Evaluation of (*Z*)-5-Decen-1-ol as an Attractant for Male Larch Casebearer Moths, *Coleophora laricella*

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Electroantennogram studies on *C. laricella* males demonstrated that the title compound, (*Z*)-5-decen-1-ol (*Z*5-10:OH), elicited the highest response among a series of straight-chain, C_{10} to C_{16} alcohols, acetates and aldehydes tested. From selective adaptation of antennal responses it was concluded that an additional type of receptor cell responded to the corresponding acetate, *Z*5-10:Ac.

Field attraction experiments were carried out by using delta-type sticky traps and rubber-cap odour dispensers. The main test sites were located in the Swiss Alps, Black Forest and northwestern Germany, and included both European and Japanese larch stands, having different degrees of *C. laricella* infestations. At all test sites pure *Z*5-10:OH proved to be highly attractive to native male moths over a range of different lure doses, with a progressive increase of captures towards the higher (100 µg, 1000 µg) attractant sources. However, following captures of 200–400 moths/trap the "retaining capacity" of the sticky surfaces of the traps were found to decline markedly; in tests of the higher attractant doses at sites of higher moth densities, this "saturation effect" frequently occurred within a few hrs of trap exposure, thus demonstrating the limitation in the use of sticky traps for monitoring economically-relevant *C. laricella* populations. Trap placement studies (using the standard attractant of 100 µg *Z*5-10:OH) showed substantial captures only for those traps placed on green branches within the host crowns.

The acetate analogue (*Z*5-10:Ac) strongly reduced male responses to the attractant alcohol (*Z*5-10:OH) when evaporated from the same trap, admixtures of 0.3% and 1% of this acetate reducing captures by 90% and almost 100% respectively. Possible functions of this inhibitor are briefly considered. No evidence for attractant synergism was obtained during the present study.

The results suggest that *Z*5-10:OH is a major constituent of the yet unidentified *C. laricella* sex pheromone.

The larch casebearer moth, *Coleophora laricella* (Hb.) (Lepidoptera: Coleophoridae), has been a "classical" study subject for European forest entomology (see [1–3]). The moth occurs throughout the growing range of larch in Europe and the western USSR, infesting both European larch (*Larix decidua*) and Japanese larch (*L. leptolepis*) [3]. Browning of needles caused by spring feeding of overwintered larvae is commonly observed in many forest stands in Central Europe but these frequent losses of annual growth have only occasionally prompted insecticide treatments [3–6]. Various degrees of feeding injury on western larch (*L. occidentalis*) have been reported from areas of secondary establishment of this moth in the northwestern US and western Canada [7, 8]. In a field study conducted in 1973 in British Columbia, McMillian and Borden [9] demonstrated strong attraction of males to traps baited with virgin *C. laricella* females, and concluded that "... mass trapping or confusion control programs may be possible if synthetic pheromone becomes available, particularly because of the relatively weak flight capability, which would retain most insects within a target area. Pheromone traps may also be useful tools in detecting spread of the *C. laricella* infestation, and in monitoring the effectiveness of

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bio-control programs ..." [9]. To our knowledge, no further reports have been published concerning the *C. laricella* female pheromone, its chemical nature remaining unknown as in other casebearer (Coleophoridae) moths.

Here we present laboratory and field results that have led to the proposal of (*Z*)-5-decen-1-ol (*Z*5-10:OH) being a candidate structure for the larch casebearer sexual attractant. This chemical, which has not previously been noted as a pheromone or attractant component in other insects, showed outstanding effectiveness in electrophysiological measurements and as a single compound strongly attracted male *C. laricella* moths. Field bioassays, conducted over various environmental conditions and population densities, will be considered in detail, in order to illustrate the potential of synthetic *Z*5-10:OH as a bait in survey traps. Also reported will be field data showing strong "inhibitory"effects on the attraction of male moths to *Z*5-10:OH sources caused by the corresponding acetate, *Z*5-10:Ac.

