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EFFECT OF PBAN ON PHEROMONE PRODUCTION BY MATED *Heliothis virescens* AND *Heliothis subflexa* FEMALES

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Abstract—Mated female *Heliothis virescens* and *H. subflexa* were induced to produce sex pheromone during the photophase by injection of pheromone biosynthesis activating neuropeptide (PBAN). When injected with 1 pmol *Hez*-PBAN, the total amount of pheromone that could be extracted from glands of mated females during the photophase was similar to that extracted from virgin females in the scotophase. The PBAN-induced profile of pheromone components was compared between mated, PBAN-injected females and virgin females during spring and fall. Virgin females exhibited some differences in the relative composition of the pheromone blend between spring and fall, but no such temporal differences were detected in PBAN-injected, mated females. Because the temporal variation in pheromone blend composition was greater for virgin females than for PBAN-injected females, PBAN can be used to determine a female's native pheromone phenotype. This procedure has the advantages that pheromone glands can be extracted during the photophase, from mated females that have already oviposited.

Key Words—*Heliothis virescens*, *Heliothis subflexa*, sex pheromone, PBAN, temporal variation.

INTRODUCTION

The sex pheromones of many moths are 12–18 carbon unsaturated derivatives of fatty acids, with an oxygen-containing functional group. Species specificity of the pheromone signal is achieved by the presence of specific pheromone components,

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and the relative amounts of these components in the pheromone blend. For example, most noctuid moths in the genera *Heliothis* and *Helicoverpa* use (*Z*)-11-hexadecenal (Z11-16:Ald) as the major component of their pheromone blends, but vary the types and relative amounts of minor components (e.g., Roelofs et al., 1974; Tumlinson et al., 1975, 1982; Klun et al., 1980a,b, 1982; Teal et al., 1981, 1984; Pope et al., 1982, 1984; Ramaswamy et al., 1985; Vetter and Baker, 1984; Heath et al., 1991; Choi et al., 2002). The closely related *Heliothis virescens* (*Hv*) and *H. subflexa* (*Hs*) both produce small amounts of hexadecanal (16:Ald), (*Z*)-7-hexadecenal (Z7-16:Ald), and (*Z*)-11-hexadecenol (Z11-16:OH). In addition, *Hv* females produce tetradecanal (14:Ald) and (*Z*)-9-tetradecenal (Z9-14:Ald) that are generally not found in *Hs*, whereas *Hs* females produce (*Z*)-9-hexadecenal (Z9-16:Ald) and three acetates, (*Z*)-7-hexadecenyl acetate (*Z*7-16:OAc), (*Z*)-9-hexadecenyl acetate (*Z*9-16:OAc), and (*Z*)-11-hexadecenyl acetate (*Z*11-16:OAc). Whereas the acetates are completely absent in the glands of *Hv*, Z9-16:Ald can be found in trace quantities. Even though a number of studies have been conducted to determine the pheromone compositions of *Hv* (Roelofs et al., 1974; Tumlinson et al., 1975, 1982; Klun et al., 1980; Pope et al., 1982; Heath et al., 1991) and *Hs* (Teal et al., 1981; Tumlinson et al., 1982; Klun et al., 1982; Heath et al., 1991), the female pheromone component emission ratios and rates, and the relative importance of various blend components to males, have not been fully resolved (Vickers, 2002).

Well-characterized pheromone systems in closely related, interfertile congeners offer a powerful tool for studying the genetic bases of both pheromone production and male response to different pheromone components. Understanding the genetic mechanisms underlying sex pheromone production can in turn enhance our understanding of the evolution of divergent sexual communication systems (e.g., Löfstedt, 1990, 1993; Butlin, 1995; Linn and Roelofs, 1995; Phelan, 1997; Coyne and Orr, 1998). Teal and colleagues (Teal and Oostendorp, 1995a,b; Teal and Tumlinson, 1997) recognized that *Hv* and *Hs* offer such a model: these are closely related species that can be hybridized (Laster, 1972; Prosholt and LaChance, 1974; Sheck and Gould, 1993, 1995, 1996), although their pheromone compositions differ. Recently, we initiated studies aimed at identifying genes that regulate the species-specific pheromone profiles of *Hv* and *Hs*. By hybridizing these species, followed by backcrossing to one of the parents, genes from one species have been introgressed into the genetic background of the other species. We used amplified fragment length polymorphism (AFLP) analysis to genotype each female (Sheck et al., unpublished), while her pheromone phenotype was determined by chemical analysis of the pheromone gland. Central to this approach were the requirements that each female be sampled for both DNA and pheromone, and that she produce viable progeny for the next round of genetic crosses. However, this approach is confounded by the temporal dynamics of pheromone production: females must mate and oviposit to produce progeny, but pheromone production is reduced

