

A Sex Attractant for the Siberian Moth *Dendrolimus superans sibiricus* (Lepidoptera: Lasiocampidae)¹

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Abstract Field trapping experiments were conducted against the Siberian moth, *Dendrolimus superans sibiricus* Butler, in Siberia, Russia, using traps baited with a virgin female moth or 100 µg synthetic mixtures of C₁₂ straight chain-length aldehydes, alcohols, and acetates alone or in combinations on rubber septa. Traps baited with a 1:1 blend of aldehydes and alcohols captured many males. The capture rate was similar to the rate of capture seen in traps baited with virgin females. The mixture of aldehydes and alcohols (64% *Z,E*-5,7-dodecadienal, 10% *Z*-5-dodecenal, 18% *E*-7-dodecenal, 8% *E*-6-dodecenal) and (64% *Z,E*-5,7-dodecadien-1-ol, 10% *Z*-5-dodecen-1-ol, 18% *E*-7-dodecen-1-ol, 8% *E*-6-dodecen-1-ol) can be used as a sex attractant to monitor endemic Siberian moth populations in Asia, and for surveillance and detection of the moth in countries where the insect might be accidentally introduced.

Key Words *Z,E*-5,7-dodecadienal, *Z,E*-5,7-dodecadien-1-ol, *Z*-5-dodecenal, *E*-7-dodecenal, *Z*-5-dodecen-1-ol, *E*-7-dodecen-1-ol, *E*-6-dodecen-1-ol

The Siberian moth, *Dendrolimus superans sibiricus* Butler, is the most destructive defoliator of conifer forests in northern Asia. Recent outbreaks of the moth caused defoliation of more than 1 million ha of fir, *Abies sibirica* Ledeb., and larch, *Larix sibirica* Ledeb., forests in the territory of Krasnoyarsk Krai in Siberia, Russian Federation. Populations of this species do not yet occur in the North America, but the moth is a potential threat to forests here (Baranchikov et al. 1997).

Conifer forests are important sources for timber products, and conifer ecosystems are important to wildlife, environmental quality and human recreation. Previous experience with insects such as the gypsy moth, *Lymantria dispar* (L.), demonstrates the serious adverse impact an invasive species can cause. Should the Siberian moth become established in North America, it could cause profound damage by direct impact on forests and products, and economic damage from restrictive internal and external quarantines on trade (USDA 1991).

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The capability to monitor potentially-damaging exotic insect populations is critical to preventing the establishment of invasive insects at possible ports of entry in countries where it might be accidentally introduced. Although some studies have been conducted on male Siberian moth sexual behavior toward females (Rozhkov 1963, Kupressova et al. 1982), the sex pheromone of the species is unknown. Therefore, pheromone-based trap monitoring techniques for this pest have not yet been implemented anywhere. We report the discovery of a male attractant for the Siberian moth that could be used for insect monitoring in Russia and provide a first line of defense for protection of U. S. forests against this exotic pest. Our report is an extension of an ongoing international study of the sex pheromone of the Siberian moth.

In 1997, combined and individual heptane extracts of the ovipositors of virgin Siberian moths were prepared and analyzed by capillary gas chromatography using procedures described by Klun et al. (1980). These analyses indicated that the extracts contained trace amounts of 12-carbon chain length pheromone-like compounds. However, the quantity of compounds in the extracts was insufficient to allow any conclusive chemical identifications. Inspection of published literature (Arn et al. 1986) on pheromonal components previously identified in four other species in the genus *Dendrolimus* showed a common chemical theme in the genus involving 12-carbon chain length aldehyde, alcohol, or esters having *Z,E*-5,7 diene unsaturation in the carbon chain. Based upon this information, we surmised that the female Siberian moth might also use one or more of these dienes as pheromone, and a plan was implemented to synthesize *Z,E*-5,7-dodecadialenal, *Z,E*-5,7-dodecadien-1-ol and *Z,E*-5,7-dodecadienyl acetate, and to field test the response of male Siberian moths to insect traps baited with these compounds.

