(*Z*,*E*)-6,8-heneicosadien-11-one: major sex-pheromone component of *Orgyia vetusta* (Lepidoptera: Lymantriidae)

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The western tussock moth, *Orgyia vetusta* Boisduval (Lepidoptera: Lymantriidae), formerly known as *Hemerocampa vetusta* (Boisduval), occurs primarily in coastal areas of central California and south into Mexico, with occasional records east of the central Sacramento and San Joaquin valleys (Ferguson 1978). Two biotypes, feeding on perennial yellow bush lupine, *Lupinus arboreus* Sims (Fabaceae), or silver dune lupine, *Lupinus chamissonis* Eschsch. (this study), and on California live oak, *Quercus agrifolia* Née (Fagaceae), respectively, have been recognized and were originally considered two separate species (Edwards 1881; Ferguson 1978). Various fruit and nut trees have also been reported as host plants (Atkins 1958).

Ferguson (1978) discusses the uncertain status of a "*vetusta* complex", which may also include the very similar and partly sympatric congeners *Orgyia cana* H. Edwards and *Orgyia magna* Ferguson. The name "western tussock moth" correctly applies to *O. vetusta* (Bosik 1997) but is confusingly also used for *O. cana* (*e.g.*, Beckwith 1978). Identification of the *O. vetusta* pheromone would help discern between, and determine the distributions of, species and biotypes of the *vetusta* complex.

Recent analyses of pheromone gland extracts of *O. vetusta* females resulted in tentative identifications of (*Z*)-6-heneicosen-11-one (*Z*6-21-11Kt), (*Z*)-6-eicosen-11-one, and (*Z*,*Z*)-6,9-heneicosadien-11-one (*Z*6*Z*9-21-11Kt) or (*Z*,*E*)-6,8-heneicosadien-11-one (*Z*6*E*8-21-11Kt) as candidate pheromone components (Liu 1999). Here we report analyses of the *O. vetusta* pheromone and field-testing of candidate pheromone components.

Orgyia vetusta eggs were collected on 22 March 1999 at Montaña de Oro State Park, San Luis Obispo Co., California, and on 5 April 1999 at Bull Point Head, Point Reyes, Marin Co., California. Eggs from both locations were sent to quarantine facilities of the Beneficial Insect Introduction Research Laboratory, Newark, Delaware. Hatching larvae were fed on standard gypsy moth diet (Bell *et al.* 1981) and were maintained at 25 °C, 60%–80% RH, and under

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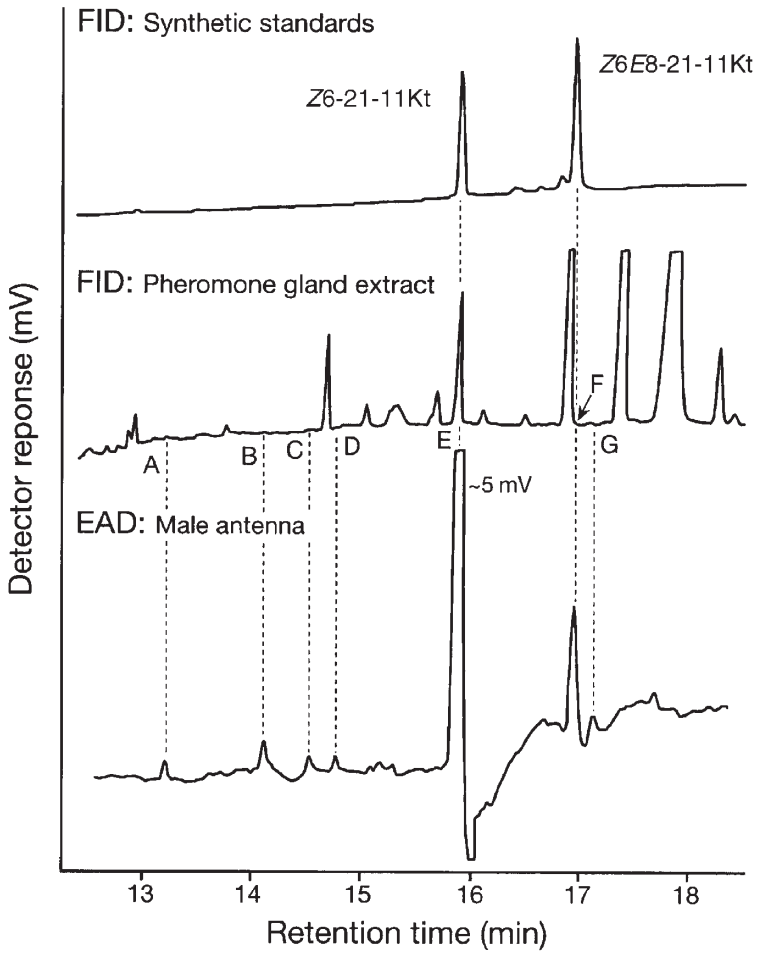
Fig. 1. Representative (n = 5) flame-ionization detector (FID) and electroantennographic detector (EAD: male *Orgyia vetusta* antenna) responses to one female equivalent (FE) of pheromone extract from female *O. vetusta*. Chromatography: DB-210 column (30 m × 0.25 mm i.d.); splitless injection; injector and FID detector: 240 °C; temperature program: 100 °C (1 min) then 10 °C/min to 220 °C (held for 10 min). A = (*Z,Z*)-6,9-eicosadien-11-ol (*Z*6*Z*9-20-11OH); B = (*Z,Z*)-6,9-heneicosadien-11-ol (*Z*6*Z*9-21-11OH); C = (*Z*)-6-heneicosen-11-ol (*Z*6-21-11OH); D = unknown; E = (*Z*)-6-heneicosen-11-one (*Z*6-21-11Kt); F = (*Z,E*)-6,8-heneicosadien-11-one (*Z*6*E*8-21-11Kt); G = unknown. Structural assignments of EAD-active components A, B, and C are tentative and are based on corresponding retention times of antennal responses and authentic standards on three gas chromatography (GC) columns (DB-5, DB-23, and DB-210). The arrow points to the expected retention time of *Z*6*E*8-21-11Kt, a compound that could not be detected by the FID in one FE of pheromone extract; the presence of *Z*6*E*8-21-11Kt was confirmed by GC – mass spectrometric analysis of a concentrated (>100 FE) pheromone gland extract (data not shown).



