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Ulrike Sölter, Uwe Starfinger und Arnd Verschwele (Eds.)

HALT Ambrosia - final project report and general publication of project findings

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

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Introduction: the HALT Ambrosia project

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Common ragweed – a troublesome invader

Ambrosia artemisiifolia (common ragweed) is a tall erect annual of the daisy family (Asteraceae) native to North America. The plant has been inadvertently imported to many countries in Europe, Asia and Australia. In Europe, the first populations were found in the mid-1800s. The species has spread over several regions in Europe, having been introduced separately to France and Northern Italy and later to South-eastern Europe from the 1900s onward. At present it is spreading through Europe and Asia. Information about the current distribution and densities of its appearance is scattered in national databases and publications. This invasive weed has established on arable and non-cultivated land like roadway sides and construction land. *A. artemisiifolia* can be a strong competitor to sunflowers, potatoes, pumpkins and legumes and can lead to high yield losses. The male flowers produce large quantities of pollen which are of high allergenic potential. Although not sufficiently shown so far, impacts on biodiversity can also not be excluded. The plant thus is a heavy burden on public health, agriculture and biodiversity with resulting high economic losses.

European Commission: DG ENV work on invasive species

In the EU Commssion, DG Environment, activities "Towards an EU Strategy on Invasive Species" date back to 2008 and before. More recently DG ENV initiated two open calls for proposals in 2010 to further support the development of policies in the field. Two projects resulted from this, "Assessing and controlling the spread and the effects of common ragweed in Europe (ENV.B2/ETU/2010/0037)" finished in October 2012 and "Complex research on methods to halt the Ambrosia invasion in Europe - HALT Ambrosia" finished in May 2014. Both these projects focussed on common ragweed which was used as a flagship species for these projects which served as pilot studies for possibilities to deal with invasive plants. Meanwhile, further developments led to the "REGULATION (EU) No 1143/2014 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species" which entered into force on 1 January 2015. This Regulation seeks to address the problem of invasive alien species in a comprehensive manner so as to protect native biodiversity and ecosystem services, as well as to minimize and mitigate the human health or economic impacts that these species can have. The Regulation foresees three types of interventions; prevention, early detection and rapid eradication, and management. A list of invasive alien species of union concern has recently been developed.

The project HALT AMBROSIA 2011-2014

The project was jointly implemented by the following institutions:

Project co-ordinator: Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Braunschweig, Germany (JKI),

Project partner: Universität für Bodenkultur Wien, Austria (BOKU)

Project partner: Plant Protection Institute, Hungarian Academy of Sciences, Hungary (PPI)

Project partner: Kaposvar University, Hungary (KU)

Project partner: Agricultural Institute of Slovenia, Slovenia (KIS)

Project partner: Aarhus University, Denmark (AU)

with support from associated partners:

CAB International (CABI), Delemont, CH,

ACW Changins CH,

Projektgruppe Biodiversität, Friedberg, DE

The overall aim of our project was to contribute to the reduction of the prevalence of the invasive alien plant *A. artemisiifolia* in European countries in order to reduce the burden on public health, agriculture and biodiversity. We developed strategy elements for the reduction of the occurrence of ragweed and its pollen in countries where the species is already established, e.g., Hungary, Slovenia, parts of Austria, and South-eastern Central Europe and for the prevention of further import and spread in countries not yet heavily infested, such as Germany, Denmark and Northern European countries. The gaps in the existing information which is needed for understanding historical successes and failures of prevention, control and eradication activities were analysed. This included:

- a fuller understanding of critical elements in the life history of common ragweed
- an evaluation of chemical, mechanical and biological control measures

Laboratory and field experiments about the germination biology and seed bank behaviour and the proportion of viable seeds found in silage and biogas plants and transported commodities such as soils were investigated. Efficacy of non-chemical control measures on Ambrosia and of combinations thereof were determined as well as the best use of herbicides. Therefore information on the best application and timing of control measures could be derived. Also the impacts of ragweed stands on other plants as well as impacts of control measures on non-target species were part of the research.

The research led to the publication of individual results in various forms, including journals, conference papers etc. The full research report as sent to and accepted by the Commission was available on the project website (no longer online) and is found on that of the Commission: http://ec.europa.eu/environment/nature/invasivealien/index_en.htm. Several project partners have expressed the wish to have all the research available in a single volume, including that not deemed suitable for publication in peer-reviewed journals.

The project has contributed to building a network of scientists who study various aspects of the ragweed issue. Even after the project has helped answer some questions, the work on common ragweed needs to continue. Project partners team up with other researchers in order to carry on with scientific studies but also to seek practical solutions for the ragweed problem: how to save people from bad health, farmers from yield losses and the environment from a potentially noxious invader. Currently many of the project partners and scores of other scientists are engaged in the COST action FA 1203 SMARTER (*ragweed.eu*) and also in the International Ragweed Society (*www.internationalragweedsociety.org*), both of which aim at helping solve the ragweed problem. For these researchers the present volume may be of value.

We are grateful that the Julius Kühn-Institut offered to produce and fund this volume. The results presented here were mainly found in the years 2011 – 2014, and the texts, including citation of references could not be brought to the newest state throughout. We still hope that the book can contribute to readers being able to access the current state of some of the knowledge on ragweed in a comprehensive form and thus carry on with the work.

A standard protocol for sampling and handling of seed material

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Introduction

Some papers concerning ragweed biology include observations or experiments with seeds of *Ambrosia artemisiifolia*. Date (season) of seed collection and post-harvest treatment (sampling, drying, cleaning, storage) can have strong influence on the seed's viability and dormancy.

Following the fate of seeds we can distinguish papers on

- seed production (timing and quantity of seeds produced during the annual life cycle or up to a defined date of the season: i.e. Dickerson 1968, Basset and Crompton 1975, Kazinczi *et al*. 2008, Pixner 2012)
- seed dispersal by different vectors (Joly *et al*,. 2011; Vitalos and Karrer, 2008; 2009, EFSA 2010)
- seed persistence under different conditions: measured as germination rates of seeds, or test of seed viability (i.e. TTC-test) following specific treatments (including soil seed bank data, seed destruction by heating, burning, etc.) (Kazinczi *et al*. 2008; Karrer *et al*., 2011)

Influence of seed collection, storage and pre-treatment:

In Hungary seeds get ripened under natural conditions at the end of September (Kazinczi *et al*., 2008). The authors do not define what they meant by "ripened": it could be germinable seeds (tested for germination with or without stratification), or viable seeds (TTC-tested directly after collection, or tested after storage at defined conditions). Dickerson (1968) used for all his experiments seeds that were stored dry in an unheated building during wintertime. It can be assumed that low temperatures near or below 0°C were able to induce stratification.

Both examples show that any experiments on germination of ragweed must define the treatment (i.e., storage conditions) before the actual germination test is performed.

Post-harvest treatment up to the time of further tests or germination test must be documented exactly for conditions of air humidity, temperature and light.

In case of the different aims of studies on seed biology of common ragweed, we propose to apply the following seed treatments:

a) <u>Number of viable seeds produced by the (living) individual plant:</u>

a1: conditions of seed collection:

If the aim is to test for the number of viable seeds produced by individual plants the seeds must be taken in fully ripened condition. Following Kazinczi *et al*. (2008) natural ripening of the seeds only can be found from "end of September" onwards. Karrer *et al*. (2011) found germinable seeds already at the end of August. Spontaneous release of seeds may happen every time from the beginning of September to spring. Most seeds drop off latest after the first frost days. Few seeds stay fixed to the plant as long the stem is not pressed down to the ground by heavy rain, wind, or snow.

As female flowering starts from beginning of August and – mostly in case of cut plants – holds on until October, the production of ripened seeds can be counted exactly in the field only with very high effort.

The two main options always have pros and contras:

(a) Picking seeds from the plant before frost may stop lately developed young embryos from ripening.

(b) Collecting seeds at the time of first frost - when all plants are killed - will also stop further development of ripening seeds.

Considering all experiences from various projects up to now we propose to sample the seeds in field experiments after the first frost killed the plants because the majority of seeds are still attached to the plants.

The number of "viable" seeds that drop off spontaneously could be counted exactly at that time, if the soil below the ragweed individuals is covered by any persistent material from early September onwards to keep all seeds for counting. This sampling net area should be twice the diameter of the individual plant. Thereby all seeds can be picked after frost has killed the plant.

a2: Conditions of storage until further experiments:

Further treatment of the collected seeds is different depending on the features to be measured.

Generally seeds should be cleaned from other vegetative parts of the plant. Such is done by different seed cleaning systems (sieves, gravity tables, upwind selection).

Commonly the seeds are dried before cleaning at room temperatures (20, 25, or 30°C) for about 1 to 8 weeks.

Some authors desiccate the seeds (i.e., at 30°C for about 2 days) before storing them at low temperatures. Such conditions might not simulate the real seed environment after seed set in the field correctly. Natural conditions in autumn include also low temperatures at night-time).

a3: Viability tests of seeds:

Viability is tested in two different ways:

By germinating the seeds, or by testing for viability by TTC-tests.

Germination tests commonly are undertaken with seeds that are stored after cleaning for at least 6 weeks under dry, cool and dark conditions (at around 0°C). Commonly used is 4°C. This treatment simulates the stratification period that is necessary to break the dormancy of ripened ragweed seeds (Payne and Kleinschmidt, 1961; Leiblein-Wild *et al*., 2014).

To study the induction of dormancy at early or later stages of development seeds must be tested directly after harvesting for germinability without any pre-germination treatment.

After collecting all seeds they may be counted for "number of viable seeds" directly after, using a standard TTC-test.

b) <u>Number of germinable seeds produced by the (living) plant:</u>

If the aim is to detect the number of germinable seeds for the next generation, the seeds must be stored immediately after collection under cool and dark conditions: 4°C in darkness is commonly used in several studies on seed persistence and soil seed bank analysis. In case of ragweed a storage period of 6 weeks is enough to stimulate germination afterwards (Karrer *et al*., 2011; Gebben, 1965). Other authors propose at least 8 weeks under such conditions (Kazinczi *et al*., 2008).

All temperatures lower than 4°C are allowed unless not deeper than minus 10°C. Very low temperatures below minus 10°C might have gradually increasing negative influence on the survival rate of ragweed seeds.

c) Number of viable seeds in the soil seed bank:

Soil seed bank can be analysed at different dates throughout the year. Generally the standard date for collecting soil samples is late winter/early spring (s. Fumanal *et al*., 2008), when dormancy of fresh or older seeds is interrupted. Kazinczi *et al.* (2008) report that winter dormancy commonly is broken already during January; such holds at least for Hungary.

New data (Schöberl and Lebernegg, 2013) show that the soil seed bank of ragweed shows some (not significant) losses during winter (4-5 months) at rates of 5 to 40 % (see trial B.2, deliverable DB2). The autumn soil sample was done in October (after first frost killed most of the plants). Soil was stored for 6 weeks at low temperature (stratification) and seeds were sieved in a wet sieving system for being counted and, directly after, tested for germinability. Spring samples from March needed no further stratification treatment and could be sieved, counted and tested directly after sampling.

The most relevant numbers for natural populations of summer annual crops are the germination rates of seeds in early spring. Thus we propose to study seed banks of ragweed always based on the early spring samples that do not need artificial stratification before germination tests. The number of seeds germinated at that time determines the success of ragweed in the raising season.

d) Long-time storage and seed exchange:

The longevity of seeds under the standard storage conditions in the lab (dry, darkness, ≤4°C) was tested also in trials B.2. Seeds from several sites and habitat types lost viability at an annual rate of about 5 to 20 % under standard storage conditions.

Experiments with buried ragweed seeds found that the seeds can survive up to 40 years (Toole and Brown, 1949). In order to standardize seed persistence measurements, seeds are tested in a joint long-term burial experiment of the HALT-Ambrosia team. In this experiment as well as in others all seeds are to be tested for germinability/viability before the start of the experiments (baseline germinability rates).

The first year data gave relatively inconsistent results with death rates (within 1 year) from 5 to 55 %. Further years will give clearer answers, hopefully.

Conclusions

Common ragweed is an interesting object for studying several physiological aspects of invasive plant seeds. Therefore, we call upon all scientists to define clearly the conditions of collection and storage of seeds used for answering different questions. For instance, analyses of the response of plants from seed lots of different geographical locations may be influenced very much by the seed treatment from field sampling up to the start of the experiment. Obviously, ragweed shows a rather complicated system of dormancy (Bazzaz, 1970). Thus the pre-experimental treatment of the seeds is expected to be very influencial.

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Germination and viability of ragweed seeds

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Introduction

Several studies in the last 50 years showed that the seed biology of common ragweed (*Ambrosia artemisiifolia)* is rather complicated. Like other typical summer annual weeds its seeds show innate dormancy after seed set in autumn and need stratification of about 4 weeks of temperatures around 0°C (Baskin & Baskin, 1998). If the conditions after stratification are not nice for germination (darkness, drought, temperature regime slightly above 0°C, low O_2 or high CO_2 concentration in the soil) enforced (secondary) dormancy can be initiated (Baskin & Baskin, 1980). As long as the conditions do not change seeds persist in secondary dormancy until spontaneous death (latest after 40 years after Toole & Brown, 1946).

Most such data were published from North American populations of common ragweed. Only few data about seed biology are available from European populations. Adaptive evolution could have changed the preferred site conditions for the regulation of germination and growth in the newly invaded area. Therefore some experiments were started to elucidate this important aspect of the life cycle within the countries covered by the HALT-Ambrosia team. The following experiments were conducted.

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Response of common ragweed (*Ambrosia artemisiifolia* L.) to soil salinity

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Introduction

Common ragweed (*Ambrosia artemisiifolia* L.) tolerance to limited growing conditiones along with disturbance facilitate spreading of this invasive species specially along the roadsides. From there it is migrating further on the agricultural surfaces and wastelands. In central Europe the vegetation on the road verges is subjected to large quantities of deicing salt, however ragweed seems to be adapted to increased salinity in the soil. The aim of the pot experiment conducted at Agricultural institdifferent growth stages of ragweed.

Material and Methods

Pot experiment was conducted in a random block design with 10 treatments (2 growth stages × 5 salt concentrations), where the main plot was weed growth stage and sub-plot was a salt concentration. The experiment was replicated eight times. The growth stages of common ragweed were based on number of leaves (-L). Salt concentration were calculated on the basis of pot mixture weight data and applied at planting and V6 growth stage in the concentrations of 0, 20, 40, 100, 200 and 400 mg/kg Na^+. Common ragweed aboveground mass was clipped at physiological maturity from each plot to collect dry weight data. Significant differences were determined using one-way Anova and means were compared with Duncan MRT test at the 5 % level of probability.

Results and discussion

Growth stage and salt addition significantly influenced ragweed dry matter production (P<0.001, Table 1), however common ragweed response to salinity varied among growth stages and salt concentrations.

Table 1. One-way Anova of the effects of salt concentration applied at planting.

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	833.534	5	166.707	**309.38**	**0.0000**
Within groups	28.02	52	0.538845		
Total (Corr.)	861.554	57			

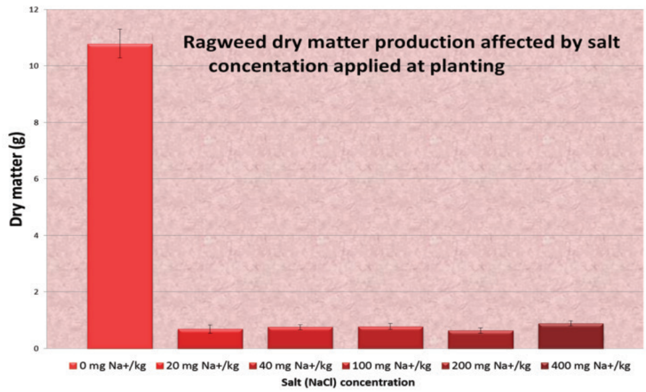

Fig 1. Effect of salt concentration applied at planting on ragweed dry matter production at physiological maturity . Values presented are means with ±SE.

Susceptibility of ragweed to salt decreased with increasing growth stage. When salt was applied at planting, significant reduction of dry matter (82-94 %) regardless of salt concentration, was observed (Fig. 1).

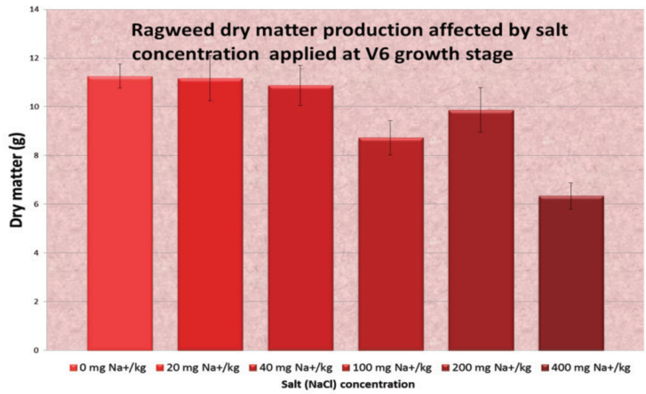

Fig 2. Effect of salt concentration applied at V6 growth stage on ragweed dry matter production at physiological maturity. Values presented are means with ±SE.

At later V6 growth stage, significant dry matter reduction (23-44 %) was observed only when higher concentration of of salt was applied (100 and 400 mg Na^+/kg, Fig. 2). Based on experiments conducted at the Agricultural Institute of Slovenia it was concluded that common ragweed is very susceptible to salt at germination and early growth, whereas inreasing tolerance to moderate salinity at vegetative growth stage (V6) was observed. These authors performed their experiments with seeds from arable fields like Leiblein *et al.* (2013). Their findings correspond to those of Di'Tommaso (2004) who found that only roadside populations were less susceptible to saline conditions but not agricultural populations.

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Triphenyl Tetrazolium Chloride Ringtest

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Triphenyl tetrazolium chloride, TTC, is a redox indicator used to indicate cellular respiration. Its solution in water is colourless but in living tissues the TTC is reduced to a red substance thus dyeing living tissues in red. The test is commonly used for testing seed quality with various instructions produced by, e.g., the International Seed Testing Association. Certain adaptations for specific seeds are commonly made. In case of common ragweed we hypothesised that the variance between seed populations collected in Hungary, Austria, and Germany would be larger than the variance between the participating labs. For our ring trial we followed in the first round our protocol 1st edition.

The experiment consisted of two rounds. In the first round, four populations of common ragweed seeds were tested. These populations were provided by JKI (Hordorf, sampled 2011, and Herbiseed, bought in 2011) and BOKU (Unterpurkla, sampled 2010, and Hagenbrunn, sampled 2010) and sent to each partner. One hundred achenes per population were required (4 replicates, each with 25 achene halves).

Materials:

- 100 achenes per population
- Tap water
- An instrument to cut achenes in halves. A nail clipper was very reliable or a surgical scalpel or similar instrument
- Distilled water
- 16 glasses of 5-10ml volume which can be covered
- Incubator or drying chamber
- Refrigerator
- 1% TTC-solution (i.e. 100 ml)
- dissecting microscope/binocular

Implementation:

- Common ragweed achenes were imbibed in tap water at room temperature for 24 hours
- The achenes were cut open with a surgical scalpel or similar instrument in such a way that the endosperm was exposed
- The biggest part of the achene is used for testing, the other part is discarded
- 25 achene halves are put into one glass and filled up with TTC solution (per replicate)

- Closing the glass tight
- Glasses are put to react at 30°C for 6 hours in absolute darkness, because TTC is light sensible, avoid unnecessary light input
- If it is not possible to keep on with the protocol after these 6h, the closed glasses can be stored in a refrigerator (6-8°C) over night
- TTC solution is poured off and halves are rinsed under distilled water.

Under a dissecting microscope, seeds were counted in three classes: a) stained (=alive), b) intermediate cases that are only lightly or partly stained, c) not stained resp. no fully developed embryo present (=dead) (Fig. 1).

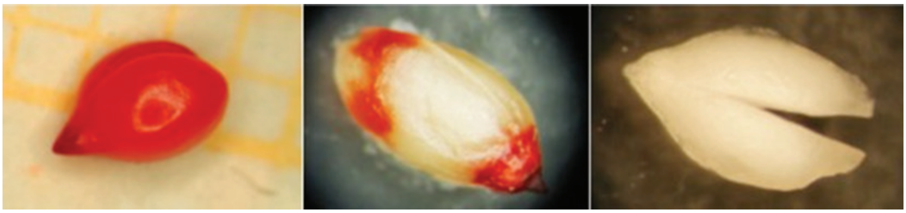

Fig. 1: Common ragweed embryo staining intensity and coding: left: stained = 1, middle: intermediate = 0.5, right: dead = 0

The differences in classification of the different seed lots by different labs were higher than the variation between the seed origins (Starfinger *et al.* 2012).

Intending to reduce variation of individual differences in classifying various stages of staining of common ragweed seeds we started a questionnaire of how differently stained seeds after TTC treatment were classified as "stained" (=class 1), "unstained" = dead (class 0), and "intermediate" (class 0.5; only parts of the embryo stained) by the various labs. The interpretations varied at high levels (Tab. 1). Before starting a second round of the TTC-test we defined the 3 classes (1; 0; 0.5) on base of this comparison of individual assessments. The results were accounted for the 2nd edition of the manual for TTC-testing, including pictures of various stages of staining and their recommended classification (last column in Fig. 2).

	figure	NL	NL 2	DK	JKI (Martina)	KU	BOKU (Felicia)	BOKU (Hannes)	BOKU(Martin)	BOKU (Nina)	BOKU (Gerhard)	KIS	Mittel	class
	1	1	1	1	1	1	0,5	1	1	1	0,5	0,5	0,86364	**1**
	2	0,5	0	0	0,5	0	0,5	0	0	0	0	0,5	0,182	**0,5**
	3	1	0,5	1	0,5	0,5	0,5	1	1	1	0,5	0,5	0,72727	**0,5**
	4	1	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,54545	**0,5**
	5	0,5	0	0	0,5	0	0,5	0,5	0,5	0,5	0,5	0,5	0,36364	**0,5**
	6	1	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,54545	**0,5**
	7	1	1	1	1	0,5	1	0,5	1	1	1	1	0,90909	**1**
	8	1	1	1	1	0,5	1	1	1	1	1	1	0,95455	**1**
	9	0	0	0	-	0	0	0	0	0	0	0	0	**0**
	Mean	0,78	0,5	0,55556	0,6875	0,38889	0,555556	0,5555556		0,61111	0,5	0,55556	0,56566	0,611111

Fig. 2: Standardisation of classification of stained embryos of common ragweed after TTC treatment

In the second round (2013) we tested seed lots provided by KU (Kaposvar, sampled in 2011) and BOKU (Zillingtal, sampled in 2011 and Unterpurkla, sampled in 2010).

The results for the BOKU trials are summarized in Fig. 3. The number of viable seeds (class 1) was high in the seed lots from Kaposvar and Zillingtal. In the seed lot from Unterpurkla (Austria, Styria) the amount of intermediates (class 0.5) was higher than the number of stained (viable) seeds.

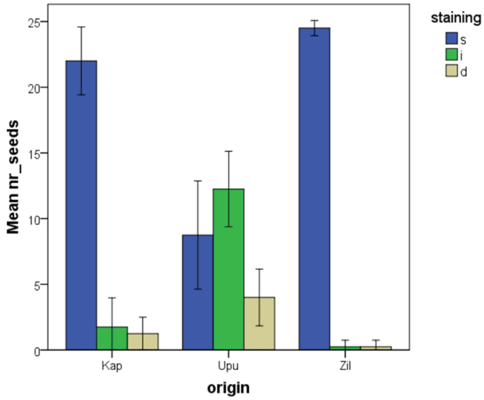

Fig. 3 : Mean number of common ragweed seeds per staining category and place of origin (Error Bars: +/- 2. SE) classified by BOKU; Seed origin: Kap = Kaposvar, Upu = Unterpurkla, Zil = Zillingtal. s – stained, I – intermediate, d – dead

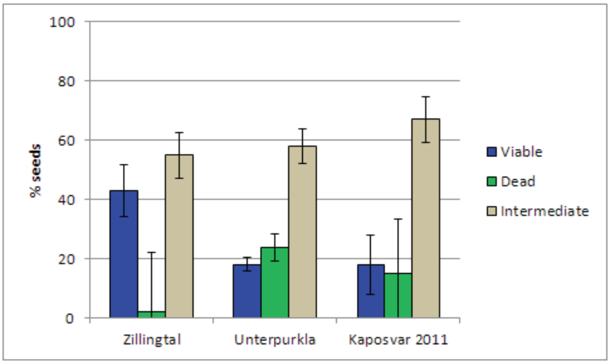

Fig. 4: Mean percentage of common ragweed seeds per staining category and place of origin (Error Bars: +/- 2. SE) classified by AU;.

The Danish lab (AU) classified the same seed lots after the same treatment rather different (Fig. 4). I. e., the number of intermediates is relatively high compared to the Austrian estimation indicating differences in the interpretation of the classification of stained embryos. .

Kaposvar and Zillingtal samples were collected in 2011 whereas the Unterpurkla seeds were collected in 2010 and stored at 4°C in darkness. The Unterpurkla seeds might have suffered from spontaneous death by ageing (Béres 2004).

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Studying the seasonal pattern of field emergence of ragweed in Hungary

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In 2011 Kazinczi observed the emergence of common ragweed on ruderal fields around Kaposvár (Hungary).

Mini (1x1m^2) quadrates were signed, heavily infested with common ragweed. From the end of March until the end of November 2011, emerged seedlings were detected every ten days. After counting the seedlings were removed. Germination peak of common ragweed was in April and first half of May. Last germination occurred at the end of July. Due to the extra dry period, common ragweed seeds failed to germinate after this time. In the first and second decades of July no germination occurred (it is presumed that secondary dormancy was induced in seeds due to the hot summer periods). It is generally believed that after germination peak emergence may be continuous with a decreasing extent until the end of vegetation (Hoffmann et al., 2010; Kazinczi and Novák, 2014). During the experimental period an average of 200 seedlings was counted for a square meter.

References

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Effect of emergence time on life cycle, shoot dry weight, pollen and seed production

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In another experiment emerged common ragweed seedlings within the same observation period (every ten days) were signed similarly in the experimental area. In order to get informations about the real biological potential of common ragweed inter- and intraspecific competition was excluded by continuous hand weeding during the experimental period (from March until November).

Emergence time obviously had some influence on final shoot dry weight, measured at the end of the vegetation period (November 2011, Table. 1).

When seeds emerged later, shoot dry weight of *A. artemisiifolia* plants considerably reduced.

Table. 1: Final shoot dry weight (means and standard deviation in g/individual) of common ragweed depending on the emergence time (months/decades e.g. 4/1: first decade of April)

Emergence time											
4/1	4/2	4/3	5/1	5/2	5/3	6/1	6/2	6/3	7/1	7/2	7/3
Shoot dry weight (g/plant)											
1840 ±609	1682 ±363	1284 ±422	972 ±349	1436 ±354	1487 ±416	914 ±0	349 ±130	404 ±246	-	-	26 ±15

Emergence time obviously also has serious influence on pollen production (number of male heads/plant: considering a mean of 17 male flowers per head and of 7148 pollen/male flower; see Reisinger and Szemenyei (2006), Table. 2, and on seed production (number of seeds per individual, Table 3). The later common ragweed germinated the quicker the individuals developed from seedling to flowering plants. Even common ragweed plants that emerged by end of July produced seeds until end of September (Table 4).

Table. 2: Number of male heads per individual of common ragweed depending on the emergence time (months/decades); average estimated pollen number per individual in green

Emergence time											
4/1	4/2	4/3	5/1	5/2	5/3	6/1	6/2	6/3	7/1	7/2	7/3
Number of male capitula/plant											
137531	125910	107435	42827	57071	94155	27552	27372	11994	-	-	817
±	±	±	±	±	±	±	±	±			±
88873	58151	96087	15230	33359	58169	0	13841	187			340
over 16 milliard pollen											~0.1 milliard pollen

Table 3: Total number of seeds (min-max in black; means in red) and number of viable seeds (green) per individual of common ragweed depending on the emergence time (months/decades)

Emergence time											
4/1	4/2	4/3	5/1	5/2	5/3	6/1	6/2	6/3	7/1	7/2	7/3
Seed number for a plant											
17778-74125	5000-94900	2125-50500	850-58493	16830-53625	19300-48200	48375	10275-33857	7921-31938	-	-	225-1700
av.	av.	av.	av.	av.	av.	av.	av.	av.			av.
33230	46185	26118	17059	32630	37100	48375	25272	19502			1144
11630	42952	16193	13647	16967	25228	44505	22998	18527			708

Table 4: Changes in phenological stages of common ragweed plants that emerged at different times; date of emergence and measurement in decades of months; developmental stages following the BBCH-scale (Hess et al., 1997; Meier, 2001).

Observation time	Emergence time									
	4/1	4/2	4/3	5/1	5/2	5/3	6/1	6/2	6/3	7/3
4/1	09									
4/3	12-14	14-16	09							
5/2	22	19	16	12-14	09-12					
6/1	32-39	26-35	16-26	20-22	18-19	16-18	10			
6/3	51-55	49-51	39-51	39-51	26-39	26-30	18	14-16	09-12	
7/2	65-69	49-61	49-61	49-51	49-51	45-48	39	26-32	19-22	
8/1	65-70	51-65	51-65	52-55	51-55	51-55	51	32-39	32-39	09-12
8/3	69-79	68-70	66-69	68-78	68-69	68-78	69	68-69	68-69	32-51
9/2	79-81	71-75	69-71	71-78	71-78	78-81	75	69-75	75-79	71-78
10/1	81-88	81-85	81-85	81-87	81-87	81-85	85	81-85	81-87	78-81
10/3	97	97	97	97	97	97	97	97	97	97

It is concluded that data of biomass production (including, shoot dry weight, pollen- and seed production for an individual plant) considerably varied between individuals (high values of standard deviations!) even inside the same emergence period. This suggests that emergence time is only one factor determining biomass (shoot dry weight, pollen, seed) production of common ragweed (Hoffmann et al., 2010).

Emergence time greatly influenced seed viability, but – irrespectively to emergence time – autumn collected common ragweed seed samples (stored in paper bags at room temperature for a half year) were in strong dormancy in spring of next year (germination percentages (4-29%) were far below to those of viability (35-95%) (Figure 1).

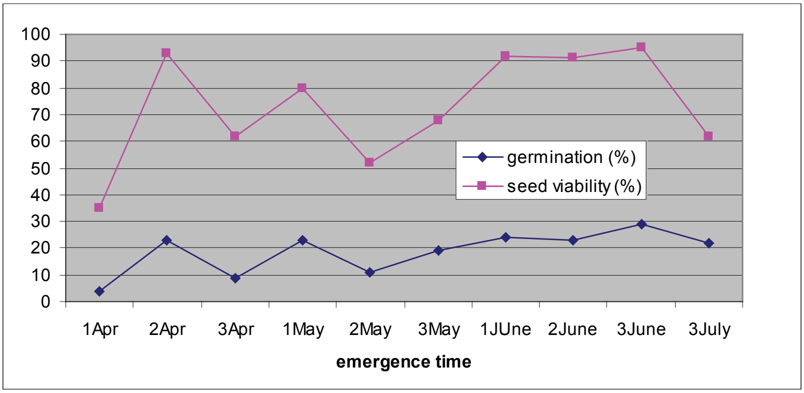

Figure. 1: Germination and viability of common ragweed seeds (KU-HU 2012).

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Soil seed bank studies I-III

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The soil seed bank of *Ambrosia artemisiifolia* (common ragweed) was analysed by Fumanal *et al*. (2007) for French populations and by Leitsch-Vitalos in Karrer (2009) for Austrian populations. No other studies focussed specifically on common ragweed soil seed banks. Further studies were performed during this project.

I. Seed bank studies in different habitat types

Introduction

Studies on the soil seed bank of *Ambrosia artemisiifolia* can help to get better insights in the population dynamics of invading populations. *Ambrosia artemisiifolia* soil seed banks show considerable spatial variation (Fumanal *et al.* 2007, Vitalos *et al*. 2009). Any control options should be evaluated in view of the age of populations and duration of accumulation of seeds into the soil seed bank. Fumanal and Vitalos studied agricultural fields, pastures, roadsides and few abandoned fields. The soil seed bank of common ragweed in near natural habitat types (meadows on military training areas and ruderal sites along rivers) were studied in 2011 at the BOKU. The aim was twofold: a) to characterize the soil seed bank of subpopulations of different age and different environmental influence near to the river Danube, and b) to describe the soil seed bank of experimental plots in an intensively invaded military training area that were managed like meadows, i.e. cut at least once a year.

Methods

In case of the embankment plots in the northern part of Vienna, we selected two areas where soil sediments produced by Danube floods have been deposited nearby the river (Fig. 1). Furthermore we sampled a roadside on the way from the flooded area to the soil deposit (Fig. 2) and the former flooded area just few meters from the river (Fig. 3 and 4). The previous year there had been a heavy flooding by the Danube that left sandy sediments in the alluvial zone with ca. 30 cm depth. The sediment was taken by machines and transported to the deposit. Before the flooding a common ragweed population was already established for about 5 years on this place, whereas the roadside population was very young (2 years) and the 2 deposit subpopulations were 2 and 4 years old.

As seeds deposited on the soil surface can by embedded into the soil from undisturbed surfaces only slowly into deeper soil horizons we expected that the populations of different age differed with respect to the distribution of common ragweed seeds by soil depth. Unfortunately, the soil at plot beside the Danube (nr. 4) turned out to be very shallow. So from this plot we only could take one soil depth layer per core. From the very young roadside population (plot 3) we took 20 soil cores from 0 to 10 cm soil depth .From the deposit plot 1 we took only 5 soil cores because the area where common ragweed was known to grow was small, from plot 2 we took 16 cores; in both cases we sampled 0-10 cm and 10-20 cm separately. Generally the sampling strategy has to follow the arrangement of the plants in the field. Therefore many populations along rivers or roadsides are linear (narrow but long) whereas anthropogenous habitats like soil deposits tend to be more rounded and can be sampled rectangular or circular.

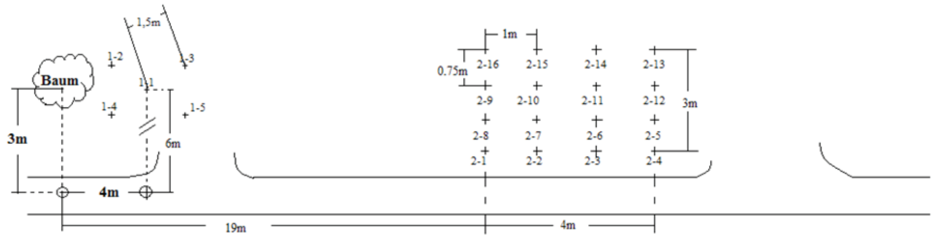

Fig. 1: Sampling design of plot 1 (left, circular sampling) and 2 (right, rectangular sampling) on the area of the artificial river sediment deposit

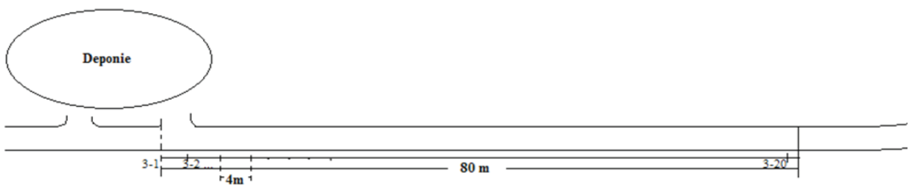

Fig. 2: Sampling design (linear transect) of plot 3 along the road between the river and the deposit

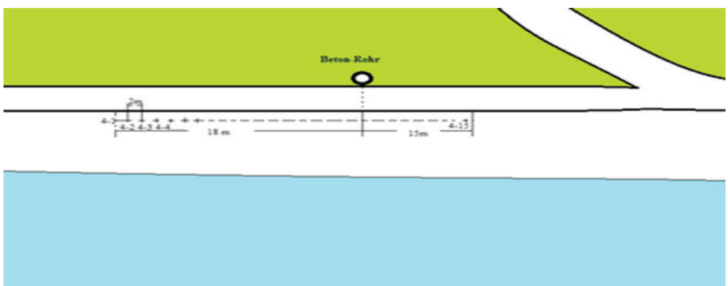

Fig. 3: Sampling design (linear transect) of plot 4 near to the river (in blue)

Fig 4: Overview (left) and detail (right) of plot 4 along the river

Fig. 5: Overview (left) and details (right) from sampling the military training area near Bruckneudorf

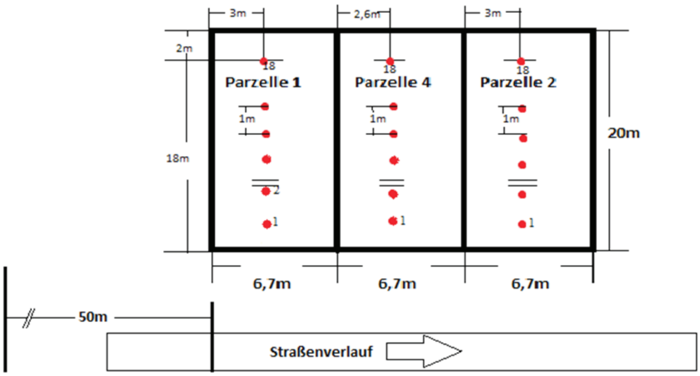

Fig. 6: Sampling design for one plot (out of 3 replicates) on the military training area near Bruckneudorf

In case of the military training area (near Bruckneudorf, Burgenland, Austria) sampling served to describe the soil seed bank of common ragweed on the experimental plots where different treatments for seed bank depletion will be tested. Therefore the design is more extensive. 3 different treatments were replicated 3 times along a meadow (Fig 5 and 6). On each treatment-subplot we sampled (linearly arranged) 20 soil cores with 6.6 cm diameter divided into layer 0-10 and 10-20 cm. The soil cores were stored for further analyses in darkness at 4°C. Common ragweed seeds were then sorted out by automated floating sieves and put into petri dishes in climate chambers (8 h light, 30°C, 16 h dark, 15°C). During the first germination turn (4 weeks) many seeds germinated and discarded. Afterwards, the remaining seeds were kept dry and cool again for 8 weeks to start a second germination turn aiming at breaking secondary dormancy of remaining seeds. Seeds that still remained dormant after the second germination round were tested with Tetrazolium dye for vitality.

Results

The two soil deposit samples had common ragweed seeds only in the upper soil layer (0-10 cm) with 467.9 and 146.22 seeds per m^2 (plot 1 and plot 2, resp.). The roadside plot 3 showed 59.76 seeds per m^2 and the river bank plot 4 226.66 seeds per m^2. On the military training area (meadows) we found on average 188 seeds per m^2, in 0-10 cm depth a mean of 129 and in 10-20 cm a mean of 59 seeds per m^2.

The germination rates of seeds from the Danube plots ranged from 100% to 88% for deposit plot 1 and 2, resp.; the seeds from the roadside plot 3 germinated by 100% and those of the river bank plot 4 by 88.23%. The seeds from the military training area gave germination rates of 55 (0-10 cm) and 68% (10-20 cm) on average. The second germination round as well as the viability test (TCC) showed that all seeds left were not dormant but dead.

The low age of the common ragweed populations from plot 1 to 3 can be deduced from the fact that there no seeds had penetrated into deeper soil layers. However, the considerable high number of seeds in the deeper soil layer of the military training area indicates that the population was established already for many years at this site.

The site Bruckneudorf (military training area) gave mean seed numbers of 626.33 seeds/m2 (SD = 864) for plot 1, 583.69 seeds/m² (SD = 800.37) for plot 2 and 12.9 seeds/m² for plot 3. These figures correspond to the seed densities given by Fumanal *et al.* (2007a, b) with 536 +/- 194 to 4477 +/- 717 seeds/m². Fumanal also found that the upper soil layer (0-5 cm) of arable fields gave lower common ragweed seed numbers than in lower soil depths (5-20 cm). On fallows and unmanaged abandoned fields the seed numbers in deeper soil was lower (cf. Fumanal *et al.* 2007b, p. 101)

References

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II. Seed bank studies: Quantification of soil seed bank losses of common ragweed between autumn and spring sampling

Introduction

The soil seed bank of *Ambrosia artemisiifolia* is commonly sampled between seed dissemination and natural germination in spring. To our knowledge nobody ever tested for differences of the natural seed banks of common ragweed between autumn and early spring. That is why we started an experiment to test the seed bank of common ragweed from 7 different experimental plots along roadsides.

Methods

We used the plots from the mowing experiment to test for the differences in seed bank composition between autumn 2011 and spring 2012 (Schöberl & Lebernegg, 2013). Thus we sampled 19 soil cores each from every treatment plot of all sites of the field cutting experiment. The soil cores were taken from the upper soil layer assuming that the intrusion of seeds to the soil at undisturbed habitats takes rather long time and differences would be assured by using the upper soil layer between 0 and 7 cm.

The soil cores from the autumn sample were stratified for 6 weeks in darkness at 4°C, the spring samples were directly analysed.

Both sets of soil cores were washed out by use of a wet sieving machine (Retsch). All obviously viable seeds were put into petri dishes and treated for 4 weeks in climate chambers at 8 h light at 30°C and 16 h darkness at 15°C. All seeds that germinated were taken out from the dishes. The remaining seeds that obviously stayed dormant were left for drying and afterwards stored again in darkness at 4°C for further stratification. After four weeks, a second germination test under the same conditions as before was performed in the climate chambers. Again all seeds that germinated were counted and deleted.

Still remaining seeds were subsequently tested for dormancy/viability via the TTC-test.

Results

We compared the total number of viable seeds (germinated + TTC-fully stained) per site between the two sampling periods and found on average an expected decrease of seeds from autumn to spring, except for one site (see Fig. 1). The unpredicted difference at the Halbenrain site might be caused by some local effect of seed introduction by very late mowers or snowplough. All other sites showed generally losses of viable seeds by 5 to 30%.

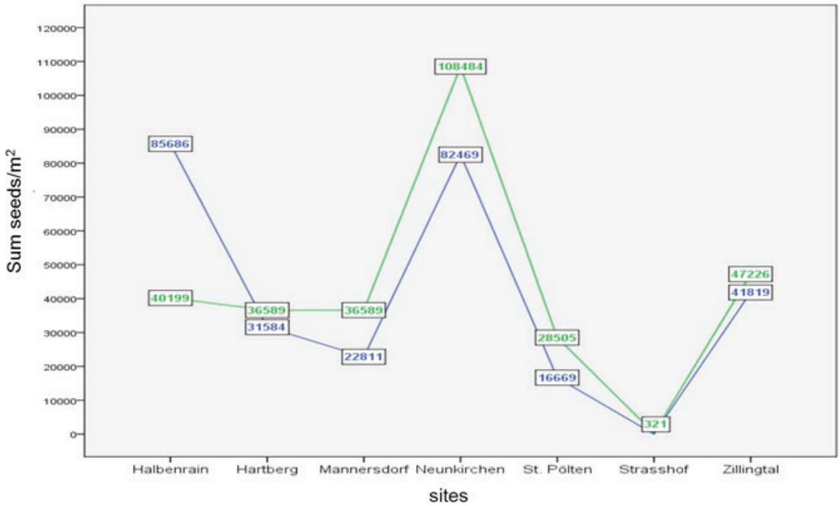

Fig. 7: Total number of viable seeds/m^2 in autumn 2011 (green) and spring 2012 (blue) at different experimental plots

In consequence comparisons of soil seed bank data as an efficacy measure should be designed in that way that the same season must be sampled. It is well known that the soil seed bank decreases in summer crop weeds from late winter/early spring to summer significantly (Fumanal *et al.* 2007) but it was not known until now that the difference between autumn and spring sampling can also be serious.

References

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III. Seed bank studies: Efficacy of various mowing regimes used for ragweed control along roadsides measured by soil seed bank

Introduction

Sampling of soil seed bank is an important efficacy measure for the different control options of common ragweed. We will use this measure to test the efficacy of different cutting regimes on those experimental plots along road shoulders of Eastern Austria that were established for the HALT Ambrosia project.

Methods

In total, 7 experimental sites in Lower Austria, Styria and Burgenland have been sampled in spring 2012 for evaluating the effects of cutting experiments. 20 soil cores (depth 7cm) per treatment and locality were taken by end of March and analysed for common ragweed seed content using a wet sieving machine (Retsch). We counted all obviously intact seeds and put them into petri dishes. In order to detect the proportion of viable seeds, first germination was induced by wetting the dishes and putting them into climate chambers at following conditions: daylight for 8 hours at 30°C and darkness for 16 hours at 15°C. After 4 weeks the first germination trial was stopped, the dishes left for drying out and stored for 4 weeks at +4°C in darkness. A second germination phase was started afterwards (mid July 2012) at the same conditions like in the first session. All seeds that did not germinate within the next 4 weeks were tested afterwards by the standard TTC-test described in B1.2 for any seeds still alive.

The results were compared to soil seed bank data of the sampling of the same experimental sites in spring 2009, before the start of the experiment (see Karrer et al. 2011). That way, it is possible to conclude on the effect of the tested mowing regimes on the soil seed bank after 3 years of application.

Statistical treatment: GLLM (in Statistica 10.0)

Results

In 2012, soil seed bank at different sites varied from 0 to 1061 seeds per m^2, for a depth of 0 to 7cm. The germination rates were generally very high (91% in average). No seeds germinated in the second germination test and no living seeds were detected by the subsequent TTC test.

After 3 years of applying different mowing regimes, the ragweed soil seed bank of treatment 1 (control, unmown) increased almost threefold, the one of treatment 2 (first cut in late June, second cut in 2nd week of September) did not change significantly, whereas it decreased by ca. 80% under treatment 3 (first cut in 3rd week of August, second cut in 2nd week of September), ca. 60% under treatment 4 (first cut in late June, second cut in 2nd week of September) and ca. 45% under treatment 5 (cut 3 times: first cut in late June, second cut in the 3rd week of August, third cut in in 2nd week of September).

Conclusion on all soil seed bank studies I-III

Because most management options act superficially, the most problematic aspect of common ragweed control is the elimination of the persistent seeds from the soil. The results of this long term experiment show that the soil seed bank can strongly be diminished by a carefully thought and adapted mowing management. The mowing management consisting of a first cut in August just about the start of appearance of female flowers and a second cut in early September), the results suggest that this management can be evaluated as very sustainable and environmentally friendly control option, as it progressively empties the soil seed bank. This way the common ragweed populations decline and can be managed easier. The most effective measure of hand-pulling of the remaining plants might become feasible after seed bank depletion.

Final comment

Based on the comparison of the soil seed bank of all experimental plots between spring 2012 and autumn 2011 (sampling done in October 2011) we found a mean loss of seeds during winter by up to 20% (Schöberl & Lebernegg 2013). The number of counted seeds showed considerable high variation. That is why we decided to do a final scientific test of the trial effects in early spring 2014 independent from the HALT-Ambrosia project. For the analysis of the experiments within the HALT-Ambrosia project the data from spring 2012 are valuable and fit better to the experimental design (comparison of spring data 2009 with spring data 2014 is better than comparison of spring samples with autumn samples using correction functions to estimate seed numbers that would be counted in spring 2014).

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Intraspecific differences of seed longevity between ragweed populations in Hungary

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Common ragweed seeds were sampled in different years and various parts of Hungary, stored under dry conditions and at room temperature and finally tested in 2012 for viability by TTC-test. Viability of seeds decreased seriously within few years. After 8 years of storage under dry and warm conditions all seeds were dead.

After 7, 6 and 4 years of dry storage seed viability was 15, 45 and 72%, respectively. Common ragweed seed viability after 3 years of dry storage varied between 67 and 90%, depending on origin of populations.

In another study viability decreased by 82% after five years for seeds stored in paper bags at room temperature (Kazinczi et al. 2011; Kazinczi and Novák, 2014).

Viability variation between origins is relatively high (Table 1).

Table 1 : Viability (% viable seeds from standard TTC-test) of seeds of common ragweed at various age stages and collected from different parts of Hungary

Year of seed collection/ age of seeds	Origin	Viability (%)
1997/15	Keszthely, waste land	0
2004/8	Szekszárd, corn	0
2005/7	Keszthely, waste land	15
2006/6	Keszthely, corn	45
2008/4	Zalaegerszeg, corn	72
2009/3	Petrivente, waste land	78
2009/3	Petrivente, waste land	67
2009/3	Keszthely, waste land	67
2009/3	Keszthely, waste land	72
2009/3	Kaposvár, waste land	68
2009/3	Keszthely, roadside	77
2009/3	Kaposvár, stubble	90
2010/2	Kaposvár, waste land	64
2011/1	Kaposvár, waste land	97

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Pre-trials on seed viability at the Julius Kühn-Institut

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Introduction

Common ragweed seeds from various sources were tested for germinability (germination test, Fig. 1) and vitality (TTC-test with different concentrations of TTC-solutions, Fig. 2). The overall germination rates varied between 42 and 76%. Those rates are comparable to French (Fumanal *et al.*, 2007), Austrian (Karrer *et al*. 2011) and American populations (Dickerson 1968).

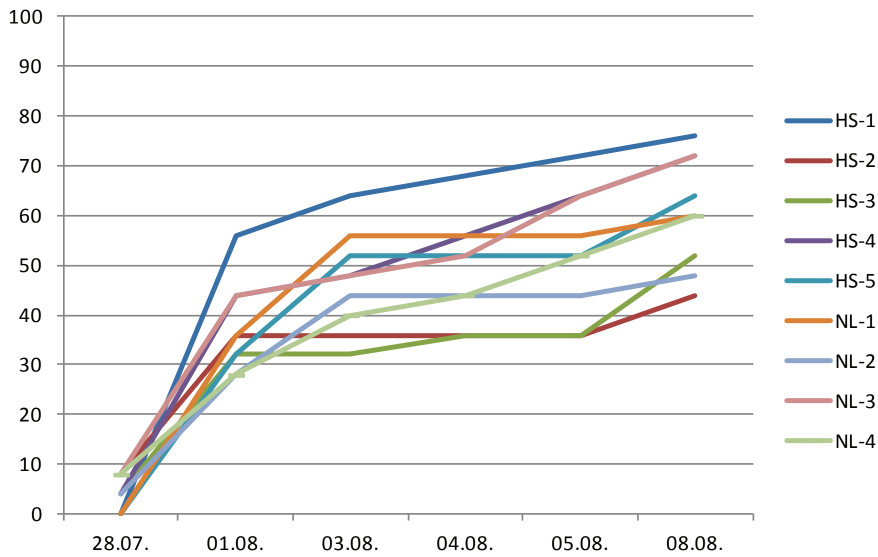

Fig. 1: Germination dynamics of 9 seed samples from two different seeds lots (HS-Herbiseed and NL-Niederlausitz) of common ragweed

0.5% (concentration 1) and 1% (concentration 2) TTC solutions were used for viability tests, incubation time was 6h at 30°C. We used two different seed lots: Herbiseed (HS) seeds were bought from a trading company (Herbiseed) in 2011 and stored in a cool (4°C), dry and dark place until they were taken out for the germination test (ca. after 3 month). Niederlausitz (NL) seeds were collected from a population found in the region Niederlausitz in Germany in 2010. Since then they were stored in an office (app. 20°C) until they were used for the germination test in 2011.

For the results (Fig. 2), we found that 1% solutions showed clearer staining and less partly stained seeds. Most seeds show positive reaction to TTC. Herbiseed samples show more dead and not fully stained seeds than Niederlausitz samples. Seeds boiled for 15 minutes show no staining.

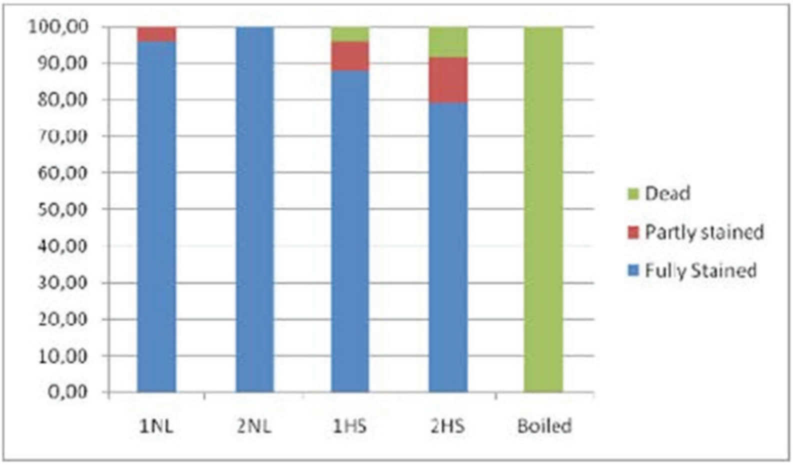

Fig. 2: TTC-staining of Niederlausitz (NL) and Herbiseed (HS) seed lots of common ragweed with two levels of TTC-content of staining solution (i.e., 1: incubated with 0.5% TTC solution; 2 incubated with 1% TTC solution)

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Pre-trials on seed viability at the University of Natural Resources and Life Sciences

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In the Austrian lab (University of Natural Resources and Life Sciences, BOKU) pre-trials on the use of the TTC-test were performed for various common ragweed seed sources (Tab. 1). Problematic is the interpretation of intermediates (partly stained seeds) that could be integrated to the sum of viable seeds or not. The two types of calculating viable seeds differ between 5 to 51%. Consequently, the question of how to count "intermediates" is to be precisely defined at the beginning of any analyses where TTC-test is used.

Tab. 1: Results of the TTC-Test for seed lots from different populations and different degrees of cleaning (Rep = replicates)

Seed source	year	rep	N	Stained (alive)	Not stained (dead)	Intermediate	Viability-stained [%]	Viability (stained + interm) [%]	Viability-stained [%] mean	Viability (stained + interm.) [%] mean	Comment	date of TTC test
Hagenbrunn	2010		100	58	19	23	58	81	58	81	well cleaned	4.1.12
Hagenbrunn	2010		100	56	27	17	56	73	56	73	less cleaned	10.2.12
Hordorf	2011		100	71	17	12	71	83	71	83		5.1.12
Herbiseed	2011		100	85	11	4	85	89	85	89		5.1.12
Hungary	2010		100	92	3	5	92	97	92	97		5.1.12
Unterpurkla	2010		100	29	38	33	29	62	29	62	well cleaned	4.1.12
Unterpurkla	2010		100	27	54	19	27	46	27	46	less cleaned	10.2.12
Hagenbrunn	2010	1	25	20	1	4	80	96	82	97	well cleaned	27.7.12
Hagenbrunn	2010	2	25	21	1	3	84	96			well cleaned	27.7.12
Hagenbrunn	2010	3	25	20	0	5	80	100			well cleaned	27.7.12
Hagenbrunn	2010	4	25	21	1	3	84	96			well cleaned	27.7.12
Hagenbrunn	2010	1	25	11	13	1	44	48	41	53	less cleaned	6.8.12
Hagenbrunn	2010	2	25	6	15	4	24	40			less cleaned	6.8.12
Hagenbrunn	2010	3	25	14	8	3	56	68			less cleaned	6.8.12
Hagenbrunn	2010	4	25	10	11	4	40	56			less cleaned	6.8.12
Unterpurkla	2010	1	25	16	2	7	64	92	63	89	well cleaned	27.7.12
Unterpurkla	2010	2	25	16	2	7	64	92			well cleaned	27.7.12
Unterpurkla	2010	3	25	15	4	6	60	84			well cleaned	27.7.12
Unterpurkla	2010	4	25	16	3	6	64	88			well cleaned	27.7.12
Unterpurkla	2010	1	25	6	17	2	24	32	38	45	less cleaned	6.8.12
Unterpurkla	2010	2	25	8	16	1	32	36			less cleaned	6.8.12
Unterpurkla	2010	3	25	16	8	1	64	68			less cleaned	6.8.12
Unterpurkla	2010	4	25	8	14	3	32	44			less cleaned	6.8.12
Hordorf	2011	1	25	21	4	0	84	84	88	90		6.8.12
Hordorf	2011	2	25	24	0	1	96	100				6.8.12
Hordorf	2011	3	25	24	1	0	96	96				6.8.12
Hordorf	2011	4	25	19	5	1	76	80				6.8.12
Herbiseed	2011	1	25	25	0	0	100	100	92	96		6.8.12
Herbiseed	2011	2	25	22	1	2	88	96				6.8.12
Herbiseed	2011	3	25	22	2	1	88	92				6.8.12
Herbiseed	2011	4	25	23	1	1	92	96				6.8.12
Hungary	2010	1	25	25	0	0	100	100	95	96		6.8.12
Hungary	2010	2	25	24	0	1	96	100				6.8.12
Hungary	2010	3	25	23	2	0	92	92				6.8.12
Hungary	2010	4	25	23	2	0	92	92				6.8.12

Viability of seeds ripened after cutting (pot experiment)

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Methods

Pot experiments were done in Germany, 2012 August-December. Common ragweed (*Ambrosia artemisiifolia*) plants were cut at different post floral stages of the female flower. After cutting, single plants were stored in paper bags at a dry place at moderate temperatures (glasshouse) for seed ripening until the control has reached BBCH 97.

Treatments: Cutting at different growth stages, defined basically on the BBCH stages:

1. First female flowers open- 30% of female flowers open
2. Full flowering: 50% of female flowers open
3. End of female flowering
4. Nearly all fruits have reached final size normal for the species and location
5. Control BBCH 97 Seeds fall off, no cutting

Replicates: 10 plants per treatment, each plant is a replicate

Pots: 50, one plant per pot, pot size 2000cm^3

Assessments: number and weight of seeds per plant; germination and viability of seeds with TTC test.

Results and discussion

There were no viable seeds produced by post ripening when cutting at BBCH 63-79 of the female flower (Fig. 1). At BBCH 81 and 97 the number of seeds and their viability increased. So cutting common ragweed at BBCH stage after 81 (beginning of fruit ripening) is critical when the cut plants will be left on the soil surface because of post ripening of their seeds and their ability to germinate.

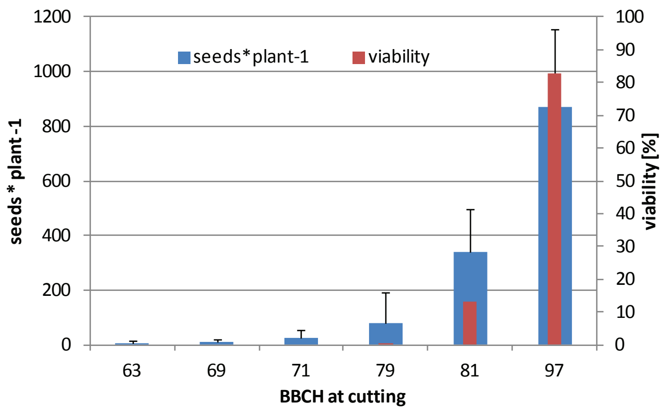

Fig. 1: Number of seeds (bars indicating standard deviation) of common ragweed and their viability at different BBCH stages of the female flower at cutting date

Post harvest seed ripening (pot experiment)

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Introduction

Machines used for mowing the shoulders and batters along any roads contribute most to the spread of common ragweed (Vitalos & Karrer 2009). Common practice of managing such habitat types is mowing or mulching few times a year whereupon leaving the biomass on the floor. Flowers on cut stems of many plants are able to develop further to a ripened stage. Therefore leaving the cut common ragweed plants on such places may not stop common ragweed from producing seeds and spread further. In this study we aim at testing the contribution of post-harvest seed ripening to the number of seeds left in habitats after cutting the invasive common ragweed.

Methods

In a cutting experiment, we studied the post-harvest ripening potential of flowers/young seeds of common ragweed cultivated at the Botanical garden of the University of Natural Resources and Life Sciences (Fig. 1). We cut branches at 5 developmental stages of female flowers (Fig. 2).

All cut branches were left on the soil surface for post-harvest ripening until end of autumn (Fig. 3). In December, the seeds yielded from the experiments were stored for 90 days at 4°C. After this stratification they were tested with regard to their germination capacity and dormancy/viability. Germination tests were run 2 times 4 weeks each term interrupted by second 5 months stratification. Seeds were germinated in petri dishes at 16/8 h darkness/light and 15/30°C. The TTC-test for viability of assumedly dormant seeds was done after the last germination trial (6 hours staining at 30°C in darkness, 1% Tetrazolium solution applied). Each treatment was tested with a subsample of 2 x 15 seeds except for those that yielded only 5 seeds each.

