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Allelopathic effects of plant extracts on common ragweed (*Ambrosia artemisiifolia* L.)

Die allelopathische Wirkung von Pflanzenextrakten auf Ambrosia (*Ambrosia artemisiifolia* L.)

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Summary

The aim of our investigations was to study the role of *Ambrosia artemisiifolia* L. as test species (recipient) under laboratory bioassay and glasshouse conditions. Sunflower (*Helianthus annuus* L.), *Convolvulus arvensis* L., *Abutilon theophrasti* Medic. and *A. artemisiifolia* were used as donor species in extract assays. Our results showed that extracts of the examined donor plants stimulated the development of *A. artemisiifolia* at low extract dilutions. The stimulating effect was stronger, when *A. artemisiifolia* plants were watered with plant extracts, as compared to a spray treatment. Measuring NPK values of the treated and non-treated *A. artemisiifolia* plants showed that *Ambrosia* plants utilised plant extracts as sources of nutrients. No autotoxicity of *A. artemisiifolia* extracts was observed. Sunflower shoot and root water extracts inhibited *A. artemisiifolia* germination only at higher concentrations, probably not due to a direct toxic effect, but perhaps due to an increased osmotic potential. The inhibitory effect of extracts on radicle length of *A. artemisiifolia* was stronger than that on germination. The observed stimulatory effects of the donor plant extracts on *A. artemisiifolia* may have a potential to promote the weed's dominance in fields.

Key words: allelopathy, bioassay, pot experiments, sunflower, weeds

Zusammenfassung

Die allelopathische Wirkung von Pflanzenextrakten aus Sonnenblume (*Helianthus annuus* L.), Ackerwinde (*Convolvulus arvensis* L.), Schönmalve (*Abutilon theophrasti* Medic.) und Ambrosie (*Ambrosia artemisiifolia* L.) auf Ambrosie wurde unter Labor- und Gewächshausbedingungen untersucht. Dabei zeigte sich, dass die Pflanzenextrakte größtenteils bei niedrigen Dosierungen eine stimulierende Wirkung auf die Ambrosie hatten. Wurden die Extrakte als Spritzapplikation auf Ambrosie appliziert, zeigte sich eine geringere Wirkung als wenn die Extrakte im Gießverfahren ausgebracht wurden. Die Bestimmung der N-, P- und K-Werte von Ambrosie zeigten, dass die Ambrosie die Pflanzenextrakte als Nährstoffquelle nutzen konnte. Weiterhin konnte keine Autotoxizität beobachtet werden. Sonnenblumenextrakte hemmten in hohen Dosierungen die Keimung der Ambrosie, was jedoch weniger auf eine direkte toxische Wirkung zurückzuführen sein dürfte, als vielmehr auf osmotische Vorgänge. Die Hemmung des Wurzelwachtums durch die Pflanzenextrakte war jedoch deutlich stärker ausgeprägt als die Keimhemmung. Die beobachtete stimulierende Wirkung der Pflanzenextrakte könnte die starke Verbreitung der Ambrosie zusätzlich fördern.

Stichwörter: Allelopathie, Bioassay, Sonnenblume, Topfexperimente, Unkräuter

1 Introduction

Common ragweed (*Ambrosia artemisiifolia* L.) is among 40 species of the genus *Ambrosia* the most dangerous invasive alien species in Europe. Hungary is considered to be the most infested country in Europe. Common ragweed's distribution, biology, ecology, harmful effects, and possibilities for control have been intensively studied all over the world (Bassett and Crompton 1975; Radics 1988; Béres 2003, 2004; Béres et al. 2006; Kazinczi et al. 2006). *Ambrosia* research is also in the centre of interest in other European countries due to human health reasons (Bohren et al. 2006).

Literature reports confirmed that *A. artemisiifolia* contains allelochemicals and has thus an allelopathic potential. The inhibitory effects of *A. artemisiifolia* extracts on different test species were proven under laboratory conditions and in bioassay studies (Brückner 1998, 2001; Béres et al. 2002). Phenolic acids, terpenes, sesquiterpene lactones and volatile compounds are supposably responsible for the allelopathic effects (Neil and Rice 1971). Furthermore, thiarubrine A is synthesized by *A. artemisiifolia* roots and has antifungal, antibacterial, and antiviral properties (Bhagwati and Hiiortso 2000). Further compounds may also play a considerable role in the plant's allelopathic potential, which may also affect fungi, bacteria and algae (Dalrymple and Rogers 1983; Fischer and Quijano 1985; Brückner et al. 2001, 2003).

No studies have yet been conducted on the sensitivity of *A. artemisiifolia* as an acceptor species. Therefore, the aim of our investigations was to study the role of this invasive weed as acceptor species (recipient) under laboratory and glasshouse conditions in order to get an insight into the sensitivity towards allelopathic effects of neighbouring crop and weed species.