dramatically after mating. Preliminary data showed that in some backcrosses, the glands of less than half of the females contained more than 50 ng of pheromone (A. Groot, unpublished data), even though the glands were extracted during the scotophase, when the pheromone titer is maximal. When gland extracts contained less than 50 ng of total pheromone, we found it impossible to accurately analyze the amount of the minor pheromone components.

Several approaches can be used to obtain pheromone before females mate, without interfering with subsequent mating and oviposition. These include (a) headspace collection and analysis, (b) solid phase microextraction (SPME) analysis, or (3) non-debilitating microdissection and extraction of a portion of a virgin female's pheromone gland. However, all are laborious, time-consuming, expensive, or preclude storage of many samples prior to pheromone analysis. Because pheromone biosynthesis activating neuropeptide (PBAN) stimulates sex pheromone production in female moths (Raina et al., 1989), it appeared promising for determining the pheromone phenotype of individual females by stimulating pheromone production *after* oviposition. Indeed, a common assay of its activity is to induce pheromone production during the photophase, when the pheromone titer is normally minimal in nocturnal moths (reviews: Rafaeli, 2002; Rafaeli and Jurenka, 2003). Furthermore, Teal et al. (1993) demonstrated that PBAN-injected *Hv* and *Hs* females were induced to produce sex pheromone during the photophase; Teal and Oostendorp (1995b) extended these observations to *Hv-Hs* hybrid females. However, only Z11-16:Ald, the major sex pheromone component, was analyzed by Teal and colleagues. Because our genetic analysis required quantification of all the pheromone components, it was important to establish whether PBAN elevated production of all pheromone components in naturally occurring ratios.

Several studies have examined variation of the pheromone blends in virgin *Hs* and *Hv* females. Pope et al. (1982) determined the temporal variation in the pheromone blend emitted by *Hv* throughout the night, and Heath et al. (1991) examined the periodicity of both pheromone gland content and its emission in both *Hv* and *Hs*. Both studies found variation in the alcohol/aldehyde ratios during the scotophase, and maximal pheromone titers and release between the 4th and 8th hour of the scotophase, although *Hs* seemed to produce maximum amounts of pheromone earlier in the scotophase than *Hv* (Heath et al., 1991). Pheromone production and composition may vary not only during the night, but also during the flight season. In this study, we aimed to determine whether the blend composition in PBAN-induced mated females at a given sampling time was similar to that found in untreated virgin females, and whether any differences between the two types of females were comparable in magnitude to differences across sampling times among virgin females. We compared the amount of pheromone produced, as well as the pheromone profile, in the glands of both *Hs* and *Hv* during two periods: spring and fall.

METHODS AND MATERIALS

Insects. Hv and *Hs* were reared on artificial diet as described in Sheck and Gould (1993, 1995). Pupae were separated by sex, and placed under a reversed light cycle (14L:10D, lights off from 04:00 to 14:00). Newly eclosed adults were collected daily and placed in plastic containers (diameter 11 cm, height 8 cm) with sugar water.

Pheromone Gland Extractions: Virgin Females. In April and May, 2002 (denoted "spring treatment"), the glands of 14 *Hs* and 7 *Hv* females were dissected 2–4 d after eclosion and 4–7 hr into the scotophase. The glands were placed in conical vials containing 50 μl hexane and 20 ng of 1-pentadecanol acetate as internal standard. After 20–60 min, the glands were removed and the extracts were stored at -20°C until analysis. Variation of pheromone composition was evaluated by repeating these extractions in September and October 2002 (denoted "fall treatment"), during which glands of 12 *Hs* females and 18 *Hv* females were dissected in a similar way.