a 14L:10D photoperiod. Pupae were extricated from their cocoons and segregated by sex (Winter 2000). Males were chilled to 13 °C to retard development. Abdominal tips with pheromone glands of 1- or 2-day-old virgin female moths were removed during calling activity (Grant *et al.* 1975) and extracted in HPLC-grade hexane. Extracts and male pupae were sent to Simon Fraser University for analyses.

Aliquots of one female equivalent (FE) of pheromone gland extract were analyzed on three gas chromatography (GC) columns (30 m × 0.25 or 0.32 mm i.d.; DB-5, DB-210, or DB-23, J&W Scientific, Folsom, California) by coupled

Table 1. Number of *Orgyia vetusta* males captured in experiments 1 (5–9 June 1999) and 2 (1–16 October 1999) in traps baited with candidate pheromone components.

Experiment No.	Treatment	No. of males captured (mean ± SE)*
1	*Z*6-21-11Kt (100)	0*b*
	*Z*6-21-11Kt (100) + *Z*6*E*8-21-11Kt (10)	2.14±0.86*a*
	*Z*6-21-11Kt (100) + *Z*6-21-11OH (10) + *Z*6*Z*9-20-11OH (10) + *Z*6*Z*9-21-11OH (10)	0*b*
	*Z*6-21-11Kt (100) + *Z*6*E*8-21-11Kt (10) + *Z*6-21-11OH (10) + *Z*6*Z*9-20-11OH (10) + *Z*6*Z*9-21-11OH (10)	0.86±0.46*ab*
	Unbaited	0*b*
2	*Z*6-21-11Kt (50)	0*b*
	*Z*6*E*8-21-11Kt (50)	9.57±1.93*a*
	*Z*6-21-11Kt (50) + *Z*6*E*8-21-11Kt (5)	2.86±0.59*b*
	*Z*6-21-11Kt (50) + *Z*6*E*8-21-11Kt (50)	11.71±3.45*a*
	Unbaited	0*b*

Note: All experiments were conducted at Montaña de Oro State Park, California. Seven replicates were conducted for each experiment. Numbers in parentheses are micrograms of pheromone; all control treatments received the equivalent amount of solvent. For compound nomenclature, see caption of Figure 1. The chemical and geometrical purity of test chemicals were >93% and >97%, respectively.

*For each experiment, numbers followed by different letters are significantly different, $P < 0.05$ (nonparametric analyses of variance (Friedman's test) followed by comparison of means by Scheffé's test; Zar 1984; SAS Institute Inc. 1988).