Table I. Electroantennogram response values of male *Coleophora laricella* to (*Z*)- and (*E*)-alken-1-ols, varying in chain length and double bond position. Values indicate equipotent stimulus amounts in a half-decadic scale (0.01 represents a range from 0.0056 to 0.018 µg; 0.03, from 0.018 to 0.056 µg; etc.).

Chemical	Amount	Chemical	Amount
*Z*3-10:OH	0.1	*E*3-10:OH	0.3
*Z*4-10:OH	0.03	*E*4-10:OH	0.3
*Z*5-10:OH	0.001	*E*5-10:OH	0.03
*Z*6-10:OH	0.03		
*Z*7-10:OH	0.03	*E*7-10:OH	0.1
*Z*8-10:OH	0.1	*E*8-10:OH	0.3
9-10:OH	0.1		
*Z*3-12:OH	0.3	*E*3-12:OH	1
*Z*4-12:OH	0.3	*E*4-12:OH	3
*Z*5-12:OH	0.1	*E*5-12:OH	0.3
*Z*6-12:OH	0.3	*E*6-12:OH	1
*Z*7-12:OH	0.03	*E*7-12:OH	0.1
*Z*8-12:OH	1	*E*8-12:OH	3
*Z*9-12:OH	0.3	*E*9-12:OH	3
*Z*10-12:OH	1	*E*10-12:OH	3
11-12:OH	1		
*Z*5-14:OH	10	*E*5-14:OH	> 10
*Z*7-14:OH	10	*E*7-14:OH	> 10
*Z*9-14:OH	3	*E*9-14:OH	10
*Z*11-14:OH	> 10	*E*11-14:OH	> 10

Antennogram studies

Electroantennogram (EAG) responses were monitored from *C. laricella* male antennae using techniques of recording, stimulation and data evaluation as in previous studies on other small moths [10–15]. Two series of experiments were carried out.

In the first series, synthetic compounds were presented to antennal preparations at a given standard amount of either 0.1 µg or 1 µg (source load). The test chemicals included known lepidopteran pheromones and approx. 200 of their synthetic analogues (varied with respect to chain length, olefinic double bonds, and functional groups). In these tests, primary alcohols of a chain length of 10 to 12 carbons, and some of their acetate and aldehyde analogues, were the only compounds eliciting substantial EAG responses. A comparative analysis showed that *Z*5-10:OH was the most effective stimulant at both levels of test amount.

A consecutive series of EAG measurements was directed at determining the relative stimulatory potencies of selected test chemicals as expressed by "equipotent stimulus amounts". Throughout this series, sources of 0.001 and 0.01 µg of *Z*5-10:OH served as the reference stimuli to which the other chemicals were related according to their equipotent amounts, referred to a half-decade scale (see [10–15]). Results of these measurements on male *C. laricella* are listed for selected primary alcohols having olefinic chains of 10, 12 or 14 carbons (Table I).

As shown by these values, the (*Z*)-5 isomer is 30 to 300 fold more effective than any of the other decenols listed. With the C_{12} chain length the (*Z*)-7 compound elicits the greatest response, followed by the (*Z*)-5 and (*E*)-7 isomers. A strong loss in stimulatory effectiveness occurs with further chain elongation; among the C_{14} alcohols only the (*Z*)-9 isomer at the amount of 3 µg was found to elicit responses equivalent to 0.001 µg of *Z*5-10:OH, with most C_{14} isomers not being effective even at 10 µg (the highest dose used in this study). These data indicate a relationship between chain length of the test molecules and the preferred position of their −C=C− double bond, comparable to structure-activity relationships described previously for alkenyl acetate receptors in some other Lepidoptera (see [11]).

