



Competitive and allelopathic interference between soybean crop and annual wormwood (*Artemisia annua* L.) under field conditions

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ABSTRACT

Annual wormwood interference on soybean crop growth and yield may result from competition and allelopathy, which are modulated by crop management. Allelochemicals released by annual wormwood (e.g. artemisinin) may affect the crop directly or indirectly through the effect on the nitrogen fixing symbiont, *Bradyrhizobium japonicum*. The objectives were (i) to quantify the crop response (i.e. biomass production, nodulation and yield) to weed interference and (ii) to determinate the relative change of competition and allelopathy interferences, when a sublethal dose of herbicide is applied. Two split plot field experiments with three replications were used. The experiment involved a factorial combination of five weed–crop density (soybean/annual wormwood, plants m^{−2}) levels: D1, pure soybean, 40/0 plants m^{−2}; D2, 40/2 plants m^{−2}; D3, 40/4 plants m^{−2} and D4, 40/8 plants m^{−2}, and D5, pure annual wormwood, 0/8 plants m^{−2}, two activated carbon (allelopathy) levels: C−, with activated carbon (reduced allelopathy) and C+, non activated carbon applied (with allelopathy) and two herbicide levels: H−, untreated and H+, treated with a sub-lethal dose of glyphosate. Activated carbon to adsorb allelochemicals (with and without activated carbon) and glyphosate application (with and no herbicide) were assigned to sub-plots. Increasing weed density did not affect crop biomass at flowering, but changed nodule number and soybean yield with a different pattern depending on carbon and herbicide treatment. Relative crop yield decreased with increasing relative weed biomass. This decrease was particularly drastic when allelopathy was reduced by activated carbon and without herbicide application. The maximum yield losses of 33% in 2006 and 17% in 2007 were observed with the highest weed density (8 plants m^{−2}). In contrast, without carbon (high allelopathy level), soybean yield remained stable within the explored range of annual wormwood biomass, despite the fact that weed biomass at high densities (D4) was high enough to generate competition. The lack of response to increasing weed density could be related to the indirect effect of allelochemicals interacting with soil microorganisms (i.e. *B. japonicum*) that positively affected the nodulation (e.g. larger nodules in 2006 and increased nodules biomass due to higher number of roots in 2007 at high densities). With herbicide application, soybean yield of both carbon treatments remained stable when biomass of annual wormwood increased. This research provided strong evidence in support of the existence of positive effect of allelopathic and competitive interactions between annual wormwood and soybean crop under field conditions that may be overridden under herbicide application.

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1. Introduction

Interference between crop and weed plants has been attributed to competition for resources, light, water and nutrients (de Wit,

1960; Aldrich, 1984). However, allelopathy, defined as the direct or indirect effect of one plant on others through the release of chemical compounds (i.e. allelochemicals) into the environment (Rice, 1984; Seigler, 1996), is also an important non-competitive interference (Inderjit et al., 2001; Callaway, 2002; Barney et al., 2005). The allelopathy encompasses both stimulatory and inhibitory interactions among neighbour plants (Molish, 1937) directly affecting growth, development, establishment and reproduction (Rice, 1984; Inderjit and Mallik, 2002) or indirectly, for example, through suppressed nodulation in legume crops (Batish et al., 2007).

Annual wormwood (*Artemisia annua* L.), a component of soybean (*Glycine max* (L.) Merr.) weed community in Argentina

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(de la Fuente et al., 1999, 2006, 2010), may release allelochemicals (i.e. artemisinin) affecting crop growth (Duke et al., 1987; Weston, 1996; Weston and Duke, 2003). However, some chemical compounds (e.g. arteannuin acid and arteannuin B) showed a stimulating effect on root formation in legume crops (i.e. mung bean) (Chen and Leather, 1990). Although artemisinin (non volatile compound) and other chemicals with allelopathic activity are present in various organs, including leaves, stems, floral and fruit parts (Delabays et al., 2001). Leaves and fruits have the most important allelopathic potential (Inderjit, 2005). These compounds are released into the soil environment by volatilization, leaching, root exudation or/and residue decomposition (Harborne, 1997). Under greenhouse conditions, soybean is susceptible to allelochemicals due to the fact that not only nodulation, and hence nitrogen fixation (Rice, 1984) are affected but also plant growth (Lydon et al., 1997) and yield (Azania et al., 2003).

Crop management imposes stress (i.e. herbicide application) on plants that can enhance not only the amount and the physiological effects of the allelochemicals produced by the donor species (Einhellig, 1999), but also the sensitivity of target plants to allelochemicals (Einhellig, 1996). Likewise, the presence of herbicides that regulate the competition between weeds and crops may interact with the activity of allelochemicals. The effect observed may be either additive, antagonist or synergistic (Einhellig, 1996). Intentional or unintentional exposure to sublethal herbicide dose is a common event in target weed populations (Vila-Aiub and Ghera, 2005). For example, *Lolium multiflorum* Lam. plants exposed to sublethal dosages of herbicide application triggered an increase of allelochemicals levels in the plants (Vila-Aiub and Ghera, 2005). On the other hand, low rates of herbicides (i.e. glyphosate) caused deleterious effect on *Bradyrhizobium japonicum* (Moorman et al., 1992; Hernandez et al., 1999) and inhibition of the nodulation (Reddy, 2000) and/or nitrogen fixation in soybean plants (Zablotowicz and Reddy, 2004).

Most studies analyzing allelopathic weed–crop interferences using annual wormwood (Lydon et al., 1997) and other allelopathic species (Batish et al., 2007) were conducted under laboratory and greenhouse conditions (Inderjit et al., 2001; Inderjit and Nilsen, 2003). Few experiments investigated the relative importance of competition and allelopathy under field conditions (Nilsson, 1994; Inderjit et al., 2001). However, knowing the allelopathic annual wormwood effect on soybean crop due to changes in weed density and glyphosate rates could be relevant to improve crop management strategies. Consequently, further studies that explore both interferences under field conditions are needed.

Within this context, the working hypothesis of this study was that allelopathic and competitive interferences in a crop–allelopathic weed system are modified by weed density and herbicide application. Thus, the overall purpose of the work was to evaluate allelopathic and competitive interferences in annual wormwood–soybean crop system modified by weed density and herbicide application under field conditions. The specific objectives were (i) to quantify the crop response (i.e. aboveground biomass production, nodulation and yield) to weed interference and (ii) to determine the relative change of competition and allelopathy interferences, when a sublethal dose of herbicide is applied.

2. Materials and methods

2.1. Field experiments

Field experiments were conducted in 2006 and 2007 at the Faculty of Agronomy in the University of Buenos Aires, Argentina (34°35'S, 58°25'W) on a silty clay loam soil classified as Vertic Argiudoll according to the USDA taxonomy (1999). Meteorological

conditions (temperature and rainfall) were recorded at meteorological station Villa Ortúzar located 34°35'S, 58°25'W (Table 1).

The soil was ploughed and refined to produce a smooth seed bed. Soybean cultivar of maturity group IV (DM 4800) was inoculated with *B. japonicum* (10^9 colony forming units/g inoculant) and sown at a rate of 45 seeds m^{-2} in plots of 16 rows width, 0.35 m apart, and 2.8 m long on January 18th in both years. The plots were N–S oriented. The sowing was carefully made in order to achieve a uniform soybean seed germination and seedling emergence. Soybean seeds (with more than 98% of germination) were placed in the rows, covered with soil, lightly compacted and irrigated. At soybean emergence (VE, Fehr and Caviness, 1977), soybean plants were thinned to 40 plants m^{-2} . Annual wormwood was previously sown in seedling trays and then transplanted to plots at soybean seedling emergence.

During the experiments plots were irrigated to supplement natural rainfall (455.4 mm in 2006 and 203.4 mm in 2007) with the objective of maintaining the soil near field capacity. Spontaneous weeds were manually removed, and all plots were sprayed with the equivalent of 0.1 L a.i. ha^{-1} of cypermethrin and 0.8 L ha^{-1} of chlorpyrifos at soybean vegetative stage (V2; Fehr and Caviness, 1977) and with endosulfan (1.5 L ha^{-1} , 35%) at reproductive stage (R6; Fehr and Caviness, 1977), to prevent pests throughout the crop cycle.