Fig. 1: *A. artemisiifolia* cultivated in pots

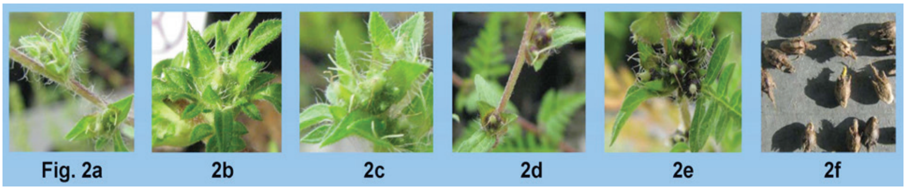

Fig. 2: Developmental stages of the female flowers of *A. artemisiifolia* in the post-harvest ripening test:

1) Young small flower with fresh whitish stigmas (Fig. 2a)

2) Flower at full size, soft, with dried stigmas (Fig. 2b)

3) Flower green and medium hard, can be compressed by fingers, capitulum with soft spines, stigma dry (Fig. 2c)

4) Flower greyish, hard and spiny, cannot be compressed by fingers; stigma dry (Fig. 2d)

5) Flower dark brown, hard, spiny, drops off when touched, stigma broken or vanished (Fig. 2e, f)

Fig. 3: *A. artemisiifolia* cut branches covered by a fine net to protect against seed predators

Results

All treatments produced at least some **ripened seeds**. Flowers cut at stages 1 and 2 (both with soft ovary) developed only 5 ripened seeds. In contrast, all ovaries cut at hard or near to hard stage (3 to 5) produced a lot of seeds that looked ripened (Fig. 4).

Flowers cut at stage 1 and 2 developed at least a few ripened seeds, probably because we overlooked few flowers that already reached stage 3 during harvest. The high number of ripened seeds in groups 3 to 5 indicates the high capacity of post-harvest ripening of common ragweed after finishing flowering and being cut off from resource supply.

The **test for germinability** (Fig. 5) provided rather different germination rates depending on the time available to finalize ripening. The few ripened seeds developed from stage 1 (cut at Aug. 18th) and 2 (cut at Sept. 9th) branches germinated by 60%. But this partition cannot be seriously interpreted because of the small sample size (n=5). Stage 3 branches (cut at Sept. 14th) provided seeds which germinated by 27%; stage 4 (cut at Oct. 1st) seeds already germinated at rates of 43%, and seeds that were cut at Nov. 15th germinated at the rather high rate of 87%. The latter differs significantly (p=0.0345, Tukey) from the germinability of seeds with less time left for post-harvest ripening.

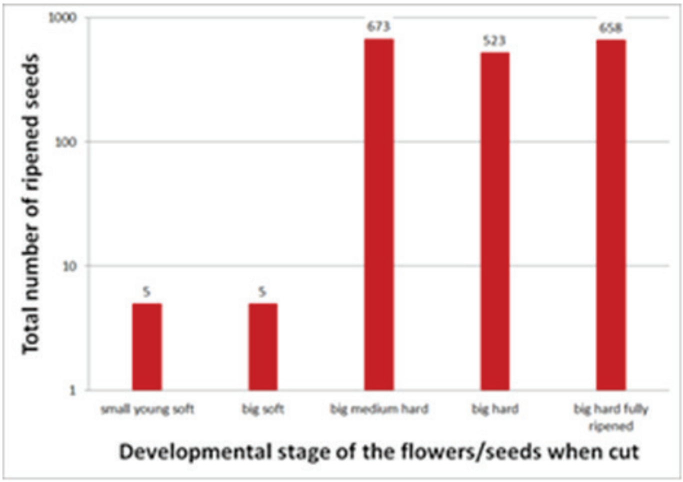

Fig. 4: Total number of ripened seeds developed from *A. artemisiifolia* branches cut at different developmental stages (log scale!)

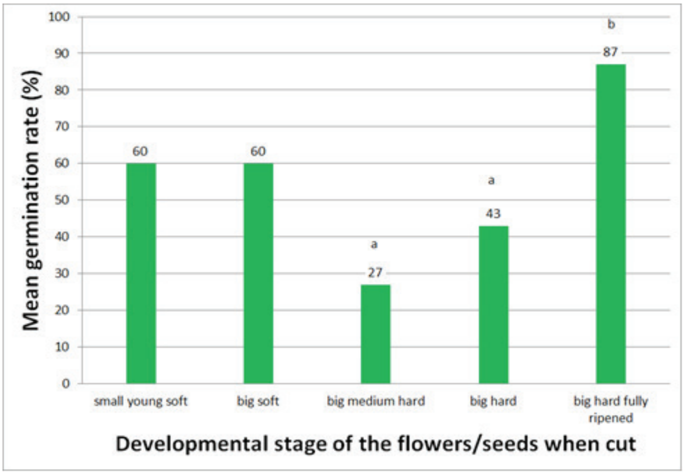

Fig. 5: Mean germination rates of ripened seeds developed from *A. artemisiifolia* branches cut at different developmental stages; stage 5 seeds differ significantly from seeds developed from branches at stage 4 and 3 (p=0.0345, Tukey); stage 1 and 2 rates cannot be interpreted seriously because of n=5.

TTC-test: The remaining non-germinated seeds were tested for viability. None of them showed to be viable.

Discussion

The traditional cutting regime of road shoulders in Middle Europe includes one or two cuts before summer (April to June), hardly any cut during summer (July and August) and one last cut in September or October. Vitalos & Karrer (2009) and Milakovic & Karrer (2010) showed that such cutting regime ends up in lots of viable seeds distributed by the mowers. It is evident that leaving cut plant biomass in September or October at the managed sites even promotes the fill-up of the soil seed bank (data not shown here) and enables further spread of common ragweed via branches with ripened seeds.

So far, post-harvest ripening of common ragweed seeds was underestimated, especially when control options like mowing were discussed (Simard & Benoit 2011). Mistakes like efforts to control common ragweed by cutting at the wrong dates get evident by the high number of viable/germinable seeds that developed from plants cut mid of September. Not only the vital resprouting capacity of common ragweed makes cutting at the wrong time rather inefficient but also leaving the cut plants in the field.

Our results indicate that the definition of seed ripening stages must consider also the hardiness of the ovaries which seem to be the best indicator for viability of young seeds.

The percentage of viable seeds (87%) within the treatment of obviously fully ripened seeds (nr. 5) is relatively high compared to other authors; i.e., Chauvel & Fumanal (2009) stated 80% as high percentage of viable seeds in populations without any stress.

Further spread of common ragweed to Northern Europe will be facilitated if the potential of producing ripened seeds earlier than given in the literature increases.

Managed populations of common ragweed obviously are able to produce viable seeds much earlier than given in the literature (Kazinczi *et al*. 2008a, b). From our results we expect the production of first viable seeds already in the second half of August (at least in the Pannonian region). Therefore, Karrer *et al.* (2011) recommend the removal and burning of any common ragweed biomass after cutting as of August.

Indeed, the post-harvest ripening potential turned out to be rather high in common ragweed. Seeds developed from inflorescences that were harvested at the beginning of September already showed germination rates of 25 to 50%. Thus it is evident that cut common ragweed biomass must be removed from habitats like road shoulders after mowing in autumn to prevent post-harvest ripened seeds from further spread and from filling up the soil seed bank. If removal of biomass is not accomplished cutting as a control measure against the invasive common ragweed is not sustainable.

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Field experiment on longevity of the seeds in the soil seed bank (Joint experiment)

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Introduction

Reports on seed longevity of common ragweed under natural conditions (i.e. in agricultural soils) are sparse. Toole and Brown (1946) found that seeds buried in soil can survive up to 49 years and that losses in the upper soil layers were high and longevity therefore shorter than at deeper burial depth. We started longevity experiments with common ragweed seeds from different origins in Germany, Slovenia, Hungary, Austria and Denmark in 2012. The experiments are planned to last for 10 years.

Methods

Each participating lab buried two common ragweed seed lots. Seeds from Hungary ('Kaposvar 2011') were buried in all countries. As second test population different labs used different local seed lots. At JKI: 'Domsdorf 2010', at BOKU and KU: 'Hagenbrunn 2011', and at KIS and AU: 'Unterpurkla 2010'. The year after the lot name indicates the year of seed sampling. The seeds were buried at two depths (5-8 and 25cm) either in early winter 2011 (BOKU) or in early spring 2012 (all other labs).

We buried the seeds in portions of 50 seeds each enclosed by a polyester tissue (net) (Fig. 1).

Fig. 1. Seed burial of common ragweed in polyester tissue (net) (KU-Hungary 2013)

During the next 10 years, seeds of the two populations and depths will be excavated on the 15th of March in each year or postponed if the soil is frozen until it is frost-free. The excavated seeds were tested for viability by germination test and a subsequent TTC-test – for comparison, together with regularly stored (dry at 4°C) seeds from the respective populations.

Test for viability of the buried common ragweed seeds was done in 2 steps:

First step: Germination test (Fig. 2): putting 25 seeds each on watered filter paper in petri dishes and left for 2 weeks in climate chambers running a cycle of 12 h light at 30^{0} C and 12 h darkness at 15^{0} C. Every second day the number of germinated seeds was counted and removed. Finally the petri dishes were opened for drying.

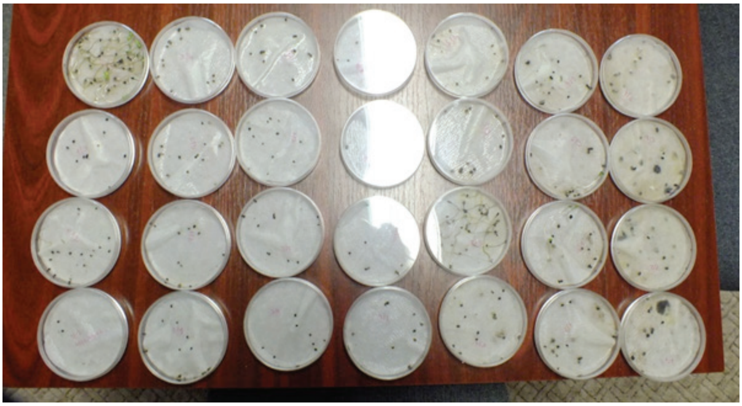

Fig 2. Laboratory germination tests in Petri dishes (KU-Hungary 2013)

Second step: non germinated seeds were subjected to a TTC-viability test following the developed protocol (see "Standard protocol for testing viability with the Triphenyl Tetrazolium Chloride Test", this issue).

The number of viable seeds was calculated as the sum of germinated seeds plus the number of fully stained non-germinated seeds (class 1) in the TTC test.

First excavation took place in March or April 2013, depending on the local climate (frozen soil).

Results

The results of the local excavations and viability tests are given here for the different labs separately.

JKI:

In Germany (JKI) more than 90% of the seeds from both populations and burial depths germinated (Fig. 3). Non-germinated seeds were tested with the TTC test and were evaluated to be dead.

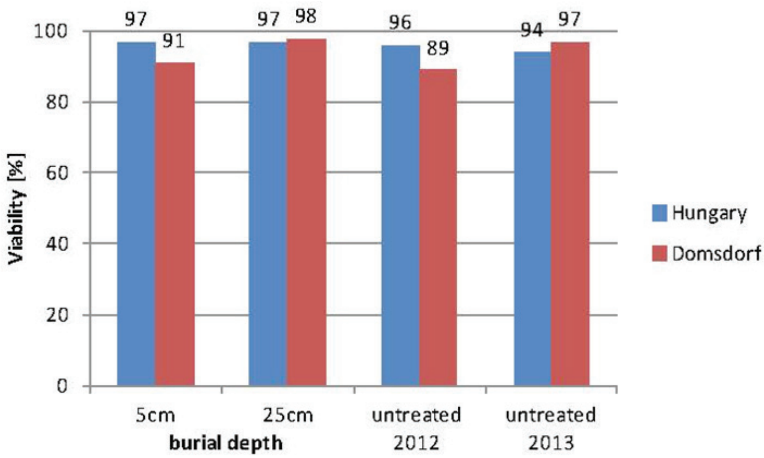

Fig. 3: Viability [%] of buried common ragweed seeds from populations (Kapsovar 2011) and Domsdorf 2010 after 1 year at 5 and 25 cm depth, and of untreated control seeds, stored at 4°C continuously (tested both in 2012 and 2013)

BOKU:

Excavation on Austrian site took place on March 22nd 2013.

Fig. 4 shows the number of viable common ragweed seeds per net from the Kaposvar 2011 burial depth of 8 versus 25 cm, differentiated by the status of viability. In both cases the number of seeds alive (germinable or positively stained) is very high. The slight tendency of higher means of viable seeds in deep soil can be recognized. This indicates the better conditions for survival of seeds in deep soil (conservation) which was documented several times for weed seeds in arable fields in the literature. The Hagenbrunn 2010 seed lot was buried in mid December but burial of Kaposvar 2011 seeds could not be continued directly afterwards because of extreme low temperatures and frozen soil.

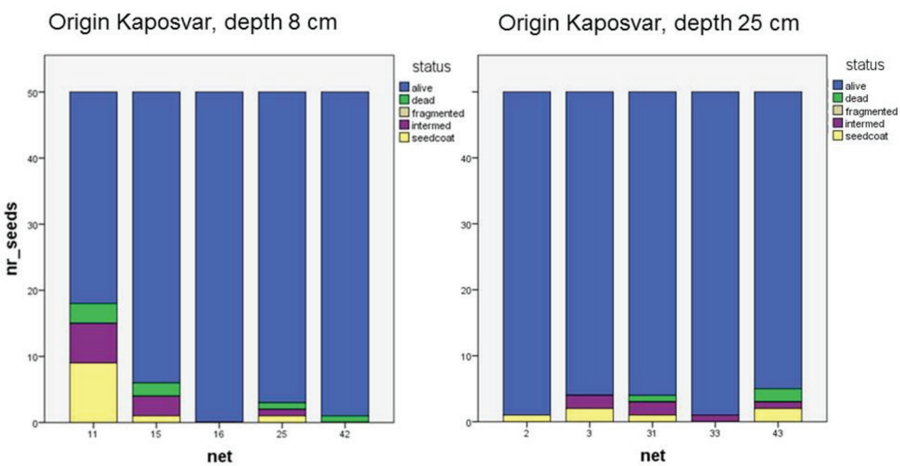

Fig. 4: Number of viable common ragweed seeds per net from the Kaposvar 2011 origin – from the burial depth of 8 cm(left) versus 25 cm (right), differentiated by the status of viability

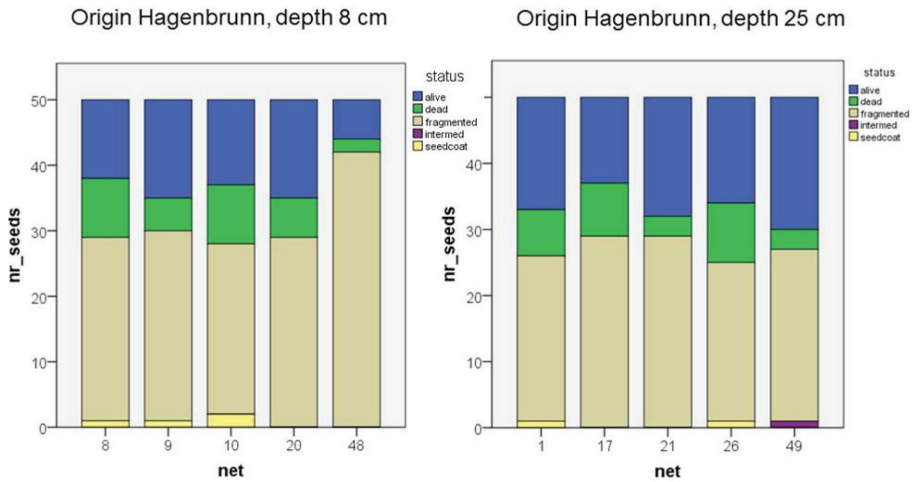

Fig. 5: Number of viable common ragweed seeds per net from the Hagenbrunn 2010 origin – from the burial depth of 8 (left) versus 25 cm (right), differentiated by the status of viability

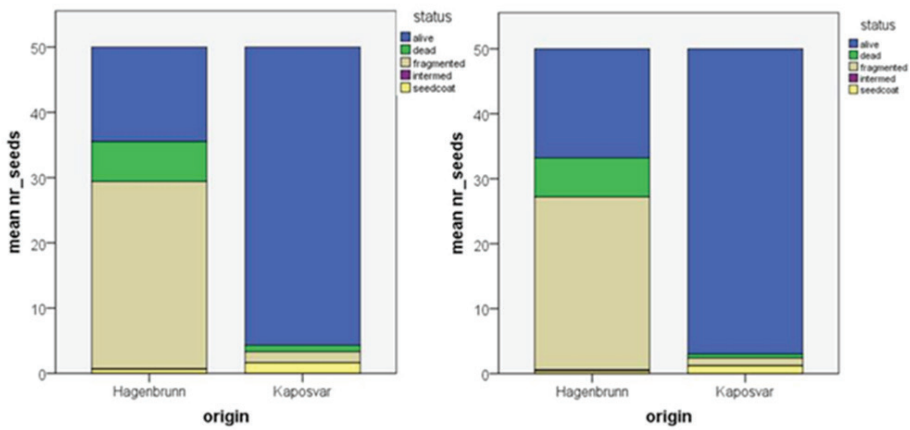

Fig. 6: Comparison of the mean number of viable common ragweed seeds per net from the origins Hagenbrunn 2010 and Kaposvar 2011 – from burial depths of 8 (left) and 25 cm (right), differentiated by the status of viability

Maybe this differing date of burial is to some extend responsible for the big difference in alive versus fragmented/dead seeds. The status "fragmented" was applied when we found only halves of seed coat or when we found not any trace of the seeds that were put into the net (lost seeds, or decomposed seeds after death or after germination in the soil substrate). The big difference of the mean number of viable seeds per net between the two origins is also illustrated by Fig. 6.

KU:

The excavated Kaposvar 2011 seeds gave different results to the Hagenbrunn 2010 ones (Table 1). The Hagenbrunn 2010 sample stored in the refrigerator gave better germination results in comparison to the seeds buried in the field.

Table 1: Germination rates and viability of common ragweed seeds buried in different depths (in % of the starting population), compared to seeds from the same seed lot stored at 4°C in darkness.

Buried (5 cm)		Buried (25 cm)		Stored in refrigerator (at 4 °C)	
Kaposvar 2011	Hagenbrunn 2010	Kaposvar 2011	Hagenbrunn 2010	Kaposvar 2011	Hagenbrunn 2010
Germination (%)					
86	30	85	48	28	50
Seed viability (%) based on TTC tests					
96	89	94	82	71	75

AU:

For the 'Kaposvar 2011' population the results from Denmark were similar to those from Germany showing a high viability of seeds irrespectively of burial depth. The results also show a tendency to seed conservation by burial compared to storage in refrigiator. The seed lot 'Unterpurkla 2010' generated much lower viability rates than 'Kaposvar 2011'.

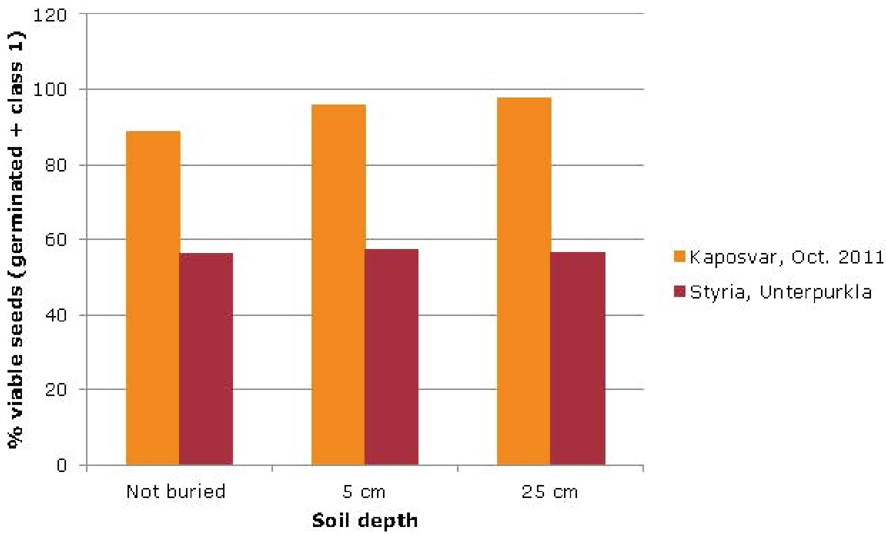

Fig. 7: Total viability (germination + TTC-test) of common ragweed seeds from 2 sources buried in 5 and 25 cm depth

KIS:

Finally, the Slovenian experimental site gave lower amount of alive seeds compared to those by the Austrian site (Fig. -11): High amounts of dead or crushed seeds.

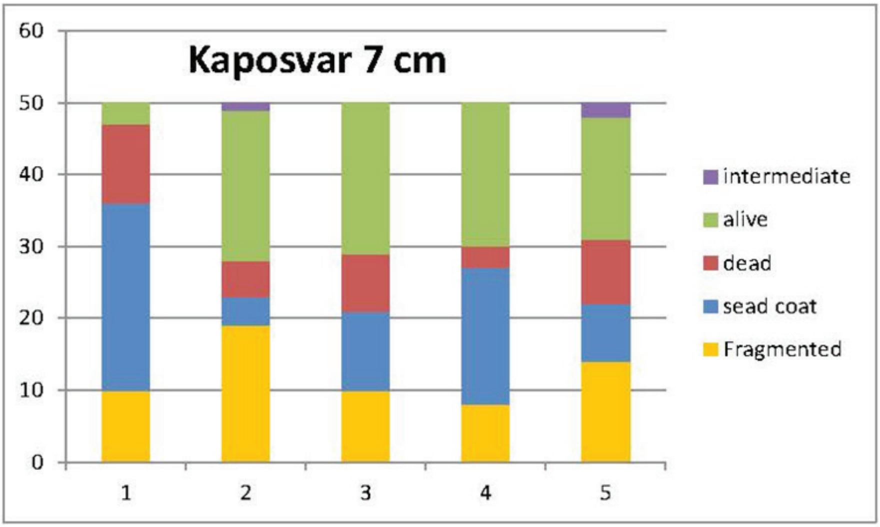

Fig. 8: Common ragweed seeds from Kaposvar 2011 buried at 7 cm in 2012 after germination and TTC test in 2013

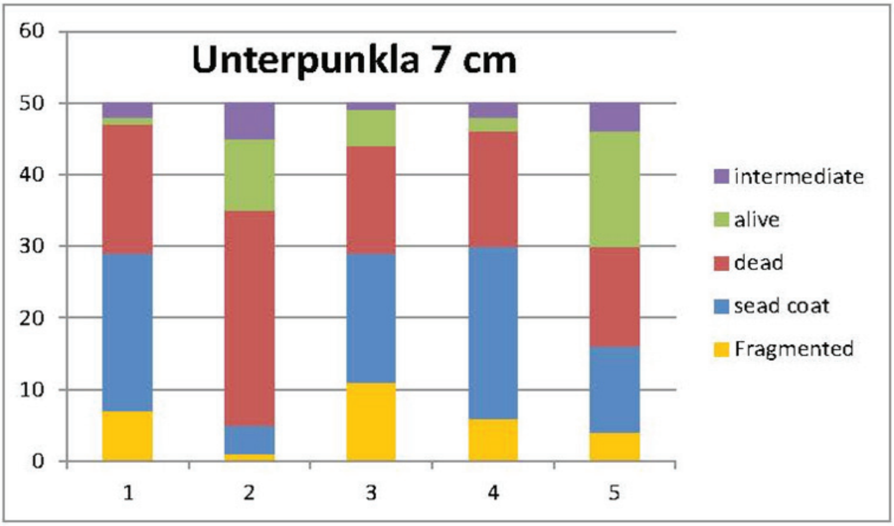

Fig. 9: Common ragweed seeds from Unterpurkla 2010 buried at 7 cm in 2012 after germination and TTC test in 2013

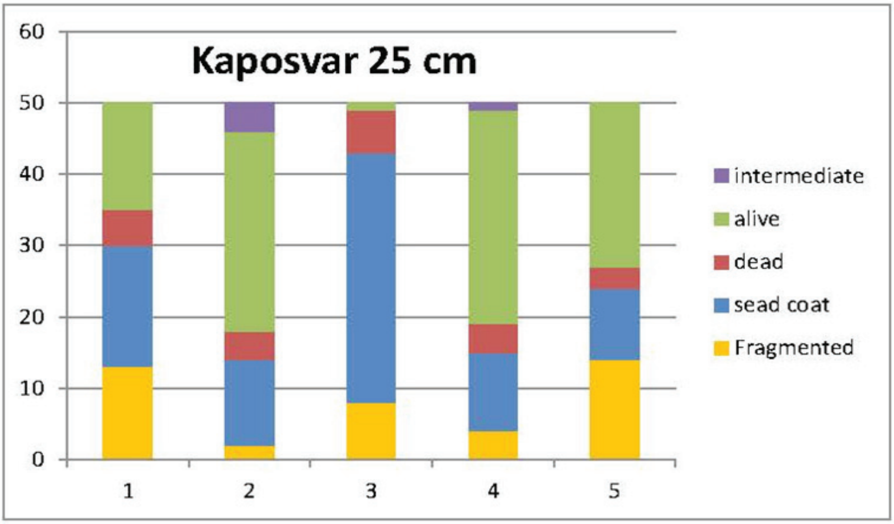

Fig. 10: Common ragweed seeds from Kaposvar 2011 buried at 25 cm in 2012 after germination and TTC test in 2013

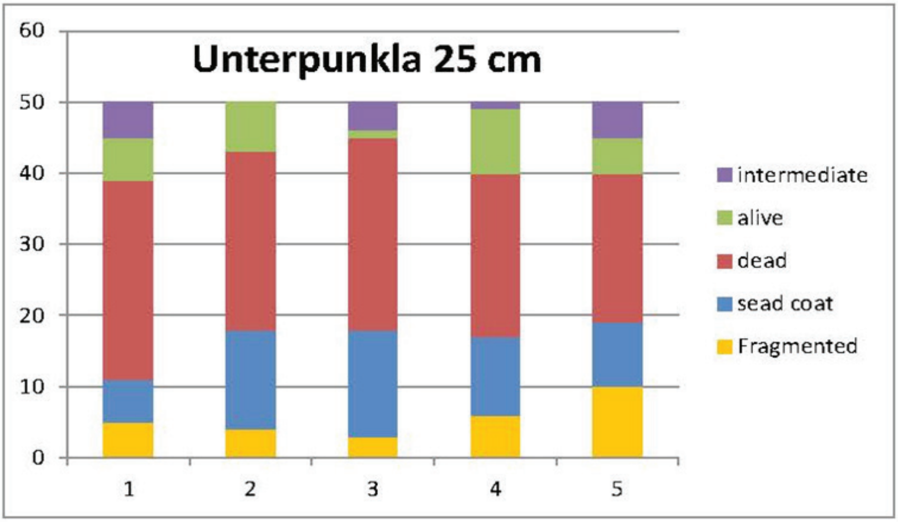

Fig. 11: Common ragweed seeds from Unterpurkla 2010 buried at 25 cm in 2012 after germination and TTC test in 2013

High variability in seed viability status of common ragweed samples was determined regardless of the seed origin and burial depth. In general the seed sample from the common ragweed population Kaposvar 2011 remained more viable compared to the Unterpurkla 2010 samples. Seed samples from both localities contained high percentage of fragmented seeds. Origin and habitat seem to be more important factors influencing viability of the seeds than burial depth.

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Field experiment on longevity of the seeds in the soil seed bank (initial seed burial experiment at the University of Natural Resources and Life Sciences BOKU)

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Introduction

In 2011 the BOKU started an initial burial experiment as the first one in the non-native region of Europe. We plan to test the viability loss of common ragweed seeds buried in a lawn at the BOKU campus in Vienna during 10 years.

Methods

70 packages (bags of fine net-like polyethylene tissue) with common ragweed seeds collected in 2010 from an arable field nearby Unterpurkla, in Styria, Austria (each net filled with 50 seeds) were buried in the soil at 10 cm depth in early spring 2011. Every year in spring the germination test procedure will be performed on the seeds of a subsample of 7 randomly selected bags. Additionally every year the germinability of seeds from the same base sample stored continuously at 4°C under dry conditions will be tested.

The excavated seeds are tested for germinability in climate chambers (8 h light at 30°C and 16 h dark at 15°C, resp.) for 4 weeks. The germinated seeds are discarded and the still dormant ones again stratified for 6 weeks under 4°C in darkness. Afterwards a second germination trial is performed under the same condition as with the first trial. Finally all remaining seeds are checked for viability with the TTC-test.

Results

The seed lot was tested for germinability/viability at the beginning of the experiment (spring 2011) following the procedure of article 6, section A. This test resulted in a 67% viability of the seed lot.

In spring 2012 the first set of 7 seed bags was dug out. We found almost all seeds or at least the seed coats of the provided seeds.

The viability test (germination and final TTC) gave a rather high number of dead seeds including empty opened seed coats (Tab. 1). The latter derived from seeds that germinated during the season between the digging date and the first excavation date one year later. Considering the high number of not viable seeds at the beginning of the experiment (33%), the loss of seeds during the first year was not extremely high (ca. 25% of the living stock at the beginning).

In 2013 again 7 nets were dug out and tested for germinability and viability. Interestingly the number of viable seeds per net in 2013 was higher than in 2012 (Tab. 1, Fig. 1). On the other hand the number of "intermediate" seeds was higher in 2013, compared to 2012.

Table 1: Comparison of mean percentages of seeds assigned to different status after germination and TTC-test from 2011 (before burial), 2012 and 2013.

	Mean percentage of seeds		
status	2011 [1)]	2012 [2)]	2013 [2)]
alive	67%	39%	54%
intermediate	?	9%	0%
dead	33%	33%	20%
empty seeds	0	19%	9%
fragmented	0	1%	17%

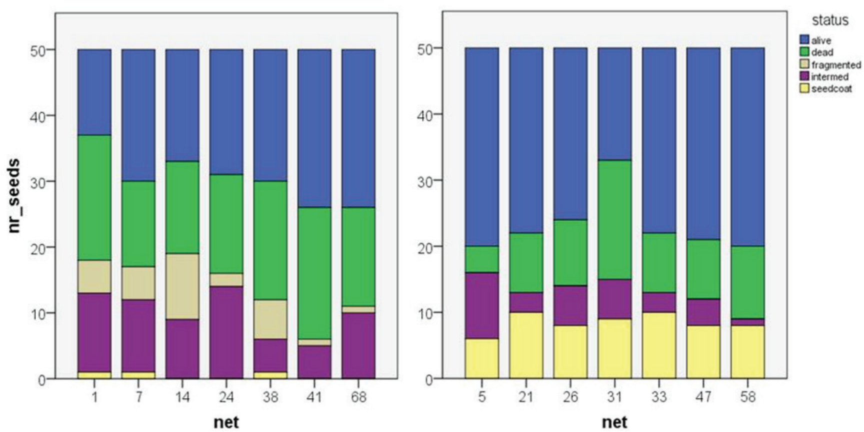

Fig. 1: Number of viable common ragweed seeds per net from Unterpurkla buried at soil depth of 10 cm, differentiated by the status of viability; test data from 2012 (left) and 2013 (right).

The overall germinability was tested when the seeds were buried in 2011 giving 66.25% germinable seeds. Interestingly, the excavated seeds from 2013 reached almost the same percentage of germinability like before being buried (Tab. 1).

Recommendations on safety of composting or use as biogas fuel of common ragweed seed contaminated material

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Introduction

Common ragweed (*Ambrosia artemisiifolia*) seeds are often found as a contaminant of different commodities, such as agricultural products (e.g., sunflower seeds), or of soil transported for construction purposes. The movement of these commodities may consequently become a pathway for the introduction of common ragweed to new areas (e.g., EFSA 2010).

In addition mechanical control measures like mowing, mulching or tillage, may yield plant material that contain viable seeds. Even after herbicide treatments with good efficacy viable common ragweed seeds can survive that and may be transported with human activities. When this plant material cannot remain in the habitat, ways of disposal are needed that are free of the risk of dispersing the seeds, but are at the same time environmentally friendly and lawful. Incinerating the material, for example, may not be lawful and creates emissions. Composting or disposing of the material in biogas plants, however, may result in residue containing viable seeds thus enhancing the risk for dispersal.

Maize is the most commonly used feedstock for biogas reactors in Germany (Westerman *et al*., 2011). If common ragweed seeds are able to survive the biogas process, this can result in another pathway of dispersal from field to field.

Before the project no detailed information was available on the ability of common ragweed seeds to survive the composting or biogas processes. Experiments on composting in the EUPHRESCO project AMBROSIA had failed to produce consistent results (Holst, 2010). In an earlier experiment with biogas fermenters the Tetrazolium test had produced ambiguous results (Heiermann *et al*., 2010). There was also no information on temperatures that common ragweed seed can survive.

Ripening of common ragweed seeds after cutting the plants

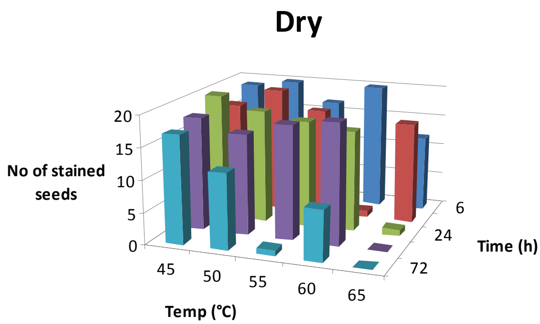
After the application of mechanical control measures, like mowing or mulching, remnants of cut plants may contain seeds already viable or finishing ripening process. In order to obtain information about the ripening process of seeds from plants cut at different postfloral stages trials in Austria and Germany were conducted, please see article 3 in Section A of this report.

Impact of heat treatments on seed viability

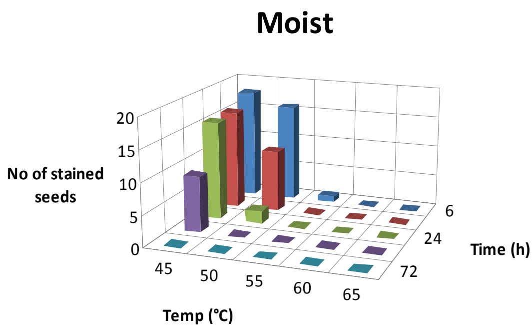
In order to recommend safe disposal of material potentially containing common ragweed seeds we conducted several series of basic laboratory experiments to determine the physiological limits of heat tolerance.

Experiment A

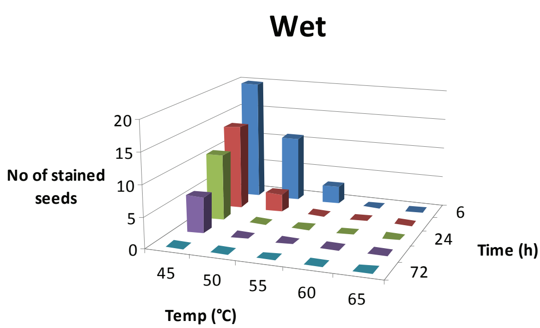
Common ragweed seeds were exposed to temperatures between 45 and 65 °C for periods of 6 to 72 hours in wet, moist, and dry conditions. Results are shown below.



a)



b)

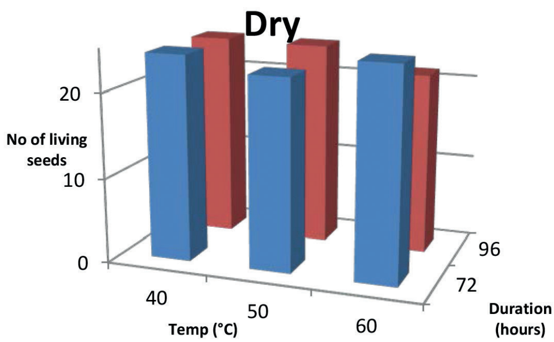


c)

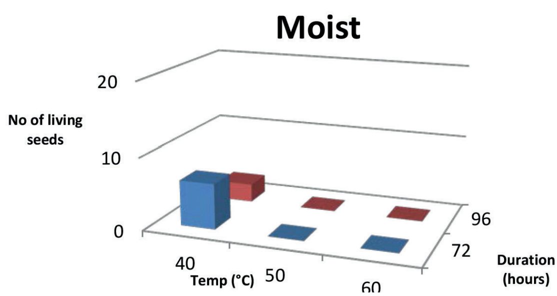
Fig. 1: Results of Experiment A: Number of common ragweed seeds surviving (out of 20) temperatures between 45 and 65°C over 6 to 72 hours under dry (a), moist (b) and wet (c) conditions

Experiment B

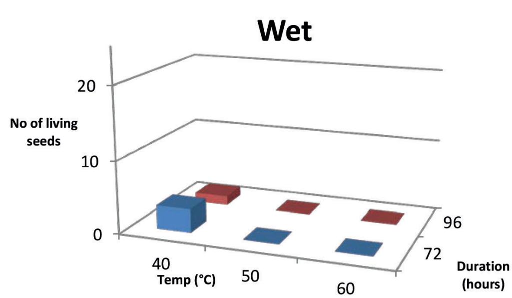
Common ragweed seeds were exposed to temperatures of 40°C, 50°C and 60°C for 72 and 96 hours in wet, moist, and dry conditions. Results are shown below.



a)



b)

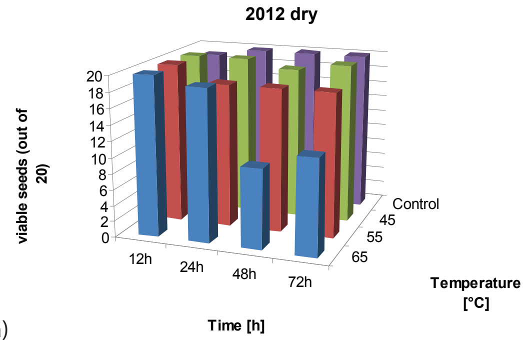


c)

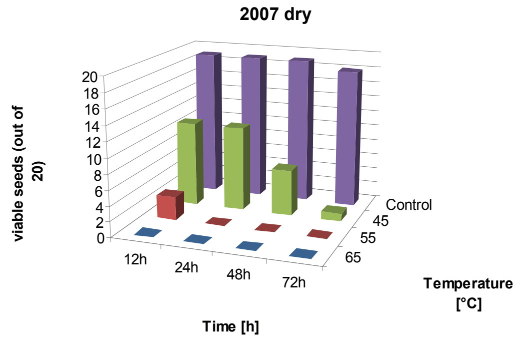
Fig. 2: Results of Experiment B: Percentage of seeds surviving temperatures of 40, 50, and 60°C over 72 and 96 hours under dry (a), moist (b) and wet (c) conditions.

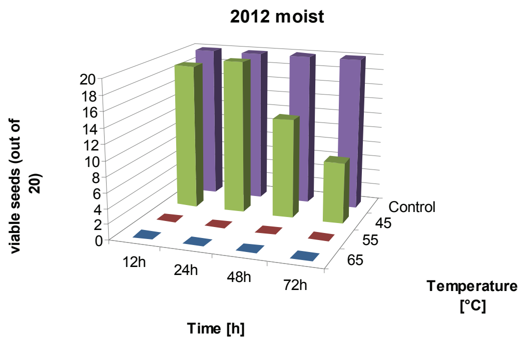
Experiment C

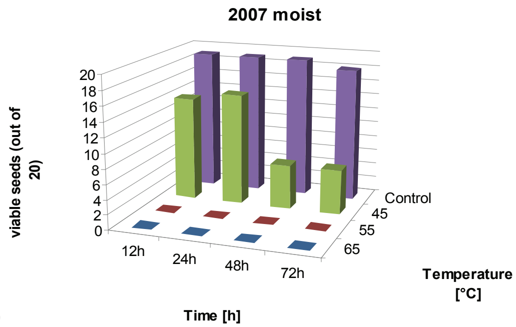
Common ragweed seeds were exposed to temperatures of 45°C, 55°C and 65°C for 12, 24, 48, and 72 hours in wet, moist, and dry conditions. Seeds of different ages: one year old (harvested in 2012) and 6 years old (harvested in 2007), were tested. Results are shown below.



a)







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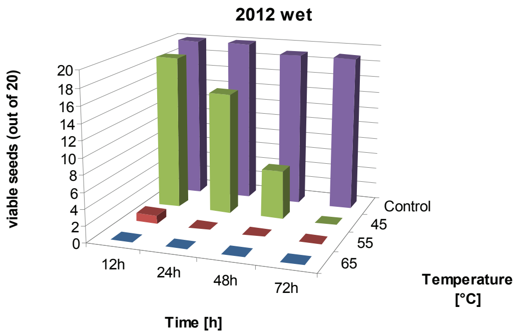

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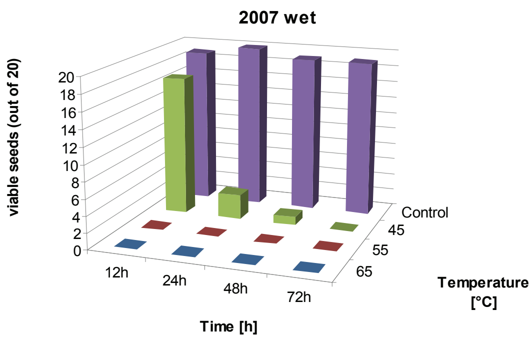

Fig. 3: Results of experiment C: Percentage of seeds of different age surviving temperatures of 40, 50, and 60°C over 72 and 96 hours under dry (a), moist (b) and wet (c) conditions. Left column: Young seeds (2012), right column: Older seeds (2007).

Result

The ability of common ragweed seeds to survive heat strongly depends on their condition:

- Dry seeds can have survival rates of 80 % after 72 and 96 hours
- Moist and wet seeds are reliably killed after 36 hours at 50°C or after 24 hours at 55°C.
- Both the viability and the ability of seeds to survive heat is reduced in older seeds.

Impact of the biogas process on seed viability

Seeds were tested in an experimental biogas fermenter (batch) at the Julius Kuehn-Institute. The fermenter was run at 37°C and shaken twice daily, the fermenting matter consisted of digestate taken from a biogas plant and water in a ratio of 1:1. Untreated seeds were stored in the fermenter for 1, 2, 4, 8, 16, and 32 days before being tested for viability with the TTC test. Some seeds were exposed to different silage processes (green rye and maize with and without additives) for 3 month, which is the normal time span for the silage process, before being tested for viability. Results are presented below (Fig. 4).

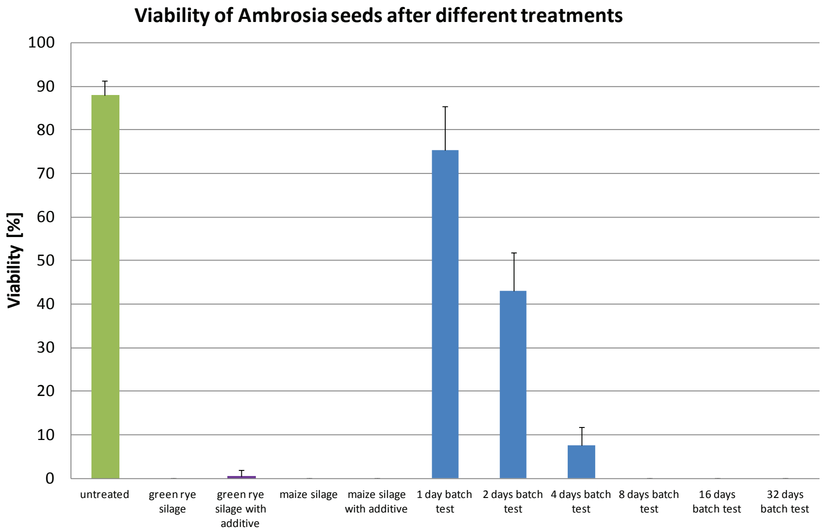

Fig. 4: Viability of common ragweed seeds after different treatments (3 month silage; 1, 2, 4, 8, 16, and 32 days in fermenter)

Ragweed seeds were also tested for germinability after exposure to a simulated biogas fermenter in an Austrian experiment (Gansberger 2011). Here, after 1 day a germination rate of 9% was found, but 0% after three days.

A series of experiments on weed seed survival in the biogas process is described by Westermann (2010).

Impact of the composting process on seed viability

As experiments in composting units were done in Austria and published after the start of HALT AMBROSIA and because our basic laboratory experiments on survival of heat stress are available, we did not conduct own experiments in composters. In the Austrian experiments (Hackl and Baumgarten, 2011), common ragweed seeds were put into polyethylene seed bags that were introduced to two types of commercial composters and at three depths (30, 60, 90, and 120 cm). The experiments were run for different lengths of time. Seeds were placed on filter paper and on water agar at 20°C/30°C (night/day) and 12h of light. Germinated seeds were counted after 7 and 21 days. In all seed lots 0 % germination was found at the first time, i.e. 10 days. In these two types of composters, temperatures of 55-60°C, and 65-80°C, respectively, were reached. The authors conclude that commercial composters are a safe way of disposing of plant material that contains common ragweed seeds, because the seeds lose their germinability.

In the Austrian experiment only a germination test was used as compared to the Tetrazolium seed viability testing applied in our experiments. The same is true for an older German study which recommended a safe disposal of common ragweed seed in composters (BGK, 2007).

A germination test alone may underestimate seed viability as dormant seeds that are viable may not germinate. The results must therefore be seen in relation to the results of our heat treatment experiments described above.

Conclusion and recommendations

Management measures against common ragweed populations may yield material that contains viable seeds. When this plant material becomes transported and disposed of without being treated in a way that reliably kills the seeds the risk of dispersal and of developing new common ragweed populations in uninfested areas arises. Management measures that aim at reducing common ragweed populations may thus miss their aim.

In general, management methods should be preferred that do not yield plant material with ripe or ripening seeds, e.g. mechanical control measures like uprooting or cutting before the onset of (female) flowering. If this is not possible, plant material resulting from mechanical control measures should remain on the site in order to avoid spillage of seeds during transport.

The example of Switzerland, where common ragweed is controlled effectively by now, demonstrates that it is necessary to increase awareness of the common ragweed problem in the building sector.

In Switzerland a special legal obligation regarding the disposal of excavated material contaminated with organic material (Neobiota) exists in the canton Zürich. The regulation says: If an invasive plant species occurs at a construction site the building owner has to fill in a declaration. Contaminated soil that cannot be used at the site has to be disposed at authorized sites.

During the construction work the contaminated material must not be mixed with clean material and it has to be separated. During the excavation a consultant has to be present at the construction site. It has to be ensured that no contaminated material is lost during the transportation. After transportation to the disposal site a form with a report has to be sent to the authorities. 1-2 month after the measure an authorized consultant has to control whether invasive plants grow back at the site.

However, there may be situations in which plant material with ripe seeds is created that cannot remain onsite. For this type of plant material, a disposal in composting or biogas facilities may be recommendable as seed viability can be destroyed by thermal and chemical conditions of treating facilities.

A recommendation for the disposal of material containing common ragweed seeds can only be given when the following conditions are met:

- During collecting and transport of the plant material, care must be taken not to spill common ragweed seeds. This may be achieved by using closed containers (e.g., plastic bags) and closed vehicles.
- The material must stay for 10 days in the biogas reactor. This is generally achieved in batch reactors but not in CSTRs (Continuous Stirred-Tank Reactor, like: single-stage, continuous flowthrough, stirred tank reactors) (Westerman *et al.,* 2010). For CSTRs, only previously ensilaged material can therefore be safely used.
- In our experiments, 55°C for 36 hours was enough to kill the seeds. In order to increase the reliability of all common ragweed seeds being killed, composters should reach 55° C for three weeks or 65° C for one week and the temperature should be monitored (cf. Schmid, 2007).
- Only industrial/commercial composting facilities can be recommended – not a private garden compost heap!
- Cutting and post ripening: safe only until the early female flowering (BBCH 63) if cut plant material is left on the soil surface

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Implications of life history for control and eradication

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Introduction

Weed control needs detailed knowledge about the biology of the target species to be efficient. In case of the invasive *Ambrosia artemisiifolia* L. (common ragweed) several studies about its biology were performed in the 20th century in North America (Gebben, 1965; Dickerson, 1968; Basset and Crompton, 1975). In the latest review about the biology of this species (Kazinczi *et al.,* 2008a) some data on population biology and habitat preferences of European populations were integrated to the pool of knowledge. Since that time the number of papers about the biology of common ragweed in European populations increased seriously.

Besides the trials within this project several new studies on *A. artemisiifolia* and other comparable invasive plants were published (see the review by Smith *et al*., 2013). We have to expect local adaptation to the new habitat (environment, co-occurring species, predators and parasites). Therefore the analysis of the most recent biological behaviour of common ragweed is essential to decide about the optimal local control measures. Scalone *et al*. (2013) already indicated that the European populations show specific adaptations to the northern climate by shifting the growth and flowering period towards July and June. During field work for the section "Biological fundamentals" and "Non-chemical and integrated control strategies" we also could find some individuals within few populations in eastern Austria that started flowering in mid-June already (Karrer *et. al*., 2011). Obviously the life cycle of common ragweed is shortened in the invasive European range.

Life cycle of Ambrosia artemisiifolia

The section "Biological fundamentals" of the HALT-AMBROSIA-project aimed at increasing the knowledge of the biological characters of common ragweed from European populations. Like any other summer annual weed the fate of the population depends very much on the seed production in the respective year so that the future generations have a realistic chance to establish and succeed. The lifespan of a single common ragweed plant (Fig. 1) begins with the barochorous release of seeds from the mother plant, followed by the phase of being part of the soil seed bank for variable times. High variation of seed morphs were found by Fumanal *et al*. (2007a, b) indicating pre-adaptation to be different distribution vectors. They found that the partition of light seeds is able to float on water for longer time and thus is prone to be spread easily along rivers.

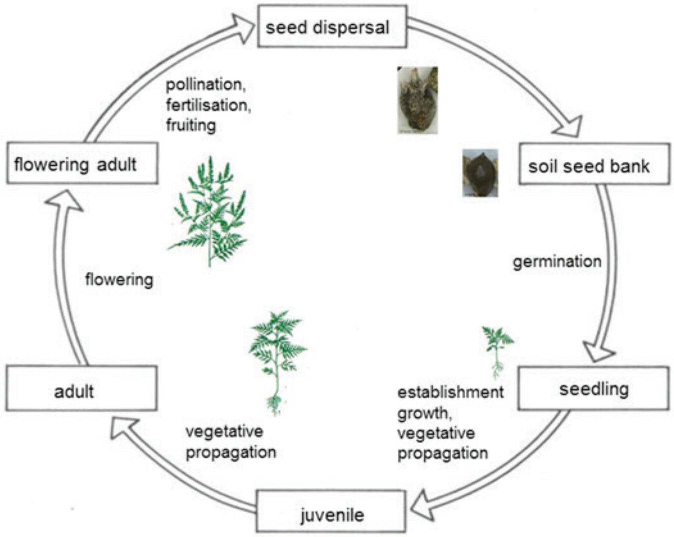

Fig. 1: Annual lifecycle (developmental stages) of *Ambrosia artemisiifolia*

Lifetime of seeds as element of the soil seed bank varies depending on the frequency of soil disturbance and dormancy. In arable fields with annual soil tillage the turnover rate of seeds is higher compared to that of abandoned fields or grassland. Consequently, the persistence of individual seeds in the soil seed bank of fields is short. In grassland, most of the seeds stay in the upper soil or on soil surface, and are integrated to the annual seed turnover, whereas the smaller partition of seeds will be integrated to deeper soil horizons by bioturbation and build the long-time persistent part of the soil seed bank.

Toole and Brown (1946) found a maximum longevity of (few) common ragweed seeds by 39 years. In their experiment the storage conditions were very good, not comparable to the very stressful conditions in the upper soil and soil surface. Seeds stored at soil surface conditions turned out to loose viability within 5 years. Our studies of the soil seed bank of common ragweed along roadsides (see section A article 3) showed losses of 20% on average when autumn and early spring samples of the sites where compared with respect to the number of common ragweed seeds in the upper soil layer (0-7 cm). Beres (2004) found that seeds exposed to field conditions (soil surface) throughout 5 years lost their viability by 100%. A screening for viability of common ragweed seeds with different age stored at dry conditions and room temperature (ca. 20°C) gave comparable results (Kazinczi in section A article 3). Considering the high variability in seed mass (Fumanal *et al.,* 2007b) one could expect that smaller seeds that show higher dormancy tend to be accumulated in the lower soil, whereas heavier seeds have better chances to stay aboveground. Fenner and Thompson (2005) state that small seeds are more likely to be buried and are more dormant. It is not known if the partition of small common ragweed seeds build up the more persistent seed bank whereas the seeds from upper soil/soil surface were bigger and less dormant but more successful by carrying more resources. Such was proved at least for other taxa (Zhang, 1993; Imbert, 1999).

Like other typical summer annual weeds common ragweed seeds show innate dormancy after seed set in autumn and need stratification of about 4 weeks at temperatures around 0°C (Baskin and Baskin, 1998) for germination. If the conditions after stratification are not suitable for germination (darkness, drought, temperature regime at low positive values, low O_2 or high CO_2 concentration in the soil) enforced (secondary) dormancy can be initiated (Baskin and Baskin, 1980). As long as the conditions do not change seeds persist in secondary dormancy until spontaneous death (latest after 39 years after Toole and Brown, 1949). Such data were published for North American populations of common ragweed. Only few data about seed biology are available from European populations (Fumanal *et al.,* 2007a; b; Beres, 2004). Adaptive evolution in the newly invaded range could have changed the preferred site conditions for the regulation of germination and growth. Therefore some experiments were started in 2012 to elucidate these important aspects of the life cycle. The burial experiment (section A article 3) will test the survival rates of common ragweed seeds buried at upper (5 cm) or lower (25 cm) soil depths. Survival rates of common ragweed seeds varied between 30 and 98% depending on the seed source. Before burial, seeds were collected and stored at various conditions, transported by postal services (airmail) at maybe less optimal temperatures. The older sample (3 years in age when buried) gave generally lower viability rates (30-80%) than the younger ones (1 year old, 70-98%).

Common ragweed individuals that germinate early in the season (March to April) grow slowly at the beginning forming a rosette-like stage with 4-6 leaves. With increasing temperatures vegetative growth is enhanced during June and July by significant stem elongation and +/- branching – depending on the resource availability (Leskovšec *et al.,* 2012 and Section B article 11) or population density (Patracchini *et al.,* 2011; Simard and Benoit, 2011). Consequently, the number of pollen as well as seeds produced per individual also depends largely on habitat features and population density.

Effects of control measures on common ragweed life cycle

If the <u>soil seed bank</u> of common ragweed is already established it can be reduced by <u>crop rotation</u> and direct control of germinated plants. On arable fields with regular ploughing a significant proportion of seeds always will be left in the soil seed bank. Switchback to summer crop cultivation will promote the common ragweed population to recover from the persistent part of the soil seed bank. Only total abandonment and succession towards forests over decades might deplete the soil seed bank by death from ageing. Depletion of soil seed bank by repeated stimulation to germinate (i.e. by soil tillage every month from spring onwards) could help to control common ragweed.

Seed production is positively correlated to biomass (Leskovšek *et al.,* 2012). Cutting aboveground biomass at early stages (May or June) is +/- compensated by rapid basal regrowth from axillary buds, often supplemented by accessory buds. Early regrowth tends to produce rather more male flowers whereas later regrowth in August or September invest more into female flowers/seeds. Regrowth from early cuts also increases the number of axillary buds positioned at lateral shoots below the cutting height. They promote common ragweed to increase even the number of lateral shoots below the cutting line that bear mostly female flowers. Based on the cutting experiments in pots (Milakovic *et al.,* 2014a) as well as in the field it can be stated that a first cut should be delayed as far as possible towards the start of female flowering. In the southern part of Central Europe (S-France, Switzerland, Austria, N-Italy, Hungary, Slovakia, Slovenia, Croatia, Serbia) such late first cut must be supplemented by at least one second cut about 3 weeks later to prohibit successful seed production from the regrowth (Karrer *et al.,* 2011; Pixner, 2012; Karrer and Pixner, 2012; Milakovic *et al.,* 2014b).

When cutting is one of the most frequent control measures against common ragweed in sensitive habitats (within villages, water resource areas, nature protection areas), application of herbicides is used often as an appropriate control tool against common ragweed in traditional farming. In graminoid crops common ragweed can be sprayed rather effectively, but herbicides have to be applied rather sophisticated if the farmers aim at very effective regulation. Several seedling cohorts (even in maize) produce enough seeds for future generations so that common ragweed continues to be present in the soil seed bank. Maybe the crop yield is not reduced but common ragweed stays in the system for long time. Furthermore, common ragweed cannot be fought chemically in some minor crops (oil pumpkin, red bean, soybean, and most sunflower breeds) because of the lack of registered herbicides in some countries (Austria, Hungary, Germany, etc).

In cereal stubbles late germinating common ragweed cohorts can even dominate. Simply spraying herbicides does not kill common ragweed by 100% at this late developmental stage (Bohren *et al.,* 2008b). But combined measures like mowing plus spraying the regrowth or simply ploughing can destroy common ragweed most efficiently (Kazinczi *et al.,* 2008b). Donald (2000) demonstrated that a combination of band applied herbicides in the crop rows and mowing twice between the crop rows was sufficient to control annual weeds like common ragweed without reducing the yield of the main crop in Australia.

The most sensitive phases of common ragweed's life cycle for appropriate application of the commonly used control measures are illustrated in Fig. 2. When optimizing the available tools with respect to timing and sequence, the effort for control can be kept low. Which kind of measure to apply, depends primarily on the habitat type infected and on the season.

Most important for hindering the invasion to not yet infected sites/countries is prevention of seed dispersal by human vectors. I.e., commodities (seed material, soil, relevant for trading and construction areas) should be kept clean as well as vehicles that move from infected to uninfected areas (most relevant in agricultural landscapes).

Once common ragweed seeds arrive on or in the soil the seed bank can be managed by depletion or long-time full abandonment. Stimulating seeds to germinate and subsequent kill is a way to decrease the presence of weeds aboveground as well as belowground (Swanton *et al.,* 2000; Murphy *et al.,* 2006). Pre-emergence herbicides would not help so much if subsequent soil disturbance provides new seeds from deeper soil horizon. Even better would be to provoke common ragweed to germinate and to kill afterwards by ploughing. During and short time after germination (seedling and juveniles up to the 4 leaf stage) is the best time for herbicide application in habitats where they are registered. Sophisticated mechanical weeding could also have high efficacy at this early stage of common ragweed development as the common ragweed seedlings and juveniles are prone to being killed by drought because of the lack of a well-developed tape root at that time of the year. The older common ragweed gets the less effective are mechanical treatments and herbicide application (Bohren *et al.,* 2008a; b; Section B).

Young adults are the best stage for hand-pulling: easy to detect and to identify, mechanically firm enough to be hold tight but the roots still not too deep. Therefore, pulling is generally the most effective control measure against common ragweed (Bohren *et al.,* 2008c) at least for small to medium sized populations (1-1000 individuals). Pulling before flowering is fine also for getting rid of the plant by use in simple humus composters. Pulling late in the year will produce individuals with ripened seeds that have to undergo a serious destruction of organic material by burning or fermentation.

Fostering competition by other plants (crops, intercrops, lawn species, tall grasses and herbs) is an admitted control option. Competition by shading green leaves or by litter can hamper already germination what can be documented easily on fallow land (Karrer *et al.,* 2011). Competition can enforce germinated common ragweed to develop quickly in height and therefore bearing only few buds for regrowth below cutting height (Milakovic *et al.,* 2014b). If germinated without competition in early spring common ragweed tends to grow only slowly in height forming almost a rosette of 4 to 6 leaves near to the ground. Consequently, such plants have very high regrowth potential from lower axillary buds after being cut. The number of available meristems for regrowth below the cutting height is also increased by the torsion of the main root and the shoot base in older common ragweed plants. This causes the indirect lowering of the shoot base with its regrowth meristems (Vitalos, unpubl.). Outcompeting regrowing lateral shoots of mown common ragweed by even faster regrowing competitors can help to keep the number of common ragweed flowers or seeds at low levels (Milakovic and Karrer, 2011). Fostering of competing vegetation after every mowing event is only possible on nutrient rich sites. Unfortunately, the substrate used to cover road shoulders since about 10 years is very unfavourable for any plant to grow. As a typical (CS)R-strategist (CSR theory: Competitors, Stress tolerators and Ruderals) common ragweed is able to establish even at such unfavourable site conditions (gravel as substrate) and it will take many years to establish a competitive vegetation cover.

