Methyl jasmonate induction of cotton: a field test of the ‘attract and reward’ strategy of conservation biological control

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Received: 6 September 2016  Editorial decision: 24 June 2017  Accepted: 11 July 2017  Published: 17 July 2017

Guest Editor: Rupesh Kariyat

Citation: Williams L III, Rodriguez-Saona C, Castle del Conte SC. 2017. Methyl jasmonate induction of cotton: a field test of the ‘attract and reward’ strategy of conservation biological control. AoB PLANTS 9: plx032; doi: 10.1093/aobpla/plx032

Abstract. Natural or synthetic elicitors can affect plant physiology by stimulating direct and indirect defence responses to herbivores. For example, increased production of plant secondary metabolites, a direct response, can negatively affect herbivore survival, development and fecundity. Indirect responses include increased emission of plant volatiles that influence herbivore and natural enemy behaviour, and production of extrafloral nectar that serves as a food source for natural enemies after their arrival on induced plants. Therefore, the use of elicitors has potential for the study of basic aspects of tritrophic interactions, as well as application in biorational pest control, i.e. an ‘attract and reward’ strategy. We conducted a field study to investigate the effects of methyl jasmonate, an elicitor of plant defence responses, on three trophic levels: the plant, herbivores and natural enemies. We made exogenous applications of methyl jasmonate to transgenic cotton and measured volatile emission, extrafloral nectar production and plant performance (yield). We also assessed insect abundance, insect performance, and parasitism and predation of brown stink bug, Euschistus servus, eggs in methyl jasmonate-treated and untreated control plots. Application of methyl jasmonate increased emission of volatiles, in particular, (+)-limonene and (3E)-4,8-dimethyl-1,3,7-nonatriene, and production of extrafloral nectar, but not yield, compared with the control treatment. Despite increased volatile and extrafloral nectar production, methyl jasmonate application did not affect plant bug performance, or mortality of E. servus egg masses, and only marginally influenced insect abundance. Mortality of E. servus eggs varied over the course of the study. Overall, methyl jasmonate treatment affected cotton plant-induced responses, but not the insects that inhabit the plants. Our results were probably influenced by reduced natural enemy colonization of cotton from adjacent non-crop habitats, and subsequent low within-field population recruitment. Much remains to be learned about the effects of exogenous application of plant-produced ‘enhancers’ on the behaviour of natural enemies before crop physiology can be manipulated to enhance pest control.

Keywords: Cotton; Euschistus servus; extrafloral nectar; methyl jasmonate; parasitism; plant bugs; plant volatile induction; Platygastridae; predation; stink bugs.

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Introduction

Conservation biological control (CBC) encompasses a variety of approaches that aim to preserve natural enemy populations and improve their efficacy through modification of the biotic environment and reduction of pesticide impacts (Ehler 1998). One such approach, provision of natural enemies with naturally occurring non-host food resources (e.g. nectar, honeydew, pollen), provides energy for maintenance, locomotion and reproduction, and thus plays an important role in sustaining natural enemy populations and fostering improved pest control (Jamont et al. 2013; Russell 2015). Extraloral nectar (EFN) production in cotton, which occurs on leaves and external parts of reproductive structures, sustains natural enemies (Wäckers and Bonifay 2004; Hagenbucher et al. 2013). In another CBC research arena, knowledge accumulated over the past 20 years has demonstrated that natural enemies use host- and plant-derived chemicals as cues for host location (Fatouros et al. 2008; Conti and Colazza 2012). In addition to the production and emission of volatiles, the attack–response signalling pathways also regulate nectar production (Heil 2011) and expression of defensive substances against herbivores (Rodriguez-Saona and Thaler 2005; Schaller and Stintzi 2008). These plant defence responses can also be induced by exogenous application of the synthetic elicitors, such as jasmionic acid or its methyl ester, methyl jasmonate (MeJA) (Thaler 2002; Wu et al. 2008) or cis-jasmonate (Bruce et al. 2003).

Our knowledge of plant volatile production and our ability to induce it have led to the use of behaviourally active herbivore-induced plant volatiles (HIPVs) to ‘herd’ or otherwise manipulate the behaviour of beneficial insects within or between crop fields to improve pest control (James 2005; Turlings and Ton 2006; Rodriguez-Saona et al. 2011, 2012; Kaplan 2012). A recently proposed strategy aims to take advantage of HIPVs and food resources by employing the two approaches in concert, the idea being to use plant volatiles to attract natural enemies to the area of interest (i.e. locations where pest control is desired), then reward the immigrants with food, i.e. nectar produced by nearby non-crop vegetation (Simpson et al. 2011a; Orre Gordon et al. 2013), or artificial food sources (Kunkel and Cottrell 2007). Thus, natural enemies will be attracted to and retained in the desired area and provide greater suppression of pest populations. However, to date, studies evaluating this ‘attract and reward’ strategy have yielded mixed results. Studies deploying synthetic HIPVs in crops adjacent to floral resources (buckwheat, Fagopyrum esculentum) yielded complicated interactions between time, different insect species and the ‘attract and reward’ approaches (Simpson et al. 2011a; Orre Gordon et al. 2013). However, in broccoli plots, a methyl salicylate-buckwheat treatment led to an increased abundance of scelionid (=platygastrid) wasps on sticky traps (Simpson et al. 2011a). Kunkel and Cottrell (2007) reported increased oviposition by a green lacewing, Chrysoperla rufilabris, in response to attractant–artificial food spray treatments in pecan, although results were inconsistent between treatments and years. The few studies that have evaluated the ‘attract and reward’ strategy suggest that the two approaches often act independently, and thus synergistic effects were infrequent; nevertheless, the evidence lends support to the idea that the strategy has potential to offer improved biological control, and further experiments are warranted.

