a very quick and reliable method, superior to other methods. However, depending on the analyst's training and application to smaller seeds, the TTC test requires a certain level of skill (Borza et al., 2007).

Different species have characteristic seed longevities and their relative longevity may vary depending on storage conditions and seed dormancy (as a genetic trait of a species; Naylor, 1983). Temperature and moisture content are considered crucial factors that primarily affect seed longevity (Priestley, 1986). Seed longevity and germination capacity have been studied in many plant species (Lueschen & Anderson, 1980; Sawma & Mohler, 2002; Uremis & Uygur, 2005; Conn et al., 2006; Csontos et al., 2016; Mercado & Delgado, 2018; Mercado et al., 2020; Wiebach et al., 2020). The oldest dry-stored seeds known to have germinated were those of *Canna compacta,* about 620 years old (Lerman & Cigliano, 1971). Due to the significant economic influence of the selected weed species (*A. retroflexus, A. theophrasti, C. album* and *D. stramonium*) on crop yields and human health (*A. trifida*) this study aimed to test the hypothesis that seed physiology and tendency to survive/propagate will preserve its germination capacity, i.e. preserve seed viability and secure weed seed longevity. Consequently, this paper reports on the viability and longevity of old seeds of some economically important agricultural weeds.

MATERIAL AND METHODS

Plant material

Seeds of *A. theophrasti, A. trifida, A. retroflexus, C. album* and *D. stramonium* were collected in the field many years ago (20/30/40 years ago), while the only exception was *A. trifida,* which has been recorded in Serbia only recently. The seeds were kept at room temperature, in dry glass containers in the laboratory, as part of a seed collection. The seeds of newer date were collected in the field and kept in paper bags at room temperature in the laboratory until further analysis.

Viability/longevity testing

Viability/longevity testing of seeds was done using three methods: crush test (Sawma & Mohler,

2002), germination test in Petri dishes (International Seed Testing Association, 1985) and TTC (3,5-triphenyltetrazolium chloride) test (International Seed Testing Association, 1985).

Crush test

The method was described by Sawma and Mohler (2002). Seeds were observed under the microscope (Stereo trinocular microscope, MICRO-SC2). Damaged seeds (incomplete or with ruptures in seed coat) were removed from the analysis. A total of 30 seeds in 3 replicates were analyzed for each tested weed species. A single seed was wrapped in a small sheet of paper and forced to break, without pulverizing it.

Germination test in Petri dishes

The test in Petri dishes (International Seed Testing Association, 1985): seeds were placed on filter paper in 9 cm Petri dishes with 5 ml of distilled water (every population containing 30 seeds/Petri dish, in 3 replicates). Petri dishes were set down to incubate at different temperatures: 15, 20 and 25 °C. The seeds were kept in the dark for 15 days and their germination was evaluated daily (with daily removal of germinated seeds). A seed was considered to have germinated when its radicle was visible (about 0.5 mm in length).

TTC test (3,5-triphenyltetrazolium chloride)

The TTC test (International Seed Testing Association, 1985): seeds were kept in distilled water for 18 hours. Subsequently, 30 seeds/test tube in 3 replicates, representing each population, were kept in 1% TTC at 30 °C for 24 h. The seeds were then dissected under the microscope (Stereo trinocular microscope, MICRO-SC2), and seeds with pink to red embryos were considered as viable (Grabe, 1970, Egley & Chandler, 1978; Leist et al., 2003).

Data Analysis

The number of viable seeds was calculated following the PS=Z1+Z2 formula (Uremis & Uygur, 2005) and viability percentage for each population according to formula PV= PS/UB×100 (out of a total of 360), with Z1 being the number of viable seeds in the germination test, Z2 the number of viable seeds in the 1% TTC test and UB the initial number of seeds. Data was processed using the analysis of variance, and Tukey's post hoc test (α=0,05) in Statistica 7.

RESULTS AND DISCUSSION

Longevity and seed germination in agriculture are pertinent because of the maximum period certain seeds can remain in the seedbank and still retain a high germination percentage. On the other hand, in invasion biology, these data can be used as criteria for assessing species invasiveness potential (Daehler et al., 2004; Hiebert, 1997). Conn and Deck (1995) determined that only 2-5 % of weed seeds (*C. album, Stellaria media, Capsela bursa-pastoris, Descurania sophia*) were viable 9,7 years after their burial. Seed viability varies among weed species (Holm et al., 1977), depending on their age, with individual differences in seed viability often being more pronounced between individual seeds than between seeds of different ages (Milberg, 1994). Additionally, their germination potential varies greatly between seed batches and, in limited data sets, no clear effect of seed age can be evident between older batches (Milberg, 1994). In preliminary testing, the crush test was shown to be the most difficult to execute and the least reliable. The seeds were wrapped in paper and crushed but the obtained products and their consistency did not adhere to the criteria described by Sawma and Mohler (2002). According to them, *A. theophrasti, A. retroflexus* and *C. album* seeds can be classified as viable if the crushed product is creamy and the flesh oily, while seeds are considered nonviable if they have deteriorated (or appear powdery) or their color is brown or black. The crush test results were not analyzed because the majority of seeds from all tested populations had deteriorated or become powdery (except *D. stramonium* seeds that could not be crushed without strong pressure, which consequently caused the seeds to become powdery). This method was only proved to be useful for the majority of tested *A. trifida* seeds. Due to the generally low germinability of all seed samples, when the results of the other seed viability methods were considered, the crush test results were not taken into consideration. The results gained by the TTC and germination tests in Petri dishes were further elaborated.

