



cis-Jasmone indirect action on egg parasitoids (Hymenoptera: Scelionidae) and its application in biological control of soybean stink bugs (Hemiptera: Pentatomidae)

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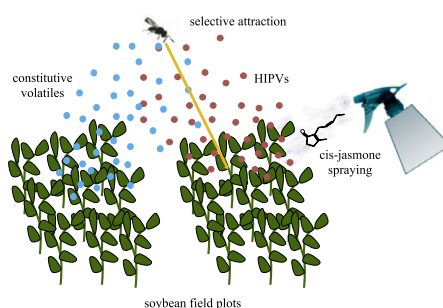
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HIGHLIGHTS

- ▶ *cis*-Jasmone is an herbivore induced plant volatile.
- ▶ *cis*-Jasmone may induce indirect defenses in soybean plants against stink bugs.
- ▶ Sprayed *cis*-jasmone increased number of Scelionidae egg parasitoids in soybean plots, when the matrix is soybean.
- ▶ Sprayed *cis*-jasmone did not change parasitism and number of stink bugs in soybean plots.

GRAPHICAL ABSTRACT



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ABSTRACT

In many plants, the secondary metabolite *cis*-jasmone activates the metabolic pathway that produces volatile organic compounds attractive to natural enemies and, sometimes, repellent to herbivores. Previous studies indicate that the feeding damage caused by the herbivore *Euschistus heros* or the exogenous application of *cis*-jasmone in soybean plants induces the release of herbivore-induced plant volatiles (HIPVs) with a similar chemical profile and these compounds can attract the stink bug egg parasitoid *Telenomus podisi* (Scelionidae). Herein we tested in field conditions the effect of exogenous application of *cis*-jasmone in soybean plants on the parasitoid and stink bug community and on stink bug egg parasitism. In two areas, one within a soybean and another within a *Crotalaria* matrix, we randomly distributed 2 m² plots, with soybean plants induced (treatment, $n = 5$) or not induced by *cis*-jasmone (control, $n = 5$) in the field. We sampled the parasitoid community weekly with yellow sticky traps ($n = 3$ /plot) and monitored parasitism with sentinel eggs of *E. heros* ($n = 150$ /plot). We also monitored the population of stink bugs weekly, by sampling each plot with shake-cloth technique. The abundance of Scelionidae was highest overall and also in treated plots during the first four weeks in the area with a soybean matrix, but decreased thereafter. The richness of parasitoid families was similar between treatment and control plots in the area with a soybean matrix, but higher in control plots in the area with a *Crotalaria* matrix. Evenness was higher in control plots in the area with soybean matrix, whereas the reverse occurred in the area with a *Crotalaria* matrix. Results suggest that treatment with *cis*-jasmone effectively attracted and enhanced the population of scelionid parasitoids, but had no effect on the occurrence and intensity of parasitism and in the number of stink bugs.

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1. Introduction

Over evolutionary time, plants developed many mechanisms to resist adverse conditions, including the production of secondary metabolites against herbivores and pathogens (Baldwin, 2010; Dicke et al., 2003). Herbivore-induced plant volatiles (HIPVs) are synthesized in plant tissues and released in response to herbivory (Dudareva et al., 2006; Hilker and Meiners, 2006). Besides repelling herbivore insects, HIPVs indirectly attract herbivore's natural enemies, providing information on the location of hosts and prey and protecting plants from subsequent attacks (Dudareva et al., 2006; Vet and Dicke, 1992).

HIPVs are complex blends of compounds, including green leaf volatiles (GLVs), aromatic compounds, mono-, homo- and sesquiterpenes. After herbivore attack, the production of HIPVs depends on metabolic pathways activated by different compounds, such as phytohormones or their derivatives (Baldwin, 2010; Dudareva et al., 2006; van Den Boom et al., 2004). When applied on plants, the naturally obtained compounds or their synthetic standards act as elicitors of HIPVs (Heil and Walters, 2009). For instance, the application of jasmonic acid on different plants promotes the emission of volatiles attractive to parasitoids (de Moraes et al., 2000; Heil, 2004; Pickett et al., 2007). Likewise, the application of *cis*-jasmone, a metabolite derived from the biosynthesis of jasmonic acid (octanodecanoid pathway, Birkett et al., 2000), induces defenses in wheat that repel pest aphids and increase their attractiveness to parasitoids (Bruce et al., 2003b). In soybean plants, *cis*-jasmone promotes the liberation of HIPVs similar to those induced by stink bug (Pentatomidae) injuries (Moraes et al., 2005, 2008), and these HIPVs attract the egg parasitoid *Telenomus podisi* (Ashmead) (Moraes et al., 2009). Thus, the induction of HIPV emission can be used in the behavioral manipulation of parasitoids that naturally occur in and around crops, or parasitoids released for augmentative biological control (Powell and Pickett, 2003). Nevertheless, an understanding of the impact of HIPV elicitors on the community structure of insect parasitoids is still wanting. This knowledge is essential to establish a solid scientific basis for the management of insect parasitoids in crops.

