Sex Pheromone of the Female Squash Vine Borer (Lepidoptera: Sesiidae)[1]

J. A. Klun, M. Schwarz, B. A. Leonhardt, and W. W. Cantelo[2]

Insect Chemical Ecology Laboratory, Beltsville Agricultural Research Center, USDA-ARS
Beltsville, MD 20705

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ABSTRACT Analysis of ovipositor extracts of the squash vine borer *Melittia cucurbitae* showed that a major component in the extracts was (*E,Z*)-2,13-octadecadien-1-ol acetate along with traces of (*Z,Z*)-and (*Z,E*)-isomers. The extracts probably also contained (*E,Z*)-2,13-octadecadien-1-ol, geometrical isomers of 3,13-octadecadien-1-ol acetate, (*Z*)-9-hexadecen-1-ol, (*Z*)-9-hexadecen-1-ol acetate, (*Z*)-11-hexadecen-1-ol acetate, hexadecan-1-ol acetate, (*Z*)-13-octadecen-1-ol acetate, and (*Z*)-13-octadecen-1-ol. Trapping tests with permutations of these compounds showed that a binary mixture of (*E,Z*)-2,13-octadecadien-1-ol acetate and (*Z,Z*)-3,13-octadecadien-1-ol acetate (99.7:0.3) was required to effectively cause capture of males. The binary mixture proved to be more effective as a lure for squash vine borer males than (*E,Z*)-2,13-octadecadien-1-ol acetate alone. Thus, behavioral evidence indicated that a 2,13- plus 3,13-isomeric combination of octadecadien-1-ol acetates quite likely is a natural element in the female sex pheromone of this moth. Physical chemical evidence for the 3,13 isomer in the female extracts was equivocal because its purported occurrence was at a trace level and absolute verification of the compound's structure was not possible.

KEY WORDS Lepidoptera; Sesiidae, squash vine borer; *Melittia cucurbitae;* (*Z,Z*)-3,13-octadecadien-1-ol acetate; (*E,Z*)-2,13-octadecadien-1-ol acetate.

The squash vine borer, *Melittia cucurbitae* (Harris), is a diurnally active sesiid moth that is a pest of considerable economic significance in pumpkin and squash crops in areas east of the Rocky Mountains from Canada to South America. Based upon trapping tests and chemical studies (Neal 1979, Schwarz et al. 1983), it was known that sesiid moths generally use octadecadien-1-ol acetates or octadecadien-1-ols with 3,13 or 2,13 sites of unsaturation and variable geometry at the sites as major constituents of their female sex pheromones. Field trapping tests (Priesner et al. 1986, Snow et al. 1987) against several species with the 3,13 and 2,13 isomers of octadecadien-1-ol acetate showed that binary mixtures of them were often more effective as male lures than either isomer alone. In case of the squash vine borer, it was known from gas chromatography-mass spectrometry (GC-MS) that the female of the species produced (*E,Z*)-2,13-octadecadien-1-ol acetate (Schwarz et al. 1983), however, the compound did not cause capture of any squash vine borer males when it was deployed in field traps. This observation and the work of Priesner et al. and Snow et al. prompted us to analyze extracts of the squash vine

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[2] Vegetable Laboratory, Beltsville Agricultural Research Center Agricultural Research Service, USDA, Beltsville, MD 20705

borer with special emphasis upon a search for minor components and positional isomers of octadecadien-1-ol acetate that might be produced by the species and synergize the male response. We report evidence that indicates (*E,Z*)-2,13-octadecadien-1-ol acetate (E,Z-2,13-18:0Ac), the major pheromonal component, and a trace of (*Z,Z*)-3,13-octadecadien-1-ol acetate (Z,Z-3,13-18:OAc) are essential components in the squash vine borer female sex pheromone.

