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# Attraction of the stink bug egg parasitoid *Telenomus podisi* to defence signals from soybean activated by treatment with *cis*-jasmone

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**Abstract**

After herbivore attack or chemical activation, plants release a blend of volatile organic compounds (VOCs) that is qualitatively or quantitatively different to the blend emitted by an undamaged plant. The altered blend of VOCs is then usually attractive to the herbivore's natural enemies. Soybean, *Glycine max* (L.) (Fabaceae), when damaged by stink bug herbivory, has been shown to emit a blend of VOCs that attracts the stink bug egg parasitoid *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae) to the plant. In this study, our aim was to investigate changes in the VOC profile of soybean (var. BR16) elicited by the naturally occurring plant activator *cis*-jasmone, and to determine whether these changes elicited the attraction of *T. podisi*. *cis*-Jasmone elicited chemical defence in soybean similar to that previously reported for stink bug damage. The main components induced by *cis*-jasmone were camphene, myrcene, (*E*)-ocimene, methyl salicylate, and (*E*,*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene. In Y-tube behavioural bioassays, *T. podisi* preferred *cis*-jasmone treated plants over untreated plants. Thus, *cis*-jasmone appears to induce defence pathways in soybean similar to those induced by stink bug damage, and this phenomenon appears to be a promising tool for the manipulation of beneficial natural enemies in future sustainable stink bug control strategies. The delay in response demonstrates that *cis*-jasmone treatment is not directly causing the response, but, more importantly, that it is causing activation of induced defence, long after initial treatment.

## Introduction

With the recent advances in our understanding of chemical communication between plants and how plants defend themselves against herbivory and pathogens, the use of semiochemicals as tools in integrated pest management (IPM) is increasingly seen as a viable alternative to conventional methods of pest control, such as pesticides. So far, promising results have been obtained through behavioural manipulation of insects in push–pull strategies (Cook et al., 2007; Hassanali et al., 2008), through the use of pheromones for monitoring or mating disruption (Cardé & Minks, 1995; Vaughn et al., 2006), and through the use of chemical elicitors to enhance direct and indirect plant defence (Birkett et al., 2000; Bruce et al., 2003a,b; Pickett et al., 2007).

Plants are equipped with several mechanisms to defend themselves against attack by herbivorous insects and pathogens. Several studies have examined the various mechanisms involved in indirect defence, including herbivore-induced plant volatiles (HIPVs) that are emitted in response to herbivory (Turlings et al., 1993; Du et al., 1998; Dicke & van Loon, 2000; Colazza et al., 2004; Moraes et al., 2005a, 2008a; Heil, 2008), or oviposition-induced plant volatiles (Meiners & Hilker, 1997, 2000; Hilker &

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Meiners, 2002). Most of these studies were aimed at understanding how plants can recognize the insects that are causing the associated damage, and how they recruit the third trophic level for protection.

An increasing number of studies is looking into how to apply these defence mechanisms to biological control, in particular whether herbivory- or oviposition-induced chemicals can be used as elicitors for plant defence activation. Methyl salicylate (MeSA) and jasmonic acid (JA) have been shown to induce plant defence and attract natural enemies, in a similar way to that described for herbivore-induced defence (Heil, 2004; Zhu & Park, 2005; Ament et al., 2006; D'Alessandro et al., 2006; Bruinsma et al., 2008). Bruce et al. (2003a,b) reported that wheat, *Triticum aestivum* (L.), treated with *cis*-jasmone resulted in reduced aphid populations when compared to untreated crops, which was subsequently attributed to changes in benzoxazinoid levels (Moraes et al., 2008b).

For soybean, *Glycine max* (L.) (Fabaceae) (var. BR16), it has been shown that plants infested by soybean aphids, *Aphis glycines* (Matsumura), emit different profiles of volatile organic compounds (VOCs) when compared with undamaged soybean plants. Methyl salicylate, a compound released in higher quantities by infested plants, elicited significant electrophysiological responses in seven-spot ladybirds, *Coccinella septempunctata* L. (Zhu & Park, 2005). Moraes et al. (2005a, 2008a) showed that an egg parasitoid of pentatomids, *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae), responds to plant volatiles induced by feeding damage caused by *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae), but not to plant volatiles released due to oviposition behaviour. The VOC profile from feeding-damaged soybean plants was different to that of the VOC profile from oviposition-damaged and undamaged plants.

Stink bugs are major pests of crops in Brazil, mainly on soybean. Around 5 million tonnes of pesticides are used annually to control stink bugs, in particular *E. heros*, *Piezodorus guildinii* (Westwood), *Nezara viridula* (L.), and *Dichelops melacanthus* (Dallas) (Panizzi, 1997). Since the 1980s, a number of other approaches that minimize the use of pesticides have been developed to control stink bugs in the field, including semiochemicals (Borges et al., 1998a,b, 2006, 2007; Moraes et al., 2005b; Pires et al., 2006) for use in monitoring traps or in mating disruption techniques.

*Telenomus podisi* is the main parasitoid of *E. heros*, and has been used with success for biological control in the south of Brazil through inoculative releases (Peres & Correa-Ferreira, 2004). To enhance the impact of biological control, induced plant defence could be considered as a route to manipulating *T. podisi* behaviour. Furthermore, studies have shown that *cis*-jasmone has the ability to induce defence in crop plants, leading to significant reductions in pest populations (Bruce et al., 2003a,b), and also to impact on the foraging behaviour of natural enemies (Birkett et al., 2000; Bruce et al., 2008). Thus, the aim of this study was to understand how *cis*-jasmone affects indirect defence activation in soybean, *G. max*. We addressed the following questions: (1) Does *cis*-jasmone application activate indirect (volatile) chemical defence in soybean? (2) If so, which individual compounds are induced by *cis*-jasmone? and (3) Does *cis*-jasmone application lead to enhanced attractiveness of the egg parasitoid, *T. podisi*, relative to untreated plants?