Materials and Methods

General. A Hewlett-Packard 6890 chromatograph fitted with a 60-m $\times$ 0.25-mm DB-WAX (J & W Scientific, Folsom, CA) and a Hewlett-Packard 5890 chromatograph fitted with a 60-m $\times$ 0.25-mm DB-1 (J & W Scientific) were used to analyze end products of organic syntheses. The monoene and dienyl acetate-ester synthetic products were isolated using preparative gas chromatography. The preparative system consisted of a Hewlett-Packard 6890 chromatograph fitted with a 30-m $\times$ 0.53-mm DB-1 megabore capillary column (J & W Scientific), HP 7673 autoinjector, and Gerstel PFC preparative trapping device (Gerstel Inc., Baltimore, MD). All samples were introduced using a splitless injection mode. The monoene acetates, isolated by preparative chromatography, were ozonized (1 min, heptane, -70°C , 1 ml O_3/min followed by treatment with excess dimethylsulfide). Mass spectra were obtained using a Hewlett-Packard 6890 gas chromatography-Hewlett-Packard mass selective detector (GC-MS). Nuclear magnetic resonance (NMR) spectroscopy was performed on a GE QE-300 NMR Spectrometer equipped with a Tecmag data system, (^1H at 300MHz and ^{13}C at 75MHz). The samples were dissolved in CDCl_3 with tetramethylsilane as an internal standard. The NMR results are reported in δ values. Infrared spectroscopy was performed using a Perkin-Elmer Model 1600 fourier transform infrared spectrometer.

Synthesis. Fig. 1 shows the synthetic route we used to prepare compounds. Starting materials were obtained from commercial sources and used without further purification, unless otherwise specified. Dry solvents, ether, Tetrahydrofuran (THF) were freshly distilled from Na/ketyl. All reactions were performed in oven-dried glass-