Starting from the initial hypothesis, germination was expected in the oldest seed samples of all tested weed species. The hypothesis regarding seed longevity was confirmed: 58-year-old seeds of *A. theophrasti* (4.72 % vitality), 49-year-old seeds of *D. stramonium* (1.67 %), 59-year-old seeds of *C. album* (3.89 %) and 53-year-old seeds of *A. retroflexus* (0.28 %) (Table 1).

Table 1. Viability percentage (PV) and number of viable seeds (PS) of the total number of 360 seeds per tested species

A. theophrasti			*D. stramonium*			*C. album*			*A. retroflexus*			*A. trifida*		
age	PV	PS	age	PV	PS	age	PV	PS	age	PV	PS	age	PV	PS
58	4.72	17	54	0	0	59	3.89	14	53	0.28	1	8	0.28	1
20	6.67	24	49	1.67	6	27	7.22	26	30	1.94	7	7	1.94	7
17	7.22	26	29	0	0	26	14.1	51	20	0.28	1	6	5.83	21
16	17.78	64	20	0.28	1	17	5.28	19	18	2.22	8	2	6.11	22
14	11.67	42	18	1.94	7	16	10.3	37	17	11.67	42	1	5.56	20
13	6.94	25	17	7.5	27	13	15.0	54	16	1.39	5			
11	10.56	38				4	5.83	21	15	7.5	27			
10	5.28	19				1	2.50	9	13	12.78	46			
9	8.06	29							7	41.67	150			
7	6.11	22												
6	4.44	16												

PV - viability percentage, PS- number of viable seeds

The longevity of these seeds can be attributed to genetic factors as a key influence in seed persistence. The survival/viability of seeds under conditions of stress (inadequate moisture and temperature) in the laboratory is thought to be a good predictor of their potential persistence in the environment (Bekker et al., 2003). Although it is clear that seedling emergence is related to seed size (Benvenuti et al., 2001) and seed energy storage (Mennan & Ngouajio, 2006), the duration of delayed germination has varied among weed species, germination rate varied from year to year, but variation was also common within the same species (Toole & Brown, 1946; Darlington & Steinbauer, 1961). Confirmed germination/seed longevity (Table 1) is of significance for agriculture, as it implies an eternal battle against weeds, while at the same time gives a clear signal of the persistence of such species in the environment. By analyzing the results, it can be concluded that each of the tested species (with the exception of *A. trifida*, whose seeds were of more recent dates) has retained its germination potential for over 50 years (Tables 1, 2, 3, 4, 5, 6). The highest vitality percentage, observed within a single species, was detected in 16-year-old *A. theophrasti* seeds (17.78 %), 17-year-old *D. stramonium* seeds (7.5 %), 13-year-old *C. album* seeds (15.00 %) and 7-year-old seeds of *A. retroflexus* (41.67 %). In contrast, small viability percentage in relatively young seeds of *A. trifida* is surprising (Table 1). Low germination rates of *A. trifida* seeds and the fact that seeds of that species are relatively short-lived (Davis et al., 2005; Harrison et al., 2007) is of equal importance for both agronomy and invasion biology (as it is an emerging invasive species in Serbia; Vrbničanin, 2015).

Germination percentage and significance of differences in the longevity of different age seeds of the tested species *A. theophrasti, A. retroflexus, A. trifida, C. album* and *D. stramonium* are shown in Tables 2, 3, 4, 5 and 6.

Abutilon theophrasti

Sixteen-year-old seeds of *A. theophrasti* germinated at all tested temperatures, while the TTC test showed 0 % vitality (Table 2). Inconsistencies in vitality percentage can be explained by the species metabolism. Vivrette and Meyr (2002) explained the absence of red/pink color by the slow metabolic rate of embryos (which can lead to erraneous, negative conclusion regarding longevity/viability). In the 9-year-old seeds, the results differed. Seeds did not germinate at the tested temperatures, while the TTC test showed 32.22 % viability/longevity (Table 2).

Seiler (2010) points to a possibility of coming to questionable conclusions as a result of using different vitality/longevity testing methods. After testing the germination of wild *Helianthus annus* and *H. petiolaris seeds* (20-year-old, kept at room temperature, 20-22 °C, 22% RH), Seiler (2010) observed low viability/germination in both species, 13 and 1.5%, respectively. Contrary to these results, the TTC test showed a high vitality percentage in the populations of 90 and 80.20 %, respectively.

