agronomy

Article

Common Ragweed (*Ambrosia artemisiifolia* L.) Causes Severe Yield Losses in Soybean and Impairs *Bradyrhizobium japonicum* Infection

Rea Maria Hall [1,2,*], Bernhard Urban [2], Helmut Wagentristl [3], Gerhard Karrer [2], Anna Winter [1], Robert Czerny [1] and Hans-Peter Kaul [1]

[1] Institute of Agronomy, University of Natural Resources and Life Sciences, Tulln an der Donau, 3430 Vienna, Austria; anna.winter27@live.at (A.W.); bertl26@gmx.at (R.C.); hans-peter.kaul@boku.ac.at (H.-P.K.)
[2] Institute of Botany, University of Natural Resources and Life Sciences, 1180 Vienna, Austria; bernhard.urban@boku.ac.at (B.U.); gerhard.karrer@boku.ac.at (G.K.)
[3] Experimental Farm Groß-Enzerdorf, University of Natural Resources and Life Sciences, Groß-Enzersdorf, 2301 Vienna, Austria; helmut.wagentristl@boku.ac.at
* Correspondence: rea.hall@boku.ac.at; Tel.: +43-664-527-2615

Abstract: *Ambrosia artemisiifolia* L. (Asteraceae), known as common ragweed, is an annual herbaceous species native to North America that has become one of the most economically important weeds in arable fields throughout Central Europe. Its large ecological amplitude enables the species to become established in several types of environments, and management options to effectively contain its spread are limited due to a lack of efficacy, high cost, or lack of awareness. In the last decade, in particular, soybean fields have been severely affected by common ragweed invasion. However, until now, information on the yield-decreasing effects of the plant has been scarce. Therefore, the aim of this study was to evaluate the competition effects of common ragweed on (1) soybean growth (aboveground/belowground), (2) the yield of two different soybean cultivars, and (3) the nodulation potential. Based on a greenhouse and biennial field trial, we found that in plots with the highest common ragweed biomass, the soybean yield loss accounted for 84% compared to the weed-free control, on average. The number of nodules, in addition to the mean nodule weight, which are tightly correlated with soybean yield, were significantly reduced by the presence of common ragweed. Just one common ragweed plant per square meter reduced the number of nodules by 56%, and consequently led to a decrease in yield of 18%. Although it has been reported that the genus Ambrosia produces and releases several types of secondary metabolites, little is known about the influence of these chemical compounds on soybean growth and nodulation. Thus, there is substantial need for research to understand the mechanisms behind the interaction between common ragweed and soybean, with a view to finding new approaches for improved common ragweed control, thereby protecting soybean and other crops against substantial yield losses.

Keywords: invasive alien species; common ragweed; rhizobia; soybean nodulation

Citation: Hall, R.M.; Urban, B.; Wagentristl, H.; Karrer, G.; Winter, A.; Czerny, R.; Kaul, H.-P. Common Ragweed (*Ambrosia artemisiifolia* L.) Causes Severe Yield Losses in Soybean and Impairs *Bradyrhizobium japonicum* Infection. *Agronomy* **2021**, *11*, 1616. https://doi.org/10.3390/agronomy11081616

Academic Editors: Jeffrey Weidenhamer, Sajid Latif, Leslie A. Weston and Ilias Travlos

Received: 18 May 2021
Accepted: 9 August 2021
Published: 14 August 2021

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the world's most important oilseed and protein crops, constituting the second largest source of vegetable oil and the largest source of animal protein feed at a global scale [1]. Due to consistent improvements in average yield through plant breeding and crop management, a significant reduction of production costs, and the introduction of genetically modified (GM) glyphosate-resistant soybean cultivars, an exponential increase in global production was observed during the past half-century, to reach its present level of over 352 million tons of soybean per year, harvested from an area of over 123.6 million ha (FAOSTAT, 2017).

Due to the role of soybean as a key protein source in livestock feed [2,3], its cultivation area has more than trebled in Europe since the 1990s, up to approximately 5.7 million ha, with an average annual production of 10.7 million tons (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 for a total ban on the cultivation of GM crops (European Commission, Directive 2015/421/EU) due to a number of concerns, including potential negative impacts on biodiversity, eco-toxicological issues, and the evolution of glyphosate-resistant super-weeds [4–6].

The last point, in particular, is currently one of the major challenges in soybean cultivation, especially in countries where the cultivation of GM soybean is banned. Attempts to increase or even maintain soybean yields face severe threats due to the rapid establishment and spread of super-weeds, such as common ragweed (*Ambrosia artemisiifolia* L., subsequently referred to as "ragweed"). The spread of ragweed has been promoted by a combination of three factors: (1) the steady extension of the cultivation area of soybean, (2) climate change (warmer and drier), and (3) a lack of efficacy in management options to effectively contain the spread of the plant [7–9]. In recent years, this annual herbaceous species, native to North America, has become not only an economically important and troublesome agronomic weed in Central Europe, but is also one of the most dominant inducers of pollen allergy [10–12]. Although it was first observed in Europe in the mid-19th century, its main naturalization and establishment has been accelerated since the beginning of the new millennium, particularly through changing climatic conditions related to climate warming [9,13].

As a thermophilic plant, the beginning of ragweed field emergence in Central Europe can be expected when the temperature in the upper soil (0–5 cm) is constantly above 10 °C [14,15]. Thus, small crops sown in late spring with wider row spacing (i.e., soybean) are particularly threatened by the presence of ragweed [7,12]. Recently, studies on the yield effect of ragweed on soybean were undertaken in the United States and showed that the presence of one plant per 110 square feet (10.2 m^2) reduced the soybean yield by 50% [16,17], which was mainly related to the influence of shading. However, several studies on the allelopathic effects of ragweed revealed that it produces and releases several types of secondary metabolites, including the sesquiterpene ambrosic acid, phenols, thiarubrines, and thiopenes. Most of these compounds have a broad spectrum of biological activity, and potentially inhibit germination and growth of plants and microorganisms [18–20]. As shown by [21], ragweed residues in soil significantly affect seed emergence and seedling growth of wheat and tomato. As revealed by [22], the presence of ragweed inhibits germination and growth of red clover due to an accumulation of dead ragweed biomass. Laboratory trials have indicated that the presence of an aqueous extract of aboveground and belowground dry matter of ragweed reduces germination rates of maize [23]. Methanol and hexane extracts from aboveground organs of ragweed lead to lower germination rates in cress, radish, and red clover [24]. However, to the best of our knowledge, no studies have focused on ragweed interference with soybean root nodulation, although previous studies with weedy species, such as *Centaurea diffusa* and *Arabidopsis thaliana*, or aromatic plants such as *Eucalyptus globulus ssp. bicostata*, revealed that soybean seedlings exposed to weeds exhibited a reduction in the number of nodules per plant. This phenomenon may be related to a decrease in the content of flavonoids, which served as the main signaling molecules to symbiotic microbes, initiating the infection process [25–27].

It must be assumed that the importance of ragweed as an agricultural weed, particularly in soybean cultivation, will further increase. Therefore, the aim of this study was to increase our knowledge of the interaction between soybean and ragweed at different densities, which is essential to quantify the effects of intra- and interspecific competition upon (1) growth (aboveground/belowground) of soybean and ragweed, and (2) the yield of soybean. In addition, we observed whether the presence of ragweed has an effect on the infection potential of soybean with rhizobial bacteria.

2. Materials and Methods

2.1. Greenhouse Trial

To test the influence of different densities of common ragweed on the growth and yield of soybean under controlled conditions, a one-season greenhouse trial was set up in the experimental greenhouse of the University of Natural Resources and Life Sciences (BOKU) in Tulln. Because we wanted to check if results vary among cultivars, two different 00-soybean cultivars (Saatbau Linz) with different growing behaviors were used. Whereas cv. Albenga is characterized by a fast juvenile development and medium growth height, cv. ES Mentor shows bushier growth, and therefore has greater stability. The plants were grown in plastic pots (18 × 18 × 25 cm) filled with a sterilized mixture of arable soil from the Groß-Enzersdorf experimental farm, 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 at a ratio of 3:2:1 to avoid soil compaction in the pots. Before seeding, soybean was inoculated with *Bradyrhizobium japonicum* (RWA Raiffeisen Ware Austria), a nitrogen-fixing bacterial species that forms root nodules specifically on soybean roots but is not sufficiently abundant in Central European soils, to ensure a successful symbiosis between soybean and the bacteria.

In each pot, one soybean was planted together with 0, 1, 3, or 5 ragweed plants (treatment labeling: R0, R1, R3, and R5) in four replications. In addition, pots with pure stands of ragweed at densities of 1, 3, and 5 plants in four replications were established to test for intraspecific competition of ragweed [13].