Cotton is subjected to herbivory by a wide variety of arthropod pests, and pest control is the single largest variable cost in cotton production (Naranjo and Luttrell 2009). Historically, the boll weevil and several species of Lepidoptera were the pre-eminent cotton pests worldwide, but changes in agroecosystem characteristics, such as the use of new crop rotation and irrigation practices, new crop species and cultivars, and implementation of broad-scale pest control initiatives (e.g. widespread use of transgenic cotton, and the boll weevil and pink bollworm eradication programmes), over the past two decades have altered the landscape of cotton pest management (Naranjo and Luttrell 2009; Bergé and Ricroch 2010). Some insects, especially those with piercing–sucking mouthparts (e.g. stink bugs), that were previously regarded as sporadic, secondary or localized pests now have increased pest status (Lu et al. 2010; Naranjo 2011; Hagenbucher et al. 2013). The ongoing evolution of agricultural production practices and continued concerns regarding health and environmental issues surrounding synthetic pesticide use have created a scenario where biorational strategies of pest suppression may be more viable than ever before (Lu et al. 2012).

The brown stink bug, Euschistus servus, causes damage to a variety of crops in the southeastern USA, including cotton, soybean, maize, sorghum, tomato and several orchard crops (McPherson and McPherson 2000). Egg parasitoids are important natural enemies of stink bugs and have shown potential for control of stink bug populations (Orr 1988; Ehler 2000). Among these, Telenomus podisi and Trissolcus euschisti (Hymenoptera: Platygastridae) are two New World species that attack E. servus (McPherson 1982). Several platygastrid wasps use host- or plant-produced volatiles to find and successfully attack their hosts (Fatoiros et al. 2008). In laboratory studies, T. podisi was attracted to plant volatiles produced after herbivory by the host, Euschistus heros (Moraes et al. 2005), to a mixture of volatiles emitted.
by cis-jasmone-induced soybean plants (Moraes et al. 2009) and to chemical residues deposited on plants by walking female hosts (Borges et al. 2003). Platygastrid wasps are benefitted by feeding on carbohydrate-rich foods (Rahat et al. 2005; Tillman 2009; Peverieri et al. 2012; Foti et al. 2016). Predators of E. servus eggs have received less attention than egg parasitoids, although evidence indicates that there is a broad range of natural enemies that attack stink bug eggs, and the potential exists to use them to suppress stink bug populations. Egg predators can inflict >75 % mortality of E. servus eggs in non-crop vegetation adjacent to Mississippi crop fields (L. Williams, USDA-ARS, Charleston, unpubl. data). Ehler (2000) found that Argentine ants, Linepithema humile, and common pillbug, Armadillidium vulgare, are important predators of Euschistus conspersus eggs in California tomato fields.

We know of no studies evaluating the ‘attract and reward’ strategy under a larger spatial scale or using induced crop plants that produce HIPVs and EFN. There are advantages to using crop-produced attractants and rewards. This approach eliminates the need to deploy or apply synthetic attractants, and to plant and maintain reward-providing non-crop plants that may complicate production practices (e.g. planting, cultivation, irrigation, harvest) and reduce acreage for production. Crops might be induced to express natural enemy attractants and rewards either by exogenous application of elicitors or via development of cultivars that express these desired traits. The goal of the present study was to assess whether treating cotton plants with MeJA stimulated the defence system of the plants, and if and how these changes, in turn, influenced the associated insect fauna. A field experiment was conducted in cotton to determine the effects of exogenous application of MeJA on plant volatile emission, EFN production, plant performance, insect abundance and herbivore mortality.

Methods

Insect collection and maintenance

A laboratory colony of E. servus was initiated from field collections in Washington, Bolivar and Sunflower counties, Mississippi. Bugs were swept using a 38.1-cm diameter net from soybean (Glycine max), cotton and non-crop hosts (e.g. Erigeron spp., Conyza canadensis, Rumex crispus, Amaranthus retroflexus) from May to October. Euschistus servus was reared in plastic buckets which included oviposition substrate (Kimwipes EX-L, Kimberly-Clark Corp., Roswell, GA, USA; product no. 34133) and food (green beans, Phaseolus vulgaris, and raw shelled peanuts, Arachis hypogaea) as described by Williams et al. (2005). Prior to 1 November, bugs were maintained at 25 ± 1 °C, 65–95 % relative humidity (RH) and 16:8 L:D photoperiod. To simulate winter conditions, bugs were held at 10 ± 1 °C, 75–95 % RH and 8:16 L:D photoperiod from 1 November to 15 February. After 15 February, bugs were returned to the 25 ± 1 °C, 65–95 % RH and 16:8 L:D photoperiod regime. During the spring and summer, cages were inspected daily; portions of paper towel with newly deposited egg masses were cut into strips (ca. 1.5 by 6 cm, one egg mass per strip). The egg masses were examined under a dissecting scope (×40), and the number of viable (not cannibalized or unfertilized) eggs on each mass was recorded, as was the dimensions (mm length × width) of each mass. Egg masses were held in the darkness at ca. 8 °C for <10 days before being used in the field study described below. Voucher specimens of E. servus are deposited in the National Entomological Collection, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

Field conditions and MeJA application

This study was conducted in a cotton, Gossypium hirsutum (var. DPL 521RR), field in Elizabeth, Mississippi. This is a transgenic variety that provides control against lepidopterans, and provides weed control without affecting cotton. The use of this variety facilitated the study because: (i) it eliminated the need for mid- and late-season insecticide applications that might disrupt natural enemy populations and (ii) it greatly reduced HIPV emission due to herbivory by lepidopterans that would be confounded with HIPV emission from MeJA application. The field was ca. 20 ha, and was planted on 9 May using row width of 1.02 m. At planting, aldicarb (4.50 kg ai ha⁻¹) and pentachloronitrobenzene (6.73 kg ai ha⁻¹) were applied in furrow. Herbicide applications were made preplant (pendimethalin, 0.38 L ai ha⁻¹), and post-emergence (fluometuron, 0.38 L ai ha⁻¹ and metolachlor, 0.19 L ai ha⁻¹) at the two leaf stage (20 May). Acephate (0.28 kg ai ha⁻¹) was also applied at the two leaf stage. The plant growth regulator meipquat chloride was applied on 23 June (eight true leaf stage) (0.05 L ai ha⁻¹), and 9 July (12 true leaf stage) (0.07 L ai ha⁻¹). These agrochemical applications were necessary to provide cotton plants that were representative of those under commercial production. The field was furrow-irrigated (7.6 ha-cm) on 17 July. Average daily maximum and minimum temperatures at the site from 25 May to 10 October were 31.2 and 19.6 °C, respectively; precipitation during this time was 46 cm. These climatic conditions were representative of normal conditions.

