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Aphid antixenosis in cotton is activated by the natural plant defence elicitor *cis*-jasmone

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ABSTRACT

Upon insect herbivory, plants can release blends of volatile organic compounds (VOCs) that modify herbivore and natural enemy behaviour. We have shown recently that cotton, *Gossypium hirsutum*, emits a blend of defence VOCs that repels the cotton aphid, *Aphis gossypii*, upon herbivory by this notorious crop pest, including (*Z*)-3-hexenyl acetate, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), methyl salicylate and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT). In this study, we investigated changes in the defence VOC profile of *G. hirsutum* induced by the naturally-occurring plant elicitor *cis*-jasmone (CJ) and whether these changes modify the behaviour of *A. gossypii*. In four-arm olfactometer assays, VOCs from untreated plants were significantly attractive ($P < 0.05$), whilst VOCs from CJ-treated plants were significantly repellent ($P < 0.05$). The VOCs induced by CJ appeared to comprise (*Z*)-3-hexenyl acetate, DMNT, methyl salicylate and TMTT. In quantitative VOC collection studies, sustained release of DMNT and TMTT was observed in CJ-treated plants over a period of five days, with levels becoming statistically significantly higher than for control treated plants on the fifth day in most cases. Despite earlier indications, no statistically significant differences were observed in levels of (*Z*)-3-hexenyl acetate or methyl salicylate between CJ and control treatments on any day. Furthermore, DMNT and TMTT emissions from CJ-treated plants were further enhanced by subsequent addition of *A. gossypii*. CJ treatment induced statistically significantly higher DMNT and TMTT expression levels as early as day three, when *A. gossypii* was present. The results in this study show that CJ can induce the production of *A. gossypii*-induced VOCs from *G. hirsutum*, with potential for deployment in novel crop protection strategies.

1. Introduction

The deployment of naturally occurring small lipophilic molecules (SLMs) as elicitors of indirect and direct defence in crop plants against insect pests is a relatively recent concept, but has started to receive more attention in recent years (Turlings and Ton, 2006; Pickett et al., 2007; Dewhirst and Pickett, 2010). The oxylipin compound *cis*-jasmone (CJ), produced naturally by plants (Loughrin et al., 1994, 1995; Rose and Tumlinson, 2004, 2005), was discovered as an elicitor of plant defence initially with field beans, *Vicia faba* L (Fabaceae), whereupon CJ treatment induced the production of defensive volatile organic compounds (VOCs) which attracted beneficial aphid parasitoids, *Aphidius ervi* Haliday (Birkett et al., 2000). These plants were more attractive to *A. ervi* in wind tunnel assays when tested 48 h after exposure to CJ had ceased. Furthermore, the signalling role of CJ was qualitatively different from that of the biosynthetically-related methyl jasmonate, and gave a long-lasting effect after removal of the stimulus (Birkett et al., 2000). Wheat plants, *Triticum aestivum* L. (Graminaceae), when exposed to CJ, showed an increase in emission of VOCs, including 6-methyl-5-hepten-2-one, which was directly associated with attraction of *A. ervi* (Pickett et al., 2007). CJ also had a direct effect on settling alate grain aphids, *Sitobion avenae* F., the numbers of which were significantly reduced on treated wheat, compared to control plants in field simulator assays. Furthermore, there was significant reduction in the mean relative growth rate (MRGR) and intrinsic rate of population increase (r_m) on treated plants

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versus untreated plants under laboratory conditions (Bruce et al., 2003). In the field, replicated experiments conducted over four seasons showed that winter wheat plots treated with CJ had significantly reduced cereal aphid populations, when compared to untreated control plots (Bruce et al., 2003). CJ has been shown recently to induce indirect defence in *Arabidopsis thaliana* L. (Brassicaceae) leading to the attraction of *A. ervi* (Bruce et al., 2008), and in soybean, *Glycine max* L. (Fabaceae), leading to the enhanced attraction of egg parasitoid natural enemies of stinkbugs (Moraes et al., 2009). In both cases, induction of production of the plant stress compound (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) was a major contributing factor in enhancing defence.

The cotton aphid, *Aphis gossypii* Glover, is a major destructive sucking pest on a global scale, with a wide host range, attacking many economically important crops (Kerns and Gaylor, 1992). Apart from direct damage, the pest is vector of many viral diseases such P (PRSV-P) and W (PRSV-W) strains of Papaya Ringspot Virus, Watermelon Mosaic Virus 2 (WMV-2), Cucumber Mosaic Virus (CMV), Celery Mosaic Virus (Francki et al., 1979) and Zucchini Yellow Mosaic Virus (ZYMV) (Blua and Perring, 1992). As for many aphid species, direct damage and virus transmission is caused by the piercing and sucking feeding behaviour, mainly on the underside of leaf surfaces. Infested leaves often become cupped downwards and may appear wrinkled. Heavy infestations on some hosts may result in wilting, whilst young plants may have reduced or stunted growth. They produce copious amounts of honeydew which serves as a medium on which sooty mold grows. Sooty mold blackens the leaf and decreases photosynthetic activity (Elmer and Brawner, 1975). Adult *A. gossypii* are usually wingless, but a high population density induces the production of winged individuals which will migrate to new food sources.