In Brazil, stink bugs are one of the most serious pests of soybean plantations (Panizzi, 1997). The biological control of these herbivores by egg parasitoids is an alternative that could reduce the use of synthetic insecticides and contribute to the conservation of natural resources, becoming an important tool for sustainable agriculture (Corrêa-Ferreira and Moscardi, 1996). Herein we evaluate in the field, the effects of induced defenses in soybean plants treated with *cis*-jasmone on the attraction of Scelionidae parasitoids of stink bug eggs in particular, and on the abundance and richness patterns of parasitoid community in general. We expected an increase in the density and richness of parasitoids in treated plots, especially scelionids, and higher prevalence and intensity of parasitism in stink bug eggs.

2. Material and methods

2.1. Study site

We conducted field experiments in two adjacent areas at Embrapa Recursos Genéticos e Biotecnologia, Distrito Federal, Brazil (15°43'50" S, 47°53'59" W). One consisted of 0.55 ha of soybean, *Glycine max* (L.) Merr. (cv-MSOY 6101; hereafter called "soybean area"), whereas the other had a total area of 0.71 ha of rattle pod, *Crotalaria juncea* L. (hereafter called "Crotalaria area"). Within each area, we randomly embedded 10 small plots with soybean (cv BR-16) between November 2008 and March 2009. We fertilized the soil, corrected its superficial acidity and inoculated seeds with

nitrogen fixing bacteria (*Bradyrhizobium* sp.) at a rate of 1 kg of inoculant to 50 kg of seeds following recommendations of Embrapa (2010).

2.2. Experimental design

In each area, we conducted experiments in the early reproductive stage of soybean plants in 10 randomly distributed plots. Each plot measured 2.0 × 1.0 m and they were far between at least 10 m. We manually sprayed soybean plants of five plots (treatment) in each area with *cis*-jasmone (TCI, Japan), using 6.0 ml/plant of a solution of 250 mg of *cis*-jasmone and 100 mg of surfactant Tween® 20 (Sigma–Aldrich, United States) diluted in 1 l of water. We based the dosage of solution and procedures of application on a previous laboratory study carried with *cis*-jasmone and the same tritrophic interaction of this study (Moraes et al., 2009). We sprayed soybean plants in the initial reproductive stage (R1-flowering, Ritchie et al., 1988), when the crop is colonized by stink bugs (Panizzi, 1997), and maintained plots until the physiological maturity of soybean, when stink bugs migrate to refuges or areas with alternative host plants.

2.3. Sampling of parasitoids and stink bugs

We sampled parasitoids during nine weeks with 15.0 × 12.5 cm rectangles of yellow sticky traps (Biotrap®, BioControle Métodos de Controle de Pragas, Brazil), attached by wire to wooden stakes about 1 m high from the ground. In each plot, we placed traps in three points along one of the diagonals, one point at the center and one at each edge of the diagonal. The first sampling was synchronized with the spraying of plants. The adhesive surface of traps was exposed in the field for three days, after which they were removed, wrapped in plastic film, and stored in refrigerator for the identification of insects. We replaced traps every week. We identified Scelionidae parasitoids to the lowest possible taxonomic rank, following the dichotomous key developed by Dr. Marta Loiacono (División Entomología del Museo de La Plata, Argentina), and other parasitoids to the family level following Gauld and Bolton (1988) and Goulet and Huber (1993).

We recorded the incidence (presence or absence) and intensity (number of parasitized eggs/number of remaining eggs) of egg parasitism weekly, by randomly placing three cardboard squares in each plot (3.0 × 3.0 cm), each cardboard square containing 50 sentinel eggs held by Arabic gum. We covered each cardboard square with tulle (silk net) ~2.0 mm diameter to avoid predation; the tulle allowed free passage of parasitoids but precluded access to eggs by predators (e.g., bugs, ants, and beetles). We obtained eggs of the brown stink bug, *Euschistus heros* (Fabricius), the main soybean pest in central Brazil, from a colony maintained at Embrapa Recursos Genéticos e Biotecnologia, following the rearing procedures described in Laumann et al. (2011). In each plot, we placed cardboards with eggs on three different plants, tied with color tape on a leaf petiole. Eggs were exposed in the field for three days and then transported to the laboratory, packed in plastic pots with lids, and maintained in a room with controlled environmental conditions (LD 14:10 h, 26.0 ± 1 °C and 65 ± 10% relative humidity). We verified parasitism by the emergence of parasitoids or by dissecting eggs and recording the presence of pupae and immature stages, when parasitoids did not complete their development. We counted parasitized eggs and identified emerged parasitoids under a Zeiss SV6 stereomicroscope. Of the 270 sentinel egg cards placed in each study area, 107 (soybean: 40%) and 152 (*Crotalaria*: 56%) were lost by the action of rain, predation (e.g., ants, beetles and other stink bugs) or mastication of the card with the tulle (e.g., Orthoptera), and these cards were not considered in statistical analyses.

To estimate stink bug density, we sampled each plot weekly with four shake-cloth (Shepard et al., 1974), a 50.0 × 100.0 cm rectangle of white flexible plastic with wooden handles attached to the sides. The shake-cloth is similar to beating trays and was used to cover the soil between the soybean rows while plants were shaken vigorously above it. In each plot, we made four samplings, identified stink bugs (species and life stage), and released them at the same site. Given that we had no replication of each matrix type (soybean, *Crotalaria*), we conducted the statistical analyses described below separately for each area.