After analyzing germination at the tested temperatures (15, 20 and 25 °C), it was concluded that *A. theophrasti* seeds have the highest germination rate at 25 °C, regardless of seed age, which was confirmed by the analysis of variance (with the exception of 17-, 14- and 6-year-old seeds, Figure 1, Table 2). However, the t-test showed that there were no significant differences between the tested temperatures in 58-, 20-, 17-, 14-, 11-,

Table 2. Germination rates and statistical significance of differences in seed vitality of *A. theophrasti*

	age	58	20	17	16	14	13	11	10	9	7	6
	15 °C	0	0	0	21.11	0	0	6.67	2.22	0	2.22	0
GT	20 °C	6.67	3.33	2.22	20	2.22	7.78	10	0	0	0	0
	25 °C	7.78	4.44	6.67	30	20	20	2.22	6.67	0	10	2.22
TTC	30 °C	7.78	18.89	20	0	24.44	0	23.33	12.22	32.22	12.22	15.56
	PS	17	24	26	64	42	25	38	19	29	22	16
						t-test						
	15-20 °C	ns	ns	ns	ns	ns	*	ns	ns	-	ns	ns
GT	15-25 °C	ns	ns	ns	*	ns	0	ns	ns	-	ns	ns
	20-25 °C	ns	ns	ns	ns	ns	**	ns	ns	-	*	ns
						ANOVA-LSD test						
	15-20 °C	ns	*	ns	ns	ns	**	ns	ns	-	ns	ns
GT	15-25 °C	*	**	ns	ns	ns	**	ns	**	-	**	ns
	20-25 °C	ns	ns	ns	*	ns	**	**	**	-	**	ns

Number of live seeds PS; ns-differences are not statistically significant; p<0.05 *, p<0.01**; ANOVA-analysis of variance; TTC test; GT–germination test

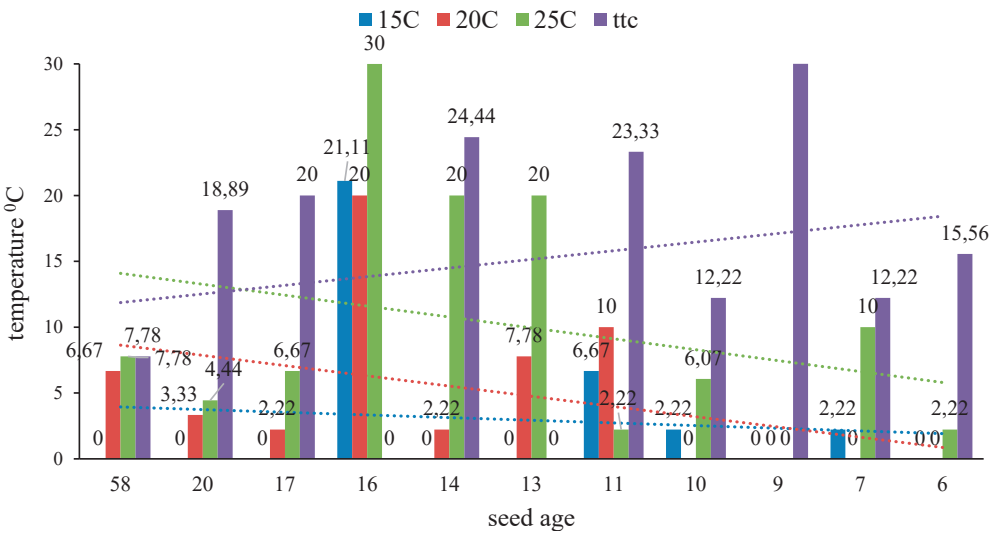

Figure 1. *Abutilon theophrasti* seed longevity

10- and 6-year-old seeds. It is interesting that 58- and 6-year-old seeds showed similar vitality (4.72% and 4.44%, respectively, Tables 1 and 2). A study done by Horowitz and Taylorson (1984) showed that the optimal germination temperature in this species is within 24-30 °C range, while germination declines above 35 °C. They highlighted that the permeability of seeds varied depending on a set of factors and their combinations, which in turn reflects on the germination process. Seed permeability changes after drying at 34 °C. In 3-year-old seeds, the seed coat remained hard, but in 15-year-old seeds, this did not affect (i.e. improve) their permeability.

Figure 1 shows vitality tendencies (in %) in *A. theophrasti* seeds of different ages. A trend analysis showed that seed vitality increases in newer seeds (TTC trend line), which was expected. However, an increase in germination rate is not evident (in all ages) with the increase in temperature (Figure 1). In older seeds (58-, 20-, 17-, 16-, 14- and 13-years-old) better germination rate was evident at 25 °C than on 20 °C, which was not the case with 11-, 10-, 9-, 7- and 6-year-old seeds. Contrary to that, Mennan (2003) recorded a significant decrease in seed vitality/longevity over time (after 1 year of storage of *Galium aparine* and 22 months of *Bifora radians*).