## Materials and methods

### Plants and insects

Soybean plants, *G. max* (var. BR16), were grown in a glasshouse with supplementary lighting to give a 14-h day at a temperature of 27 °C, until they reached the V3 stage (Fehr et al., 1971). The egg parasitoid *T. podisi* was reared in a laboratory colony originated from individuals collected in fields near Brasília, Brazil (15°47′S, 47°55′W). The parasitoids were maintained in an environmental chamber in plastic cages (tissue culture 25.0 $cm^2$ flask, angled neck; ICN Biomedicals, Irvine, CA, USA). To obtain parasitoids for bioassays, eggs of *E. heros* were exposed to adult female parasitoids for 24 h. The parasitized eggs were removed and placed in glass tubes (7.5 × 1.3 cm) for incubation at L14:D10 photoperiod, 26.0 ± 0.5 °C, and 65 ± 10% r.h. Once these parasitized eggs eclosed, parasitoids were kept in plastic cages for mating for 48 h, without any contact with host material. These parasitoids were therefore naive, and were all less than 48-h-old when used in bioassays.

### *cis*-Jasmone application

Soybean plants in the V3 stage were subjected to spray treatments, manually applied over all aerial parts of the plant. Each treatment lasted for 30 s. The sprayed plants were then used for volatile collection or bioassays either 3 (72 h) or 4 (96 h) days after spraying. This time-frame was used to avoid contamination of the samples by residual *cis*-jasmone from the spraying, and because previous works with beans and wheat (Birkett et al., 2000; Moraes et al., 2008b) showed that the plants take around 48 h to start to respond to the *cis*-jasmone/Tween-20 treatment. The following spray treatments were used: (1) distilled water as a control treatment (referred as 'untreated'), (2) Tween-20, a non-ionic surfactant (0.1% in distilled water) as a second control, and (3) *cis*-jasmone (1.4 mmol $l^{-1}$) solubilized in distilled water (100 ml) by Tween-20 (0.1%

vol/vol in distilled water, added prior to *cis*-jasmone). To avoid an induction of control plants by either *cis*-jasmone/Tween-20 or by released HIPVs, the treated and untreated plants were maintained in different chambers under the same conditions of light, temperature, and humidity (L14:D10 photoperiod at 26.0 ± 0.5 °C, and 65 ± 10% r.h.).

#### Olfactometer bioassays

An acrylic block with a Y-shaped cavity (27.5 × 21.0 cm), sandwiched between two glass plates, was used as the bioassay arena (Moraes et al., 2005a). The trunk of the apparatus measured 8 cm and each arm measured 7 cm. As the odour sources, single plants were placed in glass vessels and each vessel was connected to the olfactometer via polytetrafluoroethylene (PTFE) tubing. Charcoal-filtered, humidified air was passed through the system at 300 ml/min through each arm in a push–pull system. The behaviour of the insect was monitored by a camera [CCD camera (Sony SPT M324CE; Sony, Minato-Ku, Tokyo, Japan) fitted with a 12.5–75.0 mm/F1.8 zoom lens] coupled to SACAM software (Jorge et al., 2005) for registering behavioural parameters. A single *T. podisi* female was introduced at the base of the Y-tube and observed for 10 min, and the first choice in each arm was recorded by the software. If the parasitoid entered one arm of the Y-tube and remained for 20 s a first choice was recorded. Each individual was used only once. The following bioassays were carried out: (1) *T. podisi* response to 72-h untreated soybean vs. 72-h *cis*-jasmone/Tween-20-treated plants (n = 153), (2) *T. podisi* response to 96-h untreated soybean vs. 96-h *cis*-jasmone/Tween-20-treated plants (n = 187), (3) *T. podisi* response to 72-h untreated soybean vs. 72-h Tween-20-treated plants (n = 67), and (4) 96-h untreated soybean vs. 96-h Tween-20-treated plants (n = 67).

#### Air entrainment

Soybean plants used for air entrainment were placed in separate cylindrical glass chambers (internal volume 10 l). In order to reduce contamination by volatiles from the soil, the top of the pot was covered with aluminium foil. The tube containing the adsorbent (Super Q, 100 mg), and a PTFE tube carrying charcoal-filtered air entering the vessel at 1 l/min were secured to the apertures in the top of the vessel using screw-cap lids. The tube with adsorbent was connected via a PTFE tube to a vacuum at a flow of 800 ml/min, creating a positive pressure push–pull system. The volatiles were sampled for 24 h during the periods 72–96 h and 96–120 h after treatment. The trapped volatiles were eluted from the adsorbent using 500 µl hexane and pre-concentrated to 200 µl under an $N_2$ flow. Extracts were stored at –20 °C until used for gas chromatography (GC) and GC-mass spectrometry (GC-MS). For the GC and GC-MS analysis, an aliquot of the extract was separated (50 µl) and 2 µl of 16-hexadecanolide was added as internal standard, for a final concentration of 38 µg/ml of the internal standard.

For volatile collections, the three treatments were replicated as follows: at 72 h after treatment there were 14 *cis*-jasmone/Tween-20-treated plants, 11 untreated plants, and nine Tween-20-treated plants; at 96 h there were 17 *cis*-jasmone/Tween-20-treated plants, 16 untreated plants, and nine Tween-20-treated plants. The Tween-20 spray treatment was included as a supplementary control to account for any surfactant effect.