2.2. Treatments and experimental design

The experiment involved twenty treatments, resulting from a factorial combination of: five weed–crop density (soybean/annual wormwood, plants m^{-2}) levels: D1, pure soybean, 40/0 plants m^{-2} ; D2, 40/2 plants m^{-2} ; D3, 40/4 plants m^{-2} and D4, 40/8 plants m^{-2} , and D5, pure annual wormwood, 0/8 plants m^{-2} ; two activated carbon (allelopathy) levels: C–, non activated carbon applied (with allelopathy) and C+, with activated carbon (reduced allelopathy), and two herbicide levels: H–, untreated and H+, treated with a sublethal dose of glyphosate. Control plots were pure soybean (D1) and pure annual wormwood (D5) with (C–; H–), (C+; H–), (C–; H+), (C+; H+) treatments, respectively.

The experimental design was a split-plot factorial arranged in a randomized complete block with three replications due to the slight slope of the field used during 2006. In 2007 treatments were arranged in a factorial randomized complete design with three replications. Crop–weed density (competition) treatment was assigned to the main plots ($64 m^{-2}$) and subplots consisted of a factorial combination of activated carbon (allelopathy) and herbicide treatments.

Crop–weed density interactions were studied using an additive experimental design (Park et al., 2003). Thus soybean density was left constant (40 plants m^{-2}) in each plot and different densities of annual wormwood plants were transplanted into the inter-row, according to density treatments at soybean emergence (VE, Fehr and Caviness, 1977).

Activated carbon was used to reduce allelopathy under field conditions (Nilsson, 1994) and as a simple way to separate allelopathy and competition. Activated carbon adsorbs organic compounds with little effect on inorganic nutrients (Inderjit and Callaway, 2003). At vegetative stage of soybean V1 (Fehr and Caviness, 1977) 500 g m^{-2} activated carbon (Refil SA and Clarimex SA) was spread according to Nilsson (1994) and to a previous experiment performed to test if the allelochemicals released by annual wormwood were adsorbed by the soil. Reduced allelopathy sub-plots were covered with soil ($650 g m^{-2}$). The purpose was to avoid wind dispersal of the carbon, considering that the carbon texture is very fine (from 2 to 5 nm) and to prevent colour and soil temperature differences between allelopathy treatments (C+ and C–).

Table 1

Monthly maximum, minimum temperatures (°C) and accumulated rainfall (mm) during 2006 and 2007 recorded at Climatological Villa Ortúzar Station.

Crop month	2006			2007		
	Temperature (°C)		Rainfall (mm)	Temperature (°C)		Rainfall (mm)
	Maximum	Minimum		Maximum	Minimum	
January	36.6	14.9	33.3	35.4	12.7	70.9
February	35.0	12.6	201.4	35.8	11.0	123.2
March	30.1	7.1	149.4	31.1	11.6	0.3
April	28.4	4.6	69.6	31.4	5.2	0.0
May	24.7	−0.3	1.7	24.9	−2.1	0.0

Laboratory tests were conducted to assess the adsorption capacity of the activated carbon and the probability that it would mix with the soil in the field plots. Three artemisinin concentration levels (0, control; 0.2 mL L^{−1} and 0.5 mL L^{−1} standard artemisinin Sigma–Aldrich 98%) were tested ($n=3$). Standard solutions of artemisinin were prepared by dissolving an accurately weighed quantity (about 6 mg) of artemisinin in ethanol, and adjusting it to the 6 mL mark. Three aliquots equivalent to 0.2 and three 0.5 mL of the mentioned solution were drop-poured into six 50 mL flasks, respectively. The solutions were then added to six beakers containing 2 g of activated carbon (5 mm layer) and were kept under 20 °C for 2 days. Control treatment was made following the same steps without the addition of artemisinin. Ethanol was vacuum evaporated by a rotary evaporator. Activated carbon was washed with 10 mL to simulate rainfall and then shaken (10 min). The water extracted solution was vacuum distilled by a rotary evaporator. The residue was dissolved in ethanol and vacuum filtered. The filtrate was concentrated and transferred into a 5 mL flask. Artemisinin content in the solutions was measured by Reversed Phase HPLC following the method described by Qian et al. (2005). After laboratory tests, artemisinin content in the water solution extracted from the activated carbon was 0% for all the tested concentrations of artemisinin, thus it can be assumed that the quantity of activated carbon used in the field experiment to reduce allelopathy was enough to adsorb artemisinin and other minor weight chemical compounds lixiviated from leaves of annual wormwood.

To test whether the activated carbon layer would remain pure without mixing with the rest of the soil during the experiment, three Büchner funnels were prepared. They contained a layer of soil (10 mm), a layer of carbon (5 mm) and a layer of soil (10 mm). Both soil layers were taken from the experimental site. The combination of layers was sprayed with water up to saturation to simulate rain every second day during two weeks. Visual observation of the layers was performed so as to verify there was no mixing between soil and carbon.

At vegetative stage of soybean V4 (Fehr and Caviness, 1977) glyphosate was applied in sub lethal dose (1/8 of commercial dose, 3 kg a.i. ha^{−1}). Sub lethal dose of glyphosate was chosen by previously testing four doses of glyphosate (0, 1/2, 1/4 and 1/8 of dose, 3 kg a.i. ha^{−1}) on annual wormwood plants (3 plants per treatment) growing in the same field ($n=3$). Visual observation of weed plants was carried out after 15 days of the herbicide application.

2.3. Data collection

At flowering (R1; Fehr and Caviness, 1977), soybean plants in the central rows of the plots were sampled to determine dry weight of aboveground and root biomass and nodulation. Roots were sampled to determine nodule number and dry weight. Soil samples (2.1 m × 0.20 m × 0.25 m deep) that included the primary and secondary roots and nodules were immediately washed with water and filtered (1 mm mesh). Samples were placed in plastic bags and frozen until separation of nodules from roots.

At the end of soybean growing season (maturity: R8; Fehr and Caviness, 1977), soybean plants covering a surface of 0.75 m² in the three central rows of plots were harvested to determine aboveground biomass and seed yield. In the same sample area, 5 plants of annual wormwood were harvested to determine aboveground biomass. Dry weight of all samples was determined after oven-drying samples for three days at 68 °C until reaching constancy of weight.

2.4. Statistical analyses

Main and interaction effects of experimental factors were determined from analysis of variance (ANOVA) using the “General Lineal Model” (GLM) procedure in the Statistical Analysis System (SAS, v. 9.1, SAS Institute, Cary, NC). Block and replication were set as random effects. ANOVA assumptions were tested (random and homogenous residuals, normal distribution). Means were separated using Tukey’s test at $p<0.05$. Data was log-transformed when distribution was not normal. In this case, re-transformed means are presented in figures. Number of roots was input as co variable for ANOVA in number and biomass of nodules in 2007.

Considering there was a gradient of aboveground biomass in the experiments, analysis of regression was used to describe the relationship between soybean yield and total annual wormwood aboveground biomass at maturity. A hyperbolic model was used, as shown in Eq. (1). Hyperbolic functions have been used to describe yield with increasing weed biomass (Canner et al., 2002). To compare data obtained in both years, yield of soybean and total aboveground biomass of annual wormwood were transformed to relative values. This was done by dividing yield or biomass obtained in each subplot by the yield or biomass obtained in each control subplot each year.

$$\hat{Y}_{ijk} = \frac{(a + b \times X_{ij})}{(c + X_{ij})} \quad (1)$$

where \hat{Y}_{ijk} represents the predicted ratio between soybean yield (g m^{−2}) and soybean yield in each control plot (D1) plot for the carbon i ($i=C+$, $C-$), herbicide application j ($j=H+$, $H-$) and replication k ($k=1-3$); X_{ij} represents the ratio between aboveground biomass of annual wormwood (g m^{−2}) at maturity and aboveground biomass of annual wormwood in each control plot (D5) for the carbon i and herbicide j treatments; $-a/b$ is the amount of relative biomass of the weed as \hat{Y}_{ijk} approaches 0 and a/c is the intercept, i.e. the relative soybean yield in absence of the weed. \hat{Y}_{ijk} and X_{ij} units are g m^{−2}/g m^{−2} while units of parameter model a , b and c are g m^{−2}.

