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Arthropod communities related to different mixtures of oil (*Glycine max* L. Merr.) and essential oil (*Artemisia annua* L.) crops

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ABSTRACT

Plants can host many herbivores and their natural enemies during their growth cycles. For this reason, changes in the relative abundance of crop and weed plants in a monocropping system as well as different crop plants in an intercropping system may produce great bottom up impacts in the specific and functional structure of spontaneous communities of arthropods. The hypothesis of this study was that the combination of two contrasting species, soybean (*Glycine max*, Fabaceae, N_2 fixing plant) and annual wormwood (*Artemisia annua*, Asteraceae, VOCs plant), would be related to different spontaneous communities of arthropods depending on the proportion of each species, and this would favor crop biodiversity without compromising crop production. The objectives of the study were: (a) to analyze the differences of spontaneous communities of arthropods related to different soybean (S)–annual wormwood (W) mixtures, using standard crop management for S production in Argentina, (b) to determine S and W total biomass and W essential oil content and yield and, (c) to analyze the relationship between arthropod communities and crop productivity. Factorial field experiments with 3 replications were done during 2006 and 2007. S density was kept constant (40 plants m^{-2}) and different W densities (plants m^{-2}) were added. Treatments were pure S, S + 2W, S + 4W, S + 8W and pure W (8 plants m^{-2}). Arthropods were sampled at soybean full flowering and were classified in functional groups as herbivores and non-herbivores. S and W total and relative biomass and W essential oil content and yield from leaves and inflorescences were estimated in reproductive stage. Arthropod morphospecies abundance and richness were determined for each treatment. Data were analyzed using uni (ANOVA) and multivariate (CCA) techniques. Arthropods belonging to 7 orders presented a total richness of 48 morphospecies in 2006 and 36 in 2007, while total abundance was 379 in 2006 and 318 in 2007. The proportion of non-herbivores was higher than the proportion of herbivores. Different arthropod communities were observed according to each treatment. No differences were found among treatments in S + W and S total biomass production, while W total biomass and essential oil yield were both different among treatments. Relative biomass production of S and W was the main explanatory variable related to the contrast of arthropod communities between pure annual wormwood (W) and the rest of the treatments. Annual wormwood could be used as an accompanying essential oil crop or left as a weed in the densities tested in this work, favoring biodiversity and, eventually, pest management without compromising soybean crop yield.

1. Introduction

Plants can host many herbivores and natural enemies of herbivores during their growth cycles. For this reason, changes in the relative abundance of different plant components may produce great bottom up impacts in the spontaneous communities of arthropods (Marshall et al., 2003). Said components are crop and weed plants in a monocropping system or different crop plants in an intercropping system. As primary producers, crop and weed plants directly serve as food for herbivores and, in turn, herbivores can serve as a food source for beneficial arthropods. Weeds can also serve as alternative hosts for crop pests when the crop is absent or its phenological stage is unsuitable for the pest. Many beneficial arthropods also feed directly on plant material, such as nectar or pollen, at some stage during their life cycle. Such plant feeding, at adult or immature stages, is often required for optimum performance of the beneficial arthropods (Norris and Kogan, 2005) in addition to the herbivore prey.

Interactions between arthropods and plants are mediated by physical (color, shape, and texture) and chemical (odor and taste) characteristics of the plants (Gershenzon, 1984; Coleman, 1986; Rausher, 1992; Dicke, 1999; di Giulio et al., 2001; Norris and Kogan,

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2005). Plant traits such as leaf nitrogen and water content, toughness, thickness, color, internal and volatile organic compounds (VOCs), are known to be important determinants of arthropod behavior (Coleman, 1986; Gershenzon, 1984; Howe and Westley, 1988; Pastor et al., 1997; Tomassini Barbarossa et al., 2007) and thus, of community structure (Lenardis et al., 2007). For instance, VOCs often serve as attractants or repellents for feeding and oviposition (Städler, 1986; Bernays and Graham, 1988; Unsicker et al., 2009). Stimulus showed dose-dependent opposite effects, attraction or repellency, on odor-induced insect behavior depending on signal concentrations (Dethier and Yost, 1952, cited by Tomassini Barbarossa et al., 2007). VOCs dose is related to the genotype and the environment (Gershenzon, 1984).

VOCs emitted by crops can mix with those emitted by other species that share the habitat, for example weeds or accompanying crops (Harborne, 1987; Städler, 1992; Bernays and Chapman, 1994). Many arthropods may be very sensitive to the relative species composition in the mixture (Khan et al., 2008). The role of different weeds-crop or intercrop mixtures on spontaneous communities of arthropods may be explained considering that weeds or accompanying crops represent a diluting factor in the concentration of the predominant crop plant. The introduction of an element of biodiversity expands the spectrum of natural enemies available to colonize the crop stand depending on the availability of prey or hosts (Root, 1973). If the weed or accompanying crop is chemically or taxonomically related to the main crop, the associated fauna may be a threat to the crop. Otherwise, the herbivores may be irrelevant for the crop, unless they are a polyphagous species. The arthropod fauna on unrelated weeds or accompanying crops may harbor prey for natural enemies that are important for the crop. However, the importance assigned to plant mixtures to develop IPM and as sources of diversity should be weighed in relation to competition (i.e. crop yield loss) and their role in hosting beneficial arthropods as well as crop pests (Norris and Kogan, 2005).