PBAN-Injections and Pheromone Gland Extractions: Mated Females. Upon eclosion, single *Hs* and *Hv* females were confined with conspecific males. The mating buckets were checked daily for oviposition, and females were allowed an additional 4 d to oviposit before they were injected with PBAN. Males were kept in the mating buckets the entire period, which allowed for multiple matings.

A stock solution of *Hez*-PBAN (Peninsula Laboratories, San Carlos, CA) (200 pmol/μl in 50% methanol and 1 N HCl) was diluted in saline (Kurtti and Brooks, 1976) within 24 hr of injection to 1pmol/μl. Females were injected during the photophase with 1 pmol PBAN in 1 μl, using a 10 μl syringe (Hamilton, Reno, NV) with a 31 gauge needle that was inserted ventrally between the 8th and the 9th abdominal segments. One hr after injection, the pheromone glands were dissected and extracted as described for virgin females. This procedure was conducted in the spring and in the fall, in the same periods as the virgin females. A total of 37 mated *Hs* females were subjected to this procedure (23 and 14 in the spring and fall, respectively), while a total of 23 mated *Hv* females were used and analyzed (9 and 14 in spring and fall, respectively).

Gas Chromatography (GC) and GC-Mass Spectrometry (MS). The hexane extracts were reduced to 0.5–1.5 μl under a gentle stream of N_2. Each sample was injected into a pulsed splitless injector held at 240°C in an HP6890 GC (Agilent, Palo Alto, CA), and separated using a 30 m $\times$ 0.25 mm $\times$ 0.5 μm Stabilwax column (Restek, Bellefonte, PA) programmed from 60°C (with a 2 min hold) to 180°C at 30°C/min, then to 230°C at 2°C/min, during which all the pheromone components eluted. The column was then heated to 245°C at 20°C/min and held at 245°C for 5 min to clean it before the next analysis. The FID detector was held at 240°C. The amount of each pheromone component was calculated relative to the 20 ng of internal standard. The components we quantified were: 14:Ald, Z9-14:Ald, 16:Ald,

Z7-16:Ald, Z9-16:Ald, Z11-16:Ald, Z7-16:OAc, Z9-16:OAc, Z11-16:OAc, and Z11-16:OH. Most chromatographic analyses did not separate Z7-16:Ald from Z9-16:Ald. Therefore, we combined the peak areas of these compounds and denoted the combination as Z7/9-16:Ald, even though Z7-16:Ald is present only in low amounts in both species, and Z9-16:Ald is present in relatively large amounts in *Hs*.

The chemical identities of all pheromone components were confirmed by GC-MS. Aliquots of these extracts were injected into an HP5890 GC, and separated using a 30 m $\times$ 0.25 $\times$ 0.5 μm DB-Wax column or a DB-225 column (both from J&W Scientific, Folsom, CA) temperature programmed from 60°C (with a 1 min hold) to 230°C at 10°/min and held for 10 min. The eluted compounds were detected with an HP5972 mass selective detector, and mass spectra were compared to those of known standards injected in the same manner. Further GC-MS analysis of the 14-carbon aldehydes was conducted on a G1800A GCD (Agilent, Palo Alto, CA) equipped with a 60 m $\times$ 0.25 mm $\times$ 0.25 μm DB-23 column, programmed from 130°C (1.5 min hold) to 170°C at 6.5°/min, then to 210°C at 2.5°/min, and to 240°C at 10°/min, followed by an 11 min hold.

Statistical Analyses. First, the total amount of pheromone extracted from the pheromone glands of virgin and PBAN-injected mated *Hs* and *Hv* at the two sampling periods was compared between species, treatments (virgin vs. PBAN-injected, mated females) and periods (spring vs. fall). The total amounts of pheromone were log-transformed and subjected to 3-factor analysis of variance (ANOVA) in which the factors species, treatment, and period were treated as fixed. ANOVA was carried out using the GLM procedure of SAS (SAS, 2002). Specific comparisons between virgin females and PBAN-injected mated females were carried out as appropriate, depending on the presence of interaction between treatments and the other two factors. To test for differences in variability among individuals in the total pheromone amounts, a generalization of the Lev1:med test (Conover et al., 1981; Boos and Brownie, 1989) was used to test for main and interaction effects of the factors species, treatment, and period, on the measure of variation corresponding to the average absolute deviation from the median. This test is robust to non-normality of the data, and is essentially a 3-factor ANOVA on $Z_{ij} = |Y_{ij} - median_i|$, where Y_{ij} is the total pheromone for the jth individual in the ith group, and $median_i$ is the median of the ith group, a group being one of the eight species-treatment-period combinations.