Eq. (1) was analyzed using the non-linear modules of Sigma Plot v.10. Goodness of fit was judged by residual mean square (RMS), estimation of parameters, adjusted R^2 , and visual examination of the residuals.

3. Results

3.1. Crop response to weed interference at flowering stage (R1)

(a) *Crop biomass*: At flowering, mean soybean aboveground biomass was similar in all plots regardless of herbicide application or carbon addition to the soil (Table 2). However, in 2006 experiment herbicide sub-lethal dose application resulted on average in an 11% increase of soybean biomass production relative to the untreated plots ($p = 0.01$, Table 3). This increase was related to the nearly 16 fold reduction in annual wormwood average biomass observed in the herbicide treated in relation to the untreated plots (Table 2).

(b) *Root biomass and number*: In general soybean root number and biomass did not vary across treatments. However, in 2007 experiment root number showed a significant reduction when the weed density was 4 and 8 plants m^{-2} (D3 and D4 levels) in relation to the crop's pure stand (D1), in the plots with reduced allelopathy (with carbon) (Fig. 1). Differences observed in this and other variables (see below) among carbon treatments when soybean and annual wormwood were grown together were not related to a direct effect of activated carbon, since in pure stands plots (D1 and D5) no significant effect ($p > 0.05$) of activated carbon was observed on aboveground biomass of soybean and annual wormwood at R1 (Fig. 2A and B), biomass and number of nodules (Fig. 2C

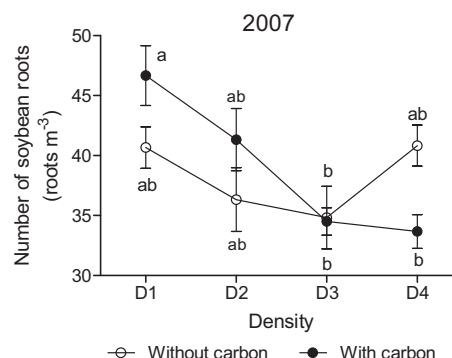


Fig. 1. Effect of density (D) and carbon (with, black symbols and without, white symbols) on number of soybean roots (roots m^{-3}) in 2007. D, (soybean/annual wormwood, plants m^{-2}); D1, 40/0 plants m^{-2} ; D2, 40/2 plants m^{-2} ; D3, 40/4 plants m^{-2} ; D4, 40/8 plants m^{-2} . Vertical lines are the SE. Different letters indicate significant differences between means according to Tukey's test at $p < 0.05$.

and D), and biomass and number of roots (Fig. 2E and F) in both years.

(c) *Nodule size and number*: The reduction of soil allelochemicals by the activated carbon affected the development of soybean nodules, with a different pattern each year (Tables 2 and 3). In 2006 the crop's roots of the plots with activated carbon treatment

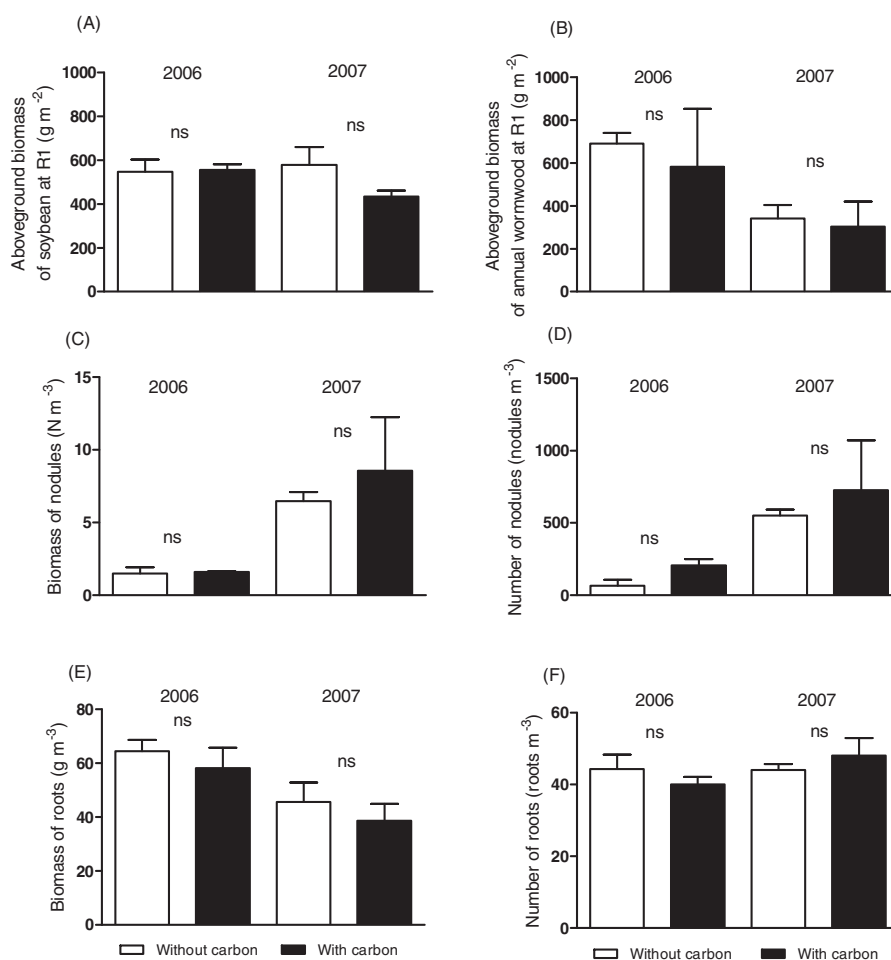


Fig. 2. Effect of activated carbon on: (A) aboveground biomass of soybean at flowering (R1; Fehr and Caviness, 1977) ($g m^{-2}$), (B) aboveground of annual wormwood at R1 ($g m^{-2}$), (C) biomass of soybean nodules ($g m^{-3}$), (D) number of nodules (number m^{-3}), (E) biomass of roots ($g m^{-2}$) and (F) number of roots (roots m^{-3}) in control plots during 2006 and 2007. Control plots were pure soybean (D1) or pure annual wormwood (D5) without carbon and untreated herbicide (white bars) and with carbon and untreated herbicide (black bars), respectively. Values of biomass of annual wormwood, number and biomass of nodules are the re-transformed means and vertical lines are SE. ns, not significant; F-test significant at $p < 0.05$.

Table 2

Mean values of aboveground biomass of soybean and wormwood at flowering, biomass and number of nodules and biomass and number of soybean roots for the density (D), activated carbon (C) and herbicide (H) treatments in 2006 and 2007.

Year	Factor	Level	Soybean					Annual wormwood	
			Aboveground biomass at R1 (g m ⁻²)	Nodules		Roots		Aboveground biomass at R1 (g m ⁻²) ^a	
				Biomass (g m ⁻³) ^a	Number (nodules m ⁻³) ^a	Biomass (g m ⁻³)	Number (roots m ⁻³)		
2006	Density	D1	571.16	144.97	1.49	60.46	41.58	–	
		D2	523.76	77.31	1.24	53.86	40.66	27.99a	
		D3	558.24	395.20	2.82	62.86	39.58	52.09b	
		D4	497.57	249.28	2.30	51.98	36.50	117.84c	
		D5	–	–	–	–	–	636.90d	
	Carbon	C–	534.67	71.21a	1.62a	56.83	38.58	216.64	
		C+	540.69	362.17b	2.30b	57.75	40.58	143.77	
	Herbicide	H–	508.58	210.82	1.94	58.09	39.37	265.01a	
		H+	566.78	222.56	1.98	56.49	39.79	16.62b	
2007	Density	D1	487.34	673.11	7.54	40.82	43.66a	–	
		D2	496.91	568.22	6.89	40.23	38.77ab	11.57	
		D3	481.07	318.56	4.54	42.19	34.55b	21.31	
		D4	496.65	518.67	5.92	38.94	37.33b	34.03	
		D5	–	–	–	–	–	316.08	
	Carbon	C–	494.52	611.72	6.71a	42.61	38.22	99.00	
		C+	486.47	427.56	5.73b	38.47	38.94	99.78	
	Herbicide	H–	504.55	536.61	6.44	41.93	39.11	109.01	
		H+	476.44	502.67	6.00	39.16	38.05	90.60	

D, density; C, activated carbon; H, herbicide. Within each column, means followed by different letters are significantly different for the same factor in each year at the 0.05 significance level based on Tukey's test.