Materials and Methods

The squash vine borer is univoltine in the vicinity of Beltsville, MD. Larvae usually develop in the vines of its preferred host species, *Cucurbita pepo,* (Howe 1949) and overwinter *ca.* 3 cm below the surface of the soil inside a tough silk-lined cocoon. Pupation occurs in the spring and adult flight takes place about the time when the crops begin to extend vines (Metcalf et al. 1951). In an effort to obtain adult virgin females for pheromonal analysis, larvae were collected from *C. pepo* in the field and placed into the fruit of white bush scallop squash by cutting a hole in the top of the fruit and inserting a larva or two into it. Larvae usually completed development within a single fruit but in some instances, the fruit began to decay and larvae were transferred to a fresh fruit. The larvae and fruit were held in the ambient conditions of the laboratory and when the larvae completed development, they bored their way to the exterior of the fruit. In all, about 100 prepupal larvae were collected and placed in soil in a screened cage that was partially buried out of doors and allowed to overwinter. The cage was transferred to an environmental chamber (16h photophase, 24°C, 85% RH) in mid-March and by the first week in May the first adults emerged. Approximately 15 adult females were obtained over the course of a month. Upon emergence, the females were placed in a small screened cage containing a seedling squash plant and the cage was positioned at a window in the laboratory. Most females were held for 48h after they emerged and were observed to begin calling (extended their ovipositors to expose their pheromone gland) between 1300h-1400h. Females that exhibited this behavior were collected from the cage and their ovipositors were excised and soaked individually in 40 microliters heptane for 1h. Individual extracts were analyzed by GC to determine which of them contained a sufficient titer of pheromonal compounds to permit analysis for trace constituents. The extracts of only 4 of the females proved to be adequate for such analysis and these were combined. The extracts of all other females showed only negligible amounts of the major pheromonal component, E,Z-2,13-18:OAc.

Gas-liquid chromatographic analyses of the ovipositor extracts were conducted using 60m × 0.25mm (ID) open-tubular columns; Supelcowax, a polar column (Supelco, Inc., Bellefonte, PA 16823) and DB-1, a nonpolar column (J & W Scientific, Inc., Folsom, CA 95630). The respective oven temperature programs for the two columns were 90°C at injection with heating at 30°C/min to 195°C and 120°C at injection with heating at 15°C/min to 200°C. Instrumentation and other operating parameters were identical to those described by Klun et al. 1982. GC-MS used the DB-1 capillary and the instrumental conditions were also described by Klun et al. 1982. GC-MS study of the female extracts was conducted after they were partitioned into polar and nonpolar components using silica. The extracts were placed on a 10mm × 2mm bed of 60-200 mesh Baker 3405 silica powder (J. T. Baker, Phillipsburg, NJ) in a disposable pasteur pipette that had been rinsed

with 2ml diethyl ether and heated at 100°C for 1h. Compounds were first eluted from the silica with ca. 200 microliters hexane: ethyl acetate (95:5) and then with 200 microliters diethyl ether. The first eluant contained acetates and the ether eluant contained long-chain alcohols according to GC analysis. The ether solution was evaporated to near dryness, then taken up in 50 microliters heptane, and acetylated using acetic anhydride-pyridine (Klun et al. 1982). The natural acetates and acetate-derivatized material were then subjected to GC-MS. The purpose of this partitioning and derivatization treatment was to ease mass spectral characterization of the alcohols in the extracts; acetates chromatograph more efficiently and are inherently more detectable than alcohols in GC-MS.

Field experiments were conducted using conical traps that have been described by Webster et al. (1986). All compounds were applied to rubber septa in 50 microliters methylene chloride. Each septum was dosed with 685 micrograms E,Z-2, 13-18:OAc plus amounts of other compounds in porportions that were indicated in extracts (Table 1) of the female. Freshly treated septa were placed in the insect traps weekly and the number of males captured in each trap was usually monitored daily. Traps were 30m apart, 1m from the ground, and were located on the Beltsville Agricultural Research Center's farmlands and in plantings of squash in community gardens nearby. Replicated trapping tests were conducted during the 1986 and 1987 summer seasons. As desirable it might have been, however, none of the tests included squash vine borer females because it was impossible to obtain the appropriate number of virgin specimens. Thus, all treatment comparisons were made between mixtures of synthetic compounds.

Table 1. Compounds indicated in the combined ovipositor extracts of four squash vine borer females according to GC and GC-MS analysis.

Compound No.	Chemical (acronym)	Percentage Composition
1	(*E,Z*)-2,13-octadecadien-1-ol acetate (E,Z-2,13-18:OAc)	68.5
2	(*Z,E*)-2,13-octadecadien-1-ol acetate (Z,E-2,13-18:OAc)	0.1
3	(*Z,Z*)-2,13-octadecadien-1-ol acetate (Z,Z-2,13-18:OAc)	0.1
4	(E,Z)-2,13-octadecadien-1-ol (E,Z-2,13-18:OH)	9.1
5	(*Z*)-13-octadecen-1-ol (Z-13-18:OH)	3.4
6	(*Z*)-9-hexadecen-1-ol acetate (Z-9-16:OAc)	0.7
7	Hexadecan-1-ol acetate (16:OAc)	0.8
8	(*Z*)-11-hexadecen-1-ol acetate (Z-11-16:OAc)	0.2
9	(*Z*)-9-hexadecen-1-ol acetate (Z-9-16:OH)	0.1
10	(*Z*)-13-octadecen-1-ol acetate (Z-13-18:OAc)	16.5
11	(*Z,E*)-3,13-octadecadien-1-ol acetate (Z,E-3,13-18:OAc)	0.2
12	(*Z,Z*)-3,13-octadecadien-1-ol acetate (Z,Z-3,13-18:OAc)	0.1
13	(*E,Z*)-3,13-octadecadien-1-ol acetate (E,Z-3,13-18:OAc)	0.2