2.4. Statistical analyses

We used a repeated measures analysis of variance (Tabachnick and Fidell, 2001) to assess differences between treatment and control plots in the total abundance of Scelionidae sampled with sticky traps and stink bugs sampled with shake-cloth. To assess the effect of treatment on the incidence and intensity of parasitism of sentinel eggs, we used generalized linear models (GLM) specifying errors with binomial and Poisson distributions, respectively (Zuur et al., 2009). We implemented repeated measures ANOVAs and GLMs with R (R Development Core Team, 2011).

We used χ^2 tests to assess the effect of treatment with *cis*-jasmonone on the pooled abundance of Scelionidae and other parasitoid families sampled with sticky traps. We determined the significance of the tests with 1000 Monte Carlo randomizations. Diversity is composed of two elements: richness, the number of taxa in a community or sample, and evenness, a measure of the distribution of relative abundance of different taxa (Olszewski, 2004; Peet, 1974). To compare richness of parasitoid families between treatment and control plots, we used a rarefaction analysis based on 1000 randomizations with the Species Diversity module of EcoSim (Gotelli and Entsminger, 2004). The rarefaction analysis generates an empirical distribution of the expected number of families in “rarefied” samples drawn randomly from the sample with higher number of individuals. We used the expectation and variance of family richness in *z* tests (Zar, 2010) to assess differences in richness between the two treatments. As an index of evenness, we used the probability of interspecific encounters (PIE, Hurlbert, 1971), which is the probability that two individuals randomly sampled from a community represent two different species (in this case, families) (Gotelli and Graves, 1996; Olszewski, 2004). We assessed the difference in Hurlbert’s PIE between treatment and control plots using rarefaction, based on 1000 randomizations, with the program EcoSim (Gotelli and Entsminger, 2004) and a *z* test (Zar, 2010). We excluded two families, Encyrtidae and Pteromalidae, from the analyses because of their exceptional high abundance in both experimental areas in comparison with other families and lack of interaction between these parasitoids and the stink bugs of soybean in Central Brazil.

To analyse the effect of treatment with *cis*-jasmonone on the community of adult parasitoids sampled with sticky traps through time (9 weeks), we used a principal response curve (PRC) analysis with package *vegan* (Oksanen et al., 2009) of R (R Development Core Team, 2011). The main result of this method, which is based on a partial redundancy analysis (RDA), is a curve representing the temporal course of community composition in experimental treatments and a scoring of each species showing their relative contribution to the community trend (Leps and Smilauer, 1999). An axis showing species scores in the corresponding RDA axis complements the curve. We assessed the significance of the treatment effect with 999 Monte Carlo permutations. We implemented two PRCs, one for the genera of Scelionidae and another for parasitoid families. We used the logarithm (base 10) of the mean parasitoid abundance per plot and per week, for each genus or family. From a total of 270 sticky traps placed in each area throughout the

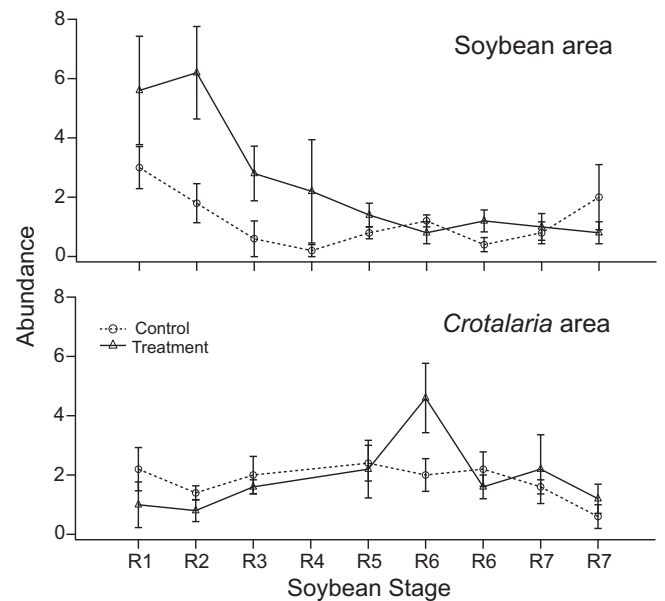


Fig. 1. Variation of the mean number (\pm SE) of Scelionidae sampled weekly with yellow sticky traps by soybean stage, in control and *cis*-jasmone treated plots in two areas.

experiment (3 traps × 5 plots × 2 treatments × 9 weeks), five and three traps were missing, respectively, from the soybean and *Crotalaria* areas. To avoid loss of information in PRC analyses, we estimated missing observations of sticky traps using multiple imputation (Quinn and Keough, 2002; Tabachnick and Fidell, 2001). Multiple imputation is regarded as the most robust approach for imputing values to missing observations, and also makes fewer assumptions about the pattern of missing observations (Rubin, 1996; van Buuren et al., 2006). We used the method of multivariate imputations by chained equations to impute missing data (van Buuren et al., 2006), with package *mice* in R (R Development Core Team, 2011). We used the significance level of 5% in all hypotheses tests.