Second, the impact of PBAN on ratios of pheromone components was tested. The relative percentage of each of the components was calculated by setting the total amount of all pheromone components to 100%. The three C_{16} acetates were summed and treated as one component, the sum of acetates, to reduce the number of comparisons. All values were then log-transformed to stabilize the variance. When we compared *Hv* with *Hs*, large differences were found between the means and the variances of most pheromone components. Therefore, only intraspecific differences were analyzed further. Within each species, the following comparisons

were made, using multivariate ANOVA (MANOVA): (a) Differences in overall pheromone composition between treatments, (b) Differences in overall pheromone composition between periods, and (c) Interaction effects between treatments and periods. These overall comparisons were followed by a univariate ANOVA for each component separately, with separation of means using Tukey's adjustment for multiple comparisons. Comparisons between virgin females and PBAN-injected mated females in the same period were of primary interest, but comparisons across periods were also of interest to provide information about the magnitude of temporal differences.

RESULTS

Our GC and GC-MS results confirmed the presence of 14:Ald and Z9-14:Ald in *Hs* females (data not shown). The retention times of each of these two compounds on both polar (Stabilwax) and non-polar (HP-1) GC columns were coincident with the retention times of the respective authentic sample. The parent ions of 14:Ald and Z9-14:Ald were not detected, but for 14:Ald, *m/z* 194 ($M^+ - 18$) and 168 ($M^+ - 44$) were diagnostic. The mass spectrum of Z9-14:Ald showed $M^+ - 18$ at *m/z* 192, which is characteristic of C_{14} monounsaturated aldehydes. Based on these results, together with the earlier identification by Klun et al. (1982), we are confident that both 14:Ald and Z9-14:Ald were present in *Hs* females.

Injection of 1 pmol of *Hez*-PBAN into mated, post-oviposition *Hs* and *Hv* females during the photophase resulted in a total amount of extractable pheromone in the range of that found in virgin females during the scotophase (Table 1). The only significant effects were the Species × Period and Species × Treatment × Period interactions ($P = 0.004$ and $P < 0.001$, respectively). Pheromone glands of virgin *Hs* females in the spring contained significantly more pheromone than those of PBAN injected, mated *Hs* females in the same period, but the difference between virgin *Hs* females in the spring and fall (i.e., a temporal effect) was of similar magnitude to the treatment difference in the spring (Table 1). In contrast to the significant temporal effect for virgin *Hs* females, the PBAN-induced pheromone titers in mated females of both species were not influenced by the sampling period. More importantly, the amount of pheromone extracted during the photophase from glands of mated, post-oviposition females injected with PBAN was sufficient, i.e., $\gg$50 ng, for accurate analyses of pheromone blend composition.

Analysis of absolute deviations from the median using the Lev1:med test (Conover et al., 1981; Boos and Brownie, 1989) yielded a marginally significant ($P = 0.058$) treatment main effect. Mean absolute deviations presented in Table 2 suggest that variation in the amount of pheromone produced among females of the same species within a period was higher in virgin females than in PBAN-induced mated females. Values in Table 1 for the coefficient of variation also suggest that PBAN reduced variation in pheromone titers.

TABLE 1. AMOUNT OF PHEROMONE EXTRACTED FROM THE GLANDS OF VIRGIN FEMALES, AND MATED FEMALES INJECTED WITH 1 PMOL PBAN AFTER THEY HAD OVIPOSITED FOR 4 DAYS[a]

Source	Period	*N*	Total pheromone per female (ng), mean ± SEM (CV)	
H. subflexa	Spring	13	Virgin	414 ± 72 (63) **a**
		23	Mated + PBAN	201 ± 21 (50) **b**
	Fall	13	Virgin	166 ± 41 (90) **b**
		14	Mated + PBAN	231 ± 28 (46) **ab**
H. virescens	Spring	7	Virgin	196 ± 44 (59) **A**
		9	Mated + PBAN	260 ± 37 (43) **A**
	Fall	18	Virgin	317 ± 46 (61) **A**
		14	Mated + PBAN	279 ± 35 (47) **A**

[a]Within a species, means without a letter in common differ significantly ($P < 0.05$), using Tukey's procedure for multiple comparisons on log-transformed total amounts. ng: nanogram, SEM: Standard error of the mean, CV: Coefficient of variation.

