Insect assemblies related to volatile signals emitted by different soybean – weeds – herbivory combinations

M. Torcat Fuentes*, A. Lenardis, E.B. de la Fuente

Universidad de Buenos Aires, Facultad de Agronomía, Departamento de Producción Vegetal, Cátedra de Cultivos Industriales, Av. San Martín 4453, C.P.1417, Buenos Aires, Argentina

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ABSTRACT

Soybean, the main crop of Argentina, has promoted spatial and temporal homogenization of the agricultural landscape accompanied by significant losses of biodiversity, along with increases in the use of chemical inputs. One of the possible solutions to overcome these effects is the diversification of the agroecosystem, avoiding the control of weed species that could as attractants of beneficial insects and deterrents of herbivory, without compromising crop yield. The aims of this work were to evaluate the composition, abundance and richness of insect assemblages in different soybean-weed (*Artemisia annua* and *Chenopodium album*)-herbivory combinations and to determine the relationship between insect assemblages and volatile signals emitted by the different combinations. Two factorial experiments were conducted during two consecutive seasons under field conditions. Factors were: i) crop-weed combinations with five levels: pure soybean (PS), soybean + 2 *A. annua* plants m^{-2} (2A), soybean + 4 *A. annua* plants m^{-2} (4A), soybean + 2 *C. album* plants m^{-2} (2C) and soybean + 4 *C. album* plants m^{-2} (4C) and ii) herbivory with two levels: with (+H) and without (−H). Volatile signals were measured with an electronic nose and insects were sampled with pitfall traps located in the center of each plot. Different insect assemblages related to treatments were identified. Richness increased when soybean was accompanied by weeds. The proportion of non-herbivores was always higher than that of herbivores. In both seasons, the same sensors detecting volatile cues were the main explanatory variables of the insect data, being responsible for the separation of *A. annua* treatments from the others. Many non-herbivore species were attracted to *A. annua* and repelled by signals captured by these sensors and, on the contrary, many herbivore and non-herbivore species were attracted to those blends associated to PS and *C. album*. PS and *C. album* could represent a food source for herbivores and alternative food or host for non-herbivores. Herbivory was not a discriminant explanatory variable. This work highlights that insect assemblage varies according to different crop-weed combinations and their volatile signals. Considering the positive impact on insect diversity of some species currently considered as weeds, could be useful to design future agro-ecosystems in which the regulation of pests is accomplished by increasing the fauna of beneficial insects and, thus, reducing the use of pesticides.

1. Introduction

Soybean (*Gycine max* L. Merr.) crop, has become the most important crop of Argentina (Aizen et al., 2009). Areas cultivated with soybean have expanded in recent decades accompanied by the use of new agronomic and innovative technologies, such as no tillage, transgenic varieties, precision agriculture and the increment in the application of herbicides and fertilizers (Satorre, 2005). These changes allowed the human appropriation of resources leading to a clear landscape homogenization and also significant losses of biodiversity and wildlife, thus, threatening the ability of agroecosystems to maintain food production and functioning (de la Fuente et al., 1999, 2006). Therefore, the current

challenge is to focus on designing sustainable cropping systems which maintain food production while reducing external inputs. One sustainable option is the agroecosystem diversification, using species that could have a favorable impact on some ecosystem services, such as regulation of pests by increasing the fauna of beneficial insects (Landis et al., 2000; Moraes et al., 2005). This strategy goes in the same direction of the "push-pull" idea, which consists of reducing pest populations by repelling them from the system or by attracting them to other neighboring plants, using a set of stimuli emitted by plants, which modify insectś behavior. Push-pull mechanisms require a precise assemblage of plant species (Hassanali et al., 2008; Malézieux, 2012). In this context, some positive roles could be identified in species often

considered as weeds. Crops coexisting with these species could benefit from the natural regulation of pests, without compromising crop yield and generating economic benefits at local scale, in addition to the positive impacts on the environment (Malézieux et al., 2009). Nicholls and Altieri (2012) highlight the importance of plant biodiversity in agroecosystems to enhance beneficial insects' population and, as a consequence, to reduce pests' pressure.

In response to insect damage, most plants release volatiles (Agrawal, 2011; Berenbaum, 1995; Heil, 2010), mainly terpenoids, allowing parasitoids and predators to distinguish between infested and non-infested plants, in addition to compound blends that make leaves more or less attractive to herbivores (Pare and Tumlinson, 1999). However, the breeding of grain crops was mainly focused on primary metabolites production (carbohydrates, proteins and lipids), indirectly reducing the production of secondary metabolites (terpens, alkaloids, etc) and thus, chemical defenses. In this context, accompanying species could play positive roles in the agricultural ecosystem, bridging these gaps of natural defenses, which are actually absent in many crop systems. For instance, neighboring weed plants can attract insects to the system or repel them, but they can also act as an alternative food source for both natural enemies and primary consumers (herbivores), representing a dilution factor in the concentration of the predominant crop plants (Bernays and Chapman, 1994; Norris and Kogan, 2000, 2005).

This idea to provide volatile organic compounds (VOC's) resources by adding accompanying species in agroecosystems is an interesting practice, but candidate plant species are not always screened for their attractiveness to insects in the system. Within the agricultural fields of Argentina, there are numerous weeds known for their allelopathic effect, such as *Artemisia annua* L. (Morvillo et al., 2011)and *Chenopodium album* L. (Leicach et al., 2006). *A. annua* produces and releases secondary metabolites (mainly terpens) and can interact positively with soybean crop, favoring insect communities richness (Lenardis et al., 2011) without reducing crop yield (Morvillo et al., 2011). *C. album* produces secondary metabolites and may be an alternative food for herbivorous insects (Sigsgaard et al., 2008; Štolcová, 2009) visiting soybean crop, thus, it can relieve the crop from herbivore pressure, diluting the concentration of host plants. Soybean crop may also release a set of chemical compounds when consumed by some herbivores (Moraes et al., 2005).

