The Journal of Experimental Biology 214, 3672-3677

doi:10.1242/jeb.059923

RESEARCH ARTICLE

Increased plant volatile production affects oviposition, but not larval development, in the moth *Helicoverpa armigera*

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Accepted 9 August 2011

SUMMARY

It is well established that herbivorous insects respond to changes in plant odour production, but little attention has been given to whether these responses relate to direct fitness costs of plant volatile production on insect growth and survival. Here, we use transgenic *Nicotiana tabacum* (tobacco) plants that produce relatively large amounts of the volatile (*S*)-linalool to study whether the responses of egg-laying herbivorous insects to linalool production relate directly to the growth and survival of offspring. In choice tests, fewer eggs were laid on transgenic plants compared with non-transformed controls, indicating that increased linalool emissions have a deterrent effect on *Helicoverpa armigera* oviposition. Larval survival and larval mass after feeding on transgenic leaves, however, was comparable to non-transformed controls. (*S*)-linalool, whether in volatile or sequestered form, does not appear to have a direct effect on offspring fitness in this moth. We discuss how the ecology of this polyphagous moth species may necessitate a high tolerance for certain volatiles and their related non-volatile compounds, and suggest that responses by adult female *H. armigera* moths towards increased linalool production may be context specific and relate to other indirect effects on fitness.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/214/21/3672/DC1

Key words: *Helicoverpa armigera*, *Nicotiana tabacum*, linalool, olfaction.

INTRODUCTION

Plant odours are comprised of mixtures of volatiles produced by different biosynthetic pathways (Pichersky and Gershenzon, 2002). These odours provide species-specific signals to herbivorous insects, which are attracted to their host plants to feed or lay eggs (Bruce et al., 2005; Dudareva et al., 2006; Hare, 2011). The number and relative concentrations of volatile compounds released by various plant organs can vary according to factors such as plant age, flowering status, nutritional status and damage through insect herbivory (Dudareva and Pichersky, 2000; Hare, 2011; Pichersky and Gershenzon, 2002), thereby carrying information relating to plant quality. Consequently, if subtle intraspecific differences in odour composition correlated predictably with differences in fitness for the developing herbivore, ovipositing adults might be under selection pressure to recognise and respond to these odour differences (Pichersky et al., 2006).

Fitness benefits in recognising the presence or absence of particular volatiles may be directly associated with the volatile itself, such as a potential toxic effect of the odour and non-volatile compounds associated with its synthesis (Abdelgaleil et al., 2009; Chang et al., 2009; Phillips et al., 2010; Rajwinder et al., 2010). Alternatively, indirect fitness benefits to volatile recognition may occur through the volatile's influence on the behaviour of other organisms, such as herbivore predators (Gershenzon and Dudareva, 2007; Hare, 2011; Kessler and Baldwin, 2001).

Here, we investigate whether increased production of the volatile monoterpene linalool influences the attraction of a herbivorous insect pest species – the moth *Helicoverpa armigera* – and measure associated direct (nutritional) costs of linalool production on offspring fitness. Linalool is a common floral volatile in many moth-pollinated plants and has been shown to have both attractant (Raguso et al., 2003; Suckling et al., 1996) and deterrent (Kessler and Baldwin, 2001) properties in ovipositing Lepidoptera. Linalool is a major component of the floral odour of tobacco (Loughrin et al., 1990), a preferred host of *H. armigera* (Firempong and Zalucki, 1990) and is also produced in small quantities from vegetative tissue volatile blends in response to herbivore attack (De Moraes et al., 2001). At high concentrations, linalool can be toxic to a variety of insect species (Abdelgaleil et al., 2009; Chang et al., 2009; Phillips et al., 2010), affecting mortality, growth, activity and feeding in lepidopteran larvae (Rajwinder et al., 2010; Singh et al., 2009). This volatile occurs naturally in two isomeric forms, and a recent study using artificial odour blends has suggested that the *R* and *S* isomers of linalool may be perceived differently by the moth *Manduca sexta*, with both isomers being attractive to nectar feeding moths, but only the *R* isomer deterring ovipositing moths (Reisenman et al., 2010).

