

# The influence of mowing regime on the soil seed bank of the invasive plant *Ambrosia artemisiifolia* L.

Ivana Milakovic<sup>1</sup>, Gerhard Karrer<sup>1</sup>

<sup>1</sup> Institute of Botany, Department of Integrative Biology and Biodiversity Research, University of Natural Resources and Life Sciences, Gregor Mendel Strasse 33, 1180 Vienna, Austria

Corresponding author: *Ivana Milakovic* (ivana.milakovic@boku.ac.at)

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## Abstract

*Ambrosia artemisiifolia* is an invasive annual herb infamous for the high allergenicity of its pollen, which is related to increasing medical costs. Additionally, it can cause serious yield losses as agricultural weed. Common ragweed seeds accumulate in the soil and can remain therein viable for decades, which poses a problem for the sustainable management of these populations. A long term management should thus target a reduction of the soil seed bank. We observed the influence of four different mowing regimes on the ragweed soil seed bank at six roadside populations in eastern Austria. The mowing regimes were based on methods from common roadside management practice and specifically adapted to reduce seed production. After three years of application, the soil seed bank was indeed reduced by 45 to 80 percent through three of the four mowing regimes tested. Therefore, we suggest that the best mowing regime for the most effective reduction of the size of the soil seed bank is the one consisting of one cut just after the beginning of female flowering (around the 3<sup>rd</sup> week of August in Eastern Central Europe), followed by a second cut 2–3 weeks later.

## Keywords

Common ragweed, invasive plant, management, mowing, roadside vegetation, seed bank, neophyte

## Introduction

Invasive alien species (IAS) are evident threats to local and regional biodiversity (McGeoch et al. 2010, Vilá et al. 2010, SBSTTA 2014). Additionally, many IAS have severe economic impact (Jeschke et al. 2014) either as weeds that reduce agricultural

yield (Oerke 2006) or by endangering human health (Reinhardt 2003, Salo et al. 2011). Control and eradication of IAS is of increasing importance for diversity conservation and environmental health (Pyšek et al. 2007, Shine et al. 2009, Smith et al. 2013).

Common ragweed (*Ambrosia artemisiifolia*) is an annual IAS, growing on disturbed sites like roadsides, fields, riversides and gardens. It is feared for the allergenic properties of its pollen, as well as a weed in agriculture, in both instances related to high financial costs (Coble et al. 1981, Buttenschøn et al. 2009, Rosenbaum et al. 2011, Smith et al. 2013). *A. artemisiifolia* is native to North-America and currently spreading through Europe and Asia (Kazinczi et al. 2008). In Europe, preferred habitats are summer crop fields in summer warm climates, but also ruderal places and roadsides.

The plant reproduces exclusively by seeds. One individual can produce up to 62000 seeds in North-America (Dickerson and Sweet 1971) or up to 18000 in Europe (Fumanal 2007). Ragweed seeds can enter primary dormancy and germinate next spring, or enter secondary dormancy after failure to germinate in spring (Bazzaz 1970, Baskin and Baskin 1980) and remain dormant in the soil seed bank for up to 39 years (Toole and Brown 1946). Ragweed dormancy is broken by stratification (Bazzaz 1970).

The persistent soil seed bank of *A. artemisiifolia* compromises the efficacy of any kind of control measure. Even if a control option succeeds in killing green plants aboveground, some part of the population remains dormant in the soil awaiting more favorable conditions to germinate. Another disadvantage of a persistent soil seed bank is that it acts as a source of further spreading of the weed in soil containments (Nawrath and Alberternst 2013, Karrer 2014). Soil is relocated from many habitats where the plant is growing, such as construction sites or roadsides to other sites. Therefore, aim of any sustainable long-term control of common ragweed should be a reduction of the soil seed bank in established populations.

