Common ragweed (*Ambrosia artemisiifolia* L.) causes severe yield losses in different soybean varieties by reducing the infection potential of *Bradyrhizobium japonicum*

*Das Beifußblättrige Traubenkraut (*Ambrosia artemisiifolia *L.) führt zu erheblichen Ertragsverlusten bei verschiedenen Sojabohnen-Sorten infolge eines reduzieren Infektionpotenzials der Sojabohne mit* Bradyrhizobium japonicum

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Abstract

Ambrosia artemisiifolia L. (Asteraceae) known as common ragweed is an annual herbaceous species native to North America which has become one of the economically most important weeds in agricultural areas throughout Middle Europe. Its large ecological amplitude enables the species to establish in several types of environment, but management options to effectively contain its spread are limited due to lack of efficacy, cost and time or lack of awareness. In the last decade especially soybean fields were severely affected by ragweed invasion, but until now information on the yield-decreasing effects of the plant are scarce for Middle Europe.

Therefore, the aim of the study, conducted in 2017 and 2018 as a greenhouse and biennial field trial, was an evaluation of the competition effects of ragweed upon 1) growth (aboveground/belowground), 2) infection potential of rhizobia and 3) yield of two different soybean varieties.

Results revealed that on plots with the highest ragweed biomass the yield loss accounted for 83.7% on average. Particularly, the numbers of nodules as well as the mean weight of the nodule, which stand in tight correlation with soybean yield, were significantly reduced by the presence of ragweed. Only one ragweed plant per square metre reduced the number of nodules by 55.8% and consequently led to a decrease in yield of 18%.

Keywords: Common ragweed, invasive alien species, rhizobia, soybean nodulation

Zusammenfassung

Das ursprünglich aus Nordamerika stammende Ragweed oder Beifuß-Ambrosia *(Ambrosia artemisiifolia L.* (Asteraceae), íst eine einjährige, krautige Pflanze. Sie hat sich in den letzten Jahrzehnten massiv ausgebreitet und zählt heute in vielen Teilen Mitteleuropas zu den wirtschaftlich wichtigsten Unkrautarten in der Landwirtschaft. Aktuell übliche Bekämpfungsmaßnahmen wie Mähen oder Herbizidapplikationen zeigen auf vielen Standorten nur sehr eingeschränkte Erfolge, sind aus zeitlichen oder wirtschaftlichen Gründen nicht umsetzbar bzw. scheitern daran, dass der Neophyt nicht erkannt wird. Speziell Sojabohnenbestände wurden in den letzten Jahren immer stärker von Ragweed befallen, jedoch gibt es bislang nur wenige Studien zur Ertragswirkung von Ragweed.

Das Ziel des zweijährigen Feld- und einjährigen Glashausversuches, die 2017 und 2018 durchgeführt wurden, war deshalb die Untersuchung der Konkurrenzeffekte von Ragweed auf 1) Wachstum (oberirdisch / unterirdisch), 2) Entwicklung, 3) Infektionspotential der Knöllchenbakterien und 4) Ertrag von zwei verschiedenen Sojabohnensorten.

Die Ergebnisse der Untersuchung zeigten, dass auf den Versuchsflächen mit der höchsten Ragweed-Biomasse der Sojaertrag um 83,7 % sank. Speziell die Anzahl und das Gewicht der Knöllchen (*Bradyrhizobium japonicum*) auf den Sojawurzeln wurden durch Anwesenheit von Ragweed stark reduziert. Eine Ragweed-Pflanze pro Quadratmeter reichte aus, um die Anzahl der Knöllchen um durchschnittlich 55,8 % zu reduzieren. Da jedoch eine effektive Infektion wesentlich zur Ertragsbildung von Soja beiträgt, führte diese Reduktion an Knöllchenbakterien bzw. deren Gewicht zu einem Ertragsverlust von 18 %.

