Know your enemy: Are biochemical substances the secret weapon of common ragweed (*Ambrosia artemisiifolia* L.) in the fierce competition with crops and native weeds?

*Kenne den Feind: Nutzt das Beifußblättrige Traubenkraut (*Ambrosia artemisiifolia *L.) im Konkurrenzkampf mit Kulturpflanzen und heimischen Unkrautarten biochemische Geheimwaffen?*

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Abstract

Following the “novel weapon hypothesis”, the invasiveness of non-native species like common ragweed (*Ambrosia artemisiifolia* L.) can result from a loss of natural competitors due to the production of chemical compounds by the non-native species that unfavorably affect native communities. In this case, native plants may not be able to tolerate compounds released by a non-native plant that has not co-evolved in the same environment. Particularly the genus *Ambrosia* produces several types of organic compounds, which have a broad spectrum of biological activities and which could be major drivers in the successful invasion and competition process of common ragweed. To 1) asses the chemical profile of the aboveground biomass of common ragweed four different extracts (H_2O, hexane extract, methanol extract and essential oil) were prepared and analysed for their content substances. In the next step a laboratory experiment was implemented to 2) determine the effects of different concentrations of these substances on germination and seedling development of three different crops (soybean, wheat, and rapeseed), native weedy species (*Chenopodium album*, *Senecio vulgaris* and *Arabidopsis thaliana*) and on common ragweed itself. Results showed that germination as well as seedling development was significantly influenced by the chemical compounds in the extracts. Even though the extracts did not affect the germination capacity of crops, severe reduction in root and shoot growth were observed with all three tested crops. The highest inhibitory effect on germination of native weedy species as well as common ragweed was observed with essential oil and the aqueous extract.

Keywords: Enemy release hypothesis, invasive alien species, novel weapon hypothesis, plant extracts

Zusammenfassung

Entsprechend der „Novel Weapon“-Hypothese, kann der Invasionserfolg von nicht-heimischen Arten wie Ragweed (*Ambrosia artemisiifolia* L.) auf einen Verlust von natürlichen Konkurrenten zurückzuführen sein. Grund für diesen Verlust sind chemische Verbindungen, die die gebietsfremde Art erzeugt und die heimische Arten aufgrund fehlender Koevolution negativ beeinträchtigen. Studien haben gezeigt, dass die Gattung *Ambrosia* unterschiedliche organische Verbindungen produziert, von denen sehr viele ein breites Spektrum an biologischer Aktivität zeigen, weshalb diese chemischen Verbindungen ein wichtiger Grund für den Invasionserfolg und die Konkurrenzkraft der Pflanze sein können. Um 1) das chemische Profil der oberirdischen Biomasse offenzulegen, wurden vier verschiedene Extrakte (wässriger Extrakt, Hexan-Extrakt, Methanol-Extrakt und ätherisches Öl) hergestellt und auf ihre Inhaltsstoffe analysiert. Im nächsten Schritt wurde ein Laborexperiment durchgeführt, dass Aufschluss darüber geben sollte 2) welche Effekte unterschiedliche Konzentrationen dieser Extrakte auf Keimung und Jungpflanzenentwicklung von drei verschiedenen Kulturpflanzen (Sojabohne, Weizen und Raps), drei heimischen Unkrautarten (*Chenopodium album*, *Senecio vulgaris* und *Arabidopsis thaliana*) sowie auf Ragweed selbst, haben. Die Ergebnisse zeigten, eine deutliche Beeinträchtigung von Keimung und Entwicklung aller getesteten Pflanzenarten. Obwohl keiner der Extrakte eine direkte Wirkung auf die Keimfähigkeit der Kulturpflanzen hatte, konnte eine deutliche Wachstumsreduktion von Wurzel und Spross bei allen Nutzpflanzen festgestellt werden. Im Gegensatz dazu, konnte man bei den heimischen Unkrautarten und Ragweed selbst eine deutlich verminderte Keimfähigkeit sowie Wachstumshemmung speziell bei der Behandlung mit ätherischem Öl und dem wässrigen Extrakt beobachten.