Experimental plots were interspersed in this field to test the effect of MeJA application to cotton foliage on
plant and insect response. Two treatments (MeJA and untreated control) were assigned to each of four replicates in an RCB design. The blocks (=replicates) were assigned to account for a slight elevation gradient in the field. Each plot measured 20 m on a side (thus encompassing ca. 0.04 ha), and each plot was at least 20 m from other plots. Each plot was thinned by hoeing in June to the equivalent of ca. 75 250 plants per hectare, thus resulting in ca. 3010 plants per plot. First bloom in the plots was observed on 1 July, as is typical for this region. MeJA (Bedoukian Research Inc., Danbury, CT, USA; product no. 399) was mixed with 800 mL of absolute ethanol which was then added to distilled water and the solution was applied until run-off with a backpack sprayer using a coverage rate of 93.5 L ha⁻¹. Each plant in MeJA plots received an average of 0.5 mg MeJA on each application date. There were a total of five application dates (24 June, 9 and 23 July, and 6 and 20 August). Applications were made at dusk to minimize drift and UV degradation from sunlight. To minimize the likelihood of contamination of control plots with MeJA, a different backpack sprayer (identical manufacturer and model as used for treatment of MeJA plots) was used to apply distilled water to the untreated controls at the same volume as in MeJA plots.

**Plant response: volatile induction and collection**

To investigate the volatile response of cotton plants to MeJA exposure, cotton plants (4- to 6-week-old with 4–6 fully expanded leaves) were treated with MeJA (Sigma-Aldrich, St. Louis, MO, USA) overnight, starting at 1800 h, in the greenhouse, by applying 20 μL of an ethanol–MeJA (9:1) solution (after Rodriguez-Saona *et al.* 2001) onto a 15-cm cotton wick. Two treated cotton wicks (total of 18 μmol MeJA/plant) were placed near cotton leaves but without physically contacting the treated plants. Plants were placed inside a 17-cm diameter and 35-cm high Plexiglas chamber (with the entire top and sides of the chamber, covered with a fine mesh for ventilation) prior to volatile collections. Control cotton plants were exposed to 40 μL ethanol, but no MeJA, and placed in separate chambers under the same conditions. Plants were held in the chambers for 16 h (25 ± 2 °C; 70 ± 10 % RH; 14:10 L:D), after which cotton wicks from the treated plants were removed and plants were placed inside volatile collection chambers. Plants of similar size, assessed visually, were assigned to each of the treatments. Elicitor and control treatments in the greenhouse were similar to those from field studies (see above).

Volatiles emitted by cotton plants were collected in a push-pull apparatus (Heath and Manukian 1994; Rodriguez-Saona *et al.* 2001). The system consisted of four independent glass chambers (Analytical Research Systems, Inc., Gainesville, FL, USA) that allowed for simultaneous collections. Purified air entered the chambers at 2 L min⁻¹ and was pulled out through a filter containing 30 mg of Super-Q adsorbent (Alltech Associates Inc., Deerfield, IL, USA) at 1 L min⁻¹. The apparatus allowed for sampling volatiles from the above-ground portion (all of its leaves) of a cotton plant, while the pot remained outside the collection vessel. Volatiles were collected for 24 h (1000–1000 h). After collection, the entire system was cleaned with tap water and 70 % ethanol. The experiment was replicated four times for each treatment, with each treatment–replicate combination consisting of a single plant.

The collected volatiles from Super-Q traps were eluted with 150 μL of methylene chloride and 400 ng of n-octane (Sigma-Aldrich) were added as internal standard (IS) to each sample. Volatiles were analysed as described in Rodriguez-Saona *et al.* (2009) and Rodriguez-Saona *et al.* (2011). For analysis, we used a Hewlett-Packard 6890 Series Gas Chromatograph (GC) equipped with a flame ionization detector and an apolar HP-1 capillary column (10 m, 0.53 mm, 2.65 μm; Agilent Technology, Santa Clara, CA, USA), with helium (39 cm s⁻¹) as carrier gas. The oven was programmed at an initial temperature of 40 °C, held for 1 min, and then raised to 14 °C min⁻¹ to 180 °C where it was held for 2 min, and then increased at 40 °C min⁻¹ to a final temperature of 200 °C, and held at this temperature for 2 min. Quantification of compounds (ng/24 h) was based on comparison of peak areas with that of the IS (n-octane).

Initial identification of compounds was based on comparisons of retention times with those of commercial standards and confirmed by mass spectrometry analysis (GC-MS). GC-MS analysis was performed on a Varian 3400 GC equipped with a Finnigan MAT 8230 mass spectrometer (MS) equipped with a C-1701 column (60 m × 0.32 mm × 1.00 μm; Supelco, USA) at the Rutgers Mass Spectrometry and Chromatography Support Facility (New Brunswick, NJ, USA). The GC oven was programmed at an initial temperature of 50 °C held for 3 min, then increased at 10 °C min⁻¹ to a final temperature of 260 °C. The MS data were acquired and processed in a Finnigan MAT SS300 data system, and compounds were identified by GC retention index, and comparison of their retention times with those of commercially available compounds, and their spectral data to those from NIST library (Linstrom and Mallard 2011).