Milakovic et al. (2014a and 2014b) and Bohren et al. (2008) found that seed production per plant could be influenced by carefully timed mowing. This study's goal is to test the effect of different cutting regimes applied for three years (Milakovic et al. 2014a) on the quantity and quality of the ragweed soil seed bank.

Regrowth of ragweed after mowing is well-documented (Barbour and Meade 1981, Bohren et al. 2005, Bohren et al. 2008, Meiss et al. 2008, Karrer et al. 2011, Patracchini et al. 2011, Simard and Benoit 2011, Tokarska-Guzik et al. 2011) and varies with season (Milakovic et al. 2014b). Timing and frequency of cutting has specific influences on the seed production of ragweed (Simard and Benoit 2011, Milakovic et al. 2014a). Higher ranked resprouts after cuts tend to produce only female flowers (Karrer et al. 2011) and, in consequence, preferably seeds that are incorporated into the soil seed bank.

Soil seed bank of plants varies by year and season. On undisturbed soil, the annual seed production of ragweed germinates to high percentages in early next spring (Dickerson 1968, Bassett and Crompton 1975, Fumanal et al. 2008, Kazinczi et al. 2008, Leitsch-Vitalos and Karrer unpubl.). Soil tillage incorporates new seeds into deeper layers of the soil (Buhler et al. 1997) and promotes long time persistency of ragweed seeds (Toole and Brown 1946).

The effects of different tillage systems were analyzed with respect to the composition of the soil seed bank of arable fields (Clements et al. 1996, Buhler et al. 1997, Cardina et al. 2000, Clay et al. 2006). Up to now, no study has considered the soil seed bank of ragweed for measuring the success of control options, even though the seeds in the soil make up a great portion of the population in annual weeds with a persistent soil seed bank. In this study, we used the soil seed bank of ragweed populations as long-term efficacy measure of non-chemical control options. We varied the mowing regime of ragweed roadside populations in Austria with respect to timing and frequency (Milakovic et al. 2014a) and analyzed the soil seed bank of ragweed at the beginning and at the end of the experiment.

## Methods

We sampled the soil seed bank of six roadside populations in Eastern Austria before and after three years of application of management practices. Austrian arterial road verges are cut at least two times a year; a first cut in spring and a second cut between July and October. This resulted in a significant spread of common ragweed along arterial roads since 2000 (Karrer et al. 2011, Essl et al. 2009).

The cutting experiment was set up in 2009 in the heavily infested parts of Austria (Lower Austria, Styria and Burgenland) (Table 1). All populations have been naturalized for about one or two decades before the experiment.

### Experimental design:

On each site, five experimental plots were installed on continuous spontaneous populations of *A. artemisiifolia* with coverages ranging from 5 to 25%. The plots were arranged along a line of 100 m, adjacent and parallel to the asphaltic surface of highways or arterial roads. Each plot sized 20 × 0.5 m and received one of the following treatments (mowing regimes), as defined in Milakovic et al. (2014a):

Treatment 1: not mown (control),

Treatment 2: first cut before the start of flowering (the last week of June), and second cut at the beginning of seed set (second week of September). Treatment 2 resembles the common roadside cutting regime in eastern Austria.

Treatment 3: first cut after the beginning of female mass flowering (third week of August), and second cut at the beginning of seed set (second week of September),

Treatment 4: first cut before the start of flowering (last week of June), second cut before the onset of male mass flowering (last week of July), and third cut at the beginning of seed set (second week of September),

Treatment 5: first cut before the start of flowering (last week of June), second cut after the beginning of female mass flowering (third week of August) and third cut at the beginning of seed set (second week of September).

**Table 1.** Location (coordinate system WGS84) and habitat characteristics (road type, road orientation, initial ragweed coverage (%)) of the experimental sites along arterial roads in Austria.