To overcome problems associated with insecticide use in *A. gossypii* control, natural plant resistance, compatible with other eco-friendly pest management practices (Biswas and Singh, 1998), has been investigated. Marker-assisted selection (MAS) offers a means to increase the efficiency of incorporating aphid resistance in new plant varieties, but although some effort has been made for melon crops (Klingler et al., 2001), for cotton, *Gossypium hirsutum* L. (Malvaceae), no progress has been reported. Furthermore, breeding approaches have been slow and difficult to achieve by traditional selection methods, and consequently the availability of sucking pest resistant cultivars is unlikely in the foreseeable future. Another option is to exploit natural plant defence pathways, including the production of volatile semiochemicals (behaviour-modifying chemicals) following insect herbivory (Bruce and Pickett, 2007). These volatiles attract both parasitic and predatory insects that are natural enemies of the herbivores. They may also induce defence responses in neighbouring plants (Pickett and Poppy, 2001). In view of our earlier studies showing the induction of defence in *G. hirsutum* following herbivory by *A. gossypii* (Hegde et al., 2011), we investigated the ability of CJ to induce the production of defence VOCs in *G. hirsutum*, using *A. gossypii* as the model insect herbivore.

2. Results

In four-arm olfactometry bioassays, alate (winged) *A. gossypii* spent significantly more time ($P < 0.01$) in the arms containing the VOCs collected over a 72 h period from untreated cotton, *G. hirsutum*, compared to the solvent control (Fig. 1), and significantly less time ($P < 0.01$) in the arms containing the VOCs collected from CJ-treated *G. hirsutum* over a similar period, compared to the solvent control (Fig. 1). Comparison of GC and GC–MS analyses of VOCs collected from water, Ethylan BV (EBV, formulation control) and CJ-treated plants revealed that the latter appeared to emit higher levels of four defence compounds, i.e. the green leaf volatile compound (*Z*)-3-hexenyl acetate, the homomonoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), the aromatic compound methyl salicylate, and the homosesquiterpene (*E*,*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) (Fig. 2).

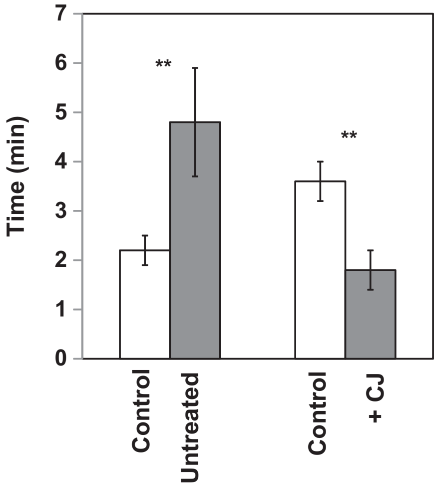

Fig. 1. Response of alate cotton aphids, *Aphis gossypii*, to VOCs collected over 72 h from untreated cotton, *Gossypium hirsutum* versus a solvent control (diethyl ether) and CJ-sprayed cotton, *G. hirsutum* versus a solvent control (diethyl ether). Data are expressed as the mean (±SE) time (min) spent in treatment and control arms, and were analysed using a paired *t*-test (one tail) with statistical software (Genstat Version 11, VSN International Ltd.) after ensuring that data were normally distributed. $^{**}P < 0.01$.

In quantification studies conducted over five consecutive days, GC analysis revealed consistently higher levels (expressed in log nanograms/gram fresh weight/hr) of DMNT and TMTT from CJ-treated plants across several days, compared to water and EBV-treated plants. Statistical analyses revealed that DMNT levels produced by CJ-treated plants were significantly higher than those from water-treated plants on the fourth and fifth days ($t_{81} < -2.21$; $P < 0.05$; Fig. 3), and were significantly higher than those from EBV-treated plants only on the fifth day ($t_{81} = -2.32$; $P < 0.05$; Fig. 3). Statistical analyses revealed that TMTT levels produced by CJ-treated plants were significantly higher than those from water-treated plants on the fourth and fifth day ($t_{81} < -2.08$; $P < 0.01$ and $P < 0.05$, respectively; Fig. 3), and were significantly higher than those induced by EBV treatment on any given day other than day three ($t_{81} < -2.09$; $P < 0.01$ for day one, $P < 0.05$ for other days; Fig. 3). Furthermore, addition of 100 *A. gossypii* to water, EBV or CJ-treated plants resulted in consistently higher levels of DMNT and TMTT in CJ treatments. Significantly higher levels of DMNT were emitted by CJ-treated plants compared to water-treated plants ($t_{81} < -1.99$; $P < 0.05$ on days three and five, $P < 0.01$ on day four, Fig. 4) and EBV-treated plants ($t_{81} < -1.99$; $P < 0.05$ on days three, four and five, Fig. 4). Significantly higher levels of TMTT were produced by CJ-treated plants, compared to water-treated plants, on days one, three, four and five ($t_{81} < -1.99$; $P < 0.01$ for days one, three and four, $P < 0.05$ on day five; Fig. 4), and compared with EBV-treated plants on all days ($t_{81} < -1.99$; $P < 0.01$ for days one, three and four, $P < 0.05$ for days two and five, Fig. 4). Despite earlier indications, there was no significant difference in the levels of (*Z*)-3-hexenyl acetate and methyl salicylate between water, EBV- and CJ-treated plants, either without or with subsequent addition of *A. gossypii* (Figs. 3 and 4, respectively) on any day, and there was no detection of CJ from water, EBV- and CJ-treated plants (data not shown). CJ treatment induced the release of an equivalent profile of defence VOCs to that elicited by *A. gossypii* ($t_{81} = 2.827$, $P = 0.003$ for DMNT; $t_{81} = 1.662$, $P = 0.05$ for TMTT;