In general, an increase in seed germination rate was observed in younger seeds (TTC test). Nevertheless, no consistency was observed in the number of viable seeds (PS, Tables 1 and 2), depending on their age. The highest number of viable seeds was documented in 16-year-old seeds and the lowest in 6-year-old. It is interesting that the number of viable seeds among 58-year-old seeds was 17, while this number in 6-year-old seeds was 16 (Tables 1 and 2). It is difficult to discern the initial germination rate in test samples, but it was presumably lower on average. This is consistent with the results of a study by Uremis and Uygur (2005), which tested the seed germination rate of several weed species following their storage in soil (over seven years). A significant reduction in germination rate (from 95% to 6.71%) was observed in *A. theophrasti*. The fact that very old seeds kept their ability to germinate can be useful in weed seed bank analyses and suggest an adequate weed control method in agricultural fields. This result is especially meaningful for sugar beet production, where herbicide-resistant *A. theophrasti* populations are found with increasing frequency (Heap, 2023). Earlier studies had also shown that seeds of this weed species are able to remain viable for up to 50 years in soil (Dorado et al., 2009).

Datura stramonium

Datura stramonium is an economically important species, causing yield losses of 26-71% at densities of 3-11 pm^{-2} in tomato fields (Monaco et al., 1981), and 15-45% losses in soybean crops (Hagood et al., 1981). It has also been shown to exhibit a very high competitive index in soybean production in Serbia (Meseldžija et al., 2020). Additionally, this species is poisonous for people and animals as it contains tropane alkaloids, principally scopolamine, hyoscyamine and atropine (Miraldi et al., 2001; Ogunmoyole et al., 2019). Data analysis showed that *D. stramonium* seeds had the lowest vitality/longevity and germination rate of all tested weed species (Tables 1 and 3). In the tested seeds of varying ages (54, 49, 29, 20, 18 and 17 years old), the first recorded germination was in 20-year-old seeds (1.1%), only at 25 °C, while the highest germination rate was characteristic for 17-year-old seeds (16.67%, Table 3 and Figure 2).

Nevertheless, the TTC test confirmed that 49-year-old seeds have preserved their vitality/longevity (6.67%, i.e. there were 6 viable seeds among 360 tested). Due to their low germination rate, data could not be analyzed further, except for 17- and 18-year-old seeds (Table 3). According to Conklin (1976), low germination rate or complete lack of germination in *D. stramonium* seeds can be attributed to: (1) unfavorable environmental conditions, (2) impermeable seed coat, (3) the presence of endogenous inhibitors, and (4) slow metabolic rate of seeds (no pink/red coloration after soaking in 1% tetrazolium solution; Vivrette & Meyr, 2002). Low germination rate of this weed species, with only 3% of

Table 3. Germination rate and statistical significance of differences in seed vitality of *D. stramonium*

	age	54	49	29	20	18	17
	15 °C	0	0	0	0	0	0
GT	20 °C	0	0	0	0	1.11	10
	25 °C	0	0	0	1.11	3.33	16.67
TTC	30 °C	0	6.67	0	0	3.33	3.33
	PS	0	6	0	1	7	27
				T-test			
	15-20 °C	-	-	-	-	ns	*
GT	15-25 °C	-	-	-	-	ns	*
	20-25 °C	-	-	-	-	ns	ns
				ANOVA-LSD test			
	15-20 °C	-	-	-	-	ns	**
GT	15-25 °C	-	-	-	-	ns	**
	20-25 °C	-	-	-	-	ns	*

Number of live seeds PS; ns-differences are not statistically significant; $p<0.05$ *, $p<0.01$**; ANOVA-analysis of variance; TTC test; GT-germination test

germinated seeds in 19.7-year-old seeds was also observed by Conn et al. (2006). In contrast, a study by Toole and Brown (1946) showed that *D. stramonium* seeds germinated after 39 years of burial 34 cm below soil surface.

Literature data shows that the optimal temperature for germination of *D. stramonium* seeds is between 20 and 35 °C (Andersen, 1968; Conklin, 1976). Therefore, the observed lack of germination at 20 and 25 °C could be explained as a complete loss of seed vitality, i.e. by the fact that the tested seeds were not viable. The occurrence of seed mortality can be explained as physiological or chemical damage (Priestley, 1986).

Chenopodium album

The tests conducted and analysis of the obtained results have shown that all *C. album* seeds germinated regardless of their age (apart from 17-year-old seeds at 20 °C, Table 4). It is especially important to highlight that 59-year-old seeds germinated in all tested temperature treatments, with a total of 14 viable seeds and viability percentage of 3.89 % (Tables 1 and 4).