Stichwörter: Knöllchenbakterien, Infektionspotenzial von Sojabohne, invasives Unkraut, Ragweed

Introduction

Due to the role of soybean (*Glycine max* (L.) Merr.) as a key protein source in livestock feeding, e. g. as full-fat soybean for pig fattening or concentrated feed in the dairy industry (ZOLLITSCH et al., 1993; VOLLMANN et al., 2000) the cultivation area of soybean (*Glycine max* (L.) Merr.) in Europe has more than trebled since the 1990s up to approximately 5.7 million ha and an average annual production of 10.7 million tons of beans (FAOSTAT, 2017). In contrast to the world's major producers of soybean, several countries within the European Union (EU) such as Austria, Germany and France have opted total ban of cultivation of GM-crops (European Commission, Directive 2015/421/EU), as there are a number of concerns resulting from the unprecedented adoption of glyphosate-resistant crops including potential negative impacts on biodiversity, eco-toxicological issues, and the evolution of (glyphosate-)resistant super-weeds (WATKINSON et al., 2000; SANDERMANN, 2006; OWEN et al., 2010).

The last point in particular, is nowadays one of the major challenges in soybean cultivation, especially in countries where the cultivation of GM-soybean is banned. For example in Austria, the cultivation area of soybean increased by approximately 134% from 2006 to 2016. In the same time, yields per unit area increased by approx. 19% due to advances in plant breeding and agrotechnical improvements (ROWNTREE et al., 2013; STATISTIK AUSTRIA, 2018). However, these increases in the average yield per unit area are severely under threat due to the rapid spread and establishment of super-weeds like common ragweed (*Ambrosia artemisiifolia* L.) which is actually promoted by a combination of three factors, 1) the steady extension of the cultivation area of soybean, 2) climate change, and 3) lack of efficacy in management options to effectively contain the spread of the plant (JOHNSON et al., 2007; KARRER et al., 2011; RICHTER et al., 2013).

Common ragweed – a threat to European soybean production

Common ragweed is an annual herbaceous species native to North America which is not only one of the most dominant inducers of pollen allergy but also a troublesome agronomic weed. (FUMANAL et al., 2007; BULLOCK, 2010; SMITH et al., 2013). Although it was first observed in Europe in the mid-19th century, its main naturalization and establishment in Europe was restricted over a long period of time mainly due to climatic factors (RICHTER et al., 2013). Thus, changing climatic conditions, particularly climatic warming accelerated the spread of the plant since the beginning of the new millennium. In Austria since the year 2000, the number of summer days which are defined as days on which the temperature exceeds 25 °C increased by approx. 67% and the number of sunshine hours more than doubled compared to the time period between 1955 and 1999 (ZAMG, 2018). The extraordinary spread of common ragweed started along the high-capacity road system from where seeds of the plant were easily transferred to the surrounding area by implements of the road maintenance services but also with water run-off and wind. Upon arrival in the field, particularly agricultural machinery contributed substantially to the further spread of the seeds across fields (FUMANAL et al., 2007; KAZINCZI et al., 2008; KARRER et al., 2011). One counting of the University of Natural Resources and Life Science in Vienna, revealed a seed potential of a harvest-combine in a medium-infested field (approx. 4.5 ha) of 7,500 seeds that may stay germinable in soil for 40 years (TOOLE and BROWN, 1946; ESSL et al., 2009; KARRER et al., 2011).

As thermophilic plant the beginning of field emergence in Central Europe can be expected when the temperature in the upper 5 cm soil layer is permanently above 10 °C (BÉRES, 2004). Thus, particularly spring-sown, smaller growing crops in wider row-spaces like soybean are mainly affected by the presence of common ragweed. Its rapid growth and its fast developing, large leaf area allows the invasive plant to compete successfully throughout the growing season (JOHNSON et al., 2007; SMITH et al., 2013). At the moment authorized herbicides against common ragweed are available for soybean and maize (active substances: Triketone, Bentazon, Sulfonylurea) but show low or highly fluctuating effects (BAYRISCHE LANDESANSTALT FÜR LANDWIRTSCHAFT, 2015). Furthermore, it has been reported that common ragweed can build up resistances within a very short time. The first scientific report of glyphosate-resistant common ragweed was from Missouri, USA, in 2004. After glyphosate application, the common ragweed plant had dead tissue on their upper portions, suggesting that the application was effective. However, re-growth appeared from the lower parts of the stem, allowing the plant to survive (POLLARD et al., 2004; JOHNSON et al., 2007; NANDULA et al., 2017). This high regeneration ability was also monitored after mechanical control measures (grooming) which is the most common practice in Austria and other countries like Germany, and even after cutting as common ragweed can resprout easily (KARRER et al., 2011).