When comparing the overall profiles per species using MANOVA, a significant difference was found between virgin females and PBAN-injected mated females, averaged over periods, for both *Hv* and *Hs* (Table 3). Overall pheromone profile differences between periods were not found in *Hv*, but were significant in *Hs*. Overall interaction effects between treatment and periods were found for both *Hv* and *Hs* (Table 3).

To determine which components contributed to these overall differences, results of separate analyses on each component are presented in Table 3. In *Hv*, significant treatment main effects were found for 14:Ald and Z11-16:OH (see Table 3 and Figure 1). Examining the means for these components showed that,

TABLE 2. VARIATION, MEASURED BY THE MEAN ABSOLUTE DEVIATION FROM THE MEDIAN, IN TOTAL PHEROMONE AMOUNTS EXTRACTED FROM THE GLANDS OF VIRGIN FEMALES, AND MATED FEMALES INJECTED WITH 1 PMOL PBAN AFTER THEY HAD OVIPOSITED FOR 4 DAYS

Source	Period	*N*	*z* mean ± SEM[a] (ng)	
H. subflexa	Spring	13	Virgin	175 ± 53
		23	Mated + PBAN	82 ± 13
	Fall	13	Virgin	103 ± 39
		14	Mated + PBAN	84 ± 16
H.virescens	Spring	7	Virgin	91 ± 30
		9	Mated + PBAN	75 ± 26
	Fall	18	Virgin	147 ± 29
		14	Mated + PBAN	107 ± 18

[a]Mean and SEM of $Z_{ij} = |Y_{ij} - \text{median}_i|$, or the absolute deviation from the median, a measure of variation within a group of the total amount of pheromone extracted (Conover et al., 1989; Boos and Brownie, 1991). See text for further explanation.

TABLE 3. MANOVA PER SPECIES TO TEST FOR OVERALL[a] DIFFERENCES IN PHEROMONE COMPOSITION, FOLLOWED BY UNIVARIATE ANALYSES FOR EACH OF THE COMPONENTS (EXPRESSED AS A PERCENTAGE OF TOTAL PHEROMONE AMOUNT). *P*-VALUES ARE REPORTED FOR TREATMENTS, PERIODS, AND INTERACTION EFFECTS

	H. subflexa			*H. virescens*		
	Treatment	Periods	Treatment × Period	Treatment	Periods	Treatment × Period
MANOVA	<0.001	<0.001	0.001	<0.001	0.30	0.035
Univariate tests						
Compound[b]						
14:Ald	<0.001	0.002	ns	<0.001	ns	0.033
Z9-14:Ald	ns	ns	0.001	ns	ns	ns
16:Ald	ns	<0.001	0.001	ns	ns	ns
Z7/9-16:Ald	0.034	0.020	ns	ns	ns	ns
Z11-16:Ald	0.040	0.007	ns	ns	ns	0.012
Acetates[c]	0.017	0.030	ns	—	—	—
Z11-16:OH	ns	0.023	ns	0.011	0.017	0.009

Note. ns: $P > 0.05$.
[a] Comparing pheromone profiles as a whole, instead of per compound.
[b] All analyses were carried out on log-transformed values.
[c] Absent in *Hv* females.

although statistically significant, the difference in the relative percentages was small for 14:Ald, i.e., 0.8%. The difference in Z11-16:OH between virgin and PBAN-injected *Hv* was bigger (6.7%), which is due to the relatively high amount of Z11-16:OH in virgin *Hv* females in spring.

In *Hs,* significant differences were found for all components except Z11-16:OH (Table 3). The percentages of 14:Ald, 16:Ald, and Z11-16:Ald were significantly lower in fall than in spring, whereas the percentages of Z9-14:Ald and Z7/9-16:Ald were significantly higher in fall than in spring (see Figure 1). Also, significant differences were found between virgin *Hs* females and PBAN-injected, mated *Hs* females (Table 3). Despite these differences, the relative percentages of the different compounds found in virgin or PBAN-injected mated *Hs* females overlapped in at least one of the periods (Figure 1).

