Influence of Pyrethroid Pesticide Formulation on Volatile Emissions from Cotton, *Gossypium hirsutum* L., Leaves[1]

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ABSTRACT Volatile organic compounds (VOCs) are known to influence numerous ecological interactions among plants, insects, and natural enemies. There is little information on the direct or indirect effects of insecticide application on the production and emission of VOCs from plants. The goal of this study was to investigate the influence of two pyrethroid formulations on the emission of VOCs from cotton (*Gossypium hirisutum* L.). Emissions were sampled from cotton leaves treated with either a capsule suspension (CS) or an emulsifiable concentrate (EC) of *lambda*-cyhalothrin, or untreated controls 24 and 96 h after application of insecticides and analyzed by gas chromatography/mass spectrometry. Results indicated that application of either formulation of pyrethroid had no significant effect on the total emission of volatiles during any sampling period. Interestingly, untreated plants did exhibit significantly greater emissions of (*E*)-2-hexenal, β-farnesene, and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetrae compared with plants treated with CS or EC formulations, seemingly due to significantly greater herbivore feeding activity on control plants compared with insecticide treated plants. The results of this study provide no indication that insecticide treatment influences VOC emission from plants; however, more controlled laboratory studies would likely provide more conclusive evidence.

In cotton, *Gossypium hirsutum* L., produced in the southeastern United States, insecticides are routinely applied to control bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), and stink bugs (Hemiptera: Pentatomidae) (Roof 1994). Although pesticides are tested for phytotoxicity and impacts on target and non-target organisms (Croft 1990, Desneux et al. 2007), their effects on secondary metabolism in plants is often overlooked.

Cotton produces a number of volatile organic compounds (VOCs) that coordinate a number of inter- and intra-specific interactions. Terpenoids are a major class of VOCs stored in lysigenous glands and are precursors to the production of heliocides and gossypol, which function as direct defenses against herbivores (Opitz et al. 2008). Herbivore-induced VOC emissions also function as indirect defenses and may facilitate biological control by attracting natural enemies (McCall et al. 1994, Paré & Tumlinson 1999). Several studies have shown that insecticides have a direct negative impact on the searching behavior

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and survival of beneficial insects (Desneux et al. 2004, 2007). Furthermore, recent data suggests that herbivore-induced VOCs may be exploited by electronic nose technology to monitor for pest damage to crop plants (Laothawornkitkul et al. 2008, Henderson et al. 2010). If pesticides influence the emission of VOCs, this may lead to changes in VOC-mediated ecological processes, and affect the accuracy of VOC-based electronic gas sensors used for pest monitoring.

Insecticides are often mixed with adjuvants, including suspension aids or surfactants, to enhance the absorption and/or activity of the active ingredient. Certain adjuvants may cause transient changes in photosynthesis (Wood et al. 1997) and may interfere directly with enzymes in secondary metabolic pathways (Lydon & Duke 1989). In cotton, methomyl applications were found to cause a 3-fold increase in phenolic compounds and a 50% increase in tannin production (Parrott et al. 1983). Formulations of pyrethroids have been shown to disrupt electron flow through photosystem II, causing reductions in the rate of photosynthesis in leaves of tobacco, *Nicotiana tabacum* L. (Bader & Shuler 1996). In contrast, Haile et al. (1999) demonstrated that the pyrethroid permethrin caused unaltered or increased photosynthetic activity in leaves of alfalfa, *Medicago sativa* L., and soybean, *Glycine max* L. Haile et al. (2000) found transient effects of several pyrethroid active ingredients as well as a non-ionic surfactant on lettuce, *Lactuca sativa* L., in which photosynthesis was reduced 4–48 hours after application, with full recovery after five days. Because VOC emissions are strongly coupled to photosynthetic activity (Paré & Tumlinson 1999), insecticides may indirectly influence VOC emissions through changes in primary metabolism. The objective of this study was to investigate the influence of different formulations of a pyrethroid insecticide on temporal changes in VOC emissions from cotton leaves.

Materials and Methods

Volatiles were collected from non-Bt (*Bacillus thuringiensis*) cotton (Stoneville 4664 RF) in a field test for mid-range recommended rates of pyrethroid insecticides. Emissions were compared between plants treated with 0.37 Kg [AI]/ha of a capsule suspension (CS) (Karate Z® - Syngenta Crop Protection, Inc., Greensboro, NC) or 0.37 Kg [AI]/ha of an emulsifiable concentrate (EC) (Lambda-CY® - United Phosphorous, Inc., King of Prussia, PA) formulation of *lambda*-cyhalothrin and unsprayed controls. Contiguous plots were established using a randomized complete block design, with four replications of each insecticide treatment in plots containing 8 rows 13 m in length. Insecticides were applied on 31 July and 7 August. Control and treated plots were subsequently sampled on 15 August for *H. zea* larvae using a canopy drop-cloth method to assess the number of larvae present on 5.5 m (18 row ft) of row per plot.