Stichwörter: Enemy release-Hypothese, invasives Unkraut, Novel weapon-Hypothese, Pflanzenextrakte

Introduction

Plants release volatile organic compounds (VOCs) into the surrounding environment from organs such as leaves, flowers and roots. This concept of "allelopathy" was first raised by MOLISCH (1937). Since then, more than 1.700 VOC have been identified, most of them having the ability to inhibit or stimulate growth of other plants. During their evolution, plants have developed different ecological means to adapt their development, physiology and life history to various biotic and abiotic factors (NINKOVIC, 2002). Chemical messages play an important role in these behavioral mechanisms. Following the „enemy release hypothesis" (KEANE and CRAWLEY, 2002) and the "novel weapon theory" (CALLAWAY and RIDENOUR, 2004) particularly the invasiveness of non-native species like common ragweed (*Ambrosia artemisiifolia* L.) can result from a loss of natural enemies due to the production of toxic compounds (growth inhibitors etc.) by the non-native species that unfavorably affect native communities (VIVANCO et al., 2004; BALEZENTIENE, 2012). In this case, the native plants may be unable to tolerate compounds released by a non-native plant that has not co-evolved in the same environment (THORPE et al., 2009).

Particularly the genus *Ambrosia* produces several types of secondary metabolites including the sesquiterpene ambrosic acid, phenols, thiarubrines and thiopenes (BLOSZYK et al., 1992). These compounds have a broad spectrum of biological activities. Some of them are allelochemicals inhibiting the growth of other plants (WATANABE et al., 1981; PAJEVIĆ et al., 2010). Aqueous extracts of *A. artemisiifolia* and *A. palmeri* inhibit the growth of several plants or soil algae (FISHER and QUIJANO, 1984; BRÜCKNER et al., 2003). Volatile oils of *A. trifida* act against crop plants (WANG et al., 2005). Accordingly, allelopathy seems to be an important factor for the successful competition of *Ambrosia* species.

Not only living plants but also dead plant residues can release allelopathic substances. As shown by VIDOTTO et al. (2013) with their study on allelopathic effects of *A. artemisiifolia* (common ragweed) the amount of *A. artemisiifolia* residues in soil significantly affected seed emergence and seedling growth of different indicator crops and other weedy species. This was also confirmed by MUTCH et al. (2003) showing that the presence of ragweed after winter wheat or barley cultivation inhibited germination and growth of the succeeding crops due to an accumulation of dead ragweed biomass and root exudates.

However, information on the active chemical compounds in the ragweed plant and how they affect germination and/or growth of other plant species is very low. Thereby it has to be taken into account that different extracts have different chemical compounds, depending on the polarity of the solvent.

Therefore the aim of the study which started in summer 2019 and will go on until winter 2020 was 1) to assess the chemical profile of the aboveground and belowground biomass of common ragweed in four different extracts (aqueous extract, hexane extract, methanol extract and essential oil), 2) to determine the effects of different concentrations of these extracts on germination and seedling development of three different crops (rapeseed, wheat, and soybean) of three native weedy species (*Chenopodium album*, *Senecio jacobaea* and *Arabidopsis thaliana*) and of common ragweed itself.

Materials and Methods

Ragweed extracts and chemical profiling

For extraction we used aboveground biomass of common ragweed (stems and leaves) harvested before flowering end of July 2019. For the preparation of the aqueous extract ragweed biomass was dried 48 hours at 40 °C. For the initial extract we stirred 1000 g of the dried biomass in 1000 mL distilled water. Beside this pure extract we diluted this stock solution to a 1% and 10% solution (Tab. 1).

Methanol and n-hexane extracts were also obtained from dried ragweed biomass. Thereby 100 g of plant material was extracted with 100 mL solvent on an orbital shaker (120 rpm) for 24 hours at room temperature. Essential oil was produced following the protocol of LUKAS et al. (2009). The stock solution was diluted to a 0.5% and 1% solution. For the control variant normal tab water was used. Analysation of the compounds was performed at University of Veterinary Medicine Vienna following protocol SOP_3.05 via gas-chromatography/mass-spectroscopy (GC-MS).

Tab. 1 Overview of the various extracts and different concentrations used in the trial, including the abbreviations used in the graphs.