2.2. Field Trial

The two yearlong field experiment was conducted on the Groß-Enzersdorf experimental farm of BOKU during the vegetation periods of 2017 and 2018. The experimental farm is located in the Marchfeld Plain, which is part of the Pannonian Basin in Eastern Austria. This region is characterized by a semi-arid Pannonian climate, with hot and intermittently dry summer periods. The mean annual temperature is 10.7 °C, the mean annual precipitation is 543 mm (1983–2012), and, on average, 1900 h per annum of sunshine are measured (APCC, 2014). In the soybean growing period of 2017 (cf. Table 1), an average temperature of 20.4 °C and a precipitation sum of 183.1 mm were recorded. In the growing period 2018, the average temperature was 21.3 °C and 305.6 mm of rainfall was measured (data from the meteorological station on the experimental farm Groß-Enzersdorf of BOKU). To avoid drought stress, all plots were irrigated throughout the growing season until an available field capacity of approx. 20–25 Vol.% was reached. Therefore, we measured the volumetric soil humidity twice per week using a permittivity sensor (“WET-2”, Delta-T Devices). Furthermore, plots were kept free from spontaneously emerging weeds through a weekly manual weed removal. As N fertilization can have a negative impact on the soybean–rhizobia mutualism, and therefore on the nitrogen-fixing efficacy [28–30], the plants were not fertilized.

Table 1. Codes for sampling, sowing, and harvest dates (DAS = date after seeding), and the BBCH stage/range of the examined soybean plants.

Sampling Dates	Greenhouse Trial 2018	DAS	BBCH	Field Trial 2017	DAS	BBCH	Field Trial 2018	DAS	BBCH
Seeding	13 February	21	16–18	23 May			17 May		
T1	6 March	39	21–29	12 June	20	21–29	6 June	20	15–25
T2	14 April	60	51–65	3 July	41	51–55	26 June	40	25–29
T3	4 May	80	69–79	27 July	65	59–65	20 July	64	51–59
T4	1 June	108	96–99	21 August	90	69–79	16 August	91	65–79
T5				22 September	122	93–99	18 September	124	96–99
					runner bean:		19 October	155	89–97

As with the greenhouse trial, we wanted to observe the individual influence of ragweed on soybean growth, yield, and nodulation. Thus, only one soybean plant per 1 m^2 plot was sown, with different densities of ragweed (0, 1, 3, or 5 plants; treatment labeling: R0, R1, R3, and R5), in four replicates to specifically dissect the impact of ragweed on soybean plants. The aim of this experimental design of exemplary nature was to exclude possible biochemical and growth-related interactions between soybean plants, hence concealing the direct impact of ragweed on soybean growth and nodulation. Furthermore, pure stands of ragweed at densities of 1, 3, and 5 plants in four replicates were established to test for the intraspecific competition of ragweed [13].

In 2017, we used the same trial setup with the cultivars Albenga and ES Mentor, as in the greenhouse trial. Due to the results that we gained in the greenhouse and in the first field trial, in 2018 the same trial setup was used, but instead of ES Mentor another legume species (scarlet runner bean (*Phaseolus coccineus*)) was included in the trial to check whether the results obtained with soybean were species specific, or could be generalized to another, more competitive legume species. As the shoots of runner bean have a vertical growing behavior, we used bamboo sticks of 120 cm length as supports for the tendrils.

Before sowing, soybeans were inoculated with the same *Bradyrhizobium japonicum* product as used in the greenhouse trial. As runner bean was introduced as a crop to Europe after the discovery of the Americas, it has a long tradition in European, and particularly Austrian, agriculture. Although commercial inoculants are available, recent common practice has not demanded inoculation, because rhizobacteria strains which form a symbiosis with runner bean are sufficiently abundant in Austrian soils [31].

2.3. Measurements

In greenhouse and field trials, five samplings (T1–T5) were performed throughout the vegetation period from complete pots or plots, respectively (Table 1), to measure the growth height, aboveground dry matter (AGDM), and belowground dry matter of soybean, runner bean, and ragweed, in addition to the number and weight of nodules on the legume roots. The growth rate, based on the AGDM, was calculated according to [32]. The root mass and number of nodules were sampled from T3 and T4 to allow for a stable establishment of the symbiosis, and to avoid possible losses due to degradation processes towards maturation. For carving out the soil in the field trials, we used standardized metal frames (20 × 20 × 20 cm). The roots in the soil samples were washed out manually. Nodules were counted, removed from the soybean roots, dried at 40 °C for 24 h, and weighed. The roots of the soybean and ragweed were separated, dried at 105 °C for 24 h, and weighed. The same drying conditions were used for the aboveground dry matter. The runner bean was harvested 31 days later than soybean since the seeds need frost to mature. At final harvest, the AGDM of soybean was separated into straw and grain, in order to determine the soybean grain yield.

2.4. Statistical Analysis

Data analysis was performed using software R, Version 3.4.4 (R Core Development Team, 2018). For the graphical visualization of the results, we used the R software and SigmaPlot, Version 14.0 (Systat Software, 2018). Analysis of variance with subsequent multiple comparisons of means according to Tukey were performed (significance level $\alpha = 0.05$). Shapiro Wilk's test was used to test the normal distribution of data, and Levene's test was used to check homogeneity of variances. If a normal distribution was not found, a Kruskal–Wallis ANOVA on ranks was performed. If homogeneity of variances was not found, a statistical analysis was executed using Welsh's unequal variances t-test. Because ANOVA only checks if there are differences due to the factors and does not show how the results could be best explained with the given variables, generalized linear mixed models (GLMMs) were constructed to find the best model to explain soybean yield and other yield-influencing parameters. Prior to this, data exploration analysis (collinearity, outlier detection, and dispersion of response variables) was executed following [33]. As collinearity of explanatory variables can lead to incorrect parameter estimation, predictors with r ≥ 0.5 were excluded from analysis in the same model. In this case, variables with higher single explanatory power were chosen for calculating the models. Models were selected by comparing the second order Akaike Information Criterion value (AICc), corrected for small sample sizes. To identify the most parsimonious model based on the lowest AICc value, we computed the AIC differences (ΔAICc) between the different candidate models [34]. As a rough rule, [35] proposed that models for which ΔAICc ≥ 2 receive substantial support, because the chance of a smaller AICc value being correct is approximately 73%.

3. Results

As the main focus of this study was an investigation of the effects of ragweed on soybean, the results focus on these research questions. Data on the runner bean are summarized in brief at the end of this section.

3.1. Growth Performance of Soybean

3.1.1. Greenhouse Trial

In the greenhouse, the height of the ragweed plants did not exceed the canopy height of either soybean cultivar. Nevertheless, the growth height of cv. Albenga was decreased by 11% on average with five ragweed plants in the pot (R5). Cv. Mentor showed a significant mean reduction in height of 26% in the R5 pots. The negative impact of ragweed density on the growth height was accompanied by a significant reduction in biomass production. In early developmental stages, significant differences in the aboveground dry matter (AGDM), particularly between the control plants grown without ragweed (R0) and the soybean plants grown at the highest ragweed density of five plants per pot (R5), were detected. Both cultivars were unable to compensate for this initial disadvantage prior to harvest. On harvest date (T5), the lowest biomass was observed with cv. Albenga in the R5 pots, at 51% less AGDM than the control plants. The lowest AGDM of cv. ES Mentor was measured in the R3 pots, where the ragweed biomass was 33% higher than in the R5 pots (Figure 1, Table S1 Supplementary Material).

Correspondingly, the soybean growth rate also decreased with increasing ragweed density. In the greenhouse trial, Albenga plants grown without ragweed competition showed the highest growth rate of 0.5 g/d between T3 and T4, which was a 90% or 87% higher growth rate than for plants grown with three or five ragweed plants per pot, respectively. In contrast, the highest growth rate of 0.6 g/d for ES Mentor was observed between T2 and T3 in the pots with highest ragweed density (R5). However, these plants were not able to maintain this; thus, the growth rate decreased sharply down to 0.02 g/d between T3 and T4 (60 to 80 days after seeding), whereas plants grown without ragweed competition maintained and further increased their growth rate during this time span. Consequently, at 0.5 g/d, the growth rate of R0 plants was 96% higher than that of R5 at T4 (Table 2).

Table 2. Growth rate of soybean and ragweed (g/d; mean ± standard deviation) throughout the growing period (T1–T5), dependent on the ragweed density (0, 1, 3, 5 plants: R0, R1, R3, and R5) and cultivar; n = 4; significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Soybean				**Ragweed**		
	T1–T2	**T2–T3**	**T3–T4**	**T4–T5**	**T2–T3**	**T3–T4**	**T4–T5**
Greenhouse trial Cultivar Albenga							
R0	0.21 ± 0.01	0.47 ± 0.13	0.02 ± 0.20	0.01 ± 0.01	n.a.	n.a.	n.a.
R1	0.18 ± 0.02	0.31 ± 0.14	0.07 ± 0.81	0.04 ± 0.34	0.02 ± 0.01	0.07 ± 0.03	−0.01 ± 0.16
R3	0.17 ± 0.02	−0.04 ± 0.05	0.10 ± 0.18	0.15 ± 0.16	0.01 ± 0.00	0.05 ± 0.00	−0.01 ± 0.01
R5	0.14 ± 0.03	0.06 ± 0.04	0.01 ± 0.06	0.12 ± 0.07	0.01 ± 0.00	0.02 ± 0.00	−0.00 ± 0.02

