Identification and Field Evaluation of a Sex Pheromone of the European Pine Moth

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The principal component of the female sex pheromone of the European pine moth *Dendrolimus pini* L. (Lasiocampidae) was identified as (*Z,E*)-5,7-dodecadienal by capillary gas chromatography, mass spectrometry, single receptor analyses, and field trapping tests. Traps initially baited with 1000 µg of (*Z,E*)-5,7-dodecadienal (> 99% *ZE*) effectively monitored low *D. pini* populations over 6 weeks without rebaiting. Captures disappeared upon addition of ≧ 1% of either (*E,Z*)-5,7-dodecadienal or (*Z,E*)-5,7-dodecadienyl acetate, the key stimulants for additional receptor cell types located in male antennal hair sensilla.

The Forest Services of central European countries with stands of Scots pine (*Pinus sylvestris* L.) endangered by the feeding of *Panolis flammea* Schiff. (Noctuidae), *Lymantria monacha* L. (Lymantriidae), *Dendrolimus pini* L. (Lasiocampidae) and *Bupalus piniarius* L. (Geometridae), are required by law to survey for population densities of these lepidopterous pests. Bogenschütz [1, 2] has emphasized the potential of traps baited with sex-attractant chemicals for early detection of population increases among low (non-damaging) populations of *P. flammea* and *L. monacha*. We report here the identification of the primary sex pheromone component produced by the female European pine moth, *Dendrolimus pini* L. We also describe a synthetic chemical formulation suitable for use in population monitoring of this species by survey traps.

Female sex pheromone components are chemically known for four species of the family Lasiocampidae. The tent caterpillars *Malacosoma disstria* Hbn. and *M. californicum* Pack. produce sex pheromones whose principal components are (*Z,E*)-5,7-dodecadienal (*Z*5,*E*7-12:Ald) and (*E,Z*)-5,7-dodecadienal (*E*5,*Z*7-12:Ald) [3, 4]. The sex pheromone of the Japanese pine moth *Dendrolimus spectabilis* Butl. was reported as (*Z,E*)-5,7-dodecadien-1-ol (*Z*5,*E*7-12:OH) [5, 6], and that of the Masson pine moth *D. punctatus* Wlk., as a combination of this alcohol with the corresponding acetate and propionate [7, 8].

All four geometrical isomers of the 5,7-dodecadien-1-ols and their acetate and aldehyde analogues were included in our study of the *D. pini* pheromone. The 5,7-dodecadienes were synthesized and purified by Wittig condensation reactions previously described [9, 10]. The purity of all final products was better than 98%; the *Z*5,*E*7-12:Ald had a purity of better than 99%.

Nerve impulse responses of single receptor cells located in hair sensilla (*S. trichodea*) on *D. pini* male antennae were recorded as described for other moth species [11]. All twelve 5,7-dodecadienes were tested over six decadic steps of stimulus amount, from 10^{-4} µg to 10 µg (source load). The recordings revealed the presence of four different cell types, each responding specifically to a particular 5,7-dodecadiene compound. These four "key compounds" were: *Z*5,*E*7-12:Ald, *E*5,*Z*7-12:Ald, (*E,Z*)-5,7-dodecadien-1-ol (*E*5,*Z*7-12:OH), and (*Z,E*)-5,7-dodecadienyl acetate (*Z*5,*E*7-12:Ac). No evidence was found for additional cell types in these sensilla. Generally, the *Z*5,*E*7-12:Ald receptor cell showed the largest nerve impulse amplitude, suggesting that this compound has primary pheromone function. These single cell data are consistent with a report by Bestmann *et al.* [12] who demonstrated high responsiveness of *D. pini* male antennae to *Z*5,*E*7-12:Ald in antennogram measurements.

To analyze the pheromone released by virigin *D. pini* females, we washed the extruded abdominal tips of 20 calling female moths into 2 ml of *n*-hexane and examined the washings by capillary gas chromatography (GC) and computerized gas chromatography – mass spectrometry (GC-MS). The following procedure was used to identify the *Z*5,*E*7-12:Ald. A standard solution containing all four geometrical isomers of the 5,7-dodecadienes with aldehyde, alcohol and acetate functions was prepared and the *D. pini* tip wash solution was

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compared to this standard using two methods. A Hewlett Packard model 5710A gas chromatograph equipped with capillary injector and a nonpolar DB-1 fused silica column (30 m × 0.3 mm id; J and W Scientific Inc., Rancho Cordova, Calif.) temperature programmed 70° to 200 °C at 4° per minute separated all four geometrical isomers. Chemical ionization mass spectra were obtained using a Finnigan model 3300 quadrupole mass spectrometer coupled to an Incos model 2300 data acquisition system. Methan was the reagent gas, hydrogen the carrier gas, and the polar DB-5 fused silica capillary column (60 m × 0.35 mm id; J and W Scientific Inc., Rancho Cordova, Calif.) was temperature programmed 100° to 180 °C at 4° per minute. This column also separates all four geometrical isomers of the 5,7-dodecadienes. The elution order of the isomers from both columns was *ZE*, *EZ*, *ZZ* and *EE*.

