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Reidentification of pheromone composition of *Sparganothis sulfureana* (Clemens) and evidence of geographic variation in male responses from two US states

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(*E*)-11-tetradecenol
(*Z*)-9-tetradecenyl acetate

A B S T R A C T

GC-EAD analyses of pheromone gland extracts of calling female *Sparganothis sulfureana* revealed at least 6 compounds that consistently elicited antennal responses from male antennae. In addition to the major pheromone compound, (*E*)-11-tetradecenyl acetate (*E*11–14:OAc), which was previously reported, the other compounds were found to be (*E*)-9-dodecenyl acetate (*E*9–12:OAc), (*Z*)-9-dodecenyl acetate (*Z*9–12:OAc), (*Z*)-9-tetradecenyl acetate (*Z*9–14:OAc), (*Z*)-11-tetradecenyl acetate (*Z*11–14:OAc), and (*E*)-11-tetradecenol (*E*11–14:OH). Tetradecyl acetate, hexadecyl acetate and hexadecenyl acetates were also present in the extracts, but elicited no EAG response from male antennae. Wind tunnel tests demonstrated that males from New Jersey responded equally well to a blend containing five pheromone components in relative to the pheromone glands of calling females. Different male-response profiles from field-trapping tests conducted in the states of Wisconsin and New Jersey were observed, respectively. Significantly higher numbers of male *S. sulfureana* were caught in New Jersey in traps baited with the binary blend of *E*11–14:OAc (30 μg) with 1% of *Z*11–14:OAc, but males from Wisconsin responded equally well to traps containing blends of *E*11–14:OAc with 0–10% of *Z*11–14:OAc. The addition of more than 10% of *Z*11–14:OAc to the primary pheromone compound reduced male captures significantly in both states. Male catches were doubled by adding *E*9–12:OAc and *E*11–14:OH to the most attractive binary blend in both states. The trapping test with caged live virgin female moths showed that males in Wisconsin preferred females from the local population than those from New Jersey. The differences in male responses observed may indicate the existence of pheromone polymorphism in this species.

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Introduction

The cranberry, *Vaccinium macrocarpon*, is a native North American crop that is commercially grown on thousands of hectares in the northeast, north central and northwest regions of the United States. The *Sparganothis* fruitworm, *Sparganothis sulfureana* (Clemens), is one of the three most important pests of cranberries in Massachusetts, Wisconsin, and New Jersey, which three states together account for almost 90% of the cranberry production in United States (Cross, 2003). In New Jersey, *S. sulfureana* is regarded as the most important cranberry pest during the post-pollination period and is the primary target for most insecticide applications. *S. sulfureana* has two generations per season (Averill and Sylvia, 1998). The first generation begins in early June with a flight period of 4–5 weeks, and the second generation emerges in the first week of August, and continues for 8–9 weeks (Cockfield et al., 1994). The second generation during late summer has been considered as the most destructive, when the larvae feed on both fruits and foliage.

Control of *S. sulfureana* in cranberry marshes relies heavily on the application of organophosphate and carbamate insecticides. However, with increasing concern about potential environmental risks such as surface water pollution, and the elimination of organophosphate insecticides due to the 1996 Food Quality Protection Act, makes it necessary to develop alternative pest management tools. Pheromone mating disruption is one of these alternatives that has been used against this pest with partial pheromone compounds, such as microencapsulated (*E*)-11-tetradecenyl acetate (*E*11–14:OAc) (Polavarapu et al., 2001). However, formulated natural blend of sex pheromone is argued to provide most robust mating disruption (Cardé and Minks, 1995).

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Field screening tests with several aliphatic alcohols and acetates have identified (*E*)-11-tetradecenyl alcohol (*E*11–14:OH) (Roelofs and Comeau, 1970) and *E*11–14:OAc (Comeau and Roelofs, 1973; Mayer and McLaughlin, 1991) as sex attractants for *S. sulfureana*. Recently it was found by two of us (Roelofs and Nojima, unpublished) that *E*11–14:OAc was present in female pheromone gland extracts. Additional field-trapping tests indicated that the addition of two other compounds, (*Z*)-9-tetradecenyl acetate (*Z*9–14:OAc) and (*E*)-9-dodecenyl acetate (*Z*9–12:OAc), increased male captures by 2- to 10-fold at apple orchards in New York State (Hill and Roelofs, unpublished). However, there are no reports to date that determine sex pheromone constituents of *S. sulfureana* based on sex pheromone gland extraction or effluvia collections. Identification of the full natural blend of sex pheromone of *S. sulfureana* will provide further insights into pheromone-mediated sexual communication, and may further optimize mating disruption technology for this cranberry pest.

In the present study, we report the results from gas chromatographic-electroantennogram (GC-EAD) analyses of pheromone gland extracts from calling female *S. sulfureana* that reveal six pheromone candidate compounds eliciting antennal responses from male antennae. Further, we report pheromone structure identification and male behavioral responses to pheromone blends containing minor pheromone components in laboratory wind tunnel studies and in cranberry fields. Different profiles of male responses in different field test sites have also been observed, which may suggest pheromone polymorphism in this species.

Methods and materials

Insects

Male and female moths of *S. sulfureana* used for the present study were emerged from pupae shipped from Marucci Center for Blueberry and Cranberry Research and Extension, Rutgers University, Chatsworth, New Jersey. The male pupae were maintained at 20 °C, 55% relative humidity in a climate-control chamber with a 14:10 L:D cycle, whereas female pupae were set in a separate chamber at 23 °C under a reversed photoperiod. Emerged adults were held in paper cartons (3.75 L), and supplied with a 5% sucrose solution until used in the experiments.