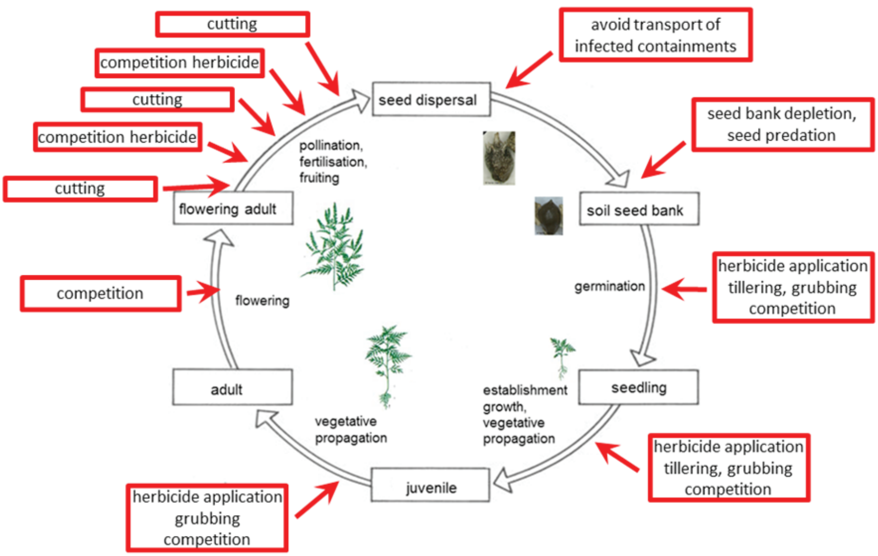

Fig. 2: Life cycle of common ragweed and the optimized timing for appropriate application of control measures.

Consequences and conclusions

The HALT-trials gave improved insight to the biology of seeds and seed production in European populations of common ragweed at different habitat types. But, we have to face new problems when common ragweed succeeds to adapt to lower temperatures for growth, to higher temperatures for stratification and to earlier initiation of flowering and seed set. Future research has to be on the qui vive when adaptive processes in common ragweed evolution call for continuous adaptation of control measures.

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A standard protocol for testing viability with the Triphenyl Tetrazolium Chloride (TTC) Test

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A standard protocol for the TTC Test was developed in order to conduct ringtests with 3 different common ragweed (*Ambrosia artemisiifolia*) populations among project partners (JKI, KU, BOKU, KIS, AU, for abbreviations, see introduction of this volume) and interested institutions (Ministry of Economic Affairs, Agriculture and Innovation; Iğdır Üniversitesi Turkey and UMR Biologie et Gestion des Adventices France). After the first ringtest the viability results showed more variability between laboratories than between the populations (Starfinger *et al.,* 2012). Therefore the protocol was adapted concerning the categories of dyed seed tissue and a second ringtest was carried out.

Triphenyltetrazolium chloride, TTC, is a redox indicator used to indicate cellular respiration. Its solution in water is colorless but in living tissues the TTC is reduced to a red substance thus dyeing living tissues in red.

The test is commonly used for testing seed quality with various instructions produced by, e.g., the International Seed Testing Association. Certain adaptations for specific seeds are commonly made. This protocol was developed after pre-trials.

Three populations of Ambrosia seeds were tested (2 from BOKU and 1 from KU). These samples were sent to each partner by BOKU and KU. 100 achenes per population are required (4 samples, each with 25 achene halves).

Materials:

- 100 achenes of each population. Choose randomly, i.e., do not exclude small or light ones that you might suppose to be less viable.
- Tap water
- An instrument to cut achenes in halves. A nail clipper was very reliable or a surgical scalpel or similar instrument
- Distilled water
- 12 glasses of 5-10ml volume which can be covered
- Incubator or drying chamber
- Refrigerator
- 1% TTC-solution (ca. 100ml)
- dissecting microscope/binocular
- Implementation:
- Ambrosia achenes are imbibed in tap water at room temperature over night (i.e., for ca. 12 -15 hours).
- The achenes are cut open with a surgical scalpel or similar instrument in a vertical line (top to base).
- The bigger part of the seed is used for testing, the other part is discarded.

- 25 achene halves are put into one glass and filled up with TTC solution (4 times per population).
- Glasses are tightly closed and put to react at 30°C for 6 hours in darkness. Because TTC is light sensible, avoid unnecessary light input.
- If it is not possible to keep on with the protocol after these 6h, the closed glasses can be stored in a refrigerator (~6-8°C) over night.
- TTC solution is poured off and halves are rinsed under distilled water.

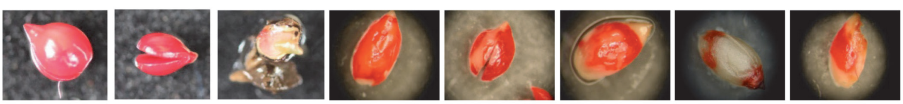
Under a dissecting microscope the seedhalves are removed from the integument (outer shell). Seeds are counted in 3 classes: a) stained (=alive), b) not stained resp. no fully developed embryo present (=dead), c) intermediate cases that are only lightly or partly stained. For the decision on intermediate see below.

Figures 1. – 4.: Illustrating the procedure

1. potential tool for cutting open the achenes (nail clipper); 2. achenes after opening; 3. well-stained embryos; 4. no embryo developed

How to assign "alive" (1); "dead" (0) and "intermediate" (0.5) to staining results

The staining of different tissues in the seed may have different implications for the interpretation of the test. A dead (= not stained) radicula in a otherwise stained seed will mean that the seed is dead. For the sake of simplicity and ease of judgment in the ring test, all seeds that are completely stained shall be deemed alive (1), seeds without any trace of staining will be deemed dead (0) and the rest intermediate (0.5). Figure 5. shows examples and how they should be rated.



1	1	0	0.5	0.5	0.5	0.5	0.5

Fig. 5.: Examples of different staining results and how they should be rated; 1 – alive, 0 – dead, 0.5 – intermediate.

Perspectives for biological control

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Summary

The recent invasion by common ragweed, *Ambrosia artemisiifolia,* has, like no other plant, raised the awareness of invasive plants in Europe. Recently, chemical and mechanical control methods have been developed and partially implemented in Europe, but sustainable control strategies to mitigate its spread into extensively managed land and to reduce its abundance in badly infested areas are lacking. One management tool - not yet implemented in Europe but successfully applied in Australia - is biological control. With the notable exception of the recently detected leaf beetle *Ophraella communa*, almost all natural enemies that have colonized *A. artemisiifolia* in Europe are polyphagous and impose only little damage, rendering them unsuitable for a system management approach. Two fungal pathogens have been reported to adversely impact *A. artemisiifolia* in the introduced range, but their biology makes them difficult for mass production and application as a mycoherbicide. In the native range of *A. artemisiifolia*, on the other hand, a number of herbivores and pathogens associated with this plant have a very narrow host-range and reduce pollen and seed production, the stage most sensitive for long-term population management of this winter annual. We discuss and propose a prioritisation of these biological control candidates for a classical or inundative biological control approach against common ragweed in Europe by considering past experiences from North America, Asia and Australia. We argue that the biological control approach should be considered as an integral part of an integrated management approach against common ragweed in Europe. Along these lines, the COST action 'SMARTER' (launched in 2012) aims at promoting biological control against common ragweed, integrating it with available chemical and physical control options, and developing habitat- and region-specific recommendations for a integrated management of common ragweed across Europe.

Introduction

Like no other plant, common ragweed, *Ambrosia artemisiifolia* L., has raised the awareness of invasive plants in Europe. First records of this plant species in western Europe date back to the mid 1800s and in eastern Europe to 1900, but it was only in the late 1920's that *A. artemisiifolia* became an increasing problem in Europe (Csontos *et al.,* 2010). The main concern regarding *A. artemisiifolia* is its large production of highly allergenic pollen that causes already rates of sensitisation among Europeans from 15% (e.g. Germany, Netherlands, Denmark) to 60% (Hungary; Rybnicek and Jäger, 2001; Taramarcaz *et al.,* 2005). This results in allergic rhinitis and severe asthma in over 20% of the population of affected areas (Kazinczi *et al.,* 2008).

The recent spread of *A. artemisiifolia* and the resulting increasing risk to human health and agriculture has resulted in a number of publications on the further invasion and potential danger of this invasive weed, its medical aspects, pollen monitoring across Europe, and control methods at a local scale (Buttenschøn *et al.,* 2009). Moreover, in 2006 the national authorities in Hungary and Switzerland established a legal basis for mandatory control of *A. artemisiifolia*. Yet, although chemical and mechanical control methods have been developed and partially implemented (Buttenschøn *et al.,* 2009), sustainable control strategies to mitigate its spread into areas not yet invaded and to reduce its abundance in badly infested areas are lacking in Europe.

One management tool that has received little attention in Europe so far is biological control. Three principal methods of biological weed control can be distinguished: (i) The classical approach aims to control naturalized weeds by a limited number of introductions of exotic control organisms from the weed's native range; (ii) The inundative method uses periodic releases of an abundant supply of a native or exotic biological control agent over the entire weed population ; (iii) The system management approach, sometimes also referred to conservation biological control, aims to increase the impact of native antagonists (Müller-Schärer and Schaffner, 2008). Based on a prioritizing scheme developed by Sheppard *et al.* (2006), *A. artemisiifolia* was identified as one of the 20 most promising species for classical biological control in Europe.

Because *A. artemisiifolia* also causes problems in the northern parts of North America, Australia and large parts of Asia, there is a significant amount of information available on the biology of this plant and on the efficacy of various control measures from other parts of the invaded range. Up to date, *A. artemisiifolia* has been subjected to classical biological control programmes in Eastern Europe, Australia, and eastern Asia with variable success (Julien and Griffiths, 1998; Reznik *et al.,* 2007; Zhou *et al.,* 2009). The information gathered in these biological control programmes may act as a solid basis to develop a biological control program for Europe. Its integration into existing short-term control measures may then lead to a sustainable management strategy of *A. artemisiifolia* and other *Ambrosia* species invasive in Europe.

This report summarizes previous attempts to control *A. artemisiifolia* using biological control worldwide and explores prospects for its application in Europe.

Natural enemies of *Ambrosia artemisiifolia*

To assess whether any natural enemies (herbivores or fungal pathogens) attacking *A. artemisiifolia* could potentially be used for biological control of this weed in Europe, we conducted a literature review to compile a comprehensive list of natural enemies associated with *A. artemisiifolia* and other *Ambrosia* species in the native range in North America and in the introduced range in Europe, and of the biological control activities that have been conducted worldwide so far. The results of the review were published in Gerber *et al.* (2011) and are outlined below.

Herbivores and pathogens associated with *A. artemisiifolia* in Eurasia

About ten species of insects, mites and fungi were recorded in Eurasia by Kovalev (1971a), several generalist fungal pathogens and insect species found in Hungary (Bohar and Vajna, 1996; Kiss *et al.,* 2008), and 28 species of insects recorded in former Yugoslavia (Maceljski and Igrc, 1989). In total, some 60 insect species (including two unidentified geometrids) are reported to be associated with *A. artemisiifolia* in Europe (Essl et al. 2015). The insect complex revealed mainly polyphagous species, some of them even known as agricultural pests. In China, the moth *Ostrinia orientalis* Mutuura & Munroe (*Lepidoptera*: *Pyralidae*) attacks *A. artemisiifolia* and was found to significantly reduce biomass and plant height (Wan *et al.,* 2003); however, the species is also recorded from *Xanthium sibiricum* and *Rumex species* (*Polygonaceae*), hence has a relatively broad host-range (Ishikawa *et al.,* 1999).

In 2013, the ragweed leaf beetle *Ophraella communa* Lesage (Col.: Chrysomelidae) was detected in southern Switzerland and northern Italy (Müller-Schärer et al. 2014). At sites where *O. communa* was found in Switzerland and Italy in 2013, up to 100% of the plants were attacked, with attack levels high enough to completely defoliate and prevent flowering and seed set of most ragweed plants (Müller-Schärer *et al.*, 2014). This oligophagous beetle is used as a biological control agent against *A. artemisiifolia* in China, but despite extensive host specificity tests, the risk of attack and the level of damage of sunflower under field conditions remain unclear (see below).

Of the 20 fungal pathogens found associated with *Ambrosia* species in Eurasia (Gerber *et al.,* 2011), most are known to have a wide host range and were found to have little impact on the plant in the field (Kiss *et al.,* 2003). Outbreaks of disease epidemics caused by two biotrophic fungal pathogens, *Phyllachora ambrosiae* (Berk. & M.A. Curtis) Sacc. and *Plasmopara halstedii* (Farl.) Berl. & De Toni, did affect *A. artemisiifolia* in Hungary in the years 1999 and 2002 (Vajna *et al.,* 2000; Vajna ,2002), but not in other years (Kiss, 2007).

A newly described species associated with *A. artemisiifolia* in Hungary (Farr and Castlebury, 2001), *Septoria epambrosiae* D.F. Farr, is also known from *A. trifida* in North America. In China, the damaging microcyclic rust *Puccinia xanthii* Schwein. has been recorded from *A. trifida* as *P. xanthii* forma specialis *ambrosiae-trifidae* Batra (Lu *et al.,* 2004), following Batra's initial classification of a host-specific *P. xanthii* accession from the same host plant in North America (Batra, 1981). This rust species is considered to comprise a number of host-specific rust populations adapted to specific *Asteraceae* hosts (Batra, 1981; Morin *et al.,* 1993; Kiss, 2007; Seier *et al.,* 2009).

Herbivores and pathogens associated with *A. artemisiifolia* in the native range

Compared to the low number of phytophagous organisms associated with *Ambrosia* species in the introduced range in Eurasia, numerous species are known from their native range. Up to date, as many as 450 species of insects, mites and fungi have been identified to be associated with *Ambrosia* species in North and South America (Goeden and Andres, 1999). On individual *Ambrosia* species as many as 113 (on *A. psilostachya*) and 88 (on *A. confertifolia*) insect species were recorded in Southern California alone (Goeden and Ricker, 1975; Goeden and Ricker, 1976b). Many of these species are not specific as they also feed on other genera in the *Asteraceae* family or are known to develop on species in other plant families. However, our survey for species potentially specific at the subtribe level (i.e. associated with *Ambrosia* and for which no other host plant record has been found outside of the subtribe *Ambrosiinae*) revealed as many as 109 specialist invertebrate and 19 specialist fungal species (Gerber *et al.*, 2011). This amounts to approximately 36 and 25% of the total number of invertebrates and fungal species recorded from the native range, respectively. Within invertebrates, Lepidoptera (40 species) largely dominate, followed by Coleoptera (28 species), Diptera (19 species) and Hemiptera (18 species). In addition, four mite species have been recorded from members of the genus *Ambrosia*. The majority of herbivores with known feeding niche are leaf-feeders (50%), followed by stem-miners (28%), seed-feeders (12%) and flower- or pollen-feeders (9%).

As observed for the invertebrate fauna, numerous fungal pathogens known to be associated with *Ambrosia* species in the native range have a wide host range, either within the *Asteraceae* or across a number of different plant families. However, some fungal species are similarly restricted to the genus *Ambrosia*, e.g. *Septoria ambrosiicola* Speg. and *Passalora ambrosiae* (Chupp) Crous & U. Braun (synonym *Cercospora ambrosiae* Chupp; see Gerber *et al.*, 2011). Other pathogen species such as the white blister 'rust', *Pustula tragopogonis* (Pers.) Thines (synonym *Albugo tragopogonis* (D.C.) Gray), and the true rust *Puccinia xanthii* have been recorded from a number of different genera within the *Asteraceae*; however, *P. tragopogonis* and, as indicated above, *P. xanthii* have been shown to comprise different formae speciales with a highly restricted host range. The existence of formae speciales is also known for the powdery mildew species *Golovinomyces cichoracearum* var. *chichoracearum* (DC.) V.P. Heluta (synonym *Erisyphe cichoracearum* DC.), and a restricted host range of accessions of this pathogen associated with *A. artemisiifolia* cannot be ruled out (Ellison and Barreto, 2003). However, this hypothesis would need to be verified through cross-inoculations and molecular studies (Evans, 2000).

Biological control of Ambrosia species

Biological control of *Ambrosia* species in their native range

Ambrosia artemisiifolia and *A. trifida* are also noxious weeds in their native range, in particular in Canada (Cowbrough, 2006) and in the Northern United States (USDA-NRCS, 2009a; USDA-NRCS, 2009b), causing allergenic hay fever (Bassett and Crompton, 1975). As the highest densities of both species are found in the most densely populated part of Canada (southern Ontario and Quebec), the feasibility of the mycoherbicide approach, i.e. the periodic inundative application of high doses of indigenous pathogens over an entire weed population, was studied in both Canada and the USA. Protomyces gravidus Davis, which attacks *A. artemisiifolia*, *A. trifida*, *Xanthium strumarium* L. and members of the genus *Bidens* (tribe *Coreopsideae, Asteraceae*), was studied in the USA (Cartwright and Templeton, 1988). The species causes stem gall disease and killed plants when these were infected systemically. However, the low rate of infection and lack of virulence when applied as a mycoherbicide strongly limits the use of this organism to control *Ambrosia* species. The project was therefore stopped. A forma specialis of *Pustula tragopogonis* has been described on *A. artemisiifolia* in Canada (Hartmann and Watson, 1980a). Host specificity tests on 59 species from 46 genera indicate that, other than *A. artemisiifolia*, disease symptoms developed only on sunflower cultivars (*Helianthus annuus* L.). Although a few pustules developed on the cultivars inoculated, the disease did not persist and sunflower is therefore considered a non-compatible host for the *P. tragopogonis* accession from *A. artemisiifolia* (Hartmann and Watson , 1980a). Attack by *P. tragopogonis* can be very damaging and significantly reduce pollen and seed production if systemic infection is achieved, as shown both in laboratory and in field trials. The rate of systemic infection obtained in the laboratory was however low (14%), and Hartmann and Watson (1980b) suggested that multi-cyclic applications of *P. tragopogonis* suspensions would be necessary to increase infection level in a field environment. Difficulties to mass produce this white blister 'rust' have so far limited its potential use (Teshler *et al.*, 2002). *Pustula tragopogonis* was accidentally introduced from Canada into the former USSR in the early 1960s where initially it caused heavy infection of *A. artemisiifolia* and reduction in biomass and seed production, but levels of damage have strongly declined since (Julien and Griffiths , 1998).

A *Phoma* species, recorded on *A. artemisiifolia* in North America, was considered as a potential mycoherbicide candidate (Brière *et al.* , 1995). A combination of this *Phoma* species and a phytophagous insect, the leaf beetle *Ophraella communa* LaSage (Coleoptera: Chrysomelidae), were synergistic and resulted in high plant mortality (Teshler *et al.* , 1996). Unfortunately, the culture of *Phoma sp.* lost its virulence and attempts to revive or re-isolate the species from natural sites have failed (Teshler *et al.*, 2002). Two plurivorous pathogens, the soil borne fungus *Rhizoctonia solani* J.G. Kuehn and the gram-negative bacterium *Pseudomonas syringae* pv. *tagetis* (Hellmers) Young, Dye & Wilkie have also been preliminarily evaluated as potential biocontrol agents for a crop management strategy against A. grayi in the USA (Sheikh *et al.*, 1999; Sheikh *et al.*, 2001; Wheeler *et al.*, 1998). Under greenhouse conditions *R. solani* was shown to cause significant disease in inoculated *A. grayi* plants seen as an increase in root necrosis and a reduction in plant emergence as well as in fresh and dry leaf weight (Wheeler *et al.*, 1998). *Pseudomonas syringae* pv. *tagetis* proved to be pathogenic towards *A. grayi* causing systemic chlorosis in infected plants during greenhouse trials. Subsequent field trials conducted in Texas showed the bacterium to be effective against the weed at relatively low concentrations and following a single application (Sheik *et al.*, 1999; Sheikh *et al.*, 2001).

The beetles *Zygogramma suturalis* and *Ophraella communa* are natural enemies of *A. artemisiifolia* in Canada and were studied as inundative biological control agents (Teshler *et al.*, 2002). The reduction or cessation of *Z. suturalis* oviposition on extensively damaged plants (as observed in the former USSR; see below) and pupation in soil are, however, an important limitation for the mass-rearing of this species (Teshler *et al.*, 2002). *Ophraella communa* is considered more promising because it is easy to mass-rear and handle (Teshler *et al.*, 2002). Under favourable conditions the beetles can completely defoliate their host plants (Welch ,1978), but generally, population densities and impact of *O. communa* in North America are low, presumably because of strong attack by predators and parasitoids by the end of summer (Teshler *et al.*, 2002). If used in inundative biological control, it was therefore suggested that releases of beetles should occur early in the growing season (Teshler *et al.*, 1996).

Classical biological control of *Ambrosia* species worldwide

There is a long history of classical biological control attempts against exotic *Ambrosia*, mainly *A. artemisiifolia*, in different parts of the world, including eastern Europe (Russia, former Yugoslavia, Georgia, Ukraine), Australia and Asia (China and Kazakhstan), resulting in the release of numerous invertebrate biological control agents (see below). To date, no studies and therefore also no intentional introductions of fungal pathogens from the native range have been made in any of the introduced ranges of invasive *Ambrosia* species (Julien & Griffiths, 1998).

Eastern Europe

Classical biological control of exotic *Ambrosia* species started in the former Soviet Union in the 1960s, when more than 30 insect species from North America were introduced into quarantine (Goeden and Andres, 1999). Host-specificity testing of the candidate natural enemies were conducted in quarantine, involving eight varieties of sunflower (*Helianthus annuus*), 18 other *Helianthus* species, and 80 species representing 46 genera and 18 families of plants (Kovalev, 1971b). By 1990, five species of insects had been released with the aim to establish a complex of natural enemies. In 1969 the release of the noctuid moth *Tarachidia candefacta* (synonym: *Ponometia candefacta*) collected on *A. artemisiifolia* in Canada and California, was the first intentional introduction of a natural enemy for the biological control of an invasive exotic plant into Europe (Kovalev, 1971b). In 1972, a subspecies of *T. candefacta* collected on *A. psilostachya* (now *A. coronopifolia*) was also released (Julien and Griffiths, 1998; Kovalev, 1971b). The species established on both *A. artemisiifolia* and *A. psilostachya* (Kovalev, 1971b), but so far *T. candefacta* has been unsuccessful as a biological control agent. Predation of the exposed larvae (Goeden and Andres, 1999) and unsuitable climatic conditions (Poltavsky and Artokhin, 2006) have been stated as potential reason for its failure. While in the past, strong frosts might have limited population growth, Poltavsky and Artokhin (2006) observed increased numbers in their study region (Rostov-on-Don) from 2003 onwards after a series of mild winters.

In 1978, the leaf beetle *Zygogramma suturalis* from Canada and the USA was released and quickly established in the North Caucasus (Julien and Griffiths, 1998), and has since spread practically over the whole area heavily infested by *A. artemisiifolia* in Russia (Reznik *et al.*, 2007). In the same year, the species was also released in Kazakhstan, Georgia and Ukraine, but establishment is only confirmed from Kazakhstan (Julien and Griffiths, 1998). *Zygogramma suturalis* was further released in 1985 and again in 1990 in former Yugoslavia (now Croatia). Prior to its release in 1985, host specificity tests under no-choice condition were conducted on 128 plant species/varieties and no feeding was reported on any other plant than *A. artemisiifolia* (Igrc, 1987). The species has established in Croatia, but so far densities of beetles in the field are low (Igrc *et al.*, 1995). In Russia, one complete and a partial second generation are produced and both larvae and adults feed on leaves and flowers of *A. artemisiifolia* from April to mid September (Reznik, pers. comm.). At first, the results obtained with this beetle in Russia were very promising (Reznik, 1991). *Zygogramma suturalis* reached densities as high as 5,000 individuals per m^2 in one locality in southern Russia and completely destroyed all of the *A. artemisiifolia* as the beetle population moved across an infested field at a rate of 3 m per day (Goeden and Andres, 1999). Reduction of the weed increased crop yield by two- to threefold (Goeden and Andres, 1999). Further investigations have however shown that *Z. suturalis* is not able to control the weed sufficiently, in particular on arable land (Reznik, 1996). Serious damage of *A. artemisiifolia* plants over large areas provoke oviposition inhibition and can result in summer diapause in female *Z. suturalis* (Reznik, 1991). Population outbreaks and complete destruction of host plant populations as reported by Kovalev (1989) can only occur during the short period in spring when young adults emerge and lay eggs, since females of the first generation show little or no reaction to the degree of damage of their host plant (Reznik, 1991). Data from field surveys conducted between 2005 and 2006 indicate that average population densities in Russia are very low and, consequently, the impact on the target weed is negligible (Reznik *et al.*, 2007). Damage to ragweed was recorded mainly in undisturbed patches, where both *A. artemisiifolia* and beetle densities were higher (Reznik *et al.*, 2007).

Further releases of North American insects into the former Soviet Union included the seed feeding fly *Euaresta bella* from Canada and the USA in 1969 and again in 1990, the pollen-feeding beetle *Trigonorhinus tomentosus* from the USA in 1977 and the leaf feeding beetle *Zygogramma disrupta* from USA in 1978, but all three species failed to establish (Julien and Griffiths, 1998).

The eriophyid mite *Eriophyes boycei* collected on *A. psilostachya* was also considered as a potential agent of *A. artemisiifolia* and was shipped to the former Soviet Union but did not survive the transport (Goeden *et al.*, 1974). Eriophyid mites have repeatedly been used in classical biological control programmes, and have contributed to the successful management of alien invasive weeds (Briese and Cullen, 2001). However, they tend to be highly host specific (Skoracka, 2006), raising doubt on whether *A. artemisiifolia* indeed belongs to the fundamental host-range of *E. boycei*.

Australia

Between 1980 and 1984, three biological control agents from Mexico were introduced into Australia for the biological control of *Parthenium hysterophorus* L., which is closely related to *A. artemisiifolia*, i.e. the leaf feeding chrysomelid beetle *Zygogramma bicolorata*, the sap sucking bug *Stobaera concinna* and the tip-galling moth *Epiblema strenuana* (McFadyen and Weggler-Beaton, 2000). All three insects also attack *A. artemisiifolia* and in particular *E. strenuana* is reported to reduce its size, abundance and pollen production. In 1990 *Z. suturalis* was introduced into Australia from the USA to increase *A. artemisiifolia* control, but the species failed to establish (Julien and Griffiths, 1998). Further, an undescribed *Liothrips* species collected on *A. elatior* (now an accepted synonym for *A. artemisiifolia*) in northern Argentina, was tested in quarantine (McFadyen and Weggler-Beaton, 2000). However, host specificity tests revealed that the species also develops on and severely damaged young sunflower seedlings. Even though this Liothrips species was not recorded to attack sunflowers in the field in Argentina, the species was rejected for field release. The species was also considered and rejected for introduction into Canada (McFadyen and Weggler-Beaton, 2000).

The gall midge *Asphondylia ambrosiae* was shipped to Australia several times, but could not be successfully reared (Goeden and Palmer, 1995). *Asphondylia* larvae feed on symbiontic fungi that line the walls of their galls, and not on the plant material directly. The host plant is inoculated with the fungi by ovipositing females. The release of *A. ambrosiae* and other fungus-feeding cecidomyid flies for classical biological control would therefore require the simultaneous importation of these symbiotic fungi, which makes the use of these cecidomyiid flies as biological control agents rather unrealistic. An alternative approach might consist of rearing *A. ambrosiae* using fungi from European gall midges; such an approach has been successfully adopted in the rearing of the fungus-feeding galling midge *Schizomyia cryptostegiae* Gagné, which was introduced in Australia as a biological control agent against rubber vine, *Cryptostegia grandiflora* R.Br. (McFadyen, pers. comm.).

Presently, the two agents *E. strenuana* and *Z. bicolorata* are known to be widespread and exerting a degree of control in most of the affected areas in eastern Australia. There has been no formal assessment of the impact of these biocontrol agents on *A. artemisiifolia*. However, according to Palmer and McFadyen (2012) there is much less *A. artemisiifolia* in southeastern Queensland and northern New South Wales than there was in the 1980s. The plant is now relatively rare and no longer causes significant allergenic symptoms in the flowering season (Palmer and McFadyen, 2012). From an economic point of view, biological control of *A. artemisiifolia* is regarded as an outstanding success in Australia (Palmer *et al.*, 2010).

Eastern Asia

Releases of *Zygogramma suturalis* in China in 1985, both from Canada and from the former Soviet Union, resulted in establishment in some locations, but failed in others (Wan *et al.*, 1995). Additional tests on 74 plant species/varieties were conducted prior to field releases and feeding was only recorded on *A. artemisiifolia*. Interestingly, the close relative *A. trifida*, a species also invasive in Europe, was not accepted as a host by the beetle (Wan *et al.*, 1989). *Euaresta bella* was introduced into China in the late 1980s, but as in Russia, this seed-feeding fly failed to establish (Wan *et al.*, 1993).

In 1991, *Epiblema strenuana* was introduced from Australia into China where additional host specificity tests were conducted (Ma *et al.*, 2003; Wan *et al.*, 1995). In contrast to results from host specificity tests conducted in Australia (McFadyen, 1985), *E. strenuana* was able to complete its development on a local sunflower variety tested (Wan *et al.*, 1995). In subsequent choice-tests (i.e. in the presence of the target weed *A. artemisiifolia*), acceptance and suitability as host varied according to test conditions: sunflowers were attacked and adults emerged from plants that were exposed under multiple choice conditions in a greenhouse (Wan *et al.*, 1995), while sunflowers were attacked but no development was found in a field cage test (Wan and Wang, 2000). Under open field condition, no eggs were laid on sunflowers but larvae moved from *A. artemisiifolia* that had died prematurely to sunflower and completed their development (Wan and Wang, 2000). Overall, Wan and Wang (2000) considered the risk of *E. strenuana* to cause economic damage to sunflowers to be low. To further avoid potential damage to sunflower, it was recommended to release the species only south of the Yangtze river, i.e. where sunflower is not a major crop (Wan and Wang, 2000). However, *E. strenuana* has also been recorded from members of the genera *Bidens* and *Chenopodium*, indicating that its host-range includes plant species outside the tribe Ambrosiinae.

In addition to the deliberate releases of biological control agents, *Ophraella communa*, a North American leaf beetle, was accidentally introduced into Japan in the late 1990s (Yamanaka *et al.*, 2007 and references therein). The beetles can cause complete defoliation and death of *A. artemisiifolia* (Dernovici *et al.*, 2006; Palmer and Goeden, 1991). In 2001 it was also found in Jiangsu province in China (Zhang *et al.*, 2005), from where good control of *A. artemisiifolia* populations is reported (Zhou *et al.*, 2009). Originally, the species was reported only from *A. artemisiifolia*, but more recently it also has been recorded in the field from several other species within the subtribe Ambrosiinae, including several *Ambrosia* and *Xanthium* species, *Parthenium hysterophorus*, *Iva axillaris* Pursh., *Ratibida pinnata* (Vent.) Barnhart (subtribe Rudbeckiinae), as well as from *Helianthus ciliaris* DC. (subtribe Helianthinae; Dernovici *et al.*, 2006; Futuyma and McCafferty, 1990; Goeden and Ricker, 1985; McFadyen and McClay, 1981; Palmer and Goeden, 1991; Watanabe and Hirai, 2004). Host-specificity tests revealed that *O. communa* can attack and complete its life-cycle on sunflower and the species was subsequently rejected as biological control agent for Australia (Palmer and Goeden, 1991). Recent studies indicate however only a low risk that *O. communa* would cause significant damage to sunflower plants in the field. *Ophraella communa* rarely lays eggs on sunflowers under choice conditions, larval mortality on sunflower is high and newly emerged adults leave the sunflower plants in search of *Ambrosia* (Dernovici *et al.*, 2006). Only if *Ambrosia* plants are completely defoliated, 1st instar larvae move to adjacent sunflower (Dernovici *et al.*, 2006). These results are in accordance with field observations from Japan where adults only occasionally feed on sunflowers and where reproduction has only been found on *A. trifida* and *A. artemisiifolia* (Watanabe and Hirai, 2004). The distribution of *O. communa* in China is predicted to only partially overlap with sunflower cultivation (Cao *et al.*, 2007). Recently, a mass rearing programme was established with *O. communa* in China with the aim to use this agent for inundative application in severely invaded habitats (Zhou *et al.*, 2009).

Prospects for biological control of *Ambrosia artemisiifolia* in Europe

While both the inundative and the system management approach (see above) are primarily aimed at crop weeds, the classical approach has traditionally and most successfully been used against invasive plants spreading over large areas of natural and semi-natural habitats, extensively managed agro-ecosystems or aquatic ecosystems (environmental weeds; Müller-Schärer and Schaffner, 2008). As outlined above, with the possible exception of the leaf beetle *Ophraella communa*, distinct virulent strains of the rust fungus *P. xanthii* as well as the two pathogens *Phyllachora ambrosiae* and *Plasmopara halstedii*, no natural enemy recorded on *A. artemisiifolia* and other exotic *Ambrosia* species in Eurasia so far appears to be sufficiently specific and/or damaging, particularly with regard to long-term and sustainable control. The apparent lack of a regular re-occurrence of epiphytotics by *P. ambrosiae* and *P. halstedii* (Kiss, 2007) raises the question whether they could be facilitated through artificial inundative application of these two fungal pathogens. However, neither of these fungi can be cultured in vitro; thus their biology makes them presently unsuitable for mass production and application as a mycoherbicide. This thus excludes a system management approach or an inundative application of European antagonists to control *A. artemisiifolia* in Europe, and leaves either classical biological control or an inundative application of exotic organisms for managing common ragweed in Europe by biological means.

When developing a biological control approach as part of an integrated management programme against *A. artemisiifolia* in Europe, priority should be given to organisms with a narrow host range and that have the potential to either negatively impact the population growth rate of ragweed, or to quickly reduce ragweed biomass. In terms of host specificity, one of the most critical issues is the close relatedness of the target to the commercially important sunflower, *Helianthus annuus*. As sunflower varieties might differ in their susceptibility to biological control candidates (Morin *et al.*, 1993), several varieties need to be included in biosafety studies, especially those that occur in the regions where *A. artemisiifolia* is abundant and specific control agents are planned to be released. Only one plant species of the subtribe Ambrosiinae is considered native to Europe, i.e. *Ambrosia maritima*, which is furthermore restricted to the Mediterranean. Such a low number of very closely related native species increases the chance of finding "safe" biological control agents (Pemberton, 2000). Thus, the occurrence and conservation status of *A. maritima* in the different parts of Europe and its susceptibility as host will be crucial in the evaluation process of potential biological control agents. On the other hand, due to the observed high within-population variation (Genton *et al.*, 2005) of *A. artemisiifolia* found in France, biological control agents should also be not too (genotype or host strain) specific in order to account for genetic differences among populations and to control all individuals in a population.

In terms of impact, flower-, pollen- and seed-feeding organisms or those that contribute to a reduction in seed output should be considered first when applying the classical biological control approach, as pollen production is the prime factor causing the high impact on human health of ragweed (see above), and a reduction in seed output is likely to translate into reduced population densities and dispersal of annuals (Ramula *et al.*, 2008). On the other hand, natural enemies that quickly reduce the biomass are expected to be especially suited for an inundative application to reduce crop losses due to competition with ragweed (Müller-Schärer *et al.*, 2000; Harrison *et al.*, 2001). There is generally a lack of information on whether ragweed specialists are able to quickly reduce biomass of *A. artemisiifolia*, but indirect evidence may come from congeneric species that are known to seriously damage their host plants (see below). Building on the information compiled above, we propose in the following an outline to tackle biological control of *A. artemisiifolia* in Europe, involving both pathogens and insects and different biological control strategies for different habitats. Our prioritization of potential biological control candidates for *A. artemisiifolia* is based on evidence of their narrow host range, their feeding niche and control efficacy, availability and suitability to rear, and past experience. This allowed us to identify 23 potential agents, seven of which were given first priority (Table 1).

1) Redistribute insects already established as biological control agents in Europe

The moth *T. candefacta* is well established in Russia but so far considered an ineffective agent in areas with harsh winters. In recent years, however, this moth has increased its distribution range and locally also in abundance (Poltavsky and Artokhin, 2006; Stojanovic et al, 2011), suggesting that *T. candefacta* might more readily establish and be more successful in controlling its host plant in regions with less severe winters. Based on the criteria listed above, we give this species high priority for further studies (Table 1). Prior to considering *T. candefacta* or any other insect tested in Russia (see below) for further relocation or for release in Europe, additional host-specificity tests need to be conducted, in particular with plant species in the family *Asteraceae*. At the time when these insects were released in Russia, the main emphasis of host-specificity tests was placed on crop plants, assuring that the species would not attack cultivated species. Because of its relatively broad host-range, *Ophraella communa* was originally not considered as a high-priority species for the biological control of common ragweed in Europe (Gerber et al. 2011). The accidental establishment of this species in northern Italy and southern Switzerland has, however, generated a lot of interest in better understanding the potential risks and benefits of using *O. communa* for the biological control of common ragweed also in Europe. Within the frame of the COST action SMARTER ("Sustainable management of *Ambrosia artemisiifolia* in Europe"), coordinated research has been initiated to address aspects such as the potential distribution and climate-dependent population dynamics of *O.*

communa in Europe, risks of non-target effects under field conditions and impact on the population dynamics of the target weed.

2) Re-evaluate insect species tested and released in Russia that failed to establish

Three insect species, i.e. *E. bella*, *T. tomentosus* and *Z. disrupta*, were found to be sufficiently specific in host-specificity tests conducted in Russia and were released, but did not establish (Julien and Griffiths, 1998). Additional releases of these insects should be attempted, in particular to establish *T. tomentosus* and *E. bella*, as these species occupy feeding niches neither exploited by native herbivores nor by the two established biological control agents *T. candefacta* and *Z. suturalis* in Russia. Larvae of *E. bella* develop in seeds, thereby directly reducing seed output. *Trigonorhinus tomentosus* feeds as adult and larva on pollen and could directly contribute to reduce pollen load in the air. The third species, *Z. disrupta*, occupies a similar feeding niche as *Z. suturalis*. Additional efforts to establish this species could be considered in case *Z. disrupta* does not display oviposition inhibition on damaged *A. artemisiifolia* as seen for *Z. suturalis*. We rank all these three species as first priority control agents (Table 1).

3) Reconsider species that have been studied but, for different reasons, were never released

Zygogramma tortuosa, originally recorded from *Ambrosia eriocentra*, was introduced for testing in quarantine in Russia, but was rejected because adults also fed on sunflower (reviewed in Goeden and Ricker, 1979). Goeden and Ricker (1979) found however that *Z. tortuosa* did not feed and females did not oviposit on sunflower in open field tests. Furthermore, first instar larvae transferred onto sunflowers were not able to complete their development. *Zygogramma tortuosa* might therefore be reconsidered as a biological control agent, in particular if it does not show a similar oviposition inhibition on damaged *A. artemisiifolia* as *Z. suturalis*. Of the three *Zygogramma* species listed in Table 1, we consider *Z. disrupta* (see above) as the most promising biological control candidate and give *Z. tortuosa* second priority.

Besides the gall forming species *Asphondylia ambrosiae*, three additional cecidomyid flies, *Contarinia partheniicola* and *Rhopalomyia ambrosiae* and the stem mining *Neolasioptera ambrosiae*, have been proposed as potential biological control agents because they are likely to be host specific (Gagné, 1975). Similar to *Asphondylia* larvae, *Neolasioptera* larvae may also rely on symbiontic fungi, while *C. partheniicola* and *R. ambrosiae* are not considered to live in symbiosis with fungi (Skuhravá, pers. com.). However, *C. partheniicola* and *R. ambrosiae* appear to be difficult to collect; despite repeated, intensive surveys in Texas and Florida, *R. ambrosiae* could not be relocated and only small numbers of *C. partheniicola* were found (Goeden and Palmer, 1995). Nevertheless, these Dipteran species may have some potential as biological control agents against *A. artemisiifolia* in Europe (Table 1).

4) Assessment of additional phytophagous organisms recorded on *Ambrosia* species in the native range

The list of organisms recorded from *Ambrosia* species in their native range is long and several species appear to have a narrow host-range and are potentially of interest for biological control (Gerber *et al.*, 2011). However, Goeden and Palmer (1995) cautioned that the knowledge of the host range information on insects associated with *Ambrosiinae* might not prove to be reliable. Based on our prioritization criteria given above, we propose several species associated with *A. artemisiifolia* in its native range to be considered as potential biocontrol agents for *A. artemisiifolia* (Table 1) or potentially any of the other invasive *Ambrosia* species in Europe, such as *Ambrosia trifida*.

Evaluation of invertebrate organisms

The high number of species in the weevil genus *Smicronyx* and the moth genera *Schinia, Bucculatrix* and *Epiblema* recorded from *Ambrosia* species (Gerber *et al.*, 2011) may indicate that speciation has occurred on common ragweed and consequently, narrow host associations can be expected. Furthermore, species in the genera *Epiblema* and *Smicronyx* have been reported to be successful biological control agents against *Parthenium hysterophorus* (McFadyen and Weggler-Beaton, 2000), indicating their potential as biological control agents for *Ambrosia* spp. Of particular interest is the seed-feeding weevil, *Smicronyx perpusillus*, which is only reported from *A. artemisiifolia*, and to which we therefore give first priority (Table 1). Also, the recent establishment of *Epiblema strenuana* in Israel (Yacooby and Seplyarsky, 2011) offers the opportunity to conduct field studies in Israel to assess its usefulness as a biological control agent against common ragweed in Europe.

Two additional presumably monophagous species are the leaf beetle *Ophraella slobodkini* and the moth *Bucculatrix agnella*, both of which feed on leaves. Provided that the European climate is suitable for *O. slobodkini* and that this species is as damaging as its congeneric *O. communa*, it could likely contribute to the control of *A. artemisiifolia* in Europe, using either the classical or the inundative approach (as with *O. communa* in China; see above). We therefore give this species first priority. Previous experiences in biological control of *A. artemisiifolia* indicate that defoliators can be effective in controlling plant populations in the invaded range (see above). *Ophraella slobodkini* is described only from *A. artemisiifolia* from northern Florida, but could also be reared on the closely related *Iva fructescens* L. in the laboratory (Futuyma, 1991). Larval survival was however lower and development time longer than on *A. artemisiifolia*, suggesting that this species is indeed more specific than *O. communa* that was accidentally introduced to China and Japan (see above).

In addition to these three species potentially monophagous on *A. artemisiifolia*, several other insect species are reported on *A. artemisiifolia* but also from other *Ambrosia* species in their native range, including the weevil *Smicronyx tesselatus*, the two dipteran flies *Callachna gibba* and *Euaresta toba* and the two moth species *Schinia rivulosa* and *Tischeria ambrosiaeella* (Table 1). Although not strictly monophagous, these species could possibly be considered as biological control agents against *A. artemisiifolia* if the risk of non-target attack on *A. maritima*, the only native congeneric species in Europe, turns out to be minimal.

Several insect and mite species listed in Gerber *et al.* (2011), including the above-mentioned *E. boyeci*, have been recorded on other *Ambrosia* species, but not on *A. artemisiifolia* under field conditions. For example, various invertebrates associated exclusively with the invasive *A. psilostachya* and *A. trifida* under field conditions might be considered as biological control agents specifically against these invasive species. Some of these herbivores may also have potential as biological control agents against *A. artemisiifolia*, provided that this plant species belongs to their fundamental host-range.

Evaluation of fungal pathogens

The potential of pathogens to impact adversely on A. artemisiifolia and its pollen production was documented during naturally occurring epiphytotics of *Phyllachora ambrosiae* and *Plasmophora halstedii* observed in Hungary in 1999 and 2002 (Kiss *et al.*, 2003; Vajna, 2002; Vajna *et al.*, 2000).

Among the range of fungal pathogens known to attack *Ambrosia* species in their native range (Gerber *et al.*, 2011), the highly damaging rust fungus *Puccinia xanthii* is the most promising candidate for biological control of *A. artemisiifolia*. The rust completes its life cycle on one single host species and while recorded from numerous Asteraceous genera (Hennen *et al.*, 2005), individual rust populations or accessions within *P. xanthii* have shown a high degree of host specialization. For example an accession of *P. xanthii* collected on *A. trifida* in North America showed high specificity to its original host but failed to infect *A. artemisiifolia* and *X. strumarium*; this accession was therefore named *P. xanthii* forma specialis *ambrosiae-trifidae* (Batra, 1981). Similarly, accessions of the rust originating from *Xanthium* species were shown to be non-infectious to *A. artemisiifolia* (Morin *et al.*, 1993, Kiss, 2007). Accessions of *P. xanthii* from *A. artemisiifolia* collected in Texas (USA) in 1989 showed evidence of an equally high host specialization; they proved to be highly pathogenic to an *A. artemisiifolia* biotype from Australia during initial evaluations, while failing to infect *P. hysterophorus* and *Xan-*

thium species (pers. comm. H.C. Evans). The significant impact *Puccinia xanthii* can have on its hosts has been documented from China when a sudden outbreak of *P. xanthii* f. sp. *ambrosiae-trifidae* on *A. trifida* caused serious die-back of infected plants in 2003 (Lu *et al.*, 2004), as well as from Australia where a strain of *P. xanthii* successfully controls a number of highly invasive *Xanthium* species of the Noogoora burr complex (Morin *et al.*, 1996). Based on the documented host specificity of individual *P. xanthii* accessions and their damaging impact we give this rust first priority. Doubts have been cast on the potential of *P. xanthii* as a biocontrol agent for *A. artemisiifolia* based on a lack of disease incidence following unsuccessful attempts to collect the rust on this host in North America in 2002 and 2003. However, these latest surveys included neither the region in Texas where the most recent collections of this rust strain were made nor the majority of other sites where previous herbarium material had been collected (Kiss, 2007). Moreover, scarcity in the native range does not preclude a fungal pathogen from becoming a successful biocontrol agent (e.g. Trujillo, 2005).

The documented host range of *Septoria ambrosiicola* and *S. epambrosiae* as well as of *Passalora ambrosiae* (synonym *Cercospora ambrosiae*) and *Passalora trifidae* (Chupp) U. Braun & Crous (synonym *Cercospora trifidae* Chupp, 1949) is restricted to the genus *Ambrosia* (Gerber *et al.*, 2011). As stated for the invertebrate candidates, these fungal pathogens could be considered for biological control if the risk of damage to *A. maritima*, the only European native congeneric species, was assessed as minimal. Based on this uncertainty as well as a lack of data about the impact of the two *Septoria* and *Passalora* species on their *Ambrosia* hosts in the native range we give them second priority. However, *Septoria* as well as *Cercospora* species have previously been evaluated and used against a number of invasive weed species and, in the case of *Septoria passiflorae*, applied inundatively to control Banana Poka Vine, y tripartita var. y, in Hawaii (Charudattan *et al.*1985; Julien and Griffiths, 1998; Trujillo *et al.*, 2001).

5) New surveys in source regions matching specific European conditions

We expect that further explorations of the natural enemy complexes associated with *A. artemisiifolia* or closely related species will reveal new candidate species, or biotypes of known species (Gerber *et al.*, 2011), for the biological control of *A. artemisiifolia* in Europe.

Most biological control agents for *A. artemisiifolia* and *A. trifida* have so far been collected in the eastern United States and Canada, where both ragweed species occur. However, the genus *Ambrosia* covers a much larger geographical area, including different climatic zones. Targeting regions with climatic conditions comparable to those in the invaded range in Europe increases the chances that biological control agents will establish and persist. The richest source of natural enemies is probably the Sonoran desert region (i.e. in the south-western United States and northern Mexico), the centre of origin and diversification of the genus *Ambrosia* (Harris and Piper, 1970). Surveys for phytophagous or pathogenic organisms in the Sonoran Desert have so far mainly been restricted to the state of California and large areas remain unexplored (Goeden and Palmer, 1995). Natural enemies from the Sonoran desert itself might be well pre-adapted to warmer climates in Sub-Mediterranean Europe, e.g. the Rhone Valley, Northern Italy and some parts of the Balkans. These organisms are, however, unlikely to become adapted to more temperate or continental areas, except if they are collected at high elevations. The most likely regions to harbour cold adapted specialized herbivore species are the mountains of Mexico adjacent to the Sonoran desert (Harris and Piper, 1970) and/or areas at higher elevation in the northern part of Mexico (Bohar and Vajna, 1996). Due to their eco-geographical separation from the southern parts of the United States because of the Sonoran desert, different organisms are likely to have evolved in these mountain ranges.

Early on in the history of biological control of *Ambrosia* species, mountain regions of South America were also highlighted as a potential source for climatically adapted phytophagous species for Canada and Europe (Harris and Piper, 1970). These regions are likely to have different natural enemy complexes because they are isolated from the Mexican mountain range by a tropical region. The presence of several *Ambrosia* species in mountain regions of South America originates from a phylogenetically early invasion, indicating that the genus might have been present there long enough to acquire specialist phytophages originating from the local fauna (Harris and Piper, 1970). Despite these recommendations by Harris and Piper (1970), few surveys have been conducted and only little information is available on species associated with *Ambrosia* in South America. In 1975-76, McFadyen (1976) conducted limited surveys on insects associated with *A. tenuifolia* (later attributed to *A. eliator*, an accepted synonym of *A. artemisiifolia*) in northern Argentina and reported several potentially specific insect species from this area. Besides the *Liothrips* species mentioned above, two stem mining beetles (*Curculionidae* and *Cerambycidae*) were sent to a quarantine facility in Canada, but the species entered diapause from which they failed to emerge and no host-specificity tests could be conducted (Maw, 1981). The weevil *Conotrachelus albocinereus* Fiedler (*Coleoptera*, *Curculionidae*) which was collected from *A. elatior* in Argentina, was released in Australia as a biological control agent of *Parthenium hysterophorus* and has proven to be highly damaging to his weed (R. McFadyen, pers. comm.). Recent collections in warm temperate, mountainous areas of southern Brazil have revealed new pathogen records on *A. artemisiifolia* (H.C. Evans, pers. comm.), confirming the recommendations made by Harris and Piper (1970).

Outlook

Herbicides and mechanical control (uprooting, cutting, ploughing) are well suited as local and short-term measures to eradicate initial and small populations and to reduce yield losses in crops. However, these control methods largely remain limited to well-managed habitat types with the main focus to protect crop yield. Yet, a large part of land infested by common ragweed in Europe is non-crop land such as riverbeds, roadsides and field borders, on which eradication of ragweed using herbicides is too expensive and/or prohibited. Additionally, the need to protect the accompanying vegetation, especially in sensitive ecosystems, does not allow large-scale application of herbicides. We therefore propose that sustainable control strategies to mitigate *Ambrosia's* further spread into areas not yet invaded and to reduce its abundance in badly infested areas in Europe need to be based on a wider combination of methods, including biological control.

With regard to biological control interventions, we see a two-forked strategy. Firstly, a classical approach for the widespread and highly infested non-crop areas such as grassland, wasteland, roadsides and riverbanks using mainly agents that reduce flowering, pollen production and seed set. A number of herbivores and pathogens associated with *A. artemisiifolia* in its native range are likely to have a very narrow host-range that is either restricted to the target species itself or to a few species within the genus *Ambrosia*. Gerber *et al.* (2011) have identified 18 insect and 5 fungal pathogens to be promising candidates for a classical biological control approach (Table 1), and the recent establishment of *O. communa* in southern Europe warrants detailed investigations also of this species, although it was originally not prioritized for classical biological control of common ragweed in Europe. Secondly, an inundative approach will be necessary for crop fields that suffer from ragweed infestations. Candidate biological control agents for mass-rearing and repeated releases against ragweed in Europe are, similar to *O. communa* in China (Zhou *et al.*, 2009), the defoliator *Ophraella slobodkini* or the fungus *S. epambrosiae* (Table 1).

Based on its history of at least partially successful biological control attempts against exotic *Ambrosia*, we argue that biological control as part of an integrated management approach (Müller-Schärer *et al.*, 2000; Müller-Schärer, 2002) will likely be needed to produce acceptable levels of overall ragweed control across different habitats in Europe. To promote such a European-wide integrated management of common ragweed, a COST action named 'SMARTER' was recently launched. The objectives of the COST Action are to: (a) make available a forum for discussing innovative long-term options for managing and monitoring ragweed; (b) train, educate and motivate skilled young scientists to work on invasive species management to meet increased demands of the society for experts on this issue; (c) to identify knowledge gaps hindering the sustainable integrated management of ragweed and promote new research to fill these gaps, (d) to develop site- and country-specific recommendations for ragweed management and promote their implementation, and (e) develop a common vision for interdisciplinary collaboration in research and monitoring of IAS, especially ragweed. Numerous scientists collaborating in the HALT Ambrosia project are also actively involved in this new action, and the findings generated in the HALT Ambrosia project will be of key relevance when developing habitat-specific recommendations for ragweed management in Europe.

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Table 1 Host-range, prioritization and management approach suggested for biological control candidates against Ambrosia artemisiifolia in Europe (see text for details; adapted from Gerber et al. 2011).

Taxon	Host range[a]: Field observations	Host range[a]: Experimental studies	Biosafety / Feasibility	Priority for Europe	Management Approach
Insecta					
Coleoptera					
Ophraella communa LeSage	*Ambrosia, Iva, Parthenium, Xanthium, Ratibida*	Heliantheae	Attack of sunflower/ Jerusalem artichoke?	2	Classical / inundative?
Ophraella slobodkini Futuyma	AMBEL	AMBEL, Ivaf		1	Classical / Inundative?
Smicronyx perpusillus Casey	AMBEL	?		1	Classical
Smicronyx tesselatus Dietz	AMBEL, *Ambrosia*	?	attack of *A. maritima?*	2	Classical
Trigonorhinus tomentosus (Say)[b]	*Acha*, FRSCO, *Ache*, AMBDU, AMBER	AMBEL[d]	attack of *A. maritima?* establishment?	1	Classical
Zygogramma bicolorata Pallister[c]	AMBEL, *Parthenium*	?	attack of *A. maritima?*	2	Classical
Zygogramma disrupta Rogers[b]	AMBEL	AMBEL[d]	establishment?	1	Classical
Zygogramma tortuosa Rogers [b]	AMBER	*Ambrosia*	attack of *A. maritima?*	2	Classical
Diptera					
Callachna gibba (Loew)	AMBEL, AMBPS	?	attack of *A. maritima?*	2	Classical
Contarinia partheniicola (Cockerell)	*Acha*, FRSCO, AMBDU, AMBER, AMBPS, Pinc	?	rare in native range?	2	Classical
Euaresta bella (Loew)[b]	AMBEL	AMBEL[d]	establishment?	1	Classical
Euaresta toba (Lindner)	AMBEL, AMBCU, AMBTE	?	attack of *A. maritima?*	2	Classical
Rhopalomyia ambrosiae Gagne	AMBEL, AMBPS	?	rare in native range?	2	Classical
Hemiptera					
Stobaera concinna Stal[c]	AMBEL, *Parthenium*	?	attack of *A. maritima?*	2	Classical
Lepidoptera					
Adania ambrosiae Murtfeldt	FRSAC, AMBEL, *Acha*, AMBER, AMBPS	?	attack of *A. maritima?*	2	Classical
Bucculatrix agnella Chambers	AMBEL	?	attack of *A. maritima?*	2	Classical
Schinia rivulosa Guenée	AMBEL, AMBPS, *Ambrosia*	?	attack of *A. maritima?*	2	Classical
Tarachidia candefacta[b] (*Ponometia candefacta* Hübner)	AMBEL, FRSCO, AMBPS	AMBEL[e]	attack of *A. maritima?*	1	Classical
Tischeria ambrosiaeella Chambers	AMBEL, AMBTE	?	attack of *A. maritima?*	2	Classical

Fungi				
Ascomycota				
Dothideomycetes				
Capnodiales				
Mycosphaerellaceae				
Septoria ambrosiicola Speg. 1910	*Ambrosia*	attack of *A. maritima?*	2	Classical / Inundative?
S. epambrosiae D.F. Farr 2001	*Ambrosia*	attack of *A. maritima?*	2	Classical / Inundative?
Passalora ambrosiae (Chupp) Crous & U. Braun 2001	*Ambrosia*	attack of *A. maritima?*	2	Classical
Passalora trifidae (Chupp) U. Braun & Crous 2003	*Ambrosia*	attack of *A. maritima?*	2	Classical
Basidiomycota				
Pucciniomycetes				
Pucciniales				
Pucciniaceae				
Puccinia xanthii Schwein. 1822			1	Classical

[a] Plant species: **EPPO (Bayer) codes used when available (see** http://eppt.eppo.org/index.php**);** FRSAC: *A. acanthicarpa*; AMBEL: *A. artemisiifolia*; Acha: *A. chamissonis*; FRSCO: *A. confertiflora*; Ache: *A. chenopodiifolia*; AMBCU: *A. cumanensis*; AMBDU: *A. dumosa*; AMBDE: *A. deltoideae*; AMBER: *A. eriocentra*; AMBPS: *A. psilostachya* (now *A. coronopifolia*)*;* AMBTE: *A. tenuifolia;* Ivaf: *Iva frutescens*; Pinc: *Parthenium incanum.*

[b] tested as classical biological control agent against *A. artemisiifolia*.

[c] released as classical biological control agent against *P. hysterophorus*.

[d] according to tests conducted in Russia but no access to data.

[e] according to tests conducted in Russia (Kovalev 1971b)

Efficacy report and guidance on options for thermal control of *Ambrosia artemisiifolia*

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Introduction

Thermal weed control is an alternative treatment where neither chemical nor mechanical control is allowed or possible. Research activities are needed to develop innovative control systems especially for non-cropping areas because herbicide uses are very restricted within the EU. Since *Ambrosia artemisiifolia* is also spreading in organically grown fields there is a strong demand to provide alternatives for organic farmers. The principle of thermal control is that temperatures above 60°C in the plant cells lead to nucleic acid denaturalization. This impact causes an irreversible damage of the plant tissue and leads to necrosis. Machinery for thermal weed control is working with flames, infrared or heated air and heated water (steam or boiling water) and hot foam, which is applied on the plants.

Materials and Methods

Based on pre-trails in 2011, two experiments on thermal control of *A. artemisiifolia* plants were conducted from June 2012- October 2012. Small plot (2x3 m) field experiments at the experimental site at JKI with transplanted *A. artemisiifolia* in gravel and grassland (10 plants per treatment, each plant was a replication). Furthermore, a large scale field experiments (0.80-1.50 x 50 m, 4 replications) on a rural roadside banquette in Brandenburg with a natural *A. artemisiifolia* infestation were carried out.

The following treatments were conducted in comparison with untreated plots:

- Thermal: Flaming 600°C (Green-Flame 850 E, Green-Flame, Vordingborg, Denmark)
- Thermal (in gravel and grassland only): Hot Air 370°C (Combi Compact, Adler Arbeitsmaschinen, Nordwalde, Germany)
- Thermal (at the roadside banquette only): Hot Water 99°C (Wave High Series hand unit, Wave Europe, Wekerom, Netherlands)
- Mechanical: mowing (with a brushcutter in gravel and grassland and with a self-driving mower by road maintenance staff at the roadside banquette)
- Chemical: Herbicide application with a hand unit (6L Banvel M /ha: 30 g Dicamba /L and 340 g MCPA /L)

The transplanted *A. artemisiifolia* plants in grassland and gravel were treated at BBCH 14-16 and 18-24 at the end of July (Table 1).

5 weeks after the treatments took place, half of the plots were mown. The roadside banquette trial was conducted at BBCH 50-65 of *A. artemisiifolia*, also at the end of July.

4 weeks after the last treatment dry matter of the remaining *A. artemisiifolia* plants in gravel and grassland and on a 0.25m^2 area at the roadside banquette were determined.

The statistical analysis was carried out with STATGRAPHICS Plus 5.1.

Table 1: experimental lay out

Habitat:	grassland and gravel	roadside banquette
BBCH stage at treatment:	14-16 and 18-24	50-65
1. treatment:	Flaming, Mowing, Herbicide Hot air	Flaming, Mowing, Herbicide Hot water
2. treatment:	Half of the plots were mown 5 weeks after 1. treatment	-
Harvest of Ambrosia DM:	4 weeks after 2. treatment	4 weeks after 1. treatment

Results

The results of the gravel and grassland experiment showed that *A. artemisiifolia* dry matter in grassland was significantly reduced by thermal control at BBCH 18-24 (Figure 1). In gravel thermal control by hot air at BBCH 18-24 led to significant lower *A. artemisiifolia* dry matter than the control, flaming however, seemed to stimulate plant growth. Flaming and hot air at BBCH 14-16 reduced significantly dry matter in grassland respectively in gravel.

Plots that had a second treatment by mowing 5 weeks after the first treatments showed very low *A. artemisiifolia* dry matter of less than 0.5 g per plant in average in all treatments This successful second treatment was independent of the kind of the first treatment (data not shown).