Table 2. *Cont.*

	Soybean				Ragweed		
	T1–T2	**T2–T3**	**T3–T4**	**T4–T5**	**T2–T3**	**T3–T4**	**T4–T5**
Cultivar Mentor							
R0	0.23 ± 0.05	0.51 ± 0.06	0.54 ± 0.25	0.01 ± 0.06	n.a.	n.a.	n.a.
R1	0.20 ± 0.02	0.60 ± 0.07	0.28 ± 0.51	−0.05 ± 0.14	0.01 ± 0.01	0.04 ± 0.00	−0.05 ± 0.03
R3	0.13 ± 0.03	0.49 ± 0.05	0.03 ± 0.32	−0.00 ± 0.04	0.01 ± 0.00	0.04 ± 0.00	0.00 ± 0.03
R5	0.09 ± 0.06	0.61 ± 0.04	0.02 ± 0.57	0.21 ± 0.24	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.01
R	***	***	n.s.	n.s.	***	n.s.	n.s.
V	***	**	n.s.	n.s.	***	n.s.	n.s.
R * V	*	***	n.s.	n.s.	n.s.	n.s.	n.s.
Field trial 2017 Cultivar Albenga							
R0	0.45 ± 0.24	0.09 ± 0.20	0.25 ± 0.60	0.12 ± 0.53	n.a.	n.a.	n.a.
R1	0.40 ± 0.21	0.25 ± 0.80	0.15 ± 1.07	−0.10 ± 0.38	2.79 ± 4.01	1.55 ± 2.11	0.34 ± 0.66
R3	0.42 ± 0.10	0.32 ± 0.38	−0.53 ± 0.50	0.05 ± 0.02	1.13 ± 1.42	0.56 ± 0.64	−0.16 ± 1.10
R5	0.23 ± 0.27	−0.01 ± 0.15	0.15 ± 0.23	−0.11 ± 0.19	0.40 ± 0.47	1.93 ± 2.46	−0.13 ± 0.18
Cultivar Mentor							
R0	0.31 ± 0.05	0.78 ± 0.17	0.38 ± 0.15	0.13± 0.28	n.a.	n.a.	n.a.
R1	0.17 ± 0.13	0.65 ± 0.47	0.12 ± 0.18	0.05 ± 0.24	3.26 ± 1.84	1.65 ± 1.74	2.11 ± 0.93
R3	0.22 ± 0.14	0.45 ± 0.26	0.01 ± 0.20	−0.06 ± 0.32	1.30 ± 1.01	1.05 ± 0.79	0.47 ± 1.26
R5	0.18 ± 0.16	0.23 ± 0.20	0.07 ± 0.30	0.03 ± 0.16	1.00 ± 1.52	0.79 ± 0.69	0.24 ± 1.42
R	*	**	n.s.	n.s.	n.s.	n.s.	n.s.
V	*	***	n.s.	n.s.	**	n.s.	n.s.
R * V	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Field trial 2018 Cultivar Albenga							
R0	0.32 ± 0.05	0.43 ± 0.5	0.30 ± 0.12	0.12 ± 0.20	n.a.	n.a.	n.a.
R1	0.28 ± 0.06	0.45 ± 0.37	0.18 ± 0.13	−0.03 ± 0.19	4.17 ± 2.90	1.84 ± 2.29	2.88 ± 4.29
R3	0.22 ± 0.06	0.38 ± 0.17	−0.05 ± 0.03	−0.01 ± 0.16	2.86 ± 1.13	1.33 ± 0.61	0.14 ± 0.85
R5	0.14 ± 0.10	0.11 ± 0.14	−0.03 ± 0.04	−0.04 ± 0.10	2.15 ± 1.81	2.55 ± 1.87	0.37 ± 1.60
R	**	***	**	n.s.	*	n.s.	n.s.

3.1.2. Field Trial

In contrast to the growth performance in the greenhouse trial, in the field ragweed succeeded in overtopping the soybean canopy of both cultivars within 90 days (T4) (Figure S1 Supplementary Material). The maximum growth height of cv. Albenga (mean ± stdv; 2017: 55 ± 12 cm; 2018: 70 ± 14) and of cv. ES Mentor (2017: 64 ± 7 cm) was observed at final harvest (T5) in the plots without ragweed competition. When five ragweed plants per plot were present, Albenga showed a mean decrease in height of 55% (2017) and 22% (2018), whereas cv. ES Mentor produced 18% shorter plants in 2017. The influence of ragweed on the AGDM of soybean became even more obvious in the field trials. The negative impact of ragweed became apparent in the early development stages of soybean.

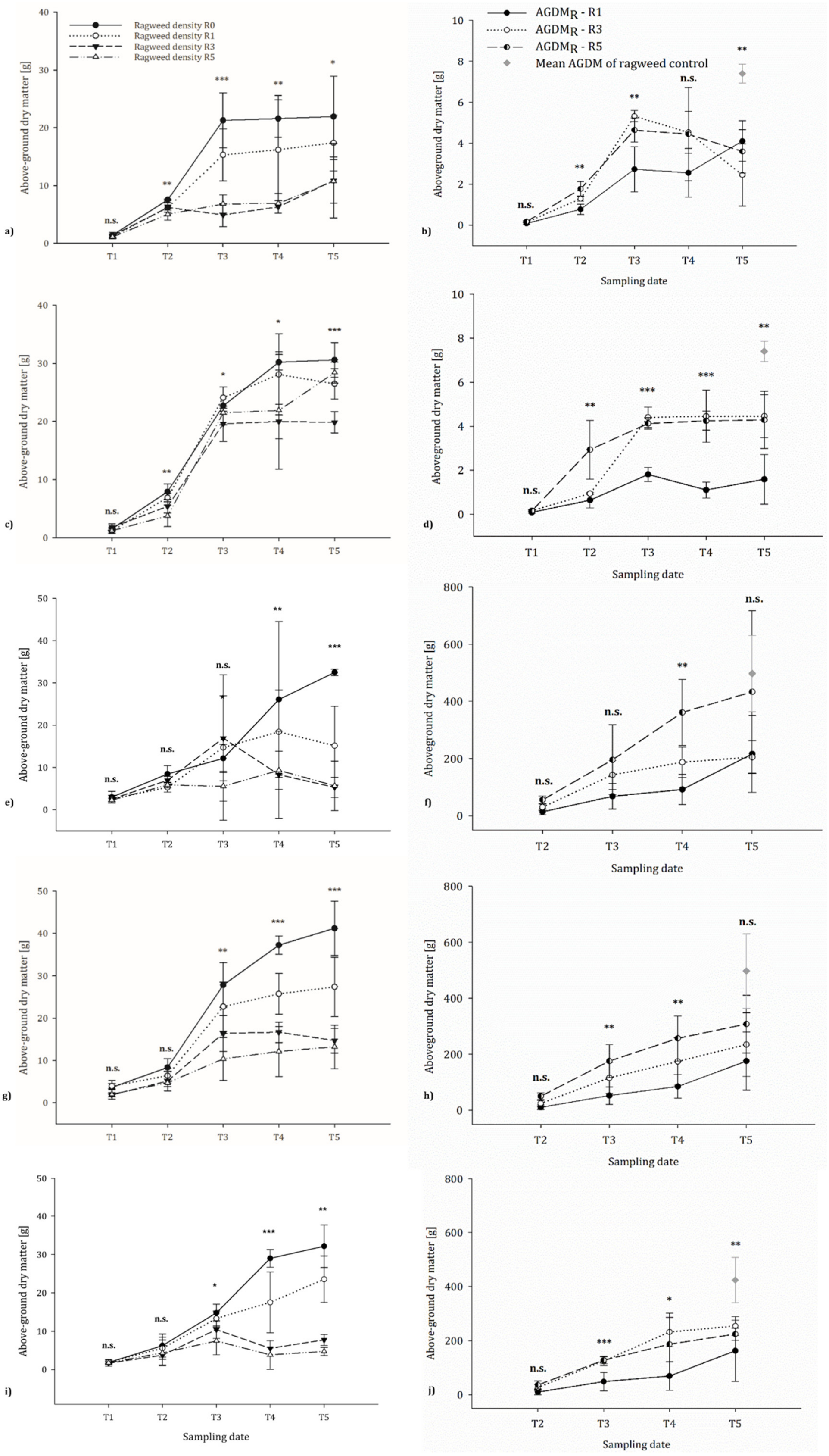

Figure 1. Above-ground dry matter (g) of soybean (left) and ragweed (right) of (**a**,**b**) Albenga (greenhouse trial, 2018), (**c**,**d**) ES Mentor (greenhouse trial, 2018), (**e**,**f**) Albenga (field trial, 2017), (**g**,**h**) ES Mentor (field trial, 2017), (**i**,**j**) Albenga (field trial, 2018), in the course of the trial period, with dependency on the ragweed density (0, 1, 3, 5 plants: R0, R1, R3, R5; n = 4; significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

On sampling date T3, which was 65 days after seeding, the biomass of cv. ES Mentor grown in the R5 plots was reduced by 45% compared to that of the control plants. Cv. Albenga was even more susceptible to the presence of ragweed. At T3, a significant reduction in biomass of 50% was observed in the year 2018 in the R5 plots (Figure 1). The negative impact of ragweed increased until harvest with both cultivars. A significant reduction in the AGDM of Albenga was observed in the R3 plots (2017: −84%; 2018: −76%) and the R5 plots (2017 and 2018: −83%). With ES Mentor only, one ragweed plant was sufficient to clearly reduce biomass production by 34%, and ES Mentor plants grown in the R5 plots showed a reduction in biomass of 68% on average (Table S1 Supplementary Material). Accordingly, in the field trials, the presence of ragweed had a severe impact on the growth rate of both cultivars until T3 (Table 2). In 2017, cv. Albenga showed the highest mean growth performance of 0.5 g/d between T1 and T2 in the R0 plots, which accounted for +49% compared to the R5 plants. In this time span, the growth rate of cv. ES Mentor in the R0 plots was 31% lower than that of cv. Albenga, but increased sharply to 0.8 g/d between T2 and T3. In contrast, with a growth rate of 0.2 g/d, the ES Mentor plants in the R5 plots showed a 71% lower growth rate during this time. Between T3 and T4, the growth rate was 81% higher than that of the R5 plants. In 2018, the highest growth rate of cv. Albenga, which again was 0.5 g/d, was found in the R1 plots between T2 and T3, and was significantly higher (+76%) than those of the R5 plants, with a growth rate of 0.1 g/d.