3. Results

3.1. Effects on the abundance and diversity patterns of parasitoids recorded in sticky traps

From the total parasitoids recorded with stick traps (soybean area = 2274; *Crotalaria* area = 3244), we identified 25 families and one superfamily in the soybean area and 24 families and two superfamilies in the *Crotalaria* area. In both areas, besides the Chalcidoidea unidentified, the family Scelionidae always was between the top 3 more abundant, with Mymaridae as the most abundant in the *Crotalaria* area. Considering the soybean area, the family Ichneumonidae was the most abundant before Scelionidae in control plots, and Bethyilidae was the most abundant after Scelionidae in treatment plots. From the Scelionidae species identified (soybean area = 164; *Crotalaria* area = 148), *T. podisi* and *Trissolcus basal* (Wollaston) were the most common.

In the soybean area, the repeated measures ANOVA revealed no effect of treatment with *cis*-jasmonone on the abundance of Scelionidae ($F_{1,8} = 4.49$; $P = 0.067$), but a significant effect of time ($F_{8,64} = 5.72$; $P < 0.001$) and of the interaction between time and treatment ($F_{8,64} = 2.33$; $P = 0.029$). During the first four weeks of the experiment, the abundance of Scelionidae was highest overall and also in treatment plots, but decreased thereafter (Fig. 1). In

the *Crotalaria* area, there was no effect of treatment with *cis*-jasmone ($F_{1,8} = 0.04$; $P = 0.85$), a significant effect of time ($F_{8,64} = 3.10$; $P = 0.009$) and no interaction between time and treatment ($F_{8,64} = 1.97$; $P = 0.076$). Overall, the abundance of Scelionidae was highest during weeks five and six of the experiment (Fig. 1). These results indicate that the treatment with *cis*-jasmone promoted an increase in the abundance of Scelionidae that lasted for about a month, but only in the soybean area.

Pooling the data from all sampled weeks, the relative abundance of parasitoid families differed significantly between treatment and control plots only in the soybean area (soybean: $\chi^2_{[NA]} = 48.73$, $P < 0.01$; *Crotalaria*: $\chi^2_{[NA]} = 28.37$, $P = 0.19$). To identify which parasitoid families mostly influenced the results, we performed an analysis of the χ^2 residuals. By selectively removing parasitoid families with largest residuals, we identified Ichneumonidae and Scelionidae as the main differences between treatment and control plots: the χ^2 test remained significant after the exclusion of Ichneumonidae ($\chi^2_{[NA]} = 36.37$, $P = 0.02$) or Scelionidae ($\chi^2_{[NA]} = 35.80$, $P = 0.04$), but not after the exclusion of both ($\chi^2_{[NA]} = 26.12$, $P = 0.19$). In plots treated with *cis*-jasmone, the abundance of Scelionidae was higher, whereas the abundance of Ichneumonidae was lower than expected by chance (Fig. 2).

The PRC analysis revealed a significant effect of the *cis*-jasmone treatment on the abundance of parasitoid families only in the soybean area (soybean: $F_{9,72} = 1.27$; $P = 0.03$, RDA1 = 5%; *Crotalaria*: $F_{8,64} = 1.04$; $P = 0.37$, RDA1 = 3%; Fig. 3). The main differences between treatment and control plots in the soybean area were in the abundance of Scelionidae and Ichneumonidae: the former were more, and the latter were less, abundant in treatment plots during the first four weeks of the experiment (soybean stages R1–R4),

whereas the reverse occurred during the last five weeks of the experiment (soybean stages R5–R7, Fig. 3). Conversely, the PRC indicated no effect of the *cis*-jasmone treatment on the abundance of Scelionidae genera in both areas (soybean: $F_{9,72} = 1.26$; $P = 0.20$, RDA1 = 1.6%; *Crotalaria*: $F_{8,64} = 1.32$; $P = 0.17$, RDA1 = 1%; Fig. 4). Nevertheless, in the soybean area there was a clear trend for all genera of Scelionidae to be more abundant in treatment plots during the first four weeks, with a steady decrease in relation to control plots subsequently (Fig. 4).

In the soybean area, the rarefaction analysis revealed no difference in the richness of parasitoid families between control and treatment plots (control and treatment: 24; $z = 0.62$; $P = 0.27$). However, the richness of parasitoid families was higher in control plots (control: 23; treatment: 21; $z = -6.901$; $P < 0.01$) in the *Crotalaria* area. The evenness of parasitoid families differed significantly between treatment and control plots in the soybean (control: 0.920, treatment: 0.914, $z = 5.29$, $P < 0.01$) and *Crotalaria* areas (control: 0.854, treatment: 0.861, $z = 2.45$, $P < 0.01$), being higher in control plots of the former, but higher in treatment plots of the latter.