Although habitat richness provides organisms that can take advantage of this habitat (Benton et al., 2003), the general trend of agricultural intensification has been the simplification and specialization of the agro ecosystem including very low richness of species (few weeded crops), genes (few cultivars) and management (few active ingredients or tillage systems) (Vandermeer et al., 1998). Moreover, high input of fertilizers and chemical pesticides has led to losses in the arthropod populations (Ewald and Aebischer, 1999; Biaggini et al., 2007), directly through toxicity and indirectly through both food availability and habitat quality (Kromp, 1999; Holland and Luff, 2000). The Pampean agroecosystem is not an exception to this trend since transgenic soybean [*Glycine max* (L.) Merr.], resistant to glyphosate and permanent no-tillage, is the main land use in Argentina (Pengue, 2005; Cerdeira and Duke, 2006). In addition to the effect of agroecosystem simplification, fields growing with herbicide resistant soybean could have fewer arthropods (Buckelew et al., 2000), probably due to the reduction of weed population and not through the direct effect of herbicide or transgenic varieties on arthropods (Marshall et al., 2003; Norris and Kogan, 2005).

Annual wormwood (*Artemisia annua* L.) is a component of the weed community in the Rolling Pampa Argentina (de la Fuente et al., 2006) that could interfere with soybean crop by competition for resources and by the release of VOCs affecting crop growth (Duke et al., 1987; Weston, 1996; Weston and Duke, 2003). However, annual wormwood is also an essential oil crop of economic value (Bagchi et al., 2003; Fulzele et al., 1995) that could be introduced as a planned component of the agroecosystem.

It is expected that the combination of two contrasting species (i.e. soybean, Fabaceae, N_2 fixing plant and annual wormwood, Asteraceae, VOCs plant) will lead to greater overall biological productivity than each species grown separately because the mixture can use resources more effectively than separate monocultures (Willey, 1979; Willey et al., 1986). In many instances, the increased resource use is not a necessary condition for intercrop advantage over separate monocultures (Vandermeer, 1989; Keating and Carberry, 1993; Ong and Black, 1994). Facilitation is frequently involved, such as when one crop attracts natural enemies to the system and thus facilitates the escape of the second crop from some fraction of pest attack (Vandermeer, 1984, 1989).

Within this context, the hypothesis of this study was that the combination of two contrasting species, soybean and annual wormwood, would be related to different spontaneous communities of arthropods depending on the proportion of each species and this would favor crop biodiversity without compromising crop production. Therefore, the objectives of the study were (a) to analyze differences in spontaneous communities of arthropods related to different soybean–annual wormwood mixtures using standard crop management for soybean production in Argentina, (b) to determine soybean and annual wormwood total and relative biomass and annual wormwood essential oil content and yield and, (c) to analyze the relationship between arthropod communities and crop productivity.

2. Materials and methods

2.1. Field experiment

Two factorial field experiments were carried out in the Faculty of Agronomy, University of Buenos Aires (34°35′5″ lat. S, 58°29′ long. W), during 2006 and 2007. Treatments were arranged in a completely randomized block design in 2006 and in a completely randomized design in 2007 with three replications. In both years a soybean cultivar of maturity group IV (DM 4800) inoculated with *Bradyrhizobium japonicum* (10^9 colony forming units/g inoculant) was sown in plots 8 m long and 2 m wide with 0.35 m inter-row spacing on 10 January. In order to synchronize soybean and annual wormwood growth, annual wormwood was sown previously and then transplanted into the inter-row, 10 days after soybean sowing.

According to an additive model, soybean density in each plot was kept constant (40 plants m^{-2}) and different densities of annual wormwood plants were added. Thus, different treatments were pure soybean (S) and annual wormwood (W) (8 plants m^{-2}), and different mixtures of soybean (40 plants m^{-2}) and 2 (2W), 4 (4W) and 8 (8W) annual wormwood plants m^{-2}.

During the experiment, spontaneous weeds were removed manually. The crop was irrigated when needed to avoid water stress. Herbivorous arthropods were chemically controlled only during vegetative stage of soybean (V2; Fehr and Caviness, 1977) with the equivalent of 100 cm^3 ai ha^{-1} of cypermethrin and 0.8 l ha^{-1} of chlorpyrifos.

2.2. Arthropod sampling and determination

In both years, arthropods were sampled when soybean was at full flowering (R2; Fehr and Caviness, 1977). This time interval was chosen based on three criteria: (1) spring–summer communities were present, (2) chemical control had already been applied, and (3) crops had achieved maximum ground cover. Arthropod sampling was done using a sweep net (Tonkyn, 1980). Fixed net sizes (30 cm diameter) and sweeping patterns were used (two net sweepings in each plot) in the central zone of each plot, avoiding margins. In both years, the sampling was carried out under similar climatic conditions (sunny and without wind), at the same time of day, between 10.00 AM and 15.00 PM (h) each day.

Arthropod determination was performed at order, family and morphospecies level in all cases, and order and morphospecies level for Araneae. Although detailed taxonomy usually improves the interpretation of results, the determination work at species level is usually time-consuming, if not impossible, due to lack of taxonomic expertise in some orders, at the study site. The analysis at morphospecies level allows the study of arthropod communities since the differences between the number of morphospecies and taxonomic species are in many cases less than 3, 3% (Derraik et al., 2002). Each family was represented by only one morphospecies.

Each arthropod morphospecies was classified in functional groups as herbivores and non-herbivores. Their habits and food preferences during the crop cycle were determined using anatomic characteristics mainly based on feeding traits and not exclusively on taxonomic membership and bibliography (Richards and Davies, 1984; Arroyo Varela and Viñuela Sandoval, 1991). Although defining functional groups in such a way is not a perfect approach, it is an efficient and useful step forward to relate diversity and agroecosystems function (Perner et al., 2005).

The arthropod specimens that document the observations are available in the author's personal arthropod collection in the Faculty of Agronomy, University of Buenos Aires.