Among the (*E*)-unsaturated alcohols the *E*5-10:OH was found to be the most stimulatory, eliciting equivalent EAG responses at approx. 30 fold the amount as needed for the (*Z*)-5 isomer. Also at the

other double bond positions investigated the (*Z*) isomer was consistently the more stimulatory; this holds for all three carbon chains (C_{10} to C_{14}) although at the longer chains the (*Z*)/(*E*) difference was often not more than half a decadic step in stimulus amount (Table I).

Di-unsaturated alcohols were not tested systematically but those included all showed a decrease in effectiveness when compared to the respective (*Z*) or (*E*) monoene. For example, the four isomeric 5,7-dodecadienols, as well as the four 7,9-dodecadienols, all were less stimulatory than the (*Z*)-7 monoene, *Z*7-12:OH. In summary, no evidence for the existence of an alcohol structure more stimulatory than *Z*5-10:OH could be obtained.

In the series on alkenyl acetates (results not listed here in Tables) the respective tests showed that *Z*5-10:Ac was the most effective structure. In order to obtain the equivalent EAG response amplitude from non-adapted *C. laricella* male antennae, this acetate was required at 10–30 times the amount of the alcohol, *Z*5-10:OH. However, following series of strong (1 µg or 10 µg) stimuli of *Z*5-10:OH, antennal preparations were observed to respond almost equally to both compounds; whereas differences in equipotent amounts of up to 300 fold were occasionally noted following strong stimulation with *Z*5-10:Ac. Selective adaptation of this kind can be considered as indicating perception of the two compounds by separate types of receptor cells (see [12, 16]).

In summarizing the EAG results, the data strongly point to *Z*5-10:OH as a candidate structure for the chemically-unknown sex pheromone produced by the female larch casebearer moth. This chemical has not yet been reported as an insect pheromone but closely-related structures are known from some moth species (see discussion, p. 963). The acetate, *Z*5-10:Ac, also deserved attention in the field work because of its outstanding EAG effectiveness in the ester series.

Field study procedures and test sites

Materials, techniques, and general operating procedures

All statements as to the behavioural effectiveness of synthetic *Z*5-10:OH and related compounds on male *C. laricella* will be based upon moth captures in sticky traps. Visual observations on the moths' flight and landing behaviours were also made but will be reported elsewhere [17].

Brown tetratraps with flaps [18], having a sticky surface of 96 × 166 mm, were used throughout all phases of this study. Their retaining capacity for *C. laricella* is in the order of 400 moths/trap, as will be pointed out in the results section. This same trap type has already been successfully used with several other forest Lepidoptera, including the pine beauty moth *Panolis flammea* [19–21], the European fir budworm *Choristoneura murinana* [15, 20–22], and the pine moth *Dendrolimus pini* [23]. The test compounds, all ≧ 99% pure, were applied as hexane solutions to the cavity of serum bottle caps, placing blends of two or more chemicals on a single cap. The caps were fixed to the inner roof of the trap at approx. 4 cm above the sticky bottom. Traps were hung from larch branches at eye level if not specified otherwise.

During the peak flight period countings were made several times per day, but the usual control intervals were 1 to 4 days. Lower catches were mostly counted in the field with the trapped insects being removed there, whereas with higher catches the sticky boards were frequently exchanged and the countings were then made in the laboratory. The experiments lasted between 10 to 20 days. Over the entire period, traps remained in the same position and caps were left unchanged.

The tests were mostly conducted as series comprising 4 to 6 traps, each loaded with a different chemical formulation. Blank traps (without chemical) were not systematically used in 1980 but were included in all test series in 1981. A given series was replicated 6 or 12 times per test site.

In the experiments at Tinizong, 1980, the 4 traps of the series were placed on the same tree. At all other locations the traps were on separate trees, spaced 5 to 15 m (see further below). Traps were not rotated during experiments but their relative positions were systematically altered between replications, thus accounting for possible interactions between neighbouring traps. Distances between replications of a test series were approx. 20 m for site B at Zuoz, and 50 m or more with all other experiments.