**Plant response: EFN production**

Extralfloral nectar was collected from 10 randomly chosen plants in each field plot on five dates (4, 12, and 25 July, and 8 and 24 August). Thus, EFN was measured...
on a total of 80 plants on each date. On each plant, the EFN from the nectary on the central vein of the fifth mainstem leaf below the terminal was collected with a calibrated glass micropipette (5–20 μL capacity) (VWR Scientific, West Chester, PA, USA; catalogue nos 53432-706, -728, -740). Immediately after each sample was collected, the length of the nectar column was measured to the nearest 0.1 mm with a dial caliper (Swiss Precision Instruments, Inc., Garden Grove, CA, USA; model no. 31-414). This number was then divided by the length of the micropipette from the tip to the calibration mark and multiplied by the capacity of the micropipette to give volume (in μL) of nectar collected. A new micropipette was used for each sample. Nectar collections were conducted from 0630 to 0745 h CST to minimize loss due to nectarivory by arthropods.

**Plant response: cotton yield**

Seed cotton (i.e. lint and seed combined) was hand-picked in October from 10 randomly chosen 1-m-row samples in each plot. (Note that 11 samples were taken in one plot.) All the seed cotton was picked from each plant in the 1-m-row sample, and the number of plants in each sample was recorded (mean = 7.56 plants, SD = 2.27). Thus, seed cotton yield was assessed on a total of 612 plants in this study. Yield (seed cotton) from each sample was placed in paper bags, air-dried in a vacuum hood for several weeks at 22 °C, and then oven-dried at 70 °C for 3 days before being weighed. Because seed cotton is a combination of seed and lint, it is not a measurement of fitness, thus precluding evaluation of MeJA treatment on cotton defence-fitness trade-offs and related topics.

**Insect response: stink bug egg mortality**

_Euschistus servus_ egg masses obtained from the laboratory colony were established in each plot beginning 32 days after the first MeJA application. These sentinel egg masses (mean number of egg masses established per plot per date = 10, range = 8–12, mean number of eggs per plot per date = 180, range = 121–224) were attached to randomly chosen cotton plants (one egg mass per plant) in each plot. Each egg mass was attached to the underside of a cotton leaf (fifth mainstem node below the terminal) with a metal hair clip. Height (in cm) of egg masses above the soil surface was recorded (mean = 62.4, SD = 10.7). Coloured plastic flagging was tied around the base of each plant harbouring an egg mass to aid in recovery of the egg mass. Sentinel egg masses were established on 25 July, 7 August and 21 August. Sample sizes (total number of egg masses, total number of eggs) exposed at each date were: 25 July = 66, 1268; 7 August = 89, 1601; 21 August = 88, 1461. The sentinel egg masses remained in the field 7 days, after which they were recovered and returned to the laboratory. Egg masses were observed under a dissecting scope (×40) and the fate of each egg was assigned to one of five categories: (i) parasitization, (ii) predation by arthropods with chewing mouthparts, (iii) predation by arthropods with piercing-sucking mouthparts, (iv) healthy (from which a nymph emerged) and (v) unviable. The number of eggs on each egg mass was counted and subtracted from the number present when placed in the field, thus giving an assessment of mortality from predators with chewing mouthparts. These predators often destroyed 100 % of the eggs, leaving only remnants of the basal portion of the chorion attached to the paper strip. Intact, but empty _E. servus_ eggs with small hole(s) penetrating the chorion were evidence of predators with piercing-sucking mouthparts. After assessment of predation, the egg masses were placed individually in ventilated Petri dishes and held for 3 weeks at 25 ± 1 °C, 65–95 % RH and 16:8 L:D photoperiod. Egg masses were inspected daily for evidence of parasitization (i.e. darkening of the eggs and development of wasp body parts), and emerging wasps were preserved in 75 % ethanol for taxonomic identification.

**Insect response: plant bug performance**

The effect of MeJA on development of tarnished plant bug, _Lygus lineolaris_ (Hemiptera: Miridae), was evaluated in the field plots in two separate trials. The first trial began on 3 July and ended on 22 July, and thus included plants that had been treated twice with exogenous MeJA; the second trial began on 23 July and ended on 14 August, and included plants with four MeJA applications. At the start of each trial, five sleeve cages (Williams et al. 2012) were established on the terminal portion of the fifth node fruiting branch of five randomly chosen plants in each plot. The enclosed plant material included 2–3 leaves and 1–3 small floral buds (2–5 mm diameter). Predators were removed from the cages and three first instar _L. lineolaris_ nymphs reared in the laboratory (Snodgrass and McWilliams 1992) were added to each cage. Thereafter, mortality and maturation to adulthood of the nymphs were assessed on three (Trial 1) and four (Trial 2) dates.

**Insect response: abundance**

Arthropod populations were monitored in the plots on seven dates (20 June, 3, 14 and 25 July, and 1, 14 and 25 August). In each plot, a sample was taken from a randomly chosen location in a 12 × 14 m area (168 m²) in the centre of each plot. A sample consisted of one 25-sweep sample using a 38.1-cm diameter net, with each sweep covering two rows of cotton. Contents of each sample were transferred to an organdy bag, and
the bags were transported to the laboratory and frozen. When samples were thawed and sorted, all arthropods were identified to the highest taxon possible. For many groups, there were insufficient numbers of specimens to conduct statistical analysis. Therefore, analysis was conducted only for the cotton aphid, *Aphis gossypii*, adult banded-wing whitefly, *Trialeurodes abutilonea*, and total Coccinellidae (*Coccinella septempunctata*, *Harmonia axyridis*, *Hippodamia convergens* and *Coleomegilla maculata*) immatures and adults.

**Data analysis**

The effect of MeJA on volatile blend composition from cotton plants was analysed using principal component analysis (PCA) ([Minitab v. 16 2013; Minitab Inc., State College, PA, USA.](https://academic.oup.com/aobpla) The score plot was used to visualize the relationship between MeJA and control treatments. We also used general linear model one-way ANOVA (Minitab) to compare total volatile emissions between MeJA and control plants and to determine which specific compounds were affected by the MeJA treatment. Volatile emission data were ln(x + 0.05)-transformed prior to analysis to satisfy assumptions of normality and homogeneity of variances.