2.3. Plant measurements

During reproductive stage (R5; Fehr and Caviness, 1977), soybean plants in 0.75 m^2 and annual wormwood plants in 1 m^2 were harvested and total aerial biomass was determined. Another sample of five annual wormwood plants per plot was kept in the freezer (−10 °C) until hydro distillation. At the moment of distillation, the stems of the plants were separated from whole aerial parts. Then the contents of essential oil from leaves and inflorescences (LI) as a whole were determined from the fresh material. A subsample of all units LI was dried at 70 °C to determine water content. Each unit LI was sliced and hydrodistilled for 1 h using a Clevenger type apparatus according to the European Pharmacopeia method (Pharmacopée Européene, 1997).

2.4. Data analyses

Data were summarized in a table considering the abundance (number of individuals by morphospecies in each treatment) and the richness (or number) of morphospecies or functions occurring in each treatment (Whittaker, 1975; Magurran, 1988).

Abundance and richness data were transformed using square root and then analyzed with analysis of variance, ANOVA, using SAS version 9.1 (SAS Institute Inc., 2003). Means were compared by the Tukey's significant difference test at the 0.05 probability level.

The presence–absence data of arthropod morphospecies in each treatment was subjected to Multi-Response Permutation Procedures (MRPP) (Mielke, 1984) using PC-ORD Multivariate Analysis of Ecological Data Version 5.0 (McCune and Mefford, 1995). This method was used to analyze differences among treatments in morphospecies composition.

Biomass and essential oil yield were analyzed using analysis of variance, ANOVA SAS Version 9.1 (SAS Institute Inc., 2003). In 2006, two samples of the essential oil of pure stand annual wormwood treatment were omitted from the analysis because of problems during hydro-distillation. In 2007, essential oil yields were transformed using square root to homogenize variance. Means were compared by the Tukey's significant difference test at the 0.05 probability level.

The relationship between arthropod abundance and explanatory variables of plants in the different treatments was analyzed using a direct gradient analysis, canonical correspondence analysis (CCA) using PC-ORD Multivariate Analysis of Ecological Data Version 5.0 (McCune and Mefford, 1995). CCA constructs those linear combinations (axes) of explanatory variables along which the distribution of the morphospecies are maximally separated (ter Braak, 1987), and it has a great potential for examining the response of communities to diverse variables (Kenkel et al., 2002). Axis scores were centered and standardized to unit variance, and were scaled to optimize representation of treatments. The explanatory variables used were total biomass (soybean + annual wormwood) and relative annual wormwood biomass (annual wormwood biomass/total biomass). To determine association between data and explanatory variables, a biplot from CCA was obtained by overlaying a vector diagram based on coefficients from the canonical functions describing each canonical axis.

3. Results

3.1. Arthropod communities

Forty-eight and 36 arthropod morphospecies were surveyed during 2006 and 2007, with a total abundance of 379 and 318 morphospecies during 2006 and 2007, respectively. In both years, arthropods belonged to the same 7 orders, Coleoptera, Diptera, Hemiptera, Hymenoptera, Araneae, Orthopthera and Lepidoptera. Functions were determined in more than 94% of the cases. In both years, total abundance and richness of non herbivorous arthropods was higher than total abundance and richness of herbivorous arthropods ($P = 0.05$). Although species composition was different, the relationship herbivores/non herbivores of morphospecies abundance and richness was similar among treatments, ranging from 0.17 to 0.29 for abundance and 0.33 to 0.46 for richness during 2006 and from 0.1 to 0.29 for abundance and 0.17 to 0.46 for richness during 2007 (Tables 1 and 2).

The combination of arthropod morphospecies and treatments resulted in 5 different arthropod communities and 10 faunistic groups in 2006 and 9 faunistic groups in 2007. Each of those communities represented treatments of pure annual wormwood, soybean and different mixtures of soybean annual wormwood (columns in Tables 1 and 2).

In both years, group I included morphospecies common to all arthropod communities, whereas the other groups characterized different communities. In 2006, the arthropod community related to pure annual wormwood was characterized by groups I, II, III and IV and pure soybean community was characterized by the presence of groups I, II, III, V and VI and the absence of the rest of the groups. The 2W mixture was characterized by groups I, II, III, IV, VI and VII; 4W by groups I, II and IX and 8W by groups I, III, VIII and X. Thus, group V was present only in soybean community; group VI in pure soybean and 2W communities; group IV mainly in pure annual wormwood community and groups VII, VIII and IX were only present in the mixtures (Table 1). According to MRPP, species composition was different between communities of pure soybean and mixtures with high proportion of annual wormwood (W and 8W, $P = 0.10$ and 0.06, respectively) and between mixtures with high (8W) and low (2W) proportion of annual wormwood ($P = 0.005$). No differences were found between treatments in total abundance and richness of arthropod morphospecies (Table 1).

In 2007, pure annual wormwood community was characterized by groups I, II, III, IV and V and the absence of the rest of the groups, while pure soybean was characterized by groups I, II and VI and the absence of the rest of the groups. The 2W mixture was characterized by groups I, II, III, VI and VII; 4W by groups I, II, III, IV, VI and IX and 8W by groups I, II, III, VI and VIII. Thus, group V was present only in pure annual wormwood community and groups VII, VIII and IX were present only in the mixtures (Table 2). According to MRPP, species composition was different ($P = 0.001$) between communities of pure annual wormwood and the rest of the treatments (S, 2W, 4W and 8W). Total abundance of arthropod morphospecies

Table 1
Faunistic group, order, family, function and abundance of arthropod morphospecies of the pure soybean, pure annual wormwood and different soybean–wormwood mixtures during 2006.