Isomers of 2,13-18:OAc were prepared in our laboratory (Schwarz et al. 1983), 3,13-18:OAc isomers were from commercial sources, and other compounds were from our laboratory's collection of compounds. All unsaturated compounds used in the field tests were rendered geometrically pure by argentation high performance liquid chromatography (Klun et al. 1982).

Results and Discussion

GC analyses of the female extracts indicated the occurrence of 13 possible pheromonal compounds (Table 1) according to coincidences of retention times with reference compounds on the polar and nonpolar capillary columns. Figure 1 shows the chromatogram obtained from an aliquot sample of the combined extracts on the Supelcowax column. The identity of each chromatographic peak, having a single number associated with it (from Table 1), was confirmed by coincidence of retention time on both the polar and nonpolar columns. However, the identity of the chromatographic peak 2,12 was either Z,E-2,13-18:OAc or Z,Z-3,13-18:OAc and the peak 11, 13 represented either Z,E- or E,Z-3,13-18:OAc according to coincidences of retention indices with reference compounds (Table 2). Subsequent field tests were designed to ascertain potential behavioral importance of each of these compounds.

Table 2. Elution sequences of 3,13-18:OAc and 2,13-18:OAc geometrical isomers from polar and nonpolar GC columns.

Kovat's Retention	Geometric Isomer	
Index	3,13-18:OAc	2,13-18:OAc
	Polar (Supelcowax)	
2531	EE	-
2534	ZE + EZ	-
2537	ZZ	ZE
2542	-	ZZ
2563	-	EE
2570	-	EZ
	Nonpolar (DB-1)	
2155	EE + ZE	-
2158	EZ + ZZ	-
2162	-	ZE + ZZ
2171	-	EE + EZ

GC-MS analysis of the acetate fraction of the female extracts from silica provided confirming mass spectral evidence for the identities of compounds 1, 6, 7, 8, and 10. the analysis also verified the presence of a trace amount of an acetate ester having mass spectrum and retention time that was the same as 3,13-18:OAC. Thus, the identity of the chromatographic peak labeled 2,12 in Figure 1 was verified to be an octadecadien-1-ol acetate. However, only subsequent field bioassays permitted inference of the probable geometry and sites of unsaturation for this trace substance.

GC analysis of the polar fraction from silica gel partitioning indicated the presence of alcohols 4, 5, and 9 and GC-MS study of these alcohols after acetylation showed that the derivatives had retention times and mass spectra identical to E,Z-2,13-18:OAc, Z-13-18:OAc, and Z-9-16:OAc, respectively.