The herbicide treatment resulted in a complete eradication of the *A. artemisiifolia* plants in grassland and gravel, both in the plots with the first treatment only and with the second treatment, too.

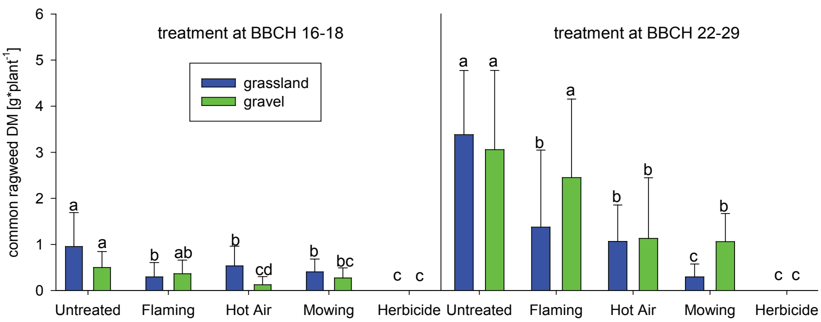

Fig. 1: *A. artemisiifolia* DM [g*plant-1] in grassland and gravel 9 weeks after treatment, columns of the same colour with different letters differ significantly at P<0,05, bars indicate standard deviation

The results of the roadside banquette trial showed that the thermal control treatments flaming and hot water led to significant lower Ambrosia dry matter than the control (Figure 2). The hot water treatment had the lowest DM which differed significantly from flaming. The following order of the treatments point out the best eradication: Hot Water > Mowing > Herbicide > Flaming.

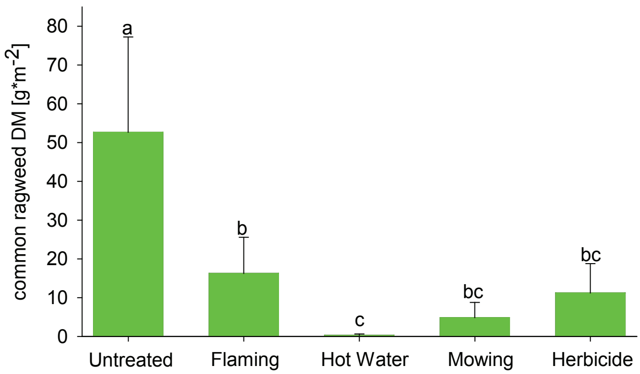

Fig. 2: *A. artemisiifolia* DM [g* m^{-2}] at roadside banquette 4 weeks after treatment, columns with different letters differ significantly at $P<0.05$, bars indicate standard deviation

Discussion and Conclusions

The results of these experiments demonstrated the efficiency of thermal control methods based on hot air and hot water. Recent investigations in Germany and other European countries could also identify hot water systems as a promising tool (Dittrich *et al.*, 2012; Rask *et al*., 2007). They concluded that at least 2 applications are necessary for a successful weed control. In general the hot water control is applied up to 4 times during the vegetation period but in our studies was carried out one time only with very promising results. However, there are still gaps of knowledge in terms of the dose-response relation for Ambrosia (e.g. propane consumption in kg/ha) and also correct timing of the application is often difficult (Ascard, 1995). Investigation of the earlier Euphresco project on Ambrosia clearly pointed out the low competitiveness of Ambrosia (Holst *et al*., 2010). Therefore any direct control method should be as selective as possible to inhibit growth of Ambrosia by the competition of the surrounding vegetation. Despite its high regrowth capacity , there are no indications that Ambrosia is less susceptible against heat treatments like most of other weed species. Additional information is still required to develop a more specific guidance which enables the practical implementation. Focusing on eradication of Ambrosia we should know more about heat effects on seed viability in non-cropping areas. A critical point of thermal control methods is the high energy input corresponding with high costs. This will require an economic evaluation specified for different uses and scenarios.

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Guidance for the Management of contaminated soil

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Introduction

In order to prevent introduction and spread of common ragweed, the knowledge of entry and spread pathways is essential. Seeds of the species are dispersed via a number of different mechanisms, several of which are aided by human activities. The introduction of ragweed from foreign countries and the spreading of already existing populations in a region may be realized with different mechanisms (Fig 1, Fig 2). A lot of information on these mechanisms is found in the literature (e.g. Kazinczi *et al.*, 2008, Alberternst *et al.*, 2006), but there is still a gap in knowledge, e.g. regarding spread of ragweed seeds with excavated material.

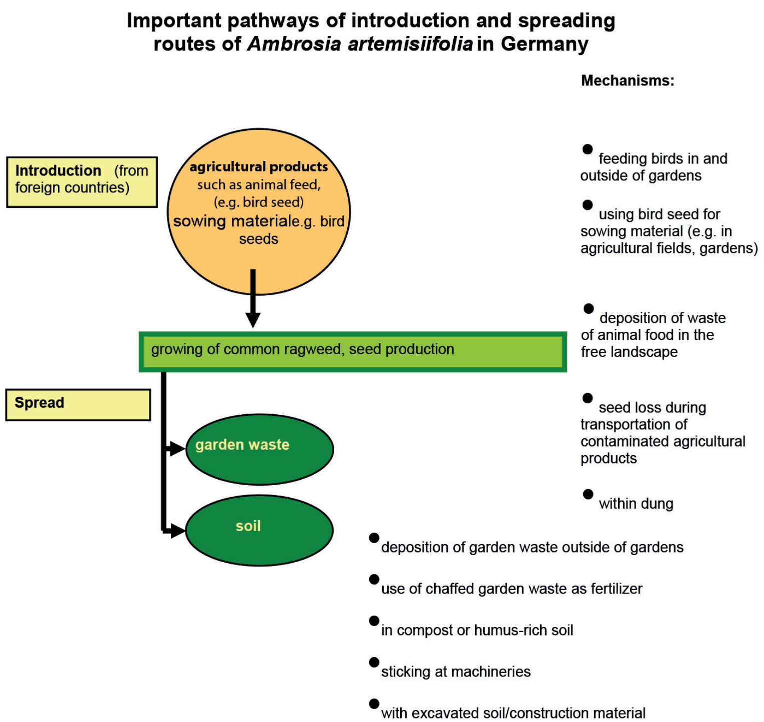


Fig 1: Pathways of introduction and spreading routes of *A. artemisiifolia* in Germany.

While the introduction with bird seed played a major role in the spreading process over the last years we currently observe a shift to an increased spread of *A. artemisiifolia* seeds within soil in Germany.

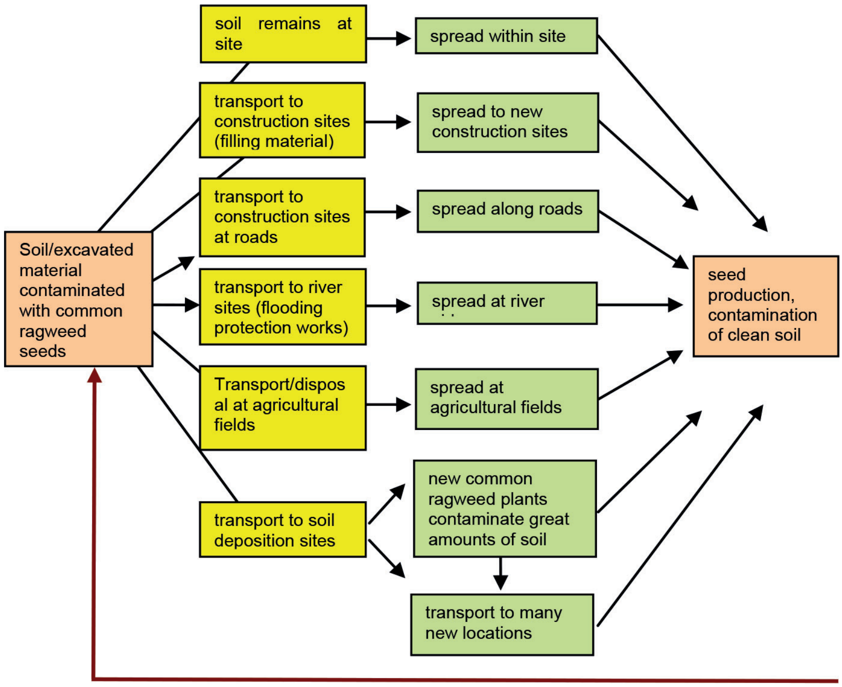


Fig. 2: Possibilities for *A. artemisiifolia* seeds to spread within excavated material.

To install effective and adequate control measures it is necessary to know the relevance of the different routes that the species uses, to enter new growing sites. Spread of *A. artemisiifolia* seeds within excavated material is an important pathway for the species to reach new locations (Bohren 2005, 2007), and thus this is investigated in this study. The aims of this part are, to learn more about:

1. the role of construction activities in the spreading process of *A. artemisiifolia* in Europe with special regard to the situation in Germany
2. measures to prevent seed dispersal with excavated material
3. methods to decontaminate soil
4. prescriptions to prevent the spread of ragweed in soil already in force in European countries.

Methods

A literature survey was conducted which showed there is little published information on the question to what extent *A. artemisiifolia* is spread with soil. So we tried to find out more using a questionnaire sent to experts in different countries. The situation in Germany is illustrated with some exemplary field studies.

Inquiry via questionnaire

In November 2012 a questionnaire (see appendix) with three questions dealing with the topic "relevance of soil and construction material for the spread of common ragweed" was sent to 103 experts currently working on the topic "Ambrosia" in 37 countries (Australia, Austria, Belgium, Bosnia-Herzegovina, Bulgaria, Canada, China, Croatia, Czech Republic, Denmark, Finland, France, Georgia, Germany, Greece, Hungary, Iran, Israel, Italy, Lithuania, Luxembourg, Macedonia, Netherlands, Norway, Poland, Romania, Russia, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom, USA). We used nearly the same email-list as for the inquiry about impacts of *A. artemisiifolia* on biodiversity. The questionnaire asked for information on the following questions:

- How important are the following spreading pathways for common ragweed in your country: building sector (construction material), building sector (seed loss during transportation), agriculture (seeds sticking at machineries), agriculture (seed loss with agricultural products), sowing material, traffic, bird seeds, others.
- Is the building sector informed about the *A. artemisiifolia*-problem? (e. g. occurrence of ragweed plants on soil depots or construction material, seed loss during soil transpor¬tations etc.)
- Are there legal or other regulations to avoid dispersal of *A. artemisiifolia* seeds with soil or construction material in your country?

We thankfully received 13 answers to the questionnaire from Maira Bonini (Italy), Bruno Chauvel (France), Bernard Clot (Switzerland), Chantal Déchamp (France), Alain Demierre (Switzerland), Peter Kotanen (Canada), Beryl Laitung (France), Arnaud Monty & Grégory Mahy (Belgium), Sergey Reznik (Russia), Baruch Rubin (Israel), Ingrida Sauliene (Lithuania), Carsten Ambelas Skjoth (Denmark), Wil Tamis (Netherlands) and added our own estimation for Germany (Beate Alberternst & Stefan Nawrath). The information given by the experts in the questionnaire is described below. The low number of replies indicates that the knowledge on this topic is currently low.

Field studies done in Germany

Field studies were conducted in Brandenburg (Niederlausitz, East-Germany) in an area where the most extended ragweed occurrence of Germany is present. Soil depots and roadsides were investigated here.

To compare the heavily infested area in the Niederlausitz with a region with relatively low ragweed occurrence, results from studies conducted in Bavaria for the Bavarian State Ministry of the Environment, Public Health and Consumer Protection (Nawrath & Alberternst 2008, 2009, 2011, 2012) since 2007 are considered in the following. In these studies pathways of introduction, spreading routes, the development of ragweed populations and the success of control measures were investigated. It was determined that ragweed already occurs at soil depots in Bavaria and that it is distributed via excavated material.

Investigations in the Niederlausitz

Soil depots

Between 20th and 24th September 2012 eleven soil depots were surveyed for ragweed occurrence (Tab 1). The depots consisted of soil that was excavated and stored in order to use it at a later date ("transient soil depots"). The area of the sites was inspected for *A. artemisiifolia* on the soil dumps and at the ruderal sites. When the soil depots were not openly accessible, owners were asked for permission. If the area was not accessible, we walked around the site and looked for *A. artemisiifolia* from outside.

Road sites

In the study area south-west of Cottbus, various roads were inspected from a car for *A. artemisiifolia* at the roadsides, with particular focus on newly built hard shoulders and roads under construction. The roads which were investigated are marked in yellow in the map in Fig 13. During the drive GPS values were taken with a navigation tool (Garmin GPS map 62s). The routes travelled were automatically registered by the navigation tool and are demonstrated in (Fig 13). Additionally, observations during the field work done for the biodiversity study in July 2011 were included.

Results

Inquiry

Question 1: Of what relevance are the following spreading routes for *Ambrosia artemisiifolia* in your country?

Nine experts from France, Italy, Switzerland, Russia, Canada, the Netherlands, and Germany answered on question 1 that the dispersal of ragweed seeds with construction material such as sand, gravel, construction waste is of high importance for the spread of the species in their country (Fig 1). This is also relevant in Israel but a score was not possible here. In Denmark where common ragweed is still rare, this pathway of spread is currently of low importance. Six experts note that a transport of common ragweed seeds sticking to agricultural machineries is a major spreading pathway in their countries (Switzerland, Israel, Russia, France, Italy). Bird seed is mentioned to be an important pathway of introduction in the Netherlands, Belgium, Denmark, and also in Germany. In Switzerland this was a crucial pathway in former times, but due to legal regulations that prohibit animal food to contain common ragweed seeds (<0,005% common ragweed seeds/kg) it is not relevant any more. In Canada, Russia, and Italy a loss of common ragweed seeds during the transportation of agricultural products is important for the spread of *A. artemisiifolia*, and this is mentioned as the most important spreading pathway in Russia (S. Reznik). In Russia and Italy the introduction of common ragweed seeds with contaminated sowing material is important for the dispersal of the species.

The answers of the experts compiled in Fig. 3 demonstrate that different pathways are relevant in the spreading process of *A. artemisiifolia*. The spread of common ragweed seeds with construction material is relevant in many countries.

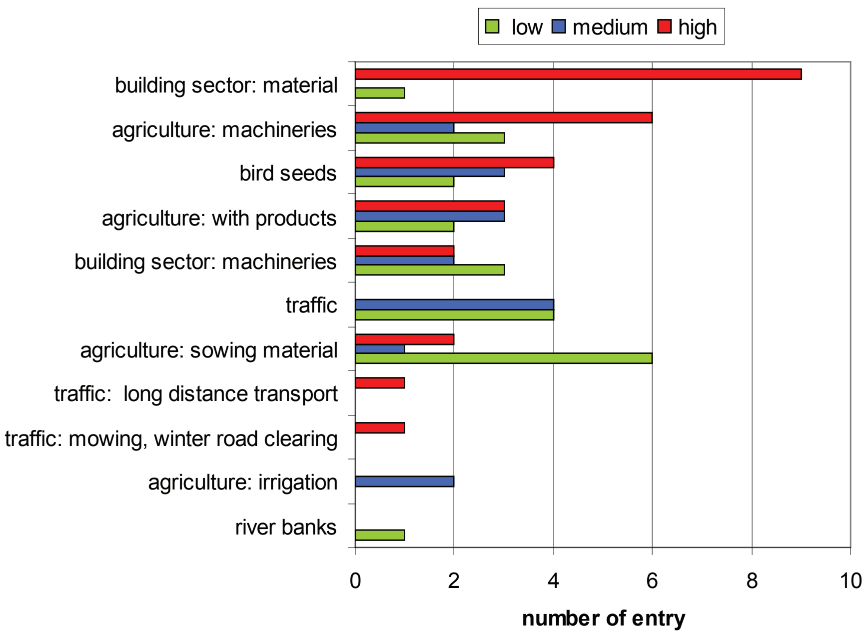


Fig. 3: Relevance of spreading routes for *A. artemisiifolia* (14 questionnaires, including 3 x France, 2 x Switzerland).

Question 2: Is the building sector informed about the *A. artemisiifolia*-problem?

Six experts mention that the building sector is not informed about the *A. artemisiifolia*-problem in their country. Two have no information about this, but doubt that the sector is informed. Six experts say that the building sector has knowledge on the problem but does not conduct special control measures against the species. In France and Italy only in a few cases special control measures against *A. artemisiifolia* are undertaken. In Italy for example operators sometimes sow out antagonistic grasses (M. Bonini).

Question 3: Are there legal or other regulations to avoid dispersal of *A. artemisiifolia* seeds within soil or construction material in your country?

Referring to the answers of eight experts, there are no or no special regulations regarding this question in Denmark, Belgium, The Netherlands, Germany, France (2x), Italy and Israel. Four persons had no information on this topic. In France regulations are set in force by local authorities in infested areas but no national regulation exist (B. Laitung). In Switzerland the use of soil contaminated with *A. artemisiifolia* seeds is prohibited (A. Demierre). In the Lombardy region in Italy *A. artemisiifolia* must be controlled by mowing between June and 20th August in general, but there are no special regulations to prevent the spread of *A. artemisiifolia* with construction material (O.P.G.R. 29th March 1999, M. Bonini).

Unfortunately, we did not receive information from experts from heavily infested south-east European countries.

The role of construction activities in the spreading process of *A. artemisiifolia*

A. artemisiifolia achenes can be transported within excavated material over long distances and can reach new growing sites and areas far away from the initial seed source. From own investigations in Germany we learned that soil excavated at a construction site is often not directly used for construction work at other places but deposited at special sites and used later (Fig. 2). Soil depositions often provide suitable growing conditions for pioneer species such as *A. artemisiifolia*, like disturbed, sunny vegetation-free sites where these species can grow and produce seeds. A great amount of soil can be infested with common ragweed seeds at the deposition sites when contaminated soil is mixed with common ragweed-free material. Contaminated material can be dispersed widely during construction works, and by this way common ragweed can be introduced to many new locations (Fig 4 c, d, Fig 2).

In some cases soil from construction sites is disposed at agricultural fields (Fig 4 a, b). If the soil is contaminated with ragweed seeds the species can be introduced there.

Fig 4: Pathways of spreading for ragweed with excavated material.
a) Soil depot with ragweed occurrence, Strullendorf, Bavaria, 29th Oct.2009
b) soil disposal at an onion field, Griesheim, South Germany, 29th July 2007
c) construction site with ragweed occurrence, Griesheim, 27th June 2006
d) ragweed occurrence at the sides of a newly built road in a reconstructed mining area near Senftenberg, East Germany (10th July 2011).

Situation in Europe

As we know from other invasive plant species such as *Fallopia japonica*, the spread via soil is very effective and can result in a wide distribution of a species. According to Bassett & Crompton (1975), the achenes of *A. artemisiifolia* are mostly dispersed by human activities with soil or seed transportation. Bohren *et al*. (2005) describe the transport of humus to construction sites and to gravel pits as an important spreading route in Switzerland. Spread with excavated material is also relevant in Switzerland. Transportation of soil and gravel between neighbouring countries is a common practice in parts of Europe, particularly between Switzerland, France and Italy, where construction materials and substrates near borders are exchanged across borders, which may lead to the establishment of ragweed on new sites (Bohren 2007, Buttenschøn *et al*. 2010). Bohren (2007) describes that machines for soil treatment are routinely exchanged between French regions of Lyon and the Swiss Basin Lemanique. Also, Essl *et al*. (2009) mention the transportation of soil as an important pathway for the spread of *A. artemisiifolia* in Austria.

Relevance of common ragweed spread within excavated material in relation to the scale of infestation with *A. artemisiifolia* – an example from Germany

The relevance of spreading routes of *A. artemisiifolia* often depends on the scale of the infestation in a country or a region. Our investigations conducted in Bavaria (where ragweed is not very common) had shown that the dispersal of *A. artemisiifolia* seeds with excavated material was of lower importance compared to introductions with bird seed (Fig 5, unpublished data; Nawrath & Alberternst 2007 to 2012). 29% of the large common ragweed stands (> 100 common ragweed plants) currently

(2012) known in Bavaria were introduced with bird seed, whereas 18% were introduced with soil/excavated material. For 109 stands at roadsides no pathway of introduction was detectable, but it is unlikely that the species came here with excavated material. However, during the last years the transfer of ragweed seed with soil has increasingly been observed in Bavaria. For regions where ragweed is common and already occurs in the system of soil transport and use, we expect that the spread with soil will become increasingly important. Common ragweed is not evenly distributed in Germany. Whereas in Bavaria the species is still relatively rare, it is common in the "Niederlausitz", an area south-east of Berlin, near the Polish border (Fig 6). In this region extensive ragweed populations occur, e.g. on agricultural fields, at ruderal sites, and also along roadsides (Brandes & Nitsche 2006, Jentsch 2007, Nitsche 2010, Lemke oral presentation 06/23/2010). To learn more about the spread of *A. artemisiifolia* with excavated material in this highly infested region, and to find out whether this spreading route is more relevant than in regions with low infestations, investigations described in the following were conducted in 2012.

Relevance of transient soil depots for the spread of *A. artemisiifolia* in Germany

Examples from the Niederlausitz (East-Germany)

Ambrosia artemisiifolia was found at 7 of the 11 (63.6%) transient soil depositions investigated in this study (Tab 1, Fig 7).

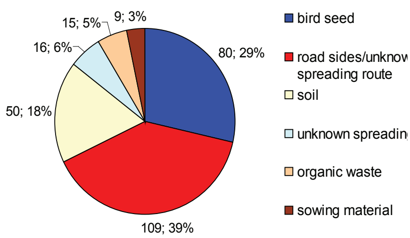


Fig 5**:** Pathways of introduction of n=279 big ragweed stands (>100 individuals) known in Bavaria till 2012.

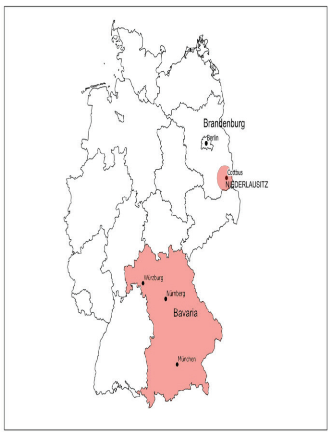
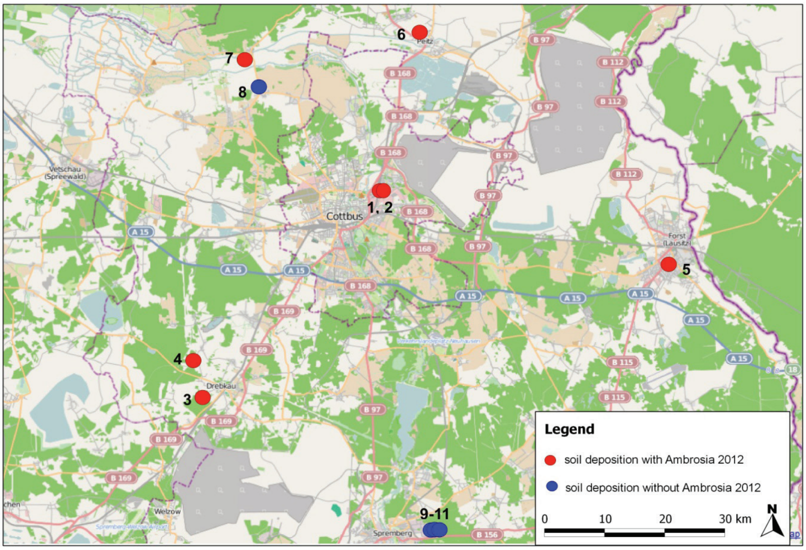


Fig 6: Map of Germany with the federal states Brandenburg and Bavaria where the investigations took place. In the red marked "Niederlausitz" the most extended common ragweed stands of Germany occur.

Tab 1**:** Transient soil depositions operated by construction companies or road maintenance services, or used during construction work investigated in September 2012.

No	**Location**	**Type**	**Size[1)]**	**Method**	**Geographic coordinates WGS84**	**Common ragweed occurrences**
1	Cottbus, Industrieal area „Am Gleis"	Depot of building material, operated by construction company	large, 24000 m^2	premises inspected	51.770099 14.378945	extensive common ragweed stand, rich in individuals, at storage place of construction material (Fig 8)
2	Cottbus, Industrieal area „Am Gleis"	Transient soil depot of building company	large, ca. 14500 m^2	premises inspected	51.771486 14.379283	extensive common ragweed stand, rich in individuals, on soil pile, (Fig 8)
3	sw of Drebkau	Transient soil depot and storage area of construction company	very large, 40000 m^2	premises inspected	51.648756 14.203109	extensive common ragweed stand, rich in individuals, on soil pile an instorage area (Fig 8)
4	w of Drebkau-Siewisch	Transient soil depot and storage area of construction company	large, 13000 m^2	premises inspected	51.677955 14.190555	extensive common ragweed stand, rich in individuals, in storage area
5	near junction Forst A 15 sw of Forst (Lausitz)	Transient soil depot of road maintenance service	small, ca. 1800 m^2	premises inspected	51.711287 14.609634	small stand, locally numerous of individuals, on soil pile
6	N of Peitz	Transient soil depot and area of construction company/concrete factory	small, 800 m^2	Partially seen from outside	51.871657 14.411245°	small stand on soil pile and in storage area
7	E of Burg (Spreewald) district Schmogrow-Fehrow, street L 501	Transient soil depot built during road construction (L 501)	small, 240 m^2	premises inspected	51.852245 14.225585	small stand
8	N of Sielow, street L 50	Transient soil depotbuilt during road construction (L 50) L 50	small, approx. 500 m^2	premises inspected	51.840701 51.840701	not found
9	E of Spremberg	Transient soil depotoperated by construction company	small, ca 1700 m^2	premises inspected	51.569115 14.413370	not found
10	E of Spremberg	Transient soil depotoperated by construction company	medium-sized, ca. 2000 m^2	premises inspected	51.571839 14.412445	not found
11	E of Spremberg	Transient soil depotand storage area operated by construction company	medium-sized, ca. 2700 m^2	premises inspected	51.578519 14.406793	not found

[1)] size= area investigated for common ragweed occurrences



map basis: OpenStreetMap

Fig 7: Location and number (compare Tab 1) of the transient soil depots investigated for ragweed occurrence in the Niederlausitz near Cottbus, East-Germany, September 2012.
At seven of the eleven sites investigated, common ragweed plants were found

At the sites 5, 6, and 7 only a small amount of *A. artemisiifolia*-plants occurred. At the sites 1 to 4 in Drebkau, Siewisch, and Cottbus hundreds of common ragweed plants were found at the transient deposition sites. The common ragweed plants mainly grew in ruderal areas and on soil piles which were not removed for at least one year (Fig 6).

The maps illustrated in Fig 9 demonstrate the distribution of ragweed at the transient soil depositions in Cottbus, Drebkau, Siewisch, and Drebkau. In Drebkau some piles of construction material with ragweed stands were present at the margins of the site (Fig 9). In Siewisch and Cottbus *A. artemisiifolia* was dispersed nearly over the whole soil deposition. In Cottbus the species was found on three different soil piles which were used by different operators. Some of these operators are also involved in construction work at roads. A pile of humus rich material which was used to fill in banquets at road margins (pers. communication with a foreman at a disposal site in 2012) was grown with *A. artemisiifolia*. This strongly indicates that common ragweed is spread with construction material from the soil depositions.

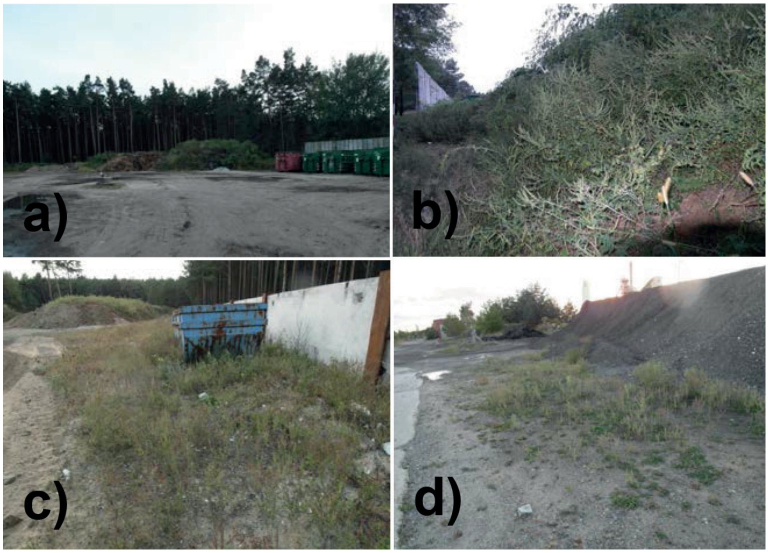


Fig 8: Common Ragweed occurrence at transient soil depot in Drebkau (a-c) and Cottbus (d), Sept 2012.

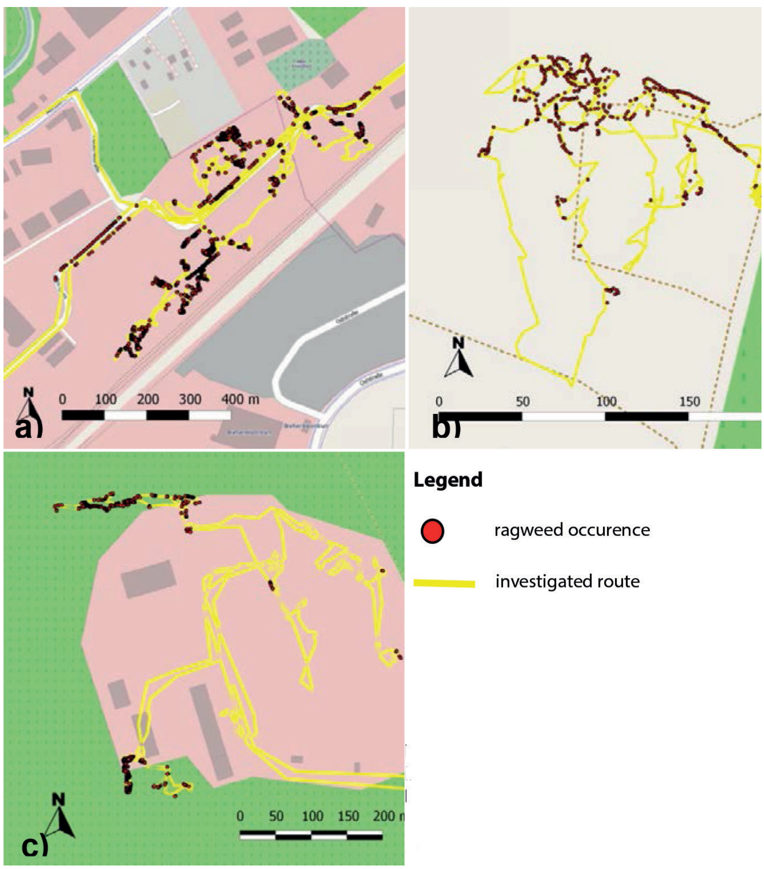


Fig 9 Occurrences of ragweed at soil depots in Cottbus (a), Drebkau-Siewisch (b) and Drebkau (c) in 2012.

Map basis: OpenStreetMap

Examples from Bavaria (South-Germany)

To compare a region with high ragweed infestations with an area with low infestations, the results from former studies in Bavaria are presented (Nawrath & Alberternst 2008, 2009, 2011, 2012). In 2009 and 2010, *A. artemisiifolia* plants occurred in 11 out of 68 (16.2%) soil depots investigated in Bavaria. The populations were small and comprised of single plants or small stands up to 40 individuals (Nawrath & Alberternst 2011). In Bavaria, where compared to the situation in Brandenburg only a small amount of ragweed occurs, the species was found in relatively small quantities at soil depots. It is not normally possible to track back the mechanism of introduction of *A. artemisiifolia* into a given site, but it may be done where the degree of infestation is low and only few pathways need to be considered. We were able to demonstrate this with the example Hilpoltstein, a village in Bavaria: In the county 25 big (> 100 individuals) ragweed stands are known. Five of these stands and one population of less than 100 plants could be traced back to a single soil depot in Hilpoltstein that supplied soil for construction works in the county (Fig 12). Sometime before that, ragweed had entered the soil depot with soil from a construction site nearby. The construction site had been used as a cut flower field with sunflowers planted with seeds from bird seed. Common ragweed was unintentionally introduced here by the farmer with the bird seed. Although it became known to the operator of the soil depot that this soil was contaminated with common ragweed seeds, the soil was still sold.

Fig 10: Excavated soil at the construction site „Am Falkenhorst" in Hilpoltstein.

In 2007 extensive ragweed occurrences grow at this construction site. Excavated material from here was transported to the transient soil depot in Hiltpoltstein.

Fig 11. Soil depot with ragweed occurrence in Hilpoltstein in Bavaria.

The transient soil depotreceived soil from a construction side nearby ("Am Falkenhorst") that was built on a former cut-flower field. Sunflowers from bird seed that was used for sowing material were cultivated here.

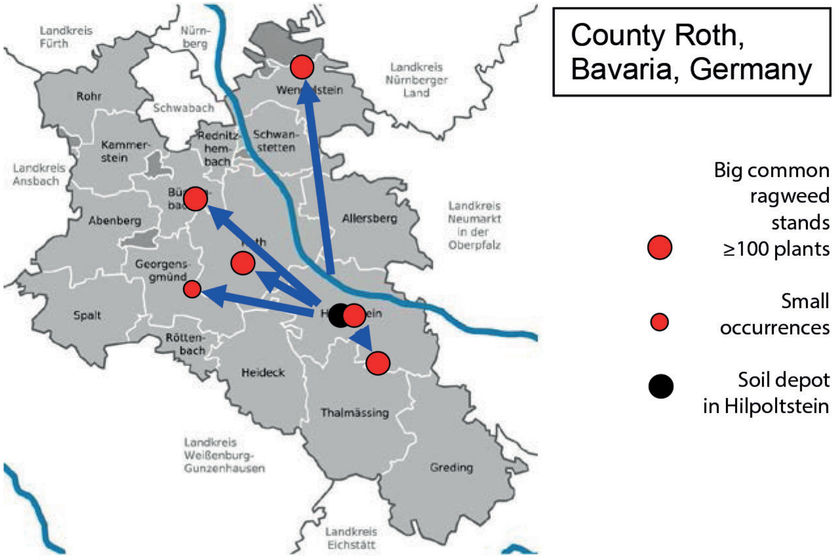


Fig 12: Transient soil depot in Hilpoltstein (black dot) that could be detected to be the origin of six new common ragweed stands (red dots) in the county Roth, Bavaria (Germany) in 2012.

Spreading of *A. artemisiifolia* promoted by road construction work - examples from the Niederlausitz

Common ragweed along roadsides in the Niederlausitz

In the study area in the Niederlausitz, common ragweed often occurs at road margins (Jentsch 2007, Nitzsche 2010, Lemke 2010). Also in 2012 extensive ragweed stands were found at roadsides (Fig 13). From the road sides *A. artemisiifolia* is able to spread into other habitats such as agricultural fields or ruderal areas.

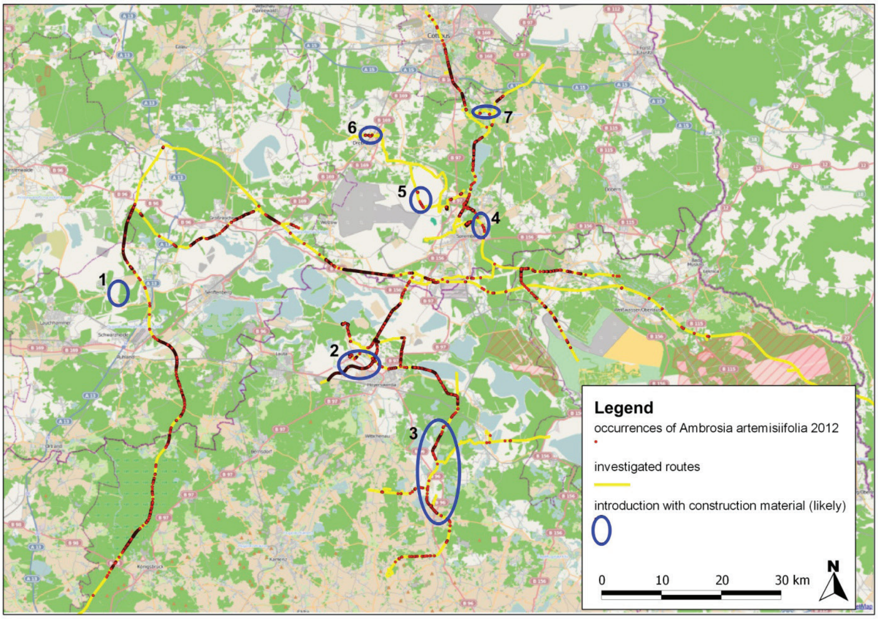
A map, provided in the internet by the Free University Berlin (FU Berlin 2013), shows the distribution of common ragweed in Berlin and Brandenburg. It can be seen that the most extended ragweed stands in the Niederlausitz occur around the town Drebkau south-west of the city Cottbus. Here many agricultural fields are heavily infested with *A. artemisiifolia*. According to the FU maps, ragweed is less common in the surrounding areas. The species is rarely found in agricultural fields and occurs predominantly at road margins. The distribution of common ragweed in this region was not mapped consistently over the whole area, existing information is mainly based on voluntary reporting. This may result in a bias with ragweed stands in agricultural fields being reported less than roadside populations. However, the very high proportion of ragweed stands at road margins indicates that roadsides are very important spreading routes and can be the gateway to new regions.

How can ragweed enter the road margins and spread there?

There are different ways how ragweed seeds can reach the road margins. *A. artemisiifolia* achenes could be lost during the transportation of agricultural products. They also could be spread by agricultural machines when seeds stick at them directly or mixed with soil and were lost during the drive on the road. Once ragweed has reached the road, it can be spread with mowing machines when these are used during the maturity of seeds (Vitalos & Karrer 2009, Nawrath & Alberternst 2011a). An important pathway of introduction at the road margins is the use of construction material which is contaminated with ragweed seeds. During our field work in 2011 and 2012 examples for this pathway of spread were found in the study area.

Introduction of ragweed with construction material

In Tab 2 (compare Fig 13) newly built roads and roads with rebuild banquets are listed. At these sites ragweed plants were found during the investigations in 2011 and 2012. Ragweed was introduced here likely with contaminated soil. Two of these roads are described more precisely below.



Map basis: OpenStreetMap

Fig 13: Occurrence of common ragweed at road margins in the Niederlausitz near Cottbus, East-Germany, September 2012.

Marked in blue: Ragweed occurrence at newly built roads and at roads with new hard shoulders. *A. artemisiifolia* was most probably introduced here with the construction material (Tab 2).

Tab 2**:** Common ragweed stands at newly built roads which were most probably introduced here with contaminated construction material.

investigations: July 2011 and September 2012

No	Location	Type	Length od colonized road section	method	geographic coordinates WGS84	Ragweed occurrence	Year of study
1	NW of Schipkau, between Klettwitz and Kostebrau	Newly built road, road deallocated in 2010	> 4,2 km	Inspected from car, partially walking	51.516357 13.838765 bis 51.535074 13.882989	dispersed, partially richt in individuels	2011
2	Near Drebkau, B169	Road construction road deallocated in 2009	ca 8 km	Inspected from car	51.666616 14.242706bis 51.630018 14.174699	Very rich in individuals	2012
3	W of Spremberg and Schwarze Pumpe, B97new	Road construction, road deallocated in 2010/11	ca 12 km	Inspected from car	51.597687 14.363081 bis 51.502121 14.332527	Single plants up to individual rich stands	2012
4	Nordöstlich Cottbus bei Merzdorf, B168neu	Road construction, road deallocated in 2012	ca. 4,4 km	Inspected from car	51.757307 14.402565 bis 51.790382 14.376286	Single plants, rich in individuals, mainly at areas aside of the road	2012
5	Sielow, „Sielower Chaussee"	Road construction ca. 2011/2012	750 m	inspected on foot	51.787729 14.309792	Single plants	2012
6	E of Schmogrow-Fehrow, „Dorfstrasse"	Road construction ca. 2011	330 m	inspected on foot	51.851869 14.230974	Medium-sized stand	2012
7	Turnow, „Wiesenweg"	Road construction ca. 2011	150 m	inspected on foot	51.872610 51.872610°	Low number of plants	2012

Example 1: A newly built road located northwest of Schipkau

At a newly built road in an extended reconstruction area between the villages Klettwitz and Kostebrau northwest of Schipkau in East-Germany ragweed was observed first in 2010, shortly after finishing the construction works (FU Berlin 2013). Also during the field work at 6th July 2011 many ragweed plants were observed at the margins of this road (Fig 14). The ragweed plants occurred predominantly at one road side whereas no or only a few plants were found at the other road margins.

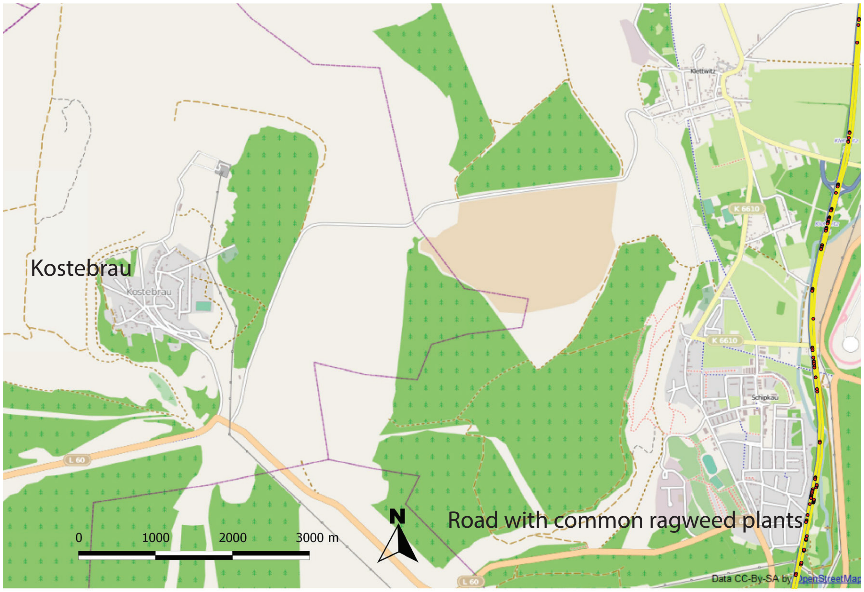


Fig 14: Newly built road in a reconstruction area connecting the villages Klettwitz and Kostebrau near Senftenberg (2011/0706).

At the roadside common ragweed which was very likely introduced with construction material occurs.

Map basis: OpenStreetMap

At the roadside where the ragweed plants occurred, a humus-rich material was used to fill the hard shoulder (Fig 15). At the opposite side of the road no or only less of this material was used. The road runs through an open, vegetation-poor area which provides good conditions for the pioneer species *A. artemisiifolia*. Unfortunately it is not allowed to walk in the reconstruction area. So only the margins of this region could be inspected (Fig 15). In the visible area, ragweed plants were only registered at the margins of the road but not in surrounding areas. This indicates that *A. artemisiifolia* was not introduced from the surroundings and supports the observation that ragweed was introduced with the humus-rich material used to fill in the road margins.

Fig 15: Newly built road in a reconstruction area connecting the villages Klettwitz and Kostebrau near Senftenberg (photo: 2011/07/06).

a) Newly built road in a reconstructed mining area.

b) Common ragweed occurs predominantly at the road side where brown humus was brought in while on the opposite road side no or less of this substrate was used.

c) Road shoulder of the new road. Here a substrate rich in humus was used. Ragweed plants occurred here.

d) Vegetation-poor reconstruction area which is not allowed to enter. Looking from the road in this area, no ragweed plants were detected. The plants only occurred at the road margins.

Example 2: A newly built road located east of Drebkau

The newly built road B169 which bypasses the city Drebkau over a length of 8 km was opened in December 2009 (BMVBS 2009). At this road millions of ragweed plants occurred in 2011 and 2012 (Fig 13). *Ambrosia artemisiifolia* was introduced here probably with construction material and spread quickly over the last few years.

Fig 16: B169 near Domsdorf. At the margins of this newly built road millions of ragweed plants are present. Ragweed was probably introduced here with construction material and dispersed during the construction work (photo 2011/07/10).

Discussion

The investigation in Germany demonstrates that ragweed is often dispersed with soil in areas highly infested with the species. Where ragweed is still rare, a spread within soil takes place to a lesser extent. The spread within excavated material is a very effective spreading route. Thus, in countries with low infestations measures to prevent ragweed spread within excavated material should be conducted in an early phase of the spread. Measures should aim in a prevention of ragweed seeds to get into the soil distribution circle of the building industry.

Measures to prevent seed dispersal with soil

There are different possibilities to avoid the dispersal of ragweed seeds within excavated material during construction works (Tab 1). In a first step it is essential to detect a contamination of a site with ragweed seeds before seeds were spread via excavated material.

a) Detection of ragweed plants and seeds

If a population of ragweed plants is present on a ground, it is very likely that the soil contains seeds of the species. The ground, construction measures are planned on, should be checked for ragweed plants before the building works start. More complicated is it to detect soils which are contaminated with ragweed seeds when the plants are not visible, e.g. when plants were outcompeted by native vegetation but the seed bank is still present. If there is a suspicion that soil could be contaminated with ragweed seeds, the soil should be investigated. Actually no standards or regulations exist on best practices how to find ragweed seeds in the soil.

There is a similar problem for farmers to detect nematodes on their farmland. Possibly methods to find these organisms could also be used to find ragweed seeds in the soil. Chambers of agriculture in Germany provide recommendations how to take soil samples for nematode investigations. The chamber of agriculture North-West (Niedersachsen, LUFA 2008) gives recommendations as follows: 30 soil samples taken in the upper 0-30 cm in steady distances should be taken per ha. Nematodes are not equally dispersed in soil – similar to the seed distribution of *A. artemisiifolia*. Thus, it is important to take many soil samples and investigate a mixed sample. 30 soil samples should be mixed in a bucket and 1 kg should be investigated for nematodes. A similar procedure could be used to find ragweed seeds. However, an adequate measure should be tested.

When a soil sample is taken, the ragweed seeds must be detected in the soil. One possibility to find the seeds is, to dry and sieve the soil, and afterwards search thoroughly for the achenes. This measure is very labour-intensive. Another option is, to put the soil in flat bowls in order to germinate all seeds. For this method ideal growing conditions for the species must be provided and it takes time before the seeds germ and results are achieved. It should be taken into consideration that *A. artemisiifolia* seeds need a stratification before they geminate properly.

It could also be an option to develop special DNA-tests to find ragweed seeds in soil samples. Currently we are lacking information on this topic.

b) Options to prevent spread of ragweed seeds in excavated material

Excavated material should be kept at the same site and it should be separated in order to avoid a contamination of clean material (see Fig 17). Newly grown ragweed plants should be removed before seed set. A cover of the contaminated soil piles with a foil could help to prevent germination and seed set of *A. artemisiifolia* plants. If possible, the contaminated soil should be used for fillings below the surface. If this is not possible, the material should only be used in areas where a combat of *A. artemisiifolia* is ensured over several years until no ragweed plants grow up any more. A control of success is necessary. If it is not feasible to keep the soil at the same site, the material could be transported to a location where it is used in civil engineering processes and is deeply buried. Instead of burying, the soil could also used for construction work in areas where no suitable growing conditions for the species are present (e.g. varnished areas, intensively used grassland). A mixture with uncontaminated soil should be avoided and the material should only be transported to a single site in order to prevent an allocation at different locations.

It is another option to finally dispose the material at a special site, or to sterilise it. A compilation of possibilities to treat contaminated soil and an assessment of efficacy and effort are given in Tab 3.

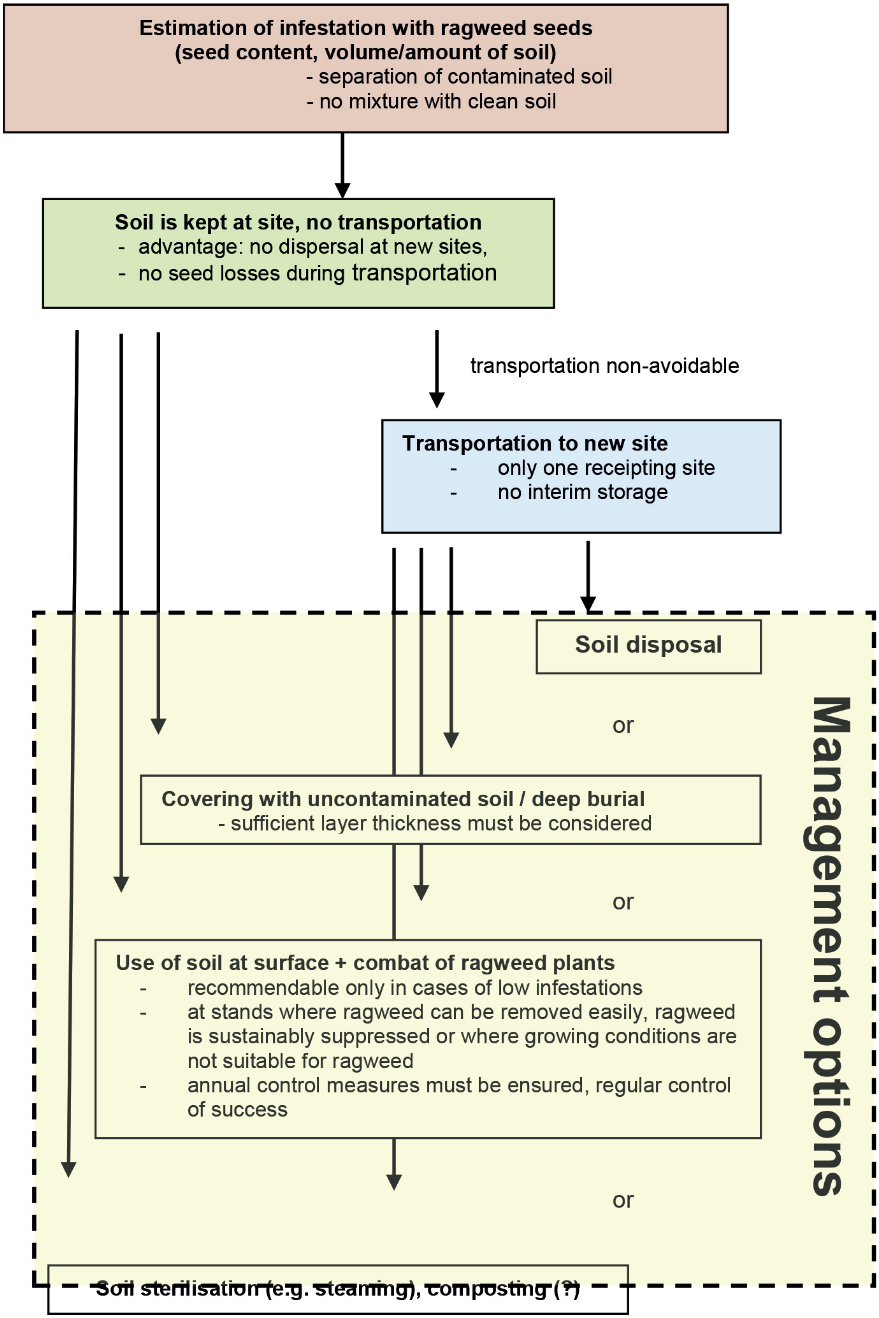


Fig 17: Options to prevent the spread of *A. artemisiifolia* in contaminated, excavated material.

Tab 3: Possibilities to treat contaminated soil and assessment and effort of measures.

measure	**assessment & effort**
final disposal at a special disposal site, no further use of soil	• effective measure • risk of seed losses during transportation • loss of top soil for further use • costs for transport and final disposal • costs for cleaning of the machines
deep burial, cover with non-contaminated material	• effective measure • loss of top soil for further use • relatively low effort when material is buried at same site • costs for transport if buried at other site • costs for cleaning of the machines • risk of seed losses during transportation
use of contaminated soil and control of *A. artemisiifolia* at site	• only advisable at same site when ragweed control is ensured for several years • at sites where no suitable growing conditions for *A. artemisiifolia* are present • only advisable at sites with small ragweed populations • not advisable at road sites, river channels • effort for combat depends on size/dispersal of the ragweed population and on consistency of control measures
sterilisation of soil	• effective • very laborious, high energy input, cost-intensive • risk of seed losses during transportation of material
In general • if possible only use of contaminated material at same site • avoidance of transport due to risk of seed losses/dispersal • separation of contaminated material in order to avoid contamination of clean material • if transport is necessary, only transport to a single site (no dispersal of contaminated material at different sites) • avoidance of seed losses during transportation, cleaning of machines • monitoring of sites where a ragweed contamination is known, control of success of control measures	

Methods to sterilise soil

An effective non-chemical method to decontaminate soil of bacteria, viruses, fungi, nematodes and weed seeds is a treatment with hot steam. Most weed seeds exposed to temperatures of 70-80 °C over 15 minutes die (Gudehus 2005). Steaming of soil is a method which is often used in horticulture. It is possible to steam soil surfaces in place, or substrata can be transported to a special steaming facility.

Steaming of soil surfaces

There are different methods to sterilise soil surfaces such as steaming with foils, vapour hoods, steam harrows, steam ploughs, and steaming with negative pressure by using drainage pipes (Lampe 2011). The following descriptions of the steaming methods base on Gudehus (2005) and Lampe (2011).

To steam areas of 15 to 400 m^2 special heat-resistant foils are put on the soil and weighed down with sand sacks. Hot steam is produced by a special steam-boiler and conducted under the foil. Depending on the condition of the soil and the air temperature it takes 1 to 1.5 h to reach 85 °C per 10 cm of soil depth.

A vapour hood is a portable equipment which is put on the soil that should be treated. Depending on the model and the size of the vapour hood the equipment is put on the area that should be treated with a tractor or by hand. It takes 30 minutes to heat the soil to 90°C up to a depth of 25 cm.

Small areas can be treated with a steam harrow. This machine is constructed with tines via those the hot steam is led into the soil.

Using a steam plough is the oldest procedure to decontaminate soil. This machine is usually used in glass houses. It is a rake-like construction that is pulled by a cable winch through the soil. Through the blades of the plough hot steam is led into the soil.

It is possible to steam soils up to a depth of 80 cm by using a special drainage system that is either installed on the soil surface or buried in the soil. The drainage pipes are used to aspirate the air in the soil. The soil that should be sterilised is covered with a special foil that is sealed at the edges. The vapour is conducted under the foil and due to the aspirated air a vacuum is build up and the hot steam flows into the soil (Gudehus 2005, Lampe 2011).

Steaming of substrata

Material such as excavated soil or compost that should be sterilised could either be transported to a steaming facility (Gudehus 2005), or a mobile steaming machine could be transported to the site. The material can be put on a sterilised ground (e.g. concrete) and then can be heated with hot steam supplied via pipe systems. Other options are to put the material in a steaming box or on a special tipping trailer where hot vapour is passed in.

Composting

In Baden-Württemberg, South Germany, soil containing rhizomes of the invasive Japanese Knotweed (*Fallopia japonica*) is composted in order to kill the rhizomes. The knotweed contaminated soil is enriched with fresh compost and afterwards composted at a temperature of 70 °C. During the composting procedure it is necessary to relocate the compost 6 to 8 times (Email B. Walser 2012/12/18). This measure might also be used to decontaminate soil containing ragweed seeds. In trials it should be ensured that the ragweed seeds were already killed by this method. If the temperature is not high enough, ragweed can survive the composting procedure as it was observed in Bavaria in 2012, where in July 2012 four vital ragweed plants were found on piles of composted material (Fig 18).

Fig 18: Piles of composted material near Salmdorf, Bavaria, 2012/07/18. Four living ragweed plants were found on top of the piles.

Regulations to prevent the spread of ragweed in soil in European countries

In our study we only found little information on legal regulations regarding the treatment of contaminated soil in European countries. Switzerland has the most comprehensive regulations, and in France legal regulations exist, but only on a regional level. The Lombardy region in Italy has regulations to control ragweed by mowing, but there are no special regulations regarding the prevention of spread with soil (O.P.G.R. 29th March 1999, N. 25522). (Kazinczi *et al.* 2008 b) give a short overview of authority arrangements in Hungary. A special regulation regarding the treatment of excavated material used for construction work is not mentioned by these authors. In Germany only voluntary programmes against *A. artemisiifolia* exist.

Switzerland

The use of soil contaminated with *A. artemisiifolia* seeds is prohibited in Switzerland. If *A. artemisiifolia* is introduced at new sites during construction measures the owner of the site is legally obliged to remove the plants before they spread (result of inquiry: information given by A. Demierre).

The combat of *A. artemisiifolia* is obligate in Switzerland. In this country the "causative principle" is used and the land owner, the user of the land, the building contractor or the common carrier is obliged to remove *A. artemisiifolia*. There are special regulations to avoid the spread of *A. artemisiifolia* in soil, humus, excavated material, compost etc. in the Kanton Graubünden. The following regulations are in force (Amt für Natur und Umwelt Graubünden 2007):

— Soil contaminated with common ragweed seeds must not be transported and reused at new sites but must be disposed or collected at controlled deposition sites where a combat is guaranteed,
— a reuse of contaminated soil is only allowed at locations where a combat is ensured for a long time (e.g. at small construction sites),
— machines must be cleaned from soil, to ensure that no common ragweed seeds sticking to the machines are dispersed,
— before construction work, recultivation or other actions including earthworks take place, it has to be clarified whether the soil is contaminated with common ragweed seeds. The import of soil from regions where extensive common ragweed stands occur (Tessin, North-Italy and the Misox which is a valley in the Kanton Graubünden) is not allowed (Amt für Natur und Umwelt Graubünden 2007).

The legal basis for the handling of *A. artemisiifolia* in Switzerland is:

— The Plant protection act (Pflanzenschutzverordnung 28th Feb. 2001, Art. 27-29, Anhang 10)
— The Environmental act (Umweltschutzgesetz 7th Oct. 1983, Art. 29a, Abs. 1)
— The order of release of organisms (Freisetzungsverordnung (FrSV) 25th August 1999, Art. 4, Abs. 1; Art. 32. Abs. 1)

France

In France regulations are set in force by local authorities in infested areas but no national regulation exist (B. Laitung). In general, soil used by operators must theoretically be protected against weed seed rain in France (B. Chauvel). However, mostly no measures to avoid the spread of *A. artemisiifolia* are conducted, and referring to an estimation of B. Chauvel from France only "powerful" structures such as mayors of big cities or motorway companies may force operators to avoid the spread of the species.

In France much information is available how to control *A. artemisiifolia* (e.g. www.ambroisie.info.fr). On that webpage special information is provided on methods how to combat *A. artemisiifolia* at construction sites (http://www.ambroisie.info/docs/fiche_6.pdf).

Italy

In the Lombardy region (Italy) owners and land users are obliged to combat *A. artemisiifolia* between the end of June and the 20th August. Mayors of municipalities affected by common ragweed occurrences are obliged to surveil the compliance of the regulations (O.P.G.R. 29th March 1999, N. 25522). There is no special prescription given to prevent the spread of *A. artemisiifolia* with excavated material used for construction work.

Germany

In Germany action programmes exist that aim at the prevention of spread and the control of common ragweed (Starfinger 2012, STMUG 2013). These programmes also provide information how to prevent the spread of common ragweed via excavated material. In Germany the control of common ragweed is not obligate and no legal regulations comparable to those in Switzerland exist. Currently there is no or only little awareness of the common ragweed problem in the building sector in Germany. Authorities have no legal options to force control measures in order to prevent a spread during construction work. Due to this, authorities often do not even try to spur the building sector into action. At soil depositions usually no weed control takes place. Special information campaigns for the building sector are of high importance in order to avoid the spread with excavated material.

Recommendations

Spread of *A. artemisiifolia* seeds with soil is very effective and can lead to the colonization of new sites and areas. Thus, concepts to avoid the spread with excavated material are needed. Experiences from Germany demonstrate that voluntary action programmes (national and federal state scale) against *A. artemisiifolia* did not raise awareness in the building sector by now. The inquiry done in this study stresses this result for other European countries where no legal regulations regarding this issue exist. Switzerland has implemented legal regulations that include an ordinance for the building sector. The example of Switzerland where common ragweed is controlled effectively demonstrates that it is necessary to create awareness of the *A. artemisiifolia* problem in the building sector. There are different possibilities to prevent the spread of *A. artemisiifolia* in excavated material as described above. However, most of these measures are cost-and/or labour-intensive and would not be done on a voluntarily basis. So, legal regulations for the building sector are needed.

