Dissertation

Optimisation of mowing regimes for the control of the invasive *Ambrosia artemisiifolia L.* on roadsides

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Eingereicht von Mag.ª Ivana Milaković

Betreuer: Ao. Univ.-Prof. Mag. Dr. Gerhard Karrer
Department für Integrative Biologie und Biodiversitätsforschung
Institut für Botanik

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Mowing of the roadside vegetation on the experimental site near Halbenrain, Styria

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Included papers

1. Milakovic, I., Fiedler, K., & Karrer, G. (2014). Fine-tuning of a mowing regime, a method for the management of the invasive plant, *Ambrosia artemisiifolia*, at different population densities. Weed Biology and Management, 14 (4), 232–241.

2. Milakovic, I., Fiedler, K., & Karrer, G. (2014). Management of roadside populations of invasive *Ambrosia artemisiifolia* by mowing. Weed Research, 54(3), 256-264.

3. Milakovic, I. and Karrer, G. (submitted). Mowing management influences the soil seed bank of the invasive plant *Ambrosia artemisiifolia*, submitted in NeoBiota

Curriculum vitae

Abstract

Ambrosia artemisiifolia L. (Common Ragweed) is an annual invasive neophyte with highly allergenic pollen. This *Asteraceae* species has been introduced from North America. It induces high costs related to human health and crop yield losses in its introduced range. In the last decades, its spreading along roadsides has been massive in Austria. Roadsides are subject to frequent mowing, which can enhance the production of male and female flowers and further spread the seeds. Persistent soil seed bank (with up to 39 year-viability), high seed production (up to 18000 seeds per individual in Europe), the re-sprouting capacities of the species and the logistical limitations imposed by road security issues make the control of common ragweed in this habitat especially challenging. Emptying the soil seed bank progressively by preventing massive seed production is a suitable management strategy in this context. I therefore searched for the optimal mowing regime, which would be logistically feasible and substantially decrease the number of produced seeds, while also reducing the number of male inflorescences in order to limit the pollen pollution. I tested cutting regimes with different timings and frequencies of cuts in a glasshouse experiment during one year, and on seven roadside populations over three years. The soil seed bank of the populations has furthermore been tested before and after the experiment on the roadsides. The results show that the cutting regime, if appropriately timed, can strongly decrease the production of both seeds and male inflorescences and have a slowing down effect on the phenological development of the plant. After three years, the tested mowing regimes proved capable of reducing the soil seed bank by up to 80%. The optimal management measure for the control of ragweed along roadsides must be adjusted to the phenological development of the plant.

For an effective and sustainable control of ragweed a first cut shortly before male flowering, to limit the quantities of released pollen, followed by subsequent cuts every three to four weeks, before the onset of new flowers on the re-sprouting lateral shoots, is recommended.

Zusammenfassung

Ambrosia artemisiifolia L. (dt. Beifußblättriges Traubenkraut, engl. Ragweed) ist ein einjähriger Neophyt mit hoch-allergenem Pollen. Dieser aus Nordamerika eingeschleppte Korbblütler verursacht hohe Kosten in den Bereichen Gesundheit und Landwirtschaft. In den letzten Dezennien hat sich Ragweed massiv entlang des Straßennetzes ausgebreitet. Straßenränder werden häufig gemäht, wobei die Mahd zum falschen Zeitpunkt oder zur falschen Frequenz eine Erhöhung der Anzahl der weiblichen und männlichen Blüten, sowie eine Weiterverschleppung der Samen verursachen kann. Die persistente Samenbank (mit einer Lebensfähigkeit bis zu 39 Jahren), die hohe Menge an Samen (bis zu 18 000 Samen pro Pflanze in Europa), die Produktion von regenerativen Trieben nach dem Schnitt und die Sicherheitsvorgaben im Straßenbereich machen das Management dieser Pflanze im Straßenbereich äußerst schwierig.

Eine progressive Entleerung der Samenbank durch die Verminderung der Samenproduktion ist in diesem Kontext eine gute Strategie für das Management. In vorliegender Dissertation wurde nach dem optimalen Schnittregime gesucht, das gleichzeitig die Anzahl der weiblichen Blüten und der männlichen Infloreszenzen verringert, logistisch durchführbar ist und die Samenbank reduziert. Verschiedene Schnittregime mit unterschiedlicher Frequenz und Zeitpunkt der Schnitte wurden zuerst ein Jahr lang im Glashaus, dann an sieben Straßenpopulationen in Ostösterreich in einem Zeitraum von drei Jahren getestet. Dazu wurde die Samenbank der Populationen vor und nach der Anwendung der Schnittregimes untersucht. Die Ergebnisse zeigen, dass ein richtig angepasstes Mähen die Produktion der Blüten verringern, die phänologische Entwicklung der Pflanzen verlangsamen, und in drei Jahren der Anwendung die Samenbank bis zu 80% verringern kann. Das optimale Schnittregime muss an die phänologische Entwicklung des Ragweeds angepasst sein. Ein erster Schnitt knapp vor der männlichen Blüte, gefolgt von weiteren Schnitten vor der Blüte der neuen Triebe, empfiehlt sich für ein nachhaltiges und effizientes Management von Ragweed.

1. Introduction

1.1 Context

Ambrosia artemisiifolia (common ragweed) is an invasive neophyte introduced to Europe from North America in the 19th century. Today, it is a major source of health and agricultural problems (DAISIE, 2009). Its current spreading all over the European continent (Kazinczi *et al.,* 2008, Bullock *et al.,* 2012, Smith *et al.,* 2013, Essl *et al.*, 2015) raises major concerns. The European Commission's project DAISIE classifies common ragweed among the "100 worst" species in terms of negative impact on biodiversity, economy and health. Its extremely allergenic pollen causes allergic rhinitis and asthma that must be treated medically. The high medical costs it entails have been estimated to €2,3 to 14,2 billion p.a. in Germany (Born *et al.,* 2012), more than €88 million p. a. in Austria (Jäger, 2006) and around CHF 350 million in Switzerland (Bohren, 2009). Common ragweed is an actual nuisance and a growing threat to all European countries, since its pollen is transported across long distances (Cecchi *et al.*, 2007).

Furthermore, the species causes substantial crop yield losses in agriculture (Brandes & Nitzsche, 2006, Zwerger & Eggers, 2008). The total costs of the impact of common ragweed on health and agriculture for the EU and neighbouring countries have been estimated to €4.5 billion per year (Bullock *et al.*, 2012). An intensification of *Ambrosia*-induced problems in agriculture and health is expected in the future, as the plant has shown positive correlations of spreading and pollen production in environments with higher concentrations of CO_2 in the air (Ziska & Caulfield, 2000, Wayne *et al.*, 2002). Moreover, models predict an expansion of the range of the species in Europe in the decades to come due to global warming (Essl *et al.*, 2009, Cunze *et al.*, 2013, Chapman *et al.* 2013, Storkey *et al.*, 2014, Hamaoui-Laguel *et al.*, 2015).

This growing problem led to the consensus that Europe-wide concerted measures should be taken to prevent the further spreading of ragweed. This was the conclusion reached by over 100 scientists from 24 countries who convened in two international congresses in September 2008 (Bohren, 2008).

Since spreading on roadsides is a problem that many countries are currently facing (Chauvel *et al.*, 2006, Essl *et al.*, 2009, Vitalos & Karrer, 2009, Joly *et al.*, 2011, Karrer *et al.*, 2011, Lemke, 2014), I focused my research on the development of an effective management method for this habitat type.

This thesis was embedded in an interdisciplinary research project on spreading mechanisms of ragweed along roadsides and in agriculture, the related biological fundamentals about ragweed, the sources and vectors of spreading, and management options for all concerned habitats (RAGWEED 2, Karrer *et al.,* 2011). After the end of this project, the research on mowing management of ragweed was continued in the framework of the European Union's project HALT Ambrosia on the management of common ragweed (Starfinger *et al.*, 2014).

The present PhD thesis aims to contribute to the understanding of the effect of particular mowing regimes on the reproductive processes of common ragweed. The results of this thesis will help improving the efficacy of non-chemical methods decreasing the production of seeds and the production of pollen while giving concrete suggestions about management practices.

1.2 Characteristics of *Ambrosia artemisiifolia L.*

Ambrosia artemisiifolia L. (common ragweed) is a summer annual *Asteraceae* native of North America (Basset & Crompton, 1975). This erect, 5- 70 (200) cm tall herb has a tap root, and its glabrous to rough hairy stems can be unbranched or bushy branched. The leaves are short-stalked, pinnately lobed (rarely unlobed) and typically opposite in the lower and alternate in the higher parts of the stem (Fig. 1). The high individual plasticity corresponds to the high (potential) RGR that gets evident on nutrient rich arable fields. On nutrient-poor sites, ragweed remains small and less branched (Karrer *et al.*, 2011).

Male flower heads contain 10-100 (200) male flowers and are arranged in spikes on the terminal parts of the axes (Fig. 2). Female flower heads are one-flowered and are situated in groups of 1-10 in the axils of the leaves. The fruits are one-seeded obovate achenes covered by the involucrum that often has some small spines in the middle part (Fig. 2). Up to 62 000 seeds (Dickerson & Sweet, 1971) and more than 18 000 seeds (Fumanal *et al.*, 2007) per individual were recorded in North America and Europe respectively.

Fig. 1: Typical habitus of an individual of common ragweed growing along roadsides.

The produced seeds are innately in primary dormancy, which is broken by stratification, i.e. winter-similar conditions of moist and chilling (Baskin & Baskin, 1980). In Eastern Central Europe, germination starts around the end of March or beginning of April, and can go until August (Szigetvári & Benkö, 2008). Seeds that did not germinate in this period fall into secondary dormancy. Those seeds accumulate in the soil, where they can remain viable up to 39 years (Toole & Brown, 1946), and germinate in the following years when the conditions are favourable. The start of male flowering is around the end of July in Austria, whereas that of female flowers occurs one week later, around the beginning of August. Those indications can vary by weeks (Bohren *et al.,* 2008a) depending on weather conditions. Meteorological temperature sums play an important role in the maturation of flowers (Laaidi *et al.*, 2004). The maturation of the first seeds is completed in September, when seeds start to drop off.

Figure 2 shows the developmental stages of male and female flowers. Figure 3 shows the life cycle of *Ambrosia artemisiifolia*.

This anemophilous herb with highly allergenic pollen grows typically on reportedly warm, ruderal sites experiencing regular disturbance, such as roadsides, cultivated fields, riversides and gardens. The regular top soil disturbance in these habitat types promotes its germination (Gebben, 1965, Fumanal *et al.*, 2008). *Ambrosia artemisiifolia* can germinate despite high soil salinity levels (Ditommaso, 2004). This gives ragweed an advantage over other salt-intolerant species on the winter-salted roadsides, where it can build dense populations. However, ragweed is not a highly competitive plant, as often suggested, as its cover decreases naturally with succession in undisturbed habitats (Lewis, 1973).

Ragweed is a highly allergenic plant. A single plant can produce between 100 million and 3 billion pollen grains during one season (Fumanal *et al.*, 2007), causing hay fever and asthma. Patients start to experience symptoms at less than 20 pollen grains per cubic meter of air (Smith *et al.*, 2013). In rare cases, the plant provokes allergic reactions through skin contact.

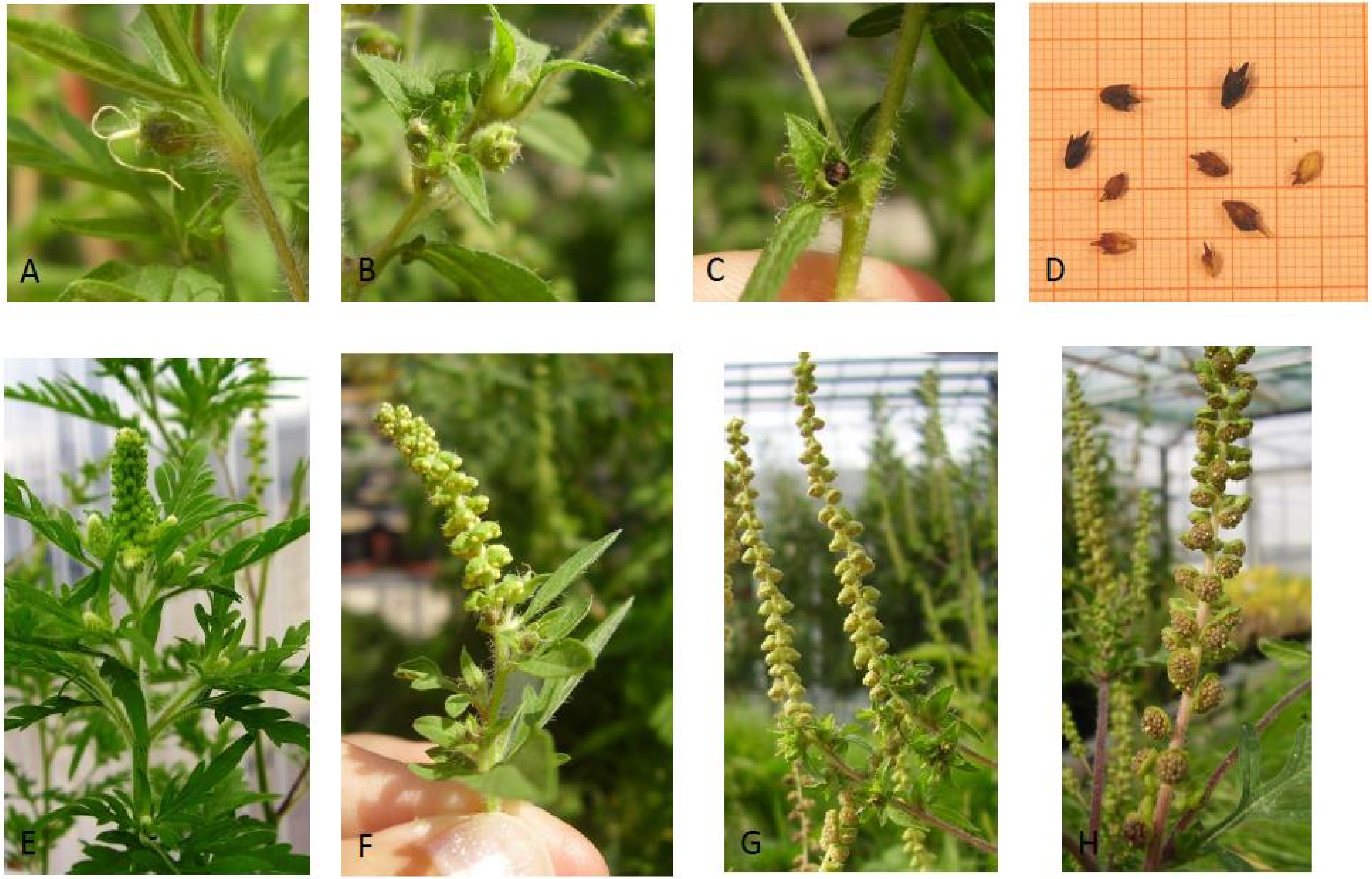

Fig. 2: Phenological stages of female flowers and male inflorescences: female flowers in flowering (A), developing/ripening seeds (B), seeds dropping off(C), seeds (D) and male inflorescences in development (E), just before flowering (F), flowering (G) and withered (H).