^a Data was log-transformed before for statistical analysis.

developed a higher number of nodules than those of the untreated plots. However, in 2007 mean nodule number was higher in the untreated plots. Only in 2006 and without carbon (with allelopathy) the average nodule's weight was larger than with carbon treatments (Table 2 and Fig. 3).

3.2. Crop response to weed interference at maturity (R8)

In both years soybean aboveground biomass was not affected by the experimental treatments (Tables 4 and 5). However, the effect of weed interference on soybean yield was different each

Table 3

Analyses of variance of aboveground biomass of soybean and annual wormwood at flowering (R1), biomass and number of nodules and biomass and number of soybean roots as affected by weed density (D), activated carbon (C) and herbicide (H) treatments and their interactions for 2006 and 2007.

Year	Factor	d.f.	p-Values of F-test					
			Soybean				Annual wormwood	
			Aboveground biomass at R1	Nodules		Roots		Aboveground biomass at R1
				Biomass	Number	Biomass	Number	
2006	Block	2	0.32	0.82	0.97	0.47	0.92	0.60
	D	3	0.36	0.54	0.77	0.35	0.66	0.01
	Block × D	6	0.14	<0.001	<0.001	0.01	<0.01	0.04
	C	1	0.78	<0.01	<0.001	0.15	0.29	0.21
	H	1	0.01	0.94	0.84	0.29	0.97	<0.001
	D × C	3	0.07	0.15	0.21	0.64	0.13	0.36
	D × H	3	0.57	0.68	0.76	0.08	0.88	0.16
	C × H	1	0.92	0.82	0.80	0.25	0.24	0.83
	D × C × H	3	0.48	0.67	0.51	0.18	0.71	0.27
	Error	22						
2007	D	3	0.97	0.91	0.79	0.85	<0.01	0.97
	Replication × D	8	0.41	<0.001	<0.001	0.42	0.82	0.41
	C	1	0.77	0.07	0.01	0.12	0.65	0.77
	H	1	0.31	0.98	0.62	0.29	0.51	0.31
	D × C	3	0.64	0.78	0.72	0.91	0.03	0.64
	D × H	3	0.63	0.44	0.37	0.95	0.53	0.63
	C × H	1	0.05	0.65	0.83	0.17	0.26	0.05
	D × C × H	3	0.54	0.20	0.26	0.65	0.56	0.54
	Error	24						

d.f., degree of freedom. Numbers in bold indicate significant differences at $p < 0.05$ based on F-test.

Table 4
Mean values of soybean yield and aboveground biomass and annual wormwood at maturity (R8) in the density (D), activated carbon (C) and herbicide (H) treatments in 2006 and 2007.

Year	Factor	Level	Soybean		Annual wormwood Biomass at R8 (g m ⁻²) ^a
			Yield (g m ⁻²)	Biomass at R8 (g m ⁻²)	
2006	Density	D1	314.97 a	638.12	–
		D2	331.57 a	682.38	143.69 a
		D3	306.52 a	633.72	244.86 b
		D4	264.90 b	573.36	287.66 c
		D5	–	–	453.23 d
	Carbon	C–	299.09	636.85	295.82
		C+	309.89	626.94	269.24
	Herbicide	H–	294.22	607.27	507.75 a
		H+	314.76	656.52	46.94 b
2007	Density	D1	237.91 ab	658.15	–
		D2	269.04 a	666.37	47.71 a
		D3	264.52 ab	657.40	98.24 a
		D4	232.16 b	615.39	223.17 b
		D5	–	–	563.74 c
	Carbon	C–	254.52	656.03	245.32
		C+	247.29	642.63	221.11
	Herbicide	H–	241.15	625.08	260.82 a
		H+	260.66	673.58	205.61 b

D × H factor interaction in both years is shown in Fig. 6. D × C × H factor interactions of soybean yield in 2006 is shown in Fig. 4. Within each column, means followed by different letters are significantly different for the same factor at each year at the 0.05 significance level based on Tukey's test.

^a Data were analysed on log-transformed data and retransformed to the original scale.

Table 5
Analyses of variance of soybean yield and aboveground biomass and annual wormwood biomass at maturity (R8) in the density (D), activated carbon (C) and herbicide (H) treatments and their interactions in 2006 and 2007.

Year	Factor	d.f.	p-Values of the F-test		
			Soybean		Annual wormwood
			Yield	Aboveground biomass at R8	Aboveground biomass at R8
2006	Block	2	0.62	0.95	0.62
	D	3	<0.01	0.34	<0.001
	Block × D	6	0.09	0.09	0.81
	C	1	0.48	0.71	0.68
	H	1	0.10	0.07	<0.001
	D × C	3	0.39	0.45	0.48
	D × H	3	0.58	0.51	<0.001
	C × H	1	0.62	0.21	0.79
	D × C × H	3	0.01	0.33	0.43
	Error	22			
2007	D	3	0.02	0.08	<0.01
	Replication × D	8	0.97	0.98	0.06
	C	1	0.69	0.69	0.11
	H	1	0.28	0.18	<0.01
	D × C	3	0.98	0.85	0.36
	D × H	3	0.85	0.69	0.03
	C × H	1	0.77	0.78	0.65
	D × C × H	3	0.77	0.92	0.48
	Error	24			

D, density; C, activated carbon; H, herbicide; d.f., degrees of freedom. Numbers in bold indicate significant differences at $p < 0.05$ based on F-test.

Table 6
Estimated parameters values obtained by fitting a hyperbolic model (Eq. (1)) describing the relationship between relative biomass (RB) and relative yield (RY) of soybean and relative biomass of annual wormwood at maturity in herbicide untreated plots with carbon. Values between parenthesis denote the SE; P_{model} = likelihood of the model and d.f. = degrees of freedom.

Treatment	Regression parameters (±SE)			RMSE (g m ⁻²)	R^2_{adj}	P_{model}	d.f.
	a (g m ⁻²)	b (g m ⁻²)	c (g m ⁻²)				
RY	–0.77 (0.06)	1.07 (0.05)	–0.73 (0.04)	0.13	0.73	0.01	7

Hyperbolic model: $\hat{Y}_{ijk} = (a + b \times X_{ij}) / (c + X_{ij})$

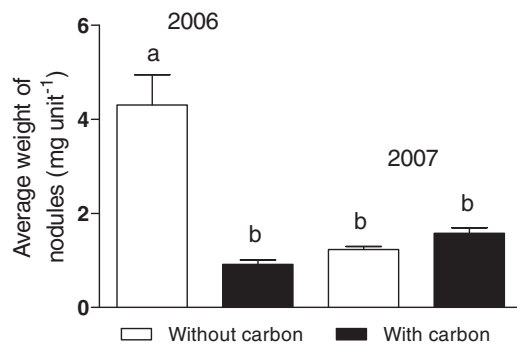


Fig. 3. Effect of carbon (with, white bars and without, black bars) on average weight of nodules (mg unit⁻¹) in 2006 and 2007. Bars represent the means values and vertical lines are the SE. Different letters indicate significant differences according to Tukey's test at $p < 0.05$. F -test significant at $p < 0.05$.

year. In the 2006 experiment soybean yield response to wormwood density increase was altered by both the addition of carbon and herbicide as accounted by the observed triple treatment interaction ($D \times C \times H$) ($p = 0.01$, Table 5). Without herbicide application, annual wormwood presence in the mixture reduced soybean yield, significantly, only at the highest weed density level (D4), and if carbon was applied to the soil (Table 4, Fig. 4).