Recently, yield losses of 70% were reported for oil seed pumpkin. A yield reduction of 30% at densities of 5 ragweed plants per m² was demonstrated for sunflower and maize (KAZINCZI et al., 2008). However, for soybean data on the yield-decreasing effects of common ragweed in Middle Europe is scarce. Currently, studies on the yield effect of common ragweed on soybean are available from the United States showing that the presence of one plant per 110 square feet (10.2 m²) reduced soybean yield by 50% (COBLE et al., 1981; GIBSON et al., 2007). Nevertheless, these results can only be transferred with caution to the present European situation because of differing climatic conditions, cropping practices and different varieties.

It can be assumed, that the importance of common ragweed as agricultural weed, particularly in soybean, will further increase. The aim of the study is to obtain a profound knowledge of the interaction between common ragweed and soybean which is essential to quantify the effects of the intra- and interspecific competition upon 1) growth (aboveground/belowground) of soybean and common ragweed, 2) infection potential of rhizobia, and 3) yield of soybean.

Materials and Methods

Greenhouse trial

The one-season greenhouse trial was conducted at the beginning of 2018 in the experimental greenhouse of the University of Natural Resources and Life Sciences in Tulln in plastic pots (18 × 18 × 25 cm) filled with sterilized mixture of arable soil from the experimental farm Gross-Enzersdorf which is classified as a chernozem of alluvial origin and is rich in calcareous sediments (pH 7.6, silty loam, 2.2-2.3% organic substance), sand and perlite in the ratio 3:2:1 to avoid soil compactions in the pots. Soybean cultivars Albenga and ES Mentor, both 00-varieties, were used in the trial. Cultivar Albenga (Saatbau Linz) is a 00-variety characterized by a fast juvenile development, and medium growth height. ES Mentor (Saatbau Linz) is characterized by a more bushy growth than Albenga and therefore has a shorter growth height but higher stability.

In each pot one soybean was planted together with 0, 1, 3, and 6 ragweed plants (treatment labeling: R0, R1, R3, and R6) in four replications. In addition, pots with pure stands of common ragweed at densities of 1, 3, and 6 plants in four replications were established to test for the intraspecific competition of ragweed alone. Before seeding, soybeans were inoculated with *Bradyrhizobium japonicum* (RWA Raiffeisen Ware Austria), a nitrogen-fixing bacterial species that forms root nodules specifically on soybean roots, which actually is not sufficiently abundant in Middle European soils, to ensure a successful symbiosis between soybean and the bacteria.

Field trials

The two-years field experiment was conducted in the Marchfeld Basin in Eastern Austria on the Experimental farm Gross-Enzersdorf of the University of Natural Resources and Life Sciences Vienna during the vegetation periods 2017 and 2018. The Marchfeld basin is strongly influenced by the semi-arid Pannonian climate, characterized by cold winters with fluctuating heavy frost periods and irregular snow cover. In contrast, the summer periods are hot and intermittently dry. The mean annual temperature is 9.6 °C, the mean precipitation is between 500 and 600 mm, and on average 1,900 hours of sunshine are annually measured (BMNT, 2019). As with the greenhouse trial, we wanted to observe the individual influence of common ragweed on soybean. Thus, we excluded the intraspecific competition of soybean by sowing only one soybean plant per 1 m² plot with different densities of common ragweed (0, 1, 3, and 6 plants = treatment labeling R0, R1, R3, and R6) in four replicates. Futhermore, also pure stands of common ragweed in densities of 1, 3, and 6 plants in four replicates were established to test for the intraspecific competition of ragweed alone.