Faunistic group	Order code[a]	Family	Function	Abundance				
				Communities				
				Pure stands		Mixtures of soybean/annual wormwood		
				W[b]	S[c]	2W[d]	4W[e]	8W[f]
	DIP	Dolichopodidae	nh[g]	12	12	12	9	9
	HYM	Serphidae	nh	9	12	17	5	2
I	COL	Coccinelidae	nh	4	4	21	1	11
	DIP	Tephritidae	nh	4	6	9	6	1
	HEM	Corixidae	h[h]	2	6	2	1	3
	HYM	Evaniidae	nh	5	7	7	5	
	DIP	Phoridae	nh	2	3	10	6	
II	HEM	Miridae	h	1	2	9	2	
	HYM	Braconidae	nh	1	2	6	2	
	HEM	Pentatomidae	h	5	1	3	1	
	HYM	Chalcididae	nh	4	6	9		3
	COL	Chrysomelidae	h	4	1	3		1
III	DIP	Lonchopteridae	nh	3	3	1		1
	ARA		nh	1	1	2		
	ORT	Tettigoniidae	h	1	1			
	HEM	Ochteridae	nh	2		3		1
IV	HYM	Vespidae	nh	1		1		
	LEP	Noctuidae	nh	1				
	LEP	Pyralidae	h	1				
	HEM	Nabidae	nh		3			
	DIP	Conopidae	nh		2			
	HEM	Gerridae	nh		2			
	COL	Tenebrionidae	nh		1			
V	DIP	Empididae	nh		1			
	DIP	Mycetophilidae	nh		1			
	DIP	Nemestrinidae	nh		1			
	HEM	Alydidae	h		1			
	HEM	Potamocoridae			1			
	HEM	Thaumastocoridae	h		1			
	HYM	Ichneumonidae	nh		3	5		
VI	COL	Scarabaeidae	h		4	4		
	DIP	Calliphoridae	nh		1	2		
	DIP	Ceratopogonidae	nh		1	1		
	COL	Staphylinidae	nh			4		
	DIP	Culicidae	nh			2		
	HEM	Tingidae	h			2		
VII	DIP	Bombyliidae	nh			1		
	DIP	Anisopodidae				1		
	DIP	Sarcophagidae	nh			1		
	HEM	Piesmatidae	h			1		
	HYM	Formicidae	nh			1		
	DIP	Sepsidae	nh					2
VIII	DIP	Cuterebridae	nh					1
	ORT	Acrididae	h					1
IX	DIP	Piophilidae	nh				1	
	DIP	Sciaridae	nh				1	
X	HEM	Ceratocombidae	h		3		2	2
	DIP	Tachinidae	nh			2		1
Total morphospecies abundance				63a	93a	142a	42a	39a
Total morphospecies richness				19a	30a	29a	13a	14a

Mean values followed by different letters within the same row are significantly different ($P < 0.05$) according to the method of Tukey.

[a] COL = Coleoptera; HEM = Hemiptera; ORT = Orthoptera; HYM = Hymenoptera; DIP = Diptera; LEP = Lepidoptera; ARA = Araneae.

[b] Pure annual wormwood.

[c] Pure soybean.

[d] Mixture of S and 2W plants m^{-2}.

[e] Mixture of S and 4W plants m^{-2}.

[f] Mixture of S and 8W plants m^{-2}.

[g] Non herbivores.

[h] Herbivores.

Table 2
Faunistic group, order, family, function and abundance of arthropod morphospecies of the pure soybean, pure annual wormwood and different soybean–wormwood mixtures during 2007.

Faunistic group	Order code[a]	Family	Function	Abundance				
				Communities				
				Pure stands		Mixtures of soybean/wormwood		
				W[b]	S[c]	2W[d]	4W[e]	8W[f]
	HYM	Serphidae	nh[g]	12	12	9	13	9
I	DIP	Dolichopodidae	nh	6	1	10	3	7
	DIP	Culicidae	nh	6	2	2	1	1
	HEM	Coreidae	h[h]	6	1	2	2	1
	DIP	Tephritidae	nh	22	1		1	2
	DIP	Phoridae	nh	15	1	1		
II	HEM	Miridae	h	16	3		1	1
	HYM	Formicidae	nh	12	1	1		2
	HYM	Chalcididae	nh	5	3	4	1	
	DIP	Empididae	nh	2	1			1
	COL	Coccinelidae	nh	13		2	1	6
III	HEM	Ceratocombidae	h	2			4	8
	DIP	Lonchopteridae	nh	2		1	7	6
	HYM	Braconidae	nh	6			3	2
	DIP	Calliphoridae	nh	4			1	
IV	DIP	Tachinidae	nh	2			1	
	HYM	Megachilidae	nh	1			1	
	LEP	Noctuidae	nh	1				
V	COL	Curculionidae	h	1				
	COL	Mordellidae	nh	1				
	HYM	Vespidae	nh		1	1		2
	COL	Chrysomelidae	h		1	1	2	1
VI	HEM	Anthocoridae			1			
	HYM	Cynipidae					1	1
	ORT	Tettigonidae	h		1		1	
	DIP	Scaptophagidae	nh			1		
VII	DIP	Simuliidae	nh			1		
	DIP	Tipulidae	h/nh			1		
	HEM	Hidrometridae	nh			1		
	DIP	Scyomyzidae	nh					12
VIII	DIP	Pipunculidae	nh					1
	HEM	Lygaeidae	h/nh					2
	HEM	Pentatomidae	h					1
	ARA		nh				3	
IX	COL	Scarabaeidae	h				1	
	DIP	Chamaemyiidae	nh				1	
Total morphospecies abundance				135a	30b	38b	49b	66b
Total morphospecies richness				20 a	14b	15b	20ab	19ab

Mean values followed by different letters within the same row are significantly different ($P < 0.05$) according to the method of Tukey.