For the same synthetic formulation and experimental site the captures were found to vary considerably with local moth densities, moth flight

periods, and the retaining capacities of the sticky traps. In order to discuss these various effects, for most experimental series the full capture figures will be presented.

Test areas

The field experiments were started in 1980 at *Tinizong* (Kt. Graubünden, 25 km west of Zuoz), Switzerland. The scattered stands of larch (*L. decidua*) in this area, at an altitude of 1300 m above sea level, are suffering from permanent, heavy infestations of *C. laricella* as apparent from the yearly decoloration of needles in spring. Single large trees (approx. 200 years old and 20 m in height) at the edges of the stands were chosen for the experiments. Based on counts of overwintering larvae, the *C. laricella* populations on these trees had been calculated as ranging between 200000 to 500000 moths/tree [24]. In these tests the 4 traps of the series were all placed on lower, green branches of the same tree. Due to the extreme weather conditions met in 1980, the moth flight in this mountain area occurred unusually late, from mid to end of July.

In 1981, the experiments in the Engadin valley were continued on two sites (A, B) near *Zuoz*, at 1800 m a.s., both with very low moth densities (not calculated for absolute numbers). On site A, the traps of the "dosage series" (see p. 958) were placed in a 200 year old larch forest using sporadic lower branches of the old trees of scattered young larch generation. Site B was a younger larch stand of 10 m in height, with a dense crown canopy; this site was used for placing the "inhibitor series" (p. 961). Trap distances within replicates were 10–15 m for site A and 5–10 m for site B. Both tests lasted from late June to mid of July, 1981.

The greater part of the results reported here were obtained in 1981 in a 600 hectare research area at *Lingen*, 50 km north of Münster, northwestern Germany. The area includes 125 hectare of Japanese larch (25 years old), stocked as fire protection belts (30–50 m in width) surrounding Scotch pine plantations. Since 1971, the population dynamics of *C. laricella* in these belts has been studied extensively, with particular emphasis on local differences in tree disposition [6]. Over this 10 year survey some plots had shown higher moth infestations for some years whereas in other parts of the area the moth populations remained constantly low; these differences in population densities were in an order of tenfold and appeared to correlate with local ground water conditions [6]. For the trapping experiments this pattern permitted to chose, to equal parts, plots of expectedly higher and lower moth densities. Moth flight in this area (as determined by monitor traps) started between May 23 and 27; peak flight occurred from June 1 to 4, and tests went on until June 9, 1981.

Also in 1981, a monitoring program using the "standard attractant" of 100 µg *Z*5-10:OH (determined in the field tests of 1980) was started in several European countries. Results obtained at a Black Forest locality will be included here (p. 962) in order to demonstrate effects of trap placement on moth captures.

(*Z*)-5-Decen-1-ol as an attractant

Tests at Tinizong, 1980

Attractancy tests with pure *Z*5-10:OH, the chemical eliciting the highest EAG response on *C. laricella* male antennae, were started in 1980 in eastern Switzerland at Tinizong. On July 15 (exposure of traps) no moths were observed in the field whereas on July 21 some traps showed high captures; in the laboratory, pupae (collected on July 15) began to release male moths on July 18, suggesting flight begin at this date.

The tests were conducted as series consisting of 4 traps, baited with different amounts of *Z*5-10:OH (99% *Z*) and placed on lower, green branches of the same tree. Replicates were run simultaneously, taking counts on July 21, 24, 29 and 31, and thereby replacing the sticky boards in the traps.

During the 2 weeks exposure the 24 traps caught approx. 20000 *C. laricella* moths. The captures are specified (Table II) to show differences between replications, test doses, and flight periods.

Considering different replicates, the capture sums over the 4 traps were highest for test tree no. 1 (total, 4532 moths) and the lower for no. 2, 3 and 4 (2501 to 2643 moths). These capture differences held for all 4 periods of the experiment (see Table II), suggesting differences in the local moth populations (see discussion, p. 964).