Exemplary proceedings regarding biologically contaminated soil in Switzerland

In Switzerland a special legal obligation regarding the disposal of excavated material contaminated with organic material (Neobiota) exist in the canton Zürich (Baudirektion Kanton Zürich 2011). This regulation especially refers to invasive species such as *Fallopia* sp., *Polygonum polystachyum*, and *Rhus typhina,* but in our opinion it is exemplary, and it could also be used to contain the spread of *A. artemisiifolia* within excavated material.

The regulation says: If an invasive plant species occurs at a construction site the building owner has to fill in a declaration in collaboration with a special consultant and has to send to the authorities. Contaminated soil that cannot be used at the site has to be disposed at authorized sites. In this case the proceeding is as follows:

a) Before construction work starts the area contaminated with an invasive species and the amount of contaminated soil has to be quantified.

b) A commitment to purchase the material has to be seeked from the operator of an authorized disposal site and a concept for the disposal has to be sent to the authorities.

c) The affected area has to be marked at the construction site in order to avoid a contamination of clean material.

d) Before construction work starts, the site has to be visited by the consultant, the foreman, the operator, and the excavator driver.

During the construction work the contaminated material must not be mixed with clean material and it has to be separated. During the excavation a consultant has to be present at the construction site. It has to be ensured that no contaminated material is lost during the transportation. After transportation to the disposal site a form with a report has to be sent to the authorities. 1-2 month after the combat an authorized consultant has to control whether no invasive plants grow back at the site (Baudirektion Kanton Zürich 2011).

Inclusion of *A. artemisiifolia* in announcements for construction work

Instructions to prevent the spread of *A. artemisiifolia* during construction work could be included in announcements. The building owner should be informed about *A. artemisiifolia* and the problematic of spreading during construction work. He could be obliged to investigate the construction site for the occurrence of common ragweed (or other invasive) plants in the vegetation period (June – October, when *A. artemisiifolia* is detectable) before any construction measures take place. The result should be sent to authorities that build up a data collection on *A. artemisiifolia*, respectively on invasive species. A building owner should be obliged to seek for information on common ragweed stands from the authorities. In case common ragweed occurs at a site, the owner has to be obliged to prevent the spread (e.g. no transportation of soil, or safe disposal at special site, or deep burial). In the performance description for building constructions of the Ministry of economy, family and youth in Austria (BMWFJ 2012) there is a regulation regarding soil depositions (no 581311A). This says that soil depositions fostered and hold free of weeds can be brought to account. Costs can be estimated in m^3 x weeks. This might also a basis for cost calculations for soil depositions kept free of *A. artemisiifolia*.

If no common ragweed is present before the building work starts, the owner could obligate the building company to make sure that no common ragweed is present after finishing the construction work. If soil with common ragweed stands was introduced and detected during the construction phase, the building company could be obliged to prevent spread from this soil (see above). In case common ragweed already occurs after finishing the construction work the construction company could be obligated to combat *A. artemisiifolia*.

This proceeding should be communicated with the building sector.

Summary

- Spreading within excavated material is an effective spreading route for common ragweed in Europe.
- The relevance of the soilpathway often increases when the infestation with common ragweed in a region increases (e.g. in Germany).
- In the Niederlausitz in East-Germany construction measures at road margins led to an increase of the common ragweed population at road sides during the last years.
- The use of soil contaminated with common ragweed seeds at soil surfaces should be avoided. Contaminated soil should be deeply buried, disposed or decontaminated. It could be used at sites, where no suitable growing conditions for *A. artemisiifolia* are present.
- It should be avoided to transport contaminated soil in order to prevent seed losses during the transportation. If a transport is not avoidable contaminated soil should be transported only to a single site (no dispersal). If contaminated soil is used at the surface an effective combat of *A. artemisiifolia* should be ensured over several years.
- In most of the European countries no special measures are conducted to prevent the spread of common ragweed within excavated material, by now. Comprehensive legal regulations currently exist in Switzerland. In many European countries the awareness of the *A. artemisiifolia* problem in the building industry is low and even if the sector is informed, without legal regulations usually no control or prevention measures occur (cost- and labour-intensive).
- Management programmes on a voluntary base often did not reach the building sector in Germany. In many cases common ragweed plants were not or not sufficiently removed (with some exceptions).

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Appendix: Questionnaire

Relevance of soil and construction material for the spread of *Ambrosia artemisiifolia*

Dear addressee,

seeds of the invasive and troublesome ragweed are dispersed via a number of pathways, several of them aided by humans. In the course of the EU funded project HALT AMBROSIA we are currently studying the role of construction activities in spreading the plant. As there is little published information available, we try to find out more with this short questionnaire. We hope you can find a few minutes to fill it in. You are also welcome to pass it on to colleagues who might know more or to give us additional contacts. Thank you very much for your help!

Name:

Institution/address:

Contact details:

Main field of work:

Please return the questionnaire to Beate Alberternst b.alberternst@online.de.

Postal address: Beate Alberternst, Hinter´m Alten Ort 9, 61169 Friedberg

1) How important are the following spreading pathways for *Ambrosia artemisiifolia* in your country? Please fill in ☒

Rele-vant 1)	Relevance				**spreading routes for *Ambrosia artemisiifolia***
	low	me-dium	high	no info	
					a) Building sector (e.g. road building, constructions): Transport of *A. artemisiifolia* seeds within soil or con-struction material (e.g. sand, gravel, construction waste; seed loss, growing/reproduction on earth fill)
					b) Building sector: Transport and loss of ragweed seeds sticking to building machineries (e.g. tires)
					c) Agriculture: Transport and loss of ragweed seeds sticking to agricultural machines (e.g. tires, mowing machines)
					d) Agriculture: Transport and loss of ragweed seeds with agricultural products (during harvest)
					e) Agriculture: Use of sowing material contaminated with ragweed seeds
					f) Traffic: Transport and loss of ragweed seeds sticking to trucks, cars etc.
					g) Bird seeds
					h) Other:
					i) Other:
					k) Other:

1) Relevant, but no estimation of importance possible

no info = no information of relevance for the spread of *A. artemisiifolia*

What is the most important spreading route of the pathways mentioned above in your country? Please note the number a), b), c) etc:

Comments:

2) Is the building sector informed about the Ambrosia-problem? (Occurrence of ragweed plants on soil depositions or construction material, reproduction and contamination of the soil with its seeds, important spreading route of *A. artemisiifolia* via transport of soil etc.)

☐ I don't know / no information

☐ No (operators are often badly informed about the problematic, no measures are undertaken)

☐ Yes:

☐ Yes, operators are informed, but mostly no measures to avoid the spread are undertaken

☐ Yes, operators are well informed, and measures to avoid the spread are mostly undertaken. Which measures are conducted? ……………………………………..

3) Are there legal or other regulations to avoid dispersal of Ambrosia seeds within soil or construction material in your country?

☐ I don't know / no information

☐ No

☐ Yes:

☐ The use of soil contaminated with *A. artemisiifolia* seeds is prohibited.

☐ It is compulsory to separate *A. artemisiifolia*-contaminated soil from clean soil at soil depositions and to decontaminate it before reuse at other sites

☐ It is compulsory to remove ragweed plants from soil depositions to avoid contamination with its seeds

☐ If *A. artemisiifolia* is introduced at new sites during construction measures, there is someone legally obliged to remove the plants before they spread (if yes, who?)

Could you give us some information on regulations (if existing), please?

(e.g. link to regulation, pdf, or expert who could give us more details)

……………………………………………………………………………………………………...

……………………………………………………………………………………………………...

Improving efficiency of mechanical ragweed control in urban areas

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Introduction

Mechanical control of ragweed is an ecologically friendly type of control and therefore widely accepted by common people. Nevertheless, it is limited in its efficacy because of the high regrowth capacity of this annual plant after being hurt. The first chapter gives an overview about the effects of cutting ragweed in general and focusses on the outcome of various cutting experiments in literature and some experiments performed in this project. In the second chapter, we describe one of the extensive experiments performed during this project in the very detail.

Control of common ragweed by mowing and hoeing

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Introduction

Common ragweed (*Ambrosia artemisiifolia* L.) is an annual species that depends on regular seed production for population persistence. By producing dormant soil seed banks (Basset and Crompton 1973, Toole and Brown 1946) this weed may overcome seasons with failure of seed production. Consequently, the only sustainable way to control common ragweed is preventing seed production (Bohren et al. 2008c, Karrer et al. 2011). Several actions and cutting experiments focus on the reduction of pollen produced by male inflorescences of ragweed (Benoit 2003). Only few aim at estimating both male and female flower regeneration (Bohren et al. 2008a, Milakovic et al. 2014b). Karrer et al. (2011) claim to focus more on control options that minimise seed production on regenerated shoots.

This overview on effects of mowing and hoeing is mainly based on a literature review and some provisional findings of the HALT experiments

Papers considering mowing as a control measure can be grouped according to their designs by explanatory factors:

A-simple designs:

cutting height,

cutting dates (timing),

cutting frequency,

+/- competition

B-mixed designs:

plant density *and* frequency,

timing *and* frequency,

height *and* frequency,

herbicide application *and* cutting,

competition *and* frequency

C-complex designs:

plant density *and* timing *and* frequency,

plant density *and* timing *and* frequency *and* competition

plant density *and* timing *and* frequency *and* competition *and* region

frequency *and* timing *and* herbicide application

Response variables were: simple resprouting, flowering of resprouts, number of male racemes on resprouts, biomass of shoots (uncut and/or resprouts), female flowers of shoots (uncut and/or resprouts), phenology of shoots/flowers (uncut and/or on resprouts), seed number of resprouts and seed viability of resprouts.

Experiments were either done under controlled conditions in the greenhouse (pots) or in the field differing in habitat type. Some experiments were performed in variable crops, others on roadsides (road shoulders).

Regeneration after cutting:

Regeneration after cut is well documented for common ragweed (Basset and Crompton 1975, Barbour and Meade 1981, Bohren et al. 2005, 2008a, Karrer et al. 2011, Tokarska-Guzik et al. 2011, Meiss et al. 2008). Generally the intensity of resprouting by lateral shoots is not limited throughout the year. Even from axils where their lateral shoots have already finished growth (spontaneously or after being cut), accessory buds can be developed prolonging the seasonal growth period (Karrer 2007, Karrer et al. 2011). Gebben (1965) found that the development of lateral shoots tend to be more intense at lower densities of *A. artemisiifolia* stands compared to crowded stands. Such can be interpreted as self-thinning process (Londsdale 1990) or suppression of lateral branches by shading neighbours of identical growth architecture (cohorts). Basset and Crompton (1975) report vegetative regrowth of plants by 80 % one week after they were cut at 5 cm (slightly above the cotyledons) end of May. They observed also 100 % ragweed regrowth 10 days after grain harvest with cutting height of 20 cm. Tokarska-Guzik et al. (2011) found 50% regeneration of ragweed individuals that were cut once in early developmental stages (max. 12 cm high) just above the cotyledonary node, both treatments that were cut above the first foliar leaf pair as well as those cut above the second foliar leave showed 100% resprouting. Meiss (2010) and Meiss et al. (2008) clipped solitary individuals at 5 cm height every month. This resulted in seven clipping dates and a significant reduction of total biomass by 40% – compared to the intact control. When added dense luzerne populations as competitors the reduction total ragweed biomass was near to 100%.

No significant effect on the allocation of reproduction (fecundity) were found after removing the apical meristem only (MacDonald and Kotanen 2010).

It is known that *A. artemisiifolia* can germinate in Europe throughout the whole vegetation period (end of March to October; Karrer et al. 2011, Kazinczi et al. 2008a). During the early season growth in height is low (Gebben 1965, Klein 2011) producing several short internodes with a dozen of foliar leaves (Karrer et al. 2011). But starting from mid of June rapid upright growth by elongating the youngest internodes und all newly developed internodes is regular under full light conditions (Klein 2011, Karrer et al. 2011). Seedling cohorts that start later in the year (May to August) generally produce less basal internodes, all of them elongated for rapid flowering. Growth in height stops at about mid of September (Kazinczi et al. 2008a, Klein 2011, Gebben 1965). Up to this date height increment of early cut specimens can be compensated by elongated lateral shoots (branches of first order) (Simard and Benoit 2011, Karrer 2012, unpublished). A comparison between mown and intact plants showed no significant differences with respect to the biomass produced all over the season, anyway if they were cut early or late (Simard and Benoit 2011).

Patraccini et al. (2011) documented that the survival rate (resprouting after cut) was generally very high: plants cut two or three times showed resprouting rates between 75 and 100%. Plants that were cut at plant height 80 cm survived by 100%, the 50 cm cut height gave also 100% and the 20 cm plants about 70% survivers. The latter were cut more often (3-4 times) as they reached the cutline earlier. Clipping even in the 4 times version resulted only in a death rate of 25 to 33%. In all clipping experiments by Milakovic (summarised in Karrer et al. 2011, Milakovic et al. 2014a and 2014b) death rates of uncut and cut plants was very low (0-5%) throughout spring and summer. Only starting from mid of September mortality increased successively until October.

Beres (2004) and Kazinczi et al. (2008b) also reported a strong allocation to shoots after early cut (in May or June) finally compensating totally the biomass loss. A later cut (in July or August) resulted in a significant decrease of total biomass.

Considering its summer annual life cycle, *A. artemisiifolia* turned out to be very vital by compensating efficiently biomass loss from cutting. However, cutting per se cannot control common ragweed.

Regeneration of male flowers after cutting:

Aiming at the reduction of ragweed pollen load in the air (Buttenschøn et al. 2010, Bohren et al. 2005, Delabays et al. 2005, Karrer et al. 2011), blooming of male flowers must be prevented. Of course cutting is an option as the male racemes are produced generally at the top of the main shoot as well as on the lateral shoots (Bassett and Crompton, 1975). Several experiments focussed on this response variable rather than on seed production.

The clipping experiment by Patracchini et al. (2011) resulted in a partial biomass reduction of the surviving plants but did not prevent flowering. In the high-stress treatment (4 times clipping at 20 cm), more than 67% of the plants survived to the last clipping and, among these, more than 97% flowered. Moreover, plants that reached 80 cm height and experienced 2 cuts survived at rates between 50 to 100%, and 100% of the survivors flowered. Flower initiation on regenerative lateral shoots happens quite quickly. Plants that were cut directly above the cotyledons failed to produce buds of male inflorescences after 2 weeks after the cutting date, but plants cut above the first or second foliar leaf pair showed already 60-80% and 80-90 %, respectively (Tokarska-Guzik et al. 2011). Such quick recovery from being cut was also demonstrated by Beres (2004), Bohren et al. (2008a), Delabays et al. (2008a), Simard and Benoit (2011), Karrer et al. (2011), Karrer and Milakovic (2011) and, Bassett and Crompton (1975).

Beres (2004) and Kazinczi et al. (2008b) found a significant reduction of male flowers by 87 % when ragweed was cut only once mid of July or even by 90 % for plants cut three times. Milakovic et al. (2014a) found in a glasshouse experiment 8 times smaller inflorescences numbers in early September in plants cut mid-August (at the beginning of male flowering), compared to the uncut control.

Simard and Benoit (2011) found that mowed plants produced generally less pollen per unit inflorescence length and increasing plant density also reduces pollen production per inflorescence unit. In total, plants cut 2 times produced 6 times less pollen than intact plants. Mowing high density plants show 3-5 times reduced lenghts of male inflorescences compared to intact single plants (low density). In general, the anthesis was delayed by mowing by 17 days, whereas higher densities had no effect (Simard and Benoit, 2011). They summarized that the total pollen production was reduced by 88.7 % when plants were mown twice (May and July). This fact, together with the experiments by Klein (2011) illustrates well that the compensatory growth of lateral shoots tends to allocate biomass to shoots primarily and less to pollen production i.e. when cut early in the year. When cut, later in the year (late July to September), they tend to allocate biomass rather to lateral shoots that bear female flowers at their lower nodes (Bohren et al. 2008a, Klein 2011, Karrer et al. 2011). Allocation of biomass to male inflorescences seems to be typical for uncut individuals in the early phase of stem elongation and initiation of inflorescences. But it makes sense that the plants allocate resources from pollen production towards the production of female flowers (ripening seeds) in late summer and autumn as the air is already overloaded with viable pollen at that time (Jäger 2000).

Production of female flowers and seeds, seed viability:

Sustainable control measures against ragweed must focus on preventing seed production (Bohren et al. 2005). Yet, only in very few experiments this response variable was measured when testing different cutting treatments.

As there is a preference of ragweed to produce female flowers in the middle and lower part of the plant (Gebben 1965) cutting near the base never can really prevent seed production by 100 %. Traditional cutting height used to manage the road shoulders rarely goes below 5 cm. On the other hand we know that common ragweed tends to germinate directly along the roadside rather early not facing tall competitors (Joly et al. 2011, Simard and Benoit 2010). In such habitats the early development of the plant is rather free from competition but not optimal with reference to relative growth ability. Those plants show short internodes at the base of their shoots and therefore several buds remain below the cutting height that are able to develop regenerative shoots. Milakovic et al. (2014a) found that early cuts (until mid of July) will not reduce total seed number, probably because the resprouts overcompensate the biomass losses from cutting and produce many axillary shoots with female flowers. In this glasshouse experiment, total seed numbers per plant were reduced by ca. 2-4 times compared to the control in cutting regimes with a late first cut mid-August. Field experiments by Milakovic et al. (2014b) showed as well that a cut in August is essential: 3-5 times smaller total numbers of seeds per plant were found in plants cut in August, compared to the uncut control.

Simard and Benoit (2011) reported that number and mean seed mass decreased 3-4 times with increasing plant density and by mowing. Mown plant seeds were 0.65 times less viable, whereas seeds from high density plants did not differ in this respect to single plants. Thus allocation to seed biomass (weight and number of seeds) was only reduced by mowing not by higher densities.

If cut once a year the timing is rather important. Bohren et al. (2005) and Delabays et al. (2005) argued that one cut only in the first half of September yielded no viable seeds on the few resprouts. In more detailed experiments from 2005 to 2007, Bohren et al. (2008a) had to revise some advices given that the year-to-year variation in the ripening dates of seeds showed the possibility that in years with optimal climatic conditions ragweed already can produce viable seeds in late August. Consequently, the first cut should be set not later than August 20th. But this enabled the resprouts to produce viable seeds between August and October.

All mowing treatments in Bohren et al. (2008a) resulted in a decrease of the total number of seeds and their viability. When cut early (i.e. in June) ragweed regenerated seeds with only 50 % viability compared to intact plants. Seed viability decreased to 30 % for shoots that developed from later cutting dates.

Vincent and Ahmim (1978) and Vincent et al. (1992) showed that seed production was significantly reduced only at very low cutting heights of 2 cm which is not realistic in the field.

Integrated treatments:

On crop fields production techniques contribute to the reduction of weeds like common ragweed: crop rotation, mowing, mulching, hoeing, harrowing and tilling systems are applied. Hoeing is only applied in specific crops mostly at early stages of development (Verschwele in the HALTAMBROSIA-project, Buttenschøn et al. 2010). Karrer et al. (2011) promote hoeing for ragweed control in oil pumpkin fields. Common ragweed is said to be easily controlled by rotary hoeing when less than 1/4" (MSU, weed science; http://www.msuweeds.com/worst-weeds/common-ragweed/).

Mechanical plus chemical treatments are generally used in crop fields; several treatments were tested in the EUPHRESCO-project (Holst 2009). Hoeing once induced the highest values for ragweed biomass produced, whereas hoeing two times did some harm. The effect of biomass loss by this treatment was about the same as herbicide application followed by hoeing. But the most effective combination was applying herbicide and afterwards hoeing. If herbicides are used as combined treatments it is most effective to use herbicide in early developmental stages followed by mechanical measures. The same was found in the U.S. (Donald 2000) for weeds in soybean where herbicides were combined with mowing. Two times mowing after herbicide treatment worked well in reducing weeds like common ragweed to a tolerable very low level.

Bohren et al. (2008b, 2008c) combined serial cuts and subsequent herbicide treatments of common ragweed. The treatment with Florasulam 10 weeks after cut on 19th of June gave high efficacy by low seed numbers and seed viability between 0.5 to 2.5 %. Other cutting/herbicide combinations gave less valuable or insufficient success.

Experiences by Kazinczi et al. (2008b), Delabays et al. (2005) and Bohren et al. (2005) indicate also that hoeing alone (i.e., if not performed intensively enough) showed poor control efficacy. Nevertheless soil disturbance by hoeing can promote further emergence of ragweed seeds.

Competition by desirable plants (crops, lawn) acting against weeds and ragweed i.e. is documented to work well (Kazinczi et al. 2008b, Holst 2009). Using competing plants against ragweed combined with mowing showed high efficacy in reducing or totally deleting all ragweed individuals in different trials. Meiss et al. (2008) and Meiss (2010) documented that ragweed grown together with high densities of Lucerne and cut 7 times was outcompeted by 100 % after few cutting dates. The same holds for the competition experiment with ragweed grown at different densities together with 3 different restoration seed mixtures by Milakovic et Karrer (2010) and (2011)) (see also Karrer et al., 2011). Almost all ragweed plants died during the first half of the experiments, obviously caused by the additive effect of damage due to cut and competition. In the glasshouse experiment conducted by Milanova et al. (2010), *Lolium perenne* and *Dactylis glomerata* showed to be successful in outcompeting common ragweed when whole turfs were planted: number of emerged common ragweed plants was decreased by 40% and 36%, respectively. The fresh biomass per pot was best reduced by *Lolium perenne* planted as whole turf or sown (96% and 97%, respectively). In this experiment Lucerne showed also an inhibitory effect on the growth of common ragweed, reducing its fresh biomass per pot by 91%.

The growth type of the competing plants must be optimally adapted to the intensive cutting regime. Therefore the seed mixtures used for the experiments consisted of 20 to 40 % *Lolium perenne* which is well adapted to frequent cuts by intensive basal tillering. This grass develops a dense lawn near the soil surface and regenerates within few days thus shading the resprouts of ragweed from its basal nodes. The very few resprouts that recovered could not produce a reliable number of seeds.

Conclusions

Control options against common ragweed comprise of herbicide applications and several non-chemical measures, both summarized by Buttenschøn et al. (2010). Hand pulling is generally the cheapest and most efficient control option against small populations (less than 100 individuals).

Fumanal et al. (2007) made clear that pollen and seed production was closely related to plant volume and biomass, thus providing a means of estimating potential pollen and seed production in given target areas. Such biological data could be integrated into population management strategies or into airborne pollen modelling.

Cutting experiments designed to decrease the pollen production do not consider the problem of seed production from regenerated shoots.

Basset and Crompton (1975) overdue their conclusion from the quick 100 % regeneration after one cut when they claim "several cuts during August". Based on the experience of Bohren et al. (2008a), Karrer et al. (2011), Simard and Benoit (2011), Karrer and Milakovic (2011) and Pixner (2012), Karrer and Pixner (2012) a three weeks interval between the cuts from July to September should be enough to prohibit the development of ripened seeds above the cutting line. Even post-harvest ripening of seeds on shoots left to the habitat could be avoided by 100 %.

Of course, the cutting height is problematic, because the regrowth from nodes below the lowermost realistic cutting height of 5 cm (Simard and Benoit 2011, Karrer et al. 2011, Milakovic et al. 2014b) can produce seeds anyway. Thus, regrowth should be counteracted by desired strong competitors like *Lolium perenne* (Karrer et al. 2011, Milakovic and Karrer 2009, Milakovic and Karrer 2010).

Preliminary Recommendations:

EPPO (2008) recommend fairly the same option for ragweed control like Bohren et al. (2008 c) and Karrer et al. (2011). A late first mowing just at the beginning or shortly after the start of male blooming is accepted by all scientists. Considering the detected post-harvest ripening of seeds on cut branches (Pixner 2012, Karrer and Pixner 2012, Karrer et al. 2012) we would recommend subsequent cuts every 3 weeks. Four (EPPO 2008) or more weeks (Bohren et al. 2008a) would enable serious seed production from cut branches. This means at least four cuts from mid/end of July until end of September.

Aiming at prohibiting the seed production a first cut latest mid of August and one or two subsequent cuts would give optimal results (Bohren et al. 2008a, Karrer et al. 2011, Karrer 2012).

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Identification of correct timing of mowing based on mowing in the most vulnerable phenological stages of ragweed

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This experiment produced efficacy data for mechanical measures (mowing) in correlation with ragweed (*Ambrosia artemisiifolia* L.) development. The influence of different mowing regimes on ragweed was investigated in this trial.

Material and methods

Pot trial was carried out to check the possibility to completely prevent the pollen and seed formation by mowing ragweed plants only twice a season. We tried to mimic the development of ragweed plants growing on the highway margins and frequency of mowing of highway vegetation performed by highway Maintenance Company.

For each treatment there were 5 pots (10 L) with 5 common ragweed plants. Mowing was performed at 3 cm above soil surface.

Experimental treatments:

3 growth stages of first mowing: 2 leaves – 1. node, 4 leaves - 2. node, 8 leaves – 3. Node.

A. Mowing regime for second mowing: no additional mowing, after 4 weeks, after 6 weeks, after 8 weeks, after 12 weeks.

B. Mowing regime for second and third mowing: after 4 weeks – after 3 weeks, after 4 weeks – after 6 weeks, after 6 weeks – after 3 weeks, after 6 weeks – after 6 weeks, after 8 weeks – after 3 weeks, after 8 weeks – after 6 weeks, after 12 weeks – after 3 weeks, after 12 weeks – after 6 weeks

Ragweed plants were grown in plastic pots (10 l). 5 plants of ragweed were grown in each pot. Plants were mowed at different developing stages (2 leaves – 1. node, 4 leaves - 2. node, 8 leaves – 3. node) using scissors and we cut them at height of 3 cm above the soil level. Mowing was performed once, twice or three times a season in different time intervals (4, 6, 8 or 12 weeks).

In total there were 40 combinations of intervals between mowing and growing stages of plants at period of first mowing. Percentage of plants producing flowers, percentage of plants developing fertile seeds, amount of seeds produced per plant (pot) and fresh plant mass per pot at the end of October was measured.

Results

- One or two mowing of ragweed plants is not sufficient to completely prevent pollen and seed production.

-Our results indicate that pollen and seed production can be largely (-90 %) prevented with two optimal cuts at proper development stage

- The reduction of produced seed is higher if the first mowing is performed at higher growth stage of plants (end of June or later).

- Ragweed plants produced less seed if time intervals between successive mowing are longer, especially in case if first mowing is performed at 2 leaves growth stage.

- If highway maintenance service decides to perform just two mowing a season, than first mowing should not be performed earlier than 3 nods growth stage and second mowing not earlier than 12 weeks after the first one.

- The most efficient system for pollen and seed production prevention is to perform first mowing at 3 node growth stage, repeat mowing after 8 weeks, and then the third one after 12 weeks.

Regrowth of common ragweed after mowing at different growing stages (II)

This experiment produced efficacy data for mechanical measures (mowing) in correlation with common ragweed development and the height of mowing. Besides the mowing, influence of the competition between common ragweed and other weed species was investigated in this trial.

Experimental treatments

1. Two mowing heights (3 cm and 6 cm above the soil surface)

2. Three growing stages (heights) of common ragweed at first mowing (20 cm, 40 cm, 60 cm)
3. Two time intervals between cuts (after 5 and 10 weeks)

4. Competition between common ragweed and other plants (no competition, competition with *Lolium* and *Chenopodium*)

Material and methods

For each treatment there will be 5 pots (10 L) with 5 common ragweed plants (and 5 weed species in case of competition). This pot trial was also performed to mimic the conditions of ragweed development on the margins of highway. The trial setup was the same like in trial one. 5 ragweed plants were competing with 5 lamb's quarters plants (*Chenopodium album*), or with 5 ryegrass plants (*Lolium perenne)*. Seeds of all plant species were sown together and thinning of seedlings in the cotyledon stage was performed.

Both ragweed and competitor plants were mowed by scissors at different ragweed plant heights (20, 40 and 60 cm high plants) at level of 3 cm above ground. At the end of season (end of October) plants were weighed, number of seeds produced per plant was determined and the portion of plants that developed seeds was calculated. Percentage of plants that producing flowers, Percentage of plants developing fertile seeds, amount of seeds produced per plant (pot) and fresh plant mass per pot were measured at the end of October.

Results

-The greatest dry matter reduction after cutting was determined, when ragweed was grown in the mixture with ryegrass

- The regeneration capacity of ragweed exposed to competition to other weeds after mowing is significantly lower when compared development to environment without competition with other plants

-Cutting height (3 and 6 cm) influenced ragweed dry matter and seed production only when ragweed in monoculture was grown in the pots; it increased at lower mowing height

-Dry matter and seed production of ragweed significantly decreased with ragweed first cut at later growth stages and increased period between two cuts

-Our results indicate that pollen and seed production can be completely prevented with two optimal cuts at proper development stage (40-60 cm and 10 week time interval).

Identification of correct timing of mowing based on mowing in the most vulnerable phenological stages of ragweed.

Mechanical control: Mowing

1a. Improving efficiency of mechanical ragweed control of urban areas based on mowing in the most vulnerable phonological stages of the plant

1b. Identification the optimal time of mowing that most effectively decreases the biomass, number of male inflorescences, pollen release and seed production of ragweed.

2. Material and methods

2.1. Ragweed mowing experiment was carried out in the experimental field of the Plant Protection Institute of Hungarian Academy of Sciences at Nagykovácsi (47º 32′ N, 18º 56′ E). The experiment was set up on a land, which was abandoned for three years with the only disturbance of autumn ploughing and seed bed preparation in April. Prior to set up the mowing experiment seed bed preparation was done in the middle of April; secondary tillage was carried out with harrow and cultivator. After emergence of ragweed plants, on 5 May 10x10 m plots were stacked out. Plots were separated with 1 m wide land stripes of boundaries. The stripes were kept weed free by regular cultivator treatments. Number of ragweed plants was counted on randomly selected 10x1 m^2 areas.

2.2. Experimental treatments included: in 2011 none-mowed control, early mowed treatment BBCH 51(inflorescences, flower buds visible), late mowed BBCH 59 (first flower petals visible) twice mowed treatment BBCH 51 and 59. In course of the mowing the plants were cut at the height of 5-7 cm in 2011 by traditional scythes and in 2012 to improve efficiency of mowing (decreased cutting height) to 1-3 cm Husqvarna, 128 R loanmower was used.

In 2012 and 2013 treatment included none-mowed control, early mowed treatment BBCH 51, late mowed BBCH 59, twice mowed treatment: first mowing BBCH 51 and the second one was made when re-growth terminal racemes reached BBCH 59 and in 2013 mowing 3 times BBCH 51, 59and 59treatments. The cutting height of the plants was 2-3 cm in 2012 and 2013 due to changing the traditional scythes into Husqvarna loan mower.

During the study plots in 4 replicates were randomly designed. Plants were sampled at weekly intervals 5 randomly selected plants were cut off at soil surface level from each plot (20 plants/treatment altogether). Plants were transferred into the laboratory, where the above ground fresh biomass and the plant height were measured, further male inflorescences and female flowers were counted.

For pollen production studies two plants on each plot were selected (4x2 plants/ treatment) to collect pollen. Transparent polyethylene bags for pollen collection were placed on the plants at BBCH 60 (first flower petals open sporadically) (Hess et al., 1997). Each plant was covered with a plastic bag that gave sufficient room for the growth. The non-mowed and early mowed plants were covered by 120x40 cm polyethylene bags. Plants of the late mowed, twice mowed treatments were covered with 80×40 cm polyethylene bags. Plants of mowing three times treatment were covered with 50 × 40 cm polyethylene bags. For ventilation purposes he bottom corners of the bags were opened on a 5 mm wide and 15 mm long surfaces, which served as ventilation holes just like the 10 randomly pricked 1.0-1.5 mm holes on each bag. The bigger holes served to fix the bags with a pulled trough string to the wire frame. The opening of the polyethylene bags were fixed to the wire frame and closed on the main stems of the ragweed plants under the lowest side shoots with the aid of an adhesive rubber. The polyethylene bags were replaced by new ones weekly, when the pollen content of the bags were washed off in 250 ml of 0.02 % Tween 20 detergent solution. The pollen containing solution was stirred by a glass rod than 5×1 ml samples were collected into Eppendorf tubes. Eppendorf tubes were labelled and stored in refrigerator until pollen counting. After thorough shaking from each Eppendorf tube 2.5µl samples were taken and individually transferred into a glass hemacytometer (MOM Budapest). Pollen grains were counted on 160 × magnification by means of a light microscope. Based on the numbers of 5 counts the number of pollen grains in 250 ml water was calculated.

Pollen production study was carried out in 2011 and 2012, because counting the pollen grains is a labour-consuming activity. We spent 5 months with counting the pollen grains during the first two years of the study.

Statistical analyses. Data were analyzed by ANOVA using STATISTCA, StatSoft, Inc., 2007 program package. The effect of the mowing treatments on the plant above ground fresh biomass, plant height, number of male inflorescences and number of female flowers during the whole season was evaluated by Tukey HSD test.

Results 2011

In the first year of the study the height of the mowing was 5-7 cm. Using Husqvarna, 128 R loan mower it was not possible to decrease the cutting height.

Fig. 1. Due to the 5-7 cm cutting height ragweed plants produced intensive side shoot formation. The higher the cut stem more internodes' are situated on it. The side shoots develop from the buds of the internodes.

The ANOVA revealed significant effect of mowing treatments on the plant above ground fresh biomass, plant height, number of female flowers, number of male inflorescences in 2011. *F* values are: 273, 687, 107, 1643, respectively (n=640). The *P* values are <0.000. Mowing treatments significantly influenced the number of released pollen grains as well *F*=72, n=32 *P*<0.000.

Fig. 2 Mowing induces intensive ramification.

The Tukey HSD test revealed the significant difference between the above ground biomass, plant height and the number of male flowers of non-mowed control plants and those of the early mowed plants (Table 1.). However, the number of female flowers and number of pollen grains did not decrease significantly due to early mowing. Due to late and twice mowing there was no significant difference between mowing treatments at the above ground fresh biomass, plant height, number of female flowers and number of male inflorescences. However, the number of pollen grains decreased in a greater extent due to double mowing compared to late mowing. The decreasing effect of twice mowed treatment reached 80 percent at the measured plant parameters (Figs. 3-6).

Non-mowed control plants released 59 million pollen grains during pollination. Although, the pollen reducing effect of the best mowing twice treatment was only 85 % mowing treatments shipped the beginning of pollen releasing period. The flowering of male inflorescences started on non-mowed control plants started on 25 August and lasted for six weeks. Early mowing postponed pollination by three weeks. However, due to late and twice mowing the pollen production started 6 weeks later and it lasted for 4 weeks. Early and late mowing not only postponed the beginning of pollination, but the intensity of pollen production also decreased significantly (Fig. 7, 8).

Table 1. The effect of mowing treatments on the above ground biomass, plant height, number of female flowers, male inflorescences, number of released pollen grains of ragweed plants and the percent reduction due to mowing treatments. Juliannamajor, Budapest 2011.

Treatment	Valid No	Mean± S. E.	Min	Max	% reduction
Above ground biomass (g)					
None-mowed	220	28.33±1.37 a	4.00	275	0.00
Early mowed	200	18.41±0.90 b	0.40	99	35.02
Late mowed	120	5.52±0.34 c	0.30	26	73.64
Twice mowed	140	7.47±0.38 c	0.60	34	80.64
Plant height (cm)					
None-mowed	220	100.60±1.13 a	47.00	146	0.00
Early mowed	200	47.36±1.10 b	4.70	103	53.03
Late mowed	120	25.45±0.68 c	0.70	47	74.80
Twice mowed	140	20.84±0.67 c	5.50	55	80.28
Number of female flowers					
None-mowed	220	636.76±12.90 a	0	6456	0.00
Early mowed	200	413.70±10.34 ab	0	1582	35.04
Late mowed	120	170.01±5.90 bc	0	687	73.30
Twice mowed	140	107.22±6.78 c	0	714	83.16
Number of male inflorescences					
None-mowed	220	2753.72±121.80 a	0	18580	0.00
Early mowed	200	1292.93±68.65 b	0	5860	53.05
Late mowed	120	328.36±16.64 c	0	1700	88.08
Twice mowed	140	181.41±19.67 c	0	595	93.12
Number of released pollen grains (millions)					
None-mowed	48	59.435±7.67a	39.32	109.47	0.00
Early mowed	32	43.460±1.13a	31.68	58.13	26.88
Late mowed	32	24.309±3.02b	14.12	35.88	51.10
Twice mowed	32	8.668±1.56c	2.91	17.59	85.42

Means with different letters are significantly different $p<0.05$ (Tukey HSD test)

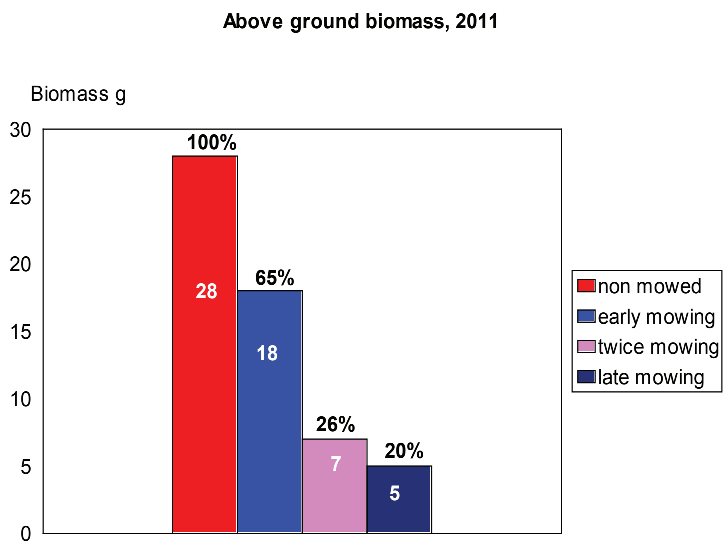

Fig. 3. The effect of mowing treatments on the development of above ground plant biomass. Budapest 2011.

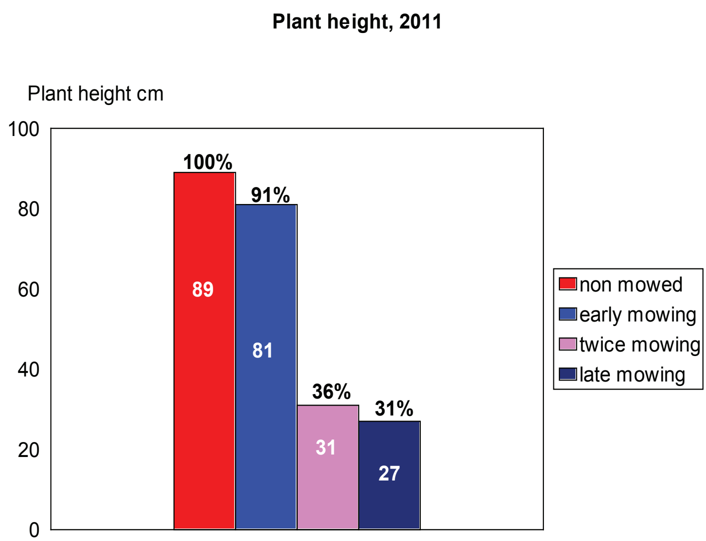

Fig. 4. The effect of mowing treatments on the plant height. Budapest, 2011.

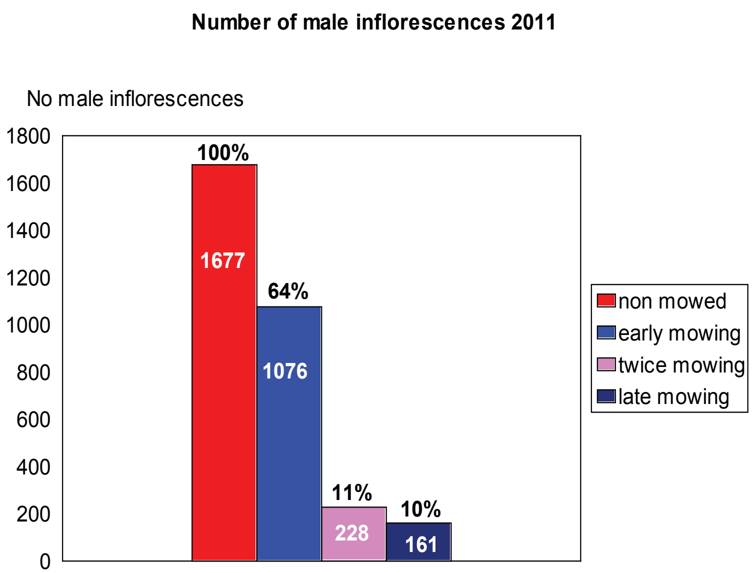

Fig. 5. The effect of mowing treatments on the number of male inflorescences. 2011.

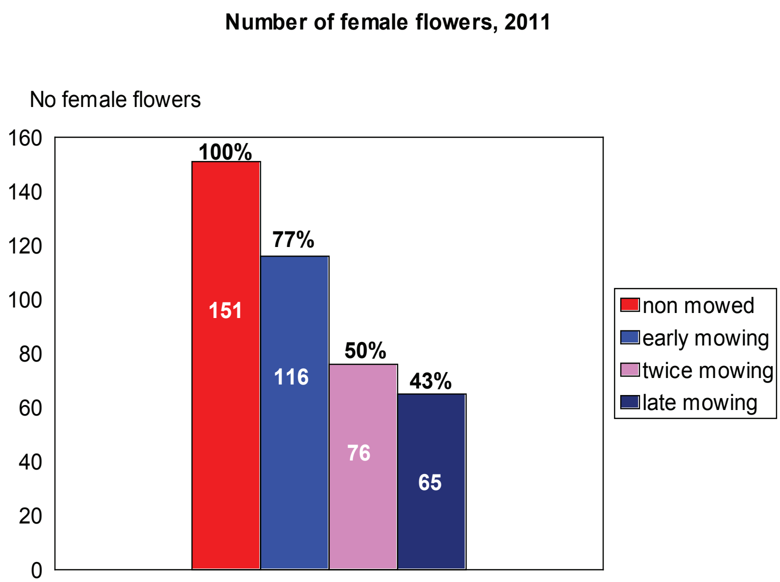

Fig. 6. The effect of mowing treatments on the number of female flowers. Budapest, 2011.

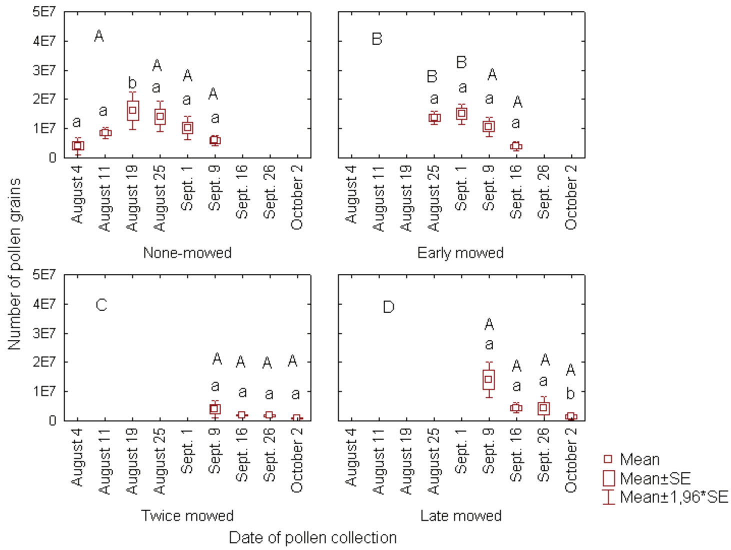

Fig. 7. The effect of mowing treatment on the number of released pollen grains and the length of the pollen production period. Budapest 2011.

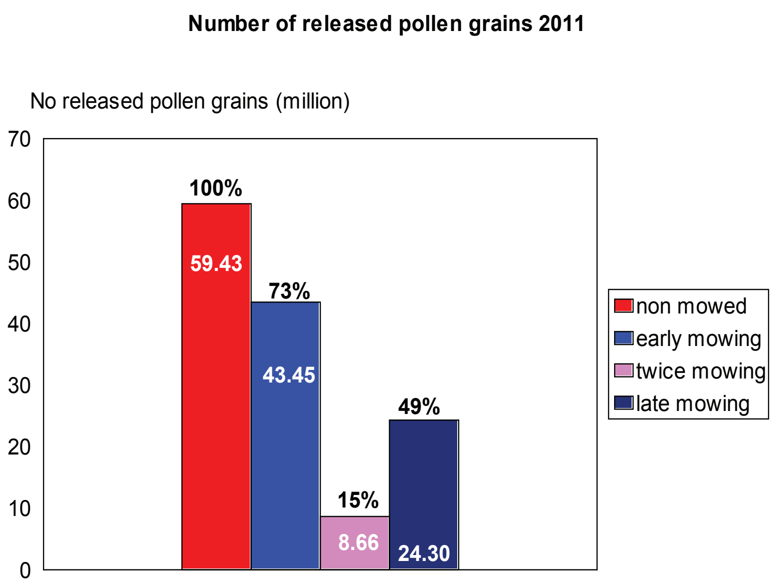

Fig. 8. Effect of mowing treatments on the number of the released pollen grains. Budapest, 2011.

Results 2012

In the second year of the study the traditional scythes was replaced by Husqvarna, 128 R loan mower . With the loan mower the cutting height of the plans could be reduced up to 2-3 cm.

Fig. 9 The early mowed plants in 2012

Due to the 1-3 cm mowing height, the mowing treatments significantly affected above ground plant biomass, plant height, number of female flowers, number of male inflorescences ANOVA. The *F* values are: 281, 163, 68, 129, respectively, n=1220 $P<0.000$. The mowing treatments significantly affected the number of released pollen grains as well $F=82$, n=40, $P<0.000$.

Fig. 10. The late mowed plants in 2012

Fig. 11. The twice mowed plants in 2012

Fig. 12. The three times mowed plants in 2012

Fig. 13. The non-mowed control plants

Table 2. The effect of mowing treatments on the above ground biomass, plant height, number of female flowers, male inflorescences, number of released pollen grains of ragweed plants and the percent reduction due to mowing treatments. Juliannamajor, Budapest 2012.

Treatment	Valid No	Mean± S. E.	Min	Max	% reduction
Above ground biomass (g)					
None-mowed	220	84.89±4.85a	2	303	0.00
Early mowed	240	15.51±0.93b	1	88	81.23
Late mowed	180	4.26±0.24c	0.2	25	94.08
Twice mowed	280	4.75±0.33c	0.2	37	94.41
Mowing 3 times	300	3.81±0.34c	0.2	65	96.52
Plant height (cm)					
None-mowed	220	82.77±1.64a	19	150	0.00
Early mowed	240	43.56±1.46b	5	93	43.38
Late mowed	180	22.66±0.72c	5	44	72.63
Twice mowed	280	19.05±0.57cd	4	56	76.45
Mowing 3 times	300	17.24±0.58d	3	65	70.92
Number of female flowers					
Non-mowed	220	663.16±75.51a	18	2550	0.00
Early mowed	240	171.11±19.49b	20	1430	74.20
Late mowed	180	68.14±6.37bc	6	480	89.75
Twice mowed	280	33.82±3.38c	2	288	95.03
Mowing 3 times	300	13.35±1.41c	2	194	97.44
Number of male inflorescences					
None-mowed	220	4638±406.91a	26	36.443	0.00
Early mowed	240	874±80. 18b	25	6877	81.16
Late mowed	180	186±18.09bc	18	1321	96.00
Twice mowed	280	55±4.97 c	14	530	98.82
Mowing 3 times	300	32±4.62 c	3	626	99.32
Number of released pollen grains (millions)					
None-mowed	8	155.295±134.492a	103.860	196.720	0.00
Early mowed	8	44.452±3.870 b	24.860	62.640	71.38
Late mowed	8	35.342±4.711 bc	61.340	22.700	73.25
Twice mowed	8	8.905±1.382 cd	17.020	4.840	94.27
Mowing 3 times	8	2.272±378 d	4.020	680	98.54

Means with different letters are significantly different *p*<0.05 (Tukey HSD test)

Due to mowing treatments the above ground biomass, plant height, number of female flowers, number of male inflorescences and number of released pollen grains significantly decreased (Table 2.). There was significant difference between early and late mowed treatments. However, there was no significant difference between twice and three times mowed plants (Figs. 14-19).

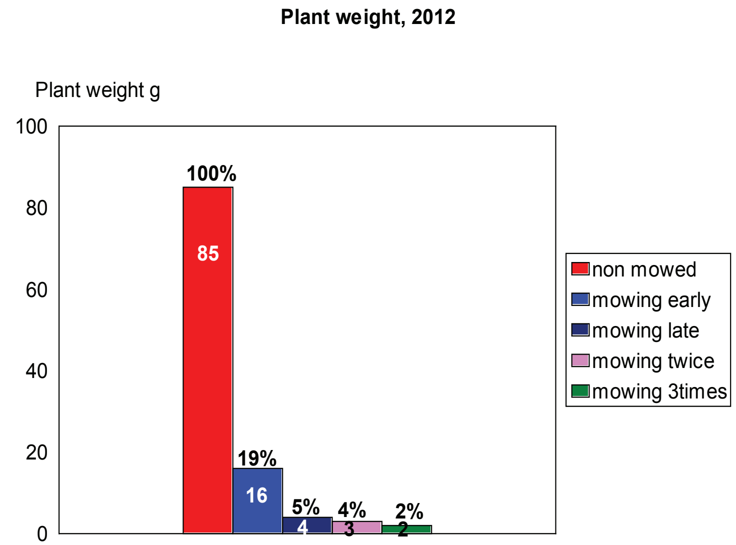

Fig. 14. The effect of mowing treatments on the development of above ground plant biomass. Budapest 2012.

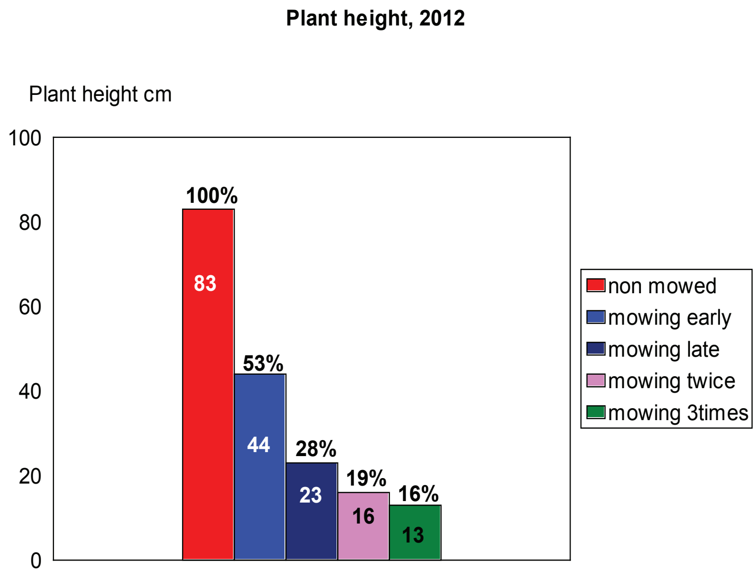

Fig. 15. The effect of mowing treatments on the plant height. Budapest, 2012.

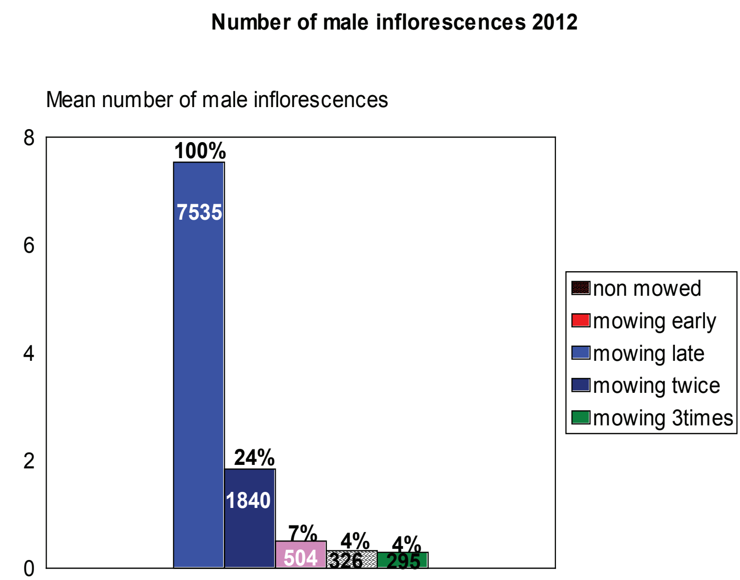

Fig 16. The effect of mowing treatments on the number of male inflorescences. 2012.

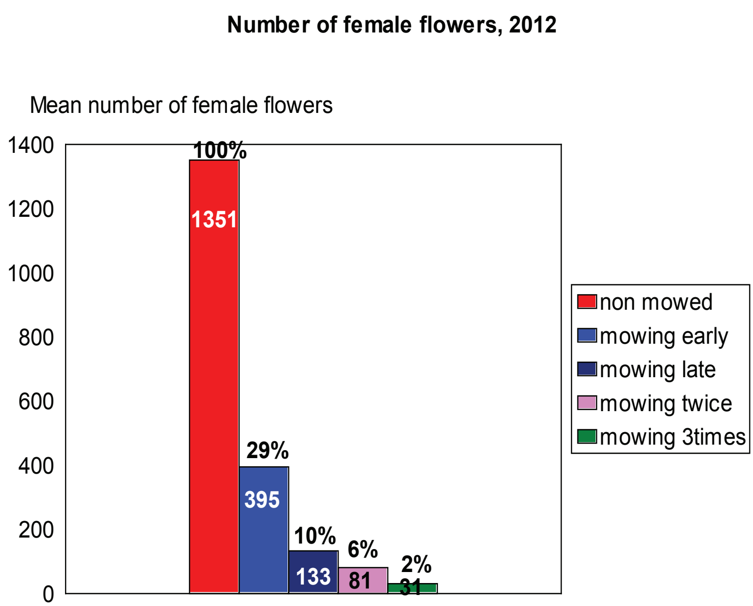

Fig. 17. The effect of mowing treatments on the number of female flowers. Budapest, 2012

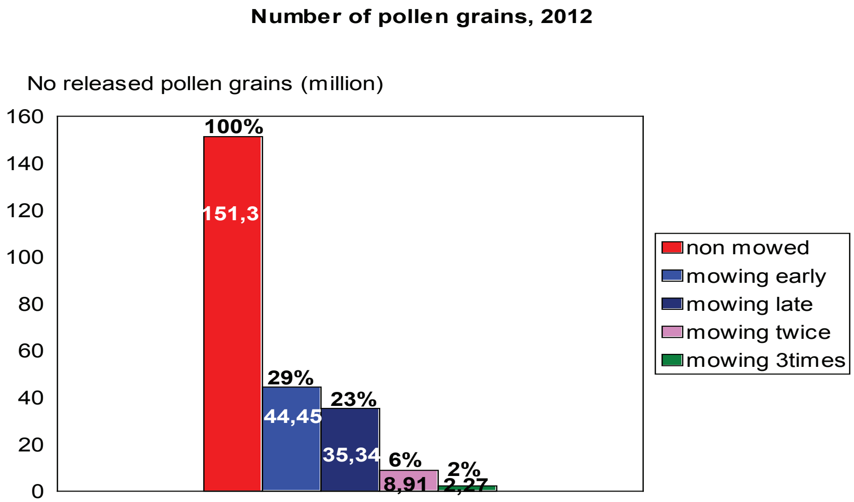

Fig. 18. The effect of mowing treatments on the number of released pollen grains. Budapest, 2012

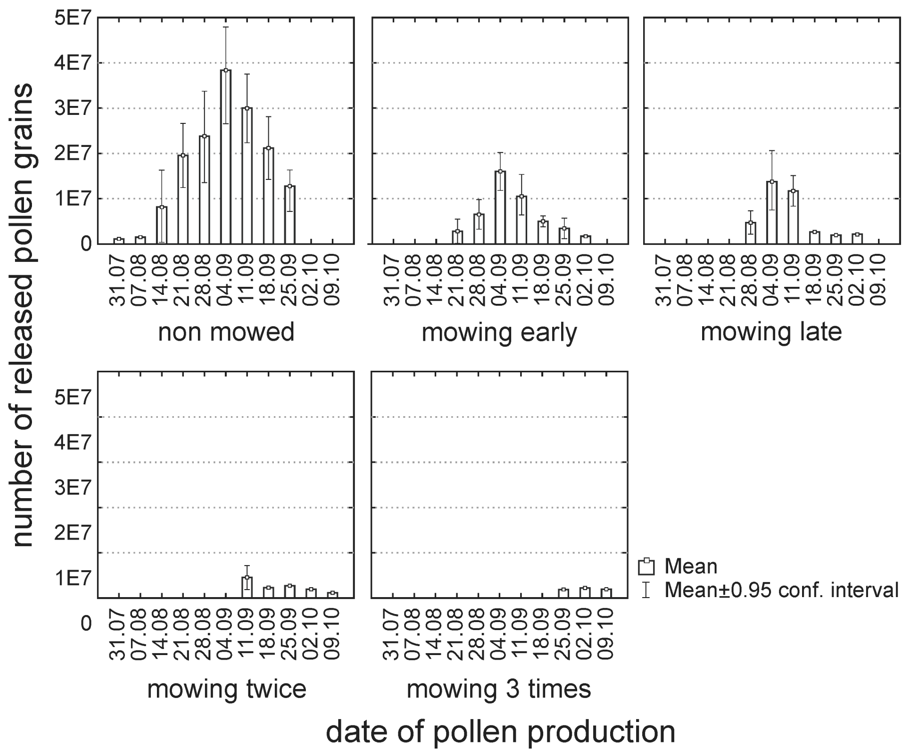

Fig.19. The effect of mowing treatment on the number of released pollen grains and the length of the pollen production period. Budapest 2012.

Results 2013

The mowing treatments significantly affected above ground plant biomass, plant height, number of female flowers, number of male inflorescences ANOVA. The *F* values are: 238, 742, 267, 68, respectively, n=1460 $P<0.000$.

Table 3. The effect of mowing treatments on the above ground biomass, plant height, number of female flowers, male inflorescences, ragweed plants and the percent reduction due to mowing treatments. Juliannamajor, Budapest 2013.

Treatment	Valid No	Mean± S. E.	% reduction
Above ground biomass (g)			
None-mowed	300	44. 48±1. 71a	0.00
Early mowed	300	19. 12±0. 71b	57.02
Late mowed	260	13. 31±0. 67c	70.08
Twice mowed	300	11. 72±0. 56c	73.66
Mowing 3 times	300	7.92±0.49d	82.20
Plant height (cm)			
None-mowed	300	90.84±1.19a	0.00
Early mowed	300	55.44±1.08b	38.07
Late mowed	260	36.93±0.99c	59.35
Twice mowed	300	30.72±0.80d	67.19
Mowing 3 times	300	23.35±0.87e	74.30
Number of female flowers			
Non-mowed	300	445.43±36.15a	0.00
Early mowed	300	187.97±12.39b	57.08
Late mowed	260	268.93±19.37c	39.96
Twice mowed	300	107.58±10.55d	75.96
Mowing 3 times	300	22.32±3.50e	95.06
Number of male inflorescences			
None-mowed	300	2099.45±91.12a	0.00
Early mowed	300	783.19±40.25b	62.70
Late mowed	260	594.90±41.74b	71.71
Twice mowed	300	207.88±20.16c	90.10
Mowing 3 times	300	72.91±11.97c	96.53

Means with different letters are significantly different $p<0.05$ (Tukey HSD test)

Due to mowing treatments the above ground biomass, plant height, number of female flowers, number of male inflorescences significantly decreased compared to none mowed control (Table 3.). Apart from the number of male inflorescences there was significant difference between early and late mowed treatments. In 2013 there was significant difference between twice and three times mowed plants except the number of male inflorescences (Figs. 20-21).