In order to understand how linalool production may relate to *H. armigera* fitness, we compare adult oviposition and larval survival on transgenic *Nicotiana tabacum* (tobacco) with increased (*S*)-linalool in vegetative and floral tissues with that on non-transformed controls. Tobacco has previously been shown to be a successful model plant for the increase and alteration of monoterpene biosynthesis (Lücker et al., 2004; Ohara et al., 2003). In addition to increased volatile release, overproduction of linalool in tobacco

could lead to the accumulation of non-volatile glycosylated forms of this monoterpene alcohol, as was shown to occur in petunia, *Arabidopsis* and potato (Aharoni et al., 2003; Lücker et al., 2001; Lücker et al., 2006).

MATERIALS AND METHODS

Plant material and growth conditions

Nicotiana tabacum var. Ti68, a rapid flowering tobacco variety, was grown on modified UC soil mix under standard glasshouse conditions or in sterile tissue culture on MS media (4.33 g l MS salts, 1×MS vitamins, pH 5.7, 20 g l sucrose). *Clarkia breweri* seed was kindly provided by Michael Wall, Rancho Santa Ana Botanic Gardens (Claremont, CA, USA).

Cloning, transformation and characterisation of transgenics

The 35S::LIS transgene expression cassette was constructed by placing a genomic copy of the (*S*)-linalool synthase gene (*LIS*) from *Clarkia breweri* (Pichersky et al., 1995) under the control of the strong constitutive cauliflower mosaic virus *35S* promoter (Odell et al., 1985) and the *Vigna radiata ACS1* translational enhancer (Wever et al., 2010) in the shuttle vector pHannibal (Wesley et al., 2001) to facilitate cloning into the plant transformation vector pUQC246 (kindly provided by B. Carroll, University of Queensland). Because of the large size of the *C. breweri LIS* gene, it was cloned into the pHannibal-5′UTR vector in two stages using a central *Xho*I site within *LIS* to facilitate cloning. The first half of the gene was amplified by PCR from *C. breweri* genomic DNA using a thermostable proofreading DNA polymerase with the primers linaloolF1, containing an *Avr*II restriction site (5′-GTACCTAGGAAACAATGGCTTCCTCCCAGCTCATAACAA-ATTTCTCC-3′), and linaloolR1 (5′-GCGTAAATACATAGCA-GATGAG-3′), and cloned into pGEM-T Easy (Promega, Sydney, NSW, Australia) before cloning into the pHannibal-5′UTR vector (*Avr*II/*Xho*I). The linaloolF1 primer incorporated translation-enhancing initiation sequences (5′-AAACAATGGCTTCCTCC-3′) upstream and downstream of the start codon. The second half was cloned similarly into the pHannibal vector (*Xho*I/*Bam*HI) to reassemble the full-length *LIS* gene, following amplification with primers linaloolF2 (5′-ACCACCTTAAACAAGACAACC-3′) and linaloolR2 (5′-GCGGGATCCTTAACTGAAACATAGTTTGAT-GTTG-3′), which contained a *Bam*HI restriction site. Each construct was verified by sequencing. *Agrobacterium*-mediated tobacco transformation was performed using the leaf disc transformation method described by Purnell and Botella (Purnell and Botella, 2007). Transgenic plants were screened for the presence of the transgene by PCR, and seed segregation analysis (based on resistance to kanamycin) was performed to select single-copy, homozygous lines for further analysis and insect experiments. Total RNA was isolated as previously described (Trusov et al., 2009). Southern and northern analyses of tobacco flowers were performed as described by Trusov et al. (Trusov et al., 2007).