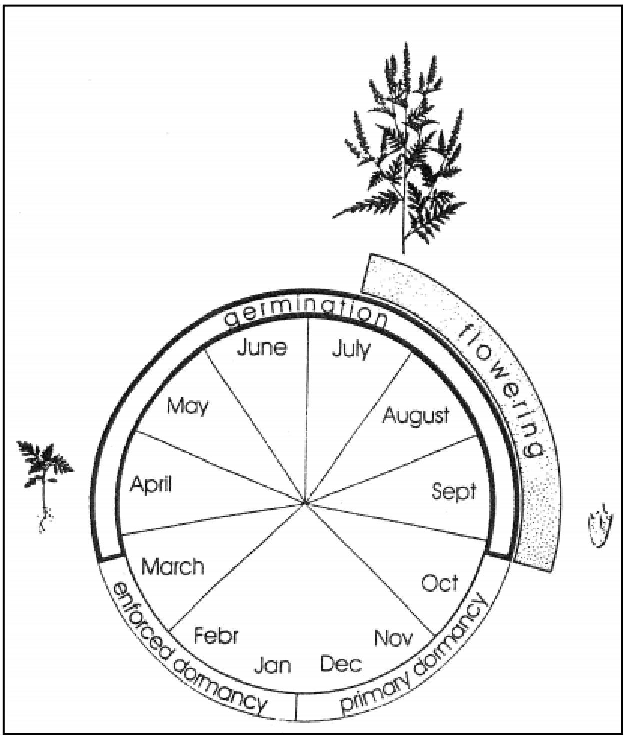

Fig. 3: Life cycle of Ambrosia artemisiifolia L. (after Beres & Biro 1993 in Kazinczi 2008, Copyright K. Biro and I. Beres).

1.3 Management

Roadsides are known to be corridors for the spread of invasive plants (Gelbard & Belnap, 2003, Von der Lippe & Kowarik, 2007, Jodoin *et al.,* 2008, Mortensen & Rauschert, 2009). These habitats have specific physical conditions caused by the traffic and road use. They experience disturbance to some extent owing to vehicles driving over, maintenance activities such as vegetation mowing or drainage ditch cleaning with the removal of the upper soil layer every few years. In cold winter regions, roads are also exposed to winter salting, which increases the salt pressure on the roadside vegetation (Blomqvist, 2001, Fay & Shi, 2012).

Past and current roadside management measures, obviously favour the spread and establishment of ragweed. The winter salting and mowing/mulching regime, in particular, foster the continuous increase of ragweed populations. The standard cutting regime on Austrian roadsides consists of 2-3 cuttings a year: an early first cut in April/May, often a second cut by the end of June and a very late third cut in autumn, in September/October. The cut material is not discarded. The first cut in spring reduces the supressing power of competing grasses and forbs, when the first seedling cohort of ragweed is tiny and not competitive yet. No cut during ragweed's flowering period in July, August or the first half of September leads to the massive release of pollen during summertime and the production of ripened seeds until mid-September. The seeds are spread by adhering to organic litter on the mowing machines (Vitalos & Karrer, 2009, Joly *et al.*, 2011).

Several methods can be considered for the control of the spread of ragweed on roadsides.

- The **hand pulling** of the plants including their roots, followed by their burning is a very efficient method, but it is very costly and can therefore only be applied on very small ragweed populations.
- The control through **herbicides** is not suitable for this habitat, because the road verges display trenches for water evacuation. These would transport herbicides into streams and pollute them. For this reason, the use of herbicides along roadsides is prohibited in many countries. Besides, many cases of resistance of *Ambrosia* to herbicides (e.g. glyphosate) have been reported (Saint-Louis *et al.*, 2005, Gerber *et al.*, 2011). The use of herbicides on roadsides, finally, is likely to damage the vegetation cover and ultimately facilitate erosion.
- **Biological control** is not developed yet, at least for Europe (Gerber *et al.*, 2011, Müller-Schärer *et al.*, 2014).
- The use of **sowing competing vegetation** proved promising under glasshouse conditions (Milakovic & Karrer, 2009) or on artificial soil from an abandoned quarry (Milanova *et al.*, 2010, Gentili *et al.*, 2015). However, it did not prove effective on roadsides within the first 3 years of field experiment (Milakovic in Karrer *et al.,* 2011, Gawalowski, personal communication). The gravel used to cover road verges often contains no or little humus. In those conditions the establishment of a closed vegetation is difficult, i.e. very slow.
- **Mowing** is the method used for the management of roadside vegetation, irrespectively of the presence of ragweed. Road verges are frequently mown in order to ensure the visibility of road signs and crossing animals. Several aspects make the management of *Ambrosia artemisiifolia* by mowing complex:
    - Firstly, the plant has the ability of re-growing quickly from the stubs after being cut (Fig. 4) (Brandes & Nitzsche, 2007, Vitalos, unpublished, Bohren *et al.*, 2008b, Meiss *et al.*, 2008).
    - Secondly, if mown too late, i.e. when the seeds drop off, the mowing machines can spread the seeds even further and thus achieve the opposite effect of expanding the populations (Vitalos & Karrer, 2009, Karrer *et al.*, 2011).

- Thirdly, a too frequent mowing can shift the regeneration to the production of seeds (Vitalos & Karrer, unpublished). Thus, the mowing of the roadside vegetation has to consider the structural development of *Ambrosia artemisiifolia* to be effective.

Fig. 4: Individuals of A. *artemisiifolia* from each cutting regime group from the glasshouse cutting experiment (experiment 1) on Sept. 18th 2009 (from left to right: 1: uncut control; 2: just after the second cut early Sept. (first cut 9.7.), 3: 10 weeks after the first cut on 9.7., 4: 1,5 weeks after the second cut (cut on 17.8. and 8.9.), 5: 4,5 weeks after the late first cut mid-August, 6: 1,5 weeks after the late first cut early September, 7: 1,5 weeks after the second cut (cut on 9.7. and 8.9.), 8: 1,5 weeks after the second cut (cut on 17.8. and 8.9.).

Little is known, though, about the impact of mowing regimes on the reproduction of ragweed when the plants are cut more than once during the vegetation period at various stages of plant phenology (Beres, 2004, Bohren *et al.*, 2008a, Patracchini *et al.*, 2011, Simard & Benoit, 2011). Besides, intraspecific competition can influence the reproductive traits of ragweed (Gebben, 1965, Simard & Benoit, 2011, Leskovšek *et al.*, 2012).

Finding the optimal mowing regime for controlling ragweed, all in all, is challenging due to the fact that the male and female flowers do not develop at the same time. It is hence difficult to reduce the numbers of both male and female flowers at the same time. Reduction of the number of male flowers is often the aim of control measures, whose goal is to reduce the quantity of allergenic pollen released in the air. But a decrease in the number of produced seeds and the consequent reduction of the soil seed bank is of higher importance in the longer term in order to reduce the populations and limit the spreading.

The number of germinating seeds varies with climatic conditions. Thus, the number of aboveground plants may not always be representative of the total population size of annual species in general and common ragweed in particular (Webster *et al.*, 2003). For ragweed, the percentage of viable seedbank germinating in one season can range from 0.1 to 38% depending on year and site (Forcella *et al.*, 1997).

The success of proposed management measures should therefore not only take into account the number of aboveground plants. It should also be evaluated by observing the influence of the management methods on the size of the soil seed bank. Reducing the number of viable seeds in the soil seed bank is the most successful management method to reduce the size of the population in the longer term. Soil seed bank can be depleted by increasing the losses or reducing the input of seeds. Classical methods of increasing the losses like stale seedbed, however, cannot be applied for roadside populations, because the vegetation cover on roadsides must remain intact to ensure the stability of road shoulders.

An optimal mowing regime should therefore aim at reducing the number of produced seeds in order to limit the spreading of the seeds and their input into the soil seed bank. It should also take into account public health considerations and aim to reduce of the number of male inflorescences produced, with the goal of reducing the quantities of produced pollen.

Therefore, this thesis' aim is to develop the optimal mowing regime for reducing the population size in the longer term, i.e. for decreasing the size of the soil seed bank. The optimal mowing regime that is searched here should moreover take into account the road security requirements and its logistical feasibility.

1.4 Research objectives

This PhD thesis aims at the improvement of non-chemical methods impeding the seed and pollen production of common ragweed. It intends to contribute conclusively to understanding the effect of particular mowing regimes on the reproductive process of common ragweed and, based on its findings, to offer concrete suggestions in terms of management practices, taking into account the practical constraints and logistical feasibility of the proposed methods. More specifically, this research aims at identifying the optimal combination in terms of timing and frequency of cuts so as to reduce the production of seeds and male inflorescences per plant as well as the soil seed bank.

This thesis, more explicitly, is given four specific goals:

- Firstly, to test experimentally the effect of different mowing regimes (including different cutting timings and frequencies) on the reproductive success of *Ambrosia artemisiifolia* and on other functional traits of the plant (i.e. production of male inflorescences) in glasshouse experiments.
- Secondly, to test experimentally the influence on the reproductive success and on the phenology of *Ambrosia artemisiifolia* of chosen mowing regimes under field conditions in seven spontaneous populations across Eastern Austria.
- Thirdly, to determine whether the use of various mowing regimes along highways over three years has an impact on the size of the soil seed bank of *Ambrosia artemisiifolia*.
- Fourthly, to recommend specific measures for controlling this weed based on the experimental findings.

The third question, i.e. the testing of the soil seed banks, was also used to measure the effectiveness of the various strategies. By comparing the soil seed bank before and after the various treatments, the research aimed at identifying which treatment most effectively led to the reduction of the soil seed bank.

2. Methodology

Three main experiments were conducted in order to answer the research questions.

Experiment 1 (Paper 1)

In the first experiment conducted in 2009, the influence of juvenile population density and of seven cutting regimes, differing in timing and frequency of cuts, on easily measurable reproductive traits, was tested. The number of male and female flowers and the phenological stage of the plants were documented on five terms during the vegetation period. This experiment was conducted during one vegetation period in collaboration with the Austrian Agency for Health and Food Safety (AGES).

Experiment 2 (Paper 2)

In the second experiment, four mowing regimes have been tested over three years (2009-2011) in field conditions on spontaneous roadside populations of A. *artemisiifolia* at seven sites across Eastern Austria. Biometrical measurements and phenological observations were done on five terms during the vegetation period, one before each cutting term and one at the end of the vegetation period. This large-scale experiment was planned and conducted in close collaboration with the authorities responsible for the management of vegetation on roadside verges (Fig. 5). The methods suggested for management could therefore be tested in a target-oriented manner, while taking into account their logistical feasibility.

Fig 5: Experimental site on highway A2 near Hartberg, Austria; in the foreground the unmown control subplot can be seen.

Experiment 3 (Paper 3)

In this experiment, the soil seed bank was sampled (Fig. 6) on the seven experimental sites of the three-year long experiment 2, before and after the experiment (i.e. in spring 2009 and spring 2012). The goal was to test the influence of four chosen mowing regimes on the soil seed banks. This experiment was used in the thesis as a measure of mid-term effectiveness of the tested mowing-regimes in controlling ragweed.

Fig. 6: Soil seed bank sampling.

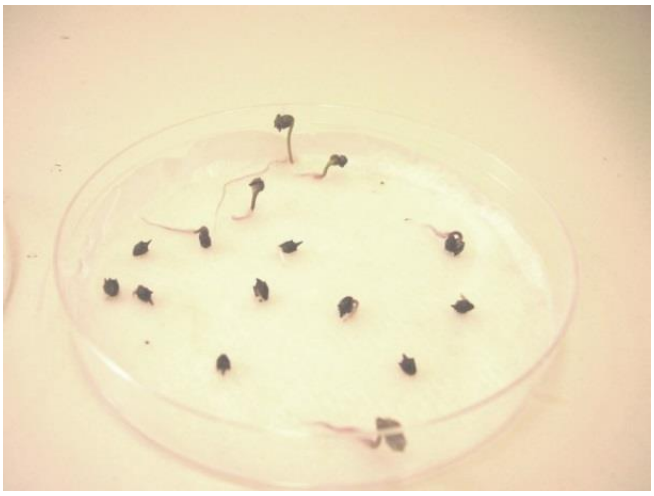

Fig. 7: Seeds of A. *artemisiifolia* germinating in a petri dish.

General remarks regarding the terms used in this thesis for describing/defining phenological/developmental stages of flowers and inflorescences (see also Fig.2):

- Female flowers in flowering: anthers visible and not dry
- Developing seeds: anthers dry, seeds green, we considered those seeds to be potentially ripened, sometimes referred to as "ripening seeds" or potentially ripened seeds
- Seeds dropping off: we considered those seeds to be ripened
- Male inflorescences in developing: inflorescences visible but not fully developed (no flowers opened yet)
- Male inflorescences flowering: flowers releasing pollen
- Male inflorescences withered: inflorescences dry, pollen release over

3. Discussion and recommendations

3.1 Biology and mowing effects

The male and female flowers of *Ambrosia* do not develop and flower at the same time. The male flowering starts around the end of July, whereas female flowering starts around one week later. Therefore the management cannot be optimal for the reduction of both kinds of flowers simultaneously. Mowing management can thus either primarily target the reduction of pollen, i.e. the number of male inflorescences, or the prevention of the production of ripened seeds, so as to prevent the further spreading of the plant.

My findings indicate that the cutting regimes tested in the glasshouse experiment can have a strong influence on the reproductive success of ragweed, as well as on its phenology. The variations in timing and frequency of cuttings had an impact on the numbers of male and female flowers and could be used to delay the phenological development of the plants.

Population density in juvenile stages did not play a role in further phenological development, but it did influence the number of male inflorescences and female flowers (see Tables 2 and 4, Paper 1). Those results have been confirmed by field experiments (Paper 2) on roadsides. This indicates that the methods tested here can be successful in field conditions and that they can be applied in the management of roadsides.

3.2 Mowing dates and response of the plant

The recommendations advanced in this thesis build on the combination of the results of the experiments, the practical knowledge gained through the realisation of the experiments, the close collaboration with practitioners and scholarly knowledge from literature. They are formulated in terms of phenological stages, and not in terms of calendar dates, so to enable their use in regions with different climatic conditions.

The timing of the first cut of the roadside vegetation is often too early (at least in Austria). Ragweed plants grow slowly in spring. A first cut before mid-June do not damage them at all, because they are still too small at that point and mostly remain below the cutting height of the machines. In case of a first cut in spring, only the surrounding competing vegetation is damaged, so that ragweed plants may even grow more vigorously. It is therefore recommended to apply a first cut when ragweed plants are more developed. This damages the plants more by depriving them from a larger proportion of their biomass. This early cut, however, should be carried out before the male flowering in order to limit the release of pollen (usually by mid-July in Middle European climatic zones).

In case of a single late cut during the female flowering (e.g. in mid-August for Austria), female flowers could be ripened or finish the ripening process after cutting (Pixner, 2012). Cutting ragweed plants at this stage would only further spread (ripened or ripening) seeds (Vitalos & Karrer, 2009), unless the material mown is discarded and burnt (which is rarely the case).

Ragweed plants have the ability to regrow new lateral sprouts after the cutting, just as perennial plants. Those lateral shoots can reach male anthesis in 17 days (Simard & Benoit, 2011) or build viable seeds in six weeks (Bohren *et al.*, 2008). Experiments from Paper 1 and 2 confirm that further cuts are necessary every 3 or 4 weeks as long as the plants are growing.

In the Paper 1 experiment, the plants in glasshouse conditions had new male inflorescences in anthesis 3 weeks after the cut. In field conditions (Paper 2), plants had already started developing new male inflorescences 4,5 weeks after the first cut in June. The same experiments show that 3 weeks after the cut, the plants already have female flowers in development; 6 weeks after the cut, those seeds can already be ripened (Paper 2).

Based on the overall results, it can be concluded that in Middle European climate zones, at least two cuts during the vegetation season are necessary. Depending on the main targets of the mowing management, two mowing regimes can be suggested:

1) <u>If the purpose is to reduce the production of pollen</u>

In this case, three cuts are recommended. The first cut must be applied just before the start of the male flowering (around mid-July in Austria). This first cut should be followed by two subsequent cuts, three to four weeks apart, in order to prevent further male flowering (and the ripening of female flowers).