[a] COL = Coleoptera, HEM = Hemiptera, ORT = Orthoptera, HYM = Hymenoptera; DIP = Diptera, LEP = Lepidoptera, ARA = Araneae.

[b] Pure annual wormwood.

[c] Pure soybean.

[d] Mixture of S and 2W plants m^{-2}.

[e] Mixture of S and 4W plants m^{-2}.

[f] Mixture of S and 8W plants m^{-2}.

[g] Non herbivores.

[h] Herbivores.

was higher in pure annual wormwood than in the rest of the treatments ($P < 0.0001$). Total richness of arthropods was higher in pure annual wormwood than in pure soybean and 2W mixture ($P < 0.01$) (Table 2).

3.2. *Crop production*

No differences were observed in total biomass between the mixtures (soybean + annual wormwood) and pure soybean in both years. Total biomass produced by pure annual wormwood was higher in pure stands than in the rest of the treatments ($P = 0.006$ in 2006, $P < 0.001$ in 2007). Mimicking differences in total biomass, essential oil yield was higher in pure annual wormwood than in the rest of the treatments ($P = 0.004$ in 2006 and $P = 0.09$ in 2007; Table 3).

3.3. *Arthropod and crop production relationship*

Arthropods and treatments were sorted in relation to the main CCA axes. In both years, axis 1 presented a contrast between pure annual wormwood (W) and the rest of the treatments (Figs. 1 and 2). In 2006, W was related to the abundance of Noctuidae, Vespidae, Lonchopteridae, Tettigoniidae, Sepsidae, Acrididae, Cuterebridae, Pentatomidae, Chrysomelidae, Ochteridae and of Araneae (Fig. 1). In 2007, W was related to Phoridae, Tephritidae, Tachinidae, Calliphoridae, Miridae, Formicidae, Mordellidae,

Table 3
Total soybean and annual wormwood biomass production and annual wormwood essential oil production in the different treatments during 2006 and 2007.

Treatments	Total biomass (g m^{-2})		Soybean biomass (g m^{-2})		Annual wormwood biomass (g m^{-2})		Essential oil (ml m^{-2})	
	2006	2007	2006	2007	2006	2007	2006	2007
W[a]	635.90	321.66	–	–	635.90 a	321.66 a	282.19 a	119.35
S[b]	551.64	506.60	551.64	506.60	–	–	–	–
2W[c]	578.54	488.49	515.36	479.82	37.93 b	8.67 b	12.82 b	5.60
4W[d]	600.93	530.42	511.27	506.92	89.65 b	23.49 b	25.06 b	8.72
8W[e]	634.05	567.92	456.05	524.84	178.00 b	43.08 b	42.83 b	25.75
P	0.88	0.08	0.17	0.82	0.006	<0.001	0.004	0.09

Mean values followed by different letters within the same column are significantly different ($P < 0.05$) according to the method of Tukey.

[a] Pure annual wormwood.

[b] Pure soybean.

[c] Mixture of S and 2W plants m^{-2}.

[d] Mixture of S and 4W plants m^{-2}.

[e] Mixture of S and 8W plants m^{-2}.

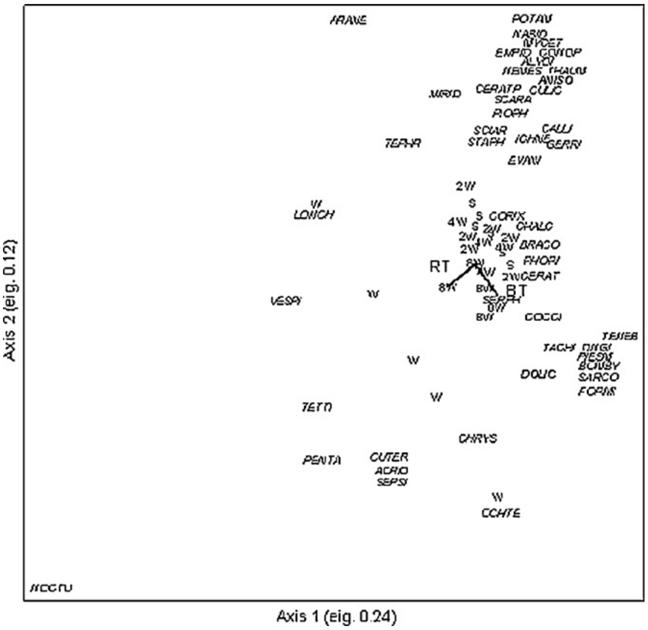

Fig. 1. Ordination diagram of morphospecies and plots in the two principal axes of CCA during 2006. Symbols: treatments (▲) and arthropod morphospecies (+). Treatment codes: pure soybean (S), pure annual wormwood (W) and their mixtures including 2, 4, and 8 plants of annual wormwood (2W, 4W, and 8W). Morphospecies codes: first 5 letters of the family name. Vectors represent explanatory variables total biomass (BT) and relative biomass (RB).