Preparation of gland extracts

Female *S. sulfureana* were observed to have a period of maximum calling between 1 to 2 h into the scotophase. Pheromone glands of 2–3 day old females were dissected at their peak calling time, and extracted in hexane (B&J high grade solvent) for 30 min. Batch extracts were pooled from 10–30 glands in 30 µl of solvent with 10 ng of internal standard (pentadecane). These batched extracts were then analyzed with gas chromatograph-mass spectrometry (GC-MS). The solvent extracts without the addition of the internal standard were saved for use in behavioral wind tunnel bioassays.

Chemical analysis

A Hewlett Packard 5890 Series II gas chromatography was equipped either with a DB-225 column or a carbowax column (30 m × 0.25 mm i.d., J & W Scientific, Folsom, CA). An effluent split at 1:1 ratio allowed simultaneous flame ionization detector (FID) and male electroantennogram (EAD) responses to female pheromone gland extracts. The extracts were injected in a splitless mode. The injector temperature was set at 250 °C, and the split valve was opened 1 min after injection. The initial column temperature was 80 °C for 1 min and then linearly increased to 230 °C at a rate of 15 °C/min. The outlet for the EAG was continuously supplied with a purified air stream flowing over the antennal preparation at a speed of 0.5 m/s. A dissected male moth antenna was used for EAG recordings. A capillary glass Ag–AgCl recording electrode filled with electrode saline (0.1 M KCl) was placed in contact with the cut tip of the antenna. Another electrode filled with the same saline was connected with the base of the antenna and served as the reference electrode. The EAG setup with a high-input DC with automatic baseline drift compensation that was used in this study was from Syntech (Hilversum, The Netherlands). A GC-EAD program (version 2.3) developed by Syntech was used to record and analyze the amplified EAD and FID signals on a Pentium III PC.

GC-MS analyses were performed by using a Hewlett Packard 5890 Series II gas chromatograph interfaced to a Hewlett Packard 5972 Mass Selective Detector (MSD), equipped with the same columns described in GC-EAD analyses. Concentrated pheromone gland extracts were injected for analyses. The GC operation conditions were same as those described above. Mass spectra were recorded from 30 to 550 a.m.u. after electronic impact ionization at 70 eV. The chemical identification of pheromone compounds was performed by comparing the retention indices and mass spectra with those of authentic standards in a mass spectral library (Wiley 138K, John Wiley & Sons, Inc., New York, USA).

All synthetic saturated and mono-unsaturated aliphatic acetates, aldehydes and alcohols (C_{12}–C_{16}) tested in the present study were purchased from the PheroBank (The Netherlands) and the purity was >99% according to analyses by GC-MS. The two groups of conjugated acetates (7,9-dodecadienyl acetates and 9,11-tetradecadienyl acetates) were generously provided by Dr. Christer Löfstedt (Pheromone Group, Lund University, Sweden) with purities described in Zhu et al. (1996).

Behavioral assays in wind tunnel

Triangular pieces of filter paper (Whatman white filter paper, 0.5 cm^2) were used as dispensers for all behavioral assays in the wind tunnel. The filter paper lure was attached to an insect pin, which was provided with a rubber septum support base and set on a wire stand approximately 40 cm above the wind tunnel floor. The filter paper was impregnated with pheromone solution 2 min before testing, and replaced every 20 min. All synthetic lures tested in the wind tunnel contained approximately 100 ng (in a volume of ~10 µl) of the major

Table 1
Comparisons of retention time between the synthetic standards and pheromone candidates from gland extracts of New Jersey female *Sparganothis sulfureana* on two different columns in GC-MS analyses.

Compounds	Carbowax		DB-225	
	Synthetics	Extracts	Synthetics	Extracts
*E*9–12:OAc	12.12	12.12	11.94	11.93
*Z*9–12:OAc	12.24	12.24	12.10	12.09
*Z*9–14:Ald	12.54	–	12.64	–
*E*11–14:Ald	12.58	12.57	12.70	–
*Z*7,*E*9–12:OAc	13.63	–	13.22	13.21
*E*7,*Z*9–12:OAc	13.75	–	13.31	–
*Z*7,*Z*9–12:OAc	13.83	*13.85*[*]	*13.37*	–
*E*7,*E*9–12:OAc	13.95	–	*13.37*	13.36
14:OAc	13.84	*13.85*	13.72	13.72
*E*11–14:OAc	14.30	*14.25–14.36*	14.05	14.06
*Z*9–14:OAc	14.26	*14.25–14.36*	13.99	14.00
*Z*11–14:OAc	14.44	14.45	14.15	14.16
*Z*7–14:OH	14.87	–	*13.63*	*13.64*
*E*11–14:OH	15.07	15.08	*13.63*	*13.64*
*Z*11–14:OH	15.21	15.21	13.80	13.80
*Z*9,*E*11–14:OAc	15.69	15.68	15.19	–
*E*9,*Z*11–14:OAc	15.80	–	15.26	15.25
*Z*9,*Z*11–14:OAc	15.89	*15.86*	*15.33*	–
*E*9,*E*11–14:OAc	16.00	–	*15.33*	–
16:OAc	15.88	*15.86*	15.46	15.47
*Z*9–16:OAc	16.17	16.14	15.81	15.79
*Z*11–16:OAc	16.29	16.28	15.91	15.90

* Retention time in italics of the two adjacent compounds means that these two compounds co-elute from the analyzing column used for analyses.

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