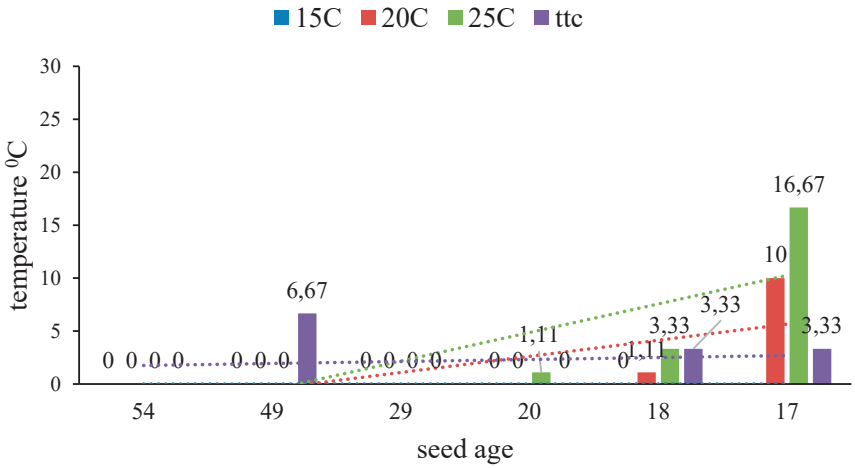

Figure 2. *Datura stramonium* seed longevity

Table 4. Germination rate and statistical significance of differences in seed vitality of *C. album*

	age	59	27	26	17	16	13	4	1
	15 °C	1.11	16.67	27.78	4.44	6.67	23.33	6.67	1.11
GT	20 °C	5.55	7.78	14.44	0	17.78	13.33	4.44	4.44
	25 °C	8.89	3.33	11.11	16.67	16.67	23.33	10	4.44
TTC	30 °C	0	1.11	3.33	0	0	0	2.22	0
	PS	14	26	51	19	37	54	21	9
					T-test				
	15-20 °C	ns	ns	ns	ns	**	ns	ns	ns
GT	15-25 °C	*	ns	*	ns	*	ns	ns	ns
	20-25 °C	ns	ns	ns	*	ns	ns	ns	ns
					ANOVA-LSD test				
	15-20 °C	ns	*	*	ns	**	ns	ns	ns
GT	15-25 °C	*	**	**	**	**	ns	ns	ns
	20-25 °C	ns	ns	ns	**	ns	ns	ns	ns

Number of live seeds PS; ns-differences are not statistically significant; p<0.05 *, p<0.01**; ANOVA-analysis of variance; TTC test; GT-germination test

The most vital seeds of *C. album* were 26 and 13 years old (14.17% and 15%, respectively). T-test and analysis of variance showed that differences in germination rates at different temperatures were not statistically significant and germination rate was higher at 25 °C (Table 4, Figure 3). In general, differences in vitality can be associated with the length of primary dormancy in this weed species. It differs between various populations and can also vary between individual plants of the same population (Holm et al., 1977).

The TTC test determined a low vitality of the tested seeds, even though the number of viable seeds (in all groups) was between 9 and 54 (Table 4). Low vitality (3%), i.e. short seed longevity in this species, was also confirmed by Conn et al (2006). As with all other tested species, no pink/red coloration after exposing seeds to 1% TTC could be linked to slow metabolic rate (Vivrette & Meyr, 2002). In general, the TTC method is not efficient enough with small seeds, as they are difficult to manipulate (cut) and the color is difficult to determine due to their small size. Nevertheless, the fact that 59-year-old seeds have germinated points to the fact that this can be a serious problem in agriculture. This is also in line with the results of Gioria et al. (2020), which showed that low-mass seeds are characterized by highly persistent seed banks. *Chenopodium album* is widely distributed, has a high seed production, its seeds are persistent and can maintain germination capacity in soil for many years (Bajwa et al., 2019). Due to the long-term application of herbicides, this species has also evolved herbicide resistance. The species is especially a problem in sugar beet production (as both species belong to the family *Amaranthaceae*), considering that the number of herbicides which are efficient in its control is decreasing.

Jursík et al. (2003) showed that *C. album* has low germination energy at temperatures between 15 and 24 °C. They found the highest germination rate (75%) at 18°C, while every subsequent temperature increase beyond 24 °C caused a decrease in its germination rate (51% at 24°C). This result supports our findings to some degree. It was concluded that germination rate was higher at 20 °C, but there were no statistically significant differences between germination rates at 20 and 25 °C (Table 4, Figure 3). The analysis of each seed age group showed that seeds germinated better at 20 °C than 25 °C (27-, 26- and 16-year-old seeds), while the opposite was true for 59-, 13- and 4-year-old seeds.