Volatile sampling and GCMS analysis

We employed a pressurised air pushing system using factory prefilled glass Tenax® TA (60/80 mesh) thermal desorption sampling tubes (Sigma-Aldrich, Sydney, NSW, Australia) for analysis of headspace volatiles. High purity instrument air (BOC Gas, Rocklea, QLD, Australia) was used to push clean air through the system and polytetrafluoroethylene (PTFE) tubing (Cole Palmer, Vernon Hills, IL, USA) was used throughout the system. A 150 mm direct reading flow meter (Cole Palmer) was placed immediately downstream of the gas cylinder, and a single Tenax® sampling tube was placed immediately downstream of this. This Tenax® tube was used to remove any impurities that may be introduced into the sampling system from the air source. Air flow was split four ways using a glass manifold (UQ Glass, Brisbane, QLD, Australia) to four 960 ml Kaptclean™ sampling jars with PTFE-lined lids (Cole Palmer). To allow air flow through the jars, two holes were drilled into the lids of each sampling jar for two 0.25 in bulkhead unions (Swagelok, Brisbane, QLD, Australia), allowing connection of an empty glass thermal desorption sampling tube (Sigma-Aldrich) at the air inlet and connection of a Tenax® sampling tube at the outlet. Pre-cleaned air was pumped into each sampling jar and volatiles released from plant samples were collected as air flowed through the Tenax® sampling tube fitted at the outlet of each jar. Air flow through the sampling jars attached in parallel was then merged again into a single stream using another glass manifold, and another Tenax® sampling tube was placed downstream of this to detect any breakthrough of volatiles that might occur from the sampling Tenax® tubes. A second flow meter was placed at the very end of the system to check that flow rate was maintained equally across the entire system and ensure that leaks did not occur.

For Tenax® trapping of headspace volatiles from intact flowers, flowers were placed in a block (5×5×3 cm) of moistened floral foam wrapped in aluminium foil within a cleaned glass sampling jar, and volatiles were sampled at an air flow rate of 100 ml min^{-1} (i.e. a maximum total system flow rate of 400 ml min^{-1} if all four sampling jars were run simultaneously in parallel). Six biological replicates were analysed for each genotype. For each biological replicate, eight flowers collected at sunset were sampled continuously by Tenax® trapping for 24 h. For Tenax® trapping of headspace volatiles from vegetative six-week-old non-flowering tobacco plants, whole intact plants were enclosed in glass jars and sampled similarly for a total of 27 h. The soil surface and plant pot was covered in aluminium foil to avoid sampling of soil volatiles.

The $CaCl_2$ acid hydrolysis method used to measure volatiles retained within plant tissues was adapted from Lücker et al. (Lücker et al., 2001). Released volatiles were measured following grinding of tissues to a fine powder in liquid nitrogen and each sample (approximately 4 g leaf tissue) was incubated while stirring for 10 min at 50°C in 65 ml of a 5 mol l^{-1} (saturated) calcium chloride solution in a cleaned glass sampling jar. Samples were allowed to cool briefly, before sampling of the released volatile compounds by Tenax® trapping (100 ml min^{-1} air flow rate) for 17 h at 30°C with continuous stirring. Measurement of volatiles released from ground floral tissues after β-glucosidase treatment was adapted from Lücker et al. (Lücker et al., 2001). Tissues were ground to a fine powder and incubated for 10 h while stirring at room temperature in 50 ml of McIlvaine's buffer (pH 7) containing 20 units of almond β-glucosidase (Sigma-Aldrich). The released volatiles were sampled by Tenax® trapping for 2 h at room temperature with continuous stirring.

Collected volatiles were eluted from Tenax® tubes with 1 ml of gas chromatography (GC)-grade hexane (>99%, Sigma-Aldrich) and 3 µl of a 1:10000 dilution of pure 2-octanone (Sigma-Aldrich) added as an internal standard. 3 µl of each sample was analysed immediately by gas chromatography mass spectroscopy (GCMS) or stored at –20°C for later analysis. For absolute quantification of (*S*)- and (*R*)-linalool, volatiles were eluted with 4 ml pentane and redistilled diethyl-ether (80:20), supplemented with up to 1010 ng (+)-3-carene as internal standard. Samples were concentrated under a slow flow of high purity nitrogen gas (99.96%, BOC Gas) to 15 µl before splitless injection of 2 µl onto the GCMS.