Tab. 1 *Überblick über die verschiedenen Extrakte und Konzentrationen inklusive der dazugehörigen Abkürzungen, die in den Grafiken verwendet werden.*

EXTRACT	Concentration	Abbreviations
Tap Water (H_2O)	--	Control
Aqueous extract	1% diluted	Aqua 1%
	10% solution	Aqua 10%
	100% undiluted	Aqua pure
Methanol	pure methanol without ragweed	MeOH 0
	150 µl of methanol extract	MeOH 150
	300 µl of methanol extract	MeOH 300
n-hexane	pure n-hexane without ragweed	Hexane
	150 µl of n-hexane extract	Hexane 150
	300 µl of n-hexane extract	Hexane 300
Essential oil	0.5% solution	EO 0.5%
	1% solution	EO 1%

Germination and seedling development – laboratory trial

The potential effects of the various ragweed extracts was studied by assessing its impact on indicator species' seed germination as well as radicle and hypocotyl elongation. As crop species we chose soybean, which is usually highly affected by ragweed competition in the field, as well as wheat and rapeseed. These two crops can be severely affected by ragweed due to their sowing date in autumn. If the preceding crop was heavily infested with common ragweed, the probability of high amounts of ragweed residues in the soil at the time of sowing and germination of wheat and rapeseed is very high and thus, can lead to a negative impairment of the crops development. Weedy indicator species were goosefoot (*Chenopodium album*) which is the most widespread and frequent weedy species in many agricultural areas in Austria, common ragwort (*Senecio vulgaris*) a toxic plant having high economic impact in the production of medicinal and aromatic plants, thale cress (*Arabidopsis thalina*) which is most common model plant in various fields of plant science and common ragweed itself. The experiment was conducted on filter paper in petri dishes. 100 seeds per extract and concentration as well as a control group in pure H2O were tested for germinability (Tab. 1). In addition, we measured the length of the radicle and hypocotyl of 50 seedlings, if available.

First results

The germination percentage of wheat, rapeseed and soybean was not significantly affected by ragweed extracts (Fig. 1 a-c). The significantly lowest germination rate of soybean was observed with pure methanol (MeOH 0) without any ragweed compounds. Despite that, ragweed essential oil significantly inhibited radicle and hypocotyl growth of all crops. The strongest effect was observed with wheat (Fig. 2) which had a 75.1% shorter radicle and 70.6% shorter shoot growth at 1% essential oil.

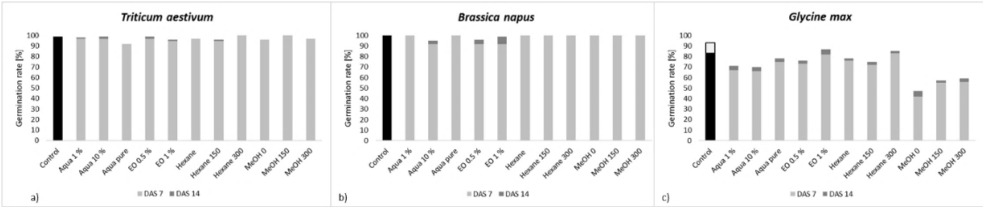

Fig. 1 a-c Germination rate (%) of a) wheat, b) rapeseed, and c) soybean as affected by four extracts of common ragweed in different concentrations (DAS = days after seeding, n = 100 seeds per species and variant).

Abb. 1 a-c *Keimung (%) von a) Weizen, b) Raps und c) Sojabohne in Abhängigkeit von vier verschiedenen Ragweed-Extrakten in unterschiedlicher Konzentration (DAS = Tage nach Ansaat, n = 100 Samen pro Art und Variante).*