Amaranthus retroflexus

It can be inferred from the results shown in Table 5 that *A. retroflexus* seeds had the best germination rate at 25 °C (showing high viability, i.e. keeping its longevity). Analysis of the obtained results has shown that seed vitality/longevity increases in younger seeds (17-, 15-, 13- and 7-years old). Furthermore, differences were detected in the seed vitality/longevity of different age groups, depending on temperature (t-test and ANOVA, Table 5 and Figure 4). It was also found that 53-year-old seeds did not germinate, even though the TTC test showed 1% longevity (1 viable seed, PV = 0.28%, Table 1) in the tested sample. Only 20-year-old seeds germinated at 25 °C (1 viable seed, PV = 0.28%, Table 1), although the TTC test showed 0% longevity (Table 5).

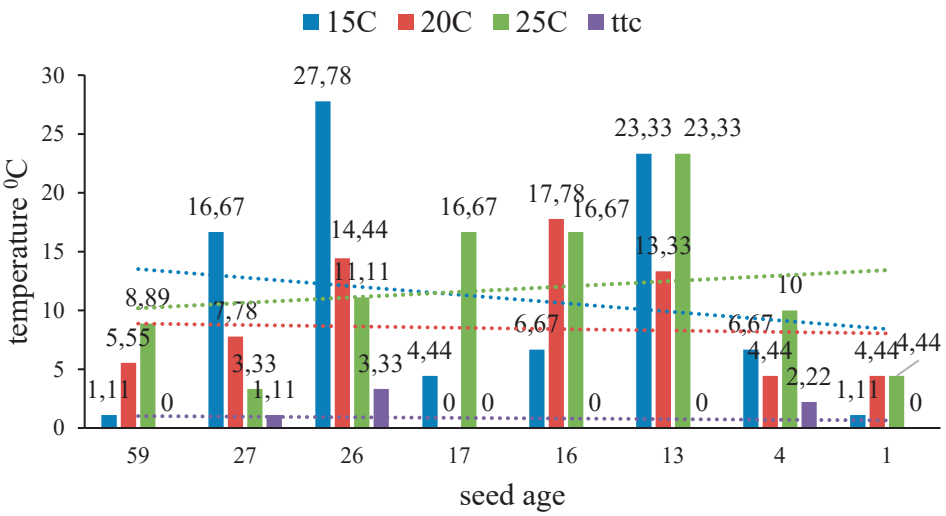

Figure 3. *Chenopodium album* seed longevity

Variations in the germination rate of *A. retroflexus* seeds depending on temperature treatments have been explained by Weaver and Thomas (1986), claiming that *Amaranthus* species seeds are relatively temperature insensitive. Given that the seeds were kept under stable conditions, at room temperature, the observed low degree of vitality/longevity was not surprising. Nevertheless, it is unclear whether, had the seeds been kept in soil, the germination rate/capacity would have been higher and enabled this weed species' persistence over longer periods of time. In general, it is a well-known fact that smaller seeds, such as those of *A. retroflexus,* have a limited food reserve to support germination (Webb et al., 1987). Of all tested weed species, *A. retroflexus* seeds showed the highest viability, with 13-year-old seeds having 46 viable seeds, and twice younger seeds (7 years old) having 3.3 times higher viability (150) (Table 5). No initial germination rate data were available for any of the tested seeds. However, according to Uremis and Uygur (2005), *A. retroflexus* seeds lose their initial germination capacity after seven years following their retrieval from soil (it reduces from 95 to 0.26%).

Table 5. Germination rate and statistical significance of differences in seed vitality of *A. retroflexus*

	samples	53	30	20	18	17	16	15	13	7
	15 °C	0	1.11	0	1.11	0	0	3.33	3.33	0
GT	20 °C	0	1.11	0	6.67	10	2.22	0	0	67.78
	25 °C	0	4.44	1.11	0	36.67	3.33	26.67	44.44	98.89
TTC	30 °C	1	1.11	0	1.11	0	0	0	3.33	0
	PS	1	7	1	8	42	5	27	46	150
					T-test					
	15-20 °C	ns	ns	ns	**	ns	ns	ns	ns	**
GT	15-25 °C	ns	ns	ns	ns	*	ns	**	**	**
	20-25 °C	ns	ns	ns	ns	ns	ns	**	**	**
					ANOVA-LSD test					
	15-20 °C	ns	ns	ns	**	ns	ns	ns	ns	**
GT	15-25 °C	ns	ns	ns	ns	**	*	**	**	**
	20-25 °C	ns	ns	ns	**	**	ns	**	**	**

Number of live seeds PS; ns-differences are not statistically significant; p<0.05 *, p<0.01**; ANOVA-analysis of variance; TTC test; GT-germination test