Headspace VOC emissions were sampled from CS- and EC-treated, as well as unsprayed control plots, between 10:00 a.m. and 12:00 p.m. from four mature leaves (*in situ*) on individual plants, three nodes below the apical meristem 24 and 96 h after the second pesticide application. For each sample, a polyacetate oven bag (Reynolds®, Inc., Richmond, VA) with an approximate volume of 1 L was placed around the leaf and loosely sealed around the petiole with a cable tie to permit air-flow through the bag. The top corner of each bag was fitted with a volatile collection trap constructed from a glass Pasteur pipette containing 35 mg

of Super Q adsorbant (Alltech, Inc., Deerfield, IL) held at the tip of each trap between two small plugs of glass wool. Air was drawn through the base of the collection bag across the leaf and onto the trap at a rate of 300 mL/min using an SKC air sampling pump (SKC Inc., Eighty Four, PA). Because ambient air was drawn through collection bags, ambient air was simultaneously sampled from the canopy in order to correct for any VOCs in the air stream drawn through collection bags. Volatiles were extracted from collection traps by washing with 200 μL of hexane collected directly into a 2 mL autosampler vial containing a 250 μL insert. For each VOC sample, 2 μL were analyzed on a Hewlett-Packard 6890 gas chromatograph (GC). Helium was used as a carrier gas at a flow rate of 1 mL/min. Injections were made in the splitless mode for 0.5 min with an injector temperature of 250°C. After injection, the column temperature was maintained at 50°C for 10 minutes, and then increased to 150°C at 5°C/min, followed by an increase to 250°C at a rate of 15°C/min, followed by a final increase to 300°C at a rate of 10°C/min and held for 5 min. Samples were subsequently analyzed by mass spectrometry (MS) using a Varian VG-70S (Waters Corp., Milford, MA) operated in electron-impact mode. The amount of volatiles was calculated by conversion of peak area units to mass (ng) based on an external standard curve of α-pinene (for monoterpenes) and β-caryophyllene (for sesquiterpenes) obtained in the laboratory. Compounds were identified by comparison with essential oil standards (Sigma-Aldrich, Inc., Milwaukee, WI) as well as solvent extracts of cotton leaf material.

A mixed model repeated measures analysis of variance (PROC MIXED) was used to analyze the differences in total emissions over time, with plant assigned as the repeated subject (SAS Institute 2009). Contrast statements were used to test for differences in individual volatile compounds at each time period (24 and 96 h post pesticide application) (SAS Institute 2009). The general linear model procedure (GLM) of SAS was used to analyze the total number of *H. zea* larvae collected from each plot, and a Tukey multiple comparison was used to test for differences in total number of *H. zea* larvae among treatments.

Results and Discussion

There was no significant difference in the total quantity of VOCs collected from control leaves and those treated with either formulation at any time period (Table 1, Fig. 1). Furthermore, there was no significant interaction between treatment and time, indicating that the effect of pyrethroids on VOC emissions did not vary significantly over the course of the experiment (Table 1, Fig. 1).

The blend of VOCs from cotton leaves was composed of several mono- and sesquiterpenes, green leaf volatiles (GLVs), and an aliphatic C9 aldehyde, nonanal (Table 2). There were no consistent differences in the composition of VOCs detected from cotton leaves treated with either formulation of *lambda*-cyhalothrin (Table 2). No differences were detected in the composition of monoterpenes from cotton leaves throughout the study (Table 2). In contrast, emissions of the sesquiterpene β-farnesene were significantly lower in plants treated with the CS formulation compared with untreated controls at both sampling periods (Table 2). Emissions of the GLV (*E*)-2-hexenal were found to be significantly lower in plants treated with the EC formulation compared with untreated controls (Table 2). Furthermore, emissions of the C11 homoterpene

Table 1. Repeated measures analysis of variance test of main effects on total quantity of volatiles collected from cotton leaves treated with a capsule suspension or an emulsifiable concentrate of *lambda*-cyhalothrin and untreated controls at 24 and 96 h after application.

