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

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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.

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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

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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

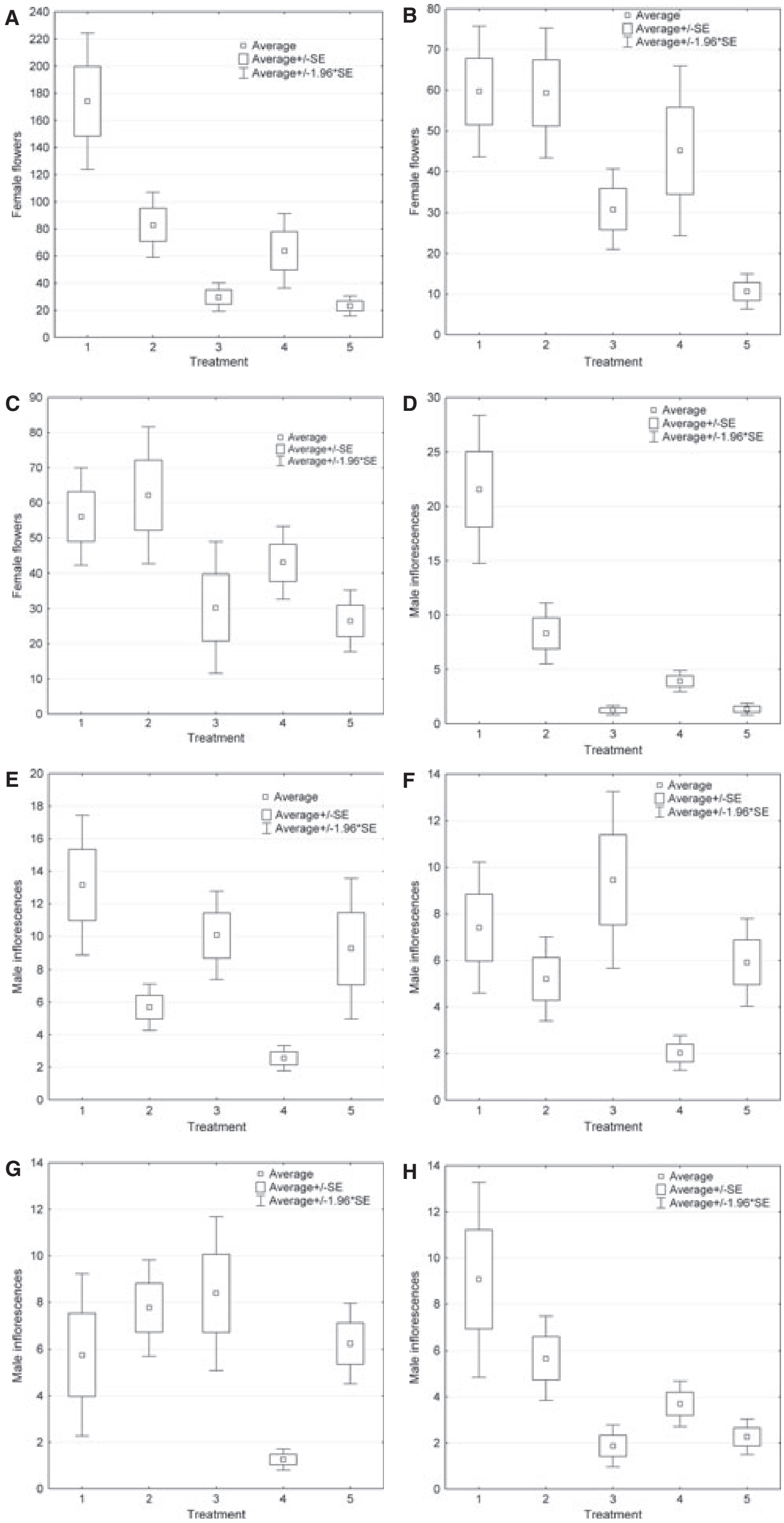

Fig. 1 Number of female flowers per *Ambrosia artemisiifolia* individual in September 2009 (A), September 2010 (B), September 2011 (C), and of the number of male inflorescences per individual in September 2009 (D), in September 2011 (H), in August 2009 (E), in August 2010 (F) and in August 2011 (G),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