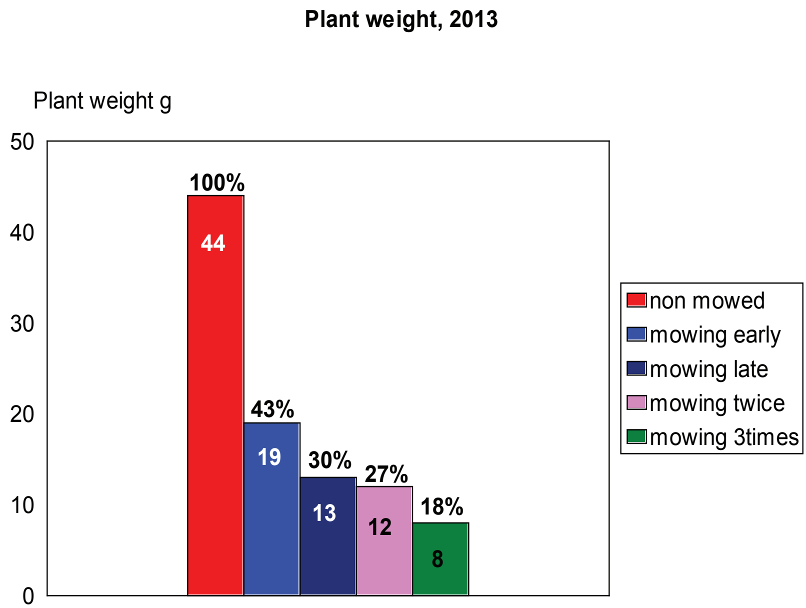

Fig. 20. The effect of mowing treatments on the development of above ground plant biomass. Budapest 2012

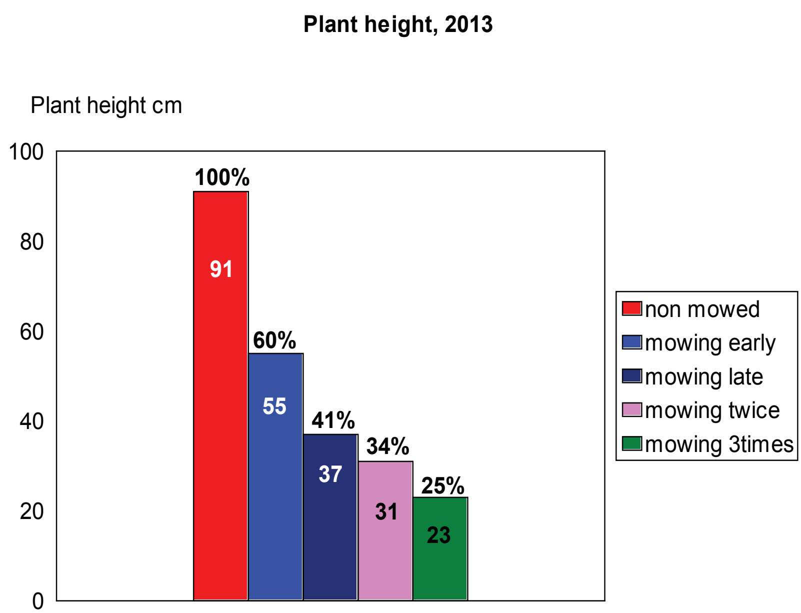

Fig. 21. The effect of mowing treatments on the plant height. Budapest, 2013.

Conclusions

We managed to decrease the cutting height up to 1-3cm by using the Husqvarna lawn mower. The low cutting height resulted in increased male inflorescence and seed decreasing efficiency. Number of female flowers, male inflorescences and pollen grains decreased more than 70 % even due to one early mowing. Late mowed treatment decreased the flowers by 90 %, but pollen grains only 77 %. Twice mowed treatment resulted in 94 % reduction of the reproductive parts. Mowing three times resulted in reduced seed, male inflorescence and pollen production between 97.7-98.5 %.

The seed production decreasing effect has great importance. Up to now results of the mowing experiments showed efficient pollen decreasing effect, however, mowing was not considered to be an efficient method to decrease seed production. The seed decreasing effect of the present study proves that mowing before flowering low (1-3 cm) cutting height results in proper seed production reduction.

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Experiments on non-chemical and integrated control strategies

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Introduction

Experiments on the competitive exclusion of common ragweed by crops or co-occurring vegetation differ by the duration. On farmland, short-term experiments help to understand the control options by integrating crop selection, seeding date and mechanical intervention. On abandoned land and restoration sites (like roadsides) intermediate- and long-term experiments are needed to select for the best control options. Within HALT-Ambrosia we set up short-term experiments on farmland and in pots as well as long-term experiments on roadsides. The latter were initiated even earlier but followed further during this project.

Suppressing common ragweed biomass with integrated farming methods

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Common ragweed can be a strong competitor to open row crops like sunflowers, maize, potatoes, pumpkins and legumes and can lead to high yield losses. But it also reacts very sensitively to competition. Therefore field trials were conducted in 2011, 2012 and 2013 with sunflower, maize and horse bean respectively. The treatments were the same for sun flower and maize: two row spacing with 35 and 70 cm widths (8 plants*m^{-2} in each case) in combination with or without undersown white clover (*Trifolium repens*). Horse bean was sown in 25 and 50 cm row widths with 40 plants*m^{-2} in each case and with or without perennial ryegrass (*Lolium perenne*). 2 g of common ragweed was sown along one metre between two rows in the middle of each plot and were thinned out at the four-leaf stage to five plants per metre (one plant every 20 cm). The common ragweed was harvested when its growth stage was in the range of beginning of budding until beginning of flowering in each year. At the same time the sunflower, maize and horse bean plants directly neighbouring on the left and right side of the 1 m common ragweed row were harvested too. Fresh matter of sunflower, maize and horse bean and dry matter of common ragweed was determined in order to detect the impact of row spacing and the undersown crop on common ragweed, sunflower, maize and horse bean biomass. The plots were irrigated if necessary.

Significantly lower (*P<0.05) dry matter of common ragweed was found in narrowly spaced sunflower and maize plots with undersown white clover compared to the other treatments. Fresh matter of sunflower and maize therefore was not affected by wide or narrow spacing or by undersown clover.

The horse bean plots showed different results: significantly lower (*P<0.05) dry matter of common ragweed was found in the plots with the undersown crop and in the narrow spacing plots. In the wide spaced plots common ragweed had the highest dry matter yield. The same was determined for the horse bean fresh matter: plots with the undersown crop and the narrow spaced rows affected the fresh matter of horse bean negatively. The results show that there is an impact of competition on dry matter of common common ragweed and it can be assumed that seed production would be reduced as well. While sunflower and maize dry matter was not affected by narrow spacing and / or the undersown crop, horse bean reacted sensitively to this integrated methods with lower fresh matter yield.

Outcompeting common ragweed by sowing different seed mixtures combined with various cutting regimes

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This experiment was originally set up during the Austrian national RAGWEED-project (Karrer et al. 2011) and continued during the HALT-Ambrosia project. Three seed mixtures (1: 15% *Festuca ovina*, 35% *Lolium perenne*, 42% *Festuca rubra rubra*, 5% *Lotus corniculatus*, 3% *Medicago lupulina*; 2: Mixture 2: 8% *Festuca ovina*, 47% *Festuca r. rubra*, 5% *Festuca r. trichophyla*, 40% *Lolium perenne*; 3: 10% *Lotus corniculatus*, 10% *Poa pratensis*, 15% *Festuca rubra*, 30% *Lolium perenne*, 25% *Festuca ovina*, 10% *Festuca arundinacea*) were combined with three different densities of common ragweed (*Ambrosia artemisiifolia*) (0 Ragweed plants/m^2,100 Ragweed plants/ m^2 und 500 Ragweed plants/m^2) and 4 different mowing regimes that were developed in agreement with the road maintenance services. Gravel was the regularly used type of soil material – not very friendly for development of vegetation. In the first year (2010) germination rates of common ragweed was very low but that of the intended competitors even lower. In the second year germination rates and biomass production of competitors increased. But common ragweed benefited by that (facilitation affect by accumulated biomass). In the 3rd and 4th year (2012, 2013) facilitation effects still can be seen but no serious competitive depression by the seeded plants.

We conclude that this experiment makes evident that utilisation of competitive effects by roadside vegetation on common ragweed is not possible if adverse soil material is used for road shoulders. These results are in contrast to pot experiments under greenhouse conditions where co-occurring vegetation was able to outcompete common ragweed almost completely (Milakovic & Karrer, 2011, Karrer *et al*. 2011).

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Mowing regime experiment on field roadside populations of common ragweed

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The vegetation of roadside shoulders is mown regularly for road security reasons. In the areas where *Ambrosia artemisiifolia* (common ragweed) is present in the roadside vegetation, several problems occur if the mowing management is not carefully timed. Common ragweed plants resprout from and come to flowering again, which can be even more massive than without cutting.

In both data analysis of data 2009-2011 and of those from 2012, mowing regimes 3 (first cut at the beginning of female flowering in the third week of August and second cut at the beginning of seed set in the second week of September) and 5 (first cut just before male flowering in the third week of July, second cut in the third week of August (at the beginning of female mass flowering) and third cut in the second week of September (at the beginning of seed set)), showed very efficient for management aiming to reduce seed production, as well as regarding their influence on the phenological development of common ragweed. These results confirm results of glasshouse experiments (Milakovic *et al*. 2013a) that showed that an August cut (just before or in the beginning of the female flowering period) is essential for management success.

In Milakovic *et al*. (2013b), we conclude that the best management solution along roadsides in order to primarily reduce seed production and simultaneously limit as much as possible the pollen release, would be a compromise between the cutting regimes 3, 4 and 5.

Considering the results of the Trial B.2-1, which show that the mowing regime 3 was by far the most efficient in reducing the soil seed bank, we conclude that a first late cut just at the beginning of appearance of the female flowers, followed by a second cut in September, before the new female flowers can be built, is the best option for long term management.

However, optimal cutting dates cannot always be brought to practice. For roadsides where the first cut must happen earlier than August for security reasons, we suggest an initial mowing at earliest in the third to fourth week of June, followed by subsequent cuts every three to four weeks as long as plants grow. It should be noted that the effect of the different mowing regimes is subject to variations in the execution of the management (in reality, deviations from plan up to one month can be expected) as well as climatic variations from year to year).

This time interval should of course be adapted depending on the speed of development of common ragweed in the respective climatic region. We strongly discourage 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.

As a general rule, we advise that common ragweed plants should be cut as low as possible, in order to exterminate most buds that might be able to resprout. Common ragweed cannot be prevented from regenerating flowers below the cutting height. In any case, to optimize efficiency any mowing plan must be finely tuned to the local phenological development by monitoring some representative populations once a week during the vegetation period. A management should not be tuned to fixed calendar dates as the climatic conditions can vary from year to year and influence the phenological development of the plants.

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The influence of different catch crops incorporated into the soil to ragweed competition in following crops

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This experiment produced efficacy data for evaluation of influence of different cover crops sown in cereal stubbles and incorporated into the soil before sowing main crops in the following year. Besides the influence of different main crops and their sowing dates on common ragweed density and development were evaluated.

Material and methods

10 different catch crops were sown into cereal stubbles in August 2010. Catch crops - plant species in Randomized Complete Block Layout trial:

1. Untreated control
2. *Fagopyrum esculentum* (Čebelica)
3. *Helianthus annuus* (PR64H45), 65.000 seeds/ha
4. *Avena sativa* (Noni)
5. *Lolium multiflorum* (KPC laška)
6. *Guizotia abyssinica* (Mungo), 10 kg/ha
7. *Camelina sativa* (12 kg/ha)
8. *Raphanus sativus* L. var. *oleiformis* Pers. (Rauola), 30 kg/ha
9. *Brassica napus* L.var. *napus f. biennis* (Starška)
10. *Trifolium incarnatum* (Inkara)
11. *Phacelia tanacetifolia* (Balo), 15 kg/ha

In 2011 the cover crops residues have been incorporated into the soil before 3 different crops have been sown. Each main plot was divided to four subplots where spring wheat (sown on 11th March 2011), spring barley (sown on 24th March 2011) and maize (sown in two different times, 16 March and 30 March 2011). Main plot size: 8 m x 17 m (136 m^2). The following parameters were reported: weed species (according to the EPPO-Code, weed number per species, total weed coverage (%) visually assessed and total weed biomass (dry matter), estimated at the last evaluation.

Results

All cover crops displayed strong suppressive effect and decreased ragweed density and coverage compared to the control plots in fall of 2010. In contrast, no significant effect of catch crops on ragweed coverage and dry matter production in wheat, barley and maize plots in the spring of the following 2011 season was determined.

Italian ryegrass and buckwheat were germinating in the spring and appearing as volunteer weeds, so their use is not recommended. In barley, wheat and maize, the greatest weed suppressive effect was exhibit by oats, buckwheat and niger seed, where weed coverage decreased compared to the control plots, where these catch crops were not incorporated.

Growth and development of common ragweed (*Ambrosia artemisiifolia* L.) under different nitrogen, water and competition levels

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Objective of the experiment was to determine effect of various nitrogen levels, soil moisture level and competition levels on the growth parameters of common ragweed (*Ambrosia artemisiifolia*).

Material and methods

Greenhouse pot experiment with randomized treatments in temporal blocks. Experiment was established as a factorial design with four replications. Two watering levels (50 % and 90 % of pot water-holding capacity), three randomized nitrogen levels (10, 50, 100 kg/ha) and three common ragweed competiton levels with no competition (one common ragweed plant in the pot), medium competition level (one common ragweed and one grass) and high competition level (one common ragweed and five grasses) were selected as factors. Italian ryegrass (*Lolium multiflorum* L.) was chosen as competitor. Five destructive harvests were conducted throughout the life cycle to determine Common ragweed morphological and physiological parameters (leaf, stem, inflorescences, total dry matter) in growth stages V6 (6 leaf), V10, V14, full flowering and physiological maturity.

Results

The leaf, stem, total dry matter and leaf area of single-grown common ragweed responded to medium and high nitrogen levels, whereas under neighbouring competition with Italian ryegrass, higher nitrogen levels were required to observe a response. Common ragweed performance was strongly decreased by interspecific competition with Italian ryegrass. Increased resource availability enhanced competition intensity. Nitrogen affected seed production only in no competition stands.

Medium competition reduced the total dry matter by up to 58 %, whereas high competition reduced it by up to 85 %. Reproductive output was also strongly affected by competition. Medium competition reduced the seed weight per plant by up to 83 %; high competition reduced it further by up to 91 %. The higher water level had a weak effect on growth parameters, but only in the absence of competition. The greatest relative growth rate was determined at early vegetative V10 growth stage. Relative growth rate was affected by competition and water level; however the relative growth rate under various nitrogen availability levels was similar. Common ragweed is not a strong competitor in resource-rich conditions, but results under moderate water stress and low nitrogen inputs showed that common ragweed growth was not greatly affected by moderate competition. Our results indicate that low-water and low-nutrient environments with an absence of competition are critical factors for the successful establishment and further spread of common ragweed.

Competitiveness of common ragweed against different plant species

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In additive pot experiment the effect of competitor plant species (*Festuca rubra, Lolium perenne, Plantago major*) was investigated on common ragweed. The densitiy of common ragweed was constant, while the density of the other (competitor) plant species was varied.

Factors:

A: plant species: *Festuca rubra* cv. Light; *Lolium perenne* cv. Lipresso; *Plantago major*.

Seeds were provided by JKI. *L. perenne* and *F. rubra* from Deutsche Saatveredelung AG, Weissenburger Strasse 5, D-59557 Lippstadt; *P.major* from Templiner Kräutergarten, Elsternest 1, 17268 Templin, Deutschland

B: densities/proportions: 100%, 50% and 25% of the three competitor plant species; common ragweed always 4 single plants in the centre of the pots (Table 1.; Figure 1.)

Table 1. Density of competitor plants

	Plantago major GA* unknown		*Lolium perenne* GA 93%		*Festuca rubra* GA 83%	
	seeds/m^2	seeds/pot	seeds/m^2	seeds/pot	seeds/m^2	seeds/pot
100%	2500	100	1970	80	2340	96
50%	1250	50	921	40	1170	48
25%	625	25	460	20	585	24

*GA: germination ability

Variants: 10

Replicates: 4

Pot size: 20 x 20 x 6 cm

Assessment: fresh and dry shoot weight of Ambrosia and other competitior plants/pot

Figure 1. Competiton between common ragweed and *Lolium perenne* in additive pot experiments. The densitiy of common ragweed is constant, while the density of the other plant species is varied.

Biomass production (fresh and dry shoot weight) of ragweed increased as the other plant's (*P. major, F. rubra, L. perenne*) density decreased. Biomass production of the other (competitor) plant's increased with the increased plant density but at higher densities the effect of intraspecific competition between individuals could be observed. *L. perenne* had the strongest competitive ability followed by *P. major* and *F. rubra* (Table 2, Figure 2).

Table 2. The change of fresh and dry shoot weight of common ragweed and competitor plants for a pot due to the different plant density (fresh shoot weight/dry shoot weight)

Densities of competitor plants	*A. artemisiifolia+P.major*		*A. artemisiifolia+L. perenne*		*A. artemisiifolia+F.rubra*	
	A. artemisiifolia	*P.major*	*A. artemisiifolia*	*L. perenne*	*A. artemisiifolia*	*F. rubra*
100%	$11.3^{c}/3.4^{d}$	$17^{a}/5^{a}$	$6.7^{c}/2.6^{d}$	$16.45^{a}/4.6^{a}$	$27.65^{c}/7.9^{c}$	$5.9^{a}/0.93^{a}$
50%	$20.8^{b}/6.5^{c}$	$10^{b}/2.5^{b}$	$18.5^{b}/5.4^{c}$	$12.7^{a/}3.9^{a}$	$61.9^{b}/16.6^{ab}$	$5.1^{a}/0.8^{a}$
25%	$24^{b}/8.4^{b}$	$7.75^{b}/0.75^{c}$	$38^{a}/7.9^{b}$	$4^{b}/1.4^{b}$	$69^{b}/18.3^{b}$	$2.4^{b}/0.39^{b}$
0%	$44.6^{a}/14.6^{a}$	-	$44.6^{a}/14.6^{a}$	-	$44.6^{a}/14.6^{a}$	-
			$LSD_{5\%}$			
	5.66/1.58	3.37/1.37	7.44/0.71	4.19/0.91	7.65/2.43	1.85/0.24

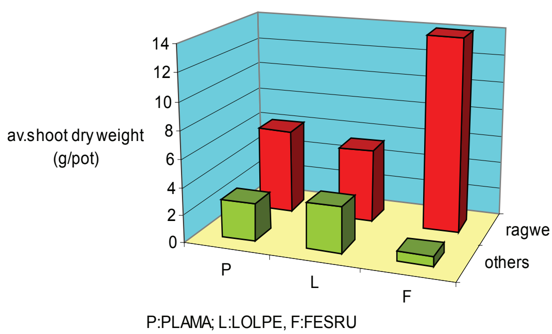

Figure 2. The average shoot dry weight/pot of *A.artemisiifolia* and competitor plants

Control of common ragweed in ALS herbicide-resistant sunflower hybrids (*Helianthus annuus*)

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Summary

Common ragweed is the number one weed in Hungary: it covers ca. 5% of the arable land, causing huge losses in row crops. In addition, because of the high allergenicity of its pollen, common ragweed is a huge burden on the health care system of the country. In 2011 and 2012 field studies were carried out in order to evaluate the common ragweed control efficacy of two acetolactate-synthase inhibitor postemergence herbicides (imazamox and tribenuron methyl) in sunflower hybrids NK Neoma and PR63E82, respectively, that carry the resistance gene against such herbicides. Common ragweed control by these herbicides was excellent: they suppressed the growth of the weed plant until the canopy closure of the crop plant (8-leaf stage). Common ragweed plants germinating after this date were unable to compete with the crop: although they survived, they remained small (ca. 75% reduction in height), produced ca. 90% less male flowers (source of the allergenic pollen), and caused no significant reduction in the crop yield. In areas where sunflower germination was poor, however, a second, mechanical common ragweed control measure was necessary to keep the weed density below damaging levels.

Introduction

The main cause of allergy and pollen asthma in North America and Central Europe is pollen from ragweed (*Ambrosia* spp.) a widespread genus in the Asteraceae (Cecchi et al., 2006). In Europe short or common ragweed (*A. artemisiifolia*) is prevalent (Grangeot et al., 2006). In Hungary, common ragweed infestation is heaviest in sunflower (*Helianthus annuus*), the third most important crop of the country (also an Asteraceae plant, thus, a botanical relative of common ragweed). In August-September common ragweed produces the overwhelming majority of allergenic pollen in the air ever in urban areas (Cecchi et al., 2006) (Cecchi et al., 2007). Increasing importance of bioenergy production together with recent advances in improving the dietary value of sunflower oil (Binkoski et al., 2005) (Edgerton, 2009) will certainly increase the production area of sunflower in the future. Thus, there is an urgent need for new tools to improve the control of common ragweed in this crop.

It is important to note that about seventy percent of the global sunflower harvest is produced in the European Union, Russia and Ukraine, where ragweed is spreading rapidly (Streit, 2012).

Recently, several new sunflower hybrids were registered in Hungary, including PR63E82 (Pioneer, Johnston, IA, U.S.A.) and NK Neoma (Syngenta, Basel, Switzerland), that are resistant to the acetolactate synthase-inhibiting herbicides tribenuron methyl and imazamox (Figure 1), respectively. These herbicides are known to control broadleaf weeds, such as common ragweed, efficiently (Merotto et al., 2009)the multilocus (tm. It is important to note that the new sunflower hybrids were developed by traditional plant breeding methods. Thus, they are not considered as genetically modified (GM) crops (Green and Owen 2011), and as a result, they can be produced in Hungary (and in other EU countries) where GM plants are not allowed.

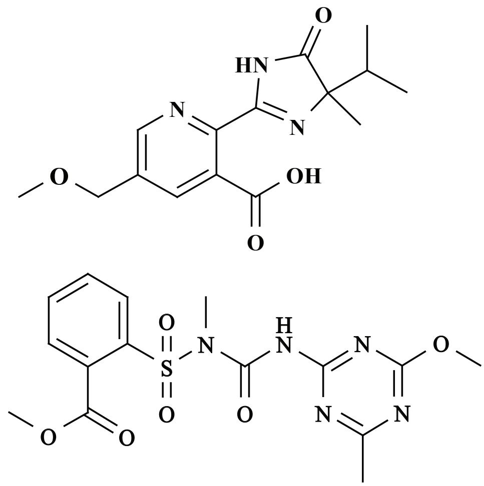

Figure 1. Chemical structures of imazamox and tribenuron methyl

Herbicide-tolerant crops were introduced into weed management practice about two decades ago. Most widely the RoundupReady™ (developed in 1994) and the LibertyLink™ (developed in 1992) GM-based technologies are used: these are founded on the application of the herbicides glyphosate (N-[phosphonomethyl]glycine) and glufosinate (2-amino-4-[hydroxymethylposphinyl]butanoic acid), respectively (Green and Owen, 2011) (Benbrook, 2012).

The first commercial imidazolinone tolerance trait (Clearfield™) in sunflowers (Sala et al., 2012)but the concomitant herbicide effect over the root system has not been reported. The objective of this work was to quantify the root biomass response to increased doses of imazapyr in susceptible (ahasl1/ahasl1 was developed from imidazolinone-tolerant wild sunflowers that were discovered in the USA in 1996 (Al-Khatib et al., 1998). Tolerance to sulfonylurea herbicides was obtained using induced mutagenesis (Streit, 2012) and led to the development of the ExpressSun™ technology (Bulos et al., 2013)SURES, and CLPlus are three herbicide tolerance traits in sunflower (Helianthus annuus L..

Materials and Methods

Field experiments

Sunflower hybrids PR63E82 and NK Neoma (21 ha each) were seeded in a commercial farm in Gyor-Kismegyer, Hungary, in Mollic Fluvisol soil (3.75% organic matter, pH 7.6) containing large seed banks of weeds and common ragweed in particular. The crops were sown between April 20 and 25 at planting density 55,000 plants ha^{-1}. Imazamox (Pulsar 40 SL, 40 g a.i. L^{-1}, BASF AG, Ludwigshafen, Germany) and tribenuron methyl (Express 50 SX, 50 g a.i. L^{-1}, DuPont, Wilmington, DE, U.S.A.) herbicides were applied post-emergence at the 4-6-leaf growth stage between May 21 and 25 at the recommended rates of 1.2 L ha^{-1} and 45 g ha^{-1}, respectively, using a Berthoud sprayer (Berthoud, Belleville, France) with an application rate of 220 L/ha, a spray pressure of 300 KPa and Visiflo TP8005 nozzles (Teejet Technologies, Wheaton, IL, U.S.A.). In the field ten 2x2 m sampling plots were randomly assigned. Half of the sampling areas were covered during the application of the herbicides: these plots served as untreated controls. Weeds at the sampling sites were surveyed as described previously(Reisinger et al., 2005).

During the weed surveys herbicide damage to the crop plants, if any, has also been recorded.

Meteorological data: During the registration of pollen counts, wind speed and wind direction were determined using a Weather Station WS-3600 instrument (Conrad Electronic SE, Hirschau, Germany).

Sunflower yields: Sunflower yields were recorded by the fields' owner.

Results

Weed surveys carried out before the application of the herbicides showed high weed densities in all fields (Table 1). Both tribenuron methyl and imazamox were highly efficient in controlling weeds in sunflower: plots planted with the herbicide-resistant sunflower remained free of common ragweed until the end of June (Table 1). Tribenuron methyl provided less control of barnyardgrass (*Echinochloa crus-galli*) and proso millet (*Panicum miliaceum*). In July and August a small number of common ragweed plants emerged (weed cover < 1%): they were ca. 75% shorter and produced more than 90% less male flowers than the untreated controls (Table 1). Established common ragweed plants were found only in untreated sampling sites and in areas where sunflower crop plants poorly germinated.

Average yields of the new hybrids (Table 1) were slightly but not significantly higher than that of the average local sunflower hybrids used in the region (2.22 ± 0.42 t ha^{-1}, provided by six growers).

Table 1: 2011. Data collected on August 31, sunflower harvest date September 17. Ragweed data in the herbicide-treated plots were determined from the few surviving plants.

Hybrid / Herbicide	Total weed cover (%)	Ragweed cover (%)	Number of male flowers per plant	Ragweed height (cm)	Sunflower yield (t/ha)
NK Neoma / Imazamox	1.2 ± 0.8	0.0	216 ± 80	27.4 ± 8.7	2.6
PR63E82 / Tribenuron methyl	4.6 ± 2.4	0.0	260 ± 118	26.0 ± 6.8	2.3
Untreated	97.0 ± 2.1	41.0 ± 14.6	3968 ± 1278	99.1 ± 19.3	n.d.

Table 2: 2012. Data collected on August 31, sunflower harvest date September 11. Ragweed data in the herbicide-treated plots were determined from the few surviving plants.

Hybrid / Herbicide	Total weed cover (%)	Ragweed cover (%)	Number of male flowers per plant	Ragweed height (cm)*	Sunflower yield (t/ha)
NK Neoma / Imazamox	1.0 ± 1.0	0.0	276 ± 113	28.4 ± 5.9	2.6
PR63E82 / Tribenuron methyl	3.7 ± 2.4	0.0	333 ± 182	27.0 ± 5.9	2.3
Untreated	97.4 ± 2.3	39.4 ± 14.6	4488 ± 753	101.8 ± 16.8	n.d.

Discussion

As regards to control of common ragweed both herbicides gave excellent results. It should be noted that various perennial and annual grasses may be poorly controlled by some sulfonylurea herbicides (Sikkema et al. 2007): in our study tribenuron methyl provided less control of barnyardgrass (*Echinochloa crus-galli*) and proso millet (*Panicum miliaceum*). Pollen production by common ragweed is at its maximum from late August to early September (Cecchi et al. 2006). Our weed surveys carried out repeatedly at the end of August showed that the few common ragweed plants (weed cover < 1%) emerging in July and August could not efficiently compete for light, water, and nutrients in established sunflower stands. Reduced common ragweed density, plant height and number of pollen-producing flowers practically halted release of common ragweed pollens from stands of herbicide-resistant sunflowers (Table 1).

In conclusion, our study clearly indicates that the new technology based on the use of sunflower hybrids resistant to ALS-inhibiting herbicides is a highly efficient tool to control common ragweed in sunflower fields and, as a result, to reduce concentrations of its allergenic pollen in the air. Major factors for the success of common ragweed control when using this technology will be 1) the management of resistance due to recurrent use of ALS-inhibiting herbicides (Délye et al., 2009) herbicide-resistant alleles","container-title":"Weed Research","page":"326-336","volume":"49","issue":"3","abstract":"Lolium species (ryegrasses, 2) the control of volunteer sunflowers (Yu et al., 2010)the effect of resistance mutations on AHAS functionality and plant growth has been investigated for only a very few mutations. This research investigates the effect of various AHAS resistance mutations in Lolium rigidum on AHAS functionality and plant growth. The enzyme kinetics of AHAS from five purified L. rigidum populations, each homozygous for the resistance mutations Pro-197-Ala, Pro-197-Arg, Pro-197-Gln, Pro-197-Ser or Trp-574-Leu, were characterized and the pleiotropic effect of three mutations on plant growth was assessed via relative growth rate analysis. All these resistance mutations endowed a herbicide-resistant AHAS and most resulted in higher extractable AHAS activity, with no-to-minor changes in AHAS kinetics. The Pro-197-Arg mutation slightly (but significantly expressing ALS-resistance genes when emerging in following crops (Breccia et al., 2013), and 3) the accuracy of herbicide application, since under extreme weather conditions the new sunflower varieties may suffer from herbicide damage.

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Efficacy of different herbicides on common ragweed in oil pumpkins (*Cucurbita pepo*)

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Oil pumpkins are economically important crop in Austria, Slovenia and Hungary. Limited choice of available herbicides and poor control of ragweed in areas infested with this species, represent a great problem for oil pumpkins growers.

The experiment produced ragweed efficacy data for 6 herbicides, which are used in oil pumpkins in Slovenia and in some other EU countries.

The experimental design was a randomized complete block with four replicates with plot size 25 m2. Following herbicides were tested:

Table 1. List of herbicide treatments for ragweed control in oil pumpkins

No	Herbicide	Test/ refer.	Active ingredients	Formul.	Rate g, ml, a.s./ha	Rate kg, l/ha
1	Centium 36 CS	R	clomazone 360 g/L	CS	90	0,25
2	Successor 600	R	pethoxamid 600 g/L	EC	1200	2,0
3	Flexidor	T	isoxaben 500 g/L	SC	375	0,25
4	Flexidor	T	isoxaben 500 g/L	SC	375	0,75
5	Centium 36 CS + Successor 600	R	clomazone 360 g/L pethoxamid 600 g/L	CS EC	90 1200	0,25 2,0
6	Centium 36 CS + Dual gold 960	R	clomazone 360 g/L S - metolachlor 960 g/L	CS EC	90 1200	0,25 1,25
7	Flexidor + Dual gold 960 + Centium 36 CS	T	isoxaben 500 g/L S - metolachlor 960 g/L clomazone 360 g/L	SC EC CS	375 1200 90	0,25 1,25 0,25
8	untreated	-	-	-	-	-

Table2. Efficacy of selected herbicides for ragweed control in oil pumpkins

No	Herbicide	Active ingredients	Rate: L, kg/ha:	Efficacy (%)	Average (%)
1	Centium 36 CS	clomazone	0,25	0 - 0	0,0 a
2	Successor 600	pethoxamid	2,0	0 - 0	0,0 a
3	Flexidor	isoxaben	0,25	15 – 20	17,5 c *
4	Flexidor	isoxaben	0,75	50 – 90	75,0 d *
5	Centium 36 CS + Successor 600	clomazone pethoxa-mid	0,25 2,0	0 – 5	1,25 b *
6	Centium 36 CS + Dual gold 960	clomazone S-metolachlor	0,25 1,25	0 - 0	0,0 a
7	Flexidor + Dual gold 960 + Centium 36 CS	isoxaben S-metolachlor clom-azone	0,25 1,25 0,25	20 – 30	25 c *
8	untreated	/	/	/	/

* Different letters indicate significant differences between treatments with Tukey HSD test (P<0,05).

Conclusions

Common ragweed in oil pumpkins was controlled only by application of higher rate of Flexidor (isoxaben) however its efficacy varied greatly.

Common ragweed cannot be sufficiently controlled with available herbicides in oil pumpkins, therefore mechanical measures have to be implemented in order to achieve sufficient ragweed control.

Efficacy of bio-herbicides against ragweed

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The results of these experiments are being prepared for journal submission, so just a short summary is given below.

Summary

Pelargonic acid and acetic acid are bio-herbicides which are registered for non-cropping uses in some countries (Germany, Switzerland, USA). Both acids are found in nature and are degraded rapidly. Pelargonic acid and acetic acid are contact herbicides which cause necrosis on direct contact with plant tissue while uncovered plant parts like the root, will stay intact.

Pot experiments were conducted in Germany, Denmark and Slovenia in 2012 using a common protocol. Pelargonic acid and acetic acid were applied simultaneously at two growth stages of *Ambrosia artemisiifolia* (BBCH 14-16 and BBCH 22-25) in a spray cabinet (Germany and Denmark) or using a hand-held sprayer (Slovenia). Each bio-herbicide was applied at 5 dosages as a single application and as a split application with 50% at the first application and 50% 10 days later. Two bio-herbicides registered for use in Germany were included: Acetic acid and pelargonic acid.

The plants were harvested four weeks after the first application. Fresh weight biomass of the above ground ragweed plants was recorded.

Results of the experiments in Germany and Denmark showed no benefit of split- compared to single application of acetic acid and pelargonic acid. Based on ED_{90} doses in L/ha, pelargonic acid was more active than acetic acid. Some results in Slovenia differed from those obtained in Germany and Denmark. The discrepancies might be related to different climatic conditions and to different application methods resulting in different coverage of plant surface.

Overall the results show, that there is a potential for bio-herbicides to control ragweed on small scale areas where synthetic herbicides and mechanical treatments are not allowed or possible.

Report on the feasibility and benefits of spot spraying

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Summary

In recent years two private farms in Zimany and in Gyor-Kismegyer (in South and North Hungary, respectively) established and systematically improved their spatial information infrastructure and generously allowed us to carry out research and development studies on site-specific weed management methods. Over the past three years, our primary goal was to improve common ragweed (*Ambrosia artemisiifolia*) control efficacy and to reduce the amounts of the herbicides used for this purpose. We tested the potential of different site-specific methods of herbicide applications to control common ragweed under field conditions. Thus, in wheat stubble we applied the non-selective (total) herbicides glufosinate and glyphosate by using WeedSeeker (NTech Industries) sensor-spot sprayers, and in maize and sunflower we used map-based site-specific application of preemergent herbicides, in combination with spot-spraying glyphosate under the leaf canopy according to the newly developed in-row treatment method that uses mechanically shielded WeedSeeker sprayers mounted on a precision cultivator (Garford Farm Machinery). Precision weed control methods showed higher than 95% weed control efficacy (resulting in fields practically free of common ragweed), and, depending on the weediness of the plot, up to 60% reductions in the amounts of herbicide used.

Introduction

During the last quarter century agriculture in Hungary has been completely restructured because of landslide political and social changes. Most importantly, small private farms replaced the large state-owned cooperatives. Unfortunately, the majority of the new enterprises lacked and many of them still lack the equipment and professional knowledge necessary for good agricultural practice. As a result, agricultural output (quantity and quality) sharply declined for many years and high weed infestations in agricultural fields became a major problem (still unsolved today: large seed banks of noxious weeds can be found in the soils of the majority of farmlands), with common ragweed (*A. artemisiifolia*) having the highest cover. Therefore, in plant protection research high priority was given to studying efficient methods of controlling common ragweed. Aiming at reducing environmental and human health hazards as well as costs of weed control by herbicides we focused our efforts on using methods of precision weed management to suppress germination and growth of common ragweed.

Generally, map-based and online techniques are used for controlling weeds in a site-specific, precision manner (Reisinger et al. 2012). The map-based method involves thorough weed scouting, preparation of precision treatment maps, and patch herbicide application. On-line techniques, on the other hand, use real-time, sensor-driven site-specific, spot spraying weed management methods (Andujar et al. 2012; Moshou et al. 2013; Torres-Sanchez et al. 2013) to overcome many of the scouting and map-making costs (Swinton 2005).

We investigated the common ragweed controlling efficacy of precision applications of the non-selective herbicides glyphosate and glufosinate using the WeedSeeker spot sprayer alone (for controlling common ragweed in wheat stubble) or as component integrated into complete weed control technologies in maize and in sunflower.

Materials and Methods

Field experiments

Investigations were carried out in 2011-2013 in Zimány (Somogy county, Hungary) and in Gyor-Kismegyer (Gyor-Sopron county, Hungary) in agricultural fields managed by Farkas, Ltd. and Megyer-Agro Ltd., respectively. The soil types in Zimany and in Gyor-Kismegyer are Eutric Cambisol and Mollic Fluvisol, respectively. Soil nutrient contents were determined in 2008 with a "one sample per 3 ha" sampling frequency. Precision application of herbicides and precision mechanical weed control followed previous lines (Reisinger et al. 2007). Fields in Zimany and Gyor-Kismegyer were well managed: the soils contained only medium levels of viable weed seeds and vegetative propagules. In both locations common ragweed was the dominant weed but in Zimany pigweed (*Amaranthus reroflexus*), lambsquarters (*Chenopodium album*), barnyard grass (*Echinochloa crus-galli*), Bermuda grass (*Cynodon dactylon*), and curly-top knotweed (*Polygonum lapathifolium*) and in Gyor-Kismegyer maple leaf goosefoot (*Chenopodium hybridum*), lambsquarters, annual mercury (*Mercurialis annua*), and jimson weed (*Datura stramonium*) were also present.

Precision weed control in sunflowers - the map-based method

2013

Sunflowers were seeded with ±2-cm accuracy (AgGPS autopilot system; Trimble, Sunnyvale, CA, USA). Immediately after seeding a herbicide combination consisting of Racer (250 g fluorochloridone) and Gardoprim Plus Gold (312 g S-metolachlor + 187 g terbutylazin; all Syngenta, Switzerland) was applied. Standard doses of the above herbicides were 2.0 and 1.25 l/ha, respectively.

Soil samples were taken with a "one sample per 3 ha" frequency. Standard methods (Reisinger et al., 2008) were used to determine the soil plasticity index of Arany (KA) and humus contents (H). These data were used to determine the herbicide doses applied at a given location in the field according to the empirical equation:

Dose = Min + 0.011(Max - Min) (KA +9.0H)

in which Min and Max are the minimum and maximum recommended doses of the herbicide and the two site-specific variables are H and KA (Reisinger et al., 2008). These parameters of the soil in the particular field were only slightly variable, resulting in minimum and maximum spray volumes of 250 and 260 l/ha, respectively, within the registered dose range of the herbicide (220 to 270 l/ha).

Herbicides were applied by a Spidotrain 2800/18 RAU machine (Kverneland Group, Kvernaland, Norway), equipped with 12004 IDKT nozzles (Lechler GmbH, Metzingen, Germany). The instruction data set was uploaded in the tractor's on-board computer. After the calibration and setup was completed, spraying was controlled by the high-accuracy DGPS system and the on-board computer.

Plants were seeded and the pre-emergent herbicide combination was applied on April 16. Precipitation was nearly equal to the average: in April, May, and June a total of 46.4 mm, 78.7 mm, and 68.3 mm rainfall was recorded, respectively.

Precision weed control in maize - the shielded sprayer method (2011)

Earlier observations, recently summarized by Novak et al. (2009), suggested that in Hungary post-emergent weed control alone may be insufficient because of the large size of the weed seed-banks in the fields. Therefore, we designed a combination of pre-emergent and post-emergent herbicide treatments, applying the latter ones against emerging perennial weeds using a sensor-spraying equipment to control the weeds growing between the crop rows.

Investigations were carried out in Zimány in a 4 ha maize fields (soil type: Eutric Cambisol) managed by Farkas, Ltd. During seeding, rows were recorded with ±2 cm accuracy. The field is infested by

Bermuda grass (*Cynodon dactylon*). For the experimental post-emergence treatments a cultivator frame (Garford Farm Machinery, Peterborough, UK) was attached to the tractor. On the frame seven plastic-container shielded WeedSeeker (NTech Industries, Ukiah, CA, USA) sensor-sprayers were mounted 76 cm apart (Figure 1). WeedSeeker sensor sprayers are optoelectronic devices, in which an optical system analyzes the wavelength of reflected infrared light. Light reflected from chlorophyll containing plants activates the spray nozzle (LU 12004, Lechler GmbH, Germany). During our experiments, sprinkler heads were shielded by 60 cm diameter flexible plastic containers (Figures 1 and 2). The tractor carried a 1000-liter water tank and an injector (Dosatron, Dallas, USA) to add formulated herbicide concentrates (glyphosate: Amega 480SL, 48% glyphosate ammonium active ingredient; Nufarm GmbH, Austria) amounts proportional to the volume of the spray solution.

Figure 1. WeedSeeker sensor-sprayers shielded by plastic container

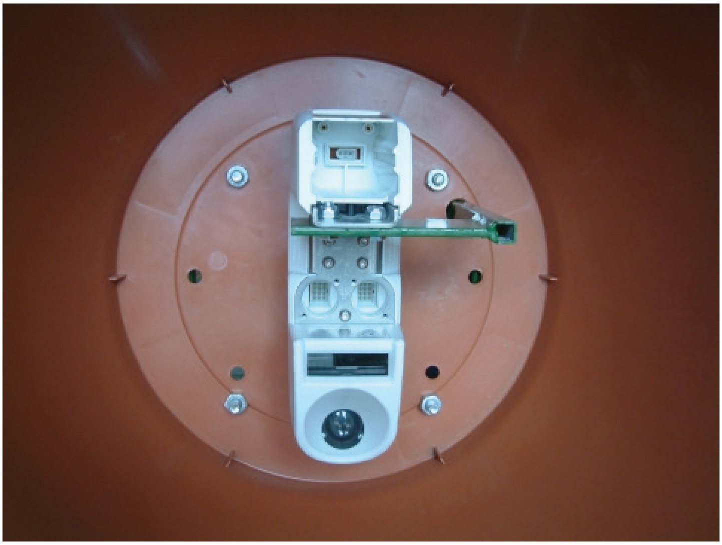

Figure 2. WeedSeeker sensor-sprayer shielded by plastic container (bottom view)

Precision weed control in wheat stubble by spot spraying

Common ragweed seedlings emerge in ripening wheat and after harvest they grow and develop very rapidly reaching 100% cover by the middle of August (Figure 3). Therefore, control of common ragweed in wheat stubble is very important.

2011

Experimental site: a 12-ha wheat stubble field in Zimany with 10 selected sampling sites (1m x 10m, characterized by GPS coordinates), 5 of which are in the herbicide treated area and 5 untreated controls. Soil: Eutric Cambisol, 2.16% organic matter, pH 6.8, containing a large weed seed bank. Winter wheat was harvested on July 05. Weed control on the stubble was done by applying glyphosate using WeedSeeker spot sprayers on August 15 (Common ragweed growth stage BBCH51). Herbicide efficacy survey: August 31.

Figure 3. Emerging common ragweed seedlings in ripening wheat.

2012

Experimental field: 14.8 ha wheat field in Zimany (plot codename: Szentgalosker). Wheat variety: 200 kg/ha Antonius. Date of wheat harvest: July 07. Wheat yield: 6.0 t/ha. Stubble tillage: cultivator on July 14. Date of herbicide application: September 02. Herbicide: Finale 14 SL (150 g/L glufosinate ammonium, Bayer Crop Science), with an application rate of 5 L/ha. Sample sites: altogether ten (2x2 m size, five sprayed with the herbicide and five untreated control). Weed control efficacy was assessed on September19.

2013

Experimental site: a 0.5 ha wheat stubble experimental field at Gyor-Kismegyer with 10 selected sampling sites (2m x 2m, characterized by GPS coordinates, 5 of which are in the herbicide treated area and 5 untreated controls). Wheat harvest: on July 08. Stubble tillage: cultivator on July 16. Weed control by applying Finale 14 SL (150 g/L glufosinate ammonium, Bayer Crop Science), with an application rate of 5 L/ha using WeedSeeker spot sprayers on August 26. (Common ragweed growth stage BBCH51). Herbicide efficacy surveys: on September 05 and September 21.

Results

Precision weed control in sunflower

Following the completion of the herbicide treatment, a spraying map was constructed using the data recorded by the tractor's on-board computer.

Weed control efficacy was first evaluated on June 05, when sunflowers were in 6-8 leaf stage. The field was completely weed-free and there were no phytotoxic symptoms on the crop plants (Figures 4 and 5).

Figure 4. Untreated control area and weed-free sunflowers

The second weed scouting was performed on July 11, during the time of sunflower blooming. Again, the field was completely weed-free.

Figure 5. Weed-free sunflowers (July 11)

Although herbicide saving in this particular field was not significant (<2%), no herbicide phytotoxicity to the crop plants was observed: their fitness was excellent and the yield high (3.6 t/ha).

Precision weed control in maize

In maize, the use of precision weed control by applying pre-emergent herbicides on 75.4 hectares led to a 14% reduction in herbicide use and to savings 10.3 €/ha. The maize field remained weed-free until the end of the growing season (Figure 7).

Figure 6. Maize field after spot-spray treatment with glyphosate (untreated area in the back)

In Hungary, pre-emergent herbicides are still used widely, although it is known that these herbicides cannot control perennial weeds (e. g. Canada thistle [*Cirsium arvense*]), and are inefficient in the absence of soil humidity. To improve weed control in such cases, we developed a method in which glyphosate is sprayed by WeedSeeker sensors directed under the canopy of the crop plant.

Figure 7. Control of Bermuda grass (Cynodon dactylon) in maize by precision application of glyphosate

It is interesting to note that the precision application of glyphosate on leaves of Bermuda grass (*Cynodon dactylon*) between the rows led to an efficient control of this weed within the row, too (Figure 8), because the herbicide was translocated within the plant to parts of the plant that were unexposed.

Following the development of the method, precision pre-emergence herbicide applications in maize were successfully used in increasing areas around Zimany, expanding to 201 hectares in 2011.

Precision weed control in wheat stubble

2011 - 2013

In all three years of the investigations a single spot spraying of the weeds growing in winter wheat stubble (Figure 8) either by glyphosate or glufosinate led to a complete elimination of the weeds and resulted in a major reduction of herbicide use (in our experiments up to 60%), depending on the weediness of the field. Very few weeds (total weed cover < 5%, efficacy of common ragweed control > 97%) grew on the winter wheat stubble following the spot spraying by glyphosate and glufosinate, and the weeds remaining were stunted and underdeveloped. As a result production of common ragweed pollen and seed in the experimental fields were completely stopped.

Figure 8. Wheat stubble: spot-spray treatment with glyphosate

Discussion

We have tested precision weed control methods (partly developed in our laboratories) to suppress common ragweed in sunflower, maize, and wheat stubble in large agricultural fields. Weed maps created in earlier years were used to design the control measures. This off-line approach was preferred because the other input data (related to soil properties) were already available. Our approach was especially successful in fields with highly variable terrain conditions: we reduced the costs of weed control and the risk of crop damage by herbicide overdose.

In sunflower and maize, failure of pre-emergent treatments because of rainfall deficit may be successfully counteracted by a post-emergent application of the non-selective (total) herbicide glyphosate sprayed under the canopy. The herbicide-saving, environment-friendly use of the WeedSeeker sensor provides a solution which combines the map-based and on-line methods. The first use of mechanically shielded WeedSeeker sensor-sprayers in order to keep fields of row-crops weed-free after pre-emergent herbicide applications by applying a non-selective herbicide revealed that the device can be applied safely and successfully. In addition, if necessary, a precision mechanical weed control could be applied by using a ridge-plough to turn a thick layer of soil in the row, thereby controlling the weeds growing in the rows, as well. This solution meets the requirements of integrated weed management.

In wheat stubble a nearly 100% control of common ragweed can be achieved by the spot-spraying application of both glyphosate and glufosinate at the standard rate using the WeedSeeker sensor-sprayer. Therefore, we suggest the inclusion of glufosinate ammonium as an alternative herbicide used in rotation with glyphosate for controlling common ragweed in wheat stubble in order to hinder and postpone the possible emergence of weed resistance to chemical management.

In summary, the site-specific control of common ragweed by spot spraying is highly efficient, allowing a reduction in herbicide use, thereby decreasing the environmental impact of weed control - a major goal of the European Union (Nordmeyer, 2006), in addition to reducing concentrations of the allergenic pollen of common ragweed in the air. A major key for the success of common ragweed control when using this technology will be the management of weed resistance due to recurrent use of glyphosate and glufosinate herbicides.

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Efficacy of imazamox on *Ambrosia artemisiifolia*

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The herbicidal active ingredient imazamox belongs to the group of imidazolinone (ALS-inhibitor). The uptake takes place through the leaves, primarily and mainly dicotyledonous weeds are affected by imazamox.

Materials and Methods

A glasshouse pot experiment with a three-factor experimental design was conducted in 2013. The impact of imazamox on *A. artemisiifolia* was investigated on two different growth stages (BBCH 21-25 and 51-55) and with four doses: 4, 8, 16 and 32 g /ha. Imazamox was applied as a single and split treatment. The split treatment was applied with 50% of the dose at the same timing as the single treatment and 50% 10 days later. The herbicide was applied in an application chamber equipped with flat fan nozzles operating at a pressure of 2,1 kPa and a velocity of 2 km/h delivering a volume of 300 L per ha.

Seeds were sown in jiffy pots at two timings to obtain two different growth stages. The seedlings were transplanted with one seedling per 2L pot at the BBCH 12. Each treatment consisted of 4 replicates.

6 weeks after application fresh matter of common ragweed plants were assessed by cutting the plant above soil surface.

Results and discussion

Lower fresh matter was observed at high herbicide doses (16 and 32 g /ha) given in single or split applications to the early growth stage at BBCH 21-25 compared to the untreated control (Figure 1 and 2). Fresh matter was higher with plants getting the herbicide application at the late growth stage (BBCH 51-55) and in low doses which can be explained by hormesis effect (Figure 1). At BBCH 51-55 even the highest dose of imazamox applied as single or splitting did not reduce fresh weight of common ragweed

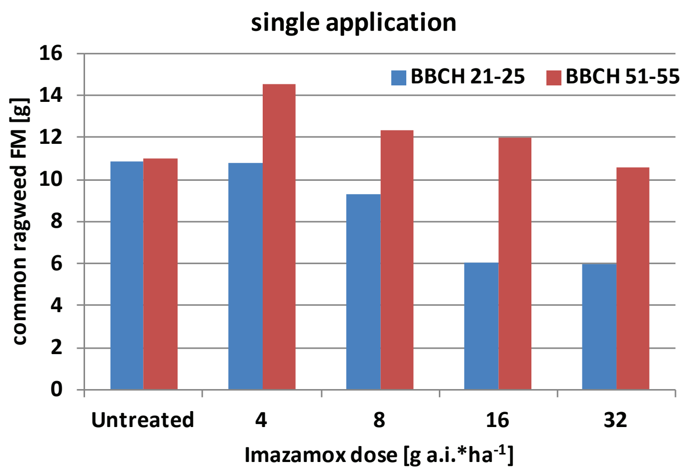

Figure 1: Common ragweed fresh matter [g] 6 weeks after imazamox single application

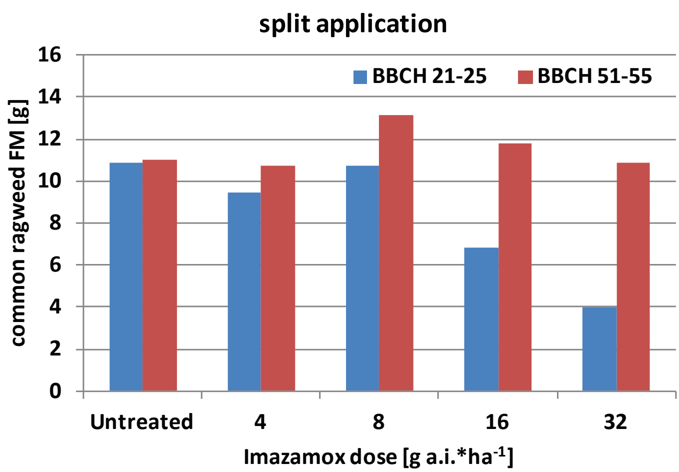

Figure 2: Common ragweed fresh matter [g] 6 weeks after imazamox split application

Conclusions

The highest dose of 32 /ha in a split application at BBCH 21-25 had the best results in reducing the common ragweed fresh matter but did not lead to plants dieback. Under field conditions it could be assumed that even these plants would be able to reshoot and produce seeds.

Effects of low herbicide dosage on production and fertility of common ragweed seeds

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Materials and methods

The impact of Callisto (mesotrione 100g /L), Primus (florasulam 50g/L), Lontrel 100 (clopyralid 100 g/L) on production and fertility of *A. artemisiifolia* (common ragweed) seeds was investigated at 20%, 40% 80% of the registered dosage. The herbicides were applied in an application chamber equipped with flat fan nozzles operating at a pressure of 2 kPa and a velocity of 2 km/h delivering a volume of 300 L /ha. The common ragweed plants were at BBCH stage 59.

A natural population from an infested farmland (cereal stubble with common ragweed infestation) in the South of Cottbus (Germany) was taken for this experiment. Plants were dug up at the end of July 2012 and transplanted with one plant per 2L pot at BBCH stage 50. The pots were placed outside. Each treatment consisted of 4 replicates.

Two month after application fresh matter and number of seeds per plant were assessed by cutting the plant above soil surface. The number of viable seeds was assessed by the TTC Test, described before in the chapter Biological fundamentals.

Results and discussion

The lowest seed production averaged over all dosages was achieved by florasulam followed by mesotrione and clopyralid (Figure 1Figure). And the lowest viability of the seeds was found in all dosages as follows: florasulam > clopyralid > mesotrione.

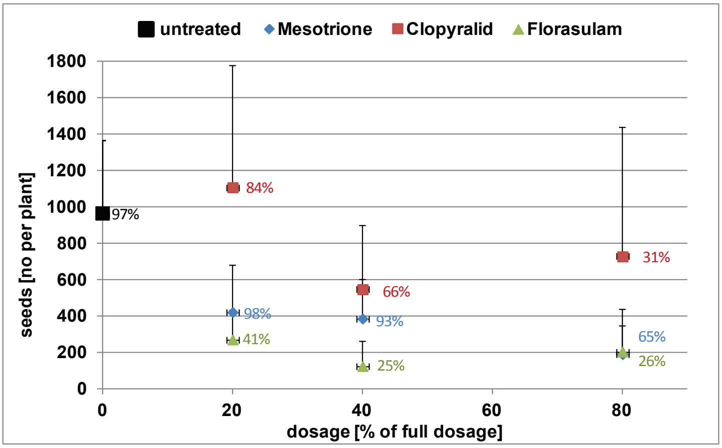

Figure 1: No. of seeds and their viability [%] after herbicide application

Conclusions

Florasulam was most effective in reducing the seed production and their viability even with low herbicide dosages.

Effects of treatment timing

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Information on herbicide efficacy and the influence of application timing is important in order to optimise control of common ragweed.

Materials and methods

Seeds of common ragweed were sown in 2 L pots in a potting mixture consisting of field soil, sand and peat (2:1:1 w/w). The pots were placed in a heated glasshouse and watered as required. After seedling emergence the number of plants per pot was reduced to 4. Herbicide treatments were carried out at four different growth stages: 4-leaf stage, 7-leaf stage, early flowering and late flowering. Five herbicides were included in the study: glyphosate, florasulam, clopyralid, mecoprop-P and mesotrione. The herbicides were applied at 4 doses (1/8N, 1/4N, 1/2N and 1 N) using a laboratory pot sprayer equipped with a boom fitted with two Hardi ISO F110-02 flat fan nozzles delivering a volume rate of ca. 150 l/ha. Each treatment was replicated three times.

Foliage fresh and dry weights were recorded 3 weeks after herbicide application.

Results

The dose requirements increased significantly for most of the herbicides when application was carried out at late compared to early development stages of common ragweed. The doses of florasulam and mesotrione had to be increased by a factor 2 to 3 and the dose of MCPP by a factor 3 to 6 if spraying was delayed from the 4-leaf stage to the 8-leaf stage. In contrast there was no need of increasing the doses of glyphosate and clopyralid. If herbicide application was further delayed until the flowering stage the doses of florasulam, mecoprop and mesotrione had to be increased by a factor 14 or more and the dose of clopyralid by a factor 5 compared to the doses at the 4-leaf stage for obtaining the same efficacy level (Table 1).

Table 1. Comparison of the doses needed to obtain the same efficacy level when spraying at late *vs.* early growth stages

Herbicide	Dose multiplication factor for delayed treatment	
	From 4- to 8-leaf stage	From 4-leaf to flowering stage
Glyphosate	0.4	1
Clopyralid	1	>5
Florasulam	2-3	14
Mecoprop-P	3-6	15
Mesotrione	1-3	>19

Conclusions

The results show that it is possible to control common ragweed - even at late growth stages - with all the tested herbicides. However the efficacy of glyphosate was much less influenced by plant growth development than the other herbicides.

Effect of sequential treatments

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Split application of one herbicide, or two in combination, can be more efficient than just one spraying with the same total dose. In this study we examined the effect of sequential treatment with the same herbicide or different herbicides on common ragweed.

Material and methods

Plants of common ragweed were sown in 2 L pots in a potting mixture consisting of field soil, sand and peat (2:1:1 w/w) and grown in a glasshouse. Prior to herbicide application the number of plants per pot was reduced to a pre-set number.

Florasulam, mecoprop-P, mesotrione, clopyralid and glyphosate were applied as single treatments at two different timings: T1 (=2-4 leaf stage) and T2 (=2 weeks after T1). In addition sequential treatments with different combinations of the herbicides were made. Each herbicide was applied at four doses and each treatment was replicated three times. Herbicide preparations were applied using a laboratory pot sprayer equipped with a boom fitted with two Hardi ISO F110-02 flat fan nozzles using a volume rate of ca. 150 L/ha.

Three to four weeks after T2 the plants were harvested and foliage fresh and dry weights are recorded. Dose-response curves were estimated using non-linear regressions and the ED_{50} and ED_{90} doses for each herbicide preparation estimated.

The Additive Dose Model was used to determine whether dose-splitting was additive i.e. that one herbicide dose applied at a specific time can be replaced by an equivalent dose ratio at another time.

Results and discussion

The experiments were analyzed using a joint-action model as dose-splitting can be considered a special case of joint action of herbicides, not as mixtures, but as staggered applications. The Additive Dose Model (ADM) which is generally accepted as the joint action reference model for mixtures of herbicides has previously been used to evaluate the efficacy of split applications. ADM implies that the ED doses of dose-splitting treatments should follow the isobole between the ED doses of the single treatments. If the calculated ED dose of a dose-splitting treatment is located above the isobole, the response to dose splitting is antagonistic and location below the isobole indicate a synergistic response (Figure 1) Most of the split treatments tested yielded a synergetic or synergetic-to-additive response. None were antagonistic (Table 1). Thus split applications with proper herbicides resulted in a higher efficacy than a single treatment, even when the total dose remained the same.

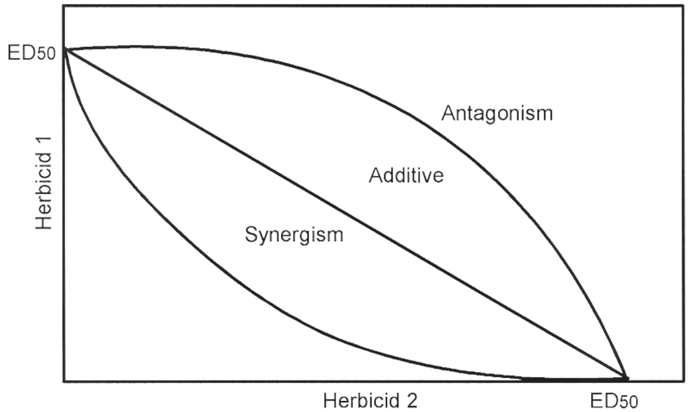

Figure 1: Schematic illustration of possible interactions between split applications according to the Additive Dose Model. The x- and y-axes represent relative doses of same or different herbicides at timing 1

Table 1. Classification of sequential treatment efficacy

Synergistic	Additive	Antagonistic
Mesotrione x 2 (T1 + T2)		
Florasulam x 2 (T1 + T2)		
MCPP x 2 (T1 + T2)		
	Florasulam (T1) + Clopyralid (T2)	
	Florasulam (T1)+ Glyphosate (T2)	
	MCPP (T1) + Clopyralid (T2)	
	MCPP (T1) + Glyphosate (T2)	

Split or sequential application of herbicides could be a recommendation to ensure effective control of early as well as late cohorts of germinating common ragweed on uncropped areas and in crops with low competitiveness. In these cases a low dose is be applied at an early growth stage and followed up by another application when new seedlings emerge. This strategy leads to repeated application on plants that have survived the first spraying. The results show that the susceptibility of plants affected by a previous herbicide treatment is equal to or higher than the susceptibility of untreated plants.