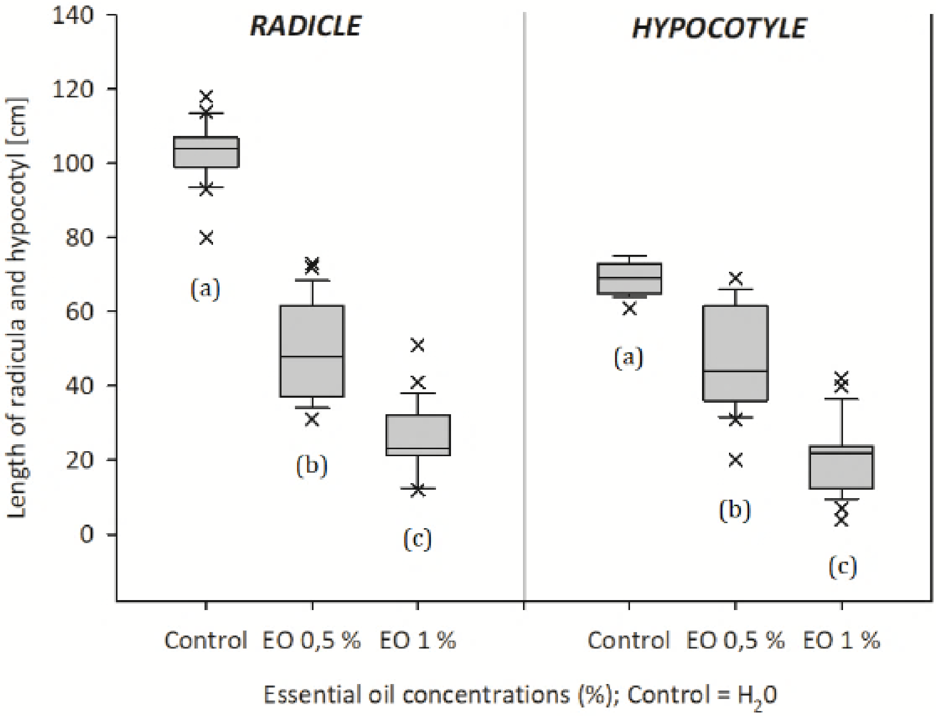

Fig. 2 Radicle and hypocotyl length of wheat after 14 days of exposure to ragweed essential oil extract in different concentrations (n = 50 seedling/variant; different letters indicate significant differences)

Abb. 2 *Radikula- und Hypokotyllänge von Weizen nach 14 Tagen Behandlung mit ätherischem Öl von Ragweed in unterschiedlichen Konzentrationen (n = 50 Keimlinge pro Variante; unterschiedliche Buchstaben zeigen signifikante Unterschiede.)*

Contrary to the crops germination of the weedy species was clearly affected by ragweed. The germination of common ragwort was significantly influenced when seeds were exposed to ragweed essential oil (Fig. 3 a). At a concentration of 0.5% germination rate decreased by 29.7%; when exposed to a 1% solution of ragweed essential oil germination rate of common ragwort dropped by 82.4%. As with ragwort, also the germination of goosefoot was significantly influenced by ragweed extracts, particularly by essential oil (Fig. 3 b). When seeds were exposed to a 1% essential oil extract the germination decreased by 72.3%. In contrast, the germination of thale cress was most affected by the presence of aqueous extract. Particularly seeds exposed to the pure aqueous extract (Fig. 3 c – Aqua pure), showed an 84.0% lower germination than the control group.

However, the strongest influence of the ragweed extracts was observed with ragweed itself. The pure aqueous extract (Aqua pure) decreased germination rate by 58.8%. The strongest inhibition of germination was observed with essential oil. A concentration of 0.5% (EO 0.5) was enough to reduce germination by 93.8% (Fig. 3 d). As with the crops, also radicle and hypocotyl growth of the weedy species were affected by the presence of ragweed extracts. A decreased growth was particularly monitored with the aqueous extracts and the essential oil.

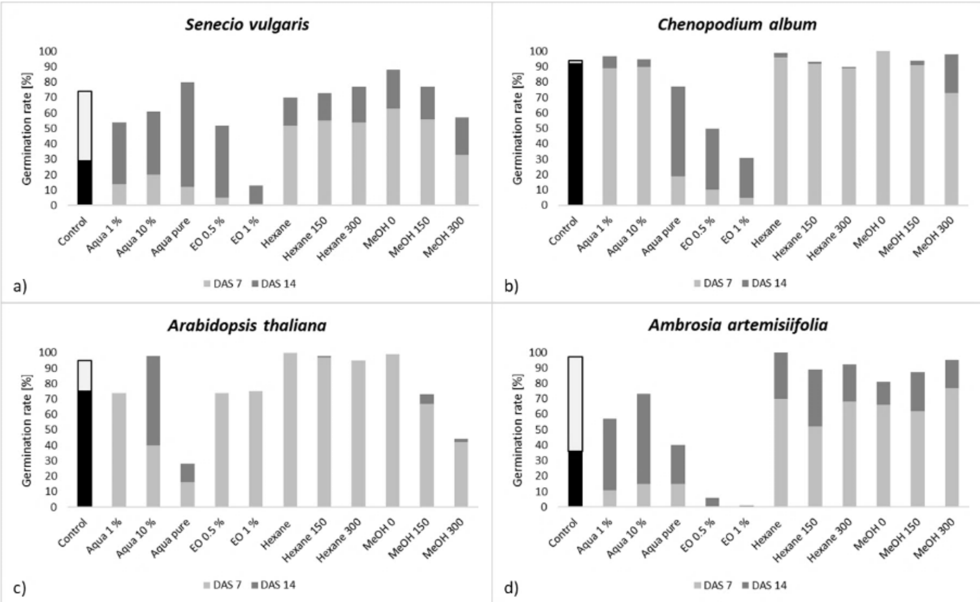

Fig. 3 a-d Germination (%) of a) common ragwort, b) goosefoot, c) thale cress, and d) common ragweed as affected by four extracts of common ragweed in different concentrations (DAS = days after seeding, n = 100 seeds per species and variant).