For GCMS analysis, samples were separated on a 30 m DB-5 capillary column coupled with a 5 m guard column (0.25 mm inner diameter, 0.25 µm thickness, Agilent Technologies, Santa Clara, CA, USA) with helium used as the carrier gas. Compounds were identified by comparison with mass spectral data (Wiley mass spectra library) and GC retention times of pure authentic standards. Peaks were quantified relative to the internal standard [2-octanone or (+)-3-carene] peak area of the total ion chromatogram. For analysis of floral headspace samples, the following GC program was used. The column inlet pressure was 88.7 kPa, with a column flow of 1.6 ml min^{-1} and total carrier flow of 71.5 ml min^{-1}. Injection was performed in the split mode with a split ratio of 42, an injection temperature of 250°C, an interface temperature of 230°C and the solvent cut time set to 3 min. The GC oven temperature was programmed for 3 min at 40°C followed by a rise to 260°C at 10°C min^{-1}, with a final hold at 260°C for 15 min. Leaf headspace and retained volatile samples were run similarly, except for the following modifications to the GC program: injection was performed in the splitless mode for 1 min and then switched to split and the temperature was held at 35°C for 2 min, and then increased to 260°C at 4°C min^{-1}, with a final hold at 260°C for 5 min. In all cases, the MS was set for a full scan from 40 to 300 mass units at an interval of 0.5 s and a scan speed of 500 amu s^{-1}. Linalool chirality (König et al., 1997) was determined on a Shimadzu GC-17A equipped with a flame ionization detector, using a 25 m, 0.25 mm inner diameter Lipodex-E column (Macherey-Nagel, Düren, Germany) with an isocratic oven temperature of 55°C and a flow rate of 2 ml min^{-1}.

Insect behavioural analyses

Helicoverpa armigera (Hübner) moths were obtained from a laboratory-reared culture maintained at the Queensland Department of Primary Industries and Fisheries (Long Pocket, QLD, Australia). Pupae were sexed and held in separate cages until emergence. Newly emerged male and female moths were transferred to 45 cm^3 holding cages containing *Cuphea hyssopifolia*, a non-host plant, which female moths readily visit for nectar. Our previous experience has shown that allowing females to forage on a floral nectar source (as opposed to *ad libitum* provision of honey solution) improved egg laying on plants (as opposed to cage walls) and prevented moths from overfeeding (J.P.C., unpublished). Moths were left for 3 days to ensure mating, after which female moths were transferred to cages for oviposition trials. Moths were studied individually, to prevent pseudoreplication effects and overestimation of numbers in the egg-laying population studied.

We conducted oviposition preference studies in a 1 m^3 mesh flight cage with a soil floor covering, housed within a glasshouse. No additional light source was used during cage experiments, although diffuse lighting was provided by nearby streetlights. In each trial, two wild-type and two LIS2-3 tobacco plants were used, matched in pairs for height and flowering status (between one and three fully open flowers), and spaced 40 cm apart in the cage. New plants were used in each trial and the position of the plants was randomised. We released one individual (mated) female moth into the flight cage at least 2 h before sunset on the night of testing, and counted the number of eggs laid on each plant the following morning. Data were collected for 20 individual females, using a total of 80 plants (40 of each line).

For larval survival experiments, we placed mated female moths in Perspex containers with a cotton nappy liner roof to collect fertilised eggs. One-day-old first instar *H. armigera* larvae were removed from the nappy liner with a fine paintbrush and placed on the upper leaves (>10 cm length) of non-flowering wild-type and LIS2-3 plants housed within the glasshouse. We used five plants of each type, placing 30 larvae on each plant (10 per leaf). Nine days later, all larvae were collected and weighed.