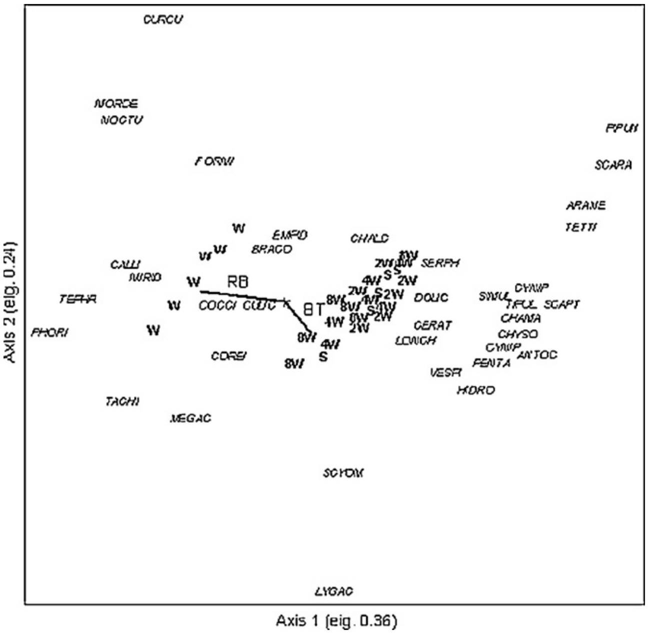

Fig. 2. Ordination diagram of morphospecies and plots in the two principal axes of CCA during 2007. Symbols: treatments (▲) and arthropod morphospecies (+). Treatments codes: pure soybean (S), pure annual wormwood (W) and their mixtures including 2, 4, and 8 plants of annual wormwood (2W, 4W, and 8W). Morphospecies codes: first 5 letters of the family name. Vectors represent explanatory variables total biomass (BT) and relative biomass (RB).

Noctuidae, Curculionidae, Coreidae, Coccinelidae, Culicidae, Braconidae, Empididae and Megachilidae (Fig. 2). In both years, relative biomass was the main explanatory variable related to axis 1 ($r = -0.67$ in 2006 and $r = -0.82$ in 2007).

4. Discussion

Different spontaneous communities of arthropods were related to the proportion of soybean and annual wormwood in the mixtures tested in this work. Crop mixtures affected arthropod community structure and diversity. This was probably due to the promotion of heterogeneity in the plant growth and the chemical composition of its tissues, in addition to the presence of refuges and the emission of odor and visual signals, generating variability in the behavior and movement of arthropods (Tilman and Pacala, 1993; Pastor et al., 1997). Soybean is a good source of food for herbivores and indirectly for their associated non-herbivores, thus promoting bottom up processes. In turn, annual wormwood is a source of food for herbivores and also an important VOCs signal emissor promoting, not only bottom up processes, but also probably top down processes via changes in mobile organisms behavior (Coleman, 1986; Gershenzon, 1984; Howe and Westley, 1988; Pastor et al., 1997; Tomassini Barbarossa et al., 2007). It is well known that VOCs play important roles in plant defense, either by direct repulsion of herbivores or attraction of herbivore enemies (Städler, 1986; Bernays and Graham, 1988; Unsicker et al., 2009). Thus, mixing oil crops, such as soybean, with VOCs crops, such as annual wormwood, could be an interesting strategy to enhance biodiversity (Marshall et al., 2003) and develop sustainable pest management (Pyke et al., 1987).

Results have shown similar total abundance of arthropods in both years (379 in 2006 and 318 in 2007) distributed according to the proportion of different plant species in the mixtures and to the relative biomass production of annual wormwood. Therefore, it is possible that annual wormwood VOCs served as attractants or repellents for feeding or oviposition (Städler, 1986; Bernays and Graham, 1988) depending on the relative proportion of VOCs species in the mixture, dose dependant response (Dethier and Yost, 1952, cited by Tomassini Barbarossa et al., 2007). High annual wormwood biomass production during 2006 could act repelling arthropods from pure or high proportion of annual wormwood mixtures (40/4, 40/8), thus explaining the trend of low arthropod abundance and richness observed in these treatments. Low annual wormwood biomass production during 2007 could act attracting arthropods to these treatments, explaining the high values of arthropod abundance and richness observed.

Mixtures did not promote a greater total biomass production than pure soybean, so the range of mixtures included in this study did not use resources more effectively than soybean monocultures (Willey, 1979; Willey et al., 1986). Moreover, soybean crop biomass production was similar between pure soybean and mixtures, thus annual wormwood could be used as an accompanying crop or left as a weed in the densities tested in this work without compromising soybean crop production. Annual wormwood acted as diluting factor in the concentration of the predominant soybean crop and introduced an element of biodiversity expanding the list of non herbivores species available to colonize the crop without compromising crop production (Norris and Kogan, 2005). Mixtures of these industrial crops allow the increment of biodiversity without compromising productivity.

Several groups of spontaneous morphospecies of arthropods were only observed when annual wormwood was present in the mixture. Many of these morphospecies belonged to the same families (Noctuidae, Mordellidae, Curculionidae and Lygaeidae) recorded in *Artemisia vulgaris* L. in previous works (Denys and Schmidt, 1998; Denys and Tscharntke, 2002). The morphospecies of Araneae and other families reported by the same authors (Tephritidae, Coccinelidae, Chrysomelidae, Braconidae, Formicidae, Pentatomidae and Miridae) were more abundant on annual wormwood in the present work. Although *A. annua* and *A. vulgaris* are morphologically different, both species produce essential oils characterized by the presence of camphor and 1.8 cineole (Mucciarelli et al., 1995), thus it is possible that VOCs signals are similar too.

5. Conclusions

Using standard crop management for soybean production in Argentina, including transgenic cultivars resistant to glyphosate, 5 different spontaneous communities of arthropods belonging to 7 orders were observed depending on the proportion of soybean and annual wormwood in the mixture.

Relative biomass production of S and W was the main explanatory variable related to the differences in the arthropod communities. Spontaneous communities of arthropods are controlled mainly by bottom up processes, when soybean is the main crop in the mixture. Meanwhile, the role of annual wormwood attracting or repelling arthropods will depend on the magnitude of the signal related, not only to the proportion of plants in the mixture, but also to the biomass produced.