3.2. Effects on the parasitism of stink bug eggs

The overall parasitism recorded in sentinel eggs was 8.15% in the *Crotalaria* area and 4.07% in the soybean area. The parasitism in the *Crotalaria* area occurred in 14 cardboards in control plots (12 *T. podisi*, 1 *Trissolcus brochymenae* (Ashmead) and 1 *Trissolcus teretis* (Johnson)) and in 8 cardboards in treatment plots (4 *T. podisi*, 1 *Eupelmus* sp., 1 unidentified Eulophidae, 1 *Tr. brochymenae* and 1 *Tr. teretis*), while we found one cardboard parasitized in control

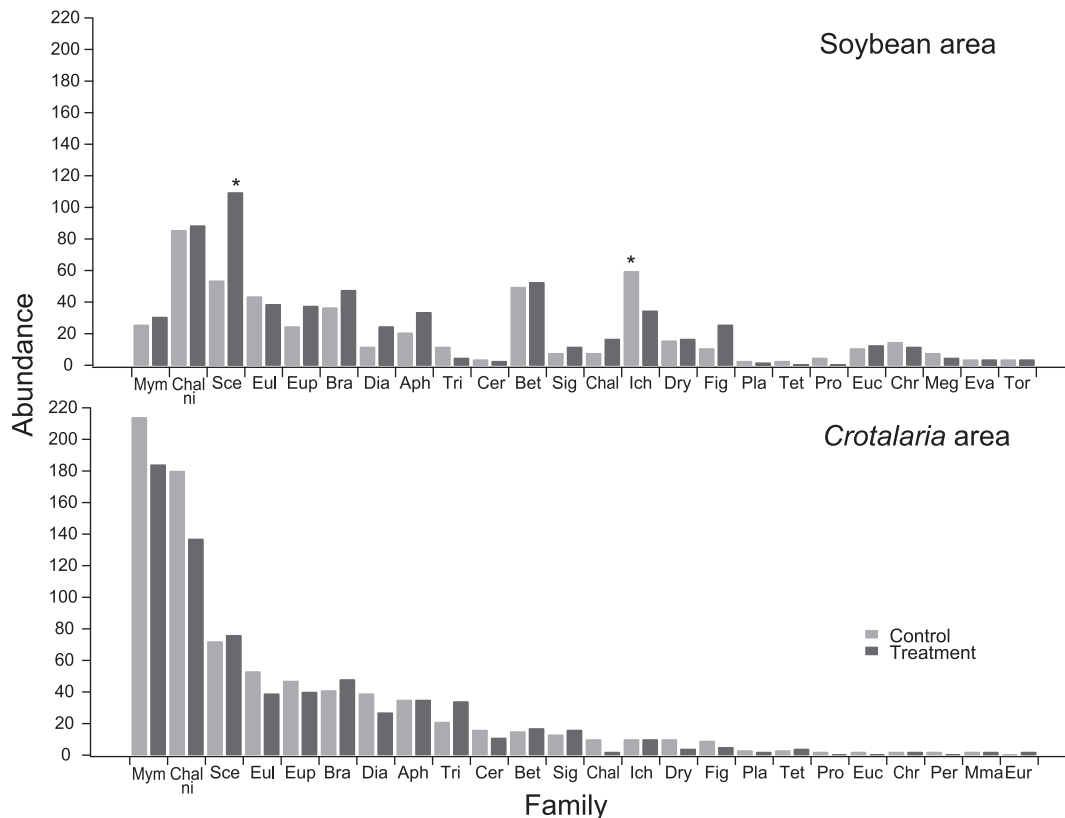


Fig. 2. Abundance of adult parasitoids sampled in soybean plots, with yellow sticky traps for nine weeks in control and *cis*-jasmone treated plots in two areas. Legend: Aph = Aphelinidae, Bet = Bethylinidae, Bra = Braconidae, Cer = Ceraphronidae, Chal = Chalcididae, Chalni = Chalcidoidea unidentified, Chr = Chrysididae, Dia = Diapriidae, Dry = Dryinidae, Euc = Eucoilidae, Eul = Eulophidae, Eup = Eupelmidae, Eur = Eurytomidae, Eva = Evaniidae, Fig = Figitidae, Ich = Ichneumonidae, Meg = Megaspilidae, Mma = Mymaromatidae, Mym = Mymaridae, Per = Perilampidae, Pla = Platygastridae, Pro = Procturidae, Sce = Scelionidae, Sig = Signiphoridae, Tet = Tetracampidae, Tor = Torimidae, Tri = Trichogrammatidae. *Indicate significant difference ($P < 0.05$).