#### Gas chromatography

The extracts obtained by air entrainment were analysed by GC for quantification of the components (Shimadzu 17A, DB-5 column, 30 m × 0.25 mm i.d., 0.25 µm film; JandW Scientific, Folsom, CA, USA), using a temperature program of 50 °C for 1 min, then a gradual increase with 15 °C/min to 250 °C, for 20 min. One microlitre of each sample was injected using a splitless mode with helium as carrier gas. The column effluent was analysed with a flame ionization detector at 270 °C. Data were collected with ClassGC software and were handled using Origin 5.0 (Originlab Coorporation, Northamptom, MD, USA). Amounts released by the plant in each 24-h period were calculated in relation to the area of the internal standard (16-hexadecanolide, 38 µg/ml). The quantification of MeSA was carried out using single ion monitoring (see below).

#### Coupled gas chromatography-mass spectrometry

For compound identification, selected extracts were analysed using a Shimadzu QP2010 instrument equipped with a quadrupole analyser, a non-polar DB-5 column (30 m × 0.25 mm i.d., 0.25 µm film, JandW Scientific) and a splitless injector, with He as the carrier gas. Ionization was by electron impact (70-eV, source temperature 200 °C). Data were collected and analysed with GC-MS solutions software. Identifications were made by comparison of spectra with library databases (NIST, 2005) or with published spectra and confirmed using authentic standards. Methyl salicylate was shown to co-elute with the aldehyde decanal. Therefore, quantification of MeSA was carried out by single ion monitoring. For this quantification, each sample was injected in triplicate with monitoring of the molecular ion (m/z 152). Quantification was carried out using an external standard method, through construction of a calibration curve using six concentration levels (0.25, 0.5, 1, 5, 10, and 50 ng $\mu l^{-1}$), replicated three times.

#### Chemicals

Super Q (80/100 mesh) was purchased from Alltech (State College, PA, USA). Hexane (95% pesticide residue) was purchased from Fisher Scientific (Loughborough, Leicestershire, UK) and redistilled before use. *cis*-Jasmone was purchased from TCI (Tokyo, Japan), 6-methyl-5-hepten-2-one from Sigma Aldrich (Gillinghan, Dorset, UK), (*Z*)-3-hexenyl acetate from Alfa Aesar (Heysham, UK), MeSA and β-caryophyllene from Sigma Aldrich (Steinheim, Germany), (*E*)-β-farnesene from Bedoukian Research (Danbury, CT, USA), and (*E*)-ocimene from Botanix (Paddock Wood, Kent, UK). (*E*,*E*)-α-Farnesene (1.66 g) was synthesized in high purity (>95% by GC) in two steps via functionalization of 3-methyl-3-sulpholene with geranyl bromide (Chou et al., 1984) and extrusion of sulphur dioxide using lithium aluminium hydride (Gaoni, 1977). (*E*)-4,8-Dimethyl-1,3,7-nonatriene (DMNT) and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) were synthesized from geraniol and (*E*,*E*)-farnesol, respectively, by oxidation to their corresponding aldehydes followed by Wittig methylenation (Leopold, 1990).

#### Statistical analysis

The choices made by the parasitoid in the bioassays were analysed by means of generalized linear mixed models, with binomial errors and a logit link function. Because several insects were tested to each individual treated plant, this clustering was controlled for by fitting plant as a random effect. The estimate of the intercept of the fixed effects (given the appropriate model constraints) gives the logit of the proportion response, and was tested by means of a Wald $\chi^2$ statistic for difference from 0 (50% response to each odour). This approach appropriately considers the nested error structure, and thus controls for the pseudoreplication caused by testing multiple insects to individual plants, which has been a subject of recent concern (Wajnberg & Haccou, 2008).

In the *cis*-jasmone induction experiments, the amounts of volatiles produced in 24 h (in ng, log-transformed) were compared between treatments using analysis of variance. To compare between treatments, two a priori orthogonal contrasts were defined. The first contrast (contrast A) compared the two controls (untreated and Tween-20-treated) together against the *cis*-jasmone treatment, giving a net effect of the treatment of interest (contrast definition: 1, 1, and –2, for the order of treatments stated above). The second contrast (contrast B) compared the untreated controls against the Tween-20 controls to determine any effect of Tween-20 on volatile release (contrast definition: 1, –1, and 0). This analysis was carried out for the total amount of volatiles produced, as well as for each compound separately.

The univariate analysis does not inform about the contribution of each compound to the total blends obtained from soybean submitted to the different treatments, namely, untreated, Tween-20-, and *cis*-jasmone/Tween-20-treated plants; therefore, a multivariate analysis of variance (MANOVA) was applied to the data, incorporating each compound as a variable and treatment as a factor. To determine the contribution of each compound to the different treatments a canonical variate discriminant analysis was carried out (Pareja et al., 2007; Moraes et al., 2008a) This analysis provides a linear combination of the variables that maximizes the differences between the treatments, and the importance of each compound in separating the treatments can be assessed by the loadings (Rencher, 2002). All statistical analyses were carried out in R (R Development Core Team, 2007).

## Results

#### Olfactometer bioassays

In Y-tube bioassays, *T. podisi* showed no preference for the odour of plants from untreated or Tween-20-treated soybean plants 72 h (Wald $\chi^2 = 0.05$, P = 0.817) or 96 h (Wald $\chi^2 = 0.08$, P = 0.773) after treatment (Figure 1B,D). Similarly, no preference was observed for the odour of *cis*-jasmone/Tween-20-treated plants over untreated plants 72 h after treatment (Wald $\chi^2 = 0.64$, P = 0.425) (Figure 1C). However, *T. podisi* responded preferentially to the odour of soybean treated with *cis*-jasmone/Tween-20 at the 96-h stage, when compared to untreated soybean (Wald $\chi^2 = 4.39$, P = 0.036) (Figure 1A).

#### Chemical analysis

Gas chromatography and GC-MS analysis of the soybean air entrainment extracts detected 31 VOC compounds (Figure 2). This chemical profile was very similar to the one obtained for soybean in other studies, but with some important differences. Moraes et al. (2008a) reported the presence of (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, hexyl acetate, and (*Z*)-3-hexenyl butyrate, which were not identified in the volatile blend presented here. Conversely, they did not detect the presence of geranyl acetate, which was identified in low concentrations, and in some samples it was present only in trace quantities, which could explain why it was not reported earlier.