In 2017, we used cultivar Albenga (Saatbau Linz), in 2018, the same trial setup was used, but in addition a second soybean cultivar (ES Mentor, Saatbau Linz) was included in the trial to check if the results gained with cultivar Albenga in 2017 were cultivar-specific or could be also transferred to another variety. Before sowing, soybean was inoculated with *Bradyrhizobium japonicum*.

For carving out the soil in the field trials, we used standardized metal frames (20 x 20 x 20 cm). The roots in the soil samples were washed out manually. Nodules were counted, removed from the soybean roots and weighed (fresh weight). Roots of soybean and common ragweed were separated, dried at 105 °C for 24 hours and weighed. The same drying conditions were used for the aboveground dry matter. In the field trials as well as the greenhouse trial, 5 samplings were performed throughout the vegetation period. Here, we only show the results of the last sampling date = harvest date. Full results are actually under revision.

Results

Influence of common ragweed on soybean yield – greenhouse trial

Ragweed density and the soybean variety had a significant impact on the yield (Tab. 1). The highest yield of 15.0 ± 3.5 g was observed with variety ES Mentor in the R0-treatment whereas variety Albenga showed the lowest average yield of 2.7 ± 2.4 g in the R6-treatment. The yield of both varieties was not significantly affected by the presence of one ragweed plant. Whereas the yield of Albenga did not change severely in the R3-treatment, the yield of ES Mentor decreased significantly in competition with three ragweed plants. The main difference between the varieties was observed in the R6-treatment. Due to intraspecific competition between the ragweed plants in the pots and interspecific competition due to its bushy growth the yield of ES Mentor could slightly increase from 7.9 ± 2.5 g in the R3-treatment to 10.0 ± 1.6 g in the R6-treatment whereas the yield of Albenga further decreased (Fig. 1a).

Tab. 1 Results of two-way-ANOVA for yield and yield parameters of one soybean plant measured at harvest in dependency of the factors ragweed density (R) and variety (V); AGDM = aboveground dry matter, n = 4.

Tab. 1 *Ergebnisse der zweifaktoriellen Varianzanalyse betreffend Ertrag und Ertragsparametern von Sojabohne zum Erntezeitpunkt in Abhängigkeit von den Faktoren Ragweed-Dichte (R) und Sorte (V); AGDM = oberirdische Trockenmasse, n = 4.*

	p-value R	**p-value V**	**p-value R ×V**
GREENHOUSE TRIAL			
AGDM soybean [g]	< 0.001 (***)	0.007 (**)	0.059 (n.s.)
Yield soybean [g]	< 0.001 (***)	0.006 (**)	0.520 (n.s.)
Number of nodules	< 0.001 (***)	0.825 (n.s.)	0.489 (n.s)
Mean weight of nodules	0.013 (*)	0.051 (n.s.)	0.209 (n.s.)
FIELD TRIAL 2017			
AGDM soybean [g]	< 0.001 (***)	--	--
Yield soybean [g]	< 0.001 (***)	--	--
Number of nodules	< 0.001 (***)	--	--
Mean weight of nodules	0.002 (**)	--	--
FIELD TRIAL 2018			
AGDM soybean [g]	< 0.001 (***)	0.060(n.s.)	0.961 (n.s.)
Yield soybean [g]	< 0.001 (***)	0.280 (n.s.)	0.617 (n.s.)
Number of nodules	0.002 (**)	0.070 (n.s.)	0.415 (n.s.)
Mean weight of nodules	0.031 (*)	0.082 (n.s.)	0.255 (n.s.)