Conclusions

Sequential treatments or split applications showed synergistic or additive effects. Most split applications were more effective than one single application (florasulam, MCPP and mesotrione) while treatments with florasulam or MCPP as the first application followed by clopyralid or glyphosate in the second application were additive. Consequently split applications can be used without loss of efficacy.

Effects of different herbicide treatments on common ragweed in winter wheat (Hungary)

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Small plot (4 x 4 m^2) experiments in four replicates were set up in winter wheat. Postemergent treatments were applied at 4-6 leaf stage of common ragweed (BBCH: 14-16) (end of May) Herbicide efficacy surveys were carried out: 2 and 6 weeks after treatments and directly before harvest based on cover percent of common ragweed.

Trials (doses according to the permission documents of herbicides)

Amidosulfuron + iodosulfuron + mefenpyr-diethyl

Cinidon-ethyl + dichlorprop + metsulfuron-methyl

Metsulfuron-methyl + fluroxypyr

Metsulfuron-methyl + bromoxynil

Metsulfuron-methyl + bromoxynil + 2,4D

Metsulfuron-methyl + fluroxypyr + MCPA

MCPA + bromoxynil

2,4D + bromoxynil

Untreated control

No evaluable data was got for common ragweed, because intensively tillering wheat suppressed lately emerged common ragweed, therefore herbicides „did not meet" with common ragweed (common ragweed was covered by wheat and other weeds). Other weed species, like *Tripleurospermum inodorum* (syn.: *Matricaria inodora*) were well-developed and dominant.

Generally, common ragweed is not a major problem in cereals. Autumn-emerged common ragweed seedlings die due to the frosts in winter, and dense-sown cereals can suppress the lately (spring) emerged common ragweed.

Effects of different herbicide treatments on common ragweed in maize (Hungary)

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Small plot (20 m^2) experiments were carried out in four replicates in order to study weed control efficacy on common ragweed; preemergent (PRE) treatments were done, when common ragweed phenological stage was 00-06 according to BBCH scale; postemergent (POST) treatments were done when common ragweed phenological stage was 12-16, according to BBCH scale]. Maize at PRE treatments was 00-05 BBCH; at POST treatments it was 14-16 BBCH.

Weed control efficacy was evaluated 1, 4, 6 and 10 WAT (week after treatments, based on cover % of common ragweed).

Seeds from the survived common ragweed plants were collected and their viability and germination percent was determined under laboratory conditions. Furthermore number of viable common ragweed seeds for a unit area was also determined (see Table 1).

Efficacy of herbicides on common ragweed greatly varied (between 60% and 98%) depending on herbicide type and application date (PRE, POST). Te efficacy of PRE treatments was better in 2012, as compared to that of previous years, resulting in less or no common ragweed seed production at all. This phenomenon was in close relation with the precipitation fell some days after application time in 2012. In earlier experiments the spring was dry and in the lack of precipitation PRE herbicides – except isoxaflutole - did not gave good weed control effect (Mezei *et al*. 2009; Kazinczi and Novák, 2014). In 2012, not only PRE but POST herbicides gave better weed control effect also as compared to that of the previous experiments. The effect of rimsulfuron was insufficient in all experiments. Among 20 treatments in case of 14 ones no viable seeds were developed in 2012.

Germination rates were much lower (1-5%) than viability percentages (56-89%), suggesting that majority of common ragweed seeds was in dormancy induced by dry storage conditions at room temperature.

No close relations between weed control efficacy and seed viability was observed; e.g. some herbicides with lower weed control efficacy (rimsulfuron, foramsulfuron) gave higher seed viability rates.

It is important observation that even in case of a "good weed control efficacy" (92%) viable common ragweed seeds can develop. Only in case of "very good weed control efficacy" (95-98%) no viable common ragweed seeds could develop in 2012. Based on previous experiments it is believed that only 100% weed control efficacy without producing any viable seeds is acceptable for the long term common ragweed control (Mezei *et al*. 2009; Kazinczi and Novák , 2014).

Table 1. Efficacy of herbicides in maize, and the effect of herbicides on the germination, seed viability and seed production of common ragweed

Treatments (doses according to the permission documents of herbicides)	**Weed control efficacy (%)*** **2012**	**Germination (%)** **2012**	**Seed viability (%)** **2012**	**Number of total/ viable seeds /m²** **2012**
dimethenamid-p PRE	92	4	56	402/225
terbuthylazine PRE	92	1	62	394/244
mesotrione PRE	98	-**	-	0
isoxaflutole PRE	97	-	-	0
flumioxazine PRE	97	-	-	0
linuron PRE	92	2	70	385/270
rimsulfuron POST	60	5	85	672/571
dicamba POST	98	-	-	0
2,4 D POST	96	-	-	0
fluroxypyr POST	97	-	-	0
bentazone POST	97	-	-	0
mesotrione POST	98	-	-	0
topramesone POST	98	-	-	0
sulcotrione POST	97	-	-	0
tembotrione POST	98	-	-	0
prosulfuron POST	96	-	-	0
foramsulfuron POST	65	4	89	423/376
tifensulfuron-methyl POST	97	-	-	0
bromoxynil POST	97	-	-	0
untreated control	0	0	70	3905/2734

*weed control efficacy: 99-100%:excellent; 95-98%: very good; 90-94%: good; 75-89%: less good; under 74%: not sufficient
-**no seeds developed

References

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Effects of different herbicide treatments on common ragweed on wheat stubble (Hungary)

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Small plot (4 x 4 m^2) experiments in four replicates were carried out. The treatments were applied according to BBCH 51 stage of common ragweed plants (end of July, directly before flowering). Evaluation of herbicide efficacy was: 7, 14 and 40 days after treatments (DAT); based on cover% of common ragweed. Six weeks after treatments we determined common ragweed seed production for a unit area. Seeds from the survived common ragweed plants were collected and their viability and germination ability was determined under laboratory conditions. Furthermore number of viable common ragweed seeds for a unit area was also given. Beside these pollen production was also estimated (number of male heads/plant: considering a mean of 17 male flowers per head and of 7148 pollen/male flower; see Reisinger and Szemenyei (2006) (Table 1).

Table 1. Efficacy of herbicides on wheat stubble and the effect of herbicides on the germination, seed viability, pollen and seed production of common ragweed

Treatments (doses according to the permission documents of herbicides)	**Weed control efficacy (%)***	**Germination (%)**	**Seed viability (%)**	**Number of total/ viable seeds /m^2**	**Pollen (million/ m^2)**
	2012	**2012**	**2012**	**2012**	**2012**
glyphosate	100	0	0	0	0
mesotrione	<50	2	89	11040/9826	2255
fluroxypyr	80	4	82	12160/9971	1306
rimsulfuron	<50	3	87	3600/3132	3221
nicosulfuron	<50	5	77	3520/2710	174
dicamba	75	1	77	8320/6406	432
rimsulfuron+nicosulfuron +dicamba	95	2	76	1600/1216	97
imazamox	75	4	84	13280/11155	676
topramesone	100	0	0	0	0
topramesone+dicamba	100	0	0	0	0
glufosinate-ammonium	95	2	62	2080/1290	24
foramsulfuron	<50	1	86	6240/5366	282
tribenuron-methyl	<50	4	83	12640/10491	7294
florasulam+clopyralid +fluroxypyr	85	2	70	2880/2016	537
florasulam+2,4 D	90	1	74	2080/1540	1305
bentazon+dicamba	95	0	76	1720/1307	233
sulcotrione	90	0	86	3040/2614	760
tembotrione	90	4	82	3200/2624	469
untreated control	0	4	86	12480/10732	8341

*weed control efficacy: 99-100%:excellent; 95-98%: very good; 90-94%: good; 75-89%: less good; under 74%: not sufficient

Weed control efficacy greatly varied depending on the treatments (<50-100%), but was similar than in previous experiments. Generally the number of viable common ragweed seeds for a square metre was higher in 2012, then in an earlier experiment (Kazinczi et al. 2010; Kazinczi and Novák, 2014). Glyphosate, topramesone, topramesone + dicamba showed 100% efficacy against common ragweed and resulted no seed and pollen production. The seed viability and number of viable seeds greatly varied due to the different herbicide treatments. No close relation between weed control efficacy, seed and pollen production could be observed; e.g. nicosulfuron and foramsulfuron effect is under 50%, but they reduced pollen production considerably (by 98 and 97%, respectively) as compared to the untreated control plots.

In some cases, when weed control efficacy was "very good" (e.g. in case of glufosinate-ammonium treatment), higher proportions of seed production/pollen production could be observed. This phenomenon may be an effective strategy for ragweed's survival under stress conditions.

Majority of common ragweed seeds were in dormancy during germination tests (germination% varied between 1 and 4%); this was due to the lack of stratification. In spite of low germination rates seed viability was high (between 62 and 89%, depending on herbicide treatments).

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Effects of different herbicide treatments for common ragweed control at different phenological stages under field conditions (Hungary)

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At the experimental area of Kaposvár University (KU) a ruderal area heavily infested with common ragweed was chosen, where small plots (2 x 2 m) were signed. Common ragweed infestation was evaluated before treatments.

Preemergent (PRE) treatments (before germination peak) were carried out on 23.04.2012. Average density: 10-15 common ragweed/m^2. Common ragweed phenology: BBCH: 05-12.

Postemergent (POST) treatments (at germination peak) were carried out on 18.05.2012. Average density: 40-50 common ragweed/m^2. Common ragweed phenology varied from cotyledonous-8 leaf stage (BBCH: 09-18).

Weed control efficacy was evaluated 1, 3, 6, 8, 12, and 18 weeks after treatments (WAT).

Treatments were the followings (doses according to the permission documents of herbicides):

Dimethenamid+pendimetalin (PRE) + imazamox (POST)

Fluorchloridon(PRE)

Fluorchloridon (PRE) + oxyfluorfen (POST)

Oxyfluorfen (PRE)

Terbuthylazine (PRE)

Flumioxazin (POST)

Rimsulfuron (POST)

Nicosulfuron (POST)

Foramsulfuron (POST)

Imazamox (POST)

Tribenuron-methyl (POST)

Nicosulfuron + rimsulfuron + dicamba (POST)

Dicamba (POST)

Fluroxypyr (POST)

Bentazon+dicamba (POST)

Mesotrione (POST)

Florasulam+clopyralid+fluroxypyr (POST)

Sulcotrione (POST)

Glufosinate-ammonium (POST)

Tembotrione (POST)

Glyphosate (POST)

Untreated control

Generally, herbicides gave a better weed control effect against common ragweed 3 WAT (Table 1). 12 WAT the efficacy of herbicides drastically decreased. The reasons of uneffectivity were that: 1.

herbicide treatments happened at different phenological stages of common ragweed (BBCH: 05-18), and it is known that majority of leaf herbicides is effective only against very young common ragweed seedlings (until max. 4 leaf stage, BBCH:14). 2. Due to the continuous germination of common ragweed some seedlings emerged only after the POST treatments, so herbicides "did not meet" with the common ragweed plants; common ragweed seedlings "escaped" from the herbicide effect.

Generally, PRE+POST combinations gave better results, than PRE and/or POST treatments alone.

ALS inhibitors (imazamox, tribenuron-methyl, nicosulfuron, foramsulfuron) were working efficiently until max. 4 leaf phenological stage of common ragweed (BBCH:14).

Non-selective leaf herbicides (glufosinate-ammonium, glyphosate) were good for older common ragweed control also, but were ineffective for lately-emerged seeds (they did not meet with the common ragweed plant).

Due to the selection pressure of auxin type (dicamba, fluroxypyr) herbicides monocot species were selected (Figure 1).

Majority of leaf herbicides have no long-term effect; lately-emerged common ragweed plants (those ones which emerged after the POST treatments) escaped from the effect of herbicides (common ragweed did not meet with the herbicides) (Kazinczi and Máté 2014) (Table 1).

Figure 1: Effect of dicamba treatment on common ragweed (left: 3 WAT, right: 12 WAT)

Table 1. Weed control efficacy of different treatments on common ragweed

Treatments	Weed control efficacy (%)*	
	3 WAT	12 WAT
Dimethenamid+pendimetalin (PRE) + imazamox (POST)	70	40
Fluorchloridon(PRE)	80	40
Fluorchloridon (PRE) + oxyfluorfen (POST)	95	60
Oxyfluorfen (PRE)	90	40
Terbuthylazine (PRE)	70	20
Flumioxazin (POST)	63	30
Rimsulfuron (POST)	68	10
Nicosulfuron (POST)	65	10
Foramsulfuron (POST)	60	10
imazamox (POST)	65	50
Tribenuron-methyl (POST)	55	20
Nicosulfuron + rimsulfuron + dicamba (POST)	60	10
Dicamba (POST)	90	95
Fluroxypyr (POST)	92	20
Bentazon+dicamba (POST)	95	40
Mesotrione (POST)	98	45
Florasulam+clopyralid+fluroxypyr (POST)	82	10
Sulcotrione (POST)	75	10
Glufosinate-ammonium (POST)	99	10
Tembotrione (POST)	90	10
Glyphosate (POST)	97	70
Untreated control	-	-

*weed control efficacy: 99-100%:excellent; 95-98%: very good; 90-94%: good; 75-89%: less good; under 74%: not sufficient

References

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Conclusions from the results of 15.5 – 15.8 experiments

Under optimal growing conditions, common ragweed did not seem to be a considerable weed problem in cereals and not even in other dense-sown autumn crops, like oilseed rape.

In maize, a lot of effective herbicides are available for common ragweed control. The efficacy of soil herbicides greatly depends on the presence of precipitation. Among leaf herbicides some sulfonyl-ureas, triketones and auxin type herbicides gave good effect for common ragweed control. Their efficacy depends on common ragweed phenology (the best is cotyledonous-2 leaf, BBCH: 09-12). The phenological stage of maize is also should be taken into consideration; e.g. late-applied auxin type herbicides can cause phytotoxicity on maize.

On uncultivated areas (waste lands, cereal stubbles) - from economic point - the application of non-selective herbicides is suggested. Other reason is that these herbicides can effectively control common ragweed plants even in more developed stages.

Continuous germination of common ragweed during the vegetation period make its control more difficult. Field emergence of common ragweed may occur when the long term effect of soil herbicides has already been ceased. At the same time common ragweeds are present at different phenological stages while most leaf herbicides are effective only against young seedlings. On the other hand, lately emerged common ragweed plants (when emergence occurs after the treatments) "escape" from the effects of postemergent leaf herbicides, regarding that they do not keep in contact with herbicides. So it is suggested to use a herbicide combination in which the soil herbicide (with a long term effect) and a leaf herbicide is combined (Kazinczi and Novák, 2014).

In case if we consider the main purpose of each control method (to prevent pollen and seed production of common ragweed) only 100% weed control efficacy is accepted!

Reference

Kazinczi, G. and Novák, R. (eds) (2014): Integrated Methods for Suppression of common Ragweed. National Food Chain Safety Office, Directorate of Plant Protection, Soil Conservation and Agri-Environment, Budapest, Hungary

Herbicide efficacy for common ragweed control after defoliation (Slovenia)

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The results of these experiments are being prepared for journal submission, so just a short summary is given below.

Summary

To determine efficacy of various herbicides applied to common ragweed immediately after cutting, pot experiment was conducted in Slovenia. Ragweed plants were grown in containers and were clipped 5 cm above soil surface at different growth sages (20, 35, 50 and 80 cm high plants). Herbicides based on glyphosate (1500 g/ha), thifensulfuron (12 g/ha), bentazon (1200 g/ha) and dicamba (385 g/ha) were applied to plants by spraying directly after clipping in such a way that only a certain portion of foliage area remaining after clipping was exposed to herbicide (10, 35, 60, 85 and 100 %). The efficacy (%) of herbicides was determined by weighing and comparing of dray mass of treated and untreated plants at the end of growing season. Results showed that efficacy of herbicides decreased significantly with increasing common ragweed development stage and decreasing leaf area exposed to herbicide application. Only treatments with glyphosate and dicamba at two early growth stages V10 and V18 stage resulted in 90 % dry matter reduction, when total (100 %) leaf area of common ragweed plants was covered with herbicide after defoliation. When very low leaf areas (20-35 %) were treated, the efficacy was low (20-50 %), however seed production of common ragweed decreased by 75-90 %. At least 40 % of leaf area previously defoliated common ragweed has to be covered with herbicide spray in order to achieve 50 % dry matter reduction and 90 % decrease of seed production.

Biodiversity impacts of common ragweed

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Introduction

Ambrosia artemisiifolia causes agricultural losses and severe health problems. Whether the species also has a negative influence on plant species richness and the composition of the vegetation is a matter of ongoing debate. The question whether common ragweed impacts biodiversity or not is of great importance as this impact may be an additional motive for the prevention of import and control. It would also have an influence as to which administrative sector is competent and responsible for these measures. In Germany, for example, where the species is not yet wide spread, the Federal Nature Protection Act provides a legal framework the management of invasive alien species. Only if ragweed would have proven negative effects on other species, communities, or habitats, could this be applied in the fight against the species.

In which way could common ragweed impact biodiversity? On the one hand the species could directly suppress other plant species, and on the other hand control measures directed against common ragweed could have negative effects on flora and fauna. A negative effect on biodiversity would be relevant if rare species or species important for the function of the ecosystem were affected. Relevant effects include the reduction of fitness of affected plants (e.g., vegetative development, flowering and seed set). or indirect effects on other trophic levels such as associated animals depending on these plants or the alteration of abiotic habitat conditions.

In the last decades *A. artemisiifolia* became the best recognised weed species in East-European countries (Kazinczi et al. 2008), and also in European countries with relatively low infestations such as in Germany. Common ragweed became well known due to various reports in the media during the last five years. In some countries this may lead, or already led to intensified control measures against common ragweed in order to protect the human population against the ragweed pollen. Control measures may have side effects on biodiversity since an intensified use of herbicides, intensified mowing, or early ploughing of stubble fields may harm accompanying species (Pal 2004, Pinke 2007, Pinke et al. 2008, Pinke et al. 2010, Pinke et al. 2011). Common ragweed is present not only on arable fields where herbicides are normally used, but also in various habitats such as field margins, abandoned fields, forest edges, field paths that could be affected by measures such as more frequent herbicide use or intensified mowing.

Against this background, this chapter is about the impact of common ragweed on biodiversity and non-target species, and the following questions are of major interest:

- Does common ragweed have biologic features that helps it to spread und suppress accompanying plant species?
- In which habitats does common ragweed occur and which are important according to nature protection issues? Which habitats could be affected in future?
- Are direct impacts of common ragweed on biodiversity currently known?
- Are indirect impacts of control measures against common ragweed on biodiversity known?

In order to find answers to these questions, a literature review was conducted. Additionally, scientists from different countries working on the topic "common ragweed" were asked for their estimation regarding direct and indirect impacts of common ragweed on other species and habitats by using a questionnaire. Furthermore field studies were conducted in the East German Niederlausitz, which is the most ragweed infested area in Germany.

Methods

Literature review and inquiry via questionnaire

In January and February 2012 a literature review was conducted. Also a questionnaire (see appendix) with six questions on direct impacts of common ragweed as well as indirect effects of control measures on biodiversity was sent to 118 experts currently working on the topic "common ragweed" in 38 countries (Australia, Austria, Belgium, Bosnia-Herzegovina, Bulgaria, Canada, China, Croatia, Czech Republic, Denmark, Finland, France, Georgia, Germany, Greece, Hungary, Iran, Israel, Italy, Lithuania, Luxembourg, Macedonia, Netherlands, Norway, Poland, Romania, Russia, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom, USA). It asked for information on the following questions:

1. In which habitats does *A. artemisiifolia* occur in your country (apart from gardens)?
2. Does common ragweed occur in habitats with high value for nature protection (not only legally protected areas)? Does common ragweed suppress rare and/or endangered species?
3. Do you have own investigations and/or relevées on the invaded vegetation which we could use? (e. g. relevées of affected nature reserves but also of field-vegetation, road sides etc.?
4. How often is common ragweed controlled in your country?
   If common ragweed is controlled: How do you estimate the impacts of control measures against *A. artemisiifolia* on biodiversity in your country?
5. Do you expect future negative impacts for biodiversity due to intensified control measures against common ragweed (if common ragweed spreads and/or if more action is taken)? Which habitats and species could be affected?
6. Please list other information that might be useful for this study (e.g. names of other experts to contact, published or unpublished information, vegetation relevées, etc.

We thankfully received 12 answers on the questionnaire from Norbert Bauer (Hungary), Maira Bonini (Italy), Dragana Bozic (Serbia), Bruno Chauvel (France), Anikó Csecserits (Hungary), Natalija Galzina (Croatia), Gerhard Karrer (Austria), Peter Kotanen (Canada), Robert Pál (Hungary), Sergey Reznik (Russia), Hana Skálova (Czech Republic), and Nicola Schoenenberger and Marta Rossinelli (Swzitzerland), and added our own estimation for Germany (Alberternst & Nawrath).

Additionally we gratefully received hints from Bernard Clot (Switzerland), Chantal Dechamp (France), Gabriella Kazinczi (Hungary), Heinz Müller-Schärer (Switzerland), Ljiljana Nikolic (Serbia), Hans Peter Ravn (Norway) and Ingrida Sauliene (Lithuania).

The information given by the experts in the questionnaire is described below.

Field work

In order to find answers on the question whether common ragweed might be a threat for biodiversity, field work was conducted in the Niederlausitz near the city Cottbus located in Eastern Germany. *Ambrosia artemisiifolia* is distributed unequally in Germany: while the species currently is rare in the northern part and relatively rare in the south-western part of the country, it occurs very often in the region south-west of Cottbus. Thus the following investigations were conducted in that region from 5th to 11th July 2011:

- Review of data from that region from literature
- Interview and field trips with two local experts
- Investigations of the vegetation with occurrences of common ragweed with special regard to rare and endangered species.

Results

Interaction between common ragweed and surrounding vegetation

In the following chapters biological features of common ragweed explaining its growing and spreading strategy, the habitats where the species occurs, and the accompanying flora and vegetation are described from the literature. Where results from the literature review and from the inquiry via questionnaire complement each other, the results are presented together in the appropriate chapter. The results from the field work are presented in an additional chapter.

Distribution and spreading capacity in the new growing range

Distribution

In its native range in North America, *A. artemisiifolia* is a very common weed (Mitich 1996) and it is assumed to be native in the Canadian Prairies (Bassett & Crompton 1975). From North America, *A. artemisiifolia* was introduced to many countries in different parts of the world such as Australia (Bass et al. 2000), Japan (Miyawaki & Washitani 2004), China (Chen et al. 2007), and Russia (Reznik 2009). It was also introduced to many European countries such as Hungary (Makra et al. 2005, Kacinczi et al. 2008), France (Dechamp & Meon 2002, Chauvel et al. 2006), Italy (Pizzulin Sauli et al. 1992, Mandrioli et al. 1998), Switzerland (Taramarcaz et al. 2005, Bohren 2005), Germany (Alberternst et al. 2006), Austria (Dullinger et al. 2009), Croatia (Galzina et al. 2009, 2010), Serbia (Kostantinovic´ et al. 2011), Ukraine (Burda & Tokhtar 1992), Poland (Tokarska-Guzik et al. 2010, 2011), Lithuania (Sauliene et al. 2012), Romania (Hodisan & Morar 2008, Hodisan et al. 2008), Czech Republic (Rybnicek et al. 2000) and even Sweden (Dahl et al. 1999, Möller et al. 2002) and Britain (Richi 1994). Currently *A. artemisiifolia* is most abundant in three main European regions: in the valley of the Rhône in France, in the northern part of Italy and in an extensive area in the south-eastern part of Europe, mainly in Hungary and surrounding countries (Rybnicek & Jäger 2001).

Spreading capacity

In Hungary, common ragweed became very common on agricultural fields and moved from the 21st place of the most important weeds in 1950 to the "number one" weed in Hungary in 1997 (Tóth et al. 2004 in Novák 2009). War and political shifts in Southeast-European countries have forced the spread of common ragweed since 1989/90 which is demonstrated by the example of Hungary where the agricultural co-operatives were closed and their land was redistributed to the former owners. In many cases these persons did not continue the cultivation of the fields for years and abandoned fields were quickly colonised by *A. artemisiifolia* (Kiss & Beres 2006, Balogh et al. 2007, Kazinczi et al. 2008). Similarly, the war in former Yugoslavia led to an increase of fallow land and waste places that favoured ragweed colonisation (Taramarcaz et al. 2005). According to Tóth et al. (2004 in Kazinczi et al. 2008) *A. artemisiifolia* infested 5.4 million hectares in Hungary. In Russia, where common ragweed was considered the most noxious invasive weed since the 1940s, the area heavily infested increased up to 6 million hectares by the end of the 1980s (Reznik 2009). In the Ukrainian Carpathian mountains and the Transcarpathian plain, common ragweed spread within a 55-year period (1942-1997) with a speed of 67.6 km^2/year (Song & Prots 1998).

Competitive ability

These examples demonstrate that *A. artemisiifolia* has a strong spreading capacity. In addition, the species builds up dense stands and is able to suppress accompanying plant species by competition for light, nutrients, and water and may influence them by its allelopathic capacity (Beres et al. 2002). Common ragweed is an "exceptionally good competitor" and can have a strong negative effect on species which are moderately good competitors such as *Agropyron repens* and *Plantago lanceolata* (Miller & Werner 1987, Callaway & Walker 1997, Callaway 2007). On arable fields common ragweed can cause substantial yield losses e.g. in maize, sunflower, soybean, beans, peanuts, which also demonstrates that common ragweed can act as a strong competitor (Chollet et al. 1999, Chikoye et al. 1995, Clewis et al. 2001, Zwerger & Eggers 2008, Kukorelli et al. 2011).

Reproductivity

A. artemisiifolia is an erect annual herb with unbranched to bushy branched stems. The plant is very variable regarding its size and leave shape and it normally reaches a height of 5 cm to 100 cm. In poor sandy soil the species stays small and mostly unbranched, while in nutrient-rich growing conditions with sufficient water supply it can grow up and built branchy stems up to 2 m high. *A. artemisiifolia* is monoecious and has flower heads with either male or female flowers. Male heads contain 10-15 male flowers (Hegi 1979) and are posed in spikes terminating the stems and branchlets. Sauliene et al. (2012) found in an experiment that cultivated ragweed plants produce approximately 36.000 male flower heads per plant. The inconspicuous female heads are one-flowered and located in small clusters or single in the axils of the upper leaves (Bassett & Crompton 1975). Common ragweed plants produce one seed per flowering head. With regard to Dickerson & Sweet (1971) small plants produce about 3000 seeds while large individuals generate up to 62,000 seeds. In Russia even 88,000 seeds per plant were observed (Fisjunov 1984 in Kazinczi et al. 2008) and according to Szigetvári & Benkö (2008) 150.000 seeds were found on a plant from Ukraine. For a branchy common ragweed plant of a height of approximately 1.3 m 44,211 seeds were found in Germany (Alberternst & Nawrath unpubl. data). The germination rate of the seeds is high (Kazinczi et al. 2008).

Seed bank and meaning of soil disturbances

In Hungary first seedlings emerge between March 15 and April 12 (Kazinczi et al. 2008). This is a period when first seedlings also occur in Germany (e.g., on March 23 in 2007, and March 20 in 2010, in Friedberg). The seeds germinate at or near the soil surface (Bazzaz 1974). An investigation carried out in Hungary demonstrates that the most seeds germinated from the upper 2.6-3 cm layers (Kazinczi et al. 2008). In a burial experiment Willemsen (1975) tested the germination rate of ragweed seeds on the soil surface and 5 cm and 15 cm below the soil surface. He also found the most ragweed seeds germinating at the soil surface.

According to Kazinczi et al. (2008) seeds of *A. artemisiifolia* on the soil surface or from the upper soil layer as well as those which were stored at room temperature can lose their viability after four years. However, seeds from deeper soil layers (35-45 cm) can keep their viability for a longer time (30-40 years). Toole & Brown (1946) showed that ragweed seeds buried in the soil remained viable for 39 year or more. Similarly the result of Dr. Beal´s seed viability experiment demonstrates that *A. artemisiifolia* stayed viable after storing 40 years in the soil (Tewelski & Zeevart 2002).

Ambrosia artemisiifolia is adapted to soil disturbances, which is clearly shown by its feature to germinate from the soil surface and the upper soil layers (Willemsen 1975). To protect the seed bank of common ragweed in the event that the site is disturbed again when the environmental conditions may be not suitable for seedling growth, *A. artemisiifolia* has developed an induced secondary dormancy (Willemsen 1975, Bazzaz 1979, Baskin & Baskin 1980). Referring to Bazzaz (1979) this is a typical strategy for early successional plants.

The biological features described above explain why common ragweed grows only on sites where disturbances resulting in open soil patches regularly occur. Due to the species´ ability to build up a persistent seed bank, common ragweed seeds can stay viable in the soil for some decades. In case of newly occurring disturbances resulting in the exposure of seeds on the top soil connected with suitable climatic conditions common ragweed can grow up quickly, flower and fill up the seed bank again.

Adaptability to habitat conditions

Ambrosia artemisiifolia has a distinct phenotypic plasticity, which allows it to tolerate a wide range of ecological conditions (Bazzaz 1974, Raynal & Bazzaz 1975). An examination of Leiblein (2008) demonstrates that common ragweed can grow and produce seeds in dry, moist and even under waterlogged soil conditions. According to Berés & Hunyadi (1991 in Kazinczi et al. 2008 a) it grows on every soil type in Hungary. It also sporadically occurs on saline soil types. In Slovakia *A. artemisiifolia* grows in saline grassland and is mentioned as a "diagnostic species" for that grassland among other species like *Artemisia santonicum*, *Cynodon dactylon*, *Plantago maritima* and *Podospermum canum*. Saline grassland is a NATURA 2000 habitat (1340* Inland salt meadows) (Seffer et al. 2002). *A. artemisiifolia* also exists in the coastal coenoflora of Ukraine (Dubyna et al. 2010). However, on strongly acid soils plants are less vigorous (Bassett & Crompton 1975).

In Hungary, where common ragweed is widely distributed, it is dominant on haplic cambisols, sandy soils and on fluvisols. Most favourable for its growing are slightly acidic, sandy adobe and muddy loam soils (Kazinczi et al. 2008). The species has a good drought tolerance and its sub-lethal water saturation deficit is high compared to other species (Kazinczi et al. 2008). Common ragweed seedlings tolerate water stress and their photosynthesis remains relatively high even at water potentials as low as -20 bars (Bazzaz 1974). Although common ragweed is a plant of open sunny habitats with a high photosynthetic rate (Bazzaz 1974), its photosynthetic light compensation is reached at a radiation intensity as low as 7 µmol $m2^{-}$ s^{-} detected for common ragweed plants from Germany, which enables the plant to grow even under shady conditions (Leiblein 2008). However, in closed plant associations, shading is found to be clearly inhibiting both the germination and the vegetative development of *A. artemisiifolia* (Szigetvári & Benkö 2008).

common ragweed plants are very variable concerning their growth. Currently common ragweed ecotypes are already present, as Dickerson and Sweet (1971) describe. Song & Prots (1998) describe a late-autumn variety called *A. artemisiifolia* var. *atropurpurea* which was found growing 730 m above sea level. In East Germany, common ragweed plants were found that flower as early as in June.

Compilation of data according biological features of common ragweed

In tab. 1 biological features of common ragweed respectively habitat conditions of suitable growing sites are compiled. Some features might promote the spread, others could be limiting factors.

Tab 1: Biological features and habitat conditions that could promote or limit the spread of common ragweed in a new range.

Biological feature/ habitat conditions	Feature could promote spread	Feature could limit spread
Spreading capacity	High spreading capacity especially at anthropogenic sites, spread mostly due to human activities (e.g. within soil, mowing machines, agricultural machines), Limited number of predators compared to natural habitat	Relatively big seeds, seeds not transported by wind (no flying capacity) Low spreading capacity without human assistance, probably except for spread in floating water
Competitive ability	Able to built high and dense stands especially in nitrogen-rich habitats, some competitive ability (e.g. can cause notable yield losses), relatively high drought tolerance	Plants usually remain small in nitrogen-poor habitats and often build light stands, but influence on water und nutrient supply not known, only sparse information about allelopathic ability. Easily displaced by perennial species
Regeneration capacity	High regeneration capacity after injuries (e.g. mowing, grazing), even small plants can produce seeds	-
Reproductivity and germination rate	Big plants mainly on nutrient rich soils can produce high amounts of seeds, high germination rate of seeds in upper soil layers in open regularly disturbed areas (by anthropogenic or natural means)	Small plants usually have little seeds, Low/no germination of seeds buried in the soil at undisturbed sites
Seed bank	Long living seed bank in seeds buried in deeper layers	Seeds in upper soil layers loose their viability earlier
Habitats grown by the species	Wide range of habitats, predominantly at sunny sites, disturbances necessary for germination	Less vigorous at shady sites, no/ low germination in undisturbed habitats
Adaptability to habitat conditions	Large phenotypic plasticity and genetic diversity, ecotypes present	-

Habitats with occurrences of common ragweed

Ambrosia artemisiifolia occurs in a wide range of open disturbed areas, as well in its native as in its anthropogenic range. It is widespread on arable land and on ruderal sites such as waste lands, railway areas, construction sites, parks, road sides, river banks, orchards and vineyards, meadows, pastures, afforestations, glades in forests, and fields located inside of forests cultivated by hunters for shelter and feeding of wild animals (Bazzaz 1974, Bassett & Crompton 1975, Galzina et al. 2009, Kazinczi et al. 2008b, Týr et al. 2009, Pinke et al. 2011, Bauer 2006, Alberternst et al. 2006). According to Szigetvári & Benkö (2008) *A. artemisiifolia* appears in Hungary in any places apart from extreme conditions or with very low isolation.

Results from the inquiry via questionnaire (question 1)

According to the answers from the questionnaire the most extended area colonized by common ragweed in many countries such as Hungary, Croatia, Canada, France, Italy and Germany are agricultural fields (Pál, Bauer, Csecserits, Galzina, Kotanen, Chauvel, Bonini). However, this habitat type does not provide the most extended area grown by the species in countries such as Switzerland, Russia, Czech Republic, and Serbia (Schoenenberger & Rossinelli, Reznik, Skalova, Bozic) where other habitats are mostly infested. Also in Austria the agricultural fields range on the second place for the most extended growing area after the road sides (Karrer).

Ambrosia artemisiifolia often grows on stubble fields of cereal crops as it is for example described from the Lombardy region by M. Bonini. In Serbia and in Russia the area most occupied by common ragweed is on fallow agricultural fields (Bozic, Reznik), whereas in East of France, Germany, Austria and Czech Republic the growing area on abandoned fields is not very extended (Chauvel, Skálová, Karrer). Common ragweed often occurs at field margins in East-France (where the species is considered to be still rare), Germany, Austria, Italy, Croatia, Hungary, Canada, and Russia whereas common ragweed was scarcely found in agricultural regions but it mostly grows in railway areas in Czech Republic (Skálová).

Road sides are often colonized by common ragweed and range on the first or second place for the most extended growing area in France (Chauvel), Austria (Karrer), Switzerland (Schoenenberger & Rossinelli), Germany (Nawrath & Alberternst), Italy (Bonini), Croatia (Galzina) and Canada (Kotanen).

Road margins play an important role in the spreading process of *A. artemisiifolia*. In rural settings of southern Québec, the species was clearly more abundant along rural roadsides than in fields or field margins in 2007 and 2008 (Simard & Benoit 2010). In southern Québec the expansion of the road network during the 20th century was probably the main factor that favoured the rapid dispersal of common ragweed (Lavoie et al. 2007). Road sides provide conditions appropriate for germination and growth of common ragweed because they receive considerable sunlight and are frequently disturbed (e.g. by the road maintance) (Vitalos & Karrer 2009). The seeds are easily dispersed along the roads by vehicles or by water in drainage ditches (Bassett & Crompton 1975). However, the type of the road is important for the occurrence of the species, which could be demonstrated by Joly et al. (2011) for Québec: at verges of paved roads the species was much more frequent than at unpaved roads. Also in Germany numerous common ragweed stands were detected along the verges of Bavarian highways since 2009. Due to the fact that ragweed stands at highways in Bavaria were nearly unknown until a few years ago, the spread of the species along roads is a new phenomenon in Germany (Nawrath & Alberternst 2010, 2011). In Austria the number of records of common ragweed stands increased most strongly on road sides between 1995 and 2005 compared to other habitats such as railways, other ruderal habitats, or fields (Essl et al. 2009). From road sides, common ragweed may spread into the surrounding vegetation and also into agricultural fields.

Based on the answers from the questionnaire, common ragweed often or relatively often grows in urban-industrial sites, and it is documented from numerous construction areas in different countries. It sometimes grows in managed grassland such as meadows and pastures e.g. in Canada, Croatia, Czech Republic, Germany, Hungary, and Russia. In Russia in some cases it is found along the borders of forests (Reznik) and it occurs in Hungary in *Robinia pseudoacacia* and *Populus x euramericana* forests (Bauer, Csecserits). According to the information given by A. Csecserits, common ragweed sometimes occurs in Hungary in oak forests at deer yards or in game fields and along the roads.

Sometimes common ragweed grows in nutrient poor grassland (inclusive sand biotopes and steppe vegetation) in Hungary (Bauer, Pál, Csecserits). In Russia, the Czech Republic, Croatia, Canada, and Germany, common ragweed is found in this habitat, but compared to the other habitats it only rarely occurs here.

According to Szigetvári & Benkö (2008) in Hungary *A. artemisiifolia* is absent from undisturbed, near-natural habitats and secondary habitats that have been in the process of regeneration for a longer time, although the species is very common in that country.

Vegetation co-occurring with *A. artemisiifolia*

Ambrosia artemisiifolia is present in various plant communities which are described below.

Segetal and ruderal plant societies

In the following segetal and ruderal plant societies described from various countries that contain *A. artemisiifolia* are presented. In some cases the phytocoenoses from arable fields and ruderal sites occur in both habitat types. Segetal plant communities have been investigated intensively in Hungary by Pinke (2000, 2007) and Pál (2004). The synoptic tables of relevés (Pinke 2000, 2007) demonstrate that common ragweed is present with high frequency (more than 50 %) in various weed communities of extensively cultivated arable fields in Hungary. The following list gives an overview of these plant communities from Hungary and also of plant communities including common ragweed described from other European countries:

Stellarietea: Segetal and short living ruderal plant societies

- Camelino microcarpae-Anthemidetum austriacae
- Aphano arvensis-Matricarietum chamomillae
- Spergulo arvensis-Anthemidetum ruthenicae
- Sisymbrio orientalis-Anthmidetum ruthenicae
- Stachyo annua-Setarietum pumilae
- Chenopodio-Oxalidetum fontanae
- Echinochloo-Setarietum pumilae
- Digitario-Setarietum pumilae
- Trifolium arvense-Ambrosia artemisiifolia-Gesellschaft
- Capsello-Descurainietum papaveretosum
- Panico-Galinsogetum
- Odontito-Ambrosietum
- Ambrosietum artemisiifoliae
- Ambrosio artemisifoliae
- Ambrosio artemisiifoliae-Xantheum strumariae
- Ambrosio-Setarietum viridis
- Ambrosia artemisiifolia-Datura stramonium community
- Sisymbrion
- Societies of Eragrostietalia
- Polygono-Chenopodietalia

Artemisietea: Perennial ruderal vegetation

- Artemisio-Tanacetum
- Arctio-Artemisietum vulgaris
- Onopordion
- Arction, Convolvulion
- Alliarion

Plantaginetea: Plant communities resulting from trampling

- Plantaginetea (incl. Agropyro-Rumicion crispi)
- Rumici acetosellae-Spergularietum rubrae

others

- Quercion
- Sambuco-Salicion,
- Festuco-Brometea
- Sedo-Scleranthetea
- Rununculo sardoi-Alopecuretum geniculati

Description of the communities

The association **Camelino microcarpae-Anthemidetum austriacae** is common in cereal winter crops but it also occurs in spring crops and is physiognomically characterized by *Anthemis austriaca* which usually is present in large quantities. Seedlings of *A. artemisiifolia* often occur in this community. Dominant and constant accompanying species are *Papaver rhoeas*, *Consolida regalis*, *Apera spica-venti*, *Galium aparine*, *Elymus repens*, and *Cirsium arvense*. The **Aphano arvensis-Matricarietum chamomillae** is the most common association of winter crops and occurs on acid, loamy and clayey soils. In the relevés of Pinke (2007) common ragweed occurs in this association with a frequency of 70 % but is often present only with low densities (mostly "+" and 1). The **Spergulo arvensis-Anthemidetum ruthenicae** is widespread on acid nutrient-poor sandy soils in the Trans-Danubian mountains as well as in the West Hungarian area where the investigations were conducted. Characteristic species are *Papaver argemone*, *Herniaria hirsuta*, *Veronica triphyllos*, *Vicia villosa,* and *Trifolium arvense*. *A. artemisiifolia* often occurs in the lower herb layer of this community which has an optimum from middle of May to middle of June. The **Sisymbrio orientalis-Anthemidetum ruthenicae** (former name Camelino-Anthemidetum sisymbrietosum) evolves on basic, sandy soils in extensive cereal crops. Here besides *Anthemis ruthenica*, *Veronica triphyllo*s, *Vicia villosa* and *Trifolium arvense*, species like *Bromus tectorum*, *Cerastium semidecandrum*, and *Silene conica* occur. *A. artemisiifolia* often is present in the lower herb layer. The community also has an optimum from middle of May to middle of June. The **Stachyo annuae-Setarietum pumilae** is a species-rich stubble plant community which has a symphenological optimum in late summer/early autumn. The community develops best in stubble of cereal spring crops. *Stachys annua*, *Anagallis foemina*, *Silene noctiflora*, *Euphorbia exigua*, *Kickxia elatine* et *spuria* are characteristic species of this community. *Ambrosia artemisiifolia* often occurs in this plant community, and according to the relevés of Pinke (2007) it has a constancy of 76 % in the typical variant respectively 92 % in the variant with Oxalis *stricta*.

Ambrosia artemisiifolia is also among the dominant constantly occurring accompanying species in the community **Chenopodio-Oxalidetum fontanae** and it occurs in the **Echinochloo-Setarietum pumilae** which is a typical association of root crops. The community develops in a relatively short time scale after the last hoe. Diagnostic species besides of *A. artemisiifolia* are *Echinochloa crus-galli*, *Amaranthus chlorostachys*, *A. retroflexus*, *Galinsoga parviflora*, *Chenopodium album* et *hybridum*, *Mercurialis annua*, *Persicaria lapathifolia*, *Convolvulus arvensis*, *Cirsium arvense*, *Stellaria media*, *Setaria pumila* et. *viridis*, *Solanum nigrum* (Pinke 2000, 2007).

Another community on stubble fields is the **Trifolium arvense-Ambrosia artemisiifolia community**. This fragmentary community often occurs on stubble fields instead of the Camelino-Anthemidetum scleranthetosum on sandy-loamy soil. *Ambrosia artemisiifolia* dominates the upper vegetation layer, whereas *Trifolium arvense* grows in the medium layer. *Polygonum aviculare*, *Setaria pumila*, *Conyza canadensis*, *Elymus repens*, *Anthemis ruhtenica* (seedlings), *Fallopia convolvulus* occur (Pinke 2000).

The **Trifolium arvense-Ambrosia artemisiifolia community** presents a stage of succession into the association Odontito-Ambrosietum (Silc 2002) described later.

common ragweed is found in the **Capsello-Descurainietum papaveretosum** which is often present on first year abandoned fields, at the edges of winter crops and at the margins of fields. In the relevés demonstrating the floristic composition, common ragweed has only low densities (Pinke 2000, Pál 2004).

Referring to Pál et al. (2006) *A. artemisiifolia* also occurs in waterlogged patches on agricultural fields in Hungary. The plant association that is present in these wet patches matches the **Rununculo sardoi-Alopecuretum geniculati** Bodrogközy 1962. *Ambrosia artemisiifolia* is included in the relevés which show the floristic composition of these wet patches but is only mentioned to be an indifferent species. Common ragweed occurs here with high consistency (value V = 75-100% of the relevés) but only with low cover at the time the relevé was taken (in June 2005, usually "+" or "1", Scale of Braun-Blanquet 1964). During an investigation in September, common ragweed was also registered in this association with a high consistency of V. The cover was higher than in spring and often achieved the values "2" (5-25 %) and "3" (25-50 %).

From the Crimea region in Ukraine another segetal weed association called **Ambrosio artemisifoliae-Cirsietum setosi** Marjuschkina et B. Sl. 1985 is described (Bargrikova 2005). The association belongs to the order Polygono-Chenopodion W. Koch 1926 em Siss. 1946 and belongs to the class Stellarietea mediae R. Tx., Lohmeyer & Preising in R. TX. Ex von Rochow 1951. The class Stellarietea mediae comprises the associations of communities with annual species that characterize the initial stages of restoration successions that follow after disturbances (Bargrikova 2005).

In Serbia, *A. artemisiifolia* occurs in the association **Panico-Galinsogetum** Tx. et Beck 1942 in potatoes crops (Ilic & Nikolic 2011). The association is built up by 29 species and it belongs to the order **Chenopodietalia albi** in the class **Stellarietea mediae**. *Ambrosia artemisiifolia* is present in this association but according to the relevés presented, only occurs with low constancy (value II) and low densities (value "+") (Ilic & Nikolic 2011).

In the Czech Republic *A. artemisiifolia* occurs in communities of the **Eragrostietalia,** which is a phytosociological order that includes thermophilous communities of therophytes on loose substrata mainly in south and southeast Europe. The communities occur on light-textured soils that dry and warm up rapidly and mostly consist of therophytes with C4 assimilation. Several species growing in these habitats have a clumped spatial distribution, a prostrate habitus, and a rich system of fine roots. Also, species with creeping rhizomes occur and others possess xeromorphic characters. The communities of this alliance became rare or even extinct in the northwest of Europe. In the Czech Republic the communities belonging to the orders Eragrostion and Salsolion ruthenicae may be relevant. In the Czech Republic the communities are on the northwest border of their distribution (Kropác 2006).

Silc (2002) and Silc & Kosir (2006) report from Slovenia that *A. artemisiifolia* is found in the association **Odontito-Ambrosietum** Jarolímek et al. 1997 that belongs to the alliance Dauco-Melilotion Görs 66. The association belongs to the coenological class **Artemisietea vulgaris** Lohm., Prsg. Et Tx. In Tx. 50. The class **Artemisietea vulgaris** describes plant communities of two-year to perennial ruderal communities, usually occurring at dump places, pathways, forest edges and river sides (Oberdorfer (2001). Referring to Silc (2002) the plant community of **Odontito-Ambrosietum** is found on recently deposited rubble, recently levelled terrain, along roads, on gravel sites and more rarely in fields or stubble in Slovenia. The stands are dominated by *A. artemisiifolia*, which constitutes the upper herb layer. Accompanying species are *Chenopodium album*, *Artemisia vulgaris*, *Amaranthus retroflexus*, *Atriplex patula*, *Conyza canadensis,* and *Lactuca serriola*. *Plantago lanceolata, P. major*, *Polygonum aviculare* agg., and *Potentilla reptans* are more or less common in the lower herb layer. Differential species of the association are *Setaria pumila*, *Amaranthus retroflexus*, *Chenopodium strictum* and *Odontites vernus*. Well represented in the association are species of the alliance **Dauco-Melilotion** and of the class **Stellarietea mediae**. Among the accompanying species there are a lot of species of the class **Molinio-Arrhenatheretea**. The syntaxonomic classification is difficult due to a similar number of species of the classes **Stellerietea mediae** and **Artemisietea vulgaris**. The author decided to classify the stands from Slovenia into the class Artemisietea (Silc 2002). Similarly, Brandes (2005) reports from Slovenia that *A. artemisiifolia* is present in the class **Stellarietea** or in the **Dauco-Melilotion**, depending on the location. According to this author, common ragweed often grows with species of the class Molinio-Arrhenatheretea, and thus it is not possible to assign the species to the **Odontito-Ambrosietum** Jarolimek et al. 1997 in every case. The **Odontito-Ambrosietum** was also found in a harbour in Czech Republic (Jehlik 2008). Jehlik et al. (2005) point out that common ragweed was growing in an association probably belonging to the association Odontito-Ambrosietum on sandy deposit of the Danube River in Slovakia.

In Romania *A. artemisiifolia* dominates ruderal phytocoenoses and is integrated in the association **Ambrosietum artemisiifoliae** Vitalariu 1973. According to Coste & Arsene (2003) the association belongs to the order Onopordetalia acanthi in the class Artemisietea vulgaris. The association is found on railway embankments, at ruderal places around the railway stations, which usually have a skeletal substratum, and it entered the fields from the vicinity of the railway embankments in Romania. In the plant community described, *A. artemisiifolia* has a foliar cover of 70 to 100 %. It often occurs with *Conyza canandensis*, *Hordeum murinum*, *Bromus tectorum*, *Bassia scoparia*, *Lactuca serriola*, *Voila arvensis*, *Capsella bursa-pastoris*, *Crepis foetida* subsp. *rhoeadifolia*. The association has characteristic species from the order **Sisymbrietalia**, and the classes **Stellarietea** and **Artemisietea** (Sirbu 2008).

From Serbia Jarić et al. (2011) describes a new association **Chenopodio-Ambrosietum artemisiifoliae** ass. nova that was recorded along the edge of roads and in abandoned fields. It is a ruderal community which is dominated by species of the segetal weed communities of cultivated areas. It is floristically rich in species. Besides common ragweed*, Erigeron canadensis*, *Lactuca serriola*, *Cirsium arvense*, *Galega officinalis*, *Daucus carota*, *Stenactis annua*, *Calystegia sepium*, *Cichorium intybus*, *Medicago lupulina*, *Convolvulus arvensis* occur in this association. These species are characterized by high levels of abundance and cover. The association belongs to the (former) class **Chenopodietea albae** Br.-Bl. 1951 em. Lohm., R. et J. Tx. 1961 (Order: **Sisymbrietalia**, Alliance: **Bromo-Hordeion murini**), which currently is united with the class Stellarietea. It has a high level of non-native plant species. Due to the fact that weeds from these ruderal habitats may invade cultivated areas, this association is important regarding plant protection in arable fields (Jarić et al. 2011).

Brandes & Nitsche (2006) describe occurrences of *A. artemisiifolia* in the **Rumici acetosellae-Spergularietum rubrae** at road sides in Brandenburg, Germany. Common ragweed occurs with species such as *Polygonum aviculare*, *Digitaria ischaemum*, *Spergularia rubra*, *Rumex thyrsiflorus*, *Artemisia vulgaris*, *Tanacetum vulgare*. The community typically is present on open gravel and hard sandy soils in settlements and in railway areas. It belongs to the sociological class **Polygono arenastri-Poetea annuae** (annual trampling communities) (Schubert et al. 1995). According to Brandes & Nitzsche (2006) common ragweed was also found along a pathway in the **Artemisio-Tanacetum**. At road sides most of the ragweed stands have a phytocoenological position between the **Sisymbrietalia**, **Dauco-Melilotion** and **Arrhenatheretalia**.

Additionally, *A. artemisiifolia* was reported to exist in the following phytosociological communities:

- **Arctio-Artemisietum vulgaris** (Tx. 1942) Oberd. et al. 1967, in Serbia, growing with *Artemisia vulgaris*, *Arctium lappa*, *Carduus acanthoides*, *Cirsium arvense*, *Lactuca serriola* (Stanković-Kalezić et al. 2009)
- **Ambrosio-Setarietum viridis** in Slovakia (Mochnacký 2005).
- **Ambrosio artemisiifoliae-Xantheum strumariae** Kost. 1991 in Ucraine (Protopopova et al. 2006).

- **Ambrosia artemisiifolia-Datura stramonium community**, in Germany. The association belongs to the alliance **Sisymbrion** and was found in a harbour at the Elbe-Müritz water way (Dömitzer Hafen). Besides common ragweed and *Datura stramonium* other species such as *Amaranthus retroflexus, Setaria pumila, Galinsoga parviflora, Sonchus oleraceus, Chenopodium album, Oenothera biennis, Berteroa incana, Cichorium intybus* and *Artemisia vulgaris* occur in this plant community (Brandes 2003).

Song & Prots (1998) give an overview of the frequency of occurrence of *A. artemisiifolia* in the most important plant communities for the Ukrainian Carpathian Mountains and the Transcarpathian plain (tab. 2). According to these authors common ragweed predominantly grows in segetal and ruderal plant communities. Much more rarely the species is found in nutrient-poor, dry grasslands (**Sedo-Scleranthetea** and **Festuco-Brometea**) and it rarely occurs in nitrophilous shrub vegetation (**Sambuco-Salicion, Alliarion**) and oak forests (**Quercion**).

Tab 2: Frequency of occurrence of A. artemisiifolia in the most important syntaxa by sociologic-ecological classification in the Ukrainian Carpathians Mountains and the Transcarpathian plain (Song & Prots 1998).

Syntaxa	**Frequency of occurrence in %**	
	Transcarpathian plain	**Ukrainian Carpathians Mountains**
Quercion	1.7	-
Sambuco-Salicion, Alliarion	1.7	-
Festuco-Brometea	5.4	10.2
Sedo-Scleranthetea	3.4	2.5
Plantaginetea (incl. Agropyro-Rumicion crispi)	12.9	18.7
Arction, Convolvulion	13.7	15.1
Onopordion	14.5	9.5
Sisymbrion	15.7	13.6
Polygono-Chenopodietalia	22.4	21.2
Naturalized adventive species (anthropophytes) with undefined phytosociological attachment and ephemerophytes	8.6	9.2

Sandy grassland

According to Bauer (2006), *A. artemisiifolia* grows in open sandy grasslands of the Bakony region in Hungary, where the Festucetum vaginatae is the dominant plant community. *Ambrosia artemisiifolia* is found growing together with *Calamagrostis epigeios* and *Euphorbia cyparissias* in the association **Festuco vaginatae-Corynephoretum**. This association mainly occurs on disturbed patches, in glades in forests, at forest edges, on road slopes of regenerating vegetation adjoining forest and on sandy surfaces on a recultivated area. *Ambrosia artemisiifolia* also occurs in *Corynephorus* grasslands that can be classified as **Thymo angustifolio-Corynephoretum**. In the relevés of Bauer (2006) common ragweed occurs with a frequency of III (25-50 %) and with low levels of folia cover ("+" or "2"). The association can be found on limeless sandy surfaces, where usually calciphobe sandy grassland is characteristic. *Corynephorus canescens*, *Jasione montana* and *Rumex acetosella* are frequent in that association in the Bakony region, whereas *Potentilla argentea*, *Hypochoeris radicata*, *Scleranthus annus* and *Thymus serpyllum* occur infrequently. The association is poor in species and may therefore easily be taken over by weeds. The high frequency of several weeds such as *A. artemisiifolia* and *Conyza canadensis* is the result of severe disturbance (Bauer 2006).

In Germany, common ragweed also grows in open, disturbed pioneer vegetation on sandy soils in the **Corynephoretum** and in the **Bromo-Corispermetum leptopteri** (alliance Salsolion, order Sisymbrietalia;Brandes & Nitsche 2006).

Plant communities in wet habitats or/and along river sides

Ambrosia artemisiifolia has a wide ecological range and is able to grow in habitats with wet soil conditions. In the relevés of Szirmai et al. (2009) the occurrence of *A. artemisiifolia* is mentioned in the plant community **Glycerietum maximae** in the Bodrogköz in Hungary. Additionally common ragweed was found in the herb layer of a stand in the Bodrogköz in Hungary dominated by the sedge *Carex riparia* and the willow *Salix cinerea*.

In South Ukraine, *A. artemisiifolia* is part of a floodplain plant community **Phragmito australis-Amorphetum fruticosae** which occupies the ridges near beds of rivers and periodically flooded territories on boggy, meadow-bog clayey and sandy soils. While the shrub layer is mainly formed by *Amorpha fruticosa*, *Eleagnus angustifolia*, *Salix alba* and *Populus nigra*, the herb layer consists of species such as *Phragmites australis* (5-25 %), *Conyza canadensis*, *Poa angustifolia*, *Xanthium strumarium* and even *A. artemisiifolia* (each up to 10 % foliar cover, Dziuba et al. 2010).

Jehlik et al. (2005) found *A. artemisiifolia* growing in the association **Bidenti-Polygonetum hydropiperis** Lohmeyer in R. Tx. 1950 on a gravel bank at the Danube River at Hamuliakovo in Slovakia. The association belongs to the alliance Bidention tripartiti. Common ragweed occurs in this plant society with species such as *Persicaria hydropiper*, *Bidens frondosa*, *Ranunculus repens*, *Carex acuta*, *Galium palustre*, and *Iris pseudacorus*.

common ragweed was found in the floodplain on the Croatian side of the Drava River (Csiky & Purger 2008) in amphibious plant communities. It occurs in the association **Polygono-Eleocharitetum ovatae** Eggler 1933 which belongs to the class Isoeto-Nanojuncetea (incl. Nanocyperion) and is present on bare surfaces of river gravel and sand banks. In the relevé presented, common ragweed co-occurs with species such as *Carex bohemica*, *Phalaris arundinacea*, *Polygonum hydropiper*, *Agrostis stolonifera, Cyperus glomeratus*, *Juncus articulatus*, *Rorippa palustris*, *Salix pupurea*, *Solidago gigantea*, *Cyperus fuscus*, *Conyza canadensis*, *Poa compressa*. In the floodplain of the Drava River many rare plant species listed in the Croatian red list are present, e.g. *Cyperus fuscus*, *Chenopodium ficifolium*, *Limosella aquatica*, *Scrophularia umbrosa*, *Leersia oryzoides,* and *Cyperus glomeratus*. *Carex bohemica* is also rare in Hungary. *Ambrosia artemisiifolia* occurs in the floodplain besides other invasive plant species such as *Solidago canadensis, Robinia pseudoacacia, Amorpha fruticosa, Ailanthus altissima, Linderia dubia* which are a potential danger for the valuables stands of the natural vegetation in the flood plain (Csiky & Purger 2007).

Influence of common ragweed on the vegetation

In the following the interference of common ragweed and the surrounding vegetation is described from the literature. Results from own investigations and the information given by the experts in the questionnaire are added.

a) Succession on abandoned fields in the native range

Ambrosia artemisiifolia is a pioneer species of open disturbed habitats where it builds up dense and extensive stands. It is able to grow in various habitats with disturbances regularly occurring, and it spreads from its growing sites predominantly by human activities. If the vegetation develops without further disturbance, in its native range common ragweed is suppressed by other plant species within a few years (Bazzaz 1968). In his study aiming in a determination of trends and rate of secondary succession, Bazzaz (1968) found *A. artemisiifolia* dominating an abandoned corn field in the first year after harvest. In the second year common ragweed was also abundant with a frequency of 97 %, but only few individuals were more than 15 cm tall. In the third year common ragweed still had a high frequency (80 %), but the plants were rather small and inconspicuous and were suppressed by other plant species. Bazzaz (1968) concluded that dominant and subdominant species on abandoned fields become established in a definite sequence. In this study winter-annual plants, especially *Erigeron annuus* and *Erigeron canadensis,* suppressed the growth of common ragweed-seedlings in undisturbed sites in the second year after abandonment. The winter annuals germinated in late summer and fall of the year following the abandonment. They developed into rosettes during fall and winter and in the following spring and summer these plants had a competitive advantage over the summer annuals that geminate in spring. This leads to progressive suppression of summer annuals such as common ragweed (Raynal & Bazzaz 1975). The population regulation of a common ragweed-stand occurs through phenotypic plasticity rather than by density-dependent mortality. This results in the survival of many stunted plants which produce only a few seeds. By this way there still remains a diversity of genotypes which can further adapt to environmental conditions (Raynal & Bazzaz 1975).

Armesto & Pickett (1985) who investigated changes in species richness and abundance following experimental disturbance in a 7th-yr old field dominated by *Solidago canadensis* and a 2nd-yr old field dominated by *A. artemisiifolia* in the species´ native range, state that common ragweed did not suppress the growth of other species early in summer, perhaps due to its slower growth rate and thinner cover compared to Solidago.

b) Succession on abandoned fields in the non-native range

Also investigations from Hungary demonstrate that common ragweed predominantly occurs in recently abandoned fields while it rarely grows in fields where closed secondary grassland established: Csecserits et al. (2011) investigated the vegetation of abandoned fields where the fields were categorized in fields that were abandoned 1-7, 8-20, and 21-57 years ago. *Ambrosia artemisiifolia* was found to be the most important non-native species in "young old fields" (abandoned 1-7 years ago), but its cover decreased significantly during the succession. Similarly Csecserits et al. (2009) found *A. artemisiifolia* occurring primarily on "young old-fields" in the Danube-Tisza-Interface in Hungary. On fields with a longer time of abandonment, where closed secondary grassland could establish, the abundance of common ragweed was lower than for example on plough-lands, and in semi-natural habitats (e.g. grasslands, forests). Here ragweed occurred only rarely and with low abundance (Csecserits et al. 2009).