2) <u>If the purpose is to reduce the production of seed</u>

In this case, two cuts are recommended. The first cut should occur at the beginning of female flowering (around mid-August in Austria). A second cut should follow three to four weeks later, in order to prevent the ripening of regrown female flowers (and further male flowering).

These indications should of course be adjusted to local populations, considering their stage of development under the local climate and seasonal weather conditions. The subsequent cuts should prevent newly build lateral shoots from reaching the flowering stage.

A one-off management intervention is not enough, in most cases, to have a controlling effect on the populations of *Ambrosia,* because of the presence of a soil seed bank (see 4.3. Soil seed bank effects). The management strategy suggested above must therefore be repeated over several years (depending on the size of the soil seed bank) until the population is under control. In an ideal case, the population is brought to such a small size, that individual plants can be pulled out manually. In the case of individual plants or small populations on the roadside, pulling out manually the plants with their roots before the flowering time, (and then burning them) as it has been done for some populations in Switzerland (Bohren 2009), is recommended.

3.3 Soil seed bank effects

The depletion of the soil seed bank is known as a valuable tool for weed control at disturbed sites (Mulugeta & Stoltenberg, 1997). Because it reflects the long-term development of the population size, the soil seed bank has been used as a measure of longer-term effectiveness in this study. The analyses of the soil seed bank before and after the application of mowing regimes (Paper 3) showed that mowing regimes can be successful in reducing the ragweed soil seed bank by up to 80% after three years of application. The soil seed bank should be sampled prior to deciding on the management measures to be applied. If the study of the soil shows the presence of a seed bank, additional measures should be taken to limit the spread of *Ambrosia*. Attention should be paid in road shoulders' maintenance procedures when taking away the upper soil layer. The soil collected in such places can contain viable ragweed seeds and form new populations where the soil is stored (Alberternst *et al.,* 2006). Also, the spreading of ragweed on roadsides could also be reduced to some extent by the simple measure of cleaning the mowing machines after work on an infected part of the roadside (Vitalos & Karrer, 2009). Attention should finally be paid to the post-harvest ripening of ragweed individuals that are cut late in the season (Pixner, 2012). They may either increase the local soil seed bank or contribute to the spreading of ragweed seeds, if the plants cut are deposited on green waste places.

3.4 Further recommendations for management praxis

Further recommendations can be articulated to improve management practices:

- Ragweed plants can re-sprout from lowest nodes and produce female flowers on low parts of the plant. Therefore, the mowing machines should be configured so that their cutting height should be the lowest possible. The cutting height, however, should not be too low either, so as to avoid scratching the soil surface. The frequent disturbance of the soil is indeed favourable to the germination of A. *artemisiifolia* (Gebben, 1965, Fumanal *et al.*, 2008).
- In habitats without soil disturbance, an absence of management can be a solution for control. *Ambrosia* has been reported to be displaced naturally through successional processes (Lewis, 1973, Raynal & Bazzaz, 1975).
- In any case, an analysis of the soil seed bank should be conducted before deciding on control measures. If a soil seed bank with viable seeds is present, the management method to be applied cannot boil down to a one-off intervention. It should instead be repeated over several years until the soil seed bank is depleted.

3.5 Implementation of the control measures: lessons learned

The late timing of the first cut hereby recommended is sometimes opposed by responsible land users or political authorities, because the vegetation may then reach a considerable height, and may prevent the drivers from seeing the delineator posts or the animals crossing the road, or because it may give the impression that the roadsides' maintenance is neglected.

The problem of the reduced visibility of delineator posts and animals crossing the road can be alleviated by yet cutting a triangle of vegetation in front of each delineator post, as done on some roads in the United Kingdom. If applied, this measure would additionally decrease the costs of maintenance of roadsides.

Cost reduction could also be achieved by postponing the very early cuts from April to June. Applying a too early cut, as a matter of fact, fosters the growth of *Ambrosia*: it does not damage the small ragweed plants, but reduces the competing vegetation, allowing ragweed plants to grow even better after the cut. An early spring cut, then, can lead to increase the spreading of *Ambrosia* along the roads. It can magnify road security risks by causing eyes irritation and sneezing among road drivers or road maintenance staff. If only one cut is possible, it should not be applied too late though, so as to avoid the risk of spreading ripened seeds (Bohren et al., 2008b).

3.6 Conclusions & outlook

A mowing management strategy carefully adapted to the phenological development of common ragweed can successfully reduce the numbers of both male and female flowers. The effect of such an adaptive mowing regime is a long-term and sustainable one: The size of the soil seed bank may be reduced by 80% in three years of application. For the control of populations with a soil seed bank, a one-off management intervention is not sufficient. Management measures must be carried on for several years until the soil seed bank is depleted.

The acceptance of the control measures by practitioners and policy makers is essential for the implementation of the management measures. Specific training courses for the personnel are necessary to enable them to recognize the different phenological stages of ragweed plants and consequently, the right timing of cutting.

In the future, it would be useful to further research the ripening processes of *Ambrosia artemisiifolia* seeds and especially its timing as influenced by cutting. A deeper knowledge about those processes would help to define the mowing timings even more accurately.

Research activities in the area of ragweed biology, impact and management are currently coordinated by the interdisciplinary network 'Sustainable management of *Ambrosia artemisiifolia* in Europe' (SMARTER, 2013–2017), funded under European COST action initiative (FA1203). The expert network includes healthcare professionals, aero-biologists, ecologists, economists and atmospheric and agricultural modellers.

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Paper 1

Fine-tuning of a mowing regime, a method for the management of the invasive plant, *Ambrosia artemisiifolia*, at different population densities

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RESEARCH PAPER

Fine-tuning of a mowing regime, a method for the management of the invasive plant, *Ambrosia artemisiifolia*, at different population densities

IVANA MILAKOVIC[1]*, KONRAD FIEDLER[2] and GERHARD KARRER[1]

[1]*Institute of Botany, Department of Integrative Biology and Biodiversity Research, University of Natural Resources and Life Sciences and* [2]*Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria*

Common ragweed (*Ambrosia artemisiifolia*) is an invasive annual plant with highly allergenic pollen. Its spread in introduced and native ranges often occurs on roadsides, where it builds stable and rapidly growing populations. The most sustainable way of controlling the population size of this species is to prevent seed production in order to deplete the soil seed bank. Populations on roadsides are submitted to regular mowing management, which can even exacerbate the situation by inducing resprouting after cutting or by accidentally spreading seeds along the road. The population density in the juvenile stages of development could play an important role in the success of cutting regimes, as it might influence the resprouting capacity of this plant. The influence of the juvenile population density and of seven cutting regimes, differing in the timing and frequency of cuts, on easily measurable reproductive traits was investigated in a glasshouse experiment. The cutting regimes had a strong influence on the reproductive success and on the phenology of the development stages of ragweed. The population density in the juvenile stages did not play a role in further phenological development, but did influence the reproductive traits. The reproduction of ragweed can be lowered by locally adapted combinations of the timing and frequency of mowing. As the optimal management option for the reduction of both the male and female flowers, the authors suggest a first cut just before the start of male flowering, followed by subsequent cuts every 3–4 weeks.

Keywords: annual, common ragweed, neophyte, reproduction, weed.

Ambrosia artemisiifolia L. (common ragweed) is an annual Asteraceae species. This ruderal plant, introduced to Europe from North America, grows on warm and disturbed sites. It shows salt tolerance at the germination stage of growth (DiTommaso 2004). This probably explains its massive occurrence in the vegetation of road shoulders, which are treated with de-icing salt and therefore represent unfavorable habitats for salt-intolerant species. This invasive plant is spreading all over Eurasia (Kazinczi *et al.* 2008); often, the spreading occurs massively along roads (Chauvel *et al.* 2006; Essl *et al.* 2009; Vitalos & Karrer 2009; Joly *et al.* 2011). *Ambrosia* has a high economical impact: its pollen causes allergies and asthma, the treatment of which can be very costly and it causes yield loss in different crops, such as sunflower, soy and pumpkin (Taramarcaz *et al.* 2005; Brandes & Nitzsche 2007; D'Amato *et al.* 2007; Zwerger & Eggers 2008).

The sustainable control of *A. artemisiifolia* is difficult because a single plant can produce ≤62,000 seeds (Dickerson & Sweet 1971). The seeds can persist in a germinable state in the soil for ≤39 years (Toole & Brown 1946). Some seeds become dormant after seed set and accumulate in the soil, forming a soil seed bank,

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**Correspondence to: Ivana Milakovic, Institute of Botany, Department of Integrative Biology and Biodiversity Research, University of Natural Resources and Life Sciences, Gregor Mendel Strasse 33, 1180 Vienna, Austria.*
Email: ivana.milakovic@boku.ac.at

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which provides seeds that can germinate in the following years when abiotic conditions are favorable. The management solutions for the control of this weed species therefore must aim at a continuous depletion of the soil seed bank. Mowing is the most common control measure in roadside vegetation. The vegetation must be mown for visibility and security reasons, as the use of herbicides is often prohibited by law because of environmental concerns. The influence of different mowing practices on the growth and reproduction of *A. artemisiifolia* is quite complex. This annual plant usually reacts to mowing like a perennial plant by resprouting from the basal buds that remain on the main axis below the cutting height (Brandes & Nitzsche 2007; Bohren *et al.* 2008). The timing of the cut can be essential. If done too early, mowing could enhance the production of male flower heads (Beres 2004). If done too late, the seeds might already have ripened before the cut (Bohren *et al.* 2008). Too-frequent mowing can cause this phenomenon as well (Vitalos M. & Karrer G., 2009, unpublished data). The spread of this plant along roadsides is massive and very quick. Vitalos and Karrer (2009) showed that the seeds that stick to the dirt on mowing machines are numerous and they possibly spread for several kilometres. The management of ragweed by mowing has been tested mostly for the effect of cutting, compared to no cutting. In contrast, very little is known as to how mowing regimes with more than one cut during the vegetation period, at various times during plant phenology, can affect ragweed reproduction (Beres 2004; Bohren *et al.* 2008; Patracchini *et al.* 2011; Simard & Benoit 2011). Also, little is known about the variation in the reproductive traits of ragweed in relation to intraspecific competition (Gebben 1965; Simard & Benoit 2011; Leskovšek *et al.* 2012).

In this study, it was hypothesized that variation in the timing and frequency of cuts and in population density can influence the plant reproductive traits of ragweed. Furthermore, it was expected that the plants that grow in dense populations in their juvenile stage would have more elongated first internodes and consequently fewer nodes remaining on the staples after mowing, so that resprouting could not be as successful as for the plants that grow in low-density populations. The high-density-grown plants therefore are expected to produce fewer flowers after cutting. The impact of the variation in the timing and frequency of mowing on the reproductive traits of ragweed individuals that originated from high-density, compared to low-density, populations was studied, as those traits serve as a basis for the development and refinement of cutting regime management methods. The hypothesis is that the precisely adapted timing and frequency of management can break the life cycle of ragweed by preventing seed production, progressively depleting the soil seed bank. The authors consider that knowledge about the reproductive traits of individual plants is essential for the successful control of the size of ragweed populations.

The following questions specifically were addressed in this study:

1. How do different mowing regimes affect the number of male inflorescences and female flowers?
2. Does the population density in the juvenile stage play a role in the reproductive success of *Ambrosia*?
3. Do the juvenile population density and cutting regime influence the phenological development of Ambrosia plants?
4. Which recommendations for management practice can be drawn from the results?

MATERIALS AND METHODS

The experiment was conducted in semi-open glasshouse conditions in 2009. The seeds that were used for the experiment were collected from a single ruderal population near Vienna, Austria (Seyring; 48°19′50.8′ ′N, 16°30′09.8′ ′E, 164 m a.s.l.) in fall 2008, naturally dried at room temperature for 1 month and then stored in darkness at 4°C. The plants were sown in early April 2009 in two different densities that are representative for natural populations along roadsides (Leitsch-Vitalos M. & Karrer G., 2009, unpublished data); that is, 153 seeds m^{-2} (low density) and 728 seeds m^{-2} (high density). The plants were grown in these densities for 12 weeks and subsequently transplanted into individual pots (17 cm high, 13 cm in diameter). The individually potted plants were positioned equally spaced on tables without further differences in density. After transplanting, seven cutting regimes (varying in the timing and frequency of cutting) and one control (no cutting) were applied to both the high-density and the low-density groups (see Table 1). Each combination of treatment and density consisted of ten *Ambrosia* individuals. The plants were cut at a height of 8 cm above the substrate surface in order to simulate the mowing conditions on roadsides. After the last cut at the end of September, most of the plants had died or no longer grew.

The following response variables were measured for all the individuals in all the groups at three dates, in mid-August, early September and at the end of September, always just before the cutting term:

1. The number of female flowers (regardless of their developmental stage) per individual.

Table 1. Plan of the cutting treatments

Cutting treatment	Cuts			
	July 9	August 17	September 8	September 29
1 (control; no cut)				
2	x†		x	
3	x			x
4		x	x	
5		x		x
6			x	x
7	x		x	x
8		x	x	x

† Cuts are represented by the letter x.

2 The number of male inflorescences per individual (each consisting of several flower heads arranged as spikes).
3 The phenological stage of the male inflorescences.
4 The phenological stage of the female flowers.

The scale that was used for the male phenology was: 1 = no flower; 2 = flowers are present but not yet in anthesis; 3 = flowering (the anthers are liberating pollen); and 4 = withered. For the female phenology, the scale that was used was: 1 = no visible flower; 2 = flowering; the stigmata are visible and not dry; 3 = seeds are developing (in the ripening process) and the stigmata are dry; and 4 = the seeds are dropping off. As most plants simultaneously possess flowers in different phenological stages, the most advanced phenological stage that was present on each individual was always recorded. The withered female flowers (i.e. "seeds") were considered to be potentially ripened and the "dropping-off seeds" were considered to be ripened.

For simplicity, in this article the term "female flowers" is used consistently, regardless of their phenological stage. At some assessment times, "female flowers" thus can refer to flowers, fruits or seeds, depending on their developmental stage.

The distribution of the phenological stages in the cutting treatments in July before the application of the cutting treatments is shown in Figure 1a.

Statistical analysis

The mean numbers of female flowers and male inflorescences were compared across treatments for August and two dates in September. The number of female flowers was compared only at the beginning and at the end of September. The data were analyzed by general linear model (GLM) procedures in the package, Statistica 10.0 (StatSoft 2011), using Gaussian distribution models for continuous predictors. As independent categorical factors, the treatment and juvenile density were always included in the models. The data for the number of female flowers per plant for early September and for the end of September were log-transformed in order to meet normality assumptions, whereas the data for the number of male inflorescences at the end of September were log $(x + 2)$-transformed. The inspection of residuals (Q:Q plots, frequency distributions) revealed a reasonable fit of the data to the statistical model assumptions in all the GLMs that were tested. The association of male and female phenology with the management treatments was analysed with contingency tables, using Pearson's χ^2-tests.

RESULTS

Male inflorescences

The number of male inflorescences per plant in August was associated with the juvenile population density (Table 2). The plants that were grown at a high population density during their juvenile stage had fewer male inflorescences per plant than the plants that initially were grown in the low-density populations (Table 3). In early September, the number of male inflorescences per plant was related to the cutting treatment (Table 2). The plants in cutting treatments 4, 5 and 8, cut for the first time in August, now had noticeably fewer male inflorescences than those in the other cutting treatments (Fig. 2b). At the end of September, the number of male inflorescences per plant was influenced by the juvenile density and the cutting treatment (Table 2). The plants that issued from the high-density populations had fewer male inflorescences than those from the low-density populations (Table 3). The plants of the uncut treatments 1 and 3 had far more male inflorescences on average than all the others (Fig. 2c).