3.2. Growth Performance of Ragweed

3.2.1. Greenhouse Trial

Despite the fact that the presence of ragweed had substantial negative effects on the biomass production and growth rate of soybean, particularly in the greenhouse trial, the performance of ragweed compared to that of soybean was relatively poor. The mean growth rate of ragweed (mean of all densities) was clearly below that of both soybean cultivars (Table 2). Consequently, the ragweed AGDM ($AGDM_R$) was clearly lower than those of soybean, even in the pots with five ragweed plants (Figure 1, Table S1 Supplementary Material).

3.2.2. Field Trials

However, in the field trials, the results were significantly different. In combination with cv. Albenga, the highest growth rate of 4 g/d of ragweed was found in the R1 plots between T2 and T3 (40 to 64 days after seeding), in 2018 (Table 2). A similar result was observed with cv. ES Mentor, where ragweed showed the highest growth rate of 3 g/d in the R1 plots between T2 and T3. Throughout the vegetation period and across all densities, the growth rates of ragweed were 13-fold higher than those of cv. Albenga and 18-fold higher than those of cv. ES Mentor. Nevertheless, we found evidence of interspecific competition between soybean and ragweed. At the plot level, the highest mean $AGDM_R$ values of 424 g m^{-2} (2017) and 303 g m^{-2} (2018) were found in the plots with a single ragweed plant, and without soybean (Figure 1). In competition with cv. Albenga, the $AGDM_R$ per plot was reduced by 48% (mean of both years). The presence of ES Mentor reduced the $AGDM_R$ by 42%. Additionally, we found clear evidence for intraspecific competition of ragweed at the plant level. In the R3 control plots without soybean intraspecific competition, we observed a decrease in the average $AGDM_R$ per plant of 68%. The presence of cv. Albenga in the R3 plots increased this biomass loss of the single ragweed plants to 77%. When ES Mentor was present, the reduction effect on the $AGDM_R$ per plant was 73% in the R3 plots. In the R5 control plots without soybean, intraspecific competition caused a decrease in the average $AGDM_R$ per plant of 84% compared to R1. In these high-density plots, both soybean cultivars failed to show any further competitive effect on the average individual plant weight of ragweed.

3.3. *Yield of Soybean*

3.3.1. Greenhouse Trial

Ragweed density and the soybean cultivar had a significant impact on individual plant yield. The highest yield of 15 g was observed with cv. ES Mentor in the R0 pots, whereas cv. Albenga showed the lowest average yield of 3 g in the R5 pots. The yield of both cultivars was not significantly affected by the presence of one ragweed plant; whereas the yield of Albenga did not drop severely in the R3 pots, the yield of ES Mentor decreased significantly in competition with three ragweed plants. The main difference between the cultivars was observed in the R5 pots. Due to intraspecific competition between the ragweed plants in the pots and a stronger interspecific competition due to its bushy growth, the yield of ES Mentor slightly increased from 7.9 g in the R3 pots to 10 g in the R5 pots, whereas the yield of Albenga further decreased (Figure 2, Table S1 Supplementary Material).

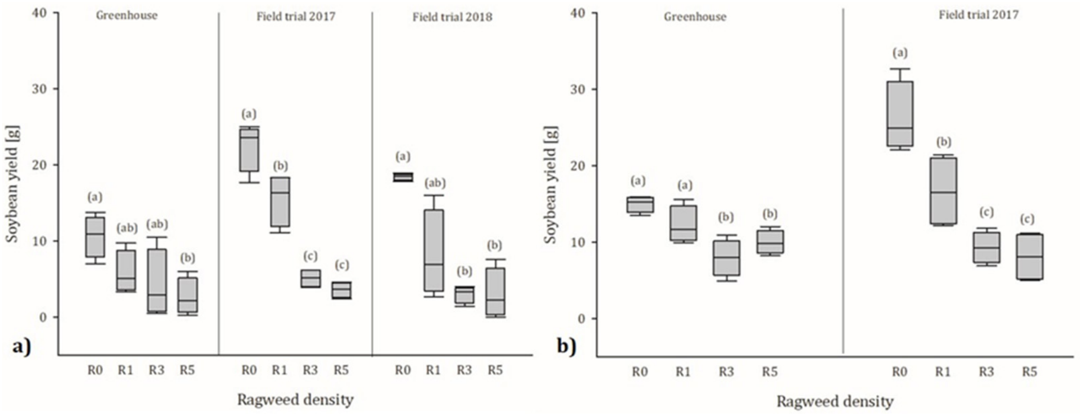

Figure 2. Soybean grain yield per plant, dependent on the ragweed density (0, 1, 3, and 5 plants: R0, R1, R3, and R5) of (**a**) cultivar Albenga and (**b**) cultivar ES Mentor, separated by trial (n = 4; different letters indicate significant differences).

3.3.2. Field Trials

In the field trials, the factors of ragweed density and cultivar had a significant influence on soybean yield (Figure 2, Table S1 Supplementary Material). The highest average yield of 26 g was observed with cv. ES Mentor in 2018, in the plots with no ragweed (R0). The lowest mean yield of 3 g was obtained by cv. Albenga in 2017 in the plots with five ragweed plants (R5). Just one ragweed plant per square meter caused an average yield loss of 44% with cv. Albenga (2017: 56%; 2018: 31%). The yield of ES Mentor was reduced by 36% when one single ragweed plant was present. With three ragweed plants per plot, the yield decreased sharply by 80% with Albenga and 65% with ES Mentor. For both cultivars, yields at R3 did not differ significantly from the yields that were obtained at the highest ragweed density level of five plants per plot. Regression analysis further revealed that not only the number of plants, but particularly an increasing amount of ragweed biomass, was responsible for the strong decrease in the yield of both soybean cultivars (overall $R^2 = 0.7$; $p < 0.001$, data not shown). This was supported by generalized linear mixed model analysis, which showed that soybean yield was tightly connected to the ragweed biomass present, in addition to the number and weight of rhizobia nodules (Table 3).

Table 3. AICc values for model selection of dependent variables; number of estimated explanatory parameters and parameter combinations = 16 based on field trial data (AICc = second order Akaike Information Criterion, ΔAICc = difference between the AICc and that of the next most parsimonious model, R^2m = marginal R^2, R^2c = conditional R^2).

Parameter	Best Explanatory Model	AICc	ΔAICc	R^2m (1)	R^2c (2)
Soybean dry matter	Null model: trial	616.5	–		
	ragweed dry matter × mean weight of nodules	524.4	0.0	0.5	0.9
	ragweed dry matter + mean weight of nodules	527.3	2.9		
Soybean yield	Null model: trial	540.7	–		
	ragweed dry matter × mean weight of nodules	446.2	0.0	0.6	0.7
	ragweed dry matter + mean weight of nodules	452.7	6.5		

(1) the proportion of variance explained by the fixed factors only; (2) the proportion of variance explained by fixed and random factors.

3.4. Nodules

Although there was a severe reduction in the aboveground biomass, we did not find any significant decreases in soybean root mass. However, particularly in the field trials, a mean decrease in soybean root biomass of 56% was observed between the R0 and the R5 variant, on average, for both years and cultivars, although the variation between replicates was large.

We detected a clear influence of the ragweed density on the number of nodules found on the soybean roots (Figure 3, Table S1 Supplementary Material). Over all trials and growing seasons, the cultivars did not differ significantly in their number of nodules, but the highest mean number of 122 ± 11 nodules was found on roots of cv. ES Mentor, without any ragweed present. In the R0 variant of cv. Albenga, 94 ± 24 nodules were counted on average. Only one ragweed plant per plot/pot was sufficient to reduce the number of nodules of cv. Mentor by 58%. With a loss of 36% of nodules, cv. Albenga was slightly more tolerant against one ragweed plant, but this advantage disappeared with increasing ragweed density. With five plants per plot/pot, the number of nodules on Albenga roots was reduced by 87% (mean: 12 nodules ± 7), whereas cv. Mentor showed a slightly lower reduction of 73% fewer nodules (33 ± 13) in the R5 variant compared to R0. Although we found significant linear correlations between the number of nodules and the yield (Mentor: $r = 0.6$, $p < 0.001$; Albenga: $r = 0.6$, $p < 0.001$), and the ragweed root dry matter and the number of nodules (ES Mentor: $r = -0.6$; Albenga: $r = -0.6$, $p < 0.001$), generalized linear mixed model (GLMM) analysis revealed that the most parsimonious model explaining the yield of soybean was the interaction of the factors of mean weight of nodules and the amount of ragweed AGDM present (Table 3). Summarizing all trials, the highest mean weight of nodules of 12 ± 1 mg was observed with cv. ES Mentor, and of 9 ± 4 mg with cv. Albenga, in the R0 variant. At ragweed densities of five plants per plot/pot, the mean weight of nodules decreased by approx. 69% with both cultivars (Figure 4).