In contrast to the situation described above, Maryushkina (1991) found that common ragweed inhibits the restoration of both annual and perennial native species, decreases the diversity of communities and delays the succession process in its new range in Ukraine. The author investigated the effect of common ragweed on species diversity on an abandoned field in Ukraine in order to analyse the influence of common ragweed on native species. Species composition and phytomass was compared on plots where common ragweed was manually removed with plots without removal of the plant. On a freshly ploughed plot on an abandoned field common ragweed significantly decreased the number of annual species, especially at the beginning of the succession process. From the results the author states that *A. artemisiifolia* inhibits the restoration of both annual and perennial native species, decreases the diversity of communities and delays the succession process in its new range. Common ragweed might profit from the fact that the number of herbivores is limited in the new range, while in the native range over 300 species of phytophagous insects feed on plants of the genus common ragweed (Kovalev 1971 a, b in Maryushkina 1991).

c) Suppression of common ragweed by perennial plants

Milanova et al. (2010) report that the plants *Lolium perenne*, *Dactylis glomerata* and *Medicago sativa* are able to suppress *A. artemisiifolia*. The turf of *Lolium perenne* was found to inhibit strongly (98 %) the production of biomass of common ragweed. *Medicago sativa* and a mixture of *M. sativa* and *Dactylis glomerata* are reliable means to suppress the growth, development and seed production of common ragweed under suitable conditions (Valkova et al. 2009). In Brandenburg (Germany) an extensive stand of *A. artemisiifolia* on an arable field was reduced nearly completely until 2011 after cultivation of a mixture of grasses and lucerne in 2009 (Jentsch 2011, pers. communication).

d) Investigations in dry grassland areas

In open grassland common ragweed is often suppressed by the closing grassland vegetation after a short time. For example in grassland which developed on an abandoned field on sandy soil in the Niederlausitz in 2007, millions of common ragweed plants were found and about 800 common ragweed plants were counted on an area of 1 square meter. Four years later common ragweed was still present in the grassland, but it occurred only to a minor extent, mainly at disturbed open patches (Fig. 1, 2). This was probably due to missing disturbance and the competition of the accompanying vegetation.

Fig 1: Grassland on an abandoned field near Leuthen, 11th August 2007. Common ragweed dominated the field. Approx. 800 common ragweed plants per m^2 were counted.

Fig 2: The same area four years later: Only a small amount of common ragweed plants was present in the meadow (9th July 2011).

According to investigations of Maryushkina (1991), common ragweed is a typical r-strategist (Grime 1979), which only is able to suppress other plants with r-properties. *Ambrosia artemisiifolia* itself is inhibited by species with K-properties. Thus, in Ukraine common ragweed usually does not penetrate into pastures which are normally dominated by species with K-properties.

Szigetvári (2004) studied the role of invasive alien plants in open sandy grassland in Hungary. The general aim of the study was to give a general description of the interaction between the most important non-woody invasive plants and the open sand grassland vegetation in the Kiskunság region in Hungary. The study took place in an area which was dominated by different types of the Pannonian open perennial sand-grassland (Festucetum vaginatae). In some patches the perennial grassland was linked to open annual grassland (Brometum tectorum), and agricultural fields as well as old fields are located between the sand-grassland areas. The area was of high conservation value. Szigetvári (2004) studied the role of invasive alien plants in the grassland and found *A. artemisiifolia* which was present in this area almost exclusively growing on the dirt roads (Szigetvári 2002, 2004). According to the author, common ragweed did not seem to have a substantial transformer impact on the essential dynamic processes and structural relations of the open sand grasslands. It was strongly related to recent disturbances and does not threaten undisturbed vegetation (Szigetvári 2004). According to Szigetvári & Benkö (2008) common ragweed occurs in near-natural sites mostly in the more frequented and thus more disturbed visitor zones, and in the buffer zones where often many newly abandoned fields providing suitable growing conditions for the species are present.

However in Bavaria (Germany) common ragweed spread on a dry inland sand dune protected for nature conservation reasons, where it was introduced unintentionally with an illegal soil deposition. The species spread in the disturbed area but was also dispersed into areas which were untouched by the soil deposition. In 2004 ca. 10,000 plants were removed by hand pulling. The ragweed population decreased to 11 plants in 2011, after regularly removal. Although the plants were displaced and thus were hindered to fill in the seed bank, the species is still present and single plants grow up till now. Possibly trampling of wild deer leads to natural disturbances and stimulates growing of common ragweed from the seed bank (pers. comm. S. Nawrath 2012/03/01). This case gives a hint that common ragweed might be able to grow in dry grassland valuable for nature protection, in case it reaches the area and natural disturbances by animals occur.

e) Impact on segetal communities

In the lowlands of Croatia the species is so frequent that it displaces all autochthonous weed species, which cannot compete with common ragweed (Pandza et al. 2001). Also in Hungary, where common ragweed is observed to build up very dense stands, it directly suppresses other weed species on arable fields (R. Pál, personal communication 2012/20/02) and thus can have severe negative impact on segetal plant communities. In Romania, a negative influence on the biodiversity of the segetal flora is expected in case of an uncontrolled expansion of *A. artemisiifolia* (Farcasescu & Lauer 2007).

Plant communities of extensive arable fields in Hungary, especially the Camelino-Anthemidetum caucalidetosum and scleranthetosum, represent refuges for many threatened weed species (Pinke 2000). Also, wet patches on arable fields have a high value for agrobiodiversity and provide a growing habitat for many endangered species such as *Elatine alsinastrum*, *Limosella aquatica*, *Linderia procumbens*, *Montia fontana*, *Peplis portula* (Pál et al. 2006). Weed species are valuable sources of food for animals and habitats for several insect and bird species (Pinke & Pal 2009, Pinke et al. 2011). Pinke et al. (2011) state that the biggest threat to the conservation of endangered weed species in Hungary is the increasing spread of *A. artemisiifolia*. According to the authors this is not only so because the species is invading more and more habitats of rare weed species but also because of eradication campaigns against common ragweed leading to a total weed control (see below).

f) Results from the inquiry via questionnaire (question 2)

According to the answers given by the experts on the questionnaire, in countries or regions with low common ragweed infestations such as Eastern France/Burgundy/Côte d´Or (invasion front; Chauvel), Czech Republic, Switzerland, and Germany it currently is not known and/or documented that common ragweed directly suppresses rare/and or endangered species. Also in Russia where common ragweed occurs in natural habitats in a few cases, no effects on biodiversity are known. However, also in countries where the species occurs more frequently data on biodiversity impacts are not or only sparsely available. A main reason for this might be that common ragweed predominantly grows in disturbed anthropogenic habitats such as road sides, construction sites, fields that are regarded as areas with relatively low nature protection value (Schoenenberger & Rossinelli, Reznik).

Field work

The field work was conducted from 5th to 11th July 2011 in the Niederlausitz in Eastern Germany. This area was chosen because it is the most common ragweed-infested area in Germany. *Ambrosia artemisiifolia* is present in this area since many decades (Hegi 1974), but the rapid spread of the species is a new phenomenon (Jentsch 2007). The study area, bordered by the villages Calau, Cottbus, Spremberg and Senftenberg, is characterized by the surface mining of brown coal, which took place during the last 100 years and is still practiced up today. Sandy soils dominate the region, and due to the mining the groundwater is lowered. On the sandy soils, often pine forests grow, and in the plains arable fields occur (BfN 2012). It was the aim of the field work to find out whether habitats or species are affected by the species.

Consultation of local experts

Due to the intensive surface mining large parts of the landscape were destroyed and are now in a recultivation process. In these areas bare soil is often present and the vegetation often shows gaps. These open areas provide good growing conditions for common ragweed. Thus, local experts were contacted to find out whether common ragweed is already spreading in the recultivation areas. The "Lausitzer Seenland Gemeinnützige GmbH" that conducts a nature conservation project in a 5760 ha big area near the city Hoyerswerda was contacted. Common ragweed occurs in that region (personal communication Dr. Alexander Harter), and during a field trip on 7th July 2011 Mr. Noak from the working group thankfully presented the plants in the field. Mostly *A. psilostachya* was present, forming extensive populations with thousands of sprouts (fig 3), but at the margin of a sandy road also a small population of *A. artemisiifolia* was detected. Occurrences of *A. artemisiifolia* were found only at the side of the dirt road. It was not known how long the species was present here, and thus it was not possible to estimate whether it enters and spreads in the open mining areas or not.

Additionally a local expert, Helmuth Jentsch, who studies the flora and vegetation including the spread of common ragweed since the 1950s (e.g. Jentsch 2007), thankfully provided a field trip at the 6th of July and demonstrated various habitats with occurrences of the species and gave an overview of the vegetation that contains common ragweed.

Fig 3a, b: Extensive stand of *A. psilostachya* growing in a recultivation area in the "Lausitzer Seenland" (demonstrated by Mr. Noak 2011/7/7). *A. psilostachya* often grows here with *Helichrysum arenarium, Jasione montana, Trifolium arvense*.

Vegetation units with occurrences of *A. artemisiifolia*

During the field work 56 relevés were conducted at sites, where *A. artemisiifolia* was found to a remarkable amount in the following habitats:

- Arable fields
- Fallow arable fields
- Field margins
- Field paths
- Forest paths and forest edges
- Road sides and food paths
- Ruderal areas
- grassland

a) Arable fields

In the investigated area *A. artemisiifolia* was often found growing on arable fields. Sometimes common ragweed occurred only at the edges of agricultural fields, in other cases the species was dispersed over the whole field (figs 4, 5). Twelve relevés were conducted on fields highly infested with the species (2 x peas, 2 x lupine, 4 x rye, 1 x sunflower, 1 x barley, 1 x maize, 1 wet patch on an arable field). 78 plant species in addition to common ragweed were found in the relevés and the mean species number was 14.5. Common ragweed was found with a cover from 3 (25-50 %) to 5 (75-100%), on average nearly 75 %. The species mostly growing with common ragweed in the fields were *Chenopodium album* and *Apera spica-venti*. Besides, *Artemisia vulgaris*, *Centaurea cyanus*, *Polygonum aviculare* agg., *Viola arvensis,* and *Lolium perenne* often occurred. Common ragweed was also found growing at a wet patch in an arable field. Here it grew with species such as *Glyceria fluitans, Rumex maritimus, Rorippa palustris, Gnaphalium uliginosum, Polygonum hydropiper*, and *P. persicaria*. Common ragweed was present in this vegetation type only with a relatively low cover of 16-25 % (value 2b).

No endangered species were detected in the sites investigated. Similarly, Nitzsche (2010) who conducted relevés in arable fields in the same region some years earlier (in 2006), did not find endangered species on these sites too, whereas Jentsch (2007) reported of *Filago arvensis* growing in arable fields together with common ragweed. *Filago arvensis* is recorded in the German Red List of endangered plant species. It is critically endangered in the Federal State Brandenburg (Ludwig & Schnittler 1996).

Fig 4: Sunflower field highly infested with common ragweed near Auras (2011/11/07).

Fig 5: Lupinus field completely infested with common ragweed near Koschendorf (2011/10/07).

Common ragweed was found predominantly at places where disturbances regularly took place. In dense vegetation with a large proportion of turf it was not present, or only rare. For example *A. artemisiifolia* was found growing with high densities in a rye field (fig 7) near Domsdorf. The field bordered on a light pine forest which had a dense grass-dominated understory. Common ragweed was not found in this vegetation unit and was also absent from the pine forest. It only occurred in the arable field which was regularly ploughed (fig 6, 7).

Fig 6: common ragweed occurs in high densities in the understory of a rye field near Domsdorf (2011/7/8).

Fig 7: common ragweed only occurs in the field regularly ploughed. It was not found in the grass dominated edge of a light pine forest that borders on the field (2011/07/08).

b) Field margins and field paths

In some cases, *A. artemisiifolia* occurs at the margins of the arable field only. In relevés of the margins of two wheat and four maize fields, 73 plant species were found apart from common ragweed. The medium plant number per relevé was 20.5, which is much higher compared with the situation inside of the field, where only 14.5 species per relevé were detected. The most common plant species growing at the field margins are *Echinochloa crus-galli, Elymus repens* and *Chenopodium album*. Once the endangered *Filago arvensis*, was found, and also *Jasione montana, Galium verum, Anchusa arvensis, Daucus carota, Lotus corniculatus* and *Vicia angustifolia* occurred at the field margins of the area investigated.

In some cases common ragweed was also present on field paths where the species usually remained small due to frequent disturbances (e.g. trampling) and occurred only in small amounts. In a relevé from a field path near Löschen, 17 plant species were found. Common ragweed grew here with species typical for trampled vegetation such as *Plantago major*, *Lolium perenne*, *Poa annua*, and *Polygonum aviculare* agg. Next to the field path investigated, dry extensive grassland with species such as *Helichrysum arenarium*, *Jasione montana*, *Artemisia campestre*, *Sedum acre* and *Festuca ovina* was present. Although this vegetation was full of gaps, common ragweed was not found here.

c) Fallow arable fields

Ambrosia artemisiifolia is able to built up extensive populations on fallow arable fields in the area investigated (fig 9). The species was recorded with cover values of 3 (25-50 %), 4 (50-75 %) and 5 (> 75%) and the plants grew up to a height of ca. 60 cm. 30 plant species were found on three fallows growing with common ragweed, and the mean species number was 23.3. *Conyza canadensis* and *Apera spica-venti* often occurred in this habitat type. During the investigation no endangered species were detected on the three fallow fields. Nitzsche (2010) also found the two species mentioned above most frequently growing with common ragweed in fallow lands and at ruderal sites. Jentsch (2007) reported from these habitats the endangered species *Filago arvensis* growing here with common ragweed. In the relevés containing Filago, common ragweed only had a cover of 2a (cover 5-15 %) or 2b (cover 16-25 %).

Comparable to the situation on the arable fields, common ragweed predominantly occurred in disturbed areas. In a ruderal field next to the fallow heavily grown with common ragweed only a small amount of small (< 10 cm) common ragweed plants was detected (fig 8, 9).

In nitrogen-poor grassland next to a fallow field where many common ragweed plants occurred, no ragweed was found, although gaps were in the vegetation of the grassland (fig 11, 12). In the grassland *Helichrysum arenarium* was present. According to the Red list of endangered species *Helichrysum* is endangered in Germany (Ludwig & Schnittler 1996, value 3), but not in the federal state Brandenburg where the study area is located.

Fig 8: Extensive stand of A. artemisiifolia on an abandoned field at Buckwitzberg (2011/8/7; pictures: Alberternst)

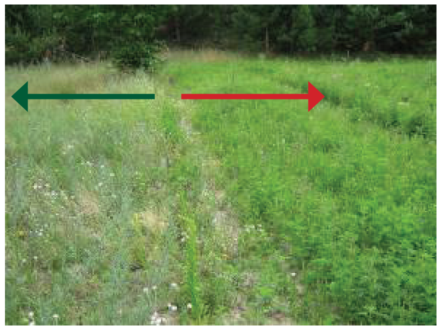

Fig 9: common ragweed predominantly occurs on the fallow field (on the right). Only a small amount of little (<10 cm) ragweed plants were found in the ruderal area (on the left) beside of the fallow field.

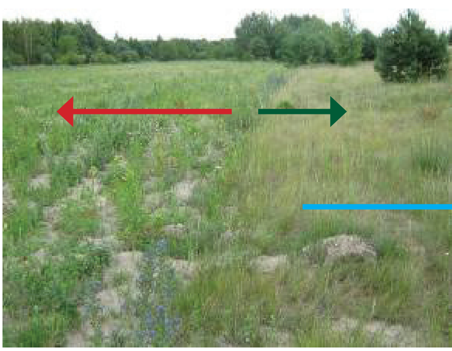

Fig 10: Fallow field with common ragweed on the left, nitrogen-poor grassland on the right side of the picture (2011/7/8). No common ragweed was found in the grassland (fig 12).

Fig 11: Nitrogen-poor grassland with gaps aside of the abandoned field with many common ragweed plants (fig 11). *Helichrysum arenarium,* which is endangered in Germany (D: 3, BB: -) grows here. No common ragweed was found in the grassland.

In own investigation of 2007, *A. artemisiifolia* is often present in stubble fields in the studied area in the Niederlausitz. At the time of the field trip in July 2011 the cereal crops had not yet been harvested, thus no relevés from stubble fields are presented.

d) Grassland

Ambrosia artemisiifolia is able to grow in grassland and pastures. In the study area it was documented from four grassland sites. In two cases common ragweed had a low cover (less than 5 %) in two other sites it reached a cover value between 50 and 75 %. In the four relevés 56 plant species were found. The medium number of plant species in the relevés was 21.5. *Achillea millefolium, Festuca rubra, Rumex thyrsiflorus*, and *Agrostis capillaris* were the most common plant species growing with common ragweed. At one site *Dianthus deltoides* was found, a species which is endangered in Brandenburg. At another site *Filago* cf *arvensis* occurred. In the grassland where common ragweed was found the vegetation was developed sparsely and showed gaps. The common ragweed plants were small (< 10 cm).

Fig 12: Grassland near Leuthen with occurrences of *A. artemisiifolia* (9th July 2011).

e) Ruderal sites

Thirteen ruderal sites located next to a newly built federal highway (B169), frequently disturbed sites in mining areas, ruderal sites next to agricultural fields or beside a parking area where common ragweed was present, were investigated. Similar to the situation in the abandoned fields *Apera spica-venti* and *Conyza canadensis* were the most frequent accompanying species. Also *Taraxacum* sect. *Ruderalia* and *Tripleurospermum perforatum* often occurred. In total, 104 species apart from common ragweed were found in the sites investigated and the mean species number per relevé was 21.3. Mostly common species such as *Elymus repens*, *Achillea millefolium*, *Agrostis capillaries,* and *Rumex acetosella* were detected, but in 7 of the 13 relevés the endangered species *Filago arvensis* was present.

Comparable to the situation in the arable fields, where common ragweed was found on wet patches as well as in relatively dry growing conditions, the species also has a wide ecological amplitude at the ruderal sites and occurred in wet as well as in dry growing conditions. In some dry and nutrient-poor growing places the whole vegetation was developed sparsely and common ragweed remained small (< 15 cm) (fig 13, 14), while in sites with a better nutrient supply common ragweed was taller and reached cover values up to 75-100 %.

Fig 13: common ragweed occurring with the endangered *Filago arvensis* at a dry ruderal site near Domsdorf (2011/07/10) (picture: Alberternst).

Fig 14: common ragweed at a dry nutrient-poor ruderal site next to a newly build highway near Domsdorf (2011/07/10, picture: Alberternst.

f) Roadsides

In the study area *A. artemisiifolia* often occurs at roadsides. The species usually grows in a distance up to ca. 50 cm (maximum 100 cm) from the paved surface. Common ragweed prefers this area next to the tarmac and was found in the vegetation away from road only in a few cases (fig 15, 16).

In seven relevés conducted at roadsides near Domsdorf, Senftenberg, and Luckaitz, 66 plant species were found in addition to common ragweed. The medium species number in seven relevés was 18.6. Common ragweed was often growing with species such as *Polygonum aviculare* agg., *Rumex acetosella*, *Festuca rubra* and *F. ovina*, *Trifolium arvense*, and *Lolium perenne*. No endangered species were found, but two species (*Armeria maritima* and *Leontodon saxatilis*) which populations currently are decreasing in Brandenburg (Rote Liste Gefäßpflanzen 2006, value: V) occurred at road sides.

According to Jentsch (2007) in the area investigated the strong appearance of common ragweed at roadsides is a recent phenomenon. Common ragweed was probably introduced into these sites with contaminated soil which was used for the filling of the road shoulders. The species could also have entered the road sides with agricultural machines loosing soil containing ragweed seeds from the agricultural fields.

Road sides are an important spreading route for common ragweed also in the area investigated: In an extensive recultivation area near Senftenberg the species was recently introduced with the construction material for a new road (fig. 18). Common ragweed grows on the shoulder of the road at a great number of sites. Whether common ragweed reaches the open vegetation in the surrounding area, for example via water flow in the drainage tubes (fig 17), needs to be studied further.

Fig 15: *Ambrosia artemisiifolia* grows in the area up to 50 cm beside of the paved road near the village Löschen. Only in a few cases the species was found growing apart from the road side.

Fig 16: common ragweed prefers the area beside of the road near Ogrosen, although the vegetation bordering on the road often is open.

Fig 17: Extensive recultivation area near Senftenberg. Many common ragweed plants were found in the banquet of a newly build road. The species was obviously introduced with the construction material for the road. Whether common ragweed spreads into the open vegetation in the surrounding e. g with water flow in the yellow drainage tubes (Fotos: Alberternst), needs to be studied.

g) Forest margins and forest paths

During the field work common ragweed was found at forest paths and at forest edges, especially at sites which bordered to arable fields. In six relevés from these habitats 78 plant species were found and the mean species number was 20.7. Common ragweed had a foliar cover between "+" (2-5 individuals, less than 5 % cover) and "4" (50-75 % cover) and it often grew with species such as *Bromus sterilis, Achillea millefolium, Taraxacum* Sect. *Ruderalia, Elymus repens, Geranium pusillum, Agrostis capillaris, Chenopodium album, Urtica dioica, Poa annua*. Usually common ragweed was not the dominant species in the vegetation. No endangered species were found at these sites during the investigation.

Fig 19: Light forest road and food path near Domsdorf with a stand of *A. artemisiifolia* at 8th of July 2011. At the beginning of July common ragweed was small, but it still was in the growing process (Fotos Alberternst).

Compilation of the vegetation data

During the field work, 194 plant species were found in the 56 vegetation relevés conducted in the Lower Lusatia at beginning of July. The compilation of data presented in table 3 demonstrates that the highest number of plant species was found in ruderal areas, whereas the number of relevés from these sites was relatively high compared with the number from other habitats. The mean number of plant species was highest in grassland, followed by ruderal areas and forest edges, whereas the smallest amount of plant species was present in abandoned and arable fields. In the ruderal areas, in grassland, at forest edges, field margins and at roadsides rare, respectively endangered species (*Filago arvensis*, *Dianthus deltoides*, *Armeria maritima*, *Leontodon saxatile*) were found. No such species were recorded from the arable fields or from the fallows during this study, whereas Jentsch (2007) detected *Filago arvensis* on agricultural fields some years earlier.

A suppression of other plant species was not obviously visible during the investigation, as it is for example in dense stands of *Fallopia japonica* in which nearly no other species exist. At the sites investigated where the mentioned rare species, or species with decreasing populations occurred, the vegetation often was sparsely developed and common ragweed usually remained small and did not dominate the stand.

Comparative studies of sites with and with removed ragweed plants

In the agricultural area of the study area common ragweed often builds up extensive and dense stands whereas spring crops, especially sunflower fields, are mostly affected by dense ragweed stands due to a low efficacy of herbicides, and the late sowing date of the crop. The investigations conducted by Drotkowski (2012) demonstrate that the cover and height of accompanying weed species are lowered by common ragweed in sunflower fields, whereas in winter crops this is not obvious due to the fact that common ragweed remains small and starts to grow in the stubble fields after harvest of the crop. Muranko (2012) did not find significant differences in the species composition in sites with and with removed ragweed plants in sunflower and winter rye fields, in grassland and at road sides in the Niederlausitz. However, the cover of ragweed in the sites investigated was relatively low and an impact might be more evident when the plant cover of ragweed exceeds 75 %.

Tab 3: Compilation of the number of plant species found in the habitats investigated. On arable fields no endangered species were found during this study, whereas the endangered *Filago arvensis* was found on arable fields in the same region by Jentsch (2007) some years earlier. The total number of plant species detected in the sites investigated was 194.

Habitat type	**number of relevés**	**total number of species**	**mean number of species**	**Rare/endangered species present**
Arable fields 13		68	14,5	Not found in this study
Fallow fields	3	31	13,0	-
Field margins	6	74	20,5	+
Forest edges	6	78	20,7	-
Grassland	4	56	21,5	+
Ruderal areas	13	105	21,3	+
Road sides	7	68	18,6	+

Data compilation on habitats colonized by common ragweed and assessment of possible effects on biodiversity

In the following, an overview of habitats grown with common ragweed, the nature protection value and possible impacts of common ragweed on biodiversity is given. The influence on biodiversity is described and data regarding biodiversity impacts are compiled. To estimate the biodiversity impact not only changes in the species composition should be considered, it should also be noted whether rare and/or habitat specific species that are important for associated species in these habitats (e.g. food resources) are affected.

Tab 4: Compilation of data on occurrence of *A. artemisiifolia* in different habitats and assessment regarding nature protection issues.

Habitat	**How often does common ragweed occur?** (in countries/regions with high infestations)	**Nature protection value of habitat**	**Influence of common ragweed on biodiversity**	**Influence regarding nature protection issue**
Agricultural fields	Very often, occurs in various plant communities	Mostly low in intensively managed areas, partly high value in extensively used areas, and in species rich habitats	Suppression of weeds in dominant ragweed stands (e.g. Pandza et al. 2001, Pál 2004, Pinke et al. 2011, Drotkowski 2012)	Negative impact when rare/endangered species or species of high value for associated species are affected (Pál 2004, Pinke et al. 2011), On abandoned fields, succession could be delayed (Maryushkina 1991)
Ruderal sites	Often, in various plant communities	Often low (e.g. in nutrient rich habitats), Could be high in nutrient-poor places when various, and also rare species are present	common ragweed could suppress weed species in dense stands e.g. at nitrogen-rich sites, Influence mostly unknown	?, in most cases probably low impact (suppression of A. a. during succession)
Roadsides	often	Mostly low	? or probably low (Muranko 2012), in very dense stands species could be suppressed	?, in most cases probably low impact
Nutrient-poor grassland (incl. sand habitats)	Rarely	high	?	?, in Germany in a single case negative influence assumed In Hungary occurrence in a grassland area only at disturbed sites (e.g. dirt roads, Szigetvari 2009) or mostly (?) in disturbed plant communities (Bauer 2006)
Newly grown meadows, pastures	sometimes	Sometimes high	?	?, probably low due suppression of common ragweed during succession
Flood plains	sometimes	Sometimes high (Czisky & Purger 2008)	?	?
forests	sometimes	Sometimes high	?	?

Discussion

Common ragweed is an annual plant and a pioneer species which needs open disturbed sites for germination and it prefers open and sunny habitats where competition with other species, especially perennials, is low. The field work in the Niederlausitz showed that common ragweed is present in the most disturbed sites and usually does not grow to a remarkable amount in the undisturbed vegetation next to extended ragweed stands. Unsuitable growing conditions in the vegetation and lacking disturbance could be the reason, but it also is possible that the species did not reach the area due to its limited spreading abilities (relatively heavy seeds, no adaptation to wind dispersal) until now. This dependence of common ragweed on disturbance limits the number of habitats where the species can grow.

In spite of its relatively low spreading capacity the species profits from human activities such as the massive transportation of soil containing ragweed seeds, e.g., during construction measures, transportation of seeds with agricultural, construction or mowing machines, resulting in an efficient and quick spread of common ragweed. However, agricultural areas, road sides and ruderal sites, where common ragweed is often present in many European countries and also in the study area in the Niederlausitz, provide suitable growing conditions for the species but these areas are usually not in the main focus of nature protection. The results from the field study show that also in these habitats sometimes rare and endangered species occur that might be affected by dense ragweed stands. In Croatia, common ragweed suppresses weed species in arable fields (Pandza et al. 2001) but it is not mentioned whether this has negative effects for nature conservation. Studies from Hungary, where common ragweed spread intensively in agricultural areas since the beginning of the 1990s, demonstrate that common ragweed suppresses also rare and endangered weed species in extensive arable field and thus is assessed as having negative impacts on the agrobiodiversity (Pinke et al. 2011, Pál 2004).

On abandoned fields but also in other habitats where plant species grow and close vegetation gaps, common ragweed itself is mostly suppressed by the establishing plant species in the native and also in the non-native range during the succession. However, a study from Ukraine shows that common ragweed inhibits the restoration of both annual and perennial native species, decreases the diversity of communities and delays the succession process probably due to a less impact of predators feeding on the plants in the non-native range.

In countries with low infestations where the intensive spread of common ragweed just started such as in Germany or in East of France no direct negative impacts of common ragweed on the biodiversity are documented, indicating that due to low infestations no conflicts with aims of the nature protection are currently present. However, also in Russia where common ragweed is very wide spread und considered to be the most noxious invasive weed since the 1940s (Reznik 2009), the impact of common ragweed on natural biodiversity is regarded to be small (Reznik, information given in the questionnaire 2012).

In general, common ragweed has a wide ecological amplitude and a great morphological plasticity that allows it to exist in various growing conditions. Thus, the species could also be expected to grow in open sites where natural disturbances such as trampling by deer or disturbance due to floating water and also dispersal by these means occur, in case the species reaches the growing habitat. It is the first step in an invasion process that the species gets in suitable habitats. The more common common ragweed becomes in anthropogenic sites, e.g. in agricultural areas, the higher is the likelihood that it reaches more natural habitats where an impact is currently not foreseeable. In the study area in the Niederlausitz *A. artemisiifolia* is present since many decades (Hegi 1974) but the rapid spread of the species just started a few years ago (Jentsch 2007), common ragweed thus might not have reached areas with higher nature protection values till now and therefore possibly no negative impacts are currently known.

In some countries common ragweed is already present in more natural habitats for example in flood plains where natural disturbances regularly take place and common ragweed spreads with the floating water. Some of the plant communities contain rare and endangered plant species (e.g. Csiky & Purger 2008). As far as we could find out currently there is only limited knowledge on the influence of common ragweed on the accompanying plant species in these, but also in many other habitats affected. Negative impacts on biodiversity are unforeseeable at the moment in countries where common ragweed spreads but has not reached all potential growing areas. Climatic changes may force the process of dispersal and thus possibly forces impacts of the species on biodiversity.

Impact of control measures against common ragweed on biodiversity

Control measures such as herbicide application and the use of mechanical control against *A. artemisiifolia* may have a negative influence on the biodiversity. Also a land use change in order to control common ragweed may cause damage to flora and fauna. In Hungary for example, since the year 2007 it has been compulsory for farmers to prevent the forming of common ragweed flower buds (Kazinczi et al. 2008). According to these authors, in crops, common ragweed must be controlled if the foliar cover of common ragweed exceeds 30 %. Specialists of "Field Offices", which exist in every large town in Hungary, conduct a survey after 30th of June. In case there is an offense against the law, a penalty can be imposed which varies between 80 and 20.000 Euros depending on the size of the infested area. This recently led to increased control acitivities against *A. artemisiifolia* by Hungarian farmers (Kazinczi et al. 2008). According to Beres (2004 in Kazinczi 2009) early stubble-stripping done in time prevents seed ripening of common ragweed, and after that, frequent soil cultivation triggers seed germination leading to a reduction of soil seed bank. Also Saric et al. (2011) recommend early and adequate stubble treatments in order to control common ragweed. Due to the fact that chemical and mechanical control methods are not species-specific, a wide range of species is incidentally affected (Pál 2004, Pinke et al. 2011). This definitely affects weed species diversity due to the fact that potential habitats of several native and archaeophytic weed species are destroyed (Pál, pers. comm. 2012).

To this background the common ragweed experts from the different countries where asked in the questionnaire for their estimation regarding the impacts of control measures on biodiversity.

Results of the inquiry

a) Implementation of control measures (question 4 a)

Fig. 20 shows the answers to the question how often common ragweed is controlled in different habitats, and it is demonstrated that common ragweed is controlled predominantly on agricultural fields. In some countries common ragweed is controlled at field margins, at road sides and on fallow land. In most countries common ragweed is never or only rarely controlled in ruderal habitats or in pastures and meadows, whereas in some countries common ragweed is rare (e.g. in Czech Republic) and thus no control measures are conducted. In some other countries there is an obligation to control the species, e.g. in Croatia where since 2004 a special legislation obliges all land owners to control common ragweed on their land. The obligation also includes public places and thus, common ragweed is more often controlled in Croatia than before the year 2004 (N. Galzija). Also in Hungary landowners are obliged to control common ragweed as described above.

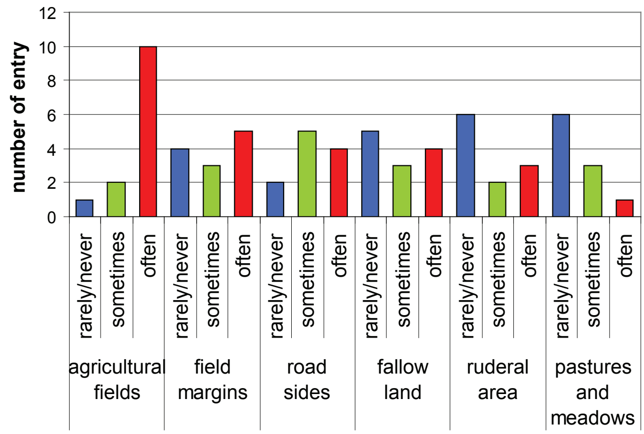

Fig 20: Answers to the question how often common ragweed is controlled depending in different growing habitats (Answers from 13 questionnaires, without answers where a question mark was put in).

b) Impact of control measures against common ragweed on biodiversity (question 4 b)

Fig 21 and fig 22 give an overview on the answers of the experts regarding the question, whether control measures against common ragweed have a negative impact on biodiversity. Additionally it was asked for available scientific studies on this question (fig 22).

In countries with low ragweed infestations no (or only little) negative impacts on biodiversity are known. Also from regions where common ragweed occurs more frequently, the impact often is regarded to be small, due to the fact that common ragweed predominantly grows in disturbed anthropogenic habitats. Some experts refer to negative impacts on biodiversity due to intensified herbicide use on arable fields as well as in non-agricultural areas such as field margins or road sides (if herbicide use is legal). Mowing of field margins or other areas to control common ragweed is thought to have a negative impact on biodiversity in some cases. Whereas early tillage in order to control common ragweed is a threat factor for rare weeds in some regions in Hungary, this is not quoted to be a problem in some other countries (sometimes due to the fact that this method is not used to control common ragweed).

However, the inquiry demonstrates that even in countries where common ragweed frequently occurs, data concerning the above mentioned question is sparse or even missing.

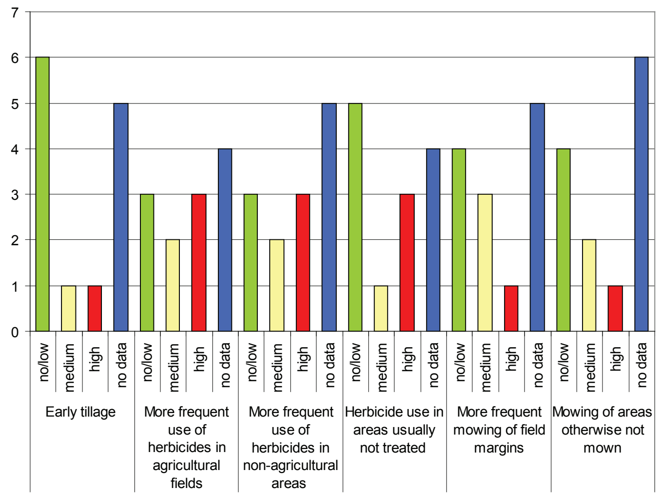

Fig 21: Estimation of the impact (no/low, medium, high impact) of control measures against common ragweed on biodiversity.

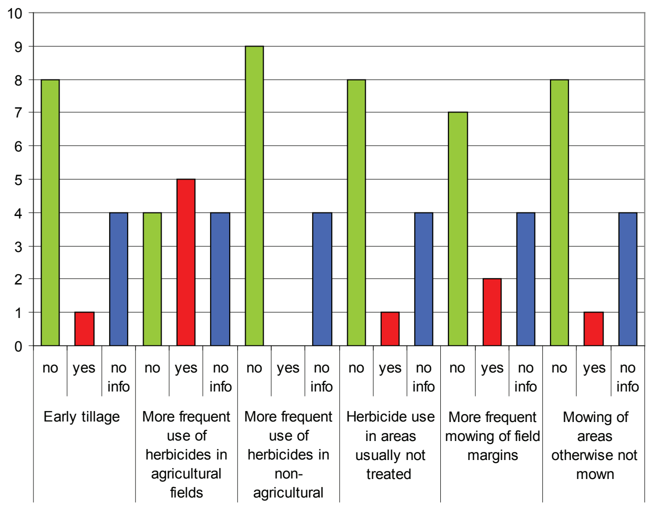

Fig 22: Availability of studies regarding the impact on biodiversity of different types of control measures against common ragweed (**no**: no studies available, **yes**: studies available, **no info**: no information given in the questionnaire).

c) Expected negative impacts in case of intensified control of common ragweed (question 5)

Some experts expect no or only minor negative impacts on biodiversity in future, even in the case of more frequent chemical or mechanical control of common ragweed, since the species predominantly grows in disturbed habitats where rare and endangered species usually are sparse or not present. Others expect adverse effects on rare and/or endangered weeds or whole vegetation units for example on extensively used arable fields or at field margins. Negative impacts due to intensified use of herbicides that could spread to other habitats (e.g. via water flow) are feared. Also rare r-strategists occurring along roadsides and railway tracks may be affected by more frequent use of herbicides. Another expert expects a negative effect for row crops. Also more frequent mowing is thought to have a negative impact on biodiversity e.g. at roadsides and forest roads. Intensified control measures in open dry grassland and xerothermic forest also could injure vegetation (information provided by N. Bauer, M. Bonini, D. Bozic, B. Chauvel, A. Csecserits, N. Galzina, G. Karrer, P. Kotanen, R. Pál, S. Reznik, H. Skálova, N. Schoenenberger, M. Rossinelli, B. Alberternst, S. Nawrath).

Possible impacts of control measures on biodiversity

Currently there is a gap in the knowledge on how control measures against common ragweed might influence the biodiversity. Due to the fact that investigations are only sparsely available, possibilities how control measures against ragweed can/could impact biodiversity, are listed here:

- Increased herbicide use could lead to an enhanced environmental burden and also kill accompanying plant species.
- Land use change as part of measures against common ragweed could have a negative impact on the vegetation. For example, early tillage may kill plants and can alter plant communities.
- Repeated mowing in order to reduce pollen production of common ragweed can lead to a reduced flower production of native species resulting in less seed set. Additionally the amount of pollen and/or nectar and also seed which provide food for insects is reduced.
- Hand pulling of ragweed plants could enhance disturbances and thus may influence the species composition.

In the following examples and results from literature and own investigations are given.

In many countries *A. artemisiifolia* predominantly occurs in agricultural fields and at field margins and thus, control measures against the species mostly occur in these habitats. Special control measures against common ragweed can impact the weed flora on stubble fields of cereal crops. Cereal fields are usually disturbed only at the beginning of the season and if the fields remain unploughed after harvest during the summer and autumn months these habitats provide the longest undisturbed growing conditions for annual weed species in arable systems (Pinke et al. 2010). Some weeds belong to the group of summer annual species which usually germinate during the spring. These species usually remain within the lower herb layer in the cereals and start to grow after harvest when the light conditions are more favourable. Intensive agricultural management with using of chemicals and early ploughing of stubbles thus has great impact on these agricultural weed communities (Pinke et al. 2010). Pinke et al. (2008) stress that *A. artemisiifolia* seriously threatens the existence of red list and other rare weed species in Hungary, not only because it is invading more and more habitats of them, but also due to intensified control measures including greater emphasis on the importance of total weed control and early ploughing of stubbles.

The following example from Bavaria demonstrate that control measures against common ragweed can also have a negative impact on the weed flora on a fallow field in Germany although common ragweed currently is not widely distributed: *Ambrosia artemisiifolia* was found on a fallow in

Georgensgmünd where endangered plant species such as *Arnoseris minima* (Red List Germany: 2), *Consolida regalis* (Red List Germany: 3) and *Filago cf. arvensis* (Fig. Red List Germany: 3) were present (fig 23). In 2010 this field was treated with herbicides especially to kill *A. artemisiifolia*. The control measure also killed the accompanying rare plants on this field.

Fig. 23: Fallow agricultural field with occurrences of *A. artemisiifolia*. Here are rare and endangered species: *Arnoseris minimia* (on the right), *Consolida regalis* and *Filago cf arvensis* occur (2011/07/04, pictures: Nawrath).

In Germany the composition of segetal plant communities drastically changed during the last 50 years. Intensified agricultural management as well as abandonment of agricultural fields especially on poor soils lead to profound changes in the floristic composition of the weed flora. The populations of many weed species strongly decreased and currently a great amount of these plants is recorded in the red list of endangered plant species in Germany (Meyer et al. 2008, Hofmeister & Garve 2006). Associated with this development, also the diversity of the fauna of arable fields drastically decreased (Heydemann & Meyer 1983).

Edges and margins of agricultural fields, and also stubble and fallow fields, often provide last refuges for segetal weed species and the associated fauna and thus are important for the agrobiodiversity (v. Elsen 2005). Common ragweed often occurs in these habitats. In the case intensified herbicide use, mowing or even ploughing takes place in order to reduce the pollen production and prevent the spread of the species, biodiversity could also be impacted at these sites. The measures might lead to reduced food resources for insects or birds or worsen habitat structures for animals living in these habitats (Pinke et al. 2011, Pál 2004). This is not only true for agricultural habitats but also for ruderal sites such as road margins, railway tracks, or industrial areas where accompanying species could be affected by control measures against common ragweed as well.

Due to its wide ecological amplitude *A. artemisiifolia* is able to grow in many different habitats such as ruderal areas, road margins, river plains, nutrient-poor dry grassland where also rare and endangered plant species could be present. Thus, intensified control measures carried out especially to remove common ragweed might also affect the accompanying plant species and the fauna associated with these species, in case nature conservations aspects are not taken into account.

Discussion

The number of studies dealing with indirect impacts of control measures against common ragweed on biodiversity is currently low and is, as far as we know, mainly confined to the work conducted by Pinke (2000, 2007), Pinke et al. (2011) and Pál (2004) in Hungary. In our opinion the indirect influences of control measures against common ragweed have a stronger negative effect on biodiversity than the direct effects. The authors from Hungary show that some of the control measures against common ragweed already have negative impacts on the biodiversity. In case of the spread of common ragweed not only in agricultural fields, but also in all habitat types suitable for the species, control measures could be intensified in order to prevent human health problems leading to negative impacts on flora and fauna.

In general, the efforts to reduce ragweed plants and populations and prevention of spread of the species in Europe should be intensified. However, nature protection as well as environmental aspects (e.g. in case of herbicide use) should be taken in consideration and accompanying plant species should be spared during the control, for example by hand pulling of small populations instead of complete ploughing or spraying of the whole site. The occurrence of *A. artemisiifolia* at a site should not be used for a justification to intensify weed control in general. Additionally, it also should not be an argument to renounce environmental friendly methods to run farming (e.g. non-ploughing cultivation methods).

Summary and conclusion

To find answers on the question whether *A. artemisiifolia* or control measures against the species have negative impacts on the biodiversity a literature review, an inquiry and field work was conducted. A review of the literature is given regarding biological features of common ragweed, the habitats and the vegetation where the species is present in Europe, and how it influences the accompanying species. The inquiry was conducted via questionnaire and 118 experts from 38 countries currently working on the topic "common ragweed" were asked for their estimation regarding the biodiversity impacts of common ragweed. The field work was conducted in the Niederlausitz, which is the most common ragweed-infested area in Germany. It was investigated in which habitats common ragweed currently is present and whether negative impacts on accompanying species can be detected. We thankfully received 12 answers on the questionnaire, whereas this number of answers is very low. This possibly reflects the limited amount of scientific information currently available on the topic.

common ragweed predominantly grows in areas where disturbances regularly occur, and it scarcely is present in undisturbed areas. The species predominantly occurs in agricultural areas and at ruderal sites and thus it mainly grows in plant communities of the phyto-sociological classes Stellarietea and Artemisietea. In general, *A. artemisiifolia* has a wide ecological amplitude, and in Europe it is present in various plant communities from wet sites, such as the Glycerietum to phytocoenoeses from dry habitats, such as the Corynopheretum and it was even found in Quercion communities (Song & Prots 1998, Bauer 2006, Szirmai et al. 2009).

In agricultural fields, on stubble fields, and on fallows common ragweed can build up dense and extensive stands. Common ragweed is a good competitor and thus causes yield losses in various crops. In Hungary common ragweed suppresses accompanying plant species by competition for light, nutrients, and water and may influence them by its allopathic capacity (Beres et al. 2002, R. Pál, personal communication 2012/20/02). This also happens in the lowlands of Croatia where *A. artemisiifolia* displaces autochthonous weed species, which cannot compete with common ragweed (Pandza et al. 2001). Not only in its alien, but also in its native range, common ragweed can have a strong negative effect even on species which are moderately good competitors such as *Agropyron repens* and *Plantago lanceolata* (Miller & Werner 1987, Callaway & Walker 1997). Studies conducted by Maryushkina (1991) on a freshly ploughed plot on an abandoned field in the Ukraine show that common ragweed significantly decreased the number of annual plant species, especially at the beginning of the succession process, and the author stated that *A. artemisiifolia* inhibits the restoration of both annual and perennial native species, decreases the diversity of communities and delays the succession process in its new range.

During the field work in the Niederlausitz *A. artemisiifolia* was found in different habitats, in arable fields, fallows, field margins, field paths, forest paths and forest edges, roadsides and food paths, ruderal areas, and in grassland. In 56 relevés from different habitats with occurrence of common ragweed 194 plant species were found. Extensive ragweed stands were present in agricultural fields, on fallows, at field margins and also at road sides and ruderal fields. Common ragweed was mainly found in disturbed areas and was rarely present in undisturbed vegetation. At some sites such as roadsides, in ruderal areas, in grassland, field margins and fallows, rare species such as *Filago arvensis*, *Helichrysum arenarium*, *Armeria maritima*, and *Leontodon saxatile* were recorded. A direct suppression of these species by common ragweed, however, could not be demonstrated. In case of more intensified control of common ragweed in these habitats, rare species could also be affected by the control measures.

According to the answers of the experts in the questionnaire, in countries with low ragweed infestations no (or only little) negative impacts on biodiversity are known. But also from regions where common ragweed occurs more frequently, the impact often is regarded to be small, due to the fact that common ragweed predominantly grows in disturbed anthropogenic habitats. Some experts report negative impacts on biodiversity due to intensified herbicide use on arable fields as well as in non-agricultural areas such as field margins or road sides (if herbicide use is legal). Mowing of field margins or other areas to control common ragweed is thought to have a negative impact on biodiversity in some cases. Whereas early tillage in order to control common ragweed is a threat factor for rare weeds in some regions in Hungary, this is actually not quoted to be a problem in some other countries (sometimes due to the fact that this method is not used to control common ragweed).

Some experts expect no or only minor negative impacts on biodiversity in future, even in case of more frequent chemical or mechanical control of common ragweed, since the species predominantly grows in disturbed habitats where rare and endangered species usually are sparse or not present, others expect adverse effects on rare and/or endangered weeds or whole vegetation units for example on extensively used arable fields or at field margins. Negative impacts due to intensified use of herbicides that could spread to other habitats (e.g. via water flow) are also expected. Also rare r-strategists occurring along roadsides and railway tracks might be affected by more frequent use of herbicides. Another expert worries about a negative effect on weeds in row crops. Additionally, more frequent mowing is assumed to have a negative impact on biodiversity, e.g. at roadsides and forest roads and intensified control measures in open dry grassland and xerothermic forest also could injure vegetation.

In general, there are many gaps in knowledge on biological impacts of common ragweed on biodiversity. Probably the species has minor direct impacts on the biodiversity at various anthropogenic sites, but the former mentioned studies, especially from the agrobiocoenoses in countries with high ragweed infestations, suggest that biodiversity could be directly affected by common ragweed respectively indirectly by control measures against the species. Additionally, common ragweed currently spreads in many countries and due to its wide ecological amplitude in future it may also occur in habitats where conflicts with aims of the nature protection could arise.

Conclusion: Due to the fact that control measures against common ragweed could have negative environmental effects the nature protection authorities should be involved in activities to control and prevent spread of common ragweed. Not only the agricultural and the human health sector, but also the nature protection sector should be involved in the development of strategies against the spread of common ragweed in Europe in an interdisciplinary task.

In order to reduce knowledge gaps regarding the direct and indirect influences of common ragweed on biodiversity, specific inquiries should be conducted.

Proposals for further research

- Comparison of vegetation types with high infestations of common ragweed (>75 % plant cover of common ragweed) with vegetation without infestations.
- Investigations in areas of high nature protection values where common ragweed is present and study of its growing behaviour.
- Inoculation and competition experiments in laboratories and at natural study sites.

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Outlook

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Common ragweed is a very prominent alien species in Europe and in other invaded ranges, mostly because of its impact on human health, but also because of the damage it causes in agriculture. Because of this, even before our project, there was plenty of information available on all aspects of the species including its biology and ecology, impacts, and control options.

As this volume shows, the project HALT Ambrosia has addressed some gaps in this knowledge base and has systemically conducted series of experiments mainly on some aspects of germination biology and on control options. In addition, some review-type chapters have comprehensively collected and discussed already existing information. The results of the project have been presented in numerous forms, as in publications and conference papers. They were specifically discussed in national conferences; proceedings of the German and Austrian conferences have been published (Karrer 2011, Starfinger *et al*. 2014).

The interest in the species is ongoing and the amount of knowledge on it is growing continuously, comprehensive overviews were recently published, e.g., Essl *et al*. 2015, Buters *et al*. 2015.

The overall conclusion from our and from other published research is that common ragweed can be successfully controlled when management measures suitable for the specific situation are chosen. If management is performed it is more a question of political will, legislative circumstances, and available resources than of applicable management measures.

Activities trying to enhance ragweed control in Europe are also ongoing. The COST action SMARTER (Sustainable management of Ambrosia artemisiifolia in Europe) provides a forum for discussing long-term management and monitoring options (ragweed.eu). The International Ragweed Society (http://internationalragweedsociety.org) aims at promoting the knowledge about ragweed and at facilitating collaboration, research, etc. in order to enhance the fight against the plant.

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Recommendations for countries affected by common ragweed invasion

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Introduction

The invasion of common ragweed, *Ambrosia artemisiifolia,* can lead to severe negative impacts depending on eco-climatic, social and habitat conditions in a country or region. It is therefore undesirable to variable degrees. Strategies to prevent its invasion and establishment, to control and eradicate the plant and/or to mitigate its impacts are therefore recommendable. They should be based on information about the presence and establishment, the climatic suitability of the country, the prediction of potential impacts and on the presence of invasion pathways. We therefore propose to design and implement such strategies depending on the presence of the plant in a given country (or other geographical unit where applicable).

Currently there is only limited knowledge on the influence of common ragweed on the accompanying plant species. In studies from Hungary it is demonstrated that common ragweed suppresses rare plants and other weed species and thus having negative impacts on the agro-biodiversity. A monitoring of these areas is recommended.

Risk assessment

Risk assessment should be carried out in order to determine whether *A. artemisiifolia* could establish in the country under current and/or under climate change scenarios. Risk assessment should follow established procedures as described in the IPPC's ISPM 11 (in particular annex 2), or the EPPO decision support scheme (EPPO PP 5/3). Climate modelling tools, such as CLIMEX may be useful.

Should the assessment result in a low risk of common ragweed invasion for a given country, the following may be of low importance. If the risk assessment shows a higher probability of common ragweed establishment and spread, preventive measures are recommended because prevention is the most cost-efficient strategy for the reduction of negative impacts.

Countries where common ragweed is not widely distributed

Common ragweed has been involuntarily introduced to many regions and countries in the world – probably including all European countries. It has established and spread to different degrees, with high infestation rates in, e.g., Hungary, Serbia, Croatia, Slovenia or parts of Austria and Slovakia, intermediate frequency in, e.g., Germany, Poland, Czech Republic, and virtual absence of established stands in Northern countries like in Scandinavia. Most of the invasions started following the introduction in the 19th century. In some countries the invasion is a more recent phenomenon, e.g., Germany, where the plant was present but not spreading for the first 100 years after its introduction. Such lag phases in biological invasions are not uncommon and underline the need to be prepared for potential ongoing range expansion. Countries that are currently free of any established common ragweed populations should still be aware of potential imminent common ragweed invasion, in particular with climate change affecting the potential naturalisation.

Countries with "intermediate invasion situations" may already begin to suffer from common ragweed impacts on human health and on agriculture. The beginning establishment in those countries points to the potential of a large increase in the damages because of the suitable eco-climatic conditions for further invasions. Therefore these countries should at the same time focus on the prevention of further spread, the timely eradication of population initials in otherwise un-infested parts of the country, and on the mitigation of impacts in more strongly invaded parts.

Prevention of import and spread of common ragweed seeds

The most important pathway for the international transport of common ragweed seed is the involuntary introduction with commodities, in particular contaminated seeds for animal consumption (bird seed) or for processing and human consumption or contaminated seeds for sowing. Animal feed including bird seed is regulated in COMMISSION REGULATION (EU) No 574/2011, but this does not apply for the same product (e.g., sunflower seed) marketed for human consumption. Even though seeds for sowing may legally contain only low amounts of seeds of other species, common ragweed's high reproduction ability may result in a problem on the farmland. Agricultural machinery and roadside mowers from common ragweed infested areas may also form an international vector of seeds. Transport of contaminated soil is an effective spreading route for common ragweed in Europe. Therefore this kind of import should be avoided. In most of the European countries no special measures are currently in place to prevent the spread of common ragweed within excavated material. Comprehensive legal regulations currently exist in Switzerland.

In countries where common ragweed is establishing, the same ways of preventing further spreading are valid like for un-infested countries.

Surveillance and early eradication

In particular where the climatic conditions are beginning to be suitable in the course of climate change, information about initial populations is essential. A surveillance programme should include the information of the public about the potential risk of contaminated bird seed and about the necessity to control common ragweed in an early invasion stage. Therefore a network of experts should be trained in identifying the plant and to take appropriate precautions in applying measures. Subsequently these areas have to be monitored for several years.

Small populations – casual or on the verge of establishing – are easily controlled by pulling the plants by hand. For the safety of the persons doing this, it is recommended to act before (male) flowering and to wear gloves in order to prevent skin irritations.

Countries where common ragweed is widely distributed and abundant

Eradication of common ragweed from these countries is not a feasible short-term option. Countries with a limited distribution should at the same time focus on the prevention of further spread, the timely eradication of population initials in otherwise un-infested parts of the country, and on the mitigation of impacts in more strongly invaded parts The negative impacts of common ragweed in countries where common ragweed is widely distributed is heavily felt, e.g., in Hungary, agricultural damage by common ragweed was estimated at 300 Mio € and 112 Mio € annually for expenditures for human health (1.2 billion € in Germany). Strategies against common ragweed in these countries should aim at minimizing the negative effects with a long-term perspective on reducing the abundance of the plant.

Containment and control

Arable fields

The pillars of the reduction of common ragweed occurrence are a) direct measures against the plant and b) the adaptation of the crop rotation system.

The basic direct measure is the application of herbicides, where the released herbicides depend on the country. The WeedSeeker™ technology is an option with future potential, because the application of the herbicide amount can be reduced which has a positive impact on the environment and the efficiency of common ragweed control is given. Tillage is a very important control strategy in cereal stubble. After the harvest, common ragweed should have time to germinate or start growing and being destroyed by any tillage system within 7-14 days after harvest.

Crop rotation should prefer crops that either have a negative effect on common ragweed germination and establishment or offer successful herbicide solutions. The former include winter cereals because the closed canopy in spring impedes common ragweed germination. The latter include maize for which a large number of suitable herbicides are available and registered. Sunflower and legumes are not recommendable for heavily common ragweed infested fields, because the wide row spacing allows common ragweed to grow without competition. Additionally no satisfying suppression of common ragweed with herbicides can be achieved. Derived from results of herbicide experiments in Hungary conducted during the HALT Ambrosia project, the following herbicides, as examples, with good to excellent common ragweed control effect in cereals are: 2,4-D, amidosulfuron, dicamba, clopyralid and mecoprop-p. And in maize: 2,4-D, bentazone, dicamba, clopyralid, prosulfuron and topramezone. Clearfield™ sunflowers can be an option in countries where this technology is permitted.

For organic farming systems control strategies by soil tillage and integrated control measures like adapted crop rotation and competitive main crops are recommended.

The suppression of common ragweed does not only prevent yield losses but also reduces population density and seed bank as a long-term effect.

Roadsides

Herbicides are not legally applicable on roadsides in many European countries. Therefore mechanical control like mowing is the commonly used control strategy. Only a strict cutting regime will lead to a successful reduction of the soil seed bank by preventing common ragweed to produce seeds: a late first cutting date at the end of July until mid of August followed by frequent cutting every 3 weeks until the end of the vegetation period. When cut plant material has to be left on the site, cutting is safe only until the early female flowering stage (BBCH 63). Mowers must be cleaned after using in common ragweed infested areas to avoid seed dispersal.

Urban-industrial habitats

The patchy mosaic of habitats in cities with different owners or managers makes it difficult to design a consistent management plan. Common ragweed may occur here in private gardens, public greens, waste places, along city streets, in industrial areas, etc. Control or – where possible – eradication of common ragweed is nonetheless important, because common ragweed populations in these habitats may emit large quantities of pollen in the direct vicinity of many people, and because these populations may serve as seed sources for the colonization of other adjacent habitats.

In such a situation concerted actions of several stakeholders and administrational bodies offer a chance to achieve control over a variety of habitats. In e.g., Berlin, Germany, an "Action Programme Ambrosia" was created with the participation of the Institute of Meteorology of the Free University, the Botanical Garden, the Plant Protection Service, the City Senate and others. Together they have organised a monitoring and eradication programme which has reduced the number of common ragweed stands in the city considerably. The participation of the general public is especially important in urban habitats; many plants like those in private gardens can only be targeted by control if the private owners are aware of the problem.

Pollen management

Eradication of common ragweed is generally focused on the prevention of seed production and thus the reduction of the seed bank. If successfully applied as a long term strategy this also leads to the reduction of pollen released into the air. There may be situations where a consequent control aimed at depleting the seed bank is not feasible for technical, legal, financial or other reasons. In such cases it may still be feasible to apply control measures in order to reduce the pollen emissions, in particular in densely settled towns or cities. When pollen reduction is the main aim, control measures like hand pulling or mowing should be applied earlier in the season, i.e. no later than at the beginning of the male flowering period. The measure should preferably be repeated.

General considerations

Precautions

Control of common ragweed stands is generally desirable. It may, however, have unwanted side-effects, like negative impacts on co-occurring vegetation. In areas with habitats or species of high nature conservation value like protected landscapes or fields with rare and endangered plant species, methods should be adopted as much as possible, e.g. by applying mechanical instead of chemical methods or hand-pulling instead of mowing.

As common ragweed is harmful for human health, including the pollen allergenicity and the potential to cause skin irritation, workers must always be protected, e.g., by protective clothing or by dust masks for work in flowering plants.

All control measures must be executed in a way that they do not result in spreading common ragweed seeds to new areas. This consists of cleaning machinery, tools, tires, etc. from seed containing soil or plant material. Common ragweed plant material containing ripe or ripening seeds should preferably not be transported as transport may lead to seed dispersal. Such plant material should be treated in a way that kills the seeds. Besides incinerating the material, disposal in professionally operated composting or biogas plants is possible.

Many experiences like those made in the current project have shown that sustainably reducing the abundance, the seed and pollen production is achievable. Control measures are available for virtually all habitat types and scenarios. But for all that the common ragweed invasions have hardly been slowed let alone stopped in most countries.

A country-wide public awareness campaign explaining the risk and the potential mitigation methods should help to join the necessary forces for a successful fight against common ragweed.

Legislation

The existence of clear legal instruments for the fight against common ragweed may be a deciding success factor. This may be in the health, the agricultural, or the environmental sector. In Switzerland, for example, the placing of common ragweed-related legal measures within the realm of plant protection seems to have helped its success.

Legislation for the fight against common ragweed should include rules for the transport of commodities contaminated with common ragweed seeds like agricultural products, obligations to report and to control common ragweed stands and rules for the safe disposal of plant material resulting from control measures.

Biological control

A complete eradication from Europe is unlikely even in the case that comprehensive control strategies are executed. The potential offered by classical biological control should therefore be regarded: if successful and safe control agents can be found, there may be suppression of common ragweed even in places where no other control is performed, e.g., because of inaccessibility. While the science of biological control of common ragweed is currently being developed by the COST action SMARTER, application through the release of control agents will need support by the authorities.

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