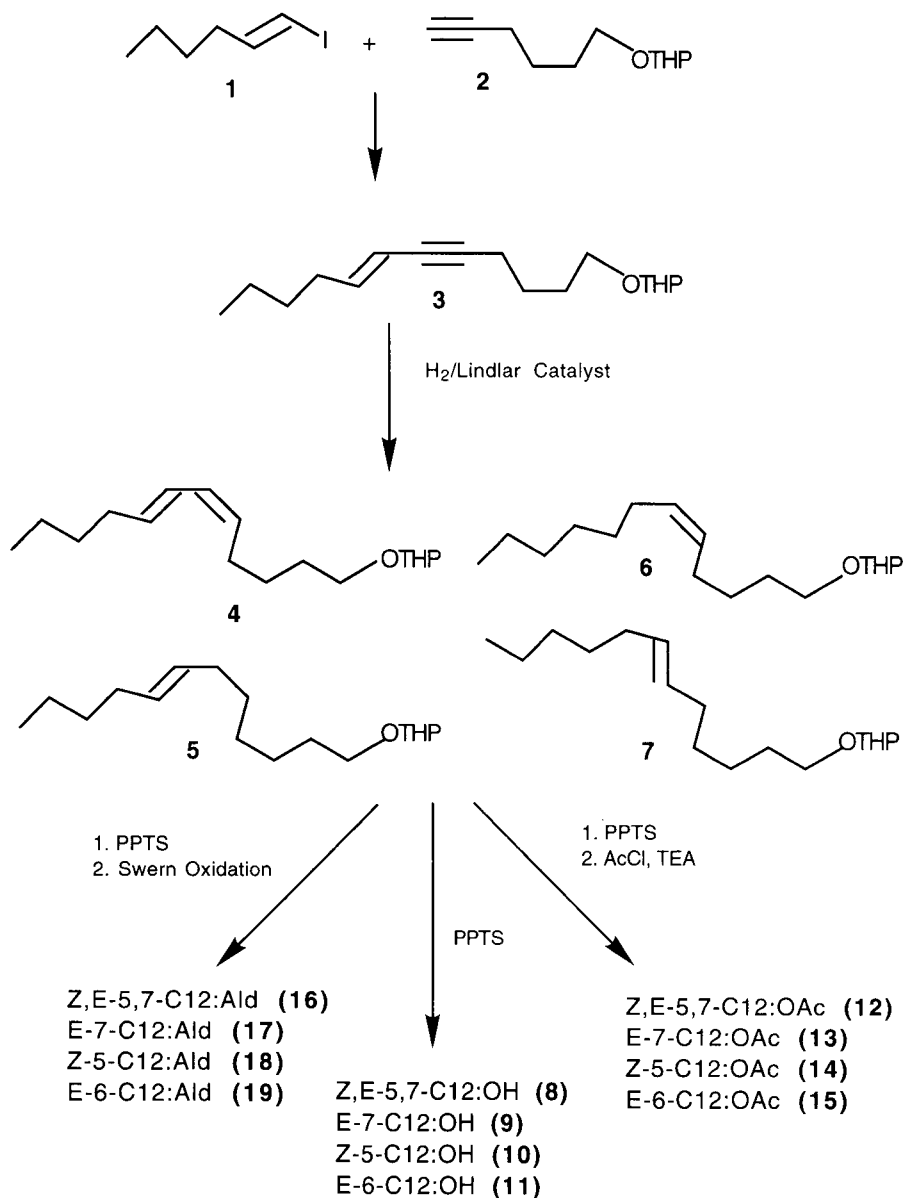


Fig. 1. Scheme for synthesis of sex attractant for the Siberian moth. Abbreviations: PPTS = pyridinium p-toluene sulfonic acid, AcCl = acetyl chloride, TEA = triethylamine. Swern Oxidation and H_2 /Lindlar Catalyst are commonly known organic chemical synthetic methods. Z,E-5,7-C12:, E-7-C12:, E-6-C12:, and Z-5-C12 refer to the geometry and site of unsaturation in twelve carbon chain length. Ald refers to aldehyde, Oac to acetate, and OH to alcohol. Bold numbers refer to compounds as they are mentioned in the text.

ware and under nitrogen atmosphere. Flash chromatography was done on silica gel (60 Å, 230-400 mesh).

(E)-1-Iodo-1-hexene (1) was synthesized in 73% yield, from 1-hexyne according to the procedure published by Stille and Simpson (1987). The spectral data of compound 1, IR, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ matched reported data. $^1\text{H-NMR}$: δ 6.49 (dt, 1H, $J = 7.3$ Hz, 14.65 Hz); 5.97 (d, 1H, $J = 14.65$ Hz), 2.06-1.98 (m, 2H), 1.25-1.38 (m, 4), 0.87 (t, 3H, $J = 7.2$ Hz). $^{13}\text{C-NMR}$: δ 146.62, 74.19, 35.66, 30.43, 21.91, 13.74. IR (neat film): 3074.8, 2957.4, 2927.3, 2856.9, 1604.9, 1463.7, 1218.2, 1179.2, 948.8, 921.0 cm^{-1} .

Tetrahydro-2-(5-hexynyloxy)-2H-pyran (2) was synthesized by mixing 10.0 g 5-Hexyn-1-ol (102 mmol) with 15.0 g dihydropyran (178mmol) at room temperature under nitrogen atmosphere. Ten drops of dichloroacetic acid were then added. The mixture was stirred for 12 h and neutralized by addition of 4 g of NaHCO_3 with stirring for 15 min. Filtration was done with the aid of dry ether. The solvent was removed, under vacuum, then flash chromatographed over 200 g silica gel using ether: pet. ether 5:95 V:V provided 18.0 g of a clear liquid (96.9 % yield). $^1\text{H-NMR}$: δ 4.58 (t, 1H, $J = 3.65$ Hz), 3.83 (m, 2H), 3.48 (m, 2H), 2.34 (t, 2H, $J = 7.2$ Hz), 1.95 (t, 1H, $J = 3.5$ Hz), 1.90- 1.54 (m, 10H). $^{13}\text{C-NMR}$: δ 98.86, 84.43, 68.41, 66.96, 62.34, 30.82, 28.88, 25.59, 25.43, 19.68, 18.32. IR (neat): 3306.4, 2939.5, 2116.6, 1649.4, 1453.5, 1351.9, 1322.8, 1260.1, 1200.1, 1119.2, 1075.0, 1034.1, 904.1, 868.8, 814.0 cm^{-1} .