Female flowers

The number of female flowers per plant in early September was associated with the cutting regime and with the juvenile population density (Table 4). The plants that were grown in the high-density populations during their juvenile stage had fewer female flowers per plant than the plants that initially were grown in the low-density populations (Table 3). The female inflorescence numbers varied across the cutting treatments in a manner

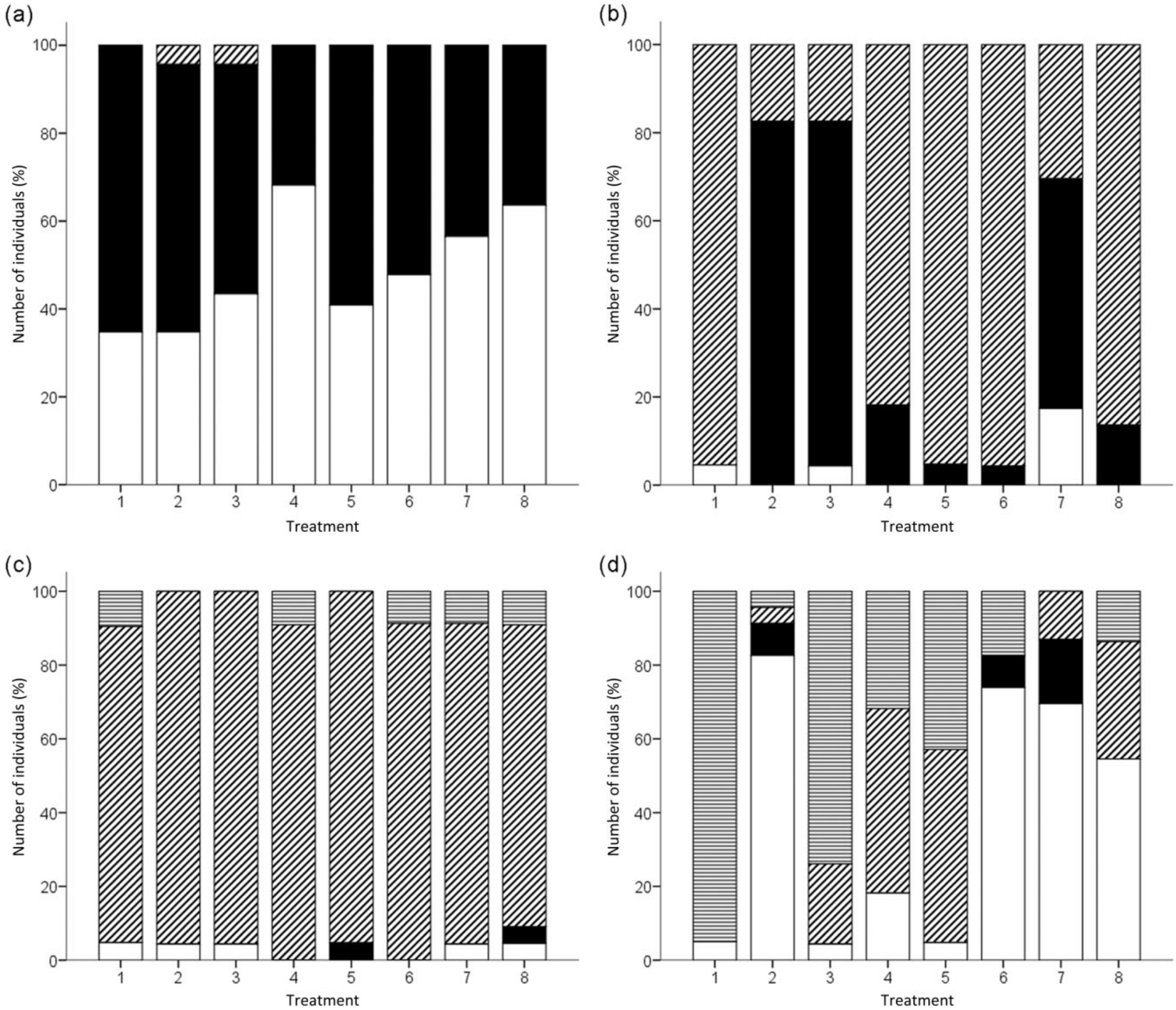

Fig. 1. Percentage of ragweed individuals in the different phenological stages of male inflorescences, according to treatment group: (a) in July, (b) in August, (c) in early September and (d) at the end of September. The vertical bars show the percentage of ragweed individuals. (□), No inflorescence; (■), inflorescences that were not yet flowering; (▨), inflorescences that were flowering; (▤), inflorescences that had withered.

Table 2. General linear model results for the number of male inflorescences per ragweed individual

Effect	August			Early September			End of September		
	d.f.	*F*-value	*P*-value	d.f.	*F*-value	*P*-value	d.f.	*F*-value	*P*-value
Treatment	7	1.49	0.173	7	20.74	**<0.001**†	7	108.12	**<0.001**
Density	1	19.48	**<0.001**	1	1.30	0.256	1	4.81	**0.029**
Treatment × density	7	1.27	0.269	7	1.68	0.118	7	1.40	0.208

† Values in bold represent significant results.

Table 3. Sample sizes, means and standard errors of the measured variables of individually potted *Ambrosia artemisiifolia* plants, according to their initial cultivation density

Inflorescence (time)	Low cultivation density			High cultivation density		
	N	Mean	Standard error	N	Mean	Standard error
Male inflorescences (August)	89	72.5	4.5	90	46.9	4.0
Male inflorescences(early September)	88	49.0	4.4	90	42.3	4.2
Male inflorescences (end of September)	87	19.0	3.4	90	14.1	2.8
Female flowers (early September)	88	281.7	25.9	90	213.9	16.7
Female flowers (end of September)	87	185.4	24.2	90	176.7	19.0

similar to that observed with the male inflorescences. The plants that were from cutting treatments 4, 5 and 8, cut for the first time in August, exhibited much fewer female flowers than those from the other cutting treatments (Fig. 2d). At the end of September, the number of female flowers per plant was significantly related to the cutting treatment and to the interaction of treatment and density (Table 4). The uncut control plants, as well as the cutting treatment 3 plants, showed up to fourfold higher the average number of female flowers than the plants in the other cutting treatments at this census term (Fig. 2e).

Phenology

The male phenology varied significantly across the cutting treatments in August and at the end of September (Table 5). In August, cutting treatment groups 2, 3 and 7, which had been cut in July (3 weeks before the August measurement), had a more delayed phenological development than those in the other cutting treatments: >70% of individuals were not yet in anthesis at this date (Fig. 1b). The plants in cutting treatments 1, 4, 5, 6 and 8 were more advanced in their phenological development of male inflorescences: >80% of individuals were already in anthesis in August (Fig. 1b). In early September, the cutting treatments did not differ significantly in the distribution of their phenological stages (Table 5, Fig. 1c). At the end of September, the plants in cutting treatments 2, 6, 7 and 8 comprised relatively more non-flowering individuals than the plants in the other cutting treatments (between 54 and 91% of individuals in these former treatments were non-flowering; Fig. 1d).

The female phenology likewise was significantly affected by the cutting treatment (Table 6). In August, cutting treatments 2, 3 and 7 (first cut in July) comprised mostly non-flowering individuals. Overall, most of the cutting treatments did not have many individuals bearing seeds at that time, except for control treatment 1 (Fig. 3). In early September, groups 4, 5 and 8 (cut in the preceding month) had the fewest individuals with seeds (Fig. 4). In cutting treatments 2, 3 and 7 (first cut in July), few individuals were already starting to have ripened seeds at this date. In contrast, the uncut cutting treatments 1 (control) and 6 (first cut just after that census) had the highest percentages of seeding individuals (Fig. 4). At the end of September, the cutting treatments with the lowest proportions of individuals bearing ripe seeds were those in which the plants were cut in early September (i.e. cutting treatments 2, 6 and 7). Cutting treatments 4 and 8, in which the plants were cut for the first time in August, had ≤50% of the individuals bearing ripe seeds, whereas cutting treatments 1 (control), 3 and 5 had 80–100% of the individuals bearing ripe seeds (Fig. 5).

At each census term, neither the male nor the female phenology was related to the initial juvenile plant density (Tables 5 and 6).

DISCUSSION

The plants that were grown in the high-density populations in their juvenile phase generally had fewer male inflorescences than those that were grown in the low-density populations. Possibly, the lower amount of available resources, caused by high intraspecific competition in the juvenile life stage, had lasting effects on the fitness of the plants. Indications in this direction have already been published by Patracchini *et al.* (2011), who observed a lower flower biomass at higher ragweed population densities, but these authors did not report on the statistical significance of their findings. An effect of plant spacing on reproductive traits was also suggested by Gebben (1965), who found evidence of higher numbers of staminate heads per plant at lower population densities, but again without indications on statistical

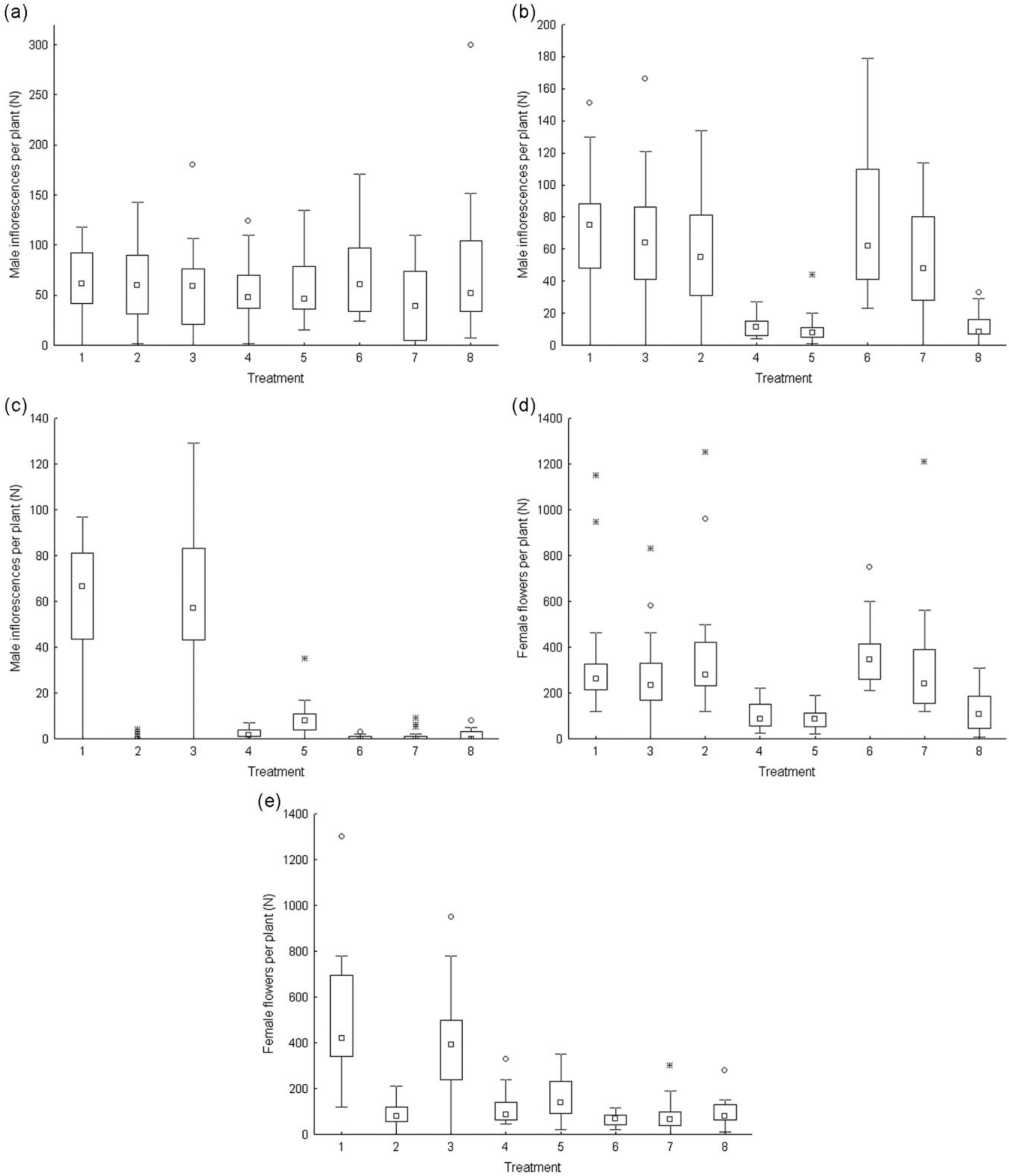

Fig. 2. Boxplots of the number of male inflorescences per ragweed individual in August (a), early September (b), end of September (c) and of the number of female flowers per plant in early September (d) and at the end of September (e) for plants under different cutting treatments. Small square, median; box, 25–75% percentiles; whiskers, area without outliers; circle, outlier; star, extreme.

Table 4. General linear model results for the number of female flowers per ragweed individual

Effect	Early September			End of September		
	d.f.	*F*-value	*P*-value	d.f.	*F*-value	*P*-value
Treatment	7	16.97	**<0.001**†	7	16.27	**<0.001**
Density	1	8.50	**0.004**	1	0.35	0.554
Treatment × density	7	1.12	0.355	7	2.70	**0.011**

† Values in bold represent significant results.

Table 5. Results of the contingency table analyses for the frequency distribution of the male phenological stages, according to the initial ragweed density and cutting treatment

Time	Density			Treatment		
	χ^2-value	d.f.	*P*-value	χ^2-value	d.f.	*P*-value
August	0.81	2	0.668	106.98	14	**<0.001**†
Early September	2.63	3	0.452	15.75	21	0.783
End of September	0.97	3	0.808	144.34	21	**<0.001**

† Values in bold represent significant results.

Table 6. Results of the contingency table analyses for the frequency distribution of the female phenological stages, according to the initial plant density and cutting treatment

Time	Density			Treatment		
	χ^2-value	d.f.	*P*-value	χ^2-value	d.f.	*P*-value
August	1.53	2	0.465	95.83	14	**<0.001**†
Early September	1.89	3	0.595	67.70	21	**<0.001**
End of September	2.04	3	0.564	99.69	21	**<0.001**

† Values in bold represent significant results.

significance. Also, Leskovšek *et al.* (2012) observed a decrease in male inflorescence biomass with increasing plant density in uncut ragweed populations; however, they did not state its significance level. In this study, the density did not play a role in male flowering phenology. The results of this study confirm the findings of Deen *et al.* (1998) and of Simard and Benoit (2011), who also did not observe any effect of density on phenological traits.