Comparisons of GC-MS analyses of the extracts obtained from untreated, Tween-20-treated, and *cis*-jasmone/Tween-20-treated soybean plants did not reveal any qualitative differences (Figure 2). The total amount of VOCs released by the three treatments did not differ at 72 h ($F_{2,31} = 1.82$, P = 0.179), whereas at 96-h the *cis*-jamone/Tween-20-treated soybean released higher quantities of

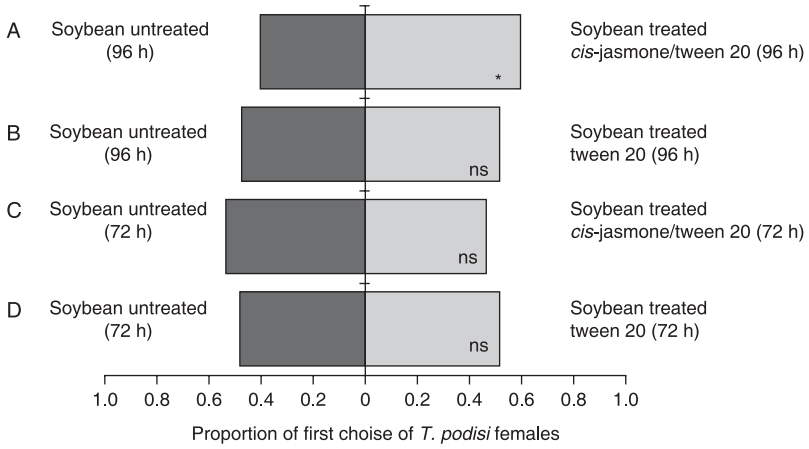


**Figure 1** Responses of *Telenomus podisi* females in a Y-tube olfactometer (% of the total choosing a test odour) to soybean plants subjected to treatments with Tween-20 or *cis*-jasmone/Tween-20 against control (water treatment). (A) Response to 96-h untreated soybean vs. 96-h *cis*-jasmone/Tween-20-treated plants (n = 187); (B) response to 96-h untreated soybean vs. 96-h Tween-20-treated plants (n = 67); (C) response to 72-h untreated soybean vs. 72-h *cis*-jasmone/Tween-20-treated plants (n = 153); and (D) response to 72-h untreated soybean vs. 72-h Tween-20-treated plants (n = 67). Asterisk indicates significant difference between treatments (Wald $\chi^2$ statistic: P = 0.03; ns, not significant).