Data from EFN production, stink bug egg mortality, insect population sampling and cotton yield were log(x + 1)-transformed prior to analysis; data from *L. lineolaris* mortality and development were arcsin-square root-transformed. EFN production and *L. lineolaris* mortality and development were subjected to repeated-measures ANOVA using Proc Mixed ([SAS Institute 2009](https://academic.oup.com/aobpla)). Main (fixed) effects were time and MeJA treatment, and replicate was a random effect. Huynh–Feldt covariance structure was used for all repeated-measures analyses. Satterthwaite approximation was used to calculate approximate degrees of freedom for *F*-tests of fixed effects. *P*-values were adjusted by Tukey’s HSD (EFN production) or Scheffe’s method (*L. lineolaris* mortality and development). The number of MeJA applications was not included as a factor in the repeated-measures analysis of *L. lineolaris* mortality and development because dates of measurement in the two trials did not match. Stink bug egg mortality were subjected to two-factor ANOVA, and cotton yield (seed cotton) data were subjected to one-way ANOVA, as were data for insect population dynamics at each date of measurement ([Zar 1996](https://academic.oup.com/aobpla)). For all dependent variables, untransformed data values are presented in results.

**Results**

**Plant response: volatile induction**

Principal component analysis resulted in a model with the first two components explaining 78.3 % of the total variation in volatile blends. The PCA score plot showed no overlap between volatile blends of MeJA and control plants (Fig. 1A). The first component explained most (54.8 %) of the variation, and the second component explained 23.5 % of the variance. The PCA results indicate that volatile blends from the MeJA and control treatments are distinct.

Methyl jasmonate-treated cotton plants emitted ~3× higher amounts of volatiles compared with control plants [total amounts (mean ± SE): MeJA-treated plants = 1552 ± 291 ng/24 h; control

**Figure 1.** PCA score plot of volatile induction (A) and headspace volatile emissions (B) from intact cotton plants treated with MeJA or untreated controls. Asterisks indicate significant differences (*P* ≤ 0.01, **P** ≤ 0.005) in volatile emissions between control and MeJA-treated plants; otherwise, differences between treatments were not significant (*P* > 0.05).
plants = 570 ± 119 ng/24 h \left( F_{1, 6} = 16.06; P = 0.007 \right) (Fig. 1B). Two volatiles were emitted in significantly greater quantities in the MeJA treatment than in the control treatment (Fig. 1B). These were: (+)-limonene \left( F_{1, 6} = 13.87; P = 0.010 \right) and (3E)-4,8-dimethyl-1,3,7-nonatriene \left( F_{1, 6} = 23.83; P = 0.003 \right). Compounds collected as headspace volatile emissions, and their Kovats’ retention indices, from MeJA-induced and untreated control plants are presented in Table 1.

Plant response: EFN production
There was a statistically significant effect of time \left( F_{4, 24} = 181.05; P < 0.0001 \right) and MeJA application \left( F_{1, 7.9} = 48.43; P < 0.0001 \right) on nectar production (Fig. 2). A significant time by MeJA application interaction was also observed \left( F_{4, 24} = 6.04; P = 0.0016 \right). EFN production in MeJA-induced cotton was greater than in untreated controls on three of the five dates of measurement (Fig. 2).

Plant response: cotton yield
Methyl jasmonate-induced plants produced a numerically, but not significantly \left( P > 0.05 \right), greater amount of seed cotton than untreated controls. When expressed on a 1-m-row basis, MeJA-induced plants yielded 214.6 g seed cotton versus 201.1 g for untreated controls; when expressed on a per plant basis, MeJA-induced plants yielded 31.2 g versus 27.5 g for untreated controls.

Insect response: stink bug egg mortality
Preliminary analyses indicated that predation over the entire study was greater by arthropods with chewing mouthparts than by those with piercing-sucking mouthparts \left( T_{46} = 1.20; P = 0.0025 \right); (mean ± SD) chewing predators = 17 ± 15.7 %; piercing-sucking predators = 6 ± 7.85 %. However, there was no difference in predation between control and MeJA-induced treatments \left( P > 0.05 \right) for either group of predators over the entire study or by the date of assessment, so data were combined into ‘total predation’ for further analysis. Results of ANOVA revealed a statistically significant effect of time on total predation \left( F_{2, 23} = 15.32; P < 0.0001 \right) of E. servus eggs (Fig. 3A). However, there was no MeJA treatment effect \left( P > 0.05 \right) for predation. When predation was assessed on a per egg basis, there was an increase from ca. 8 % (25–31 July) to 44 % (21–29 August) (Fig. 3A). We found a similar trend when predation was assessed on a per egg mass basis (25–31 July, 14 %; 7–14 August, 35 %; and 21–29 August, 56 %). Parasitism of E. servus eggs was attributed to T. podisi and Tr. euschisti, with preliminary analyses showing no difference \left( P > 0.05 \right) and was combined into ‘total parasitism’ for analysis. Analysis of variance of parasitism of E. servus eggs revealed a significant effect of time \left( F_{2, 23} = 6.02; P = 0.0100 \right) (Fig. 3B). As observed for predation, there was no difference in parasitism \left( P > 0.05 \right) between MeJA and untreated controls. Parasitism on a per egg basis ranged from ca. 5 % (7–14 August) to 22 % (25–31 July) (Fig. 3B). We found a similar trend when parasitism was assessed on a per egg mass basis (25–31 July, 29 %; 7–14 August, 10 %; and 21–29 August, 30 %).