The number of male inflorescences was strongly related to the cutting treatment in this study. In August, all the cutting regimes had rather high numbers of male inflorescences, but cutting treatments 2, 3 and 7 were more successful than the others as they had a delay in phenology: 60–80% of the plants in those cutting treatments were not releasing pollen yet. Thus, if management aims at the reduction of pollen production, then a first cut in August is too late, as ≥80% of the plants that had not been cut before August now already were releasing pollen. In early September, the plants that were cut in August had five–eightfold less inflorescences than those in the other cutting treatments. In late September, the plants that were cut 3 weeks before had practically no male inflorescences, suggesting that within 3 weeks they could not develop new male inflorescences. As some of the cutting treatments were most effective in August and others were shown to be more effective in early September, it was concluded that the most effective regime for reducing the number of male inflorescences should combine the tested treatments. The plants

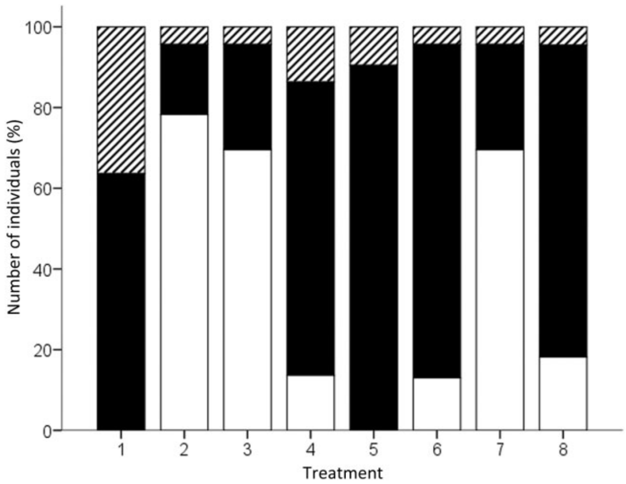

Fig. 3. Percentage of ragweed individuals in the different phenological stages of female flowers, according to the treatment group, in August. (□), No visible flower; (■), flowering; (▨), seeds.

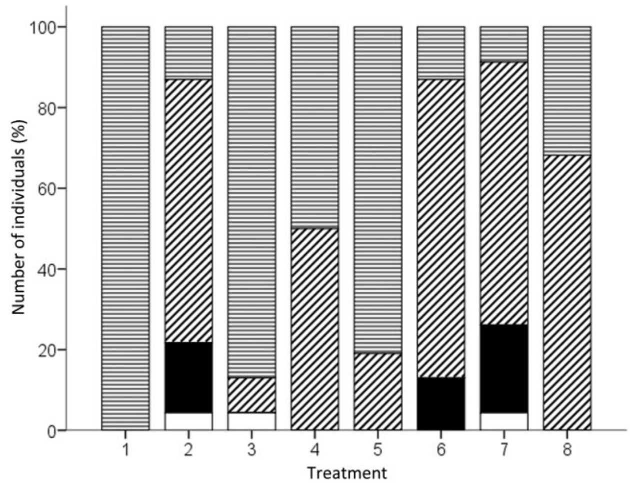

Fig. 5. Percentage of ragweed individuals in the different phenological stages of female flowers, according to the treatment group, at the end of September. (□), No visible flower; (■), flowering; (▨), seeds; (▤), seeds falling out.

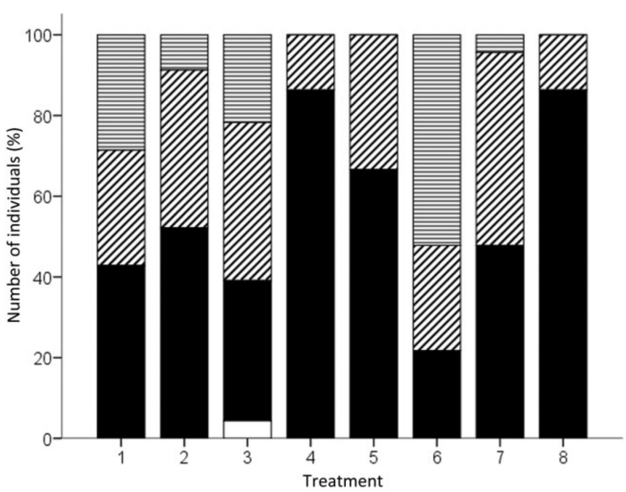

Fig. 4. Percentage of ragweed individuals in the different phenological stages of female flowers, according to the treatment group, in early September. (□), No visible flower; (■), flowering; (▨), seeds; (▤), seeds falling out.

should be cut in July just before the male flowering and then the cuts should be repeated every 3–4 weeks in order to prevent new male inflorescences from reaching anthesis.

For the female flowers, the cutting treatments were found to be effective at both relevant measuring terms (the beginning and end of September). The patterns were the same as for the male inflorescences. In early September, cutting treatments 4, 5 and 8 (all plants cut for the first time in mid-August) had the fewest numbers of female flowers per plant. Although the average number of female flowers per plant was not negligible in those cutting treatments, their phenology was not yet very advanced: in cutting treatments 4 and 8, only ~15% of the plants had seeds by early September. Therefore, the authors still consider that these cutting treatments are quite successful in controlling the number of seeds being produced.

At the end of September in all the cutting treatments (except 1 and 3), the number of female flowers was lowered. But, when taking into account the phenological stage of the flowers, cutting treatments 2, 6 and 7 were the most successful. They were associated with the lowest numbers of plants (~10–15%) bearing seeds that might have ripened. The authors suggest that this amount could be lowered even further by cutting plants 1 or 2 weeks earlier in September.

In none of the cutting treatments could the level of flower production be reduced to zero, most probably because of the height of the cut, which allowed regenerative shoot growth and a certain number of seeds to be produced below the cutting height. The phenological development, however, of the regrown organs was influenced by the cutting regime. Cutting treatments 2, 4, 7 and 8 were the most successful for sustainable ragweed control, but always at only one of the measurement dates. Cutting treatments 4 and 8, with a late first cut in August, could be improved by later successive mowing every 3 weeks, as the ragweed plants were able to mature the remaining seeds quickly after this cut.

Probably, the plant stubs and roots that were developed by the time of this first cut were already relatively rich in stored resources and allowed for the quick recovery and development during the following month, unless interrupted by a subsequent mowing.

In searching for the best management solution (aiming at a combination of reduced pollen release and prevention of seed production), the authors conclude that the most effective cutting regime under the climatic conditions of eastern Austria should be a combination of the herein-tested cutting regimes. Ragweed plants should be cut first in July, just before the anthesis of the first male flowers, and then cut twice subsequently (every 4 weeks) after this first cut. The total prevention of seed production by cutting will never be possible in natural ragweed populations as a cutting height below the cotyledonar node cannot be implemented in management practice.

In habitats with dense vegetation that is competing with ragweed, it can be assumed that the intraspecific competitive effect of a high population density can be extrapolated to the interspecific competition with the surrounding vegetation (Milakovic & Karrer 2011). Hence, the frequent practice of early spring mowing should be avoided; rather, the first cut should be delayed as far as possible towards male anthesis, ideally until the end of July in eastern central Europe. This method also could decrease the mowing costs in spring and at the same time it would help to minimize the number of male inflorescences on the plants in the critical flowering period.

The phenological status of ragweed plays a very important role in the improvement of management strategies. The results of this study show that the phenological stage of ragweed can be modulated by cutting, but this is not related to the juvenile population density. The cutting dates that were used in this experiment were developed as a compromise between biological optimization and the practical aspects of applicability in the field. For efficient management, the phenological development of the target populations must be monitored to set the cutting dates correctly. For instance, the date of the first cut (to prevent male flowering) might vary across regions by 4 weeks and across years within one region by 3 weeks.

As the plants in roadside populations grow with limited resources in comparison to glasshouse conditions, it can be supposed that their recovery after mowing would not be as vigorous and that their growth and phenological development would not be as fast. Also, the cutting height in field populations might vary, depending on the regularity of the soil surface and the machines that are used. These factors can strongly influence the success of ragweed control by cutting under field conditions (Milakovic *et al.* 2014).

In order to find the most effective and sustainable management solution, a compromise between several aspects must be found. Reducing the number of flowering male inflorescences and preventing seed production simultaneously might result in contradicting solutions. Optimal results can be achieved by setting the first cut just before the start of male anthesis, followed by subsequent cuts every 3–4 weeks, depending on the favorability of the habitat. This joins the basic idea of Bohren *et al.* (2008) of one late mowing. However, a very late first cut, for instance in September, is not a reasonable solution for roadside populations, as it will induce the spread of already-ripened seeds or the post-harvest ripening of seeds (Karrer *et al.* 2012); that is, if the biomass is not removed after the cut. Besides, in many countries, the vegetation along roads must be maintained below a certain height for traffic security reasons.

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Paper 2

Management of roadside populations of invasive *Ambrosia artemisiifolia* by mowing

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Management of roadside populations of invasive *Ambrosia artemisiifolia* by mowing

I MILAKOVIC*, K FIEDLER† & G KARRER*

*Institute of Botany, Department of Integrative Biology and Biodiversity Research, University of Natural Resources and Life Sciences, Vienna, Austria, and †Department of Botany & Biodiversity Research, University of Vienna, Vienna, Austria

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Summary

Ambrosia artemisiifolia (common ragweed) is a highly allergenic alien weed in Europe, which spreads rapidly along roadsides. Road verges are subject to frequent mowing, which further increases the spreading of the plants' seeds. *Ambrosia artemisiifolia* reacts to cutting by producing new shoots, which are able to develop flowers and ultimately new seeds. An effective mowing regime that would decrease the production of seeds and their dispersal is desirable to control the spread of the plant, but an appropriate way of mowing has yet to be found. In this study, we explored how the reproductive traits of *A. artemisiifolia* plants in seven spontaneous roadside populations reacted to the application of different mowing regimes over 3 years. The mowing regimes that were applied differed in the timing and frequency of cuttings. We found that the cutting regime, if appropriately timed, can strongly influence the production of male inflorescences (i.e. allergenic pollen), of female flowers (i.e. seeds) and had an impact on the phenological development of the plant. Based on our findings, we suggest that the optimal management of the plant along roadsides must be adjusted to its phenological development. The most effective mowing method of control consists of a first cut shortly before male flowering, to limit the quantities of released pollen, followed by subsequent cuts before the onset of new flowers on the resprouting lateral shoots.

Keywords: common ragweed, annual plant, weed control, cutting, neophyte, reproduction, traits.

Milakovic I, Fiedler K & Karrer G (2014). Management of roadside populations of invasive *Ambrosia artemisiifolia* by mowing. *Weed Research* **54**, 256–264.

Introduction

Ambrosia artemisiifolia L. (common ragweed) is an invasive neophyte that is now spreading all over Europe. This annual plant, accidentally introduced from North America, grows in disturbed sites such as roadsides, arable fields, riversides and gardens. It is a major threat for human health because its pollen induces severe hay fever and asthma (Hirschwehr *et al.*, 1998). Further, it causes serious crop yield losses both in its native and introduced range. This species produces up to 62 000 seeds per individual in North America (Dickerson & Sweet, 1971) or up to c. 18 650 seeds per individual in France (Fumanal *et al.*, 2007). Seeds are known to remain viable in the soil seedbank for up to 39 years (Toole & Brown, 1946). Seeds do not have specialised dispersal adaptations and are spread by water, animals and in soil or in crop seeds transported by man (Gebben, 1965). Generally, the process of spread is active at all range borders

Correspondence: I Milakovic, Institute of Botany, Department of Integrative Biology and Biodiversity Research, University of Natural Resources and Life Sciences, Gregor Mendel Strasse 33, 1180 Vienna, Austria. Tel: (+43) 1476543178; Fax: (+43) 1476543180; E-mail: ivana.milakovic@boku.ac.at

throughout the world. Spread along roadsides is considered a rapidly increasing problem (Chauvel *et al*., 2006; Essl *et al*., 2009; Vitalos & Karrer, 2009; Joly *et al*., 2011; Karrer *et al*., 2011). Vitalos and Karrer (2009) showed that contaminated mowing machines carry considerable numbers of seeds during the autumn mowing and disperse them along roads. The frequent soil disturbance in these habitats is favourable for the germination of *A. artemisiifolia* (Gebben, 1965; Fumanal *et al*., 2008a). The practice of winter salting (DiTommaso, 2004), as well as the high ecological tolerance of the species (Fumanal *et al*., 2008b), gives it advantage over many other salt-intolerant roadside plants.

Road security regulations generally require the vegetation of road shoulders to be kept at a low height for safety so that delineator posts remain visible and animals crossing the road can be seen from afar. In Austria, for instance, roadsides are mown twice a year, once in spring, once in autumn. The Europe-wide practice of mowing roadsides without considering *A. artemisiifolia* can increase the production and the spread of the seeds (Vitalos & Karrer, 2009).

The resprouting capacity of *A. artemisiifolia* after being cut is extremely high (Basset & Crompton, 1975; Barbour & Maede, 1981; Bohren *et al*., 2008; Meiss *et al*., 2008; Karrer *et al*., 2011; Patracchini *et al*., 2011; Tokarska-Guzik *et al*., 2011). Beres (2004) showed that a cut set too early in spring can enhance the production of flowers. The later the cut is set, the more female flowers and seeds are produced. A cutting date late in the year might cause further spread of already ripened seeds (Bohren *et al*., 2008; Vitalos & Karrer, 2009). I. Milakovic, K. Fiedler and G. Karrer (pers. comm.) found that the timing and the frequency of cuts and the density of juvenile *A. artemisiifolia* plants can influence their morphological and reproductive traits under glasshouse conditions.

Mowing is the favoured control option for ragweed along roadsides in many countries, including Austria and Switzerland. Herbicide application, even though allowed in some countries (e.g. in Germany and Hungary), is not permitted in sensitive areas and is often socially not acceptable. Moreover, mowing is applied on road shoulders for safety reasons (visibility of traffic signs and wild animals) and is thought to be effective also for controlling *A. artemisiifolia*. Unfortunately, inappropriate timing of mowing can create the opposite effect (Vitalos & Karrer, 2009; Joly *et al*., 2011). In our study, we sought a mowing regime that would at the same time meet road safety requirements and prove as efficient in controlling *A. artemisiifolia*.

We hypothesised that the timing and the frequency of cuts would have an influence on growth and reproduction of this annual plant in field populations. The mass flowering of male flowers of *A. artemisiifolia* does not happen at the same time as the female mass flowering. Therefore, mowing once can never be optimal for reducing the number of male and female flowers at the same time. An effective long-term control of the spread of *A. artemisiifolia* might best be secured by preventing seed production in order to avoid population growth and to deplete any soil seedbank. Therefore, we focussed on finding the best management for decreasing the number of produced seeds. Nevertheless, the cutting regimes should not increase the number of male inflorescences either, in order not to increase pollen emission. In this study, we analysed the response of reproductive traits of *A. artemisiifolia* plants to mowing regimes differing in timing and frequency of cuts, in seven spontaneous roadside populations throughout invaded regions in Austria. We tested these effects also for three consecutive years, to follow trends at the population level. The main aim of this study was to define regionally adaptable mowing regimes that would decrease the number of seeds and the number of male flowers in the medium term.

The following questions were addressed in this study:

1. How do the different, mowing regimes affect the numbers of male inflorescences and female flowers and the phenological development of *A. artemisiifolia* individuals in roadside populations?
2. Do different sites affect those traits and the treatment effects?
3. Which management measures based on mowing can be recommended for the control of *A. artemisiifolia* on roadsides, in Europe and generally?

Materials and methods

The experiment was conducted in seven roadside populations across eastern Austria from 2009 to 2011 (Table 1). Site 9 was established in 2010. Four populations were situated along national main roads, three along international highways.

In June 2009, 100 × 0.5 m experimental plots were installed on each of the six sites, and in June 2010 on the seventh site. Each plot had a continuous spontaneous population of *A. artemisiifolia* with coverages that ranged from 15% to 50% between plots. The replication level in this experiment was the individual plant, which was our unit of analysis (n = 20, see below). Every plot was subdivided into five 20-m-long subplots each of with received one of the following different treatments (mowing regimes):

1. Treatment 1: not mown (control).

Table 1 Location and habitat characteristics of the experimental sites

Site ID	Longitude (E)	Latitude (N)	Altitude (m)	Road type	Road orientation	Surrounding landscape structure
3	15°57 21.21	46°42 59.81	212	National	NW-SW	Fields
4	16°3 9.65	47°16 33.61	381	Highway	SW-NE	Forest
5	16°50 41.91	48°26 46.51	170	National	N-S	Fields
6	16°5 31.96	47°42 17.61	379	Highway	SW-NE	Fields/trees
7	15°40 4.61	48°10 54.87	296	Highway	SW-NE	Meadow/fields
8	16°36 18.83	48°18 40.06	162	National	W-E	Fields
9	16°25 45.02	47°48 29.52	208	Regional	NW-SE	Fields

2 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.
3 Treatment 3: first cut after the beginning of flowering (third week of August) and second cut at the beginning of seed set (second week of September).
4 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).
5 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).