All 4 test doses, from 1 µg to 1000 µg, revealed high captures: over the 2-weeks exposure the 6 traps

Table II. Captures of *C. laricella* males in tetratraps baited with four different doses of *Z*5-10:OH. Tinizong, July 15 to 31, 1980; six replicates.

Amount of *Z*5-10:OH [µg/trap]	Replicate No. 1	2	3	4	5	6	Σ	[%]	Total	[%]
Period I (July 15–21)										
1	209	59	11	20	85	9	393	(10.4)		
10	357	188	7	20	200	107	879	(23.2)		
100	278	234	192	176	247	221	1348	(35.5)		
1000	265	109	137	157	237	270	1175	(30.9)		
Σ	1109	590	347	373	769	607	3795			
Period II (July 22–24)										
1	125	84	68	87	221	97	682	(15.7)		
10	198	193	66	155	198	142	952	(21.9)		
100	328	230	162	239	221	209	1389	(31.9)		
1000	201	193	228	230	216	256	1324	(30.5)		
Σ	852	700	524	711	856	704	4347			
Period III (July 25–29)										
1	354	141	168	142	417	386	1608	(17.2)		
10	576	396	319	271	379	596	2537	(27.2)		
100	712	371	422	426	473	504	2908	(31.2)		
1000	254	200	530	410	370	513	2277	(24.4)		
Σ	1896	1108	1439	1249	1639	1999	9330			
Period IV (July 30–31)										
1	39	5	4	2	14	8	72	(2.8)	2755	(13.8)
10	172	24	7	14	31	77	325	(12.8)	4693	(23.4)
100	264	50	207	130	199	91	941	(37.0)	6586	(32.9)
1000	200	24	115	126	387	356	1208	(47.4)	5984	(29.9)
Σ	675	103	333	272	631	532	2546			

baited with the 1 µg dose caught a total of 2755 moths; and the traps with the higher doses, 4693, 6586 and 5984 moths, respectively (Table II).

Considering, for the same test period, traps belonging to the same replicate (*i.e.*, positioned on the same tree), the catch by the 100 µg trap was higher than the respective 1 µg catch in 22 (of the 24) readings, and above the 10 µg catch in 21 readings. The catch by the 10 µg trap exceeded the 1 µg catch in 19 readings although in some replicates (during period I and IV) the captures by these two doses are apparently to low to warrant closer evaluation. The captures obtained by the 100 µg and the 1000 µg trap were not significantly different from each other over any test period.

However, as established by experiments conducted in 1981 (see p. 959), with these higher attractant doses the evaluation of captures can be strongly affected due to the limited "retaining capacity" of the traps. With tetratraps, such as used here, the holding capacity for *C. laricella* declines markedly after capturing 200–300 moths [17], catch figures of 400 usually indicating "trap filling" (only few traps containing 500 moths or more). As will be reported further below, at peak flight the "trap filling" effect may be reached within less than 24 h. Accordingly, with test intervals extending over several days, such an effect occurring at initiation of the control period could drastically falsify the results, by showing the continuous captures only for the less effective (not-saturated) traps. Such an effect could have happened in particular during period III of the present experiment:

Over this period, July 25 to 29, the 24 traps caught between 141 to 712 moths/trap (Table II); for 7 of these the catch was between 300–400, and

for 11 traps, > 400. Captures of > 400 moths/trap were never observed during the other periods thus indicating peak flight occurring on all 6 trees during period III. On July 30, sticky boards were replaced in all traps and the tests carried on for another 24 h. In this period IV, only 2 traps (both baited with the 100 µg dose) revealed captures of > 300 moths, most other traps being at a stage of still unlimited holding capacity (Table II). Differing from period III, in this period IV only few moths (2.8% of the total) were observed in the 1 µg traps, and the highest doses were by far the most effective. We therefore conclude that in period III the captures had gone through a similar stage (characterized by a 100 µg/1 µg capture ratio of > 10/1) followed by rapid "saturation" of the traps with the higher attractant doses (see also discussion, p. 963).