2 Materials and methods

2.1 Pot experiments

A. artemisiifolia seeds were collected from maize fields at Keszthely (Zala county, Hungary; 46°46' N, 17°14' E, 116 m a.s.l.) in October 2004. After cleaning the collected material, the seeds were stored at 8 °C for 6 months, before use. In 2005, fresh shoots and roots of sunflower (*Helianthus annuus* L.), *Convolvulus arvensis* L., *Abutilon theophrasti* Medic. and *A. artemisiifolia* were collected at the beginning of flowering, supposing that the concentration of the presumed allelochemicals has reached a maximum at that phenophase. Harvested shoots and roots were cut into small pieces with a grinder. After grinding, 25 g fresh biomass was stirred in 100 ml distilled water and left for one day at room temperature. Then the water extracts were filtered once through filter paper (MN 640w). Pot experiments were set up under glasshouse conditions so that the test plants (*A. artemisiifolia*) were sprayed daily with a hand sprayer (volume: 2 dl) until the water extracts covered the shoots. In a second treatment

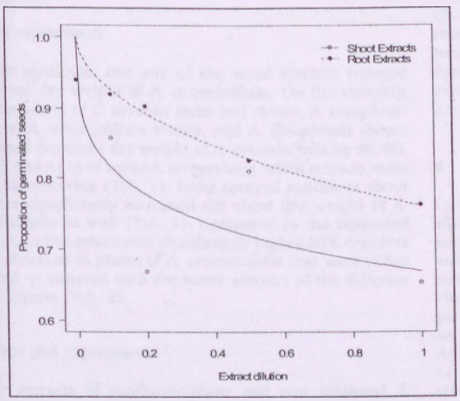

Fig. 1: Inhibition of germination of *Ambrosia artemisiifolia* seeds in Petri dishes by water extracts of sunflower (extract dilution relative to stock solution).

Abb. 1: Hemmung der Keimung von Ambrosia artemisiifolia *Samen in Petrischalen nach Behandlung mit wässrigem Sonnenblumenextrakt (Verdünnungsreihe relativ zur Stammlösung).*

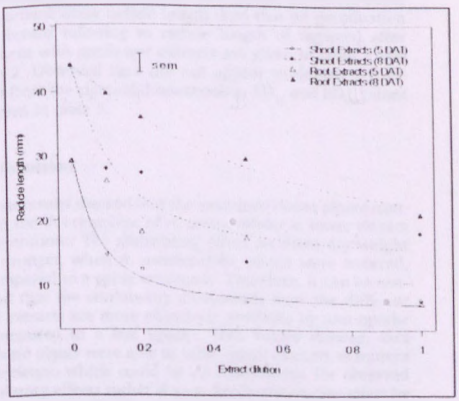

Fig. 2: Reduction of radicle length of *Ambrosia artemisiifolia* after treatment with sunflower extracts in Petri dishes (extract dilution relative to stock solution).

Abb. 2: Reduktion des Wurzelwachstums von Ambrosia artemisiifolia *in Petrischalen nach Behandlung mit Sonnenblumenextrakten (Verdünnungsreihe relativ zur Stammlösung).*

the test plants were watered daily (50 ml water extract pot^{-1}) with the plant extracts from their 2–4 leaf stage on until the end of the experiment at the 16–20 leaf stage. The soil mixture of the pots was composed of sand (pH 6.96, humus 0.27%) and peat (pH 6.78, humus 9.98%) in a ratio of 1:1. Sixty days after the first application the dry weight, nitrogen (N), phosphorus (P) and potassium (K) contents (in percentage of dry matter content) of *A. artemisiifolia* shoots was determined.

2.2 *Petri dish experiments*

In laboratory bioassays, 25 g of fresh biomass of sunflower shoots and of roots were stirred each in 100 ml distilled water. This initial extract served as stock solution. Stock solutions were diluted 2-, 5- and 10-times to prepare a set of decreasing extract concentrations for each type of extract resulting in a dilution series of 1, 0.5, 0.2 and 0.1 dilution of stock solution. The solutions were labelled and stored for one week at 8 °C in a refrigerator before and during the bioassays. After a week new stock solution and dilutions were prepared similar to the previous method. Hundred seeds of *A. artemisiifolia* were placed on two layers of filter paper in Petri dishes (120 mm diameter) in four replications for each treatment and 13 ml of the different extract solutions was added. Petri dishes treated with distilled water only were used as controls. Petri dishes were placed in an incubator at 22°C with 16 hours light and 8 hours dark during the entire trial period. During the bioassay, filter paper was kept continuously wet by periodical adjustment of extract losses. The number of germinated seeds was recorded 5, 8, 13, 17 and 21 days after the first treatments, until no further germination could be observed. The radicle length of *A. artemisiifolia* was also measured 5 and 8 days after the beginning of the bioassay.