In 2007 experiment there were no treatment interactions (Table 5) and high wormwood density (8 plants m⁻²) significantly decreased grain yield of soybean ($p = 0.02$, Table 5).

No direct effect of carbon was also observed at this phenological stage. Soybean and annual wormwood biomass and soybean yield in pure stands (D1 and D5) were similar between carbon treatments in both years (Fig. 5).

Weed biomass did not increase as expected with density in 2006 but was significantly reduced in herbicide-treated plots (Fig. 6). Without herbicide, wormwood biomass production was equal across density treatments when growing in mixture with soybean. However, in D5 plots (with 8 plants per m² of the weed growing in pure stand) biomass production almost doubled. Herbicide reduced the weed biomass in all treatments, but changed the pattern of response to density, as wormwood biomass increased both with the increase in density and the elimination of soybean interference (Fig. 6). In 2007 wormwood biomass production increased with weed density in the mixture, but showed no change to the elimination of soybean interference. Herbicide treatment changed this pattern because in the absence of soybean (D5) despite the herbicide application, the weed produced around a fivefold biomass increase relative to the mixed stands (Fig. 6).

The regression analysis showed that relative crop yield decreased with increasing relative weed biomass (Table 6). This decrease was particularly drastic when allelopathy was reduced by activated carbon and without herbicide application (Fig. 7A). The

best fit of the hyperbolic model was obtained with data from plots with carbon but no herbicide ($p = 0.01$, $R^2_{adj} = 0.73$, Table 6). In these plots grain yield decreased when annual wormwood increased from 266 to 535 g m⁻² (0.34 and 0.69 relative biomass, respectively, Table 7) in 2006 and from 127 to 256 g m⁻² (0.23 and 0.62 relative biomass, respectively, Table 7) in 2007. The maximum yield losses of 33% (0.67 RY) in 2006 and 17% (0.83 RY) in 2007 (Table 7) were observed with the highest density (8 plants m⁻²) of annual wormwood. In contrast, without carbon (high allelopathy level), soybean yield remained stable within the explored range of annual wormwood biomass, despite the fact that weed biomass at high densities (D4) was high enough to generate competition (Fig. 6 and Table 7).

Interestingly soybean yield was higher in D2 (low weed density plots) than controls in all carbon and herbicide treatments in both years (e.g. in C– and H–, soybean mean yield even increased 13% in 2007), except without carbon and no herbicide application in 2006 (Tables 7 and 8). In herbicide treatments, soybean yield of both carbon treatments remained stable when biomass of annual wormwood increased (Fig. 7B) and the biomass of annual wormwood was lower with herbicide application in both years (Fig. 6), suggesting that the level of competition of annual wormwood on soybean crop was lower in H+ than H– treatments (see Tables 7 and 8).

4. Discussion

This experimental design included the manipulation of density by adding weed plants to a crop stand and the reduction of weed growth without density variation by adding sub-lethal herbicide doses. In both years the density and herbicide treatments aimed at modulating weed interference levels produced an interaction effect, resulting in a wide range of variation in wormwood biomass. However, at flowering stage, soybean aerial and root biomass, and root density remained unchanged in all treatments. Only during 2006 with herbicide, in which weed biomass in the mixed stands, was dramatically reduced there was an increase in soybean aerial biomass production. This relative poor response to treatments was also found at maturity, since aerial biomass production was unaffected by variations in weed density and biomass among treatments. On the other hand, soybean yield diminished at the highest weed density of 8 plants per m², and the reduction in weed biomass due to herbicide application caused a significant increase in the average yield. In the 2006 experiment there was a triple interaction indicating that soybean yield was affected not only by the weed's density or biomass but also by the release of allelochemicals to the soil environment. At the D4 density level of the weed, yield was reduced only if carbon was applied to the soil, suggesting that weed interference (i.e. competition) was reduced if the allelochemicals were present in the soil. This assertion is sustained on the field experiments that included the use of activated carbon to reduce the input of wormwood allelochemicals to the soil environ-

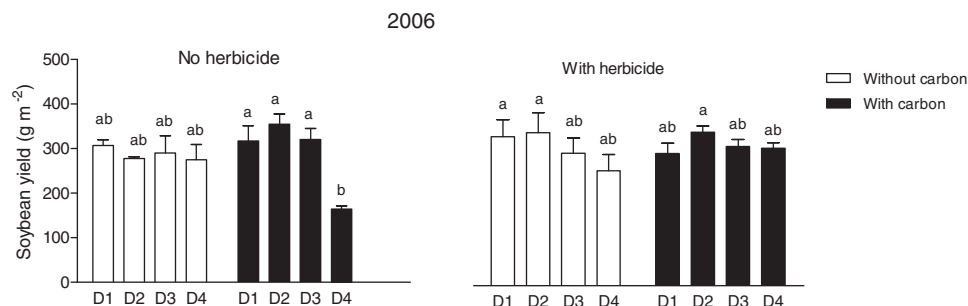


Fig. 4. Interaction effects of density (D), herbicide (with and no herbicide application) and carbon (with, black bars, and without, white bars) treatments on soybean yield (g m⁻²) in 2006. Vertical lines are SE. Different letters indicate significant differences according to Tukey's test at $p < 0.05$. F -test significant at $p < 0.05$.

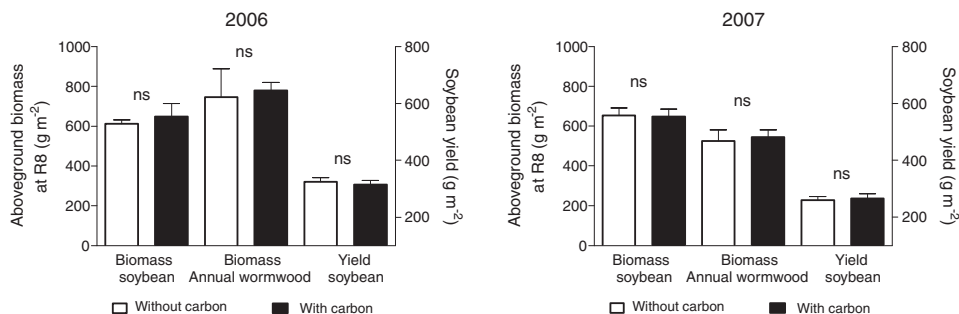


Fig. 5. Effect of activated carbon on soybean and annual wormwood aboveground biomass at maturity (g m⁻²) and soybean yield (g m⁻²) in control plots during 2006 and 2007. Control plots were pure soybean (D1) or pure annual wormwood (D5) without carbon and untreated herbicide (white bars) and with carbon and untreated herbicide (black bars), respectively. In soybean variables, values represent means and vertical lines are SE. Values of annual wormwood are the re-transformed means and vertical lines are SE. ns, not significant; *F*-test significant at $p < 0.05$.