DISCUSSION

Our results demonstrate that mated *Hs* and *Hv* females injected with 1 pmol PBAN during photophase produced pheromone at levels and ratios comparable to those produced by virgin females during the scotophase. It is likely, based on dose-response studies (Raina et al., 1989; Abernathy et al., 1996), that injection of more PBAN would have stimulated mated females to produce more pheromone.

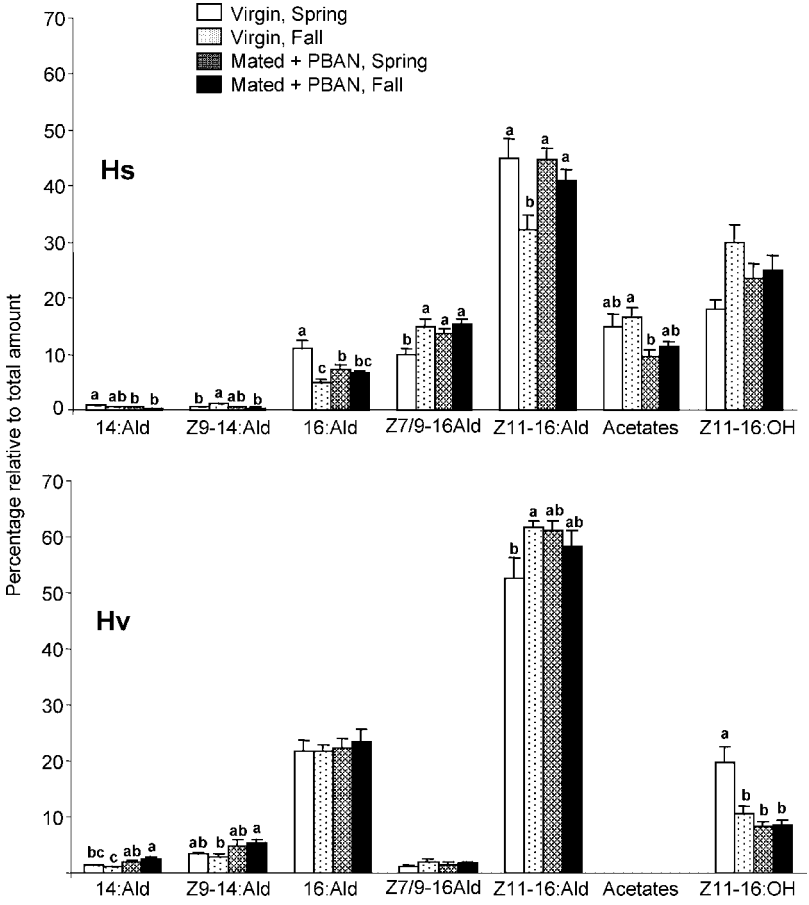

FIG. 1. Pheromone profiles (mean ±SEM) of *H. subflexa* (***Hs***, upper graph) and *H. virescens* (***Hv***, lower graph) when the relative abundance of each component is calculated as a percentage of the total amount of pheromone. Different letters within each component indicate significant differences ($P < 0.05$), using a Tukey adjustment for multiple comparisons. No letters: not significant. See text for further explanation.

Our results also demonstrate that PBAN-induced mated females produce and accumulate pheromone of comparable blend quality to that of virgin females. This is not surprising given our current understanding of the enzymatic steps affected by PBAN. In most species, including *H. zea*, PBAN appears to affect steps in or prior to fatty acid synthesis (e.g., Jurenka et al., 1991; Jurenka, 1996; Teal et al., 1996; reviews: Tillman et al., 1999; Rafaeli, 2002; Rafaeli and Jurenka, 2003). In

some species, reduction of the fatty acyl moiety to alcohol appears to be controlled by PBAN (Arima et al., 1991; Ozawa et al., 1993; Fabrias et al., 1994). One study using *Sesamia nonagrioides* (Mas et al., 2000) found an activation by PBAN of acetyl transferase, converting alcohols to acetates. In heliothines, it appears that PBAN controls either the biosynthesis of fatty acids, their reduction, or both.