The dates for cutting were set on the basis of the phenological development of the local populations but are described by monthly weeks. The phenological stages of the plants within populations at the time of cutting are given in Figs 2–4 in the Results section and in Figures S1–S5.

Cutting was conducted by the road maintenance authorities, with the mowers used in everyday practice to guarantee the most realistic treatments. The management plans were followed with 1-week precision, although in a few cases, one cut was locally omitted or cutting occurred 1 week too early or 1 week too late. These rare mowing plan irregularities unintentionally reflected the expected variance if the proposed cutting regimes would be applied on a regular basis. Data that were possibly influenced by such mistakes were excluded from statistical analyses.

Following response variables were measured at 20 randomly selected plant individuals per each subplot (i.e. treatment) per site just before the cutting dates:
1 Number of female flowers (independent of their developmental stage) per plant.
2 Number of male inflorescences (consisting of several flower heads arranged as spikes) per plant.
3 The phenological stage of male inflorescences.
4 The phenological stage of female flowers.

If there were fewer than 20 individuals per subplot left, all of them were measured. The stages for male phenology were defined as follows: 1 = no flowers, 2 = flowers present but not flowering (anthers not liberating pollen), 3 = flowering (anthers liberating pollen), 4 = withered. The stages for female phenology were as follows: 1 = no visible flowers, 2 = flowering, stigmas visible and not dry, 3 = developing seeds (potentially ripe), stigmas dry, 4 = ripened seeds dropping off. As most plants possess flowers in different phenological stages, we recorded consistently the most advanced phenological stage present on each individual. We considered the withered female flowers, that is, ‘seeds’ to be potentially ripened, and the ‘dropping off seeds’ to be ripened. In this study, the term ‘female flowers’ is being consistently used in the morphological sense, independent from its phenological stage. Thus, the term ‘female flowers’ can refer in the text to blooming flowers as well as to ripe seeds, independent of their developmental stage. Regrowth of plants after the last cut at the end of September was negligible; these plants were dying off rather than growing.

Statistical analysis

Numbers of female flowers and male inflorescences per plant individual were analysed for the months of the main flowering period. Hence, the number of male inflorescences was analysed for August and September, whereas the number of female flowers for September only. Data were analysed by linear mixed model (LMM) procedures in the package Statistica 10 (Stat Soft Inc., Tulsa, OK, USA). Data for number of female flowers per plant for September 2009 and for September 2010 were log(x + 2)-transformed to meet normality assumptions, whereas other counted data were square root-transformed. Inspection of residuals revealed a reasonable fit of data to the statistical model assumptions. Each year of the experiment was analysed separately, to circumvent distortions caused by different numbers of sites available in each year. Cutting treatment was used as fixed factor, whereas site was defined as random variable in LMMs, with treatment nested in sites. The results were controlled for a false discovery rate (Waite & Campbell, 2006). The association of male and female phenology with the management treatments was analysed with Pearson's chi-square tests, comparing frequencies of plants in different phenological stages across treatments. Data from sites where mowing mismatched the planned schedule for more than 1 week were excluded for further analysis in the respective year.

Results

Linear mixed models showed that the number of female flowers in September was strongly associated with treatment in 2009 and 2011. In 2010, this association was nominally significant, but did not persist after controlling for a false discovery rate. The average number of female flowers per plant was consistently smallest in treatments 3 and 5 in all 3 years (Fig. 1A–C). A highly significant relationship between the number of male inflorescences in September and cutting treatment was found in 2009 and in 2011; this effect was close to significance in 2010. As for female flowers, the lowest averages were found in treatments 3 and 5 (Fig. 1D and H). The number of male inflorescences in August was related to the treatment consistently over the 3 years of experiment. In all 3 years, plants in treatment 4 had fewer male inflorescences than all other groups (Fig. 1E–G).

A highly significant effect of treatment on male and female phenology was found at all sites in all years of the experiment. Moreover, the treatment was related to the phenology at almost all sites (Tables S1–S3).

For the phenology of female flowers in September, the proportions of phenological stages per treatment were similar in 2009 and 2011, when treatments 3 and 5 had the smallest fraction of individuals (*c*. 35–40%) bearing ripe or potentially ripened seeds (Fig. 2A and C). In September 2010 (Fig. 2B), treatment 3 followed this tendency with ca. 35% of potentially ripened seeds, whereas treatment 5 differed, having only 18% of plants that carried potentially ripe seeds. Proportions of phenological stages of the male inflorescences in September showed the same pattern in 2009 and 2011, when treatments 3 and 5 had highest percentages of individuals not yet flowering (around 60–70%; Fig. 3A and C). In 2010, this percentage was considerably higher in treatment 5 with over 80% of individuals not yet flowering. In contrast, treatment 3 exhibited *c*. 60% of individuals in those stages, similar to the situation in 2009 and 2011 (Fig. 3B). For the phenology of male inflorescences in August, identical patterns were found in all years of the experiment (Fig. 4A–C). Plants in treatment 4 had consistently the highest percentages, over 90%, of individuals not flowering yet (Fig. 4A–C).

Discussion

Our results show that the production of male inflorescences and seeds in *A. artemisiifolia* along roadsides can strongly be influenced by mowing management, if dates and frequency of cutting are rightly tuned to plant development.

Plants in treatments 3 and 5, with the lowest numbers of female flowers per plant in September, had both been cut in August. In September, they showed not only much lower numbers of female flowers than all other groups, but they comprised also the lowest fractions of individuals bearing potentially ripened or already ripened seeds (Fig. 2). Before the cut in August, the proportion of individuals bearing potentially ripened seeds was negligible (Figure S3). Therefore, those treatments can be evaluated as very efficient for management aiming to reduce seed production. These results are confirmed by glasshouse experiments (I. Milakovic, K. Fiedler and G. Karrer, pers. comm.) that showed an August cut is essential for management success. We further suggest that this cut would be even more effective if cutting is carried out 1 week earlier. In this case, we expect that no potentially ripened seeds at all would be present in September.

The coverage of *A. artemisiifolia* differed at the beginning of the experiment across sites between 5% and 60%. In 2011, it decreased to ca. zero on plots with significant reduction or to 45% on plots with ineffective treatment (G. Karrer & G. Rosei, pers. comm.). We took into account the problem of high year-to-year variation in annuals' coverage depending on annual climatic conditions and therefore rather focused on the number of female flowers and ripened seeds as measure of efficacy. As the seeds of *A. artemisiifolia* end up in the soil seedbank of roadsides, soil seedbank is a good measure for the comparison of the efficacy of treatments (SMARTER, 2013). Furthermore, the depletion of soil seedbank is known as a valuable tool for weed control at disturbed sites (Mulugeta & Stoltenberg, 1997) such as roadsides.

As the management of *A. artemisiifolia* often also aims to reduce pollen release, the number of male inflorescences, especially of those not flowering yet, should be considered. In September, we found that again treatments 3 and 5 were the most efficient in reducing the number of male inflorescences in two of 3 years of

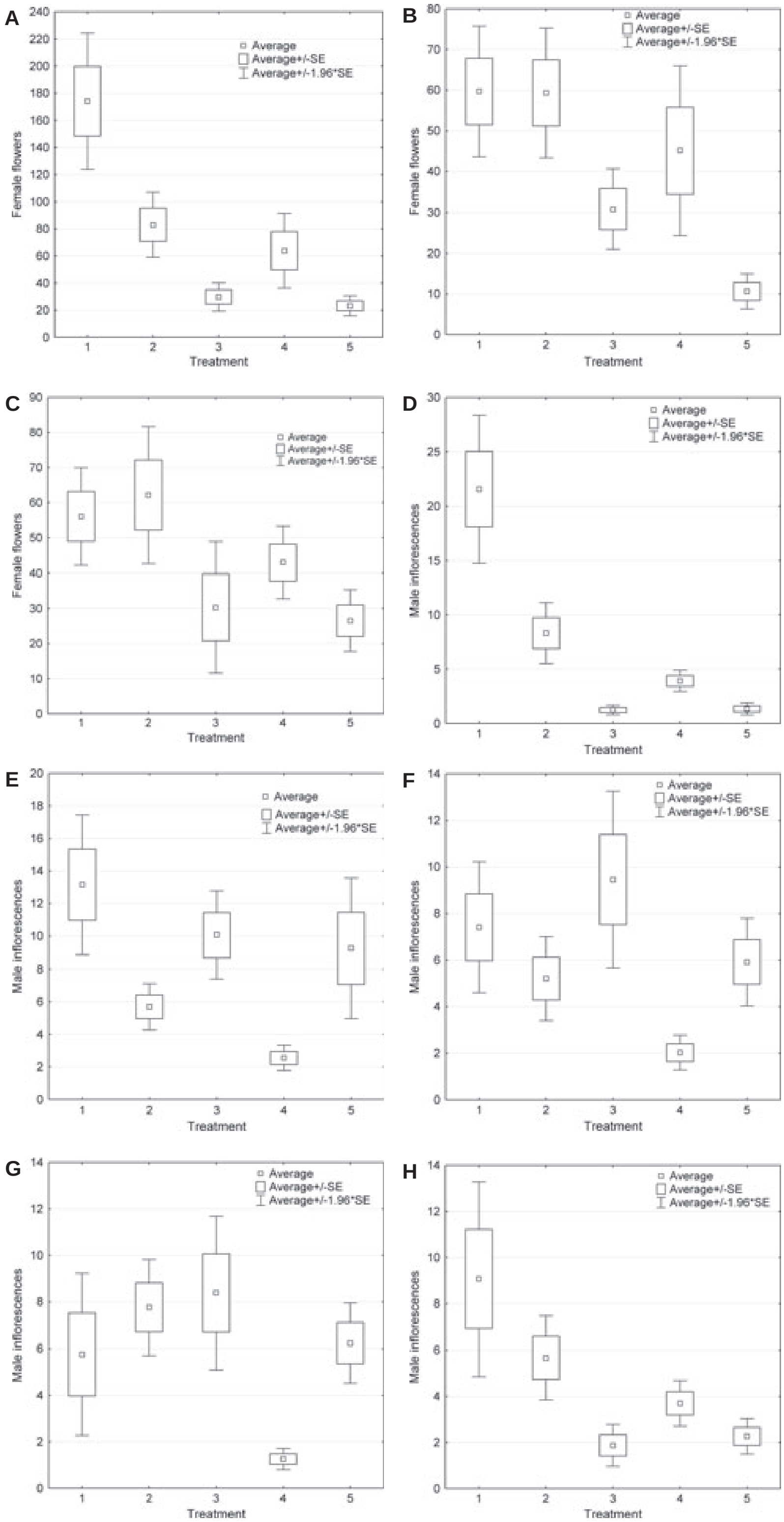

Fig. 1 Number of female flowers per *Ambrosia artemisiifolia* individual in September 2009 (A), September 2010 (B), September 2011 (C), and of the number of male inflorescences per individual in September 2009 (D), in September 2011 (H), in August 2009 (E), in August 2010 (F) and in August 2011 (G), on Austrian roadside populations in different cutting treatments.

the experiment (Fig. 1A–C). Those treatments were not only successful in reducing the number of inflorescences, but also the percentage of individuals at the flowering stage was lowest. In August, the treatment group 4 had extremely low numbers of male inflorescences per plant (Table S4) and the lowest percentage of individuals not flowering yet (>90%; Fig. 4). Thus, we conclude that a first cut in the third week of June and then a second cut 5 weeks later in the end of July is the most efficient for controlling the number of male inflorescences (and the amount of released pollen) in August.

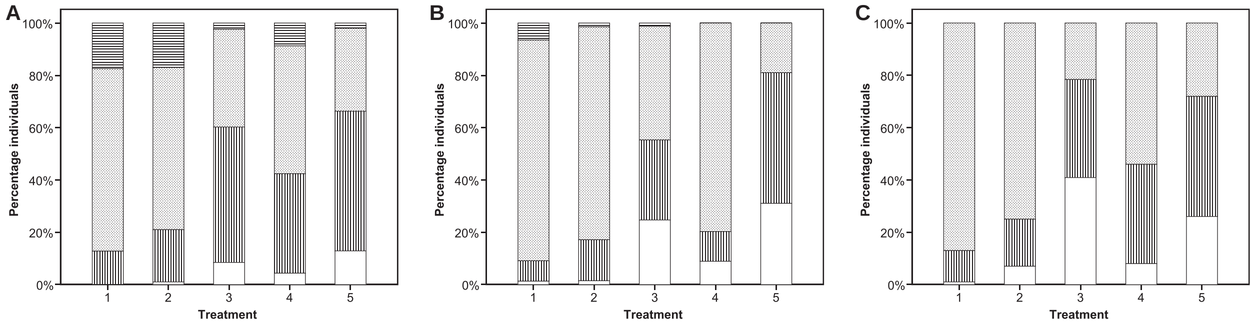

Fig. 2 Percentages of *Ambrosia artemisiifolia* individuals in different phenological stages of female flower development per cutting treatment in September 2009 (A), 2010 (B) and 2011 (C) in Austrian roadside populations. White fraction = no visible flowers; shaded by vertical lines = flowering; dotted fraction = developing seeds; shaded by horizontal lines = seeds dropping off.

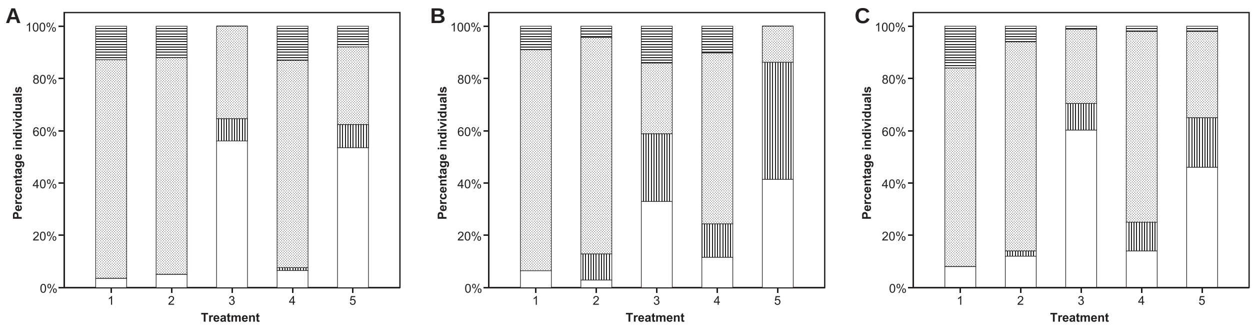

Fig. 3 Percentages of *Ambrosia* individuals at different phenological stages of male inflorescence development per cutting treatment in September 2009 (A), 2010 (B) and 2011 (C) in Austrian roadside populations. White fraction = no inflorescences; shaded by vertical lines = inflorescences not flowering; dotted fraction = inflorescences flowering; shaded by horizontal lines = inflorescences withered.