No differences were found among treatments in S + W and S total biomass production, while W total biomass and essential oil yield were different among treatments. Thus, annual wormwood could be used as an accompanying essential oil crop or left as a weed in the densities tested in this work, favoring biodiversity and, eventually, pest management without compromising soybean crop yield.

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References

Arroyo Varela, M., Viñuela Sandoval, E., 1991. Introducción a la Entomología. Ediciones Mundi-Prensa Madrid, Spain, p. 449.

Bagchi, G.D., Haider, F., Dwivedi, P.D., Singh, A., Naqvi, A.A., 2003. Essential oil constituents of *Artemisia annua* during different growth periods at monsoon conditions of subtropical north Indian plains. J. Essent. Oil Res. 15 (4), 248–250.

Benton, T.G., Vickery, J.A., Wilson, J.D., 2003. Farmland biodiversity: is habitat heterogeneity the key? Trends Ecol. Evol. 18, 182–188.

Bernays, A., Chapman, R., 1994. Behavior: the process of host-plant selection. In: Bernays, A., Chapman, R. (Eds.), Host-plant Selection by Phytophagous Insects. Chapman & Hall, New York, USA, pp. 95–205.

Bernays, E., Graham, M., 1988. On the evolution of host specificity in phytophagous arthropods. Ecology 69, 886–892.

Biaggini, M., Consorti, R., Dapporto, L., Dellacasa, M., Paggetti, E., Corti, C., 2007. The taxonomic level order as a possible tool for rapid assessment of arthropod diversity in agricultural landscapes. Agric. Ecosyst. Environ. 122, 183–191.

Buckelew, L.D., Pedigo, L.P., Mero, H.M., Owen, M.D.K., Tylaka, G.L., 2000. Effects of weed management systems on canopy insects in herbicide-resistant soybeans. J. Econ. Entomol. 93, 1437–1443.

Cerdeira, A., Duke, S.O., 2006. The current status and environmental impacts of glyphosate-resistant crops: a review. J. Environ. Qual. 35, 1633–1658.

Coleman, J.S., 1986. Leaf development and leaf stress: increased susceptibility associated with sink-source transition. Tree Physiol. 2, 289–299.

de la Fuente, E., Suárez, S., Ghersa, C.M., 2006. Soybean weed community composition and richness between 1995 and 2003 in the Rolling Pampas (Argentina). Agric. Ecosyst. Environ. 115 (1–4), 229–236.

Denys, C., Schmidt, H., 1998. Insect communities on experimental mugwort (*Artemisia vulgaris* L.) plots along an urban gradient. Oecologia 113, 269–277.

Denys, C., Tscharntke, T., 2002. Plant-insect communities and predator-prey ratios in field margin strips, adjacent crop fields, and fallows. Oecologia 130, 315–324.

Derraik, J.G.B., Closs, G.P., Dickinson, K.J.M., Sirvid, P., Barratt, B.I.P., Patrick, B.H., 2002. Arthropod morphospecies versus taxonomic species: a case study with Araneae, Coleoptera, and Lepidoptera. Conserv. Biol. 16 (4), 1015–1023.

Dethier, V.G., Yost, M.T., 1952. Olfactory stimulation of blowflies by homologous alcohols. In: Dethier, V.G. (Ed.), The Hungry Fly. Harvard University Press, Cambridge. J. Gen. Physiol. 35, 823–839.

di Giulio, M.D., Edwards, P.J., Meister, E., 2001. Enhancing insect diversity in agricultural grasslands: the roles of management and landscape structure. J. Appl. Ecol. 38, 310–319.

Dicke, M., 1999. Direct and indirect effects of plants on performance if beneficial organisms. In: Ruberson, J.R. (Ed.), Handbook of Pest Management. Marcel Decker, Inc., New York, USA, pp. 105–145.

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.

Ewald, J.A., Aebischer, N.J., 1999. Pesticide use, avian food resources and bird densities in Sussex. JNCC Report No. 296. JNCC, Peterborough.

Fehr, W.R., Caviness, C.E., 1977. Stages of Soybean Development. Special Report 80. Iowa State University, Ames, IO, p. 11.

Fulzele, D.P., Heble, M.R., Rao, P.S., 1995. Production of terpenoids from *Artemisia annua* L. plantlet cultures in bioreactor. J. Biotechnol. 40, 139–143.

Gershenzon, J., 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. In: Timmerman, B.N., Steelink, C., Loewus, F.A. (Eds.), Phytochemical Adaptations to Stress. Recent Advances in Phytochemistry, vol. 18. Plenum Press, New York, USA, pp. 273–320.

Harborne, J., 1987. Chemical signals in the ecosystem. Ann. Bot. 60, 39–57.

Holland, J.M., Luff, M.L., 2000. The effects of agricultural practices on Carabidae in temperate agroecosystems. Integr. Pest Manage. Rev. 5, 109–129.

Howe, H., Westley, L.C., 1988. Ecological Relationships of Plants and Animals. Oxford University, Oxford, p. 273.

Keating, B., Carberry, P., 1993. Resource capture and utilization in intercropping: radiation. Field Crops Res. 34, 273–301.

Kenkel, N.C., Derksen, D.A., Thomas, A.G., Watson, P.R., 2002. Multivariate analysis in weed science research. Weed Sci. 50, 281–292.

Khan, Z.R., James, D.G., Midega, C.A.O., Pickett, J.A., 2008. Chemical ecology and conservation biological control. Biol. Control 45, 210–224.

Kromp, B., 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agric. Ecosyst. Environ. 74, 187–228.

Lenardis, A.E., van Baren, C., Di Leo Lira, P., Ghersa, C.M., 2007. Plant–soil interactions in wheat and coriander crops driving arthropod assemblies through volatile compounds. Eur. J. Agron. 26, 410–417.