Some further variables possibly modifying the trapping results (such as the aging of the attractant sources over test periods, the variations among local moth populations, and the competitive effects between neighbouring traps) will be considered later, in retrospect to the different series of experiments conducted in the two-years study.

Prompted by the captures obtained with the lowest dose of 1 µg, blank traps were set out on July 25. Until July 31 (end of experiment) 3 of these controls caught respectively 1, 3 and 10 moths. The 1 µg traps on the same trees contained between 144 to 393 moths during the same time, showing that this lowest lure dose had retained considerable attractivity even after 2 weeks of exposure.

Tests at Zuoz, 1981

In 1981, attractancy studies with pure *Z*5-10:OH as a sinlge test compound were carried on both in northern Germany (see further below) and in the Engadin valley at Zuoz. In this area (see p. 956), over the past years the larch stands had shown no detectable feeding by *C. laricella*, indicating a far lower moth population than at Tinizong. The test series included the same 4 doses of *Z*5-10:OH (99% *Z*) as used in 1980, supplemented by a blank (control) trap. Traps were set out at site A on June 23, checked in 1 to 4 day intervals, and collected up on July 13.

Over this 3 weeks of exposure, the 24 traps baited with synthetic *Z*5-10:OH caught a total of 840 *C. laricella* moths. This corresponds to 4% of the catch obtained with a similar dosage series at Tinizong in 1980.

Table III. Captures of *C. laricella* males in tetratraps baited with four different doses of *Z*5-10:OH. Zuoz, June 23 to July 13, 1981; six replicates.

Amount of *Z*5-15:OH [µg/trap]	Total catch	[%]
0	6	(0.7)
1	13	(1.5)
10	104	(12.3)
100	261	(30.9)
1000	462	(54.6)

The 6 traps baited with 1 µg contained 13 moths, thus not differing from blank trap captures (Table III). The 10 µg captures exceeded those of the 1 µg traps in all 6 replications (single values not specified here) and accounted for 12.3% of the total catch. The two highest test doses, 100 µg and 1000 µg, revealed captures corresponding to 30.9% and 54.6% of the total, respectivly. Thus, in this experiment, conducted on a very low moth population, the lowest (1 µg) test dose of *Z*5-10:OH was not significantly effective in capturing male moths, whereas the highest dose of 1000 µg was the most attractive.

Tests at Lingen, 1981

In this area, the same series of pure *Z*5-10:OH as at Zuoz were tested from May 27 to June 9, 1981. Plots of known lower and higher moth densities (see p. 956) were used for positioning the traps in 12 replicated series.

On May 27, 4 replications were set out on two different sites. These 20 traps caught a total of 2737 *C. laricella* moths until June 1 (Table IV). Capture totals for replicates No. 1 and 2 (placed

Table IV. Captures of *C. laricella* males in tetratraps, baited with four different doses of *Z*5-10:OH, in 4 replicates at Lingen, May 27 to June 1, 1981.

Amount of *Z*5-10:OH [µg/trap]	Replicate No.				$\sum$ [a]	[%]
	1	2	3	4		
0	0	1	0	1	2	(0.1)
1	101	61	23	14	199	(7.3)
10	275	298	107	40	720	(26.3)
100	302	296	203	172	973	(35.5)
1000	271	168	148	256	843	(30.8)

Table V. Captures of *C. laricella* males in tetratraps, baited with four different doses of *Z*5-10:OH, in 12 replicates at Lingen, June 1 to 2, 1981.