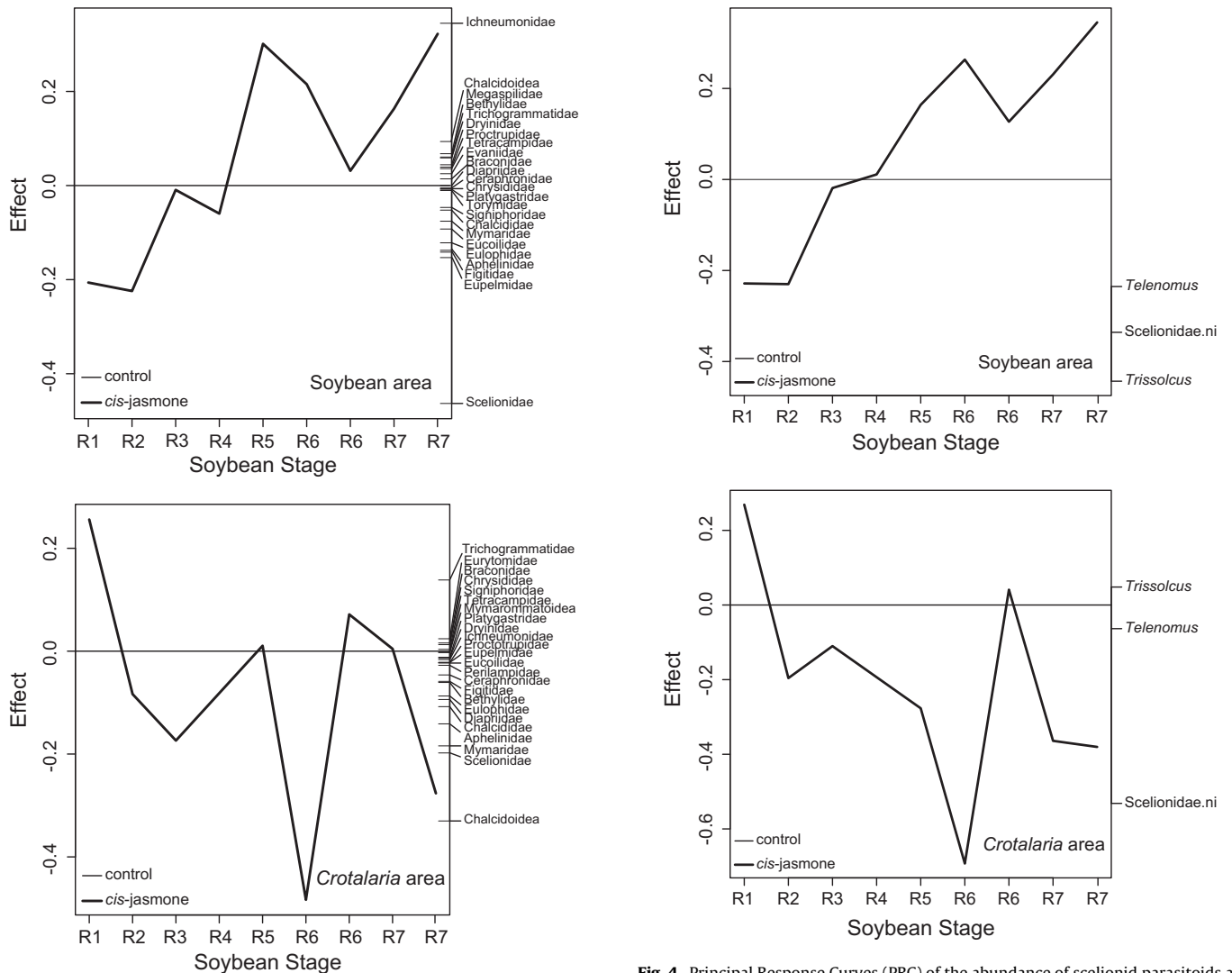


Fig. 3. Principal Response Curves (PRC) of the abundance of adult parasitoids as a function of treatment with *cis*-jasmone during nine weeks, labeled according to the reproductive stages of soybean (R1–R7), in two areas. The left axis represents community scores on the first principal component, which are linear combinations of the abundance of parasitoid families. The right axis depicts the relative contribution (weight) of each family to community scores. The figure represents the temporal variation in the community structure of treatment plots in relation to control plots (solid horizontal line). Soybean matrix: 23 families and one superfamily; *Crotalaria* matrix: 22 families and two superfamilies.

plots (*T. podisi*) and 10 cardboards in treatment plots (all *T. podisi*) in the soybean area.

Sentinel eggs recovered from the soybean area were exclusively parasitized by *T. podisi*. Likewise, *T. podisi* was the main parasitoid in the *Crotalaria* area, but *Tr. brochymenae*, *Tr. teretis*, and an unidentified Eulophidae also parasitized sentinel eggs. The application of *cis*-jasmone had no effect on the incidence (soybean: $\chi^2_{[1]} = 0.16$; $P = 0.69$; *Crotalaria*: $\chi^2_{[1]} = 0.26$; $P = 0.61$) or intensity of parasitism (soybean: $F_{1,8} = 0.98$; $P = 0.35$; *Crotalaria*: $F_{1,8} = 0.17$; $P = 0.69$) in both areas.

3.3. Effects on the abundance of stink bugs

The stink bugs species sampled with shake-cloth in the soybean area were represented by *Piezodorus guildinii* Westwood (3 males, 3 females, 20 nymphs), *Chinavia impicticornis* (Stål) (2 nymphs), *Edessa mediatubunda* (Fabricius) (1 male) and *Dichelops melacanthus*

Fig. 4. Principal Response Curves (PRC) of the abundance of scelionid parasitoids as a function of treatment with *cis*-jasmone during nine weeks, labeled according to the reproductive stages of soybean (R1–R7), in two areas. The left axis represents the assemblage scores on the first principal component, which are linear combinations of the abundance of scelionids. The right axis depicts the relative contribution (weight) of each species of Scelionidae to the assemblage scores. The figure represents the temporal variation in the assemblage structure of treatment plots in relation to control plots (solid horizontal line).