For statistical analysis of radicle length, bioassay data for roots and shoots extract and for each time period (5 or 8 days after treatment, DAT) were fitted into the log-logistic model proposed by Streibig et al. (1993):

$$Y = C_i + \frac{D_i - C_i}{1 + \exp\{b_i[\log(X) - \log(a_i)]\}}$$

Y is the response (radicle length) of the test plant as a function of extract dose X (relative to stock solution), D is the upper asymptote (response of the untreated control), C is the lower asymptote (response at extremely large dose), a is the dose that gives a response half way between upper and lower asymptotes, b is the slope around the inflection point and i is the combination between root and shoot extract and assessment time.

Preliminary analysis had shown that data transformations were not needed to meet basic assumptions for the regression analysis. The goodness of fit was assessed by graphical analyses of residuals and F-test was assessed for lack of fit. Consecutive F tests showed that the two curves for each assessment time were parallel, i.e. sharing common asymptotes and slopes. Fitted equations were used to calculate the doses required to give 50 and 90% inhibition (ED_{50} and ED_{90}) of radicle length growth by sunflower extracts.

For germination, data observed at 5 and 8 DAT were not considered because there was no clear dose-response relationship. The dose-response relationship was however well established 13 days after treatment. Thus, regression analysis was performed only on germination data obtained at 13 DAT. A logistic regression (with probit link) was fitted to the observed data, to obtain the ED_{20} values (dilution that gave 20% inhibition of germination) for shoot and root extracts. The ED_{20} values were calculated because all the higher ED levels were beyond the observed range of responses. Non-linear and logistic regressions were performed by using *R* (*R* Development Core Team 2006) with the add-on package *drc* developed by Ritz and Streibig (2005).

3 Results

3.1 Pot experiments

Neither sunflower nor any of the weed extracts reduced the shoot dry weight of *A. artemisiifolia*. On the contrary, water extracts of *C. arvensis* roots and shoots, *A. theophrasti* roots, *A. artemisiifolia* shoots, and *A. theophrasti* shoots increased the shoot dry weight of *A. artemisiifolia* by 88, 80, 56, 56 and 41% of control, respectively, when extracts were used for watering (Tab. 1). Daily sprayed sunflower shoot extracts significantly increased the shoot dry weight of *A. artemisiifolia* as well (Tab. 1). Compared to the untreated control, in the most cases significantly higher NPK contents were observed in plants of *A. artemisiifolia* that were either sprayed or watered with the water extracts of the different donor plants (Tab. 2).

3.2 Petri dish experiments

Water extracts of sunflower shoot and root inhibited *A. artemisiifolia* germination only at higher concentrations. This is probably due to an increased osmotic potential (Fig. 1) and not due to a direct toxic effect. However, this hypothesis still needs to be proven with a series of non-toxic control treatments having equal osmotic pressures as the extracts. The ED_{20} values were 0.31 ± 0.05 s.e. for shoot extracts and 1.10 ± 0.20 s.e. for root extracts.

Water extracts of sunflower had a greater inhibitory effect on *A. artemisiifolia* radicle length than that on germination rate. Results referring to radicle length of ragweed after treatment with sunflower extracts are given in table 3 and figure 2. Observed data did not appear to deviate significantly from the sigmoidal relationship. ED_{50} and ED_{90} values are given in table 3.

4 Discussion

Current results showed that the examined donor plants stimulated the development of *A. artemisiifolia* at lower extract concentrations. The stimulating effect on shoot dry weight was stronger, when *A. artemisiifolia* plants were watered, as compared to a spray treatment. Therefore, it can be concluded that the stimulatory compounds from the different plant extracts are more effectively available by root uptake as compared to a leaf uptake. NPK values showed, that *Ambrosia* plants were able to utilise plant extracts as sources of nutrients, which could be an explanation for observed stimulatory effects rather than a direct stimulatory effect by extract compounds. No autotoxicity of *A. artemisiifolia* was observed.

Our results emphasized, that the stimulatory and/or promoting effects of the donor species extracts seem to be strongly dependent on the test species (acceptor, recipient). The phytotoxicity of *C. arvensis* (Kazinczi et al. 2007), *A. theophrasti* (Mikulás et al. 1990) and sunflower (Kazinczi et al.

Tab. 1: Increase of dry weight in g of shoots of *Ambrosia artemisiifolia* treated with various plant extracts; watered (LSD = 0.27), sprayed (LSD = 0.37); pot trial in the glasshouse.