Influence of common ragweed on soybean yield – field trials

Also in the field trials the factors ragweed density and variety had significantly influenced the soybean yield. The highest average yield of 26.2 ± 4.6 g was observed with variety ES Mentor in 2018 on the plots with no ragweed occurrence (R0; Fig. 1b). The lowest mean yield of 3.0 ± 1.12 g was obtained by variety Albenga in 2017 on the plots with six ragweed plants (R6). Only one ragweed plant per square meter caused an average yield loss of 43.7% with variety Albenga (2017: 55.9%; 2018: 31.0%). The yield of ES Mentor was reduced by 36.4% when one single ragweed plant was present. With three ragweed plants per plot yield decreased sharply by 80.4% with Albenga and by 64.5% with variety ES Mentor. Both yields at treatment R3 did not differ significantly from the yields which were obtained at the highest ragweed density level of six plants per plot. These results were supported by regression analysis, indicating that not only the number of plants but primarily the increasing amount of ragweed biomass was responsible for the strong decrease of the yield of both soybean varieties (overall $R^2 = 0.67$; $p < 0.001$).

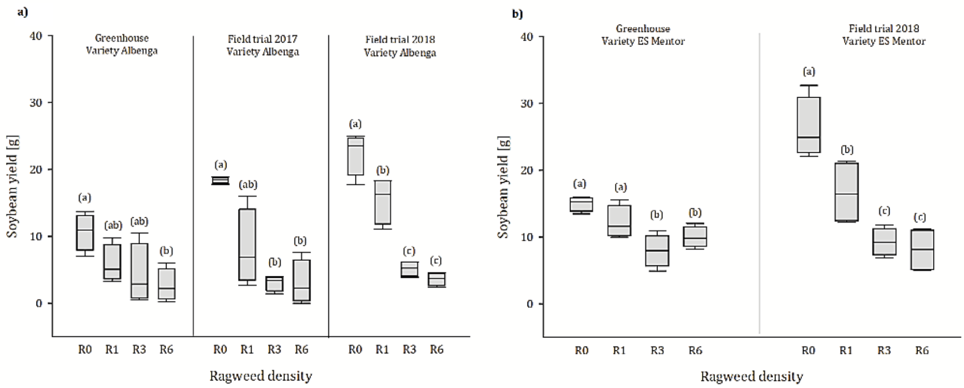

Fig. 1 a + b Soybean yield in dependency of the factor ragweed density (0, 1, 3, and 6 plants = R0, R1, R3, and R6) of a) variety Albenga and b) variety ES Mentor, separated by trial (n = 4; different letters indicate significant differences).

***Abb. 1 a + b** Sojabohnenertrag in Abhängigkeit vom Faktor Ragweed-Dichte (0, 1, 3 und 6 Pflanzen = R0, R1, R3 und R6) von a) Sorte Albenga und b) Sorte ES Mentor kategorisiert nach Versuch (n = 4; unterschiedliche Buchstaben stehen für signifikante Unterschiede).*

Common ragweed decreases number and mean weight of nodules and therefore the yield of soybean

Cultivars did not differ significantly in their number of nodules but we detected a clear influence of the ragweed density on the number of nodules found on the soybean roots (Fig. 2). Over all trials the highest mean number of 121.25 ± 10.9 nodules was found on roots of cultivar ES Mentor without any ragweed present. In the R0-variant of cultivar Albenga 94.3 ± 24.2 nodules were counted on average. Only one ragweed plant per plot/pot was sufficient to reduce the number of nodules of cultivar ES Mentor by 57.9%. With a loss of 36.0% of nodules cultivar Albenga was a little bit more tolerant against common ragweed. This advantage, however, disappeared with increasing ragweed density. With six plants per plot/pot the number of nodules was reduced by 86.8% (mean: 12.4 nodules ± 7.2), whereas cultivar ES Mentor showed a slightly lower loss of 72.9% less nodules (32.8 ± 12.5) in the R6-variant.

As depicted in Figure 3 the yield of both cultivars was significantly correlated with the number of nodules (ES Mentor: $R^2 = 0.64$, $p < 0.001$; Albenga: $R^2 = 0.58$, $p < 0.001$). Nevertheless, as revealed by generalized linear mixed model (GLMM) analysis, the most parsimonious model explaining the yield of soybean was the interactions of the factors mean weight of nodules and the amount of ragweed dry matter present.