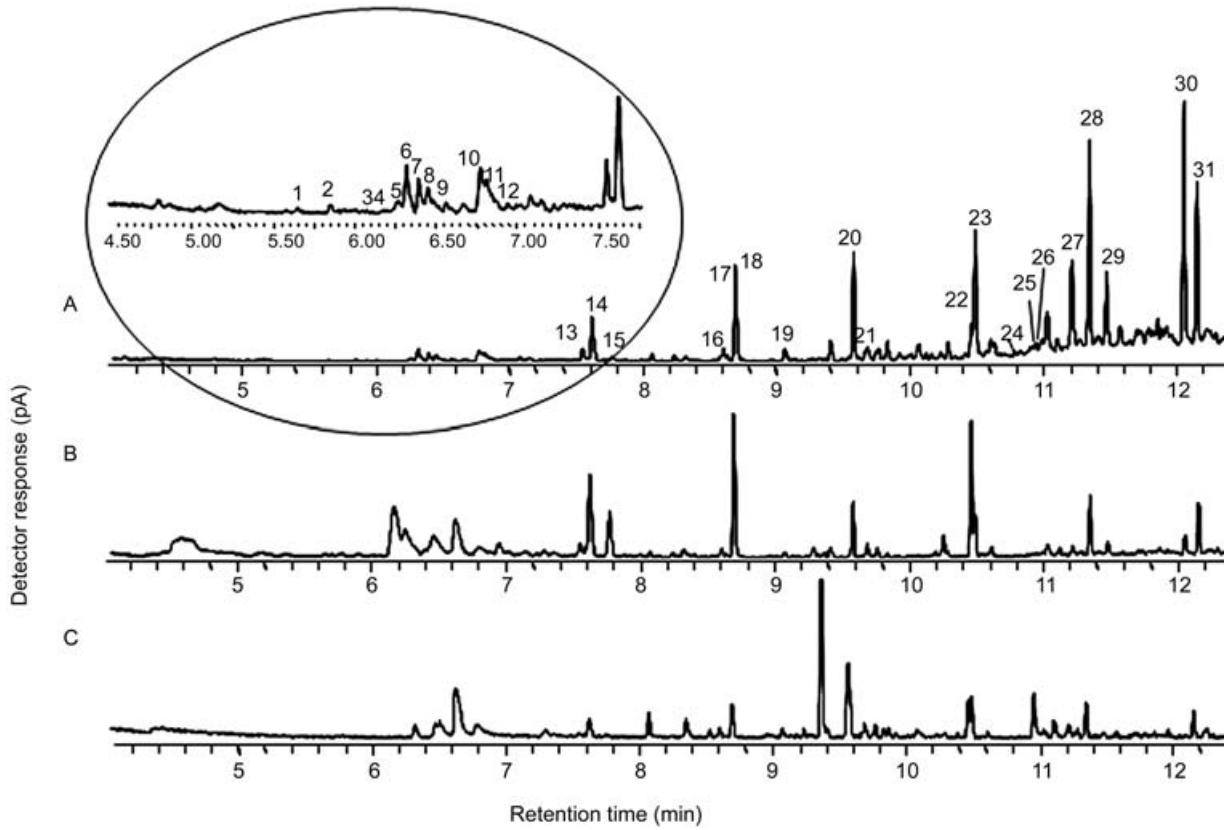


**Figure 2** Typical flame ionization detector chromatograms (pA = picoAmpère) obtained from volatile collections from soybean plants submitted to different treatments after 96 h (A) *cis*-Jasmone/Tween-20-treated soybean plants, (B) untreated soybean plants, and (C) Tween-20-treated soybean plants. 1, α-pinene; 2, camphene; 3, β-pinene; 4, 6-methyl-5-hepten-2-one; 5, myrcene; 6, decane; 7, octanal; 8, undecane; 9, (*Z*)-3-hexenyl acetate; 10, 2-ethyl hexanol; 11, limonene; 12, (*E*)-ocimene; 13, undecane; 14, nonanal; 15, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT); 16, dodecane; 17, methyl salicylate (MeSA); 18, decanal; 19, benzothiazole; 20, tridecane; 21, indole; 22, tetradecane; 23, decanoic acid, ethyl ester; 24, β-caryophyllene; 25, (*E*)-β-farnesene; 26, geranyl acetone; 27, unknown sesquiterpene; 28, pentadecane; 29, (*E,E*)-α-farnesene; 30, (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT); and 31, hexadecane.

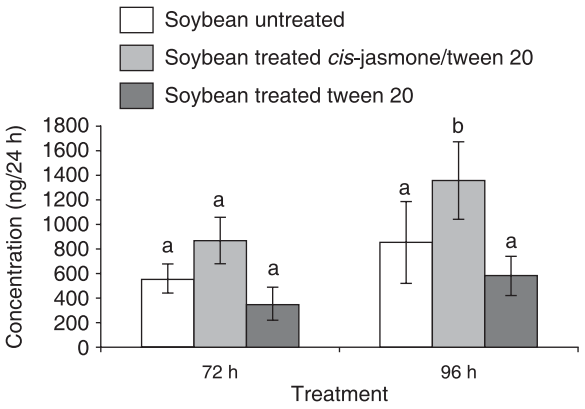


**Figure 3** Total amount of volatiles (mean ± SE) obtained from untreated soybean (distilled water) (n = 16 for 96 h treated plants; n = 12 for 72 h treated plants), *cis*-jasmone/Tween-20-treated soybean (n = 17 for 96-h treated plants; n = 14 for 72-h treated plants), and Tween-20-treated soybean (n = 9 for 72- and 96-h treated plants). The increase in the amount of volatiles released by *cis*-jasmone-treated plants was not significant at 72 h ($F_{2,31} = 1.82$, P = 0.179), but was significant at 96 h ($F_{2,39} = 6.48$, P = 0.004).

VOCs ($F_{2,31} = 6.48$, P = 0.004; contrast A: t = 3.59, d.f. = 39, P<0.001; contrast B: t = 0.86, d.f. = 39, P = 0.393) (Figure 3).

Comparison of individual components identified from the 72-h stage showed three significant differences (Table 1): limonene was released in higher quantities by *cis*-jasmone-treated plants than in the two controls ($F_{2,31} = 3.57$, P = 0.040; contrast A: t = 2.67, d.f. = 31, P = 0.012; contrast B: t = 0.04, d.f. = 31, P = 0.973); indole was released in higher amounts in Tween-20-treated plants ($F_{2,31} = 3.34$, P = 0.049; contrast A: t = 1.50, d.f. = 31, P = 0.145; contrast B: t = 2.20, d.f. = 31, P = 0.036); and β-farnesene was released in larger amounts in *cis*-jasmone-treated plants ($F_{2,31} = 3.32$, P = 0.049; contrast A: t = 2.46, d.f. = 31, P = 0.020; contrast B: t = 0.596, d.f. = 31, P = 0.555). Quantitative analysis of the 96-h stage revealed significant increases in *cis*-jasmone-treated plants for the following compounds (Table 1): α-pinene ($F_{2,39} = 3.73$, P = 0.033; contrast A: t = 2.25, d.f. = 39, P = 0.031; contrast B: t = 1.94, d.f. = 39, P = 0.060), camphene ($F_{2,39} = 4.67$, P = 0.015; contrast A: t = 2.86, d.f. = 39, P = 0.007; contrast B: t = 1.58, d.f. = 39, P = 0.123), myrcene ($F_{2,39} = 5.69$, P = 0.007; contrast A: t = 3.05, d.f. = 39, P = 0.004; contrast B:

**Table 1** Mean (± SE) amounts (ng/24 h) of main volatiles collected from untreated soybean plants or plants that were treated with *cis*-jasmone/Tween-20 or Tween-20