Tab. 1: Zunahme des Sprosstrockengewichts in g von Ambrosia artemisiifolia *behandelt mit verschiedenen Pflanzenextrakten, watered (gewässert) LSD = 0,27, sprayed (gespritzt) LSD = 0,37, Topfversuch im Gewächshaus.*

	control	plant extract							
		H. annuus		*C. arvensis*		*A. theophrasti*		*A. artemisiifolia*	
		shoot	root	shoot	root	shoot	root	shoot	root
watered	1.28±0.10	1.42±0.23	1.28±0.28	2.30±0.41	2.40±0.26	1.80±0.20	2.00±0.31	2.00±0.25	1.30±0.16
sprayed	1.28±0.10	1.70±0.17	1.34±0.14	1.44±0.09	1.03±0.12	1.40±0.21	1.02±0.17	1.10±0.15	1.10±0.19

P=0.05; ± standard error

Tab. 2: Effects of various plant extracts on N-, P- and K-content of shoots of *Ambrosia artemisiifolia* (spr. = sprayed, wat. = watered); watered (LSD = 0.68; 0.03 and 0.72 for NPK, respectively), sprayed (LSD = 0.37; 0.02 and 0.69 for NPK, respectively); pot trials in the glasshouse.

Tab. 2: Wirkung von verschiedenen Pflanzenextrakten auf die N-, P- und K-Gehalte von Ambrosia artemisiifola *(spr. = gespritzt, wat. = gewässert); watered (LSD = 0,68; 0,03 and 0,72 for NPK, respectively); sprayed (LSD = 0,37; 0,02 and 0,69 for NPK, respectively); Topfversuche im Gewächshaus.*

	control		*H. annuus*				*C. arvensis*				*A. theophrasti*				*A. artemisiifolia*			
			shoot		root		shoot		root		shoot		root		shoot		Root	
	spr.	wat.	spr.	wat.	spr.	wat.	spr.	wat.	spr.	wat.	spr.	wat.	spr.	wat.	spr.	wat.	spr.	wat.
N%	1.85± 0.12	1.92± 0.21	2.99± 0.07	4.24± 0.13	2.19± 0.07	3.52± 0.09	3.36± 0.09	4.56± 0.16	2.59± 0.10	5.01± 0.16	3.22± 0.08	3.61± 0.08	1.89± 0.13	3.44± 0.09	2.76± 0.11	4.16± 0.13	2.75± 0.10	3.20± 0.08
P%	0.26± 0.03	0.27± 0.02	0.42± 0.04	0.66± 0.07	0.37± 0.04	0.66± 0.05	0.41± 0.03	0.81± 0.08	0.35± 0.01	0.89± 0.03	0.42± 0.04	0.44± 0.06	0.33± 0.03	0.68± 0.02	0.29± 0.02	0.61± 0.04	0.38± 0.02	0.61± 0.06
K%	2.37± 0.09	1.77± 0.07	3.78± 0.11	4.28± 0.07	3.79± 0.04	3.48± 0.08	3.26± 0.09	3.57± 0.11	3.35± 0.05	2.29± 0.02	3.71± 0.06	2.98± 0.03	2.72± 0.12	3.27± 0.06	3.48± 0.10	3.05± 0.11	4.28± 0.12	5.04± 0.14

P=0.05; ±standard error

Tab. 3: Influence of sunflower shoot- and root-extracts on radicle length of *Ambrosia artemisiifolia* at 5 and 8 days after treatment (DAT); Petri dish trial.

Tab. 3: Einfluss von Wurzel- und Stängelextrakten der Sonnenblume auf die Länge der Keimwurzeln von Ambrosia artemisiifolia *nach 5 und 8 Tagen nach der Applikation (DAT); Petrischalenversuch.*

Type of extract	DAT	ED50	s.e.	ED90	s.e.
Shoot	5	0.069	0.1052	0.397	0.6619
Shoot	8	0.112	0.1450	0.646	0.9573
Root	5	0.289	0.0330	1.668	0.7378
Root	8	0.470	0.0395	2.716	1.2157

s.e. = standard error; ED_{50} = dose (relative to stock solution) required to decrease radicle length by 50%; ED_{90} = dose (relative to stock solution) required to decrease radicle length by 90%

2004) on other test species than common ragweed has been proven, but instead of the expected inhibition as in other test species, stimulatory effects were observed in case of *A. artemisiifolia*. However, it is well known that allelopathic effects can be either inhibitory or stimulatory (Rice 1984). The observed favourable stimulatory effects of allelopathic plant extracts on *A. artemisiifolia* growth offer the possibility to promote the dominance of this weed under field conditions. However, although *A. artemisiifolia* may profit from allelochemicals released by neighbouring weeds, competition – as one of the main interactions among higher plants under field conditions – and its consequences can greatly modify such favourable effects (Kovács et al. 2006, Varga et al. 2006). The role and impacts of stimulatory allelopathic interaction will thus be a subject for future research in allelopathy.

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