Site ID	Longitude (E)	Latitude (N)	Altitude (m)	Road type	Road orientation	Initial ragweed coverage
3	15°57'21.21"	46°42'59.81"	212	National	NW-SW	15
4	16° 3'9.65"	47°16'33.61"	381	Highway	SW-NE	5
5	16°50'41.91"	48°26'46.51"	170	National	N-S	14
6	16° 5'31.96"	47°42'17.61"	379	Highway	SW-NE	25
7	15°40'4.61"	48°10'54.87"	296	Highway	SW-NE	17
8	16°36'18.83"	48°18'40.06"	162	National	W-E	5

### Soil seed bank sampling

All sites have been sampled for soil seed bank before the start of the mowing experiment in spring 2009 and after three years of the experiment in spring 2012. The sampling was always performed just before or at the very start of the germination period in the field. First sampling was done in March 2009 preceding the different treatment of the plots: 20 soil cores (depth 7cm, 285cm<sup>3</sup>, equally distributed over 100m of the experiment plot) were taken at each site. After three years of applying the various treatments, in March 2012, 19 soil cores were taken per plot on each site.

The soil cores were analyzed for ragweed seed content using a wet sieving machine (Retsch). We counted all intact seeds and put them into wetted Petri dishes. In order to detect the proportion of viable seeds, first germination was induced by putting them into climate chambers at the following conditions: daylight for 8 hours at 30 °C and darkness for 16 hours at 15 °C. We stopped the germination trial after 4 weeks, left the dishes for drying out and stored them for 4 weeks at +4 °C in darkness, in order to overcome secondary dormancy by additional stratification. Afterwards, a second germination period was started at the same conditions like in the first session.

All seeds that did not germinate within the second germination session were tested for vitality by a standard staining (TTC-test with 1 % solution of 2,3,5 triphenyl tetrazolium chloride in pure water). For that, *Ambrosia*-achenes were first imbibed in tap water at room temperature for 24 hours. The achenes were then cut open with a scalpel to expose the embryo. The bigger part of the achene was used for testing, the other part was discarded. Achene halves were put into petri dishes, covered with TTC solution and left at 30 °C for 6 hours in absolute darkness. Finally seeds were evaluated under a dissecting microscope. All fully stained seeds were classified vital.

The soil seed bank samples in 2009 were taken from the whole sites that were covered consistently with *A. artemisiifolia*, and can therefore be used as baseline data for comparison to the soil seed bank counting at the differently treated plots three years later. That way, it is possible to observe the effect of the tested mowing regimes on the soil seed bank after three years of application.

Data were analyzed by GLM (generalized linear model) using Poisson distribution procedures and a log link in the package Statistica 10 (StatSoft 2011). Treatment was included in the model as independent categorical factor and seed number per m<sup>2</sup> as dependent variable. Pairwise differences between treatments were judged at 95% confidence intervals. We compared the overall most effective treatment with the initial seed bank of the populations of each site by Kruskal-Wallis Tests.

## Results

### Soil seed bank at different sites

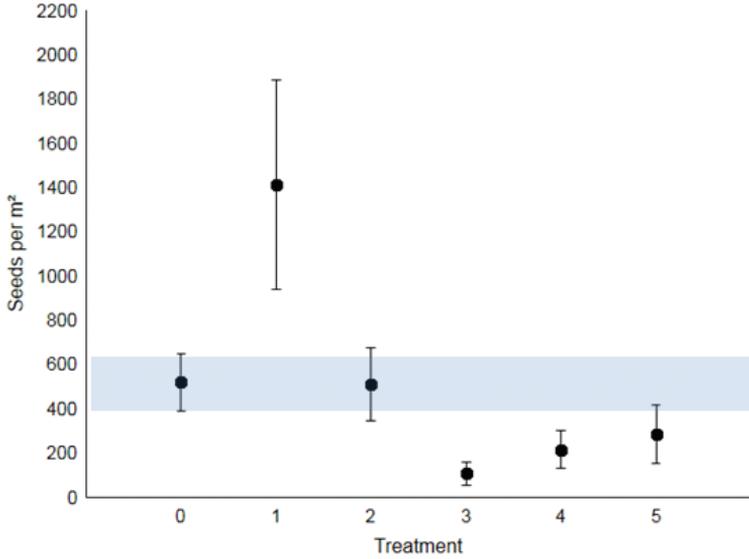
In 2009, soil seed bank varied from 123 to 823 (522 in average) seeds per m<sup>2</sup> at all sites (Table 2), with germination rates varying from 53 to 100% (mean 80%). In 2012, soil seed bank at different sites varied from 0 to 1061 seeds per m<sup>2</sup>. The germination rates were generally very high (mean 91%). From the 2012 samples, no seeds germinated during the second germination test, and no living seeds could be detected by the subsequent TTC test.