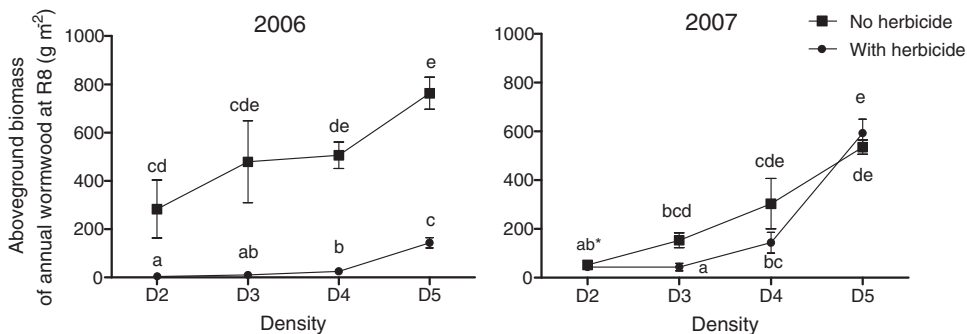


Fig. 6. Effect of density (D) and herbicide (with and no herbicide application) on aboveground biomass of annual wormwood (g m⁻²). D, (soybean/annual wormwood, plants m⁻²); D2, 40/2 plants m⁻²; D3, 40/4 plants m⁻²; D5, 0/8 plants m⁻². The symbols represent treatment means and vertical lines are SE. Different letters indicate significant differences according to Tukey's test at $p < 0.05$. *F*-test significant at $p < 0.05$.

ment. Although activated carbon is considered an effective tool for studying allelopathy under field conditions (Inderjit and Callaway, 2003), studies of allelopathy and competition interferences must be carefully interpreted, particularly when using activated carbon to separate both processes. In these experiments, activated carbon layer was experimentally tested as an effective way of capturing allelochemicals without direct measurable effects on the growth of the crop or the weed. The quantity of activated carbon used and the way in which it was applied did not directly influence the biomass and yield of soybean and the biomass of annual wormwood at both sampling development stages (R1 and R8), suggesting that the dif-

ferences among carbon treatments were not directly related to the presence or phytotoxicity of activated carbon but to the differences in allelochemicals in the soil. These results are in contrast with previous finding suggesting that activated carbon increased plant biomass (e.g. in *Festuca campestris*, *Poa compressa* and *Bromus tectorum*) even in the absence of the potentially allelopathic agent (Lau et al., 2008). Wurst and Van Beersum (2009) found a negative impact of activated carbon on growth and flowering of the legume. The presence of activated carbon also caused a significant decrease in the ability of *Larrea* sp. roots to inhibit the elongation of neighboring roots (Mahall and Callaway, 1991). Differences among

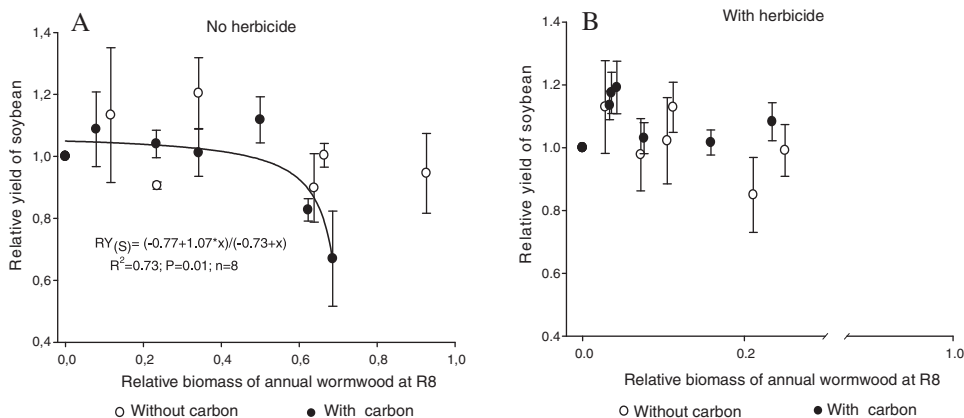


Fig. 7. Relationship between relative yield (RY, g m⁻²/g m⁻²) of soybean and relative biomass (RB, g m⁻²/g m⁻²) of wormwood at maturity, with and without carbon, untreated (A) and with herbicide application (B) during 2006 and 2007. The symbols represent treatment means and vertical lines are SE. In (A), solid line represents a hyperbolic model (Eq. (1)). Relative yield of soybean = 1 represents the values of soybean yield in the both non herbicide control plots: 306 and 317 g m⁻² for without and with carbon and in 2006 and 228 and 237 g m⁻² in 2007, respectively. In (B) soybean yield D1 was 337 and 299 g m⁻² for without and with carbon respectively in 2006 and 252 and 233 g m⁻² in 2007 respectively. Relative biomass of annual wormwood = 1 is the biomass of annual wormwood in D5 was 143 and 143 g m⁻² without and with carbon in 2006 and 577 and 607 g m⁻² in 2007.

Table 7

Means, standard error and relative values of soybean yield and aboveground biomass of annual wormwood at maturity of herbicide untreated plots in 2006 and 2007.

No herbicide application									
Year	Density level	With herbicide application				With carbon			
		Soybean		Annual wormwood		Soybean		Annual wormwood	
		Yield (SE) (g m ⁻²)	RY (g m ⁻² /g m ⁻²) ^a	Aboveground biomass (SE) (g m ⁻²)	RB (g m ⁻² /g m ⁻²) ^b	Yield (SE) (g m ⁻²)	RY (g m ⁻² /g m ⁻²) ^a	Aboveground biomass (SE) (g m ⁻²)	RB (g m ⁻² /g m ⁻²) ^b
2006	D1	306.50 (13.43)	1.00	0(0)	0	317.04 (34.41)	1.00	0(0)	0
	D2	277.62 (3.64)	0.91	175.78 (56.64)	0.24	354.49 (23.54)	1.12	390.14 (239.00)	0.50
	D3	289.76 (39.39)	0.95	691.55 (306.11)	0.93	320.77 (24.15)	1.01	266.69 (79.04)	0.34
	D4	275.25 (33.91)	0.90	476.50 (119.25)	0.64	212.30 (7.56)	0.67	535.26 (6.39)	0.69
	D5	0(0)	0	746.22 (141.43)	1.00	0(0)	0	779.89 (40.43)	1.00
2007	D1	228.13 (17.39)	1.00	0(0)	0	237.40 (2.53)	1.00	0(0)	0
	D2	258.50 (9.38)	1.13	61.34 (24.70)	0.12	258.24 (28.69)	1.09	42.96 (14.40)	0.08
	D3	274.67 (26.12)	1.20	179.52 (38.92)	0.34	246.93 (10.53)	1.04	127.02 (48.01)	0.23
	D4	228.95 (8.67)	1.00	349.00 (160.37)	0.66	196.37 (8.55)	0.83	256.82 (160.32)	0.62
	D5	0(0)	0	525.33 (54.75)	1.00	0(0)	0	544.60 (36.08)	1.00

D, (soybean/annual wormwood, plants m⁻²); D1, 40/0 plants m⁻²; D2, 40/2 plants m⁻²; D3, 40/4 plants m⁻²; D4, 40/8 plants m⁻²; D5, 0/8 plants m⁻².^a RY, Relative yield of soybean was calculated dividing soybean yield obtained in each subplot by the yield obtained in each control subplot in each year.^b RB, Relative biomass of annual wormwood was calculated dividing biomass obtained in each subplot by the biomass obtained in each control subplot in each year.**Table 8**

Means, standard error and relative values of soybean yield and aboveground biomass of annual wormwood in herbicide treated plots.

With herbicide application									
Year	Density level	Without carbon				With carbon			
		Soybean		Annual wormwood		Soybean		Annual wormwood	
		Yield (SE) (g m ⁻²)	RY (g m ⁻² /g m ⁻²) ^a	Aboveground biomass (SE) (g m ⁻²)	RB (g m ⁻² /g m ⁻²) ^b	Yield (SE) (g m ⁻²)	RY (g m ⁻² /g m ⁻²) ^a	Aboveground biomass (SE) (g m ⁻²)	RB (g m ⁻² /g m ⁻²) ^b
2006	D1	337.09 (38.62)	1.00	0(0)	0	299.24 (23.50)	1.00	0(0)	0
	D2	346.30 (45.21)	1.13	4.02 (0.50)	0.03	347.87 (14.02)	1.13	4.82 (1.83)	0.03
	D3	299.73 (35.21)	0.98	10.31 (1.59)	0.07	315.81 (15.24)	1.03	10.91 (3.71)	0.08
	D4	260.50 (11.17)	0.85	30.36 (7.88)	0.21	311.57 (12.29)	1.02	22.75 (5.66)	0.16
	D5	0(0)	0	143.30 (31.78)	1.00	0(0)	0	607.44 (90.65)	1.00
2007	D1	252.49 (52.47)	1.00	0(0)	0	233.60 (36.37)	1.00	0(0)	0
	D2	285.00 (20.22)	1.13	64.73 (0.14)	0.11	274.41 (15.29)	1.17	21.81 (9.64)	0.03
	D3	258.09 (34.72)	1.02	60.54 (28.18)	0.10	278.37 (19.47)	1.19	25.87 (12.58)	0.04
	D4	250.33 (20.76)	0.99	144.48 (94.21)	0.25	252.99 (14.13)	1.08	142.37 (19.57)	0.23
	D5	0(0)	0	577.60 (90.17)	1.00	0(0)	0	143.49 (35.82)	1.00

D, (soybean/annual wormwood, plants m⁻²); D1, 40/0 plants m⁻²; D2, 40/2 plants m⁻²; D3, 40/4 plants m⁻²; D4, 40/8 plants m⁻².^a Relative yield of soybean was calculated dividing soybean yield obtained in each subplot by the yield obtained in each control subplot in each year.^b Relative biomass of annual wormwood was calculated dividing biomass obtained in each subplot by the biomass obtained in each control subplot in each year.

these works and ours may be related to the quantity (thin layer) and conditions (covered with soil) in which the activated carbon was used.