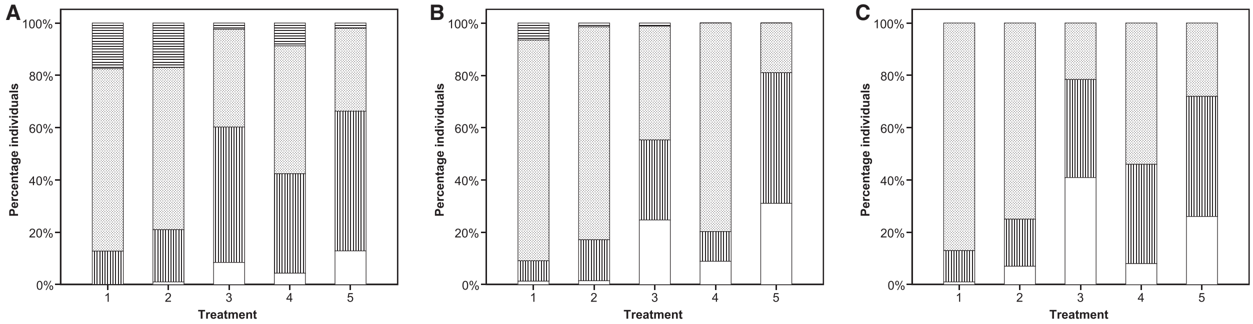

Fig. 2 Percentages of *Ambrosia artemisiifolia* individuals in different phenological stages of female flower development per cutting treatment in September 2009 (A), 2010 (B) and 2011 (C) in Austrian roadside populations. White fraction = no visible flowers; shaded by vertical lines = flowering; dotted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

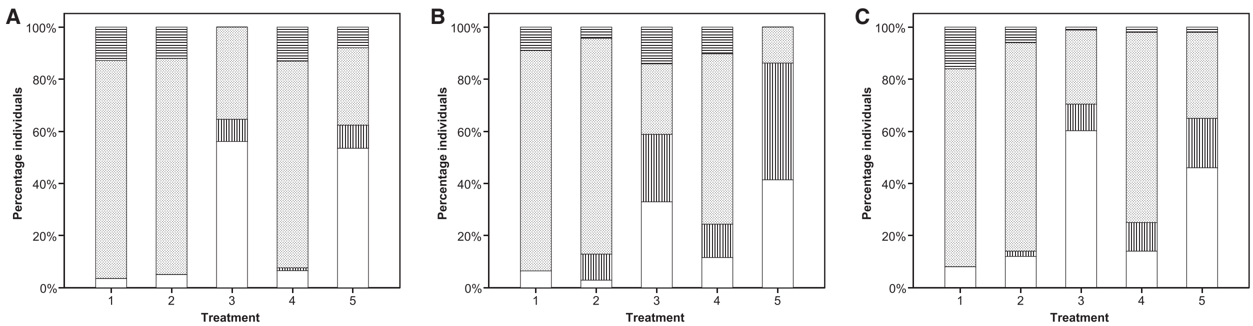

Fig. 3 Percentages of *Ambrosia* individuals at different phenological stages of male inflorescence development per cutting treatment in September 2009 (A), 2010 (B) and 2011 (C) in Austrian roadside populations. White fraction = no inflorescences; shaded by vertical lines = inflorescences not flowering;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

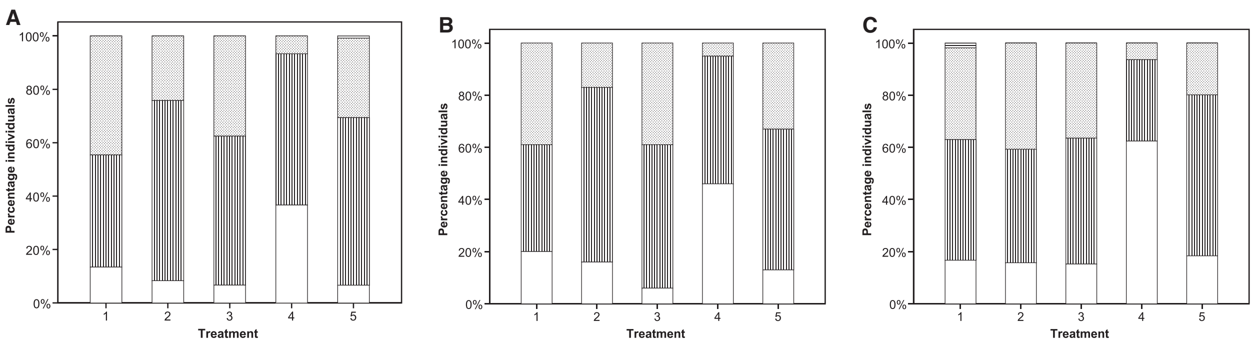

Fig. 4 Percentages of *Ambrosia* individuals at different phenological stages of the male inflorescences per cutting treatment in August 2009 (A), 2010 (B) and 2011 (C) in Austrian roadside populations. White fraction = no inflorescences; shaded by vertical lines = inflorescences not flowering; dotted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