Pheromone composition is usually reported either relative to the mass of the major component (here, Z11-16:Ald), or as a percentage of each component relative to the total mass of pheromone recovered. The first approach, expressing the secondary components as a percentage of the main component, may facilitate comparisons between species that differ in the number of secondary components. The second approach, expressing all components as a percentage of the total amount of pheromone produced, can show intraspecific treatment effects on the main component. We calculated and analyzed the relative amounts of the different pheromone components in both ways. Because similar trends emerged, we have shown only the pheromone profiles in which all components are expressed as a percentage of the total amount of pheromone produced.

The fact that we found statistically significant differences in the relative amount of 14:Ald produced in both species may not be biologically significant, because the quantities produced were very low. The overall significant difference in Z11-16:OH between virgin *Hv* and PBAN-induced mated *Hv* females, and the overall significant difference in the amount of acetates produced between virgin *Hs* and PBAN-injected mated *Hs* females, are harder to explain. However, the difference in relative percentages of these components between virgin females and PBAN-induced mated females was no greater than the difference between periods (i.e., temporal changes) for virgin females of the same species.

We did not find any of the acetates in *Hv* females, in agreement with previous analyses of *Hv* (Roelofs et al., 1974; Tumlinson et al., 1975, 1982; Klun et al., 1980; Pope et al., 1982; Heath et al., 1991). However, acetate esterases, which convert acetates to alcohols (Bjostad and Roelofs, 1983; Roelofs and Wolf, 1988; Wolf and Roelofs, 1989), appear to be present in both *Hv* and *Hs*. When Teal et al. (1989) applied acetate esters to the surface of the pheromone glands of both *Hs* and *Hv* females, they found more of the corresponding alcohols and aldehydes in *Hv* than in *Hs*. Whether acetyl transferases that convert alcohols to acetate esters (Bjostad and Roelofs, 1983; Morse and Meighen, 1986) are present in *Hv* as well remains to be determined.

Both species had Z11-16:OH, but it was present in much higher amounts in *Hs* than in *Hv*. In *Hs*, this alcohol has been found to be an essential component for the attraction of conspecific males (Heath et al., 1991; Vickers, 2002), whereas *Hv* females apparently do not emit this compound (Teal et al., 1986; Heath et al., 1991).

We confirmed an earlier report by Klun et al. (1982) that 14:Ald and Z9-14:Ald were present in *Hs* females. Because other studies did not find these compounds in *Hs* (Teal et al., 1981; Tumlinson et al., 1982; Heath et al., 1991),

it was questionable whether they were produced by *Hs* at all (Vickers, 2002). Not only did we consistently find both compounds in both virgin and PBAN-injected mated *Hs* females, but the percentage of 14:Ald relative to the main component was similar to that found in virgin *Hv* females. In *Hv,* these compounds, especially Z9-14:Ald, are essential for attracting conspecific males (Vickers et al., 1991). *Hs* males do not seem to be attracted or repelled by Z9-14:Ald (Vickers, 2002), although receptor cells responding to Z9-16:Ald also respond to Z9-14:Ald in *Hs* (Baker et al., 2004).

Z7/9-16:Ald were almost absent in *Hv*, but present in relatively large amounts in *Hs*. Z9-16:Ald is one of the major pheromone components that provides species-specificity; *Hs* males are not attracted to a blend lacking this component (Vickers, 2002), whereas attraction of *Hv* males is independent of the presence or absence of Z9-16:Ald (Tumlinson et al., 1982; Teal et al., 1986; Vickers et al., 1991). Z9-16:Ald can be formed through Δ9-desaturase acting on the 16:acid, or through Δ11-desaturase acting on the 18:acid (e.g., Choi et al., 2002). A Δ11-desaturase is present in both species, because it is involved in formation of the major pheromone component, Z11-16:Ald (Jurenka, 1996; Choi et al., 2002). We hypothesize that in *Hv,* low amounts of Z9-16:Ald are formed through Δ11-desaturase acting on the 18:acid, whereas *Hs* females possess a Δ9-desaturase which allows them to produce Z9-16:Ald through 16:acid as well. We are currently investigating whether *Hv* and *Hs* differ in how the Δ9-components are produced.

Because there was considerable similarity in blend composition between virgin and PBAN-injected mated females, we conclude that PBAN can be used in determining ratios of pheromone components in backcross females. PBAN injection has several major advantages over other possible methods, because glands can be extracted during the photophase, from relatively old (>6 d old) and/or mated females, which makes this method time- and age-independent.

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