The importance of allelochemicals in modulating the impact of weed interference on soybean crop yield became apparent when the data of the two experiments were normalized and subjected to a regression analysis.

As expected, according to the weed–crop competition model (Cousens, 1991), soybean RY was dependent on annual wormwood RB (weed density) responding to a hyperbolic model when allelopathy was reduced (with carbon). These results are in agreement with different authors (e.g. Oreja and Gonzalez-Andujar, 2007; Ryan et al., 2009), who showed that hyperbolic relationship is one of the major models describing the crop yield losses from additive experiments. However, other experiments conducted in pots showed that soybean biomass decreased as *A. annua* leaf tissue incorporated into the soil increased, following an exponential model (Lydon et al., 1997). At the same time, residue amendment of soil of *Chenopodium murale* L., resulted in a drastic reduction of growth in legume crops (chickpea and pea) (Batish et al., 2007). These results demonstrate the occurrence of allelopathy as a mechanism of interference under controlled conditions, but these responses may differ in complex, natural field systems (Ridenour and Callaway, 2001). In the field, allelopathy through allelochemicals released from aerial plant parts would depend on many factors, such as plant densities (Weidenhamer et al., 1989) and microbial activity (Ridenour and Callaway, 2001).

In this study, when competition and allelopathy occurred simultaneously, soybean RY was not affected by the presence of annual wormwood in the range of crop–weed densities studied, although weed densities were high enough to generate competition with soybean crop. Moreover, growing annual wormwood together with soybean and without carbon showed a positive effect on the average weight of nodules. This suggests that a possible mechanism explaining the apparent stability in soybean crop, in spite of weed competition, could involve positive and indirect interactions between the annual wormwood allelochemicals and the soil microorganisms, such as *B. japonicum*, generating positive changes in soybean nodulation. Positive and negative effects could happen simultaneously when species interact (Callaway, 1994) and the net effect of one species on the other may be the addition of both effects (Callaway, 1995). Direct effects of allelopathy on plant nodulation could be as important as indirect effects, altering nitrogen cycling through the effect on the chemical environment in which soil microorganisms grow (Blanco, 2007). Its consequences may be more diverse than previously assumed, since stimulatory effects may exist regularly in the field. Besides, the balance between the stimulation and inhibition depends on both plant density and the allelochemical concentration in the soil (Sinkkonen, 2001). Thus the stable soybean yield as increasing weed density could be mainly an indirect consequence of increase in the average unit of nodules (i.e. nodules were higher without carbon than with carbon in 2006). This higher weight per unit was associated with higher nodules that are localized in primary roots, which usually fix more nitrogen. In 2007, the greater number of root at D4 contributed to increase biomass of nodules in plots without carbon (750 g m^{-3}) compared to carbon treatments (287 g m^{-3}). These results do not agree with previous studies reporting a reduction of plant growth and nodulation related to the incorporation of allelopathic plant residues into the soil (Wardle et al., 1994; Batish et al., 2007). The differences between both studies may be related to either the type of allelochemicals involved (terpenoids vs. phenolic acids), the source of allelochemicals used (lixiviation from the plant vs. residue incorporation) or the nodulation process evaluated (nodule weight vs. leghemoglobin content). Thus, the presence of allelochemicals in a plant is not strong evidence for direct crop–weed allelopathy.

This is because the observed growth and nodulation may be due to the influence of these compounds on soil ecological processes rather than direct effects on the target plants (Inderjit and Weiner, 2001). The addition of plant litter to soil may also influence nutrient mobilization and soil pH, which can further influence nutrient immobilization and microbial activity (Facelli and Pickett, 1991).

Interestingly, when a sublethal dose of herbicide was applied in these experiments, mimicking spray drift in field applications, soybean RY in both carbon treatments remained stable as RB of annual wormwood increased. But at high densities of the weed (D4) soybean RY showed a decreasing trend in treatments without carbon (Soybean yield was reduced 85%, in 2006). These results suggest that, the positive effect of allelochemicals on nodulation was erased by the herbicide, decreasing soybean RY (at high RB of the weed). A negative effect of glyphosate on nitrogen fixation processes, observed in previous works, (Zablotowics and Reddy, 2004) may support this hypothesis.

Different studies have focused on allelopathic weed–crop interference including annual wormwood (Lydon et al., 1997) and other allelopathic species (Batish et al., 2007) with experiments under laboratory and greenhouse conditions (Inderjit et al., 2001; Inderjit and Nilsen, 2003). However, few experiments investigated the relative importance of competition and allelopathy under field conditions (Nilsson, 1994; Inderjit et al., 2001) where both or one of the competitive and allelopathy interferences could override the effect on another. To our knowledge, this is the first study showing allelopathic effects of annual wormwood on a coexisting soybean crop exposed to real spatio-temporal variation of weed density and herbicide sub-lethal doses. This study shows that the use of the additive experimental design and activated carbon to manipulate chemical effects in a realistic agroecological approach is an effective tool to investigate competition and allelopathy. Biotic and abiotic variations in natural soils create a great deal of conditionality in chemicals effects, and can either buffer or catalyze chemical effects (Inderjit and Callaway, 2003). Therefore, physical, chemical and biological processes in the soil environment, interacting with allelochemicals (Inderjit et al., 2001) may produce contrasting effects on soybean crop nodulation and the response of soybean RY.

Despite the difficulties in studying and clearly evaluating allelopathic interference in natural environments, this research provided strong evidence in support of the existence of positive effects of allelopathic and competitive interactions between annual wormwood and soybean crop under field conditions. In the future, it is necessary to develop a more holistic approach integrating allelochemicals into crop–weed management strategies. Multidisciplinary efforts involving plant ecology, physiology, biochemistry, soil science and microbiology, can address this complex research area to answer the key questions relating allelopathy and competition under field conditions.

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References

- Aldrich, R.J., 1984. Weed Crop Ecology: Principles in Weed Management. Breton publishers, USA.