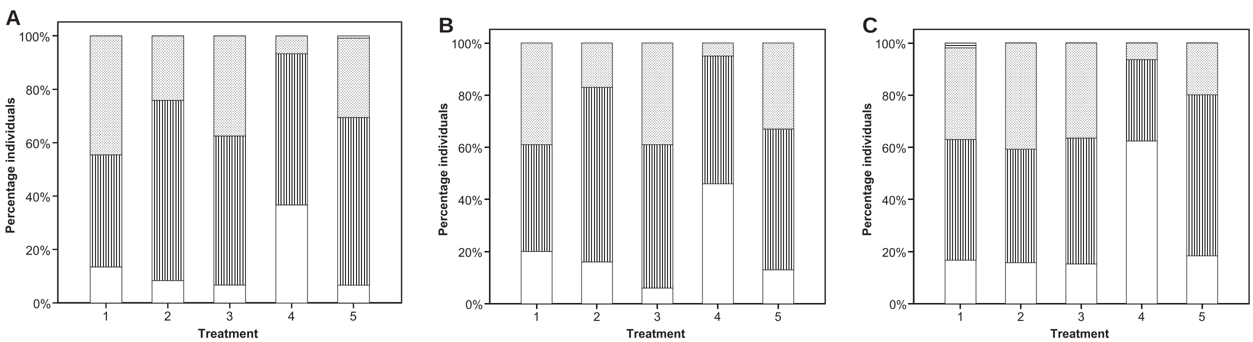

Fig. 4 Percentages of *Ambrosia* individuals at different phenological stages of the male inflorescences per cutting treatment in August 2009 (A), 2010 (B) and 2011 (C) in Austrian roadside populations. White fraction = no inflorescences; shaded by vertical lines = inflorescences not flowering; dotted fraction = inflorescences flowering; shaded by horizontal lines = inflorescences withered.

Site differences were far weaker and less consistent in comparison with the strong treatment effects. Different influences acting at different sites could be responsible for the site effects found. Of course, the bioclimatic conditions of the experimental plots vary by year and region. Across our study sites, a gradual increase in summer precipitation occurs from 600 mm annual rainfall in the north to 900 mm in the south. Varying substrates and ages of the roadside vegetation and the *A. artemisiifolia* populations and unknown random activities of the road maintenance services may also have contributed to differences between sites with regard to the reproductive traits. We also cannot exclude the selection of ecotypes that might have adapted already to the local conditions. *Ambrosia artemisiifolia,* as a preferably outcrossing annual herb, is expected to be highly flexible with respect to several characters determining the success at the population level. Population genetics indicate such high evolutionary flexibility does exist (Genton *et al.*, 2005; Blöch *et al.*, 2011).

Pulling out *A. artemisiifolia* manually is the most effective, but also the most costly control option. Thus, it is economically justified only if the populations are very small. Herbicides are commonly applied for *A. artemisiifolia* control in agriculture throughout Europe, but rarely along roadsides. Herbicide use is problematic in habitat types, such as roadsides, because the road shoulders are always associated with water runoff ditches that can facilitate movement to water systems. For this reason, the use of herbicides along roads is often prohibited or not welcomed by the general public. As herbicides have to be applied several times a year to control all cohorts of *A. artemisiifolia* seedlings, their use is also rather expensive. Moreover, the use of certain herbicides can cause a loss of vegetation cover and, consequently, an increase in erosion problems. Biological control is not developed yet, at least for Europe (Gerber *et al.*, 2011), but see Müller-Schärer *et al.* (2014). For the habitat-type road shoulders, mowing is the most suitable option for control when the populations are large. For road safety, vegetation on verges has to be mown anyway, even without the purpose of controlling *A. artemisiifolia*. In Switzerland, Bohren *et al.* (2008) propose to decrease established populations by mechanical or chemical control to a very low population density and to then pull out by hand the remaining few individuals.

In conclusion, the best management solution along roadsides to primarily reduce seed production and simultaneously limit as much as possible pollen release would be a compromise between the cutting regimes 3, 4 and 5. According to I. Milakovic, K. Fiedler and G. Karrer (pers. comm.), we suggest that the first cutting should best be carried out as late as possible, but anyway before male anthesis, which usually falls into mid-July in Eastern Central Europe (Kazinczi *et al.*, 2008). Our findings confirm those of Vincent and Ahmim (1985), conducted outdoors but not in a naturalised population, who suggested in the case of one cutting management that the single cut should ideally occur during the stage where flowers are already present, but before male anthesis. Optimal cutting dates cannot always be achieved in practice. For roadsides where mowing must occur earlier for security reasons, we suggest an initial mowing in the third week of June, followed by subsequent cuts every 3–4 weeks as long as plants grow, considering that mowing delays male anthesis by 17 days on average (Simard & Benoit, 2011). This time interval should of course be adapted depending on the dynamics of regenerative development of *A. artemisiifolia* in the respective climatic region. We strongly discourage the application of an even earlier first cut, as the results of Beres (2004) show that this might induce the compensatory production of additional male inflorescences. The current common practice of cutting first already in late April or in May is considered to be very favourable for the growth of *A. artemisiifolia* along roadsides, because the biomass of potential competitors that would shade young *A. artemisiifolia* individuals is removed. On the other hand, a very late first cut [i.e. in September, see Bohren *et al.* (2008)] might be highly disadvantageous as well, as many seeds are already mature at that time and are then spread further along the road by mowing machines (Vitalos & Karrer, 2009).

As a general rule, we advise that *A. artemisiifolia* plants should be cut as low as possible, in order to reduce the number of buds that might be able to resprout. *Ambrosia artemisiifolia* cannot be prevented from regenerating flowers below the cutting height. To optimise efficiency, any mowing plan must be finely tuned to local phenological development by monitoring some representative populations once a week during the vegetation period. Management should not be timed by fixed calendar dates, as the climatic conditions can vary from year to year and influence the phenological development of the plants. Also, tuning of management according to plant height, as shown by (Patracchini *et al.*, 2011), does not affect the percentage of flowering plants and is thus not useful.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Results of contingency table analysis for the frequency distribution of female phenological stages of *A. artemisiifolia* on roadsides in September according to cutting treatment in different years, hyphens stand for missing data.

Table S2 Results of contingency table analysis for the frequency distribution of male phenological stages of *A. artemisiifolia* on roadsides in September according to cutting treatment in different years, hyphens stand for missing data.

Table S3 Results of contingency table analysis for the frequency distribution of male phenological stages of *A. artemisiifolia* on roadsides in August according to cutting treatment in different years, hyphens stand for missing data.

Table S4 Sample sizes, row means and standard deviations for the variables: number of female flowers per plant in September, number of male inflorescences per plant in September and in August of *A. artemisiifolia* on roadsides, according to cutting treatment, for the years 2009, 2010 and 2011.

Figure S1 Percentage of *A. artemisiifolia* individuals in different phenological stages of the female flowers per cutting treatment in June 2009 (A), 2010 (B) and 2011 (C). White fractions: no visible flowers.

Figure S2 Percentage of *A. artemisiifolia* individuals in different phenological stages of the female flowers per cutting treatment in July 2009 (A), 2010 (B) and 2011 (C). White fractions: no visible flowers.

Figure S3 Percentage of *A. artemisiifolia* individuals in different phenological stages of the female flowers per cutting treatment in August 2009 (A), 2010 (B) and 2011 (C). White fractions: no visible flowers; shaded by vertical lines: flowering; dotted fraction: seeds; shaded by horizontal lines: seeds falling out.

Figure S4 Percentage of *A. artemisiifolia* individuals in different phenological stages of the male inflorescences per cutting treatment in June 2009 (A), 2010 (B) and 2011 (C). White fraction: no inflorescences; shaded by vertical lines: inflorescences not flowering.

Figure S5 Percentage of *A. artemisiifolia* individuals in different phenological stages of the male inflorescences per cutting treatment in July 2009 (A), 2010 (B) and 2011 (C). White fraction: no inflorescences; shaded by vertical lines: inflorescences not flowering; dotted fraction: inflorescences flowering; shaded by horizontal lines: inflorescences withered.

Supplementary material

Supplementary Table S1 Results of contingency table analysis for the frequency distribution of female phenological stages of *A. artemisiifolia* on roadsides in September according to cutting treatment in different years, hyphens stand for missing data

	2009			2010			2011		
	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*
All sites	96.45	12	<0.001	123.03	12	<0.001	149.49	8	<0.001
Site 3	59.58	12	<0.001	53.59	12	<0.001	68.89	8	<0.001
Site 4		-		44.59	8	<0.001		-	
Site 5	37.20	12	<0.001	55.97	12	<0.001	36.47	8	<0.001
Site 6	23.59	12	0.02		-		46.93	8	<0.001
Site 7	21.80	12	0.04		-		18.89	4	<0.001
Site 8	27.48	12	0.007		-			-	
Site 9		-		50.76	12	<0.001	71.69	8	<0.001

Supplementary Table S2 Results of contingency table analysis for the frequency distribution of male phenological stages of *A. artemisiifolia* on roadsides in September according to cutting treatment in different years, hyphens stand for missing data

	2009			2010			2011		
	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*
All sites	186.77	12	<0.001	151.58	12	<0.001	173.05	12	<0.001
Site 3	54.23	12	<0.001	44.90	8	<0.001	43.49	12	<0.001
Site 4		-		63.37	12	<0.001		ok	
Site 5	71.98	12	<0.001	56.10	12	<0.001	68.61	12	<0.001
Site 6	50.83	12	<0.001		-		42.18	12	<0.001
Site 7	23.05	12	0.027		-		9.11	8	0.33
Site 8	30.01	12	0.003		-			-	
Site 9		-		51.79	12	<0.001	90.40	12	<0.001

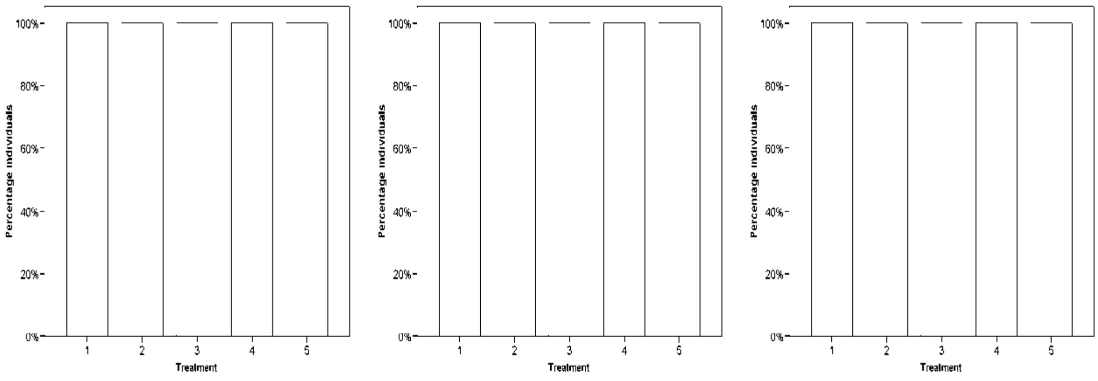
Supplementary Table S3 Results of contingency table analysis for the frequency distribution of male phenological stages of *A. artemisiifolia* on roadsides in August according to cutting treatment in different years, hyphens stand for missing data

	2009			2010			2011		
	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*
All sites	101.58	12	<0.001	87.26	8	<0.001	152.68	12	<0.001
Site 3	40.51	8	<0.001	39.77	8	<0.001	28.28	8	<0.001
Site 4	19.89	8	0.011	17.08	8	0.03	25.34	8	0.001
Site 5	21.05	8	0.007	26.46	8	<0.001	39.58	12	<0.001
Site 6	27.67	8	<0.001		-		37.35	8	<0.001
Site 7	20.18	12	0.064	19.35	8	0.013	29.18	12	0.004
Site 8	38.58	8	<0.001		-		12.73	8	0.12
Site 9		-		87.26	8	<0.001	74.47	8	<0.001

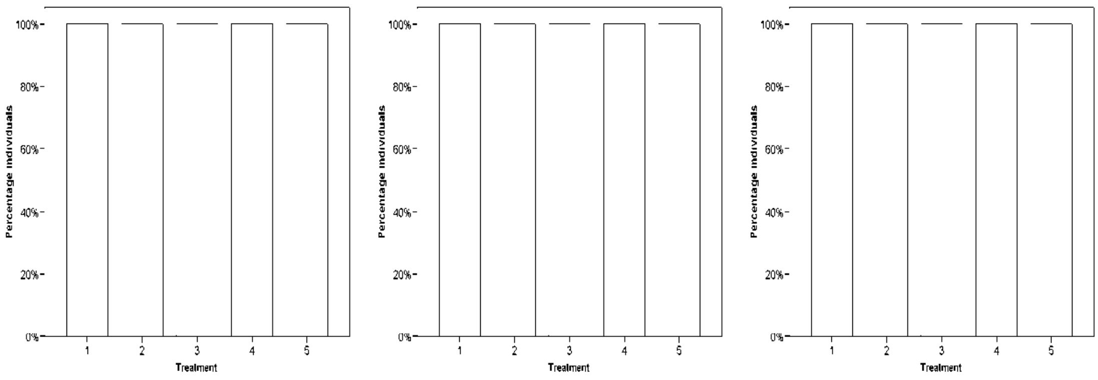
Supplementary Table S4 Sample sizes, row means and standard deviations for the variables number of female flowers per plant in September, number of male inflorescences per plant in September and in August of *A. artemisiifolia* on roadsides, according to cutting treatment, for the years 2009, 2010 and 2011

	Treatment	n	mean	SD
Female flowers September 2009	1	86	174.1	238.2
	2	100	82.9	122.2
	3	83	29.8	48.6
	4	92	63.9	134.7
	5	101	23.3	37.3
Female flowers September 2010	1	78	59.7	72.5
	2	70	59.3	68.3
	3	85	30.8	46.2
	4	79	45.2	94.4
	5	58	10.6	16.8
Female flowers September 2011	1	100	56.0	70.8
	2	100	62.1	99.6
	3	88	30.3	89.3
	4	100	43.0	52.4
	5	100	26.5	44.7
Male inflorescences September 2009	1	86	21.6	32.2
	2	100	8.3	14.3
	3	83	1.2	2.1
	4	92	3.9	4.8
	5	101	1.3	2.8
Male inflorescences September 2010	1	78	7.2	8.6
	2	70	12.0	16.0

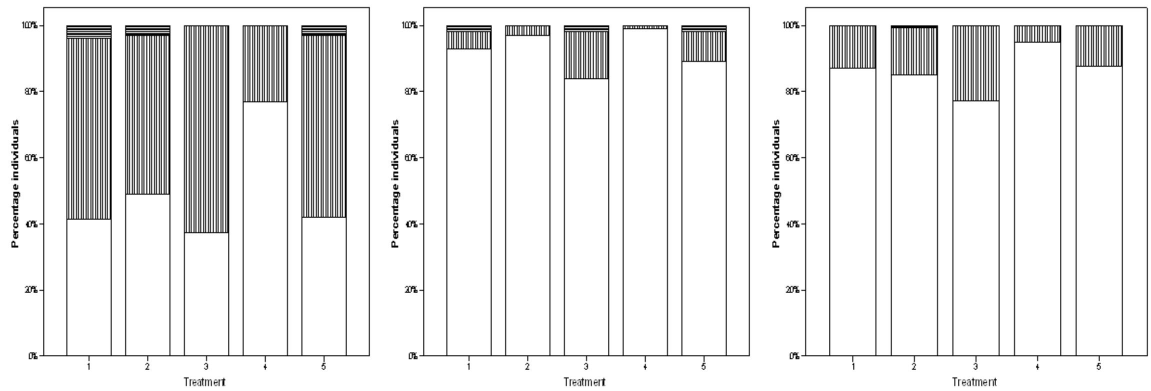
	3	85	8.5	17.8
	4	79	4.1	6.0
	5	58	1.6	1.8
Male inflorescences September 2011	1	100	9.1	21.5
	2	100	5.7	9.3
	3	88	1.9	4.3
	4	100	3.7	5.0
	5	100	2.3	3.9
Male inflorescences August 2009	1	119	13.2	23.8
	2	120	5.7	7.9
	3	120	10.1	15.0
	4	120	2.6	4.3
	5	121	9.3	24.1
Male inflorescences August 2010	1	100	7.4	14.3
	2	97	5.2	9.1
	3	100	9.5	19.4
	4	100	2.0	3.8
	5	100	5.9	9.6
Male inflorescences August 2011	1	108	5.8	18.4
	2	140	7.8	12.4
	3	118	8.4	18.2
	4	141	1.3	2.7
	5	136	6.2	10.2