Table 1. Main constituents identified in cotton plants after exogenous application of MeJA. Kovats’ retention indices: published values in Sigma-Aldrich (St. Louis, MO, USA), and mean of experimental values.

| No. | Retention time (min) | Compound                        | Compound class      | Kovats’ indices1     |
|-----|---------------------|---------------------------------|---------------------|---------------------|
|     |                     |                                 |                     | Published         | Experiment       |
| 1   | 6.74                | α-Pinene                        | Monoterpene         | 903               | 903              |
| 2   | 6.93                | β-Pinene                        | Monoterpene         | 920               | 920              |
| 3   | 7.45                | (Z)-3-Hexenyl acetate           | Green leaf volatile (ester) | 966               | 966              |
| 4   | 7.74                | (E)-β-Ocimene                   | Monoterpene         | 992               | 992              |
| 5   | 8.05                | (+)-Limonene                    | Monoterpene         | 1021              | 1021             |
| 6   | 8.54                | Linalool                        | Monoterpene         | 1068              | 1068             |
| 7   | 8.77                | (3E)-4,8-Dimethyl-1,3,7-nonatriene | Homoterpene     | 1090              | 1090             |
| 8   | 9.29                | Hexenyl butyrate                | Ester               | 1141              | 1141             |
| 9   | 10.12               | Indole                          | Amine               | 1227              | 1227             |
| 10  | 12.07               | β-Caryophyllene                 | Sesquiterpene       | 1422              | 1422             |
| 11  | 12.48               | α-Farnesene                     | Sesquiterpene       | 1453              | 1453             |
Insect response: plant bug performance

Results of repeated-measures ANOVA showed a statistically significant effect of time on the rate of plant bug mortality for Trial 1 ($F_{2, 12} = 4.33; P = 0.0385$) and Trial 2 ($F_{3, 18} = 63.57; P < 0.0001$). A similar effect was observed for the rate of development to adulthood for Trial 1 ($F_{1, 6} = 13.51; P = 0.0104$) and, marginally, for Trial 2 ($F_{1, 6} = 5.74; P = 0.0536$). The effect of time on plant bug mortality, and development to maturity over the course of the trials, was expected. MeJA treatment did not affect plant bug mortality or rate of development to adulthood ($P > 0.05$).

Insect response: abundance

Application of MeJA had variable effects on abundance of the insect species that were analysed (Fig. 4). Data for *A. gossypii* were analysed on two dates (14 and 25 July, corresponding to population peaks for alate and apterous aphids, respectively) due to preponderance of zero values on other dates. On 14 July, alate *A. gossypii* populations were lower in MeJA-treated cotton than in untreated controls ($T_3 = 3.83; P = 0.0203$) (Fig. 4A). A similar, but non-significant ($P > 0.05$), trend was observed on 25 July for apterous *A. gossypii*. Coccinellid populations peaked on 25 July; there was no treatment effect ($P > 0.05$) at either time (Fig. 4C).

**Discussion**

Synthetic elicitors, such as MeJA and JA, are useful tools to study plant reactions to stress at several levels (molecular, biochemical and organismal) and to understand the interactions between elicitation of plant response and response of pest and beneficial insects.
this study, we evaluated whether application of an elicitor of insect defence responses, MeJA, influenced plant and insect responses under field conditions. Consistent with previous studies, exogenous application of MeJA increased emission of certain volatiles (limonene and (3E)-4,8-dimethyl-1,3,7-nonatriene) compared with the control. This represents MeJA activation of two isoprenoid pathways of volatile synthesis: (i) the mevalonic acid pathway that produces sesqui- and homoterpenes ((3E)-4,8-dimethyl-1,3,7-nonatriene) and (ii) the methylerithritol phosphate pathway that produces monoterpenes (limonene) (Paré and Tumlinson 1999; Dudareva et al. 2013). Rodriguez-Saona et al. (2001) reported that MeJA-induced cotton emitted the above compounds at similar levels as in the present study, as well as other terpenes, green leaf volatiles and aromatics. Induction of volatiles was greatly diminished if MeJA was applied only once, suggesting that induction is temporally ephemeral and dependent on multiple applications of MeJA. Induction of plant volatiles using MeJA, JA or chemically related plant compounds has also led to emission of volatile blends similar to those induced by herbivores in other plant species, including tomato (Thaler et al. 2002), Vaccinium species (Rodriguez-Saona et al. 2009; Rodriguez-Saona et al. 2012), Brassica oleracea (Bruinsma et al. 2009), soybean, G. max (Moraes et al. 2009), and maize, Zea mays (Ozawa et al. 2000).

Although volatiles from the present study were not collected under field conditions, our results are consistent with those of a previous study (Rodriguez-Saona et al. 2001) and show that cotton plants respond to MeJA by increasing volatile emissions.

In addition to increasing HIPV production, we also found that MeJA application led to an increase in cotton EFN production on three of the five sample dates. The overall decline in EFN production in August was expected, as the plants began to transition from vegetative growth to fruit maturation, commonly referred to as ‘cutout’ in the USA. To our knowledge, this is the first study evaluating cotton EFN response to an elicitor. In other studies with cotton, EFN production was induced by herbivory (Wäckers et al. 2001; Wäckers and Bezemer 2003; Wäckers and Bonifay 2004). EFN production varied with time after initiation of herbivory and returned to pre-treatment levels 4 days after removal of Spodoptera littoralis larvae (Wäckers et al. 2001); this suggests that EFN production in the present study might have fluctuated during the ~14-day interval between MeJA applications. The increases in EFN production reported by Wäckers and Bezemer (2003) and Wäckers et al. (2001) are greater than that we found in the present study, where the greatest increase was ~3×, and suggests a
fundamental difference in plant response between herbivory and artificial induction. In the present study, we did not assess sugar composition; however, Wäckers et al. (2001) reported that sugar composition did not differ between EFN from S. littoralis-damaged plants and untreated controls, indicating that plants responded to herbivory by increasing the amount of carbohydrates available in EFN, as opposed to water or other compounds that have lower nutritional value for arthropods.