Amount of *Z*5-10:OH [µg/trap]	Replicate No.[a]												Total	[%]
	1	2	3	4	5	6	7	8	9	10	11	12		
0	1	0	0	1	1	0	16	12	1	2	0	1	35	(0.3)
1	54	65	6	6	2	96	399	128	4	18	21	21	820	(7.0)
10	441	265	138	103	102	489	698	241	82	146	132	28	2905	(24.7)
100	466	442	148	184	164	> 600	624	428	81	213	174	219	> 3743	(31.9)
1000	507	375	97	364	263	> 600	565	557	167	349	205	195	> 4244	(36.1)
$\sum$	1469	1147	389	658	532	>1785	2302	1366	335	728	532	464		

[a] Replicates 1–4 are the same as in Table IV.

on a „higher-density" plot) were approx. twofold of those of No. 3 and 4 (the "lower-density" ones). The traps baited with 1 µg *Z*5-10:OH caught between 14 and 101 moths, compared to only 2 moths found in the 4 blank traps. A strong increase in captures is noticeable for the 10 µg dose, in all 4 replicates; and a further increase, for 100 µg in No. 3 and 4 (Table IV). However, as demonstrated in various visual observations, in this initial period of flight the arriving males release a dense layer of scales thus "saturating" the traps at the relatively low level of 200–300 captured moths [17]. Apparently, at inspection on June 1, all traps in replicates No. 1 and 2 baited with the 3 higher attractant doses were at that stage, thus not more showing the initial capture proportions (see discussion, p. 963).

In the early evening of June 1, these 4 replicates were supplemented by 8 further ones, positioned again on plots of presumed lower and higher moth densities. By the end of the experiment on June 9, the 60 traps had caught a total of approx. 30000 *C. laricella* moths. Results are presented here for the 24 h period of June 1 to 2 (Table V).

At inspection on June 2, 17.00–18.30 h, of the 48 traps baited with synthetic attractant 12 were found to contain > 400 moths (with ≧ 500 moths in 7 traps). Such values represent the level of "trap filling" typical for periods of maximum moth flight [17]. In the 5 "higher-density" replicates (No. 1, 2, 6, 7, 8) the 100 µg and 1000 µg traps, and even some of the 10 µg traps, were all evidently at this stage (Table V). This shows that, at peak flight, tetraps baited with the higher lure doses may "saturate" within less than 24 h.

From June 2 to 9, approx. 17200 additional *C. laricella* moths were recorded in the 60 traps, in the following percent proportion: blank traps, 0.4%; 1 µg traps, 6.8%; 10 µg traps, 25.2%; 100 µg traps, 32.3%; and 1000 µg traps, 35.3% of total catch. Again, as in the initial phase of the experiment (Table IV) and the one-day interval (Table V), the higher-dosage traps were frequently at the "saturation" stage. In all 3 periods the capture values obtained thus show a dose-response relationship, "flattened" in comparison to the actual male arrivals at the different lure doses as indicated by visual observations [17] (see discussion, p. 963).

(*Z*)-5-Decenyl acetate as an inhibitor

Tests at Tinizong, 1980

At Tinizong, a further series of experiments were aimed at demonstrating possible "synergistic" or "inhibitory" properties shown by further chemicals when added to sources of *Z*5-10:OH. Included were two analogous alcohols (*Z*7-10:OH, *Z*7-12:OH) and one ester (*Z*5-10:Ac), selected due to their effectiveness in the EAG measurements (see p. 954). Individual rubber caps contained 10 µg of one of these compounds plus 100 µg of *Z*5-10:OH. These 3 binary mixtures were tested in comparison to 100 µg of *Z*5-10:OH alone, using experimental arrangements similar to those followed in the concurrent "dosage series" (see p. 956). Tests began on July 15 and counts were made on July 21, 24, 29, and 31 (the same dates as in the dosage series). The trapping results obtained in the 4 test periods are detailed in Table VI.