### Soil seed bank in different treatments

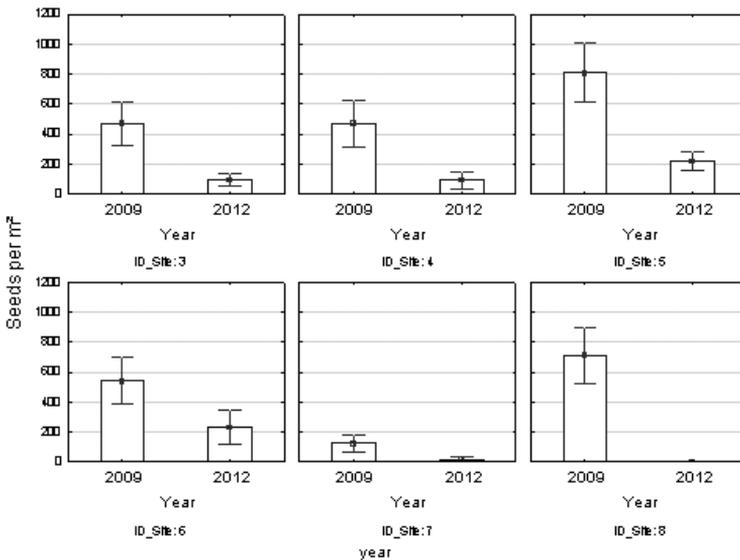
After 3 years of applying different mowing regimes, significant differences in the soil seed bank under different treatments were found (Wald  $\chi^2(5) = 188795$ ;  $p \leq 0,01$ ). The soil seed bank of treatment 1 (control, unmown) was three times higher than the soil seed bank of the population before the experiment (Figure 1). The soil seed bank of treatment 2 did not differ significantly from the soil seed bank of the population in 2009 (Figure 1). The soil seed bank of the treatments 3, 4 and 5 decreased by ca. 80%, 60% and 45%, respectively, compared to the magnitude order before the experiment (Figure 1). Efficacy of treatment 3 is obviously highest in controlling the ragweed populations sustainably. The soil seed bank decreased on all sites on the plots of treatment 3 (Figure 2), at most sites significantly (Table 3).

**Table 2.** Number of *Ambrosia artemisiifolia* seeds per m<sup>2</sup> (means and standard deviation (SD) calculated from 20 soil cores) in spring 2009 and in spring 2012 (calculated from 95 cores) at six experimental sites.

Site ID	Mean number of seeds/m <sup>2</sup> in 2009	SD	Germination rate (%)	Mean number of seeds/m <sup>2</sup> in 2012	SD	Germination rate (%)
3	467	652	66	1002	2069	98
4	467	699	53	394	1045	76
5	823	866	100	369	1102	98
6	541	702	77	1061	1181	98
7	123	246	90	205	565	86
8	713	836	95	0	-	-



**Figure 1.** Means and confidence intervals of the number of seeds of *Ambrosia artemisiifolia* per m<sup>2</sup> (depth 7cm) after 3 years of different mowing treatments (1–5) in 2012 compared to the soil seed bank of the population before the experiment in 2009 (“Treatment” 0 = baseline)



**Figure 2.** Mean numbers (and SE) of *A. artemisiifolia* seeds per m<sup>2</sup> (depth 7cm) in the plots of treatment 3 at six different sites in 2012 compared to the soil seed bank before the experiment in 2009

**Table 3.** Kruskal-Wallis test for the differences between the soil seed bank (seeds per m<sup>2</sup>) in plots of treatment 3 in 2012 and the soil seed bank of the respective populations in 2009, differentiated by sites.