Main Effect	Numerator df	Denominator df	*F* value	*P* value
Treatment	2	9	1.34	0.3092
Time	1	5	0.04	0.8471
Treatment*Time	2	8	0.04	0.9597

(*E*)-4,8-dimethyl-1,3,7-nonatriene and the C16 homoterpene (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene were found to be significantly lower in plants treated with either pyrethroid formulation compared with untreated controls (Table 2).

A significantly greater number of larvae of *H. zea* were collected in control plots compared with plots treated with insecticides (Fig. 2). No differences were

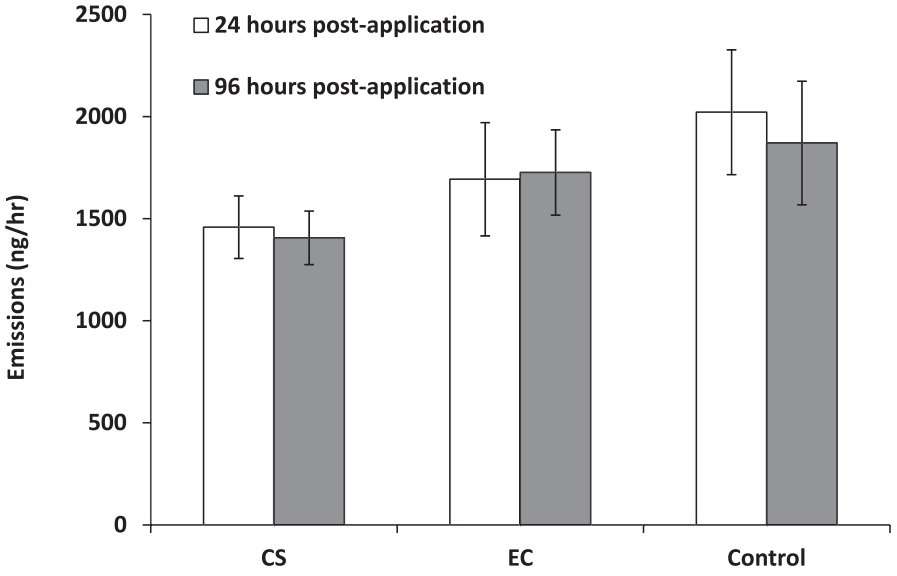

Fig. 1. Total volatile organic compound (VOC) emissions collected from cotton leaves treated with a capsule suspension (CS) or an emulsifiable concentrate (EC) of *lambda*-cyhalothrin and untreated controls 24 and 96 h after application. Bars represent mean ± SE of 5 replicates for CS formulation, 6 replicates for EC formulation, and 5 replicates for control plants.

Table 2. Composition of volatile blends collected from cotton leaves 24 and 96 h after treatment a capsule suspension (CS) or an emulsifiable concentrate (EC) of *lambda*-cyhalothrin, or untreated controls.

	24 h post-application			96 h post-application		
	CS	EC	Control	CS	EC	Control
Compound	Mean ± SE[a]	Mean ± SE[b]	Mean ± SE[a]	Mean ± SE[a]	Mean ± SE[b]	Mean ± SE[a]
(*Z*)-2-Hexenal[c]	87.1 ± 14.5	64.8 ± 7.0*	101.7 ± 19.0	64.1 ± 12.3	45.2 ± 7.9	73.9 ± 10.1
(*Z*)-3-hexenol[c]	145.5 ± 4.0	114.9 ± 16.5	130.7 ± 20.7	132.3 ± 13.5	116.7 ± 7.5	142.5 ± 19.0
α-Pinene[d]	181.5 ± 57.8	213.0 ± 53.1	262.5 ± 95.3	177.5 ± 42.3	186.8 ± 48.0	208.4 ± 42.3
βPinene[d]	30.4 ± 6.6	29.2 ± 8.0	41.7 ± 16.7	37.9 ± 7.9	38.3 ± 6.9	26.0 ± 10.0
Myrcene[d]	38.6 ± 13.1	37.4 ± 10.3	66.7 ± 24.8	38.6 ± 13.1	41.7 ± 23.7	43.9 ± 15.7
(*Z*)-3-Hexenyl acetate[c]	509.4 ± 83.4	526.7 ± 82.8	467.4 ± 84.3	496.4 ± 65.5	477.7 ± 41.8	386.9 ± 65.6
Hexyl acetate[c]	121.4 ± 34.3	117.6 ± 22.7	202.8 ± 46.4	121.4 ± 34.3	135.4 ± 26.9	241.7 ± 34.4
Limonene[d]	170.3 ± 39.3	197.8 ± 23.9	157.9 ± 41.8	131.9 ± 27.0	147.2 ± 17.8	167.0 ± 41.8
Nonanal[e]	33.2 ± 1.2	49.3 ± 11.6	50.6 ± 7.2	48.5 ± 9.6	57.5 ± 8.5	59.4 ± 9.7
(*E*)-4,8-dimethyl-1,3,7-nonatriene[f]	12.0 ± 3.4*	17.0 ± 0.6*	40.6 ± 8.2	21.7 ± 6.0	14.8 ± 1.8	30.6 ± 7.5
β-Caryophyllene[g]	45.2 ± 18.8	54.6 ± 13.2	75.3 ± 24.0	40.2 ± 12.6	61.0 ± 12.9	63.8 ± 15.5
a-Humulene[g]	15.9 ± 7.5	14.9 ± 5.1	38.4 ± 19.3	21.9 ± 9.1	17.4 ± 3.8	29.1 ± 4.7
(*E*)-β-Farnesene[g]	4.9 ± 1.1*	19.8 ± 4.6	26.4 ± 3.4	6.9 ± 2.3*	16.3 ± 4.0	23.4 ± 2.3
(*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene[f]	3.8 ± 1.0*	3.2 ± 1.0*	16.0 ± 5.5	6.6 ± 2.1	11.4 ± 2.2	12.8 ± 3.1