Abb. 3 a-d *Keimung (%) von a) Gewöhnlichem Greiskraut, b) Weißem Gänsefuß, c) Ackerschmalwand und d) Ragweed in Abhängigkeit von vier verschiedenen Ragweed-Extrakten in unterschiedlicher Konzentration (DAS = Tage nach Ansaat, n = 100 Samen pro Art und Variante).*

Outlook

Even though the germination of crops was not affected by the presence of ragweed compounds, we detected significant negative effects on the growth performance (radicle and hypocotyl) of crop seedlings. Contrary, the germination rate of native weeds was severely reduced, particularly by essential oil and aqueous extracts of ragweed. Thus, negative impacts of ragweed compounds on any neighbors, irrespective if crop or weed could be expected. Therefore, the third part of experiments should reveal interaction effects of different substrate types and the extracts. The aim is to test if various substrates retard or promote the effectiveness of the chemical compounds on germination and/or seedling growth of the same plant species used in the laboratory experiment.

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References

BALENZENTIENE, L., 2012: Inhibitory effects of invasive *Heracleum sosnowskyi* on rapeseed and ryegrass germination. Allelopathy Journal **30**, 197-208.

BLOSZYK, E., U. RYCHLEWSKA, B. SZCZEPANSKA, M. BUDESINSKY, B. DROZDZ, H. HOLUB, M., 1992: Sesquiterpene lactones of *Ambrosia artemisiifolia L.* and *Ambrosia trifida L.* species. Chemical Communications **57**, 1092–1102.

BRÜCKNER, D.J., A. LEPOSSA, Z. HERPAI, 2003: Inhibitory effect of ragweed (*Ambrosia artemisiifolia L.*) inflorescence extract on the germination of *Amaranthus hypochondriacus L.* and growth of two soil algae. Chemosphere **52**, 525-519.

CALLAWAY, R.M., W.M. RIDENOUR, 2004: Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment **2**(8), 436-443.

FISHER, N.H., L. QUIJANO, 1984: Allelopathic agents from common weeds: *Ambrosia palmeri* and *Ambrosia artemisiifolia* and related weeds. ACS Symposium Series **268**, 133–147.

KEANE, R.M., M.J. CRAWLEY, 2002: Exotic plant invasions and the enemy release hypothesis. Trends in Ecology and Evolution **17**, 164-170.

MOLISCH, H., 1937: Der Einfluss einer Pflanze auf die andere –Allelopathie. G. Fisher, Jena.

MUTCH, D.R., T.E. MARTIN, K.R. KOSOLA, 2003: Red clover (*Trifolium pratense*) suppression of common ragweed (*Ambrosia artemisiifolia*) in winter wheat (*Triticum aestivum*). Weed Technology **17**, 181-185.

NINKOVIC, V., 2002: Volatile communication between barley plants affects biomass allocation. Journal of Experimental Botany **54**, 1931-1939.

PAJEVIĆ, S., M. BORIŠEV, D. ORČIĆ, P. BOŽA, N. NIKOLIĆ, 2010: Photosynthetic and Biochemical Characteristics of Invasive Species (*Ambrosia artemisiifolia L., Ambrosia trifida L. and Iva xanthifolia Nutt.)* depending on soil humidity and phenological phase. Russion Journal of Ecology **41**(6), 498-505.

THORPE, A.S., G.C. THELEN, A. DIACONU, R.M. CALLAWAY, 2009: Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. Journal of Ecology **97**, 641-645.

VIDOTTO, F., F. TESIO, A. FERRERO, 2013: Allelopathic effects of *Ambrosia artemisiifolia L.* in the invasive process. Crop Protection **54**, 161-167.

VIVANCO, J.M., H.P. BAIS, F.R. STERMITZ, G.C. THELEN, R.M. CALLAWAY, 2004: Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. Ecology Letters **7**, 285-292.

WANG, P., W.J. LIANG, C.H. KONG, Y. JIANG, 2005: Allelopathic potentials of volatile allelochemicals of *Ambrosia trifida L.* on other plants. Allelopathy Journal **15**, 131-136.

WATANABE, S., A. KOBAYASHI, K. YAMASHITA, 1981: Growth and Germination Inhibitors in Hog-Weed. Agricultural and Biological Chemistry **45**(12), 2919-2920.