(Dallas) (1 male). The *Crotalaria* area included *C. impicticornis* (1 male, 1 female, 14 nymphs) and *D. melacanthus* (4 males).

The repeated measures ANOVA indicated no significant effect of treatment with *cis*-jasmone (soybean: $F_{1,54} = 0.46$; $P = 0.50$; *Crotalaria*: $F_{1,54} = 0.35$; $P = 0.55$), time (soybean: $F_{8,54} = 0.15$; $P = 0.99$; *Crotalaria*: $F_{8,54} = 1.05$; $P = 0.41$) or the treatment and time interaction (soybean: $F_{8,54} = 0.18$; $P = 0.99$; *Crotalaria*: $F_{8,54} = 0.57$; $P = 0.80$) on the number of stink bugs sampled with the shake-cloth in both study areas (Fig. 5).

4. Discussion

4.1. Effects on the on the abundance and diversity patterns of parasitoids recorded in sticky traps

A growing body of evidence indicates that applications of HIPVs in field conditions are attractive to natural enemies (parasitoids and predators) of herbivores (James, 2003, 2005, 2006; James and Grasswitz, 2005; Simpson et al., 2011a; Thaler, 1999; Yu

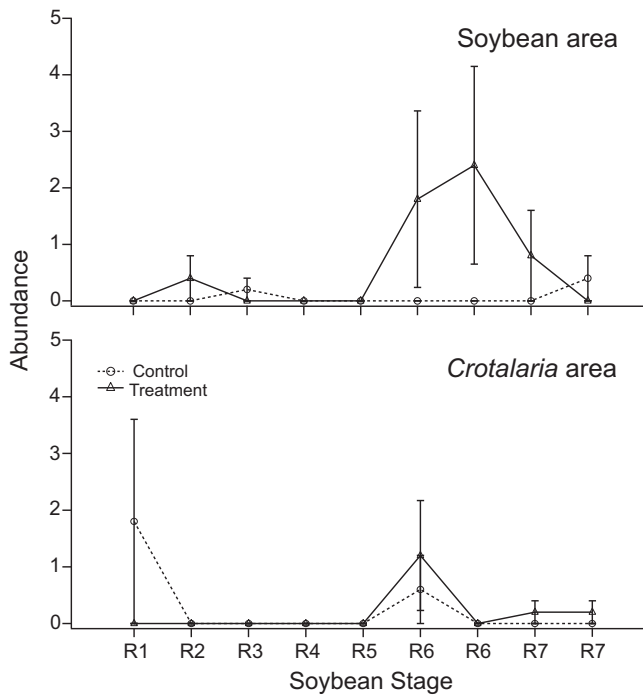


Fig. 5. Variation of the mean number (\pm SE) of Pentatomidae sampled weekly with shake-cloth by soybean stage, in control and *cis*-jasmone treated plots in two areas.

et al., 2008). We observed that the application of *cis*-jasmone promoted significant changes in the community structure of parasitoids in soybean plots. Immediately after the application of *cis*-jasmone, there was a selective increase in the abundance of Scelionidae associated with a decrease in the evenness of the parasitoid community, but no changes in the richness of parasitoid families. The effects of the *cis*-jasmone application lasted for about a month, but decreased steadily after that. No such effects were recorded in soybean plots within a *Crotalaria* matrix.

This is the first report on the potential attraction of scelionid wasps to soybean plants exposed to *cis*-jasmone in the field. The induction of HIPV production in soybean plants exposed to *cis*-jasmone and the attraction of the egg parasitoid *T. podisi* to these volatile blends was previously demonstrated in laboratory experiments (Moraes et al., 2009). *cis*-Jasmone also triggers indirect defenses in others crop plants. Birkett et al. (2000) demonstrated that plants of *Vicia faba* L. exposed to *cis*-jasmone are attractive to the predator *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) and to the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae); later, it was demonstrated that *cis*-jasmone also induces indirect defenses in wheat, reducing the infestation by the aphid *Sitobion avenae* (Fabricius) (Hemiptera: Aphelinidae) and increasing the activity of *A. ervi* (Bruce et al., 2003a, 2003b). These results evidence that *cis*-jasmone may show the same effects in different crop species.

Our results indicate that plants exposed to *cis*-jasmone selectively attract scelionid wasps. The attractiveness to scelionid wasps by soybean plants exposed to *cis*-jasmone lasted for about a month, after the single application of *cis*-jasmone, and decreased thereafter. In the same way, the attraction effect of sprayed HIPVs on vineyard, corn and broccoli occurred, but it was short lived (<10 days) (Simpson et al., 2011a).

We observed differences in the response of scelionid parasitoids on the treatment with *cis*-jasmone between experimental units, allocated in unequal matrices. Treatment with *cis*-jasmone increased the abundance of Scelionidae only in the area within a soybean matrix. This can be in agreement with the notion that a more

diverse composition of plant species may lead to increased complexity and diversity of volatiles released (Harvey and Fortuna, 2012; Randlkofer et al., 2010), and probably affects parasitoid searching activity in the *Crotalaria* matrix area.