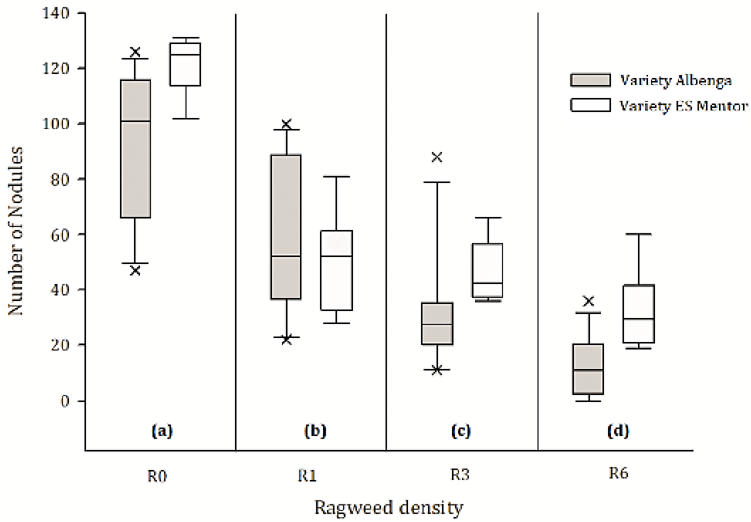

Fig. 2 Number of nodules of soybean cultivars Albenga and ES Mentor averaged over all trials in dependency of the ragweed density (0, 1, 3, and 6 plants; different letters indicate significant differences).

Abb. 2 *Anzahl der Knöllchen der beiden Sojabohnensorten Albenga und ES Mentor gemittelt über alle Versuche, in Abhängigkeit vom Faktor Ragweed-Dichte (0, 1, 3 und 6 Pflanzen, unterschiedliche Buchstaben stehen für signifikante Unterschiede).*

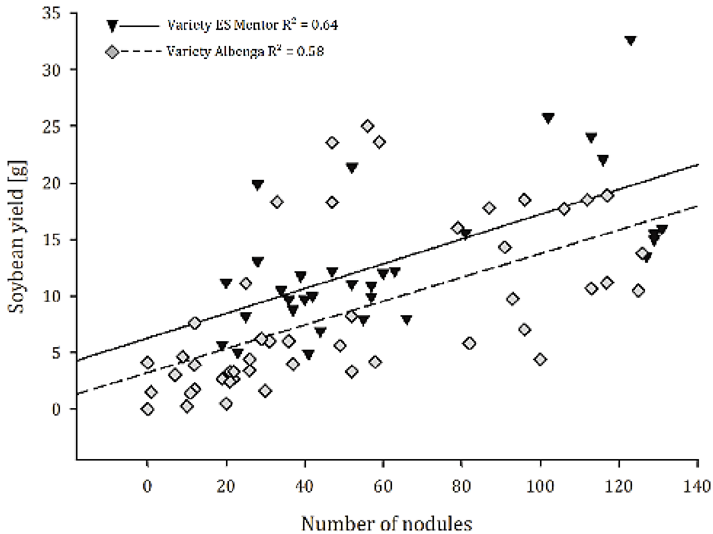

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Fig. 3 Correlation between soybean yield of variety Albenga and variety ES Mentor and the number of nodules (regression analysis, n = 32 (ES Mentor), n = 48 (Albenga).

Abb. 3 *Korrelation zwischen dem Ertrag der Sojabohnensorten Albenga und ES Mentor und der Anzahl der Knöllchen (Regressionsanalyse, n = 32 (ES Mentor), n = 48 (Albenga).*

As shown in Figure 4 soybean yield increased significantly with increasing mean weight of nodules (R^2 = 0.65, $p < 0.001$) and was clearly negatively affected by increasing ragweed dry matter (R^2 = 0.45, $p < 0.001$), irrespective of the trial year and cultivar. However, the highest mean weight of nodules of 12.4 ± 1.3 mg was observed with cultivar ES Mentor, and 8.6 ± 4.1 mg with cultivar Albenga in the R0-variant. At ragweed densities of six plants per plot/pot the mean weight of nodules decreased by approx. 69% with both cultivars.