Site ID	H	P
3	5.72	<0.05
4	6.65	<0.01
5	7.54	<0.01
6	3.04	0.08
7	3.74	0.53
8	14.7	<0.001

## Discussion

The number of ragweed seeds per m<sup>2</sup> found in populations along Austrian roadsides before the start of treatments in 2009 indicate that those are all well-established populations that cannot be controlled by a one time management action. The aboveground assimilating part of the *A. artemisiifolia* population varied between the sites at the beginning of the experiment (Table 1) but showed similar dynamics to the soil seed bank towards the end of the experiment. Compared to the soil seed bank of other ruderal habitats (waste lands and set-asides) our roadside populations showed relative low seed densities. Fumanal et al. (2008) describe seed densities ranging from 510–3324 seeds per m<sup>2</sup> in the upper 5 cm of soil. This indicates that the Austrian roadside populations are relatively young but ‘active’ populations. Corresponding to the high population turnover rates, most seeds accumulate in the uppermost soil layer and germinate at high rates to produce many new seeds every generation. The fraction of old seeds from former population establishment phases that might have lower germination rates, seems to be relatively low as the overall germination rates of the seeds in the soil is considerably high (Table 2).

The seed bank densities of ragweed along Austrian highways are generally lower than in European arable fields (Vitalos and Karrer 2008). Habitat types that have been infested by ragweed for decades, like abandoned fields in N-America, have a load of 0–200 ragweed seeds per m<sup>2</sup> even when sampling only the persistent soil seed bank in summer (Rothrock et al. 1993). Bigwood and Inouye (1988) found on average 36 ragweed seeds per m<sup>2</sup> in the upper soil (0–8 cm) and 57.6 seeds per m<sup>2</sup> at a depth of 8–16 cm in an old field in Maryland (US). Raynal and Bazzaz (1973) counted means of 64 ragweed seeds per m<sup>2</sup> in maize fields on former forest soil and 4.8 seeds per m<sup>2</sup> on former prairie soil, when analyzing the upper soil (0–5 cm) in early spring; autumn samples did not contain ragweed seeds. Considering that the Austrian ragweed seed populations along highways are concentrated at the upper horizons of the road shoulder soil, they can be classified as very active and contribute to an increasing infestation.

Because most management options act on the green parts of the plant, they are not sustainable. The most desired aspect of ragweed control is the successful elimination of persistent seeds from the soil. The results of this long term experiment show, that the

soil seed bank can be diminished vigorously by a sophisticated mowing management. The mowing regime should consist of a first cut in August, just at the first appearance of female flowers, and a second cut in early September, just before fertility of the female flowers on the regrowth from the base (Milakovic et al. 2014a). According to our results, we suggest to rate this mowing regime as the most sustainable and environmentally friendly control option, because it progressively leads to indirect depletion of the soil seed bank. This way the ragweed populations decline and can be managed easier. Hence the biologically most effective control measure of pulling out the remaining few plants by hand (Bohren et al. 2008) might become economically feasible.

We advise analyzing the soil seed bank of ragweed before installing a field experiment or defining a management regime for ragweed control, as well as after the activity. Thus sustainability can be proven. The knowledge about the status of soil seed bank is particularly important for ragweed populations growing on roadsides, as the upper soil is prone to transportation elsewhere, which contributes to further dispersal of ragweed seeds and creates new populations.

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