Statistical analyses

Two-tailed Student's *t*-tests were used for statistical analysis of volatile emissions. For adult oviposition and larval development studies, results were analysed using non-parametric statistics: Wilcoxon signed-rank test, Spearman's rank correlation, chi-squared (χ^2) and Mann–Whitney *U*-test. Means are displayed ± standard error.

RESULTS

Molecular characterisation of transgenic lines

A number of independent 35S::LIS transgenic *N. tabacum* Ti68 lines were produced by *Agrobacterium*-mediated transformation. Northern analysis was performed on putative homozygous, single-insertion lines (according to segregation analysis) and a line showing high linalool transcript levels in flowers (line LIS2-3) was selected for further studies (supplementary material Fig. S1A). Southern analysis confirmed the presence of a single transgene insertion in this line (supplementary material Fig. S1B). Correct splicing of the linalool synthase mRNA from the introduced genomic DNA sequence was confirmed by PCR amplification of the cDNA from transgenic plants followed by sequencing (results not shown). No differences in plant morphology or growth were observed in any of the transgenic lines compared with wild-type plants.

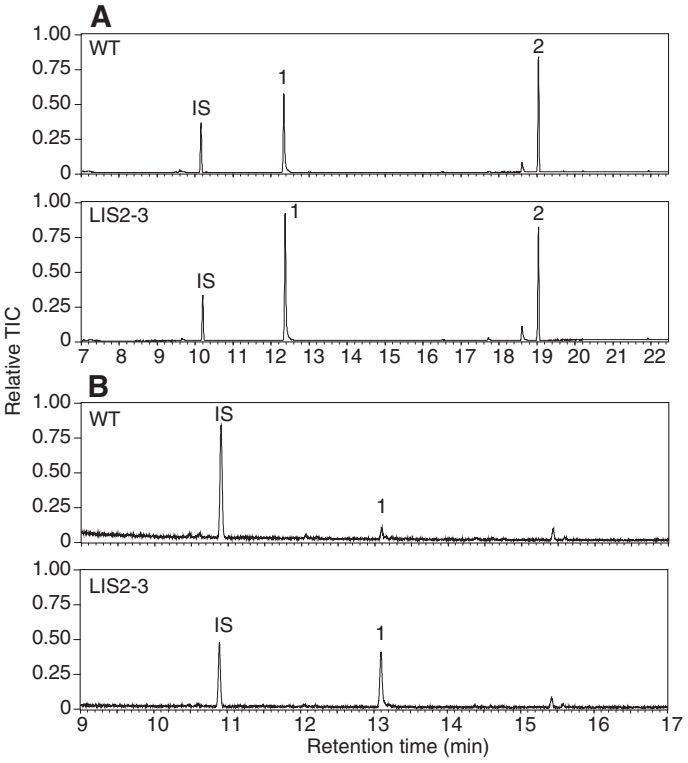

Fig. 1. Headspace volatile analysis of wild-type (WT) and transgenic LIS2-3 tobacco plants. Representative chromatographs obtained by Tenax® trapping of (A) floral headspace volatiles and (B) volatiles released by six-week-old non-flowering intact tobacco plants. Chromatographs display relative total ion count (TIC). Peaks are labeled: IS, internal standard [(+)-3-carene]; 1, linalool; 2, β-caryophyllene. Note: floral and leaf volatiles were resolved using different gas chromatography mass spectrometry programs.