Although, there is plenty of evidence about the role of plant volatiles in the interactions among plants and their surrounding biota, this role has so far been studied mainly under controlled conditions (Gols et al., 2012; Gouinguené and Turlings, 2002; Heil and Kost, 2006; Michereff et al., 2011; Moraes et al., 2005) and to a lesser extent under field conditions (Gil et al., 2002; Lenardis et al., 2011). The information obtained at organ or plant level cannot always be directly scaled up to crop or agroecosystem scale. There are relatively few field experiments that include different levels of biological integration with new insights into the role of volatiles in ecological assemblies (Pierik et al., 2014). Jessing et al. (2014) highlight this lack for *A. annua*, pointing out that there is evidence from laboratory assays about the effect of *A. annua* leaf extracts (dry o fresh) and essential oil on insects' behavior, but there is limited information at field scale.

The role of *A. annua* attracting or repelling insects will depend on the proportion of plant species and the biomass of each species (Lenardis et al., 2011). On the basis that crop diversification generates different chemical signals and insects' assemblies, the hypotheses of this work are i) "the presence of accompanying weed species releasing volatile cues will be associated with more abundant and diverse insects' assemblies than monocultures" and ii) "the volatile signals will attract more non herbivores in mixtures containing weeds producing terpenes than in monocultures". This work evaluates the relationship between volatile signals released in mixtures of soybean with *A. annua* and *C. album* weeds in the presence of herbivory and natural enemies under field conditions. In this context, the objectives were i) to evaluate the composition, abundance and richness of insect assemblages in different soybean-weed (*Artemisia annua* and *Chenopodium album*)-herbivory combinations and ii) to determine the relationship between insect assemblages and volatile signals emitted by the different combinations.

2. Materials and methods

2.1. Field experiments

Field experiments were conducted at the experimental field of the Faculty of Agronomy, University of Buenos Aires, Argentina (34˚35S, 58˚25W) during two consecutive years. Soybean DM 4670 of maturity group IV and indeterminate growth habit was sown on January 21st 2013 in season one (S1), and on February 1st 2014 in season two (S2). Soybean seeds were planted into $1 m^2$ plots at a density of 40 plants m^{-2}. They were planted along three rows per plots, leaving one row between plots as border. Seeds were previously inoculated with *Bradyrhizobium japonicum*.

The experiments were arranged in a completely randomized factorial design with three replications. The factors were: i) crop-weed combinations with five levels: pure soybean (PS), soybean+ 2 *A. annua* plants m^{-2} (2A), soybean+ 4 *A. annua* plants m^{-2} (4A), soybean+ 2 *C. album* plants m^{-2}(2C) and soybean + 4 *C. album* plants m^{-2} (4C) and ii) herbivory of *A. gemmatalis* with two levels: with (+H) and without (−H) natural herbivory in S1, and simulated herbivory in S2. In both seasons, each plot was surrounded by a tull net to avoid plot-to-plot movement among experimental units of introduced caterpillars (Fig. 1). *A. annua* (annual wormwood) seeds were provided by the State University of Campinas (UNICAMP) Sao Paulo, Brazil. *C. album* (common lambsquarters) seeds were obtained from the Faculty of Agronomy in the University of Buenos Aires (FAUBA). Given that both weeds have slow initial growth, *A. annua* and *C. album* were sown in plastic seedbeds two months before the crop, to synchronize the establishment of crop and weeds. Then, weed seedlings were transplanted to pots to avoid allelopathic effects and competition with the crop for soil resources. Pots were placed between central rows of each plot. The whole area was initially sprayed with a combination of two insecticides (cypermethrin and chlorpiriphos) at their commercial doses to ensure that the caterpillars present in the assay were those introduced.

Natural herbivory was planned using eggs of *Anticarsia gemmatalis* (velvetbean caterpillar) provided by the Institute of Microbiology and Agricultural Zoology (IMYZA) of the National Agricultural Technology Institute (INTA). They remained in the FAUBA laboratory at room temperature until they hatched. All larvae were preserved in plastic trays covered with tull net to ensure the air exchange and were fed daily with soybean leaves from the borders of the field assay. Simulated herbivory was performed by several cuts on leaf edges mimicking a 15% natural defoliation. Both herbivory treatments, natural in S1 and simulated in S2, were made in late March, imitating the peak of incidence of defoliators in soybean fields (Aragón et al., 2002).

The experimental area was totally covered with a net (35% light interference) to limit the unwanted herbivory during the first part of the assay. It was later removed, in mid-April, after the effect of natural and simulated herbivory occurred, to allow the entry of insects.

During the experiment, unwanted weeds were removed manually, and plots were irrigated through drip irrigation to supplement natural rainfall with the objective of maintaining the soil near field capacity.

2.2. Measurements

Once the covering net was removed, insect assemblies were evaluated in advanced reproductive stages of soybean (R5, Fehr and Caviness, 1977). Insects were sampled using "pitfall traps" filled with soap and water and located in the center of each plot (Fig. 1). Insects were taxonomically determined at species level, when possible, or otherwise at morphospecies level. The analysis at morphospecies level allows the study of insect assemblies since the differences between the