To a 100 µl aliquot of the *D. pini* tip washings was added dodecane and heptadecane standards and the volume reduced to 2 µl was chromatographed on the DB-1 column. Immediately following the standard solution was chromatographed and the retention times of the 5,7-dienes relative to dodecane and heptadecane was noted. The chromatograph of the *D. pini* washings contained a major peak that corresponded exactly with the *Z*5,*E*7-12:Ald and was different from the *EZ*, *ZZ* and *EE* aldehydes; no other 5,7-dodecadienes with aldehyde, alcohol or acetate functional groups were detected.

Mass spectra was obtained from a 600 µl aliquot of *D. pini* washings (containing dodecane and heptadecane standards) which was reduced to 2 µl before injection onto the DB-5 column. The reference standards containing the synthetic 5,7-dodecadienes was chromatographed and spectra and retention times were obtained. In the case of the tip washings a major peak was obtained whose retention time and mass spectrum coincided with that of authentic *Z*5,*E*7-12:Ald. In addition to low molecular weight ions (whose masses and intensities corresponded with the authentic material), $[M+1]^+$, $[M-1]^+$, $[(M+1)-18]^+$ and $[(M-1)-18]^+$ were all present. The latter are diagnostic ions for aldehydes. No other 5,7-dodecadienes were detected.

Another aliquot of the *D. pini* washings was treated with N,O-bis-(trimethylsilyl)-acetamide to derivatize the alcohols if present. No 5,7-diene alcohols were found. However, the *Z*5,*E*7-12:OH could be present in quantities below our level of detection.

A quantitative GLC analysis using external standard method gave an estimated concentration of *Z*5,*E*7-12:Ald in the *D. pini* washings of 1.3 ng per 1 FE. In the receptor recordings 1 FE of this extract elicited responses from the type A cell roughly corresponding to 1 ng of synthetic *Z*5,*E*7-12:Ald.

Field trapping tests using the four "key compounds" as lures was begun in southern Germany at Seewiesen (40 km south of Munich) and at Breisach (west of Freiburg i. Br.). Tetratraps [13] with rubber cup lure dispensers [14] were suspended at eye level in stands of Scots pine and Norway spruce. Only *Z*5,*E*7-12:Ald attracted *D. pini* males to the traps. Table I shows that a 1% addition of either *E*5,*Z*7-12:Ald or *Z*5,*E*7-12:Ac to the *Z*5,*E*7-12:Ald lure abolished captures. In contrast, in another test series, adding *E*5,*Z*7-12:OH to the attractant aldehyde in ratios up to 30:100 had no marked effect on trap captures. This alcohol is the "key compound" of another cell type established in the *D. pini* male antennal receptor system. Several other analogues including *Z*5,*E*7-12:OH and the *Z*5 and *Z*7 monoenes, which did not reveal corresponding specialist cells during receptor recordings, did not modify trap captures either. However, the *Z*5,*E*7-12:OH has been reported as a primary pheromone component for both *D. spectabilis* and *D. punctatus* [5–8].

The field tests show that both *E*5,*Z*7-12:Ald and *Z*5,*E*7-12:Ac are powerful attraction inhibitors for *D. pini*, the specialist cells responding to these compounds apparently representing "inhibitory cells", as found in other male Lepidoptera [11, 15–17]. These receptor cells may assist in maintaining reproductive isolation of *D. pini* from other

Table I. Captures of *Dendrolimus pini* males in tetratraps baited with 100 µg *Z*5,*E*7-12:Ald and a varying amount of *E*5,*Z*7-12:Ald or *Z*5,*E*7-12:Ac. Seewiesen, July 8 to 29, 1981; four replications per treatment.