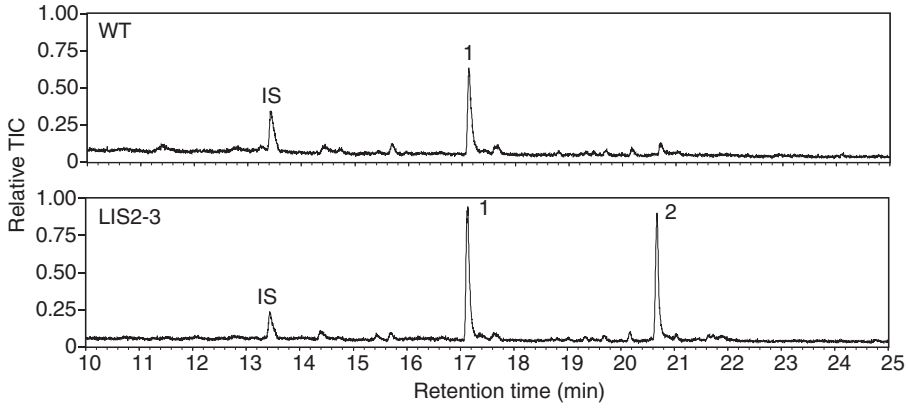

Fig. 2. Terpenoid compounds retained within leaves of wild-type (WT) and transgenic LIS2-3 tobacco plants. Representative chromatographs obtained by Tenax® trapping of volatiles released from ground leaf tissues after $CaCl_2$ acid hydrolysis treatment of leaf samples. Chromatographs display relative total ion count (TIC). Peaks are labeled: IS, internal standard [(+)-3-carene]; 1, linalool; 2, α-terpineol.

Headspace volatiles and leaf substrate analysis

As with other tobacco varieties, the predominant floral volatiles produced by the Ti68 variety are the monoterpene linalool and the sesquiterpene caryophyllene, with minor amounts (<5% of total volatiles) of other monoterpenes and sesquiterpenes (Fig. 1A). Compared with wild-type flowers, linalool emissions increased twofold in the floral headspace of fully opened (stage 12) (Koltunow et al., 1990) transgenic LIS2-3 flowers (t_6=–5.43, P<0.005), whereas no significant changes in β-caryophyllene emissions were detected (t_6=–1.15, NS; Fig. 1A). The enantiomeric excess of (*S*)- over (*R*)-linalool was 86.03±0.91% [i.e. 92.96% (*S*)-linalool and 6.93% (*R*)-linalool] in LIS2-3 flowers compared with 76.06±2.87% in wild type flowers (t_3=–4.44, P<0.005; supplementary material Fig. S2).

Leaves of wild-type plants emit only trace levels of linalool and sesquiterpene volatiles including caryophyllene, the levels of which could not be quantified by Tenax® trapping (Fig. 1B). In contrast, transgenic non-flowering six-week-old LIS2-3 plants were found to emit 220±16.31 ng of linalool per gram fresh mass over a period of 24 h (Fig. 1B). The enantiomeric excess measured for the linalool emitted from the leaves of LIS2-3 plants was 100% (*S*)-linalool; no (*R*)-linalool was detected (supplementary material Fig. S3).

Presence of sequestered terpenoids in leaves was determined by sampling the headspace of ground leaf tissue in saturated 5 mol l^{-1} $CaCl_2$. Transgenic LIS2-3 plants consistently showed higher amounts of sequestered linalool than wild-type plants (Fig. 2). In addition, acid hydrolysis released substantial amounts of α-terpineol from LIS2-3 leaves, indicating the presence of sequestered glycosylated compounds associated with linalool production (Lücker et al., 2001). These results were confirmed by treatment of ground leaf tissue from LIS2-3 plants with a β-glucosidase enzyme, which prevented release of α-terpineol (results not shown).

Helicoverpa armigera oviposition trials, larval development and survival

Adult oviposition

Egg counts for 20 individual female moths caged overnight with wild-type and LIS2-3 plants are displayed in Fig. 3A. Each moth laid a mean of 179.10±17.02 eggs on the plants per night, 64±6% on inflorescences 8and 36±6% on leaves. Significantly more eggs were laid on wild-type plants compared with transgenic LIS2-3 plants (Wilcoxon signed-rank test, Z=2.95, P<0.005); the mean percentage oviposition on LIS2-3 plants was 41±2% and 58±2% for wild-type plants. The proportion of eggs laid on flowers compared with leaves on the two plant lines was closely correlated (Spearman's rank, r_S=0.80, P<0.001).