(E)-Tetrahydro-2-(7-dodecen-5-ynyl)oxy-2H-pyran (3) was obtained by coupling of (E)-1-Iodo-1-hexene (1) to 5-hexyn-1-ol-THP (2) according to the procedure reported by Rossi and Carpita (1983). (E)-1-Iodo-1-hexene (5.0 g, 23.5 mmol) and 5-hexyn-1-ol-THP (2) (4.5 g, 24.7 mmol) was added to a mixture of benzyltriethylammonium chloride (0.23 g, 1.24 mmol), tetrakis(triphenylphosphine) palladium, $(\text{Ph}_3\text{P})_4\text{Pd}$, (1.06 g, 0.92 mmol) and CuI (0.56 g, 2.94 mmol) in benzene at room temperature under nitrogen atmosphere. Then 10% aq. NaOH was added. The mixture was stirred at room temperature for 12 h. The reaction was treated with water and excess saturated NH_4Cl water solution; the resulting mixture was extracted with hexane. The hexane extracts were washed with the NH_4Cl solution to neutrality, dried (Na_2SO_4), filtered, stirred with activated charcoal and filtered. The solvent was removed under vacuum. Flash chromatography over silica gel, hexane:ether 7:3 provided a clear liquid 3.84 g (61.2%) of (E)-tetrahydro-2-(7-dodecene-5-ynyl)oxy-2H-pyran (3). $^1\text{H-NMR}$: δ 6.05 (dt, 1H, $J = 6.5$, 16.5 Hz), 5.44 (d, 1H, $J = 16.5$), 4.58 (t, 1H, $J = 4$ Hz), 3.86-3.72 (m, 2H), 3.57-3.38 (m, 2H), 2.32 (t, 2H, $J = 5.0$ Hz), 2.102 (q, 2H, $J = 6.5$ Hz), 1.23-1.92 (m, 14 H), 0.89 (t, 3H, $J = 7.3$ Hz). $^{13}\text{C-NMR}$: δ 144.33, 109.91, 98.83, 88.27, 79.52, 67.05, 62.27, 32.66, 31.05, 30.82, 29.04, 25.75, 25.59, 22.20, 19.68, 19.26, 13.87. IR (neat): 3018.4, 2928.4, 2870.4, 2216.3, 1454.1, 1351.9, 1260.4, 1137.0, 1128.5, 1054.5, 956.2, 905.6, 869.1, 815.0 cm^{-1} .

Lindlar reduction. The reduction procedure of Oliver et al. (1994) was followed. (E)-Tetrahydro-2-(7-dodecene-5-ynyl)oxy-2H-pyran (3) (3.5 g, 13.1 mmol) was dissolved in freshly distilled cyclohexene. Lindlar catalyst (0.5 g 5% Pd on CaCO_3 -Pb poisoned; Strem Chemicals Inc., Newburyport, MA) was added, then the mixture was put on a hydrogenator under hydrogen at 1 atmosphere. The mixture was shaken for 2 h. Filtration and removal of the solvent provided a clear colorless liquid that was flash chromatographed over silica gel, using 7:3 hexane:ether, to give 3.4 g liquid. The sample was treated with pyridinium-para-toluenesulfonate (PPTS) to hydrolze the tetrahydropyranyl ether. Aliquots of the resulting alcohol were converted to acetate ester or oxidized to aldehyde using established methods.

GC and GC-MS analyses of the alcohols (8, 9, 10, 11), acetates (12, 13, 14, 15), and aldehydes (16, 17, 18, 19) derived from the Lindlar reduction showed that the reaction had proceeded in a manner that yielded a mixture of diene (4) and three monoenes (5, 6, 7) (Fig. 1). The acetates generated in the synthesis were used to document the geometry, positions of unsaturation, and percentage composition of the reduction products.

GC-MS analysis showed the major constituent had a mass spectrum that was consistent with *Z,E*-5,7-dodecadien-1-ol acetate (12) as compared to an archived spectrum; m/z (abundance); 224(M^+ , 10), 164($M^+ - CH_3$, COOH, 14.2), 136(23.5), 121(25.8), 107(22.2), 93(47.4), 79(base), 67(49.9), 55(18.8), 43(66.6), 41. GC analyses on polar and non polar capillary columns showed that the retention time of the major compound was coincident with reference *Z, E*-5,7-dodecadien-1-ol acetate from the Insect Chemical Ecology Laboratory's chemical library. The analyses also showed that the diene comprised 64% of the Lindlar reduction products. The sequence of elution for the four isomers of the conjugated dienyl acetate was *ZE*, *EZ*, *ZZ* and *EE*, and analyses of our product showed that the proportion of isomers was 93:1:1:4.

E-7-dodecen-1-ol acetate (13). GC-MS showed that one of the minor products (18%) of the Lindlar reduction had a mass spectrum that was consistent with an archived spectrum of a mono unsaturated 12-carbon chain length acetate. m/z 166($M^+ - CH_3COOH$, 23.7), 138(6.4), 123(10.6), 109(31.4), 95(59.3), 81(82.1), 67(96.1), 55(69.8), 54(45.0) 43(100). The ratio of relative abundances of m/z 55 to m/z 54 for the compound (1.55) was identical to that of authentic *E*-7-dodecen-1-ol acetate. Leonhardt et al. (1982) has shown that the m/z 55 to m/z 54 ratio is indicative of position of unsaturation in mono-olefinic long chain acetates, and this result confirmed that the site of unsaturation in the compound was at carbon seven. GC analyses showed that the compound's retention times on two liquid phases was coincident with *E*-7-dodecen-1-ol acetate.