Plants may incur a trade-off between production and deployment of secondary metabolites for defence and primary metabolic needs (e.g. growth, development and reproduction) (Stamp 2003). In our study, we did not directly assess fitness of the plants, but yield of seed cotton (lint plus seed) was not affected by secondary metabolite production after MeJA induction. Recent theoretical work suggests that the plasticity of plant biochemical machinery may offset metabolic losses incurred due to plant defence (Neilson et al. 2013).

Application of elicitors in the field has led to variable effects on herbivorous insects and their natural enemies. These effects can be direct impacts, i.e. behavioural (Halitschke et al. 2008), developmental (Cooper and Goggin 2005) or operate indirectly via natural enemies (Thaler 1999). Although our data for insect abundance revealed a treatment effect only for cotton aphid, our results were consistent with earlier studies. Peak densities of cotton aphid alatae were >2.5× lower in MeJA plots than control plots, suggesting that indirect induced responses (volatiles) reduced attraction or direct induced responses (secondary compounds) affected aphid development in MeJA plots. Application of cis-jasmone, like MeJA, a catabolite of jasmonic acid, was repellent to and slowed the population development of S. littoralis (Homoptera: Aphididae) (Bruce et al. 2003). Soybean aphid, Aphis glycines, population growth was reduced on MeJa-treated soybean (Selig et al. 2016). Birkett et al. (2000) reported that cis-jasmonate-treated plants were attractive to C. septempunctata (Coleoptera: Coccinellidae) and aphid parasitoids, suggesting the potential for increasing impact on aphid hosts. We found peak densities of ladybird beetles to be ~2× higher in MeJA plots. Banded-wing whitefly densities in MeJA plots peaked later than in control plots, suggesting that direct effects of MeJA slowed whitefly development, as has been reported for T. vaporariorum (Risal et al. 2008).

Effects of exogenous application of elicitors on natural enemy behaviour are varied. In the present study, increased volatile emission and EFN production in the MeJA treatment did not lead to increased stink bug egg mortality by parasitoids and predators. Some studies reported increases in natural enemy attack in response to elicitors (Thaler 1999; Kessler and Baldwin 2001; Bruce et al. 2003; Vieira et al. 2013), while other studies did not find a treatment effect (von Mérey et al. 2012). In laboratory studies, T. podisi was attracted to plant volatiles produced after herbivory by the host, E. heros (Moraes et al. 2005; Michereff et al. 2013), and to a mixture of volatiles emitted by cis-jasmone-induced soybean plants (Moraes et al. 2009). Little is known about the lag time between induction, whether it be by elicitor, herbivore, or mechanical damage, and the phenotypic expression of defence responses, or the duration of the responses. Volatile production, and concomitant response of wasps, differed with time after cis-jasmone application; volatile blends emitted 3 days after induction were not attractive to T. podisi, while volatiles produced 4 days after induction were attractive to the wasps (Moraes et al. 2009). Several compounds produced by soybean, including (3E)-4,8-dimethyl-1,3,7-nonatriene, appeared to influence the behaviour of T. podisi (Moraes et al. 2009). Vieira et al. (2013) extended this work to the field, where they assessed abundance of parasitoids, stink bugs and parasitism of stink bug, E. heros, eggs for 8 weeks after cis-jasmone application to soybean. They found that exogenous application of cis-jasmone to soybean attracted platygastrid parasitoids, but did not affect stink bug abundance or the incidence or intensity of parasitism of stink bug eggs. Overall parasitism during the study was <10%, with T. podisi being the most abundant species reared from stink bug eggs, followed by Trissolcus spp. (Vieira et al. 2013). These parasitism levels are similar to what we recorded in the present study (ca. 14%) by T. podisi and Tr. euschisti. Simpson et al. (2011a, b) reported that platygastrid wasps and other parasitic hymenopterans were sometimes attracted to maize, broccoli and grapes induced by exogenous application of MeJA and other elicitors. Trissolcus basalis was attracted to odours from buckwheat flowers with nectar that most-benefitted the wasp’s fecundity (Foti et al. 2016). The differences in egg mortality over time that we observed may have been due to community-wide processes occurring throughout the field that operated independently of MeJA effects. For example, the increasing predation trend might have resulted from increase population densities of predators due to field colonization and within-field recruitment, and fluctuation of parasitoid densities due to asynchrony with host densities may have resulted in the irregular parasitism rates (Kaplan 2012).

Much remains to be learned about the foraging behaviour of natural enemies before CBC practices can be used to successfully suppress pest populations. Fatouros et al. (2008) proposed a behavioural hierarchy for host searching by egg parasitoids wherein volatiles...
provided a food resource sufficient to support the natural enemy populations, then the additional EFN in MeJA plots did not retain wasps in MeJA plots long enough to increase attack rates. Low overall host and parasitoid densities in the field may have resulted in lack of host cues and thus disrupted the density-dependent threshold (Underwood 2000; Kaplan 2012). If EFN production in the surrounding cotton, including the untreated plots, provided a food resource sufficient to support the natural enemy populations, then the additional EFN in MeJA-induced plants might have been rendered unnecessary, thus compromising its function as a ‘reward’.