Particularly the relative soybean yield increased significantly with increasing mean weight of nodules, and was clearly negatively affected by increasing ragweed AGDM, irrespective of the trial year, greenhouse or field trial type, and cultivar (Figure 5). In the greenhouse trials, the threshold above which the yield was higher than the average was at a mean weight of nodules of 7 mg, and approx. 3 g of ragweed dry matter or less. In the field trials, these threshold values were 7 mg mean nodule weight and approx. 150 g of ragweed dry matter in 2017. Plants in 2018 were more susceptible to ragweed; 100 g of ragweed dry matter per square meter was enough to push the yield below the average yield. Furthermore, the threshold for the mean weight of nodules to produce an above-average yield was 10 mg.

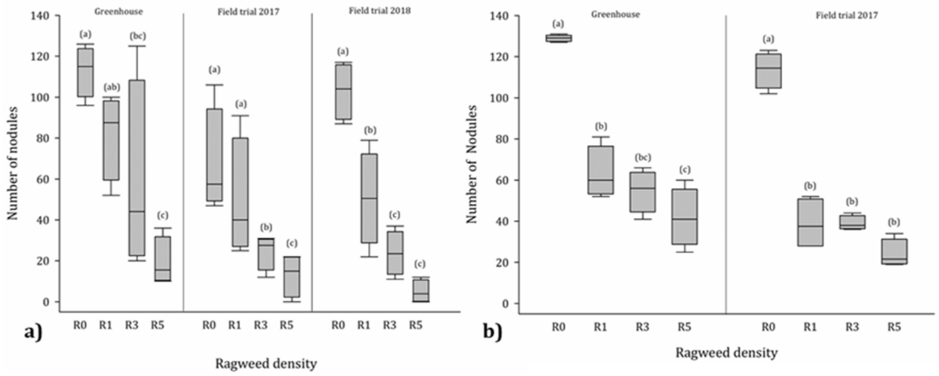

Figure 3. Number of nodules dependent on the ragweed density (0, 1, 3, and 5 plants: R0, R1, R3, and R5) of (**a**) cultivar Albenga and (**b**) cultivar ES Mentor, separated by trial (n = 4; different letters indicate significant differences).

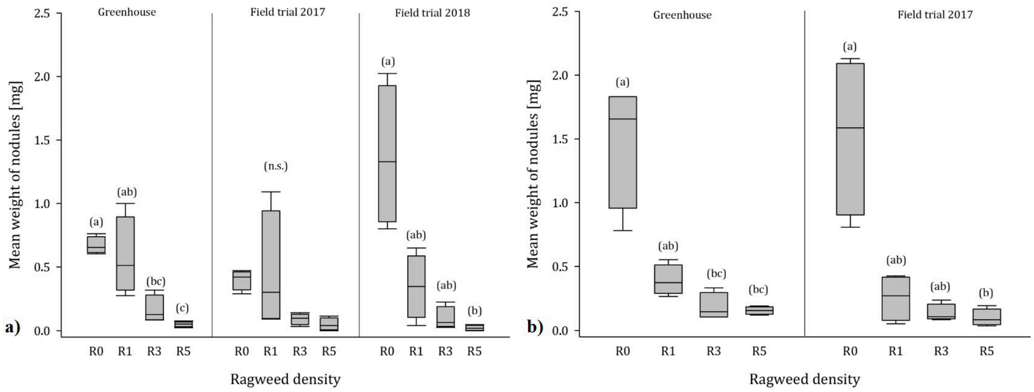

Figure 4. Mean weight of nodules [mg dry matter] dependent on ragweed density (0, 1, 3, and 5 plants: R0, R1, R3, and R5) of (**a**) cultivar Albenga and (**b**) cultivar ES Mentor, separated by trial (n = 4; different letters indicate significant differences).

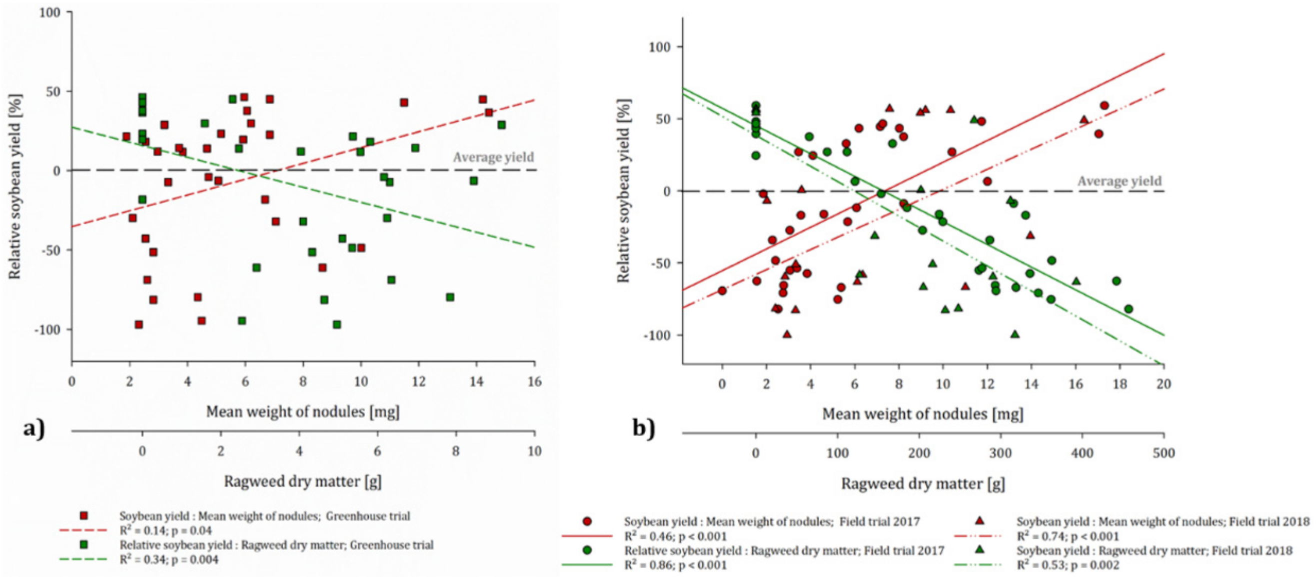

Figure 5. Regressions between the relative soybean yield in (**a**) greenhouse trials and (**b**) field trials (2017 and 2018), the mean weight of nodules (upper *x*-axis), and the amount of ragweed above ground dry matter present (lower *x*-axis; regression analysis, greenhouse trial: n = 32, field trial 2017: n = 32, field trial 2018: n = 16).

3.5. Growth and Yield of Runner Bean

In contrast to soybean, the growth height of the tendrilled runner bean in 2018 was on average 79% higher than that of ragweed, irrespective of the ragweed density. At harvest, the shoots of runner bean grown without ragweed reached a length of 293 ± 40 cm, which was 26% longer than the climbers of runner bean grown together with five ragweed plants. The maximum length of 70 ± 22 cm of ragweed plants was observed in the R5 plots. Although the foliage of the ragweed did not overtop the canopy of runner bean, we found a significant reduction in the AGDM of runner bean dependent on the ragweed density. At final harvest, runner bean plants that were competing with five ragweed plants produced 70% less AGDM than the R0 plants. Thus, for runner bean, severe grain yield losses due to the presence of ragweed were observed (Figure S2 Supplementary Material). Just one ragweed plant per plot was enough to reduce the yield of runner bean by 58%. The highest ragweed density of five plants per plot yielded a reduction of 74%. As with soybean, we found a significant impact of ragweed on the number of nodules (Figure S3 Supplementary Material). On roots grown without ragweed competition (R0), on average, 107 ± 30 nodules were counted. One ragweed plant per plot caused a reduction of 63% (mean: 39 ± 15 nodules). Five ragweed plants per plot led to a decrease in the number of nodules by 85% (mean: 16 ± 16 nodules). The correlation between the number of nodules and yield that we found for soybean was confirmed by the results of regression analysis for runner bean ($R^2 = 0.9$, $p < 0.001$). Furthermore, we detected a significant decrease in the average weight of the nodules with increasing ragweed density. In the R5 variant, individual nodules were 92% lighter than those of the R0 plants.