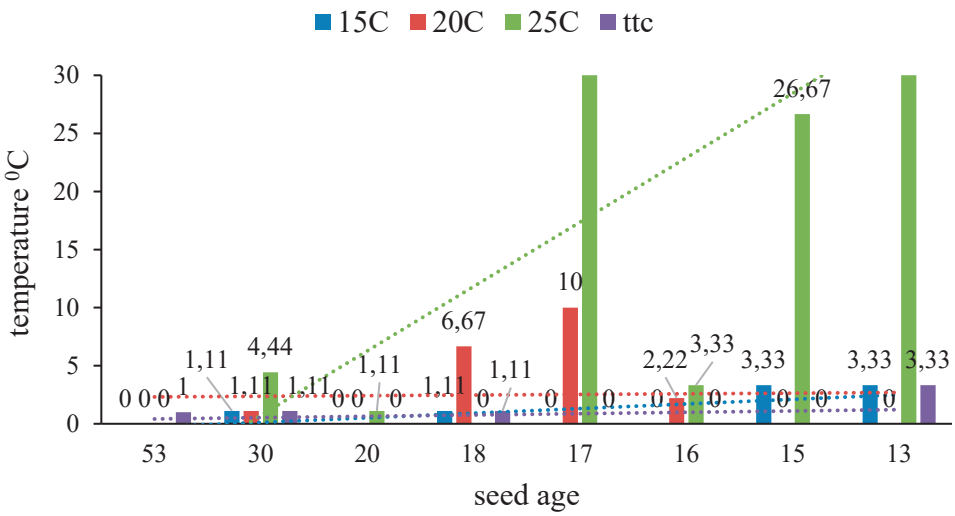

Figure 4. *Amaranthus retroflexus* seed longevity

Ambrosia trifida

Ambrosia trifida, as one of the most competitive weeds in maize and soybean production in the USA, is also a threat to human health (alergies), crop production and natural plant communities (Harrison et al., 2001; Kil et al., 2004; Schutte, 2007). The species is currently spreading in Serbia (Vrbničanin, 2015; Savić et al., 2019). Due to its habitus, the presence of one *A. trifida* plant per m^{-2} can lead to maize and soybean yield losses of over 50 % (Baysinger & Sims, 1991; Harrison et al., 2001). All new scientific data pertaining to this invasive weed species are equally important for agriculture, invasion biology and healthcare system (Follak et al., 2013). Even though this species is known for its relatively low fecundity (Harrison et al., 2001) and transient seed bank characteristics (Abul-Fatih & Bazzaz, 1979; Leck, 1989), its vitality has not been studied sufficiently, which is therefore very important to do. An analysis of results showed that the number of viable seeds (PS) increases with decreasing seed age (Table 1). Harrison et al. (2007) concluded that some *A. trifida* seeds recovered their viability after 9 years of burial (20 cm burial depth). The optimal temperature for seed germination was shown to be 10-24 °C (Abul-Fatih & Bazzaz, 1979), which is in line with the results obtained in the present study. *A. trifida* seeds had the highest germination rate at 15 °C temperature (Tables 1 and 6; Figure 5). In general, both tests (TTC and germination test) have confirmed low germination rates of the seeds of this species.

Table 6. Germination rate and statistical significance of differences in seed vitality of *A. trifida*

	samples	8	7	6	2	1
	15 °C	0	0	11.11	14.44	14.44
GT	20 °C	0	6.67	6.67	5.55	4.44
	25 °C	1.1	1.1	0	1.11	0
TTC	30 °C	0	0	5.56	3.33	3.33
	PS	1	7	21	22	20
			t-test			
	15-20 °C	ns	ns	ns	*	*
GT	15-25 °C	ns	ns	*	*	**
	20-25 °C	ns	ns	ns	ns	ns
			ANOVA-LSD test			
	15-20 °C	ns	*	**	**	**
GT	15-25 °C	ns	ns	**	**	**
	20-25 °C	ns	*	**	*	ns

Number of live seeds PS; ns-differences are not statistically significant; p<0.05 *, p<0.01**; ANOVA-analysis of variance; TTC test; GT–germination test