GC–electroantennographic detection (GC–EAD) (Arn *et al.* 1975) and GC – mass spectrometry, employing equipment and procedures recently reported in detail (Gries *et al.* 2002). GC–EAD analyses revealed two components (E and F in Fig. 1) that elicited strong responses from male antennae. The retention indices (RI, relative to straight-chain alkanes; Van den Dool and Kratz 1963) of components E (DB-5, RI = 2258; DB-23, RI = 2744; DB-210, RI = 2618) and F (DB-5, RI = 2300; DB-23, RI = 2913; DB-210, RI = 2710) suggested that they were *Z*6-21-11Kt (E) and *Z*6*E*8-21-11Kt (F). These structural assignments were confirmed by comparing the mass spectral data (Smith *et al.* 1975; Jury *et al.* 2003) of the insect-produced compounds (>100 FE) and synthetic standards that were available from previous work (Gries *et al.* 1997, 2003, 2005). Additional candidate pheromone components (*Z*,*Z*)-6,9-eicosadien-11-ol (*Z*6*Z*9-20-11OH), (*Z*,*Z*)-6,9-heneicosadien-11-ol (*Z*6*Z*9-21-11OH), and (*Z*)-6-heneicosen-11-ol (*Z*6-21-11OH) (A, B, and C in Fig. 1) were tentatively identified based on corresponding retention times of antennal responses and authentic standards on three GC columns (DB-5, DB-23, and DB-210).

Field experiments were conducted at Montaña de Oro State Park in foredune areas with abundant *L. chamissonis*. Delta-type traps made from 2-L milk cartons (Gray *et al.* 1984) and coated with Tanglefoot (The Tanglefoot Company, Grand Rapids, Michigan) were affixed to *L. chamissonis* shrubs at heights of 0.5–1 m and spacings of 15–25 m in complete randomized blocks. Traps were baited with gray rubber septa (The West Company, Lionville, Pennsylvania) impregnated with candidate pheromone components. Experiment 1 tested the hypothesis that *Z*6-21-11Kt is the major pheromone component and that its attractiveness is enhanced by the diene ketone *Z*6*E*8-21-11Kt, the three alcohols *Z*6*Z*9-20-11OH, *Z*6*Z*9-21-11OH, and *Z*6-21-11OH, or both the diene ketone and the alcohols (Table 1). Taking into account that only lures with *Z*6-21-11Kt and *Z*6*E*8-21-11Kt captured males in experiment 1, experiment 2 tested these two components singly and in combination.

Strong attractiveness of *Z*6*E*8-21-11Kt in experiment 2 clearly indicated that it is the major sex pheromone component[4] of *O. vetusta*, rather than a rearrangement product of *Z*6*Z*9-21-11Kt (Gries *et al.* 1997; Grant *et al.* 2003).

[4]Major pheromone component is defined here as a component that, by itself, is the most attractive to male moths.

Indeed, congeneric whitemarked tussock moths, *Orgyia leucostigma* (J.E. Smith), utilize *Z*6*Z*9-21-11Kt as a major pheromone component (Grant *et al.* 2003); the pheromone's rearrangement product, *Z*6*E*8-21-11Kt, is not attractive and seems to reduce the response of males to *Z*6*Z*9-21-11Kt (Grant *et al.* 2003). Whether *Z*6-21-11Kt is a pheromone component in *O. vetusta* would have to be investigated further by testing it in combination with *Z*6*E*8-21-11Kt at the very same ratio as that found in the effluvia of calling females.

The fact that a barely detectable pheromone component (F in Fig. 1) can be most effective in attracting males (Table 1) has also been shown in pheromone gland extract analyses of, and field experiments with, tentiform leafminers, *Phyllonorycter mespilella* (Hübner) (Lepidoptera: Gracillaridae). In pheromone gland extracts of *P. mespilella*, a dodecadienyl acetate was at least 100 times less abundant than a dodecenyl acetate, but when equal amounts of the acetates were tested in field experiments, the dodecadienyl acetate was significantly more effective in attracting males (Gries *et al.* 1993). However, unlike in *O. vetusta*, in *P. mespilella* the monoene pheromone component was more attractive than unbaited control traps.

Although *Z*6*E*8-21-11Kt is the major pheromone component in *O. vetusta*, it is a synergistic pheromone component in the conifer-feeding Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough), strongly enhancing attractiveness of the major component *Z*6-21-11Kt (Gries *et al.* 1997). Despite these similar sex pheromones, *O. vetusta* and *O. pseudotsugata* remain reproductively isolated through differences in host plant preference, altitude occurrence, and geographic distribution (see Beckwith 1978).

With the major pheromone component of *O. vetusta* now known (this study), and pheromone identifications under way for *O. cana* and planned for *O. magna*, we will soon be able to discern better between, and delineate the distributions of, species within the "*vetusta* complex".

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