4. Discussion

The trial period did not have a strong impact on the results, but nevertheless we found slight discrepancies in the morphological development of the soybean and ragweed plants. Despite lower precipitation levels compared to the growing season in 2018, on 10 July 2017, there was a hail event which caused some morphological damage to the soybean and ragweed plants. Although none of the plants were damaged past the point of recovery, they had to catch up in their growth, which led to a slightly shifted growth performance compared to the soybean and ragweed plants grown in 2018. However, throughout all trials (greenhouse and field trials), we found consistent results and detected not only a severe reduction in soybean growth height, but also in aboveground biomass (AGDM) development, consequently having a negative impact on the yield. A reduction in AGDM was previously measured in the early development stages of the soybean when exposed to high ragweed densities under controlled growing conditions in the greenhouse. This trend was also seen in the growth rate of both cultivars, which was significantly lower at high ragweed densities until T2 (39 days after seeding: DAS).

In contrast, in the field trials, only small effects of ragweed on these growth parameters were observed at the beginning of the season. Until T3 (40 DAS), the soybean plant height, growth rate, and AGDM were similar among ragweed densities. This is in accordance with other field studies that have shown that direct interference, i.e., touching, shading, etc., between soybean and weeds in the early development stages is not necessarily reflected in differences in soybean biomass [36–38]. For example, [39] revealed that weed removal must be undertaken no later than 8 weeks after soybean emergence to avoid significant losses in biomass when soybean was grown with interference by Palmer amaranth (*Amaranthus palermi*). A rare study dealing with soybean interference from ragweed [16] showed that weed removal is essential to prevent yield loss after 4 weeks at the latest.

4.1. Growth Performance of Ragweed

In addition to the interference effect of ragweed on both soybean cultivars, we also observed inter- and intraspecific competition effects for ragweed that led to large differences in growth height and AGDM. This corroborates other studies illustrating ragweed's extremely high morphological plasticity, which allows the majority of plants to complete their lifecycle even in very dense stands [12,40]. Particularly in plots with medium ragweed density (R3), the plants produced on average 2/3 less biomass compared to plots where only one ragweed plant was grown. Soybean had also a reduction effect on $AGDM_R$ of around 50% when grown together with one ragweed plant. However, this growth effect disappeared in plots with higher ragweed densities, which has also been confirmed by various studies on the competition between soybean and weedy species, showing that soybean can withstand low densities of weeds without severe growth and yield reductions [16,41,42].

4.2. Soybean Yield

In the present study, the critical period during which AGDM of both soybean cultivars was significantly affected by ragweed was 5 weeks in the greenhouse and 9 weeks in the field trials. After this period, the reduction in AGDM increased with increasing ragweed density, consequently leading to severe yield losses, particularly for soybean plants growing under high ragweed densities. Over all trials, the average yield of cv. ES Mentor grown together with five ragweed plants was 56.1% lower than that of the soybean plants grown without ragweed. The yield of cv. Albenga was even more reduced, by 80.2% in the presence of five ragweed plants per pot/plot. In this context, refs. [38] and [43] showed that, especially at very high weed densities, interference even at 4–6 weeks after soybean emergence is enough to severely reduce the yield.

Particularly in the field trials, only one ragweed plant per square meter was enough to reduce the yield of both cultivars by approximately one-third. Similar results were obtained by [44], who studied the interference between soybean and giant ragweed (*Ambrosia trifida*), showing that interspecific interference between adjacent weeds and soybean began at relatively low weed densities. Particularly for giant ragweed, the injury threshold of two plants per 9 m of row, which was enough to cause a yield loss of up to 50% in full-season interference, was quite low. Similar results were found by [45] for other weedy species, such as entireleaf morning glory (*Ipomoea hederacea*) and common cocklebur (*Xanthium strumarium*), which are both troublesome weeds for soybean cultivation in the USA. The conclusion reached by [38] was that, for weeds that grow taller than the crop, the main competitive factor is shading. This statement could be only partly confirmed by the present study, because particularly in the field the ragweed overgrew cv. Albenga approx. 9 weeks after sowing—the time from which the AGDM was negatively affected by the presence of ragweed. At T3 (60 DAS), the ragweed plants were 27.0% taller than the Albenga plants grown at the highest ragweed density but were on average 14% smaller than other Albenga plants grown at lower ragweed densities. The same was true for cv. ES Mentor plants in the R5 plots, which were also exceeded in height by the ragweed by 17% at T3, whereas the plants in lower ragweed densities were on average 11% taller than the ragweed.

4.3. Influence of Ragweed on Nodulation

GLMM and regression analyses were performed to identify the best model to explain soybean yield. These analyses showed that not only ragweed dry matter, but also the number of nodules and, in particular, the mean weight of the nodules, had a notable influence not only on the yield, but also yield-influencing parameters, such as the AGDM. In all our experimental environments, the soybean cultivars neither differed in their number of nodules nor in the mean weight of their nodules, but ragweed density had a significant negative impact on both parameters. Five plants per plot/pot reduced the number of nodules of cv. Albenga by 87%, averaged over all experiments. Cv. ES Mentor showed a slightly lower reduction of 73% fewer nodules in the R5 variant. Runner bean growing together with five ragweed plants had 84.7% fewer nodules, which were also 92% lighter than those of the runner bean grown without ragweed competition. As a consequence, similar to soybean, a yield reduction of 74% for the runner bean was observed. Thus, we found clear evidence that the presence of ragweed has a severe impact on growth performance and yield of soybean, mainly by influencing nodulation and nodule growth.

However, studies dealing with the interaction between weeds in general and soybean, and how these weeds affect nodulation, are scarce. One reason might be the complexity of the process, as not only soil properties and chemical interactions, but also biotic and abiotic environmental factors, may have a direct or indirect impact on legume–rhizobia symbiosis [46]. Although the effect of abiotic stressors, such as drought, salinity, or extreme temperatures, on root morphology and related physiological processes can be excluded from the present study, in particular, the factor of light must be paid closer attention. As shown by [27], the red:far red light ratio (R:FR) had a significant impact on root morphology and the chemical composition of root exudates, particularly the hydrogenperoxide (H_2O_2) and the malondialdehyde (MDA) content. Both substances play an important signaling role in the establishment and the functioning of the interaction between legumes and rhizobia [47]. In addition, it can be assumed that competition with neighboring plants can also induce the synthesis of general stress regulators such as phytohormones [48]. Abscisic acid (ABA), in particular, plays a pivotal role in many physiological processes and, therefore, also has an indirect impact on nodulation [49,50]. As shown by [51] and [52], increasing levels of ABA in plant roots had significant negative effects on nodules. Furthermore, it was shown that ABA markedly decreased the production of three isoflavonoid compounds in soybean, which in consequence reduced the attraction of the roots to rhizobial bacteria [53,54]. In this context, the presence of negative allelopathic agents can also have a severe negative impact on rhizobium reproduction, on the "communication" between soybean roots and rhizobia, and on nodule formation [55–57]. Recently, most of the studies dealing with allelopathic effects of weeds on the legume–rhizobia interaction has been executed using residues of weedy species. For example, [46] reported that residues of couch grass (*Elymus repens*) when incorporated into the soil decreased the nodule number, nodule fresh weight, and nitrogen fixation in soybean and bean. The residue of *Carduus nutans* has been shown to affect the nitrogen fixation in *Trifolium repens* [58]. Similar effects were reported by [59] with nettled-leaved goosefoot (*Chenopodium murale*) and chickpeas. In soil amended with weed residues, not only the number of nodules, but also their weight was significantly reduced, mainly by four phenolic acids, viz. protocatechuic, ferulic, *p*-coumaric, and syringic acid. All these phytotoxins are reported to inhibit growth, chlorophyll content, and biomass accumulation [60,61].

In the present study, we found clear evidence that the presence of ragweed has a severe impact on the growth performance and yield of soybean, mainly by influencing the nodulation behavior of soybean and/or rhizobia. Thus, in an ongoing study on soybean and ragweed interactions, aqueous extracts of aboveground and belowground biomass, in addition to the essential oil of ragweed, were prepared and analyzed. In total, we found more than 60 chemical compounds in the plants, i.e., very high concentrations of the sesquiterpene Germacrene-D, which is known for its antimicrobial properties, and α-caryophyllene, which is highly reactive with various other molecules such as reactive oxygen species (ROS), which thrive in the successful interaction between plant roots and rhizobia [21,24].

However, regarding the quantification of the impact of allelopathy on the soybean–ragweed interference, either independently or synergistically with other competitive factors such as light and/or biotic and abiotic factors, a large gap in knowledge remains. Due to the complexity of the symbiotic process, the detection of the major allelochemicals of ragweed is essential. In a further step, mechanistic studies on the impact of these allelochemicals on the root growth of soybean are highly recommended to obtain a better understanding of the interaction processes between these allelochemicals and root growth, and how this interference directly and/or indirectly affects nodulation.