Larval development and survival

Of 300 first instar larvae placed on five wild-type and five LIS2-3 plants, 207 (69%) were found 9 days later. The percentage of larvae remaining on wild-type plants (68%) was not significantly different from those remaining on LIS2-3 plants (70%, χ^2=0.14, d.f.=1, NS). Mean larval masses are shown in Fig. 3B. No significant differences in larval mass were found after 9 days development on wild type compared with LIS2-3 plants (Mann–Whitney U-test, U=0.93, P>0.05).

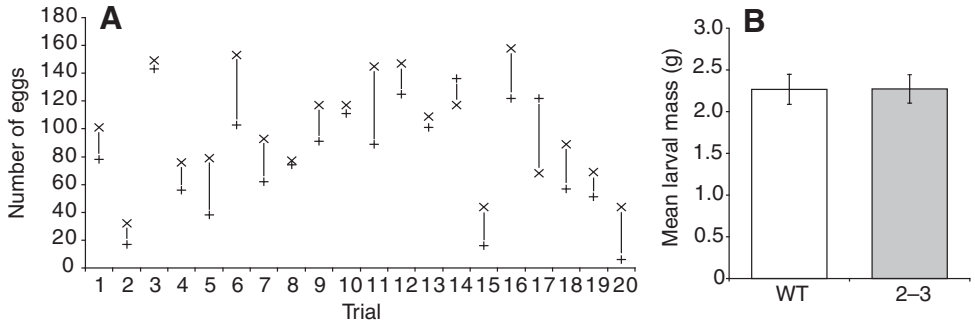

Fig. 3. Ovipositional preferences and larval growth of *Helicoverpa armigera* on wild-type and transgenic LIS2-3 tobacco plants. (A) Total number of eggs laid on wild-type (×) and transgenic LIS2-3 (+) plants in 20 replicated cage trials using individual female *H. armigera* moths. Mean egg numbers on LIS2-3 and wild-type plants are significantly different (Wilcoxon signed-rank test, P<0.005). (B) Mean larval mass (N=207 insects total) after 9 days development on wild-type (WT) and LIS2-3 (2-3) tobacco leaves. Mean larval masses are not significantly different between plant types (Mann–Whitney U-test, P>0.05).

DISCUSSION

Adult *H. armigera* moths laid significantly fewer eggs on transgenic LIS2-3 tobacco plants producing higher amounts of (*S*)-linalool, compared with wild-type controls, suggesting that an increase in (*S*)-linalool production decreases the attractiveness of tobacco to ovipositing *H. armigera* moths. Our study did not, however, find any evidence to support the hypothesis that avoidance of plants with higher (*S*)-linalool emissions may have evolved as a result of the possible toxic effects of this volatile, or non-volatile compounds related to its production. There were no significant differences in *H. armigera* larval survival or larval mass after 9 days development on LIS2-3 compared with wild-type plants, despite substantial sequestration of (non-volatile) glycosylated linalool in LIS2-3 tissues.

Given that we could not find a relationship between adult choice and offspring survival, why might females avoid plants that have increased linalool emissions? One possible answer is that linalool emission from leaf tissue influences insect fitness indirectly, through increased predation. Linalool has been shown to be emitted in response to caterpillar herbivory in host plants of *H. armigera* (Rose and Tumlinson, 2004; Turlings and Tumlinson, 1992), including tobacco (De Moraes et al., 2001), and the presence of linalool within a plant's volatile profile may attract larval predators and parasitoids (Dicke, 2009; Tumlinson et al., 1993; Turlings et al., 1995). It may seem counterintuitive that *H. armigera* can be deterred by the presence of a volatile such as linalool, which is often emitted in large quantities from the flowers of many of its host species, but contextual effects (the combination of volatiles that appear together in an odour blend) could allow insects to respond differently to increased (*S*)-linalool depending on its site of emission (Lei and Vickers, 2008; Pinero and Dorn, 2007; Tasin et al., 2010). In our study, the relative change in (*S*)-linalool production in LIS2-3 plants was much higher in leaf tissue than in floral tissue, and the decreased attractiveness of LIS2-3 to *H. armigera* moths could be due to the effects of the specific combination of green leaf volatiles and linalool in evoking behavioural responses.