Amount [µg] of added		Total catch
*E*5,*Z*7-12:Ald	*Z*5,*E*7-12:Ac	
0	0	23
0.3	0	5
1, 3, 10, 30	0	0
0	0.1	12
0	0.3	1
0	1, 3, 10, 30	0

Dendrolimus spp. in areas of sympatry at its eastern range of distribution. Of 40 recognized species of the genus found in the Peoples Republic of China [18], one species, *D. punctatus*, is known to use *Z*5,*E*7-12:Ac as a primary sex pheromone component [7, 8]. Comparative studies of the pheromone receptor systems of eastern Palearctic *Dendrolimus* spp. have shown a great diversity of cell type combinations; the pheromone receptor system of *D. punctatus*, for example, is composed of specialist cells for *Z*5,*E*7-12:OH, *E*5,*Z*7-12:OH, *Z*5,*E*7-12:Ac, *E*5,*Z*7-12:Ac and *Z*5,*E*7-12:propionate [19], thus having only two cell types in common with *D. pini*. These receptor data provide additional evidence of a crucial role of sex pheromones in reproductive isolation among the pine moths.

Several tests were set in the field to determine the effect of lure dose on the capture of *D. pini* males over an entire flight period. Purity of the *Z*5,*E*7-12:Ald was better than 99% when placed in the field. The results from the test at Seewiesen in 1982 are presented in Table II. Traps were set out in six replicated series on June 25, when monitor traps indicated flight begin, and lure sources were left unchanged until mid August. The 1 µg and 10 µg lures gave low captures over the first 3 weeks of exposure and then declined to zero. The traps baited with the 1000 µg lure were the most effective, capturing 74 moths (56.6% of the total) over the entire period (Table II). Traps freshly baited with 1000 µg *Z*5,*E*7-12:Ald set in the field in early August did not reveal markedly increased moth captures over traps previously set out. These results show that traps baited initially with 1000 µg of *Z*5,*E*7-12:Ald will effectively monitor *D. pini* populations over a 6 week flight period without rebaiting.

The release rate of *Z*5,*E*7-12:Ald from rubber septa is known to decrease exponentially with time [20]. The rate of isomerization of *Z*5,*E*7-12:Ald over time is as yet unknown but the present data suggest that isomerization was lower than found, for example, with 8,10-dodecadienals [21]. As indicated by the data presented in Table I, the presence of 1% of *EZ* isomer should have greatly reduced trap captures of *D. pini* males.

In 1982 we started a survey to establish relationships between pheromone trap captures and data obtained from conventional methods for sampling population densities of *D. pini*. Potential *D. pini* outbreak areas in different parts of Germany, Austria and Switzerland were included in this survey. The last outbreaks in these areas data back 20 years or more [22–26] and populations are presently at a low endemic stage, with the numbers of pine moth larvae found during the yearly soil sampling not exceeding 0.01 per m^2. The main trap type used was a vane trap with plexiglas wings and with a 1 litre collection receptable containing conservation liquid [27]. A set of 15 vane traps and 3 tetratraps, each baited with 1000 µg *Z*5,*E*7-12:Ald, were routinely used. The traps were set out in early July, when the flight season usually begins in Central Europe [25], and were inspected weekly.

At all test sites the captures were low, as expected. A typical result from the Breisach area is presented in Table III. Over the 6 week period the 18 traps caught a total of 194 *D. pini* males. In this experiment, the beginning of the flight season was apparently missed, but the mid-July peak was covered by the test. The capture range among traps

Table II. Weekly captures of *Dendrolimus pini* males in tetratraps baited with four different amounts of *Z*5,*E*7-12:Ald. Seewiesen, 1982; trap exposure June 25; six replications per treatment.

Dose [µg]	June	July				August			Total catch
	29	6	13	20	27	3	10	17	
1	0	2	3	0	0	0	0	0	5
10	2	6	9	1	0	0	0	0	18
100	2	6	12	10	3	0	1	0	34
1000	1	11	25	19	13	2	3	0	74
	3.8	19.1	37.4	22.9	12.2	1.5	3.1	0%	

Table III. Weekly captures of *Dendrolimus pini* males in 18 traps baited with 1000 µg *Z*5,*E*7-12:Ald. Breisach, 1983; trap exposure, July 4.

Trap type	*n*	July			August			Total catch
		12	19	26	2	9	16	
Vane trap	*15*	30	67	45	14	2	2	160
Tetra trap	*3*	8	9	10	4	0	3	34
		19.6	39.2	28.3	9.3	1.0	2.6%	

was from 2 to 20 moths; capture means were 10.7 moths for the vane trap and 11.3 for the tetra-trap. This value is already beyond the "saturation" level of the tetratrap (sticky surface 145 cm^2) for these large moths, whereas the vane traps used have the capacity to hold more than 500 *D. pini* males. Not a single pine moth larva was found in the Breisach area (and other areas in southwestern Germany as well) by the routine soil sampling from 1982 to 1984.