5. Conclusions

In the present study, we showed that an infestation with ragweed can have a severe impact on the growth performance and yield of soybean. However, it must be taken into account that we used an experimental design of exemplary nature to exclude possible intraspecific interactions of soybean, to specifically dissect the impact of ragweed on soybean plants. Thus, it can be assumed that the effects presented in the study will be somewhat defused in practical field stands, because soybean density also affects crop performance and has a competitive effect on ragweed. Nevertheless, we found clear evidence that the presence of ragweed has no significant effect on the root growth of soybean, but substantially impairs the nodulation performance of rhizobia, which will have a significant impact on the vegetative growth and yield of soybean and other legumes. Due to changing climatic conditions, limited management options, and the high plasticity of the plant, it can be assumed that the spread of ragweed will further increase. Thus, it is important to understand the mechanisms behind the interaction between ragweed and crops such as soybean, in order to reveal new management options. In this context, allelopathic interference represents a broad field of research, because little is known about the chemical properties of the plant and how it affects crops by changing growth patterns, morphological traits, and/or plant-microbe symbiosis.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11081616/s1, Figure S1. Growth height (cm) of soybean and ragweed from (a) Albenga (greenhouse trial), (b) ES Mentor (greenhouse trial) (c) Albenga (mean result of field trials 2017 and 2018), (d) ES Mentor (field trial, 2017) in the course of the trial period, dependent on the ragweed density (0, 1, 3, 5 plants: R0, R1, R3, R5; n = 4; significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Table S1. Results of a two-way ANOVA for yield and other growth parameters measured at harvest of soybean, dependent on ragweed density (R) and cultivar; V1 = Albenga, V2 = ES Mentor; AGDM = aboveground dry matter; BGDM = belowground dry matter; n = 4; significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; interactions between main factors are not visualized because no significances were detected. Figure S2. Runner bean grain yield per plant, dependent on ragweed density (0, 1, 3, and 5 plants: R0, R1, R3, and R5; n = 4; different letters indicate significant differences). Figure S3. (a) number of nodules and (b) mean weight of nodules [mg] on runner bean, dependent on ragweed density (0, 1, 3, and 5 plants: R0, R1, R3, and R5; n = 4; different letters indicate significant differences).

Author Contributions: Conceptualization, R.M.H. and H.-P.K., Methodology, R.M.H. and H.-P.K., Formal analysis, R.M.H., B.U., Monitoring, R.M.H., B.U., A.W., R.C., Data analysis, R.M.H., A.W., R.C., Writing—original draft preparation, R.M.H., B.U., Writing—review and editing, G.K., H.W., H.-P.K., Visualization, R.M.H., Supervision, H.W., G.K., H.-P.K., Project administration, H.W., H.-P.K., Funding acquisition, R.M.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Austrian Academy of Science, through the DOC scholarship.

Acknowledgments: We would like to thank the staff members of the Experimental Farm Groß-Enzersdorf, in particular Susanne and Michael Stickler, for their support and help during the greenhouse experiment and the field trials.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Pagano, M.C.; Miransari, M. The importance of soybean production worldwide. In *Abiotic and Biotic Stresses in Soybean Production*, 1st ed.; Miransari, M., Ed.; Elsevier Academic Press: Cambridge, UK, 2016; pp. 1–24.
2. Zollitsch, W.; Wetscherek, W.; Lettner, F. Use of differently processed full-fat soybeans in a diet for pig fattening. *Anim. Feed Sci. Technol.* **1993**, *41*, 237–246. [CrossRef]
3. Vollmann, J.; Fritz, C.N.; Wagentristl, H.; Ruckenbauer, P. Environmental and genetic variation of soybean seed protein content under Central European growing conditions. *J. Sci. Food Agric.* **2000**, *80*, 1300–1306. [CrossRef]
4. Watkinson, A.R.; Freckleton, R.P.; Robinson, R.A.; Sutherland, W.J. Predictions of biodiversity response to genetically modified herbicide-tolerant crops. *Science* **2000**, *289*, 1554–1557. [CrossRef]
5. Sandermann, H. Plant biotechnology: Ecological case studies on herbicide resistance. *Trends Plant Sci.* **2006**, *11*, 324–328. [CrossRef]

6. Owen, M.D.K.; Pedersen, P.; De Bruin, J.L.; Lux, S.J.; Franzenburg, D.; Grossnickle, D. Comparison of genetically modified and non-genetically modified soybean cultivars and weed management systems. *Crop Sci.* **2010**, *50*, 2597–2604. [CrossRef]
7. Johnson, B.; Loux, M.; Nordby, D.; Sprague, C.; Nice, G.; Westhoven, A.; Stachler, J. *Biology and Management of Giant and Common Ragweed*; U.S. Department of Agriculture: Washington, DC, USA, 2007; pp. 4–16.
8. Karrer, G.; Milakovic, I.; Kropf, M.; Blöch, C.; Dlugosch, A.; Leitsch-Vitalos, M.; Hackl, G.; Follak, S.; Fertsak, S.; Schwab, M.; et al. *Ausbreitungsbiologie und Management Einer Extrem Allergenen, Eingeschleppten Pflanze—Wege und Ursachen der Ausbreitung von Ragweed (Ambrosia artemisiifolia) Sowie Möglichkeiten Seiner Bekämpfung—Endbericht*; Bundesministerium für Landwirtschaft, Regionen und Tourismus: Vienna, Austria, 2011; pp. 31–44.
9. Richter, R.; Berger, U.E.; Dullinger, S.; Essl, F.; Leitner, M.; Smith, M.; Vogl, G. Spread of invasive ragweed: Climate change management and how to reduce allergy cost. *J. Appl. Ecol.* **2013**, *50*, 1422–1430. [CrossRef]
10. Fumanal, B.; Chauvel, B.; Sabatier, A.; Bretagnolle, F. Variability and Cryptic Heteromorphism of *Ambrosia artemisiifolia* Seeds: What Consequences for its Invasion in France? *Ann. Bot.* **2007**, *100*, 305–313. [CrossRef] [PubMed]
11. Bullock, J. *Assessing and Controlling the Spread and the Effect of Common Ragweed in Europe*; Centre for Ecology & Hydrology: Wallingford, UK, 2010; pp. 26–46.
12. Smith, M.; Cecchi, L.; Skjøth, C.A.; Karrer, G.; Šikoparija, B. Common ragweed: A threat to environmental health in Europe. *Environ. Int.* **2013**, *61*, 115–126. [CrossRef] [PubMed]
13. Hall, R.M.; Wagentristl, H.; Karrer, G.; Winter, A.; Czerny, R.; Kaul, H.-P. *Common Ragweed (Ambrosia artemisiifolia L.) Causes Severe Yield Losses in Different Soybean Varieties by Reducing the Infection Potential of Bradyrhizobium Japonicum*; Tagungsband: 29. Deutsche Arbeitsbesprechung über Fragen der Unkrautbiologie und-bekämpfung; Julius Kühn-Institut: Braunschweig, Germany, 2020; pp. 72–79.
14. Baskin, J.M.; Baskin, C.C. Ecophysiology of secondary dormancy in seeds of *Ambrosia artemisiifolia*. *Ecology* **1980**, *61*, 475–480. [CrossRef]
15. Baskin, J.M.; Baskin, C.C. Temperature requirements for after-ripening in buried seeds of four summer annual weeds. *Weed Res.* **1987**, *27*, 385–389. [CrossRef]
16. Coble, H.D.; Williams, F.M.; Ritter, R.L. Common ragweed (*Ambrosia artemisiifolia*) interference in soybean (*Glycine max*). *Weed Sci.* **1981**, *29*, 339–342. [CrossRef]
17. Gibson, D.J.; Millar, K.M.; Delong, M.; Connolly, J.; Kirwan, L.; Wood, A.J.; Young, B.G. The weed community affects yield and quality of soybean (*Glycine max* Merr.). *J. Sci. Food Agric.* **2007**, *88*, 371–381. [CrossRef]
18. Brückner, D.; Lepossa, A.; Herpai, Z. Inhibitory effect of ragweed (*Ambrosia artemisiifolia* L.)-inflorescense extract on the germination of *Amaranthus hypochondriacus* L. and growth of two soil algae. *Chemosphere* **2003**, *51*, 515–519. [CrossRef]
19. Wang, P.; Liang, W.J.; Kong, C.H.; Jiang, Y. Allelopathic potentials of volatile allelochemicals of *Ambrosia trifida* L. on other plants. *Allelopath. J.* **2005**, *15*, 131–136.
20. Pajević, S.; Borišev, M.; Orčić, D.; Boža, P.; Nikolić, N. Photosynthetic and biochemical characteristics of invasive species (*Ambrosia artemisiifolia* L., *Ambrosia trifida* L. and *Iva xanthifolia* Nutt.) depending on soil humidity and phenological phase. *Russ. J. Ecol.* **2010**, *41*, 498–505. [CrossRef]
21. Vidotto, F.; Tesio, F.; Ferrero, A. Allelopathic effects of *Ambrosia artemisiifolia* L. in the invasion process. *Crop Prot.* **2013**, *54*, 161–167. [CrossRef]
22. Mutch, D.R.; Martin, T.E.; Kolola, K.R. Red clover (*Trifolium pratense*) suppression of common ragweed (*Ambrosia artemisiifolia*) in winter wheat (*Triticum aestivum*). *Weed Technol.* **2003**, *17*, 181–185. [CrossRef]
23. Bonea, D.; Bonciu, E.; Niculescu, M.; Olaru, A.L. The allelopathic, cytotoxic and genotoxic effect of *Ambrosia artemisiifolia* on the germination and root meristeme of Zea mays. *Caryologia* **2018**, *71*, 24–48. [CrossRef]
24. Molinaro, F.; Mozzetti, C.; Ferrero, A.; Tabasso, S.; Negra, M. Bioherbicidal activity of a germacranolide sesquiterpene dilactone from *Ambrosia artemisiifolia* L. *J. Environ. Sci. Health B* **2016**, *51*, 847–852. [CrossRef]
25. Treutter, D. Significance of flavonoids in plant resistance: A review. *Environ. Chem. Lett.* **2006**, *4*, 147–157. [CrossRef]
26. Green-Tracewicz, E.; Page, E.R.; Swanton, C.J. Light quality and the critical period for weed control in soybean. *Weed Sci.* **2012**, *60*, 86–91. [CrossRef]
27. Gal, J.; Afifi, M.; Lee, E.; Lewis, L.; Swanton, C.J. Detection of neighbouring weeds alter soybean seeling roots and nodulation. *Weed Sci.* **2015**, *63*, 888–900. [CrossRef]
28. Devine, T.E.; Kuykendall, L.D. Host genetic control of symbiosis in soybean (*Glycine max* L.). *Plant Soil* **1996**, *186*, 173–187. [CrossRef]
29. Blumenthal, J.M.; Russelle, M.P.; Vance, C.P. Localized and internal effect of nitrate on symbiotic dinitrogen fixation. *Physiol. Plant.* **1997**, *101*, 59–66. [CrossRef]
30. Kiers, E.T.; Hutton, M.G.; Denison, R.F. Human selection and the relaxation of legume defences against ineffective rhizobia. *Proc. R Soc. B* **2014**, *274*, 3119–3126. [CrossRef] [PubMed]
31. Sessitsch, A.; Ramirez-Saas, H.; Hardarson, G.; Akkermans, A.D.; de Vos, M. Classification of an Austrian rhizobia and the Mexican isolate FL27 obtained from *Phaseolus vulgaris* L. as *Rhizobium gallicum*. *Int. J. Syst. Bacteriol.* **1997**, *47*, 1097–1101. [CrossRef] [PubMed]
32. Hunt, R.; Causton, D.R.; Shipley, B.; Askew, A.P. A modern tool for classical plant growth analysis. *Ann. Bot.* **2002**, *90*, 485–488. [CrossRef]