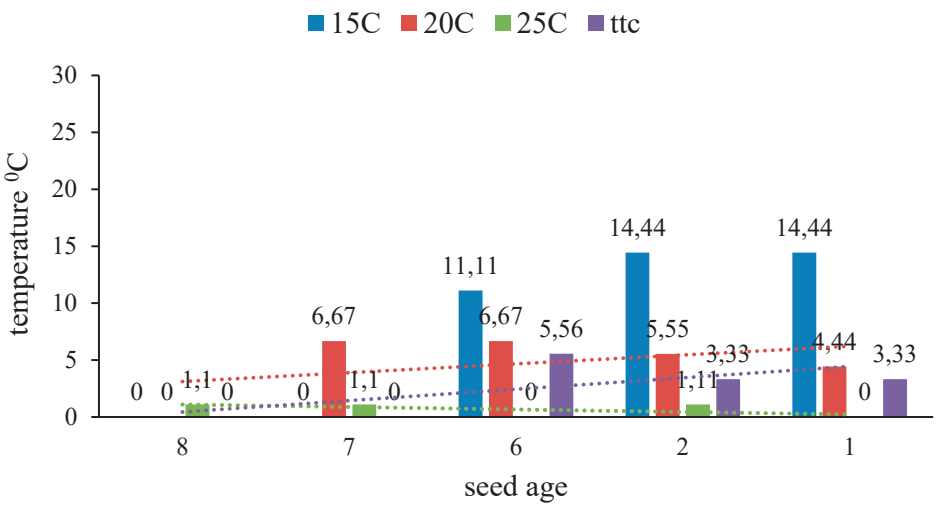

Figure 5. *Ambrosia trifida* seed longevity

The oldest *A. trifida* seeds had the lowest number of viable seeds (8-year-old seeds had 1 viable seed out of 360 in total, viability percentage PV=0,28 %), while the 6-, 2- and 1-year-old seeds had a similar number of viable seeds: 21, 22 and 20, respectively (PV=5.83%; 6.11%; 5.56%, respectively; Table 1). The analysis of variance confirmed statistically significant differences in germination rates, depending on temperature, in 7-, 6-, 2- and 1-year-old seeds (Table 6). Figure 5 shows that the seeds of this weed species have the best germination rate at 15 °C, regardless of seed age, while 25 °C temperature treatment leads to a reduction in germination rate. The results of the germination and TTC test have aligned only for *A. trifida* of all analyzed weed species. The trend line of seed germination rates at 25 °C shows a tendency of reduction, unlike the trend lines in the TTC test treatments at 15 °C temperature. Similar is also true for the trend line at the temperature treatment of 20 °C (Figure 5). Such results can be explained by the fact that *A. trifida* seeds germinate at lower temperatures, and in a wide range of soil depths and soil moistures (Abul-Fatih & Bazzaz, 1979). Due to all of the above, the fact that seeds of this weed species germinate early (before those of the other tested species), and its strong competitiveness, *A. trifida* becomes a dominant species in weed communities that it invades. The fact that this species can also show a delayed emergence by adapting to crop field conditions (Clements et al., 2004) is of particular importance, especially bearing in mind the fact that it also possesses mechanisms to develop herbicide resistance (to glyphosate and acetolactate synthase inhibitors; Heap, 2020).

CONCLUSION

Overall, this study has shown that the seed germination potential of different weed species varies greatly. The highest vitality values were observed in 7-year-old seeds of *A. retroflexus* (41.67 %), 16-year-old *A. theophrasti* seeds (17.78 %), 13-year-old *C. album* seeds (15%) and 17-year-old *D. stramonium* seeds (7.5 %). Furthermore, the most important result of this research was the finding that each of the tested weed species (excepting *A. trifida*) has preserved its germination potential for half a century. Remarkable seed longevity was found in the tested weed species: 49 years in *D. stramonium,* 53 years in *A. retroflexus*, 58 years in *A. theophrasti,* and 59 years in *C. album*, despite the relatively unfavorable conditions of their storage. Such results on weed seed viability/longevity are of critical importance for agriculture, especially organic agriculture, essentially demonstrating that such exceptional longevity and strong seed vitality imply an eternal uphill battle in weed control, giving us irrefutable proof of these weed species' persistence in the environment.

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Vijabilnost starih semena poljoprivredno značajnih korovskih vrsta

REZIME

Prisustvo banke semena je podjednako važno za poljoprivrednu praksu i oblast bioloških invazija. U poljoprivredi vitalnost semena predstavlja borbu protiv korova, a u oblasti invazivne biologije to je signal invazivnog potencijala vrste. Uzimajući u obzir smanjenje prinosa zbog prisustva vrsta *Amaranthus retroflexus, Abutilon teophrasti, Chenopodium album* i *Datura stramonium* u poljoprivrednoj praksi i značaj invazije *Ambrosia trifida* u Evropi, cilj rada je bio da se ispita vitalnost i dugovečnost starih semena navedenih ekonomski značajnih vrsta. Urađena su tri testa za proveru vitalnosti/dugovečnosti semena: test gnječenja, test klijanja u Petri posudama i tetrazolijum test (3,5 trifeniltetrazolijum hlorid-TTC). Dobijeni rezultati su pokazali velike varijacije potencijala klijavosti. Najveća vitalnost je utvrđena za seme *A.retroflexus* starog 7 godina (41,67%), zatim semena *A. teophrasti* starog 16 godina (17,78%), trinaestogodišnjeg semena *C. album* (15%) i sedamnaestogodišnjeg semena *D. stramonium* (7,5%). Takođe, utvrđena je izuzetna vitalnost semena testiranih vrsta (osim *A. trifida*) starog više od pola veka. Zabeležena je klijavost 49 godina starog semena *D. stramonium*, 53 godina starog semena *A. retroflexus*, 58 godina starog semena *A. teophrasti* i 59 godina starog semena *C. album,* čime se potvrđuje njihova perzistentnot u životnoj sredini.

Ključne reči: klijanje semena, dugovečnost semena, perzistentnost semena, vitalnost semena, seme korova