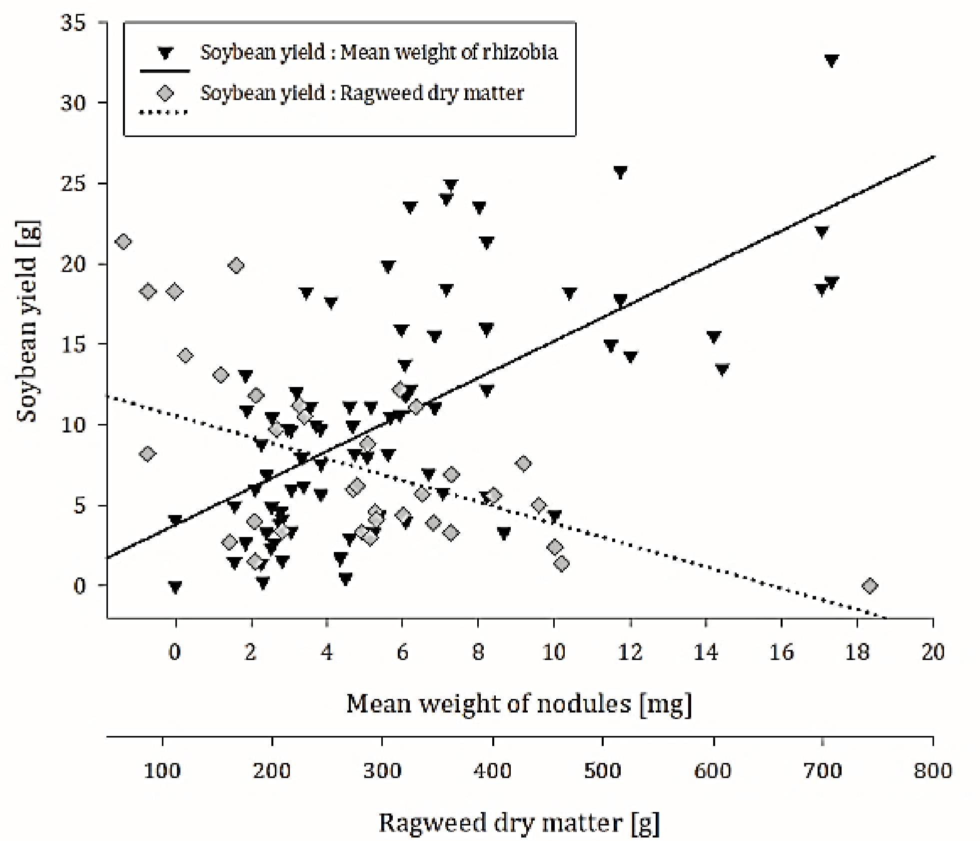

Fig. 4 Correlation between soybean yield of both varieties, the mean weight of nodules (upper x-axis), and the amount of ragweed dry matter present (lower x-axis; regression analysis, n = 32 (ES Mentor), n = 48 (Albenga)).

Abb. 4 *Korrelation zwischen dem Ertrag beider Sojabohnensorten, dem Durchschnittsgewicht der Knöllchen (obere x-Achse) und der vorhandenen Ragweed-Trockenmasse (untere x-Achse; Regressionsanalyse, n = 32 (ES Mentor), n = 48 (Albenga)).*

Outlook

Results revealed that soybean performance was severely affected by the presence of ragweed. Beside the negative effect of the aboveground competition on soybean performance, analysis showed that particularly the presence of ragweed roots had severe impact on root growth and on the infection potential of rhizobia (*Bradyrhizobium japonicum*) and thus the number of nodules which stood in high correlation with the biomass production and the yield of soybean. In an ongoing trial we will try to reveal if volatile organic compounds in the root exudates of ragweed could inhibit or disturb prenodulation signals which are used by legumes and rhizobia in the "partner finding" process. As this study should give a full assessment of the biochemical properties of ragweed aboveground- and belowground biomass, the results could serve as a basis for further research in a broad field of science, e. g. plant breeding, plant protection, ecology and many more environmental-related research sectors.

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