Z-5-dodecen-1-ol acetate (14). GC-MS showed that a second minor product (10%) of the Lindlar reduction had a mass spectrum that was the same as an archived spectrum for a dodecen-1-ol acetate. m/z 166(19.8), 123(8.9), 138(19.1), 110(28.3), 109(24.3), 95(56.1), 82(68.0), 81(62.2), 67(78.2), 55(45.5), 54(51.1), 43(100). The m/z 55 to m/z 54 ratio (0.890) for the compound was the same as the ratio found in authentic *Z*-5-dodecen-1-ol acetate, and the compound had retention times on polar and non-polar capillary GC's coincident with the *cis* isomer.

E-6-dodecen-1-ol acetate (15). A third monounsaturated acetate appeared as a chromatographic shoulder on 14. Its mass spectrum was the same as 14. Ozonolysis of preparative-collected monoene acetates yielded three acetoxy aldehydes whose retention times were coincident with 5-acetoxypentanal, 6-acetoxylhexanal, and 7-acetoxylheptanal standards on polar and nonpolar capillary columns. These results fixed the double bond in 15 at carbon six, and confirmed the double bond positions for 13 and 14. Retention characteristics of 15 and 11 on the capillary columns was consistent with a *trans* configuration.

The evidence proves that 4 (64%), 5 (18%), 6 (10%) and 7 (8%) were the products of the Lindlar reduction, and that the aldehydes and alcohols generated from the reduction products would necessarily have the same isomeric composition as the acetates 12, 13, 14 and 15. The diene and monoene over-reduction products we identified here were also observed by Ando et al. (1982) when they conducted the reduction of 3. Therefore, the Lindlar reduction of 3 characteristically and consistently

yields a predictable mixture of diene and three monoenes. Duplication of the synthetic mixtures prepared and used in this study is therefore possible by simply reducing 3 as we have described.

Preparation of synthetic chemicals for the field. The mixtures of compounds generated in the synthesis were used without attempting resolution and isolation of individually pure stereoisomers because of a time constraint. Flight of the Siberian moth was imminent, and we rationalized that it would be better to test the mixtures of compounds, as such, rather than miss a window of opportunity (once a year in approximately July) to conduct a field test. Therefore, heptane solutions of the aldehyde, alcohol and acetate mixtures were prepared (10 $\mu\text{g}/\mu\text{L}$) and used to treat the interior cup portion of 1 $\times$ 2 cm grey rubber septa (West Co., Kearney, NE) with 100 μg alone or in four combinations; [aldehydes + alcohols + acetates], [aldehydes + acetates], [alcohols + acetates], and [aldehydes + alcohols]. The respective treatments on septa were sealed in screw cap vials and carried to Russia by a special courier for the field test.

The trapping study was conducted over 13 consecutive nights, 16 to 28 July 1998, during the adult flight of the Siberian moth near the village of Ozernyy, Shira Region, Republic of Khakassiya, Siberia, Russia. Traps were standard green USDA milk-carton type gypsy moth traps (Schwalbe and Mastro 1988) that were modified to accommodate the male Siberian moth. Males of this species are considerably larger than the gypsy moth, having a wing span of 4 to 7 cm. Therefore, 7 cm wide and 3 cm high rectangular slots were cut in all traps at the points where two standard apertures for gypsy moth entry existed on each side of the trap. The modified USDA gypsy moth traps were each fitted with a rubber septum impregnated with synthetic compound(s). The septum was suspended on a wire at the center of the trap and within the openings of the trap. Moths entering the traps were killed by an insecticidal (4 $\times$ 1.3 cm Vapona[®]) strip that was placed in the bottom of the trap.