| | Treatment 72 h | | | Treatment 96 h | | |
|---|---|---|---|---|---|---|
| Compounds | Untreated | *cis*-Jasmone/ Tween-20 | Tween-20 | Untreated | *cis*-Jasmone/ Tween-20 | Tween-20 |
| (1) α-Pinene | 6.1 ± 2.5 | 33.9 ± 17.9 | 11.1 ± 5.2 | 21.5 ± 10.2 | 47.3 ± 17.6[1] | 1.3 ± 0.3 |
| (2) Camphene | 27.1 ± 14.5 | 78.01 ± 43.9 | 10.1 ± 6.2 | 39.5 ± 23.1 | 176.6 ± 94.2[2] | 1.6 ± 0.7 |
| (3) β-Pinene | 20.8 ± 14.8 | 35.9 ± 25.2 | 3.1 ± 1.9 | 21.2 ± 13.4 | 24.5 ± 10.3 | 20.7 ± 19.1 |
| (4) 6-Methyl 5-hepten-2-one | 8.7 ± 3.9 | 48.8 ± 20.2 | 2.8 ± 1.1 | 99.4 ± 66.2 | 51.4 ± 20.1 | 11.7 ± 11.2 |
| (5) Myrcene | 12.3 ± 6.5 | 31.8 ± 18.2 | 10.5 ± 7.4 | 52.1 ± 36.1 | 56.8 ± 19.9[1] | 4.4 ± 3.7 |
| (9) (*Z*)-3-Hexenyl acetate | 34.6 ± 24.9 | 23.5 ± 9.7 | 5.9 ± 2.4 | 25.1 ± 11.3 | 66.7 ± 32.2 | 6.1 ± 2.1 |
| (11) Limonene | 17.5 ± 8.6 | 52.7 ± 20.5[1] | 8.7 ± 3.5 | 32.7 ± 19.7 | 72.2 ± 26.1[1] | 8.9 ± 3.9 |
| (12) (*E*)-Ocimene | 2.3 ± 0.9 | 11.6 ± 4.6 | 2.3 ± 0.8 | 17.9 ± 13.2 | 21.4 ± 5.3[1] | 3.4 ± 1.8[2] |
| (15) DMNT | 121.3 ± 72.5 | 94.8 ± 36.1 | 29.6 ± 19.5 | 57.2 ± 14.6[2] | 139.6 ± 74.9[1] | 6.7 ± 2.5 |
| (17) Methyl salicylate | 33.7 ± 6.2 | 36.1 ± 9.7 | 27.6 ± 10.8 | 38.0 ± 5.9 | 160.3 ± 24.9[1] | 23.3 ± 5.1 |
| (19) Benzothiazole | 56.3 ± 25.1 | 59.1 ± 23.1 | 6.9 ± 2.9 | 24.1 ± 10.7 | 43.9 ± 15.5 | 51.9 ± 16.1 |
| (21) Indole | 18.1 ± 8.1 | 33.6 ± 23.1 | 68.4 ± 34.7[2] | 16.8 ± 9.1 | 29.9 ± 12.9 | 3.1 ± 0.8 |
| (24) β-Caryophyllene | 8.3 ± 2.5 | 8.6 ± 3.9 | 15.1 ± 7.3 | 6.6 ± 4.2 | 23.5 ± 11.2 | 18.2 ± 4.3[2] |
| (25) (*E*)-β-Farnesene | 17.8 ± 5.6 | 32.2 ± 5.6[1] | 21.8 ± 8.5 | 19.5 ± 8.8 | 21.8 ± 8.5 | 51.4 ± 8.2[2] |
| (26) Geranyl acetone | 29.8 ± 9.1 | 26.9 ± 6.7 | 20.5 ± 9.1 | 29.1 ± 15.2 | 45.7 ± 14.6 | 21.8 ± 7.6 |
| (29) (*E*,*E*)-α-Farnesene | 42.8 ± 12.1[1] | 69.9 ± 21.7 | 69.2 ± 35.3 | 110.7 ± 34.6 | 318.2 ± 102.6 | 48.1 ± 11.9 |
| (30) TMTT | 27.3 ± 9.4 | 56.0 ± 32.7 | 7.4 ± 4.6 | 15.7 ± 5.6 | 47.2 ± 10.3[1] | 29.6 ± 61.8 |

Numbers in brackets before the name of the compounds correspond to those in Figure 2.

[1]A significantly higher amount released by *cis*-jasmone-treated plants relative to controls, as determined by contrast A (see Material and methods).

[2]A significantly higher amount released as determined by contrast B (comparison between untreated and Tween-20-treated plants; see Materials and methods). Note that in contrast B, the *cis*-jasmone treatment is not included. DMNT, (*E*)-4,8-Dimethyl-1,3,7-nonatriene; TMTT, (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

t = 1.96, d.f. = 39, P = 0.057), limonene ($F_{2,39}$ = 3.49, P = 0.040; contrast A: t = 2.61, d.f. = 39, P = 0.013; contrast B: t = 0.91, d.f. = 39, P = 0.368), (*E*)-ocimene ($F_{2,39}$ = 5.26, P = 0.010; contrast A: t = 3.24, d.f. = 39, P = 0.002; contrast B: t = 0.46, d.f. = 39, P = 0.649), MeSA ($F_{2,39}$ = 23.03, P<0.001; contrast A: t = 6.79, d.f. = 39, P<0.001; contrast B: t = 1.18, d.f. = 39, P = 0.246), and TMTT ($F_{2,39}$ = 5.53, P = 0.008; contrast A: t = 3.22, d.f. = 39, P = 0.003; contrast B: t = 0.23, d.f. = 39, P = 0.821). DMNT showed no significant effect of *cis*-jasmone, but a reduction in the Tween-20-treatment ($F_{2,39}$ = 3.61, P = 0.037; contrast A: t = 1.18, d.f. = 39, P = 0.246; contrast B: t = 2.59, d.f. = 39, P = 0.014). Finally, β-farnesene ($F_{2,39}$ = 5.62, P = 0.007; contrast A: t = 2.08, d.f. = 39, P = 0.044; contrast B: t = 2.97, d.f. = 39, P = 0.005) and β-caryophyllene ($F_{2,39}$ = 5.59, P = 0.007; contrast A: t = 1.22, d.f. = 39, P = 0.231; contrast B: t = 2.84, d.f. = 39, P = 0.007) showed increases in the Tween-20-treated plants. For other compounds identified, there was no significant difference at either the 72-h or 96-h stage (Table 1).