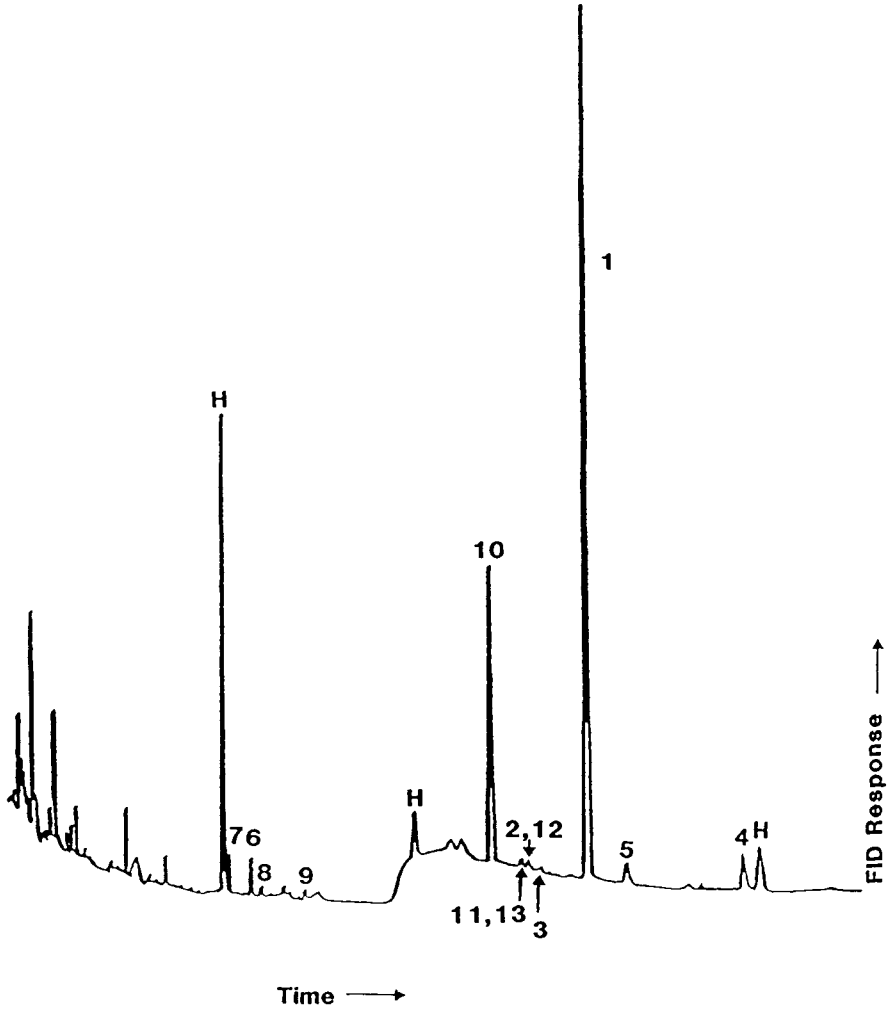

Fig. 1. Chromatogram an aliquot sample of the extracts of four female squash vine borer ovipositors obtained by using a Supelcowax capillary column. Numbered peaks had retention times that were coincident with compounds listed in Table 1. H = hydrocarbon. The three hydrocarbons were $C_{23}H_{48}$, $C_{25}H_{52}$, and $C_{27}H_{56}$.

Field trapping experiments showed that maximal flights of the squash vine borer in central Maryland generally took place during the first week of July in both years of the field testing (Figure 2). The trapping results (Table 3) obtained in Tests 1 and 2 of 1986 indicated that a trace of Z,Z-3,13-18:OAc (12) plus the major pheromonal component, E,Z-2,13-18:OAc (1), was an essential combination for the capture of males. Compound 2 had no discernable effect. Therefore, by inference only, the chromatographic peak labeled 2,12 in Figure 1 represents ZZ-3,13-18:OAC. The behavorial importance of this component was evident because, whenever it was deleted from a treatment male captures fell off drastically. On the other hand, the probable pheromonal function of component 11,13 and other compounds identified in the ovipositor extracts remains unknown because they had no detectable effects on male captures. These facts were verified in 1987 when a mixture of E,Z-2,13-18:OAc (1) and ZZ-3,13-18:OAc (12) proved to be as effective as the mixture of all 13 compounds and was significantly more effective than E,Z-2,13-18:OAc alone. Thus, the trapping data analyses indicate that these two positional isomers are crucial components in attraction of males.

Table 3. Captures of male squash vine borer and *Synanthedon acerrubri* in traps baited with compounds indicated in female extracts. Mean values in each test that are followed by the same letter are not significantly different from one another according to Duncan's Multiple Range Test (Alpha = 0.05).

Treatment*	Mean male capture/day	Total capture
Test 1 (June 12-25, 1986)	Squash vine borer	*S. acerrubri*†
A 1-13	4.9a	0
B 1,3-10,12,13	3.9a	1
C 1,2,4-11	1.3b	10
D 1,4-10	1.1b	16
Test 2 (June 26-July 15, 1986)		
B 1,3-10,12,13	3.2a	
E 1,3,10,12,13	4.7a	
F 1,3,12,13	5.6a	
G 1,10,12	3.4a	
H 1,10,13	0.9b	
I 1,3,10	0.4b	
J 1	0.8b	
Test 3 (June 22-July 13, 1987)		
A 1-13	2.4a	
J 1	0.02b	
K 1,12	2.6a	

* Compounds from the set of compounds listed by number in Table 1. The ratios of compounds tested were from same as ratios in Table 1.

† Test 1, 4 replicates; Test 2, 2 replicates; Test 3, 3 replicates. *S. acerrubri* was not captured after June 23.