Virgin female baited traps served as control in the test. For traps to be baited with live virgin females moths, the insecticide strip could not be used. Therefore, the modified USDA traps were fitted with an inverted open cone that was positioned below the entrance holes of the trap. The trap was also made deeper by taping a 13 cm high bottom portion, cut from a second trap, to the bottom of the modified trap. This trap design is essentially the same as the Otis cone trap developed by Mastro et al. (1997). Female pupae were collected from the field and isolated in the laboratory under ambient conditions. After emergence, the virgin female adults were placed in a 6 cm wide $\times$ 3 cm high cylindrical screen cage. The cage was positioned just above the open cone within the trap. Males that entered the trap fell through the cone and were retained below.

There were eight treatments in the field test; three septa treated individually with aldehydes (Ald), alcohols (OH), and acetates (OAc) alone, three binary combinations of functional groups, a mixture of all three functional groups, and a virgin female. Eight traps containing these treatments were deployed using six replicates over 13 nights in a complete randomized block design. The line of traps was along a road in a typical Siberian larch forest. Traps were positioned about 1.5 m from the ground on trees with 100 m between traps. Four-day-old females were used on the first night of the test. These females were replaced with 2-d-old females on the second night, and this set of females remained in the traps for the duration of the test. All females were alive at the end of the test, and each female had chaotically laid 10 to 40 sterile eggs in its cage. Fresh septa were placed in these traps after 7 nights.

During 19 to 25 July 1998, a second set of ten traps were deployed. Five were modified USDA traps and five were of the Otis cone trap design in which virgin female moths had been set up. The two designs were randomly positioned in a line 100 m apart and baited with Ald + OH septa. The purpose of this set of traps was to compare the male trapping efficiency of the two trap designs. Moths captured in all traps were removed and counted daily.

Results and Discussion

The numbers of insects captured by each trap were summed to obtained a total number captured by a given trap during the 13-d experiment. The dataset consisted of 48 observations: the total insects captured by each of the six replicate traps per treatment. Preliminary statistical analyses indicated that treatments (Table 1) C, D, E, G, and H all exhibited similar variability among the number of insects captured; but treatments A, B and F exhibited larger variability. The error variance was partitioned into 4 groups associated with the observed error variation to improve the precision of the analysis of variance analysis and subsequent mean comparisons using SAS® Proc Mixed (SAS Institute 1994). Significance of treatment effects was evaluated by $P > F < .05$. The mean responses of Siberian moth males to traps baited with virgin females and synthetic chemicals are shown in Table 1. The results show that a 1:1 mixture of Ald:OH was attractive to the males. The mean male capture/trap/night (3.31) in this treatment was similar to the response of males to the virgin females (3.21). During the 5-night experiment with the modified USDA traps and Otis cone traps baited with Ald + OH, mean male captures, 1.56 ± 0.27 and 1.80 ± 0.28 , in the respective traps were statistically identical ($P > F < 0.1$). Therefore, we conclude that male responses to Ald + OH were equivalent to the response of males to Siberian moth virgin females.

Table 1. Response of *D. superans sibiricus* males to virgin female moths, 100 µg mixtures of Z,E-5,7-dodecadienal + monoene aldehydes (Ald), Z,E-5,7-dodecadien-1-ol + monoene alcohols (OH), and Z,E-5,7-dodecadienyl acetate + monoene acetates (OAc) evaporated alone or in combination in traps. Means followed by the same letter are not significantly different ($P > F < 0.05$).

Treatment	Mean captures/trap/night	Mean Standard Error
A. Virgin female	3.21a	1.08
B. Ald	0.44b	0.13
C. OH	0.00c	0.01
D. Oac	0.00c	0.01
E. Ald + Oac	0.04c	0.01
F. Ald + OH	3.31a	0.54
G. OH + Oac	0.00c	0.01
H. Ald + OH + Oac	0.03c	0.01

When OAc was added to the Ald:OH, captures were significantly reduced compared to Ald:OH without OAc. This shows that the acetates deterred males from entering the traps. By itself, the Ald was moderately attractive while the OH or OAc alone or in combination caused no male captures. Additional study will be necessary to determine exactly which compounds in the ALD:OH synthetic mixture are responsible for the attraction of males.

The overall results support a hypothesis that the pheromone system of the Siberian moth is similar to other species within the genus *Dendrolimus*, and involve 12 carbon chain-length aldehyde and alcohol with 5- and 7-sites of unsaturation. Definition of the actual nature of the species' pheromone will require further study. However, until identification of the pheromone of the Siberian moth is complete, the Ald:OH mixtures on rubber septa deployed in the modified USDA gypsy moth trap can serve as a useful tool for monitoring and detection of populations of this destructive and potentially invasive pest.

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