When the data were analysed by MANOVA, significant effects of the treatments were observed. For the 72-h treatment, the bi-plot obtained showed a separation of the treatments, but it was not significant (approximate

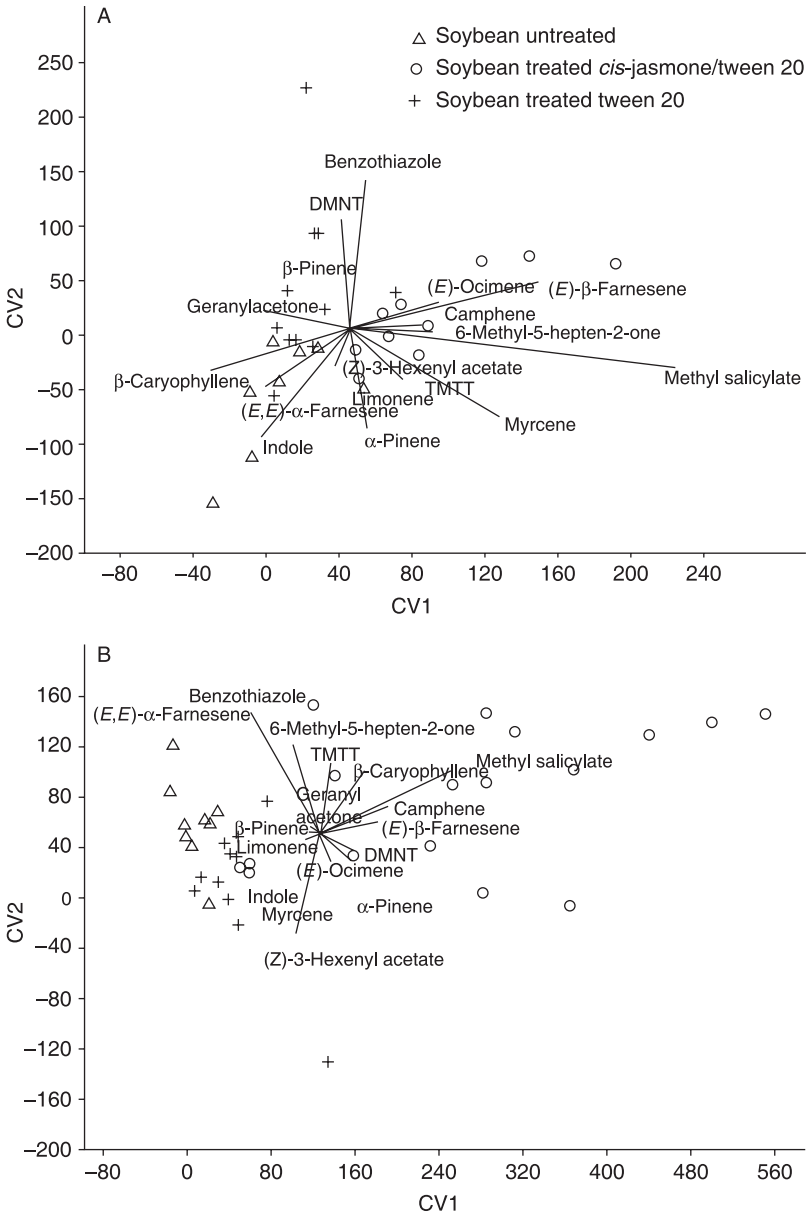


**Figure 4** Canonical variate analysis (CVA) bi-plot for (A) 72-h treated soybean plants, and (B) 96-h treated soybean plants. The vectors are the loadings for each compound, and the length of the vectors represents the relative magnitudes of the importance of each compound in differentiating treatments in the two dimensions. ○, soybean treated with *cis*-jasmone/Tween-20; +, soybean treated with Tween-20; △, untreated soybean.

$F_{34,22} = 1.54$, $P = 0.144$, Wilk's $\lambda = 0.08$), whereas for the 96-h treatments the MANOVA separated the three treatments and the statistical analysis was significant (approximately $F_{34,50} = 2.65$, $P = 0.042$, Wilk's $\lambda = 0.214$). (Figure 4A,B). The vectors of (*E*,*E*)-α-farnesene, β-caryophyllene, geranyl acetone, and DMNT at 72 h were more associated with untreated and Tween-20-treated soybean (Figure 4A), whereas at 96 h the direction of the vector from these compounds changed to *cis*-jasmone/Tween-20-treated soybean (Figure 4B); on the other hand, β-farnesene, limonene, (*Z*)-3-hexenyl acetate, and 6-methyl-5-hepten-2-one were more associated to *cis*-jasmone/Tween-20-treated soybean for 72-h treated plants and at 96 h were more associated to untreated and Tween-20-treated plants. The compounds benzothiazole, indole, and β-pinene at 72 and 96 h were more related to untreated and Tween-20-treated plants, and the vectors of the compounds (*E*)-ocimene, α-pinene, camphene, MeSA, and TMTT at 72 and 96 h were related to *cis*-jasmone/Tween-20-treated plants.

## Discussion

Treatment of *G. max* with *cis*-jasmone, a plant activator, did not change qualitatively the chemical profile of the plant, but rather the quantities of the compounds varied between the treatments. This change elicited the attraction of the egg parasitoid *T. podisi*, a natural enemy of major stink bug pests of soybean in Brazil. The volatile profile obtained in this study was similar to that reported previously for soybean variety BR16 (Moraes et al., 2008a). Zhu & Park (2005) reported linalool and 2-phenylethanol in the chemical profile of transgenic soybean, but these differences might be specific to different varieties, because in two other soybean varieties, Silvana and IAC-100, 2-phenylethanol was also found in airborne extracts (MCB Moraes, MFF Michereff, RA Laumann, & M Borges unpubl.).

The absence of most green leaf volatiles (GLVs), including (*E*)-2-hexenal and (*Z*)-3-hexen-1-ol, in the chemical profile of soybean can be attributed to the lack of mechanical damage, and to the collection of VOCs 72 h and 96 h after treatment, because GLVs are released in a rapid initial burst at the beginning of herbivore damage (D'Auria et al., 2002). Collections were made at these later stages so as to avoid collection of residual *cis*-jasmone following spray treatment, and to allow time for induced defence to be activated (Moraes et al., 2008b). (*Z*)-3-Hexenyl acetate was identified in the chemical profile of the studied plants, but was only of minor importance, as shown in the bi-plots (Figure 4). The alcohol (*Z*)-3-hexen-1-ol was not detected, probably because it is rapidly converted to (*Z*)-3-hexenyl acetate and does not accumulate in plants (Koch et al., 1999; D'Auria et al., 2002, 2007; Matsui, 2006).