33. Zuur, A.F.; Ieno, E.N.; Elphick, C.S. A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* **2010**, *1*, 3–14. [CrossRef]
34. Posada, D.; Buckley, T.R. Model selection and model averaging in phylogenetics: Advantages of Akaike Information Criterion and Bayesian Approaches over Likelihood Ratio Tests. *Syst. Biol.* **2004**, *53*, 793–808. [CrossRef] [PubMed]
35. Burnham, K.P.; Anderson, D.R. *Model Selection and Inference: A Practical Information-Theoretic Approach*, 1st ed.; Springer: New York, NY, USA, 2002; pp. 192–196.
36. Bussan, A.J.; Burnside, O.C.; Orf, J.H.; Ristau, E.A.; Puettmann, K.J. Field evaluation of soybean (*Glycine max*) genotypes for weed competitiveness. *Weed Sci.* **1997**, *45*, 31–37. [CrossRef]
37. Bensch, C.N.; Horak, M.J.; Peterson, D. Intereference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. *Weed Sci.* **2003**, *51*, 37–43. [CrossRef]
38. Zimdahl, R.L. *Weed-Crop Competition: A Review*, 2nd ed.; Blackwell Publishing: Oxford, UK, 2007.
39. Monks, D.W.; Oliver, L.R. Interactions between soybean (*Glycine max*) cultivars and selected weeds. *Weed Sci.* **1988**, *36*, 770–774. [CrossRef]
40. Essl, F.; Biró, K.; Brandes, D.; Broennimann, O.; Bullock, J.M.; Chapman, D.S.; Chauvel, B.; Dullinger, S.; Fumanal, B.; Guisaon, A.; et al. Biological Flora of the British Isles: *Ambrosia artemisiifolia*. *J. Ecol.* **2015**, *103*, 1069–1098. [CrossRef]
41. Barrentine, W.L. Common cocklebur competition in soybeans. *Weed Sci* **1974**, *22*, 600–603. [CrossRef]
42. Datta, A.; Ullah, H.; Tursun, N.; Pornprom, T.; Knezevic, S.Z.; Chauhan, B.S. Managing weeds using crop competition in soybean [*Glycine max* (L.) Merr.]. *J. Crop Prot.* **2017**, *95*, 60–68. [CrossRef]
43. Berti, A.; Dunan, C.; Sattin, M.; Zanin, G.; Westra, P. A new approach to determine when to control weeds. *Weed Sci.* **1996**, *44*, 496–503. [CrossRef]
44. Baysinger, J.A.; Sims, B.D. Giant ragweed (*Ambrosia trifida*) interference in soybeans (*Glycine max*). *Weed Sci.* **1991**, *39*, 358–362. [CrossRef]
45. Mosier, D.G.; Oliver, L.R. Soybean (*Glycine max*) interference on common cocklebur (*Xanthium strumarium*) and entireleaf morningglory (*Ipomoea hederacea var. integruscula*). *Weed Sci.* **1995**, *43*, 402–409. [CrossRef]
46. Weston, L.A.; Putnam, A.R. Inhibition of growth, nodulatoin, and nitrogen fixation of legumes by Quackgrass. *Crop Sci.* **1985**, *25*, 561–565. [CrossRef]
47. Puppo, A.; Pauly, N.; Boscari, A.; Mandon, K.; Brouquisse, R. Hydrogen peroxide and nitric oxide: Key regulators of the Legume-Rhizobium and mycorrhizal symbiosis. *Antioxid. Redox Signal.* **2013**, *18*, 2202–2219. [CrossRef] [PubMed]
48. Pierik, R.; Mommer, L.; Voesenek, L.A.C.J. Molecular mechanisms of plant competition: Neighbour detection and response strategies. *Funct. Ecol.* **2013**, *27*, 841–853. [CrossRef]
49. Swamy, P.M.; Smith, B.N. Role of abscisic acid in plant stress tolerance. *Curr. Sci.* **1999**, *76*, 1220–1227.
50. Finkelstein, R.R.; Rock, C.D. Abscisic acid biosynthesis and repsonse. In *The Arabidopsis Book/American Society of Plant Biologists*; ASPB Member Center: Rockville, MD, USA, 2002; p. e0058. [CrossRef]
51. Suzuki, A.; Akune, M.; Kogiso, M.; Imagama, Y.; Osuki, K.-I.; Uchiumi, T.; Higashi, S.; Han, S.-Y.; Yoshida, S.; Asami, T.; et al. Control of nodule number by the phytohormone abscisic acid in the roots of two leguminous species. *Plant Cell Physiol.* **2004**, *45*, 914–922. [CrossRef]
52. Farooq, U.Z.M.A.; Bano, A. Effect of abscisic acid and chlorochloine chloride on nodulation and biochemical content of *Vigna radiata* L. under water stress. *Pak. J. Bot.* **2006**, *38*, 1511–1518.
53. Cho, M.J.; Harper, J.E. Effect if abscisic acid application on root isoflavonoid concentration and nodulation of wild-type and nodulation-mutant soybean plants. *Plant Soil* **1993**, *153*, 145–149. [CrossRef]
54. Liu, H.; Zhang, C.; Yang, J.; Yu, N.; Wang, E. Hormone modulation of legume-rhizobial symbiosis. *J. Integr. Plant Biol.* **2018**, *60*, 632–648. [CrossRef] [PubMed]
55. Long, S.R. Genes and signals in the rhizobium-legume symbiosis. *Plant Physiol.* **2001**, *125*, 69–72. [CrossRef]
56. Makarova, L.E.; Latysheva, S.E.; Putilina, T.E. The effect of the phenolic compounds exuded by pea roots in darkness on the reproduction of Rhizobium. *Appl. Biochem. Microbiol.* **2007**, *43*, 429–434. [CrossRef]
57. Makarova, L.E.; Smirnov, V.I.; Klyba, L.V.; Petrova, I.G.; Dudareva, L.V. Role of allelopathic compounds in the regulation and development of legume-rhizobial symbiosis. *Appl. Biochem. Microbiol.* **2012**, *48*, 355–362. [CrossRef]
58. Wardle, D.A.; Nicholson, K.S.; Ahmed, M.; Rahman, A. Interference effects of the invasive plant Carduus nutans L. against the nitrogen fixation ability of *Trifolium repens* L. *Plant Soil* **1994**, *163*, 287–297. [CrossRef]
59. Batish, D.R.; Levanya, K.; Singh, H.P.; Kohli, R.K. Phenolic allelochemicals released by *Chenopodium murale* affect the growth, nodulation and macromolecule content in chickpea and pea. *Plant Growth Regul.* **2007**, *51*, 119–128. [CrossRef]
60. Rice, E.L. Allelopathy—An Overview. In *Chemically Mediated Interactions between Plants and Other Organisms*; Cooper-Driver, G.A., Swain, T., Conn, E.E., Eds.; Springer: Boston, MA, USA, 1984; Volume 19. [CrossRef]
61. Mizutani, J. Selected Allelochemicals. *Crit. Rev. Plant Sci.* **1999**, *18*, 653–671. [CrossRef]