The critical population level of *D. pini* in Central Europe has been indicated to be 10 larvae per m^2 [24, 25]. In areas with low annual precipitation these numbers may be reached from the endemic stage in 3 to 4 years [1, 25]. "Warning traps" used to survey European forest Lepidoptera populations are designed to indicate population increases at an early (progradation) stage [1, 2]. It remains for further field studies to determine "warning threshold" values for pheromone trap captures of the European pine moth, by correlating local trap captures with local population estimates obtained by conventional sampling methods.

[1] H. Bogenschütz, Proc. 2nd IUFRO Conf. on Dispersal of Forest Insects (A. A. Berryman and L. Safranyik, eds.), p. 51, Sandpoint 1979.
[2] H. Bogenschütz, Z. Pflanzenkrankh. Pflanzenschutz **89,** 585 (1982).
[3] M. D. Chisholm, E. W. Underhill, W. Steck, K. N. Slessor, and G. G. Grant, Environ. Entomol. **9,** 278 (1980).
[4] E. W. Underhill, M. D. Chisholm, and W. Steck, Can. Ent. **112,** 629 (1980).
[5] M. H. Vu, T. Ando, N. Takahashi, S. Tatsuki, A. Yamane, T. Ikeda, and S. Yamazaki, Agric. Biol. Chem. **44,** 231 (1980).
[6] T. Ando, M. H. Vu, S. Yoshida, N. Takahashi, S. Tatsuki, K. Katagiri, A. Yamane, T. Ikeda, and S. Yamazaki, Agric. Biol. Chem. **46,** 709 (1982).
[7] T.-M. Chen, Orally presented in the 15th Int. Congr. of Entomology held at Kyoto, Japan, August 1980.
[8] X.-Z. Meng, Kexue Tong. **28,** 141 (1983).
[9] M. D. Chisholm, W. F. Steck, B. K. Bailey, and E. W. Underhill, J. Chem. Ecol. **7,** 159 (1981).
[10] P. Palaniswamy, M. D. Chisholm, E. W. Underhill, D. W. Reed, and S. J. Peesker, J. Econ. Entomol. **76,** 1159 (1983).
[11] E. Priesner, Z. Naturforsch. **38c,** 874 (1983), and references therein.
[12] H. J. Bestmann, K. H. Koschatzky, H. Platz, J. Süss, O. Vostrowsky, W. Knauf, G. Burghardt, and I. Schneider, Liebigs Ann. Chem. 1982, 1359.
[13] H. Arn, S. Rauscher, and A. Schmid, Mitt. schweiz. entomol. Ges. **52,** 49 (1979).
[14] The chemicals were applied to red serum bottle caps (∅ 18 mm) *via* dilutions in *n*-hexane and approx. 10 mg of 2,6-ditert-butyl-4-methylphenol was added as antioxidant.
[15] E. Priesner, in: Insect Neurobiology and Insecticide Action (Neurotox 79), p. 359, Soc. Chem. Ind., London 1980.
[16] E. Priesner, Ann. Zool. Ecol. anim. **11,** 533 (1979).
[17] E. Priesner, Z. Naturforsch. **39c,** 849 (1984).
[18] P.-H. Tsai, T.-C. Hou, and F.-S. Hwang, Acta entomol. Sinica **22,** 45 (1979).
[19] E. Priesner, T.-M. Chen, and M. D. Chisholm, unpublished results.
[20] Weathering of rubber septa loaded with *Z*5,*E*7-12:Ald in a fumehood at 21 °C has shown an exponential decrease in the amount of aldehyde remaining on the septa, by one-half every six days (M. D. Chisholm and D. W. Reed, unpublished results). According to these data a 1000 µg cap should have fallen to approx. 30 µg after 1 month, thus still retaining considerable attractivity to *D. pini* males.
[21] Under field conditions *Z*8,*E*10-dodecadienal in rubber septa isomerizes to other geometrical isomers; after 7 days 1.1% and after 14 days 5.4% of *E*8,*Z*10-dodecadienal was produced (M. D. Chisholm and D. W. Reed, unpublished results).
[22] E. Jahn, Z. ang. Ent. **54,** 108 (1964).
[23] P. Benedek, Acta Phytopath. Acad. Sci. Hung. **4,** 305 (1969).
[24] R. Gauss, Z. ang. Ent. **77,** 429 (1975).
[25] G. Wellenstein, in: Die Forstschädlinge Europas (W. Schwenke, ed.), p. 435, Verlag P. Parey, Hamburg 1978.
[26] W. Altenkirch, Allg. Forstz. **33,** 367 (1978).
[27] R. Albert, Mitt. Forstl. Versuchs- u. Forschungsanstalt Baden-Württemberg **108,** 157 (1984).