After 96 h, *cis*-jasmone/Tween-20-treated soybean plants emitted several terpenoids as well as MeSA in significantly higher quantities than untreated plants and Tween-20-treated plants. Thus, a similar process of induction could be occurring in shikimic acid and isoprenoid biosynthesis. The compounds induced by *cis*-jasmone, such as MeSA, and TMTT are involved in indirect defence in a number of plants, such as maize [*Zea mays* (L.)], tomato (*Solanum lycopersicum* L.), lima bean (*Phaseolus lunatus* L.), and broad bean (*Vicia faba* L.), and are also induced by treatment with jasmonic acid, 12-oxo-phytodienoic acid (OPDA), or linoleic acid (Boland & Gabler, 1989, Turlings et al., 1998; Koch et al., 1999; Heil, 2004; Ament et al., 2006). Homoterpenoids such as TMTT are formed from the higher corresponding terpene by oxidative cleavage and loss of a four-carbon unit, whereas MeSA is the inactive form of salicylic acid, derived from the shikimate pathway. The induction of these compounds upon *cis*-jasmone treatment further highlights the wide-impacting biological effects that this activator has on soybean defence. Jasmonic acid and methyl jasmonate are well known activators of plant defence (Beale & Ward, 1998; Koch et al., 1999; Heil, 2004; Ament et al., 2006; Ozawa et al., 2008) and have been shown to induce volatiles from different metabolic origin. *cis*-Jasmone is a volatile compound produced in the final step of the jasmonate pathway, but it is also possibly generated via a novel pathway involving the oxylipin compound tetrahydrodicranenone B (iso-OPDA) (Dabrowska & Boland, 2007), and in a similar way to jasmonic acid, appears to affect multiple pathways.

Although Tween-20 was used as a surfactant to help the dissolution of *cis*-jasmone, this compound also appeared to change the chemical profile of soybean plants when compared to untreated or *cis*-jasmone-treated plants. DMNT was released in lower quantities and β-farnesene and β-caryophyllene were identified in higher quantities in Tween-20-treated plants. The mechanism remains to be studied, but presumably relates to disruption of plant lipid membranes, which is a common effect of surfactants (Kirkwood, 1999; Hess & Foy, 2000).

It is well known that natural enemies use HIPVs as cues to locate their hosts, and probably a specific blend of compounds triggers this behaviour (Vinson, 1985; Dicke, 1994; Colazza et al., 2004; Heil, 2004; Kost & Heil, 2006; Moraes et al., 2008a; Ozawa et al., 2008; Williams et al., 2008). The results obtained in this study suggest that several compounds produced by the plants, such as MeSA, (*E*)-ocimene, and TMTT, could influence the behaviour of *T. podisi*. The response of *T. podisi* to 96-h *cis*-jasmone/Tween-20-treated plants is in accordance with previous results, showing that this parasitoid responds to plants damaged by insect herbivores (Moraes et al., 2005a).

Although the main compound related to herbivory was shown previously to be (*Z*)-3-hexenyl acetate, other compounds such as MeSA and TMTT were also emitted by herbivory-damaged plants (Moraes et al., 2008a).

Terpenoids such as TMTT and DMNT, (*E*)-ocimene, and β-caryophyllene (De Moraes et al., 1998; Turlings et al., 1998; Colazza et al., 2004; Heil, 2004; Röse & Tumlinson, 2005; Zhu & Park, 2005; Kost & Heil, 2006; Williams et al., 2008), GLVs such as (*Z*)-3-hexenyl acetate, (*E*)-2-hexenal (De Moraes et al., 1998; Turlings et al., 1998; Heil, 2004; Kost & Heil, 2006; Pareja et al., 2007; Ozawa et al., 2008; Williams et al., 2008), and shikimate-derived compounds such as MeSA and indole (Turlings et al., 1998; Zhu & Park, 2005; D'Alessandro et al., 2006; Moraes et al., 2008a) are important HIPVs in many tritrophic systems. Recently, D'Alessandro et al. (2006) reported that indole, the main compound in *Spodoptera*-induced maize volatiles (Turlings et al., 1998; Hoballah & Turlings, 2005), did not have an effect on behaviour in the braconid wasp *Cotesia marginiventris* (Cresson) and had a repellent effect in another braconid, *Melipona rufiventris* Lepeletier (D'Alessandro et al., 2006). It is likely that parasitoids and predators do not use all HIPVs as cues for habitat and host location, but they are capable of separating the qualitative and quantitative chemical difference in the blends that indicate different types of damage to the plant. Different varieties of soybean release different profiles of HIPVs, but *T. podisi* appears to be capable of recognizing the blend of volatiles from undamaged plants from the HIPVs from these different varieties (Moraes et al., 2005a, 2008a). Further studies are necessary to identify which compounds are essential in the induced blend for the attraction of the natural enemy, and to understand how *T. podisi* achieves this specific response to soybean HIPVs from different varieties in the context of a complex volatile blend (D'Alessandro et al., 2006; Maffei et al., 2007; Heil & Ton, 2008; Heil, 2008; van Dam & Poppy, 2008).

In summary, the results suggest that *cis*-jasmone plays a role in affecting different biosynthetic pathways related to defences in soybean plants. These changes affect the behaviour of the parasitoid *T. podisi*, and this has great potential for the development of new strategies for stink bug control using natural enemies in soybean crops. Further laboratory and field studies are underway to evaluate how *cis*-jasmone can be used in a productive agricultural context, not only with soybean but with other economically important Brazilian crops.

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