Despite the accumulating evidence that HIPVs are important in multitrophic interactions, little is known about several components of this system that are critical to understanding both applied and fundamental aspects of this phenomenon (Kaplan 2012; Heil 2014). Numerous factors can affect the physiological perception and behavioural response by arthropods to volatiles under field conditions. Temporal components of volatiles (e.g. composition and quantity of blends, their emission and degradation), distances at which they are bioactive, physiological state of arthropods that are the putative volatile receivers, are all important biotic factors, and can interact with abiotic factors, such as wind speed and direction, to affect arthropod response. Moreover, these biotic and abiotic factors are constantly changing, and thus, arthropod responses are probably also dynamic (Braasch and Kaplan 2012; Kaplan 2012). Field odorscapes, i.e. the field-scale mixture of volatile plant-produced organic compounds, differ with plant species composition (Leppik and Frérot 2014) and time of day (Leppik et al. 2014), and these plant volatiles appear to affect insect behaviour at the field scale (Leppik and Frérot 2014; Cornu et al. 2015). Studies on the distances at which HIPVs are bioactive indicate that activity ranges using point-source lures or individually induced plants vary from <2 m (Mallinger et al. 2011; Rodriguez-Saona et al. 2011) to >8 m (Braasch and Kaplan 2012) to 10 m (Bernasconi Ockroy et al. 2001). Moreover, in a field study, Braasch and Kaplan (2012) reported redistribution of braconid wasps closer to HIPV lures and with reduced wasp abundance at greater distances from the lures. These studies suggest that volatiles can influence arthropods at a field scale. However, a simulation study evaluating the effect of HIPV enhancement on biological control suggested that recruitment of a predator declined exponentially as field edge–core ratio decreased (Kaplan and Lewis 2015). Thus, deployment of HIPVs in small- to moderate-sized fields may result in greater attraction of predators, and perhaps parasitoids, than in larger fields, although this remains to be tested. The ‘attract and reward’ approach is theoretically supported (Kaplan and Lewis 2015), but to date there is little experimental evidence in support of this strategy. Compared with other studies, ours used MeJA-induced cotton plants in relatively large plots (0.04 ha, with ca. 3010 plants per plot) where all the plants were induced and producing HIPVs and EFN. This represents the maximum expression possible of attraction (HIPVs) and reward (EFN); nevertheless, we recorded no evidence of emigration into MeJA plots from surrounding cotton, or subsequent retention in the plots.

The temporal component of volatile release by plants may be an important factor in the perception and behavioural response by parasitoids. Available data indicate that the daily periodicity of volatile release after treatment with an elicitor or herbivory is concurrent with the photoperiod (Rodriguez-Saona et al. 2001; Rodriguez-Saona et al. 2012). Furthermore, Loughrin et al. (1994) and Turlings et al. (1998) found that cotton volatile emission in response to lepidopteran herbivory was also greatest during the photoperiod, in particular, at the time of foraging by parasitoids of the herbivores. The daily activity patterns of platygastrid wasps vary (Arakaki 1990; Vogt and Nechols 1991; Gazit et al. 1996), but are generally coincident with the daily cycle of volatile release by plants.

Few studies have assessed the interplay between synthetic elicitor-induced plant volatile emission over a
longer time scale, e.g. a time course of several days, and the subsequent response by natural enemies. In a wind tunnel assay, *Cotesia glomerata* wasps were attracted to JA-induced *B. oleracea* plants beginning at 3 h after treatment to at least 4 days after treatment (Bruinsma et al. 2009). Trap catches of an unidentified platygastrid were greater 2–4 days after application of a synthetic HIPV blend in a vineyard (Simpson et al. 2011b). Trap catches of a complex of platygastrid species (mostly *T. podisi* and *Tr. basalis*) were greater in the first 4 weeks after application of cis-jasmone to soybean, declining in the subsequent 5 weeks of the study (Vieira et al. 2013). The variability of these results suggests the presence of unknown sources of variation in attraction of platygastrid wasps by HIPVs, but is nevertheless promising, and underscores the need for further research.

Dispersal of natural enemies into agricultural fields is an important factor affecting subsequent levels of pest control (Wissinger 1997; Kean et al. 2003). This dispersal is especially important in annual cropping systems, such as the cotton agroecosystem, due to the annual destruction of plants and repeated disturbance of the soil. Thus, pest control in cotton typically depends on annual recolonization by natural enemies of fields from other habitats adjacent to or perhaps further from the fields, as well as subsequent within-field recruitment. We believe that low population densities of *T. podisi*, *Tr. euschisti* and predators resulting from reduced colonization of the cotton field were limiting factors affecting stink bug egg mortality and population levels of natural enemies in our study. This is supported by a concurrent study adjacent to the cotton field where the present study was conducted, where we found considerably higher levels of stink bug egg mortality (60–90 %) in non-crop vegetation growing along the field border (L. Williams, USDA-ARS, Charleston, unpubl. data). This suggests that: (i) the effectiveness of the sentinel egg masses that we used was not compromised (Aquino et al. 2012; Jones et al. 2014) and (ii) dispersal of platygastrid parasitoids and predators from non-crop habitat into the cotton field was not sufficient to attain the host/prey–natural enemy thresholds suitable for effective foraging by the natural enemies, thus leading to the lack of treatment effect in our study.

**Conclusions**

Our results indicate that cotton plant response to exogenous application of MeJA (increased volatile emission and EFN production) did not lead to a concomitant response by insects (increased attack of stink bug eggs by natural enemies and changes in population dynamics of aphids, whiteflies and ladybird beetles). Natural enemy colonization of crops from non-crop habitats and within-field population recruitment is a necessary prerequisite for successful CBC, and were probably factors limiting success of the present study. Only after suitable populations of natural enemies are present in fields can their behaviour be effectively manipulated. This begs for additional work on the interplay between arthropod dispersal behaviour and plant-derived cues. In particular, a better understanding of the temporal and spatial dynamics of field odorscapes and the subsequent effects on arthropod behaviour will undoubtedly inform future studies on biorational pest management.

**Sources of Funding**

USDA-ARS in-house appropriated funds (L.W.).

**Contributions by the Authors**

L.W. conceived the experiments. L.W., C.R.S. and S.C.C. designed and performed the experiments. L.W. and C.R.S. contributed reagents/materials/analysis tools, analysed the data and wrote the paper.

**Conflicts of Interest**

None declared.

**Acknowledgements**

Technical assistance was provided by R. Holdcraft, O. Houston, C. Li, D. Rice, L. Santucci and S. Zhu. We are grateful to S. Colazza, I. Kaplan and anonymous reviewers for helpful comments on the manuscript. We thank T. J. Henry (USDA-ARS Systematic Entomology Laboratory) for confirming our identification of *E. servus*. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use. The USDA is an equal opportunity provider and employer. The U.S. Government has the right to retain a non-exclusive, royalty-free license in and to any copyright of this article.

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