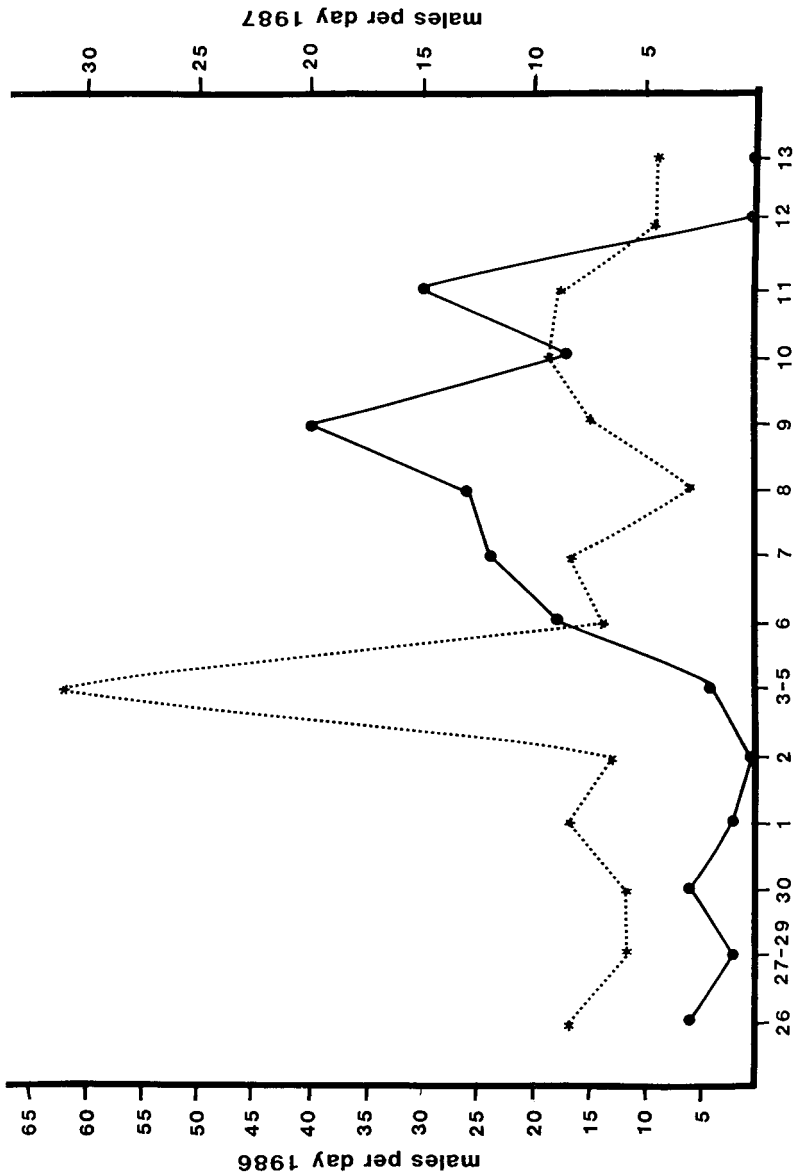

Fig. 2. Adult male squash vine borer flight periods in 1986 and 1987 according to total daily captures across all traps baited with pheromonal compounds. Dashed line presents male captures in 1986.

As a sidelight related to pheromonal specificity in the Sesiidae, we observed that *Synanthedon acerrubri* (Engelhardt) adult flight was concurrent with that of the squash borer during mid-June (Table 3, Test 1). Schwarz et al. (1983) found that this species was effectively attracted to E,Z-2,13-18:OAc alone. During the course of our testing, males of *S. acerrubri* were captured, although in small numbers, only in treatments of C and D. Circumstantially, trace amounts of compounds 3, 12, or 13 deterred the response of the species to the E,Z-2,13-18:OAc, and this suggests that specificity of pheromonal signals in the family may also be dependent upon isomer composition.

Snow et al. (1987) have shown that a trace of Z,Z-3,13-18:OAc (1%) added to E,Z-2,13-18:OAc (99%) also makes for an effective lure for the male grape root borer, *Vitacea polistiformis* (Harris). Their finding suggests that the pheromones of the squash vine borer and grape root borer may be similar to one another with regard to the use of the 3,13- and 2,13-isomer mixture. The two species are separated temporally in Maryland; grape root borer flight takes place in late July. Thus, the apparent similarity between their pheromones does not constitute a problem of pheromonal specificity. However, in other regions of the country, particularly the Southeast, flights of the two species may overlap and specificity may be dependent upon other factors such as temporal differences in periods of mate-seeking activity or species-specific visual cues.

Determination of whether or not the E,Z-2,13- and Z,Z-3,13-18:OAc isomer mixture is completely representative of the squash vine borer female sex pheromone will have to await a time when the responses of males to virgin females and the mixture can be compared. In the meantime, the binary mixture should be prove useful for monitoring adult flight activity of the pest.

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