- Azania, A.A.P.M., Azania, C.A.M., Alves, P.L.C.A., Palaniraj, R., Kadian, H.S., Sati, S.C., Rawat, L.S., Dahiya, D.S., Narwal, S.S., 2003. Allelopathic plants. 7. Sunflower (*Helianthus annuus* L.). Allelopathy J. 11, 1–20.
- Barney, J.N., Hay, A.G., Weston, L.A., 2005. Isolation and characterization of allelopathic volatiles from mugwort (*Artemisia vulgaris*). J. Chem. Ecol. 31, 247–265.
- Batish, R.D., Lavanya, K., Singh, H.P., Kohli, R.K., 2007. Phenolic allelochemicals released by *Chenopodium murale* affect the growth, nodulation and macromolecule content in chickpea and pea. J. Plant Growth Regul. 51, 119–128.
- Blanco, J.A., 2007. The representation of allelopathy in ecosystem-level forest models. Ecol. Modell. 209, 65–77.
- Callaway, R.M., 1994. Facilitative and interfering effects of *Arthrocnemum Subterminale* on winter annuals. Ecology 75, 681–686.
- Callaway, R.M., 1995. Positive interactions among plants. Bot. Rev. 61, 306–349.
- Callaway, R.M., 2002. The detection of neighbors by plants. Trends Ecol. Evol. 17, 104–105.
- Canner, S.R., Wiles, L.J., McMaster, G.S., 2002. Weed reproduction model parameters may be estimated from crop yield loss data. Weed Sci. 50, 763–772.
- Chen, P., Leather, G.R., 1990. Plant growth regulatory activities of artemisinin and its related compounds. J. Chem. Ecol. 16, 1867–1876.
- Cousens, R., 1991. Aspects of the design and interpretation of competition (interference) experiments. Weed Technol. 5, 664–673.
- de la Fuente, E.B., Suárez, S.A., Ghera, C.M., Leon, R.J.C., 1999. Soybean weed communities: relationships with cultural history and crop yield. Agron. J. 91, 234–241.
- de la Fuente, E.B., Suárez, S.A., Ghera, C.M., 2006. Soybean weed community composition and richness between 1995 and 2003 in the Rolling Pampas (Argentina). Agric. Ecosyst. Environ. 115, 229–236.
- de la Fuente, E.B., Perelman, S., Ghera, C.M., 2010. Weed and arthropod communities in soybean as related to crop productivity and land use in the Rolling Pampa, Argentina. Weed Res. 50, 561–571.
- de Wit, C.T., 1960. On competition. Versl. Landbouwk. Onderz. 66, 1–82.
- Delabays, N., Simonnet, X., Gaudin, M., 2001. The genetics of artemisinin content in *Artemisia annua* L. and the breeding of high yielding cultivars. Curr. Med. Chem. 8, 1795–1801.
- Duke, S.O., Vaughn, K.C., Croom, E.M., Elsohly, N.H., 1987. Artemisinin, a constituent of annual wormwood (*Artemisia annua*), is a selective phytotoxin. Weed Sci. 35, 499–505.
- Einhellig, F.A., 1996. Interactions involving allelopathy in cropping systems. Agron. J. 88, 886–893.
- Einhellig, F.A., 1999. An integrated view of allelochemicals and multiple stresses. In: Inderjit, Dakshini, K.M.M., Foy, C.L. (Eds.), Principles and practices in Plant Ecology: Allelochemical Interactions. CRC Press, pp. 479–494.
- Facelli, J.M., Pickett, S.T.A., 1991. Plant litter: light interception and effects on an old-field plant community. Ecology 72, 024–1031.
- Fehr, W.R., Caviness, C.E., 1977. Stages of soybean development. Special Report 80. Iowa State University, Ames, Iowa, USA.
- Harborne, J.B., 1997. Biochemical plant ecology. In: Dey, P.M., Harborne, J.B. (Eds.), Plant Biochemistry. Academic Press, San Diego, USA, pp. 503–516.
- Hernandez, A., Garcia-Plazzola, J.I., Becerril, J.M., 1999. Glyphosate effects on phenolic metabolism of nodulated soybean (*Glycine max* L. Merr.). J. Agric. Food Chem. 47, 2920–2925.
- Inderjit, Weiner, J., 2001. Plant allelochemical interference or soil chemical ecology? Perspect. Plant Ecol. Evol. Syst. 4, 3–12.
- Inderjit, Kaur, M., Foy, C.L., 2001. On significance of field studies in allelopathy. Weed Technol. 15, 792–797.
- Inderjit, Mallik, A.U., 2002. Can *Kalmia angustifolia* interference to black spruce (*Picea mariana*) be explained by allelopathy? Forest Ecol. Manage. 160, 75–84.
- Inderjit, Callaway, R.M., 2003. Experimental designs for the study of allelopathy. Plant Soil 256, 1–11.
- Inderjit, Nilsen, E.T., 2003. Bioassays and field studies for allelopathy in terrestrial plants: progress and problems. Crit. Rev. Plant Sci. 22, 221–238.
- Inderjit, 2005. Soil microorganisms: an important determinant of allelopathic activity. Plant Soil 274, 227–236.
- Lau, J.A., Puliafico, K.P., Kopshever, J.A., Steltzer, H., Jarvis, E.P., Schwarzländer, M., Hufbauer, R.A., 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. New Phytol. 178, 412–423.
- Lydon, J., Teasdale, J.R., Chen, P.K., 1997. Allelopathic activity of annual wormwood (*Artemisia annua*) and the role of artemisinin. Weed Sci. 45, 807–811.
- Mahall, B.E., Callaway, R.M., 1991. Root communication among desert shrubs. Ecology 88, 874–876.
- Molish, H., 1937. Der einfluss einer pflanze auf die andere-Allelopathie. Fischer, Jena.
- Moorman, T.B., Becerril, J.M., Lydon, L., Duke, S.O., 1992. Production of hydroxybenzoic acids by *Bradyrhizobium japonicum* strains after treatment with glyphosate. J. Agric. Food Chem. 1002 (40), 289–293.
- Nilsson, M.C., 1994. Separation of allelopathy and resource competition by the boreal dwarf shrub *Empetrum hermaphroditum* Hagerup. Oecologia 98, 1–7.
- Oreja, F.H., Gonzalez-Andujar, J.L., 2007. Modelling competition between large crabgrass (*Digitaria sanguinalis*) and glyphosate-resistant soybean in the Rolling Pampas of Argentina. Commun. Biom. Crop Sci. 2, 62–67.
- Park, S.E., Laurence, R.B., Watkinson, A.R., 2003. The theory and application of plant competition models: an agronomic perspective. Ann. Bot. 92, 741–748.
- Qian, G.P., Yang, Y.-W., Ren, Q.-L., 2005. Determination of artemisinin in *Artemisia annua* L. by reversed phase HPLC. J. Liq. Chromatogr. Relat. Technol. 28, 705–712.
- Reddy, K.N., 2000. Effect of glyphosate on growth, chlorophyll content and nodulation in glyphosate-resistant soybeans (*Glycine max*) varieties. J. New Seeds 2, 37–52.
- Rice, E.L., 1984. Allelopathy. Academic Press, New York, Orlando, USA.
- Ridenour, W.M., Callaway, R.M., 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. Oecologia 126, 444–450.
- Ryan, M.R., Smith, R.G., Mortensen, D.A., Teasdale, J.R., Curran, W.S., Seidel, R., Shumways, D.L., 2009. Weed crop competition relationships differ between organic and conventional cropping systems. Weed Res. 49, 572–580.
- Seigler, D.S., 1996. Chemistry and mechanisms of allelopathic interactions. Agron. J. 88, 876–885.
- Sinkkonen, A., 2001. Density-dependent chemical interference—an extension of the biological response model. J. Chem. Ecol. 27, 1513–1523.
- USDA, 1999. Keys of Soil Taxonomy, 8th ed. Pocahontas Press, Blacksburg, VA.
- Vila-Aiub, M.M., Ghera, C.M., 2005. Building up resistance by recurrently exposing target plants to sublethal doses of herbicide. Eur. J. Agron. 22, 195–207.
- Wardle, D.A., Nicholson, K.S., Ahmed, K.S., Rahman, A., 1994. Interference effects of the invasive plant *Carduus nutans* L. against the nitrogen fixation ability of *Trifolium repens* L. Plant Soil 163, 287–297.
- Weidenhamer, J., Hartnett, D., Romeo, J., 1989. Density-dependent phytotoxicity: distinguishing resource competition and allelopathic interference in plants. J. Appl. Ecol. 26, 613–624.
- Weston, L.A., 1996. Utilization of allelopathy for weed management in agroecosystems. Agron. J. 88, 860–866.
- Weston, L.A., Duke, S.O., 2003. Weed and crop allelopathy. Crit. Rev. Plant Sci. 22, 367–389.
- Wurst, S., Van Beersum, S., 2009. The impact of soil organism composition and activated carbon on grass-legume competition. Plant Soil 314, 1–9.
- Zablotowicz, R.M., Reddy, K.N., 2004. Impact of glyphosate on the *Bradyrhizobium japonicum* symbiosis with glyphosate-resistant transgenic soybean: a minireview. J. Environ. Qual. 33, 825–831.