Alternatively, the observed (deterrent) response to higher linalool emissions may be a remnant from the insect's evolutionary history. Being a polyphagous insect, *H. armigera* is capable of detoxifying a broad spectrum of volatile and non-volatile plant metabolic compounds. As this moth shows a strong preference for the flowering stages of its hosts (Zalucki et al., 1986), we would predict a low fitness cost associated with detoxification of compounds associated with floral volatiles, particularly linalool, which is a common volatile in moth-pollinated flowers and in the host species of *H. armigera* (Del Socorro et al., 2010; Raguso et al., 2003). The decreased response to plants with high linalool emissions may exist because of constraints to the evolution of insect behavioural responses, such as neurological constraints to sensory mechanisms (Bernays, 2001; Egan and Funk, 2006; Janz, 2003). Metabolic (fitness) costs of the volatile, or its non-volatile precursors, may have existed in ancestral insect populations, but have since been overcome (Bernays and Chapman, 1994; Steiger et al., 2011).

Interestingly, our findings on *H. armigera* oviposition responses to linalool differ markedly from a recent study on *Manduca sexta* moths, which used synthetic volatile blends to mimic *Datura wrightii* floral odours, and demonstrated that only blends with higher levels of (*R*)- isomer had deterrent effects on oviposition, and that increased (*S*)-linalool within blends was more attractive to nectar-feeding adult *M. sexta* moths (Reisenman et al., 2010). The difference in behavioural responses to increased (*R*)- and (*S*)-isomers of linalool in *H. armigera* and *M. sexta* could be due to intraspecific differences in insect olfactory responses; both species feed and lay eggs on flowering tobacco, but they differ in the breadth of their host ranges. *Helicoverpa armigera* has a host range that spans many plant families (Zalucki et al., 1986), whereas *M. sexta* is a specialist on the Solanaceae. Natural selection would therefore be expected to act differently upon the olfactory responses of these two moth species. Experimental methodology may, however, be responsible for the contrasting responses shown in these two moth studies. Reisenman et al. (Reisenman et al., 2010) conducted their study using artificial flowers and simple synthetic odour blends, whereas our study investigated odour responses in the context of whole plants, where increased linalool production was a constituent of both floral and vegetative odour blends.

In this study, we measured an insect's response to a transgenic plant that has higher emissions of a specific volatile. Two important conclusions can be drawn. Firstly, changing levels of individual volatiles, presented in the natural context of a living flower, can influence insect behaviour – that is, the insect's olfactory system is sensitive enough to detect such changes in volatile emissions. Secondly, no simple association between linalool emissions and larval survival can be found. Bearing in mind that linalool occurs in *H. armigera* host species, which differ in nutritional quality, as well as in non-host species (including floral species that may attract nectar foraging adult moths but have unsuitable oviposition sites), it may be erroneous to put too much emphasis on a specific evolutionary role for this single volatile.

ACKNOWLEDGEMENTS

We thank the Queensland Department of Primary Industries and Fisheries at Long Pocket for providing *H. armigera* pupae, Michael Wall (Rancho Santa Ana Botanic Gardens) for providing *C. breweri* seeds, Asst Prof. Bernie Carroll (University of Queensland) for the binary vector pUQC246, David Chakravorty for Southern analysis, and Dr Chris Moore for advice on volatile sampling.

FUNDING

E.J.M. was supported by an Australian Postgraduate Award, a UQ Research Scholarship and a Daphne Elliott Bursary. The work was funded by the Australian Research Council [grant number DP0988150].

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