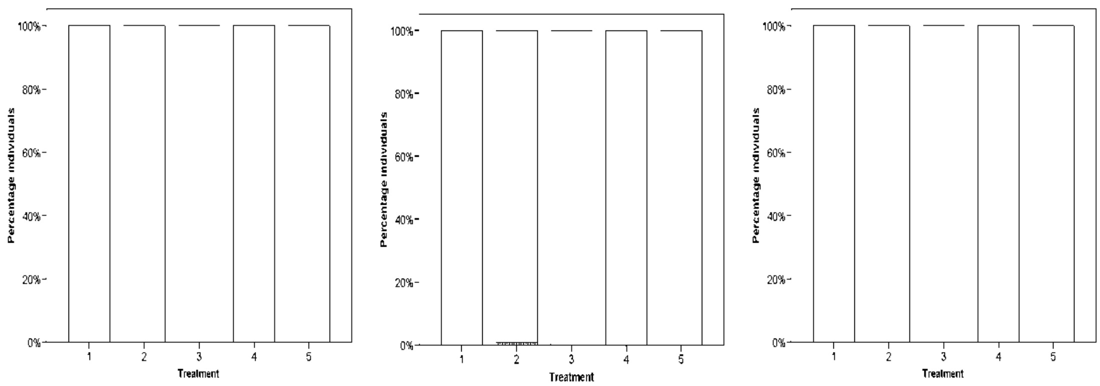
Supplementary Fig. S1 Percentage of *A. artemisiifolia* individuals in different phenological stages of the female flowers per cutting treatment in June 2009 (A), 2010 (B) and 2011 (C). white fractions: no visible flowers



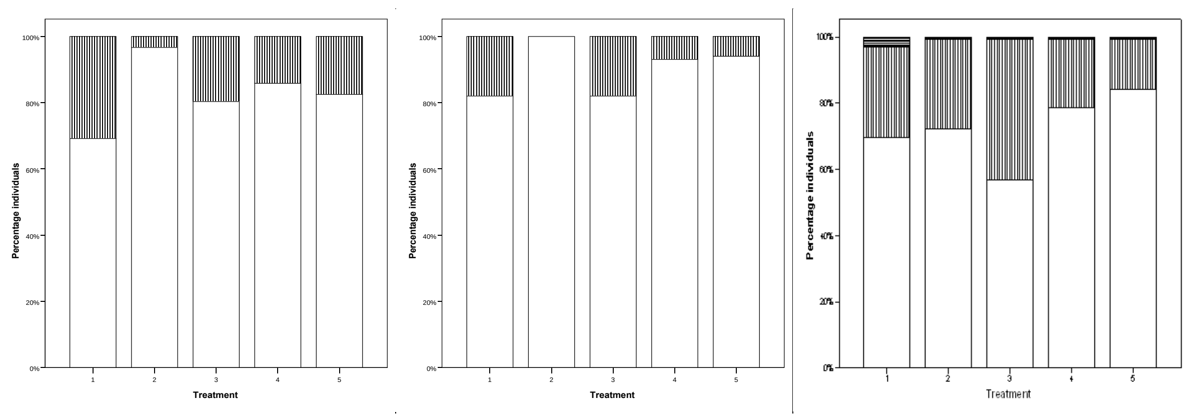
Supplementary Fig. S2 Percentage of *A. artemisiifolia* individuals in different phenological stages of the female flowers per cutting treatment in July 2009 (A), 2010 (B) and 2011 (C). white fractions: no visible flowers



Supplementary Fig. S3 Percentage of *A. artemisiifolia* individuals in different phenological stages of the female flowers per cutting treatment in August 2009 (A), 2010 (B) and 2011 (C). white fractions: no visible flowers; shaded by vertical lines: flowering;, dotted fraction: seeds; shaded by horizontal lines: seeds falling out



Supplementary Fig. S4 Percentage of *A. artemisiifolia* individuals in different phenological stages of the male inflorescences per cutting treatment in June 2009 (A), 2010 (B) and 2011 (C)., white fraction : no inflorescences; shaded by vertical lines: inflorescences not flowering



Supplementary Fig. S5 Percentage of *A. artemisiifolia* individuals in different phenological stages of the male inflorescences per cutting treatment in July 2009 (A), 2010 (B) and 2011 (C). white fraction: no inflorescences; shaded by vertical lines: inflorescences not flowering; dotted fraction: inflorescences flowering; shaded by horizontal lines: inflorescences withered

Paper 3

The influence of mowing regime on the soil seed bank of the invasive plant Ambrosia artemisiifolia

Milakovic, I. and Karrer, G. (submitted). Mowing management influences the soil seed bank of the invasive plant *Ambrosia artemisiifolia*, submitted in NeoBiota

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

Running head title:

Mowing influences the soil seed bank of Common Ragweed

Authors: Ivana Milakovic, Gerhard Karrer

Academic adresse: 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
Full address: Institute of Botany, Department of Integrative Biology and Biodiversity Research, University of Natural Resources and Life Sciences, Gregor Mendel Strasse 33, 1180 Vienna, Austria
Email: ivana.milakovic@boku.ac.at,
Tel.+431476543178, Fax. +431476543180

Keywords: *Ambrosia artemisiifolia*, invasive plant, management, mowing, roadside vegetation, seed bank, weed

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 3rd week of August in Eastern Central Europe), followed by a second cut 2-3 weeks later.

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. 2011, 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, Basset and Crompton 1975, Fumanal et al. 2008, Kaczinczi 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. 2000Clay 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 x 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 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, 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 where 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^2 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^2 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^2. 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.

Table 2: Number of *Ambrosia artemisiifolia* seeds per m^2 (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^2 in 2009	SD	Germination rate (%)	Mean number of seeds/m^2 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	-	-

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 χ^2 (5) = 188795; p≤ 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 (Figure1). 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).

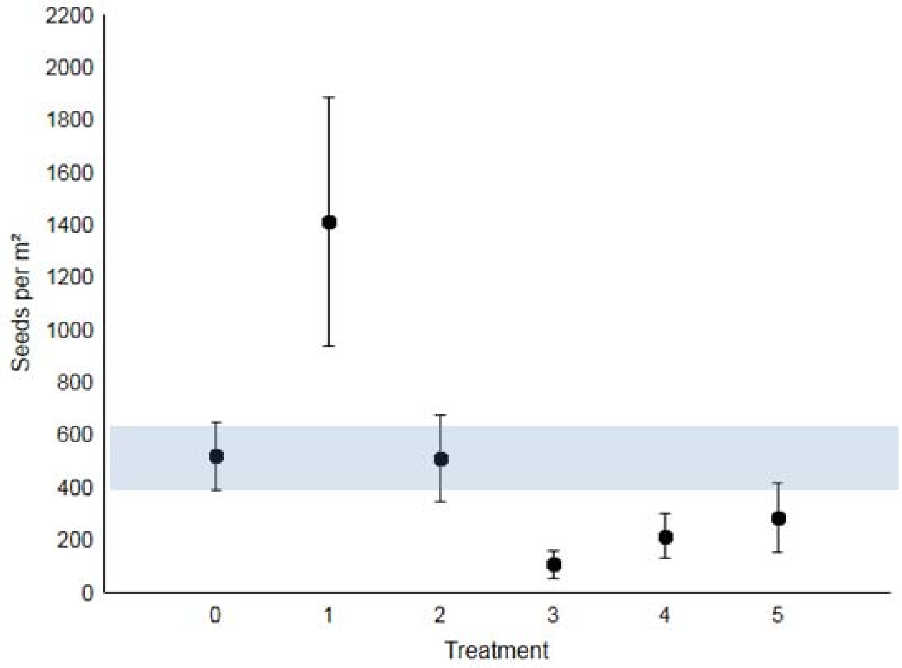


Fig. 1 Means and confidence intervals of the number of seeds of *Ambrosia artemisiifolia* per m² (depth 7cm) after 3 years of different mowing treatments (1-5) in 2012 compared to the soil seed bank of the population before the experiment in 2009 (“Treatment” 0 = baseline)

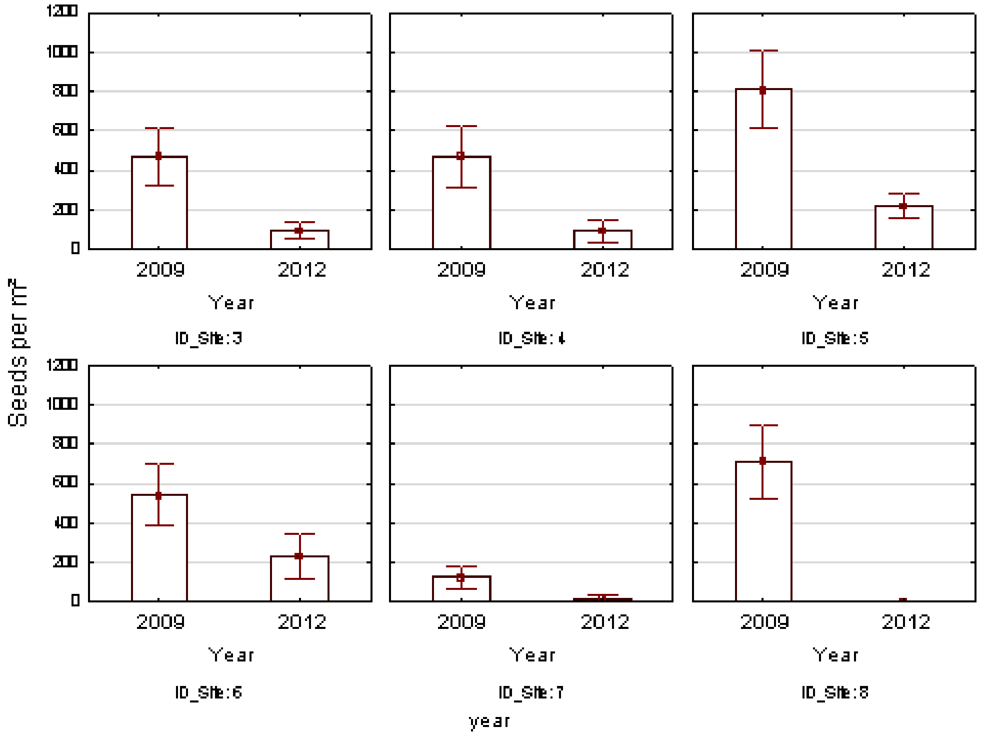


Fig. 2 Mean numbers (and SE) of *A. artemisiifolia* seeds per m² (depth 7cm) in the plots of treatment 3 at six different sites in 2012 compared to the soil seed bank before the experiment in 2009

Table 3: Kruskal-Wallis test for the differences between the soil seed bank (seeds per m^2) 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^2 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 (cutting once a year). 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 m² 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 m² 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^2 in the upper soil (0-8 cm) and 57.6 seeds per m^2 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^2 in maize fields on former forest soil and 4.8 seeds per m^2 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.

Acknowledgement

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Conflict of interest

None.

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Curriculum Vitae

Mag. [a] Ivana Milakovic
Scherzergasse 1/17, 1020 Vienna, Austria
ivana.milakovic@boku.ac.at

Born May 24th 1982 in Banja Luka (Bosnia-H.)
Nationality: Luxembourg

Education

2009-2015 **PhD studies Agriculture, Institute of Botany, University of Natural Resources and Life Sciences, Vienna, Austria**
Title: Optimisation of mowing regimes for the control of the invasive *Ambrosia artemisiifolia* L. on roadsides Supervision: Prof. Dr. Gerhard Karrer

2004-2009 **Master studies in Ecology, University of Vienna.** Thesis at the Department of Conservation Biology, Vegetation- and Landscape Ecology: „Measures for conservation of plant species from wet meadows (Molinietum) based on their population biology and growth strategy". Supervision: Prof. Dr. Gerhard Karrer und Prof. Dr. Georg Grabherr

2005-2007 Studies of Sinology, University of Vienna, Austria

2003-2004 Studies in Life Sciences, University Louis Pasteur, Strasbourg, France

2002-2003 Studies in Biology, Centre Universitaire du Luxembourg, Luxembourg

1998-2002 Lycée Athénée de Luxembourg, Luxembourg

1995-1998 Lycée Sainte-Marie d'Arlon, Belgium

Working experience

Since 10/2015 Nature protection and biodiversity expert, Environment Agency Austria

03 – 10/2015 Project associate in nature protection projects at Umweltdachverband, Vienna

10/2013-03/2015 External consultant in nature protection and rural development projects, SUSKE Consulting, Vienna

08-09/2014 Intern, United Nations Environment Programme (UNEP), Vienna Office

07/2014 Habitats mapping, Freiland Enviromental Consulting, Vienna

04/2014 Session chair, Biodiversity and LEADER Conference, Umweltdachverband, Vienna

02/2009-02/2014 Researcher, Institute for Botany, University of Natural Resources and Life Sciences, Vienna (BOKU)

2007-2009 Teaching assistant in "Biology of plants for conservation" and "Forest Botany", University of Natural Resources and Life Sciences, Vienna, Austria

09/2007	Field work in South-West China for the University of Natural Resources and Life Sciences, Vienna in collaboration with Chinese Academy of Sciences, Kunming, China
4/2006-6/2007	Field work in the Natura 2000 Region Lainzer Tiergarten in the framework of the Master Thesis, University of Vienna
2005-2010	Assistance in organisation of international conferences: XVII International Botanical Conference in Vienna 2005 and several conferences organised by the Austrian-French Centre in Opatija 2005, Sarajevo 2007 and Zagreb 2010.
6-7/2004	Traineeship in the National Museum of Natural History, Luxembourg, Departments of Ecology and Population Biology**.**

Awards and grants

2009	Scientific award of The Municipal Department for Environmental Protection of the City of Vienna (MA22)
2009	Financial support for the master thesis by the Biosphere Reserve Wienerwald
2009-2011	PhD grant, National Research Fund (FNR) Luxembourg
2002-2004	Studies grant, Fondation Mathieu Luxembourg

Languages

German: fluent
English: fluent
Bosnian, Croatian, Serbian: mother tongue

French: mother tongue level
Luxembourgish: fluent
Mandarin: communicative

Peer-reviewed papers in scientific journals

MILAKOVIC, I., FIEDLER, K., KARRER, G. (2014). Fine-tuning of a mowing regime, a method for the management of the invasive plant, Ambrosia artemisiifolia, at different population densities. Weed Biology and Management, 14 (4), 232–241.

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MILAKOVIC, I., KARRER, G. (2009): Sowing of competing vegetation as a control measure for *Ambrosia artemisiifolia* L. (Eds.) International Congress on Biological Invasions, Nov. 2009, Fuzhou, China.

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TV-contributions

-ORF2, Burgenland Heute, Interview about Ragweed, 27.1.2010
-ORF2, Wien Heute, Interview about Ragweed. 6.9.2011
-Servus-TV, Na Servus-Das Wetter: Auf den Spuren des Ragweed. 10.9.2011
-ORF2, Dobar dan Hrvati, Interview about Ragweed in Serbo-croatian. 12.8.2012
- Servus TV, Servus Krone, Interview about Ragweed, 26.6.2015