*Significantly difference from untreated control at $P = 0.05$ (SAS contrast).
[a]5 replications.
[b]6 replications.
[c]green leaf volatile.
[d]monoterpene.
[e]C9 aliphatic aldehyde.
[f]homoterpene.
[g]sesquiterpene.

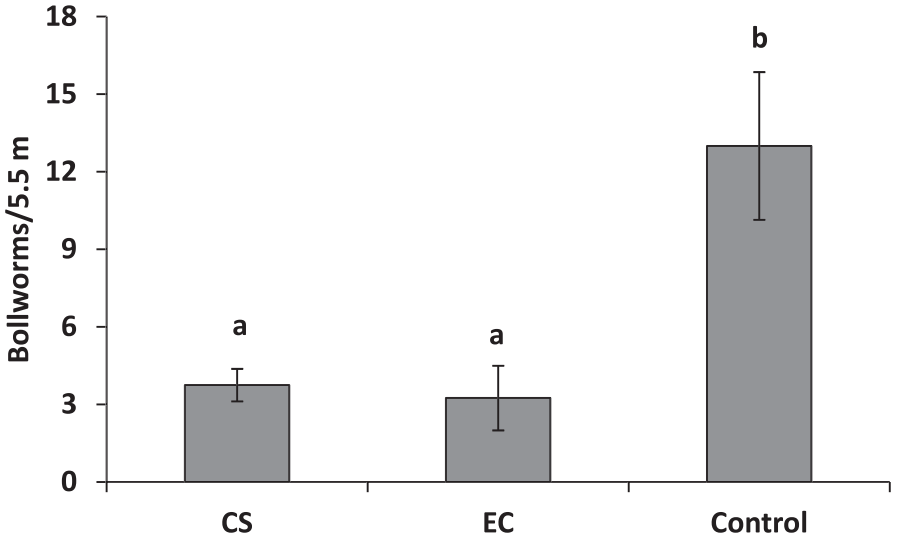

Fig. 2. Mean number of bollworms (*Helicoverpa zea*) collected on 15 August from canopy of cotton plants treated with a capsule suspension (CS) or an emulsifiable concentrate (EC) of *lambda*-cyhalothrin and untreated controls. Bars represent mean ± SE of 4 replicates. Different letters indicate significant difference ($P < 0.05$) among treatments.

detected in the number of larvae collected from plots treated with either formulation of *lambda*-cyhalothrin (Fig. 2).

Homoterpenes, GLVs, and the sesquiterpene β-farnesene were released in significantly higher concentrations from control plants compared with plants from one or both pyrethroid treatments. However, the VOC emissions detected in this study showed no consistent differences between plants treated with the two different formulations of pyrethroids. Studies have demonstrated that these compounds are commonly induced by herbivore feeding damage in cotton (Loughrin et al. 1994, Röse et al. 1996, Paré & Tumlinson 1997, 1999, Hedge et al. 2011). While herbivory was not quantified among the treatments, greater numbers of *H. zea* larvae were collected in control plots compared with pesticide-treated plots. Most likely, increased herbivore feeding damage was sustained in control plots, explaining the elevated emission of compounds from control leaves, rather than direct or indirect effects of pyrethroids on secondary metabolism in treated leaves. A more in-depth analysis of the effect of pyrethroid formulations under controlled conditions may provide direct confirmation of the influence of insecticides on VOC synthesis and/or emission.

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