4.2. Effects on the parasitism of stink bug eggs

We observed no differences in the incidence and intensity of parasitism between soybean plants exposed to *cis*-jasmone and control plants, despite the heightened abundance of parasitoids in the former. Likewise, field application of methyl jasmonate on maize did not increase the parasitism rates on *Spodoptera frugiperda* (Smith) larvae when rates were measured seven days after spraying (von Mérey et al., 2012).

Several factors may be responsible for this pattern, including the lack of resources such as pollen and nectar for adult parasitoids, that may act as a reward to retain them in the area, increasing the parasitism (Simpson et al., 2011b), and density-dependent thresholds in the parasitoid/host interaction (Kaplan, 2012; Underwood, 2000). These results may also be related to the difficulty in locating egg cues by parasitoids.

Because eggs are not easily detectable resources, egg parasitoids could use cues indirectly related to the host (Fatouros et al., 2008). Plant and host volatiles are olfactory cues over long distances (Fatouros et al., 2008; Steidle and van Loon, 2002; Vinson, 1998). In short distances, more specific volatiles, visual and physical cues may be involved in egg location and host recognition and selection by parasitoids, for an effective parasitism (Fatouros et al., 2008; Steidle and van Loon, 2002). In addition to HIPVs, scelionids use different indirect cues during host searching (Colazza et al., 2004; Moraes et al., 2005, 2008), such as sex pheromones or defensive compounds from adult stink bugs (Borges et al., 1998; Bruni et al., 2000; Laumann et al., 2009; Silva et al., 2006), footprint trails (Borges et al., 2003; Colazza et al., 2009) and mating vibratory signals (Laumann et al., 2007, 2011). Additionally, the availability of eggs on an artificial substrate (cardboards) used in our experimental setup was not sufficient to give the local cues from plants or hosts exploited by parasitoids in short distances. Therefore, the attraction of parasitoids is just the first among several steps in the process of host searching and on promoting their effectiveness in the control of crop herbivores (Simpson et al., 2011b; Vinson, 1998).

4.3. Effects on the abundance of stink bugs

We observed no differences in the abundance of stink bugs between plants exposed to *cis*-jasmone and control plants. Because the chemical profile released by soybean plants treated with *cis*-jasmone is similar to that released by plants damaged by herbivores, and both different from the volatiles released by undamaged plants (Moraes et al., 2009), we expected that plants exposed to *cis*-jasmone would have any effect (repel or attract) on stink bugs. In laboratory, stink bugs were also not responsive to soybean plants damaged by *E. heros*, but were attracted to undamaged plants (Michereff et al., 2011). The lack of *cis*-jasmone effect on stink bugs in treated plants may also be related to the low abundance of stink bugs in the experimental areas, which have no history of land use for soybean cultivation.

Results from field application of HIPVs on different crop species in some cases reveal that herbivore populations increases on treated plants. Pope et al. (2007) demonstrated that application of *cis*-jasmone on host plants increase the colonization by the aphid *Phorodon humuli*. Schrank Maize plots treated with a mixture of green leaf volatiles (GLVs) showed higher frequency of colonized plants by *Diabrotica* beetles and *S. frugiperda* larvae and the damage on plants exposed to GLVs was higher than in non exposed

plants (von Mérey et al., 2011). Contrastingly, maize plants induced with methyl jasmonate did not show higher infestations of *S. frugiperda* (von Mérey et al., 2012). Simpson et al. (2011a) reported that sweet corn and broccoli plants treated with different HIPVs were more attractive to thrips than non-treated plants. The attractiveness of HIPVs to different herbivorous insects is variable. HIPVs seem to be attractive to some groups, such as Coleoptera (Halitschke et al., 2008; Hammack, 2001; Ruther and Hilker, 2003) and Thysanoptera (Imai et al., 2001; Teulon et al., 2007), but repellent to others, such as Lepidoptera (de Moraes et al., 2001; Huang et al., 2009) and Aphididae (Birkett et al., 2000; Bruce et al., 2003a, 2003b).

These results suggest that the action of volatiles induced by HIPVs on herbivores need to be evaluated in each case before their incorporation in pest management programs.

5. Conclusion

The chemical composition of volatiles emitted by plants can vary quantitatively and qualitatively, depending on the stage of plant development and herbivore species (de Moraes et al., 1998; Takabayashi et al., 1995), the variety of the plant (Heil, 2008; Takabayashi et al., 1991) and even the type of feeding damage caused in the plant tissues or by oviposition (Heil, 2008; Hilker and Meiners, 2006). Furthermore, the responses of plants to induction by HIPVs could be affected by different biotic and abiotic stress (von Mérey et al., 2011, 2012) and by different weather conditions along the year (Simpson et al., 2011a; von Mérey et al., 2011). Our results suggest that induction of defense by *cis*-jasmonate in soybean plants attracts scelionid parasitoids, despite the lack of response on parasitism rates and on host population.

Following experiments with replication that take into account differences in environmental conditions (temperature, air relative humidity, precipitation, area size, vegetation matrix) and biotic stress (presence of damage by other herbivores, physiological stress of the plants) should be conducted to verify the weight of these variables on the fluctuation of parasitoids and their host populations, to achieve a more solid data base and to test how different dosage or application technique could help to extend the effects of *cis*-jasmonate on parasitoid recruitment and parasitism rates.

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