Magurran, A.E., 1988. Ecological Diversity and its Measurement. Princeton University Press, Princeton, NJ, p. 179.

Marshall, E.J.P., Brown, V.K., Boatman, N.D., Lutman, P.J.W., Squire, G.R., Ward, L.K., 2003. The role of weeds in supporting biological diversity within crop fields. Weed Res. 43 (2), 77–89.

McCune, B., Mefford, M.J., 1995. PC-ORD. Multivariate Analyses of Ecological Data. Version 2.0. MJM Software Design, Gleneden Beach, OR, p. 126.

Mielke Jr., P.W., 1984. Meteorological applications of permutation techniques 16 based on distance functions. In: Krishnaiah, P.R., Sen, P.K. (Eds.), Handbook of Statistics, vol. 4. Elsevier, London, pp. 813–830.

Mucciarelli, M., Caramiello, R., Maffei, M., Chialva, F., 1995. Essential oils from some *Artemisia* species growing spontaneously in North-West Italy. Flav. Frag. J. 10 (1), 25–32.

Norris, R.F., Kogan, M., 2005. Ecology of interactions between weeds and arthropods. Annu. Rev. Entomol. 50, 479–503.

Ong, C.K., Black, C.R., 1994. Complementary of resource use in intercropping and agroforestry systems. In: Monteith, J.L., Scott, R.K., Unsworth, M.H. (Eds.), Resource Capture by Crops. Nottingham University Press, Loughborough, UK, pp. 255–278.

Pastor, J., Moen, R., Cohen, Y., 1997. Spatial heterogeneities, carrying capacity, and feedbacks in animal-landscape interactions. J. Mammal. 78, 1040–1052.

Pengue, W., 2005. Transgenic crops in Argentina, the ecological and social debt. Bull. Sci. Technol. Soc. 25 (4), 314–322.

Perner, J., Wytrykush, C., Kahmen, A., Buchmann, N., Egerer, I., Creutzburg, S., Odat, N., Audorff, V., Weisser, W.W., 2005. Effect of plant diversity, plant productivity and habitat parameters on arthropod abundance in montane European grasslands. Ecography 28, 429–442.

Pharmacopée Européene, 1997. Third ed. Maisonneuve, Sante Ruffine.

Pyke, B., Rice, M., Sabine, B., Zalucki, M., 1987. The push-pull strategy behavior control of Heliothis. Aust. Cotton Grower 4, 7–9.

Rausher, M., 1992. Natural selection and the evolution of plant–insect interactions. In: Roitberg, B., Isman, M. (Eds.), Insect Chemical Ecology. Chapman & Hall, New York, USA, pp. 20–88.

Richards, O.W., Davies, R.G., 1984. Tratado de Entomología Imms. Vol 2: Clasificación y Biología. Editorial Omega, S.A. Barcelona, Spain, p. 998.

Root, R.B., 1973. Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). Ecol. Monogr. 43, 95–124.

SAS Institute Inc, 2003. SAS/STAT User's Guide, Version 9.1. SAS Institute Inc., Cary, NC, USA.

Städler, E., 1986. Oviposition and feeding stimuli in leaf surface waxes. In: Junniper, B.E., Southwood, T.R.E. (Eds.), Insects and the Plant Surface. Edward Arnold, London, UK, pp. 105–121.

Städler, E., 1992. Behavioral responses of insects to plant secondary compounds. In: Rosenthal, G., Berenbaum, M. (Eds.), Herbivores. Their Interactions with Secondary Plant Metabolites. Academic Press, San Diego, NY, USA, pp. 45–88.

ter Braak, C.J.F., 1987. Ordination. In: Jongman, R.H.G., ter Braak, C.J.F., van Tongeren, O.F.R. (Eds.), Data Analysis in Community and Landscape Ecology. , first ed. Pudoc, Wageningen, The Netherlands, pp. 91–173.

Tilman, D., Pacala, S., 1993. The maintenance of species richness in plant communities. In: Ricklefs, R., Schluter, D. (Eds.), Species Diversity in Ecological Communities. The University of Chicago Press, Chicago and London, pp. 13–25.

Tomassini Barbarossa, I., Muroni, P., Setzu, M.D., Angioy, A.M., 2007. Dose-dependent nonassociative olfactory learning in a fly. Chem. Senses 32, 535–541.

Tonkyn, D., 1980. The formula for the volume sample by a sweep net. Ann. Entomol. Soc. Am. 73 (4), 452–454.

Unsicker, S.B., Kunert, G., Gershenzon, J., 2009. Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. Curr. Opin. Plant Biol. 12, 1–7.

Vandermeer, J., 1984. Plant competition and the yield-density relationship. J. Theor. Biol. 109, 393–399.

Vandermeer, J., 1989. The Ecology of Intercropping. Cambridge University Press, Cambridge, p. 237.

Vandermeer, J., van Noordwijk, M., Anderson, J., Ong, C., Perfecto, I., 1998. Global change and multiple-species agroecosystems: concepts and issues. Agric. Ecosyst. Environ. 67, 1–22.

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 (3/4), 367–389.

Whittaker, R.H., 1975. Communities and Ecosystems. Mac-Millan, New York, USA, p. 383.

Willey, R.W., 1979. Intercropping. Itĭs importance and research needs. Part 1. Competition and yield advantages. Field Crop Abstr. 32 (1), 1–10.

Willey, R.W., Natarajan, M., Reddy, M.S., Rao, M.R., 1986. Cropping systems with groundnut: resource use and productivity. In: Agrometeorology of Groundnut. Proceedings of International Symposium ,. ICRISAT Sahelian Center, Naimey, Nigeria, pp. 193–205.