Over the 2 weeks exposure the 24 traps caught a total of approx. 20000 *C. laricella* moths. This is the same overall catch as obtained in the concurrent

Table VI. Captures of *C. laricella* males in tetratraps baited with 100 µg of *Z*5-10:OH alone or in binary combinations with 10 µg of *Z*7-10:OH, *Z*7-12:OH, or *Z*5-10:Ac. Tinizong, July 15 to 31, 1980; six replicates.

Chemical [10 µg] added to 100 µg of *Z*5-10:OH		Period I (July 15–21)									
		Replicate No.						Σ	[%]		
		1	2	3	4	5	6				
none		289	372	126	298	128	232	1445	(39.7)		
*Z*7-10:OH		298	200	118	202	77	229	1124	(30.9)		
*Z*7-12:OH		263	133	121	199	183	148	1047	(28.8)		
*Z*5-10:Ac		11	1	1	4	3	2	22	(0.6)		
	Σ	861	706	366	703	391	611	3638			
		Period II (July 22–24)									
none		323	351	249	276	203	258	1660	(39.8)		
*Z*7-10:OH		255	199	214	203	193	221	1285	(30.8)		
*Z*7-12:OH		317	247	103	164	135	182	1148	(27.5)		
*Z*5-10:Ac		27	6	11	9	16	9	78	(1.9)		
	Σ	922	803	577	652	547	670	4171			
		Period III (July 25–29)									
none		653	547	415	500	418	562	3095	(34.5)		
*Z*7-10:OH		611	518	424	500	296	592	2941	(32.7)		
*Z*7-12:OH		477	643	204	450	548	537	2859	(31.8)		
*Z*5-10:Ac		20	17	9	20	14	13	93	(1.0)		
	Σ	1761	1725	1052	1470	1276	1704	8988			
		Period IV (July 30–31)								Total	[%]
none		203	379	53	151	105	110	1001	(43.5)	7201	(37.7)
*Z*7-10:OH		178	118	50	75	100	204	725	(31.5)	6075	(31.8)
*Z*7-12:OH		91	52	115	44	144	120	566	(24.6)	5600	(29.4)
*Z*5-10:Ac		1	1	1	4	1	2	10	(0.4)	203	(1.1)
	Σ	473	550	219	274	350	436	2302			

series on the different *Z*5-10:OH doses. Similar to that series (see Table II for comparison), the captures in the present experiment differed up to twofold between replications and were again highest during period III (Table VI).

Throughout all 4 test periods the traps baited with 100 µg of pure *Z*5-10:OH alone revealed the highest catches, whereas for the binary mixture with 10 µg *Z*5-10:Ac the capture values were in the order of only 1% of the total (Table VI). Though blank traps were not included in this test series, the constantly-low level of captures with this mixture, that did not increase even during the "high-density period" III, suggests that "inhibition" of male attraction was nearly complete.

"Trap saturation" was frequently observed also in the present experiment. This effect apparently accounted for the change in capture proportions observed with the binary alcohol mixtures over the different periods: in period IV, pure *Z*5-10:OH and the two alcohol mixtures caught respectively 1001, 725, and 566 moths, thus following a 100:72.4:56.6 percent proportion; whereas in periods I and II these proportions were 100 : 77.8 : 72.5% and 100:77.4:69.1%, and in period III, 100:94.8:92.2%. Considering "saturation" of most traps during period III (Table VI), we believe that the relative effectiveness of the three lures is more adequately reflected by the values obtained in period IV with its proportionally lower catch.

Based on the present results, the two analogous alcohols (*Z*7-10:OH, *Z*7-12:OH) might be tentatively classified as "weak inhibitors", whereas the acetate analogue (*Z*5-10:Ac) evidently is a "strong inhibitor" of the attraction response of *C. laricella* males to *Z*5-10:OH sources.

Tests at Zuoz and Lingen, 1981

The "inhibitory" effect of *Z*5-10:Ac was more closely studied by test series conducted in 1981 at Zuoz (Engadin valley) and Lingen (northwestern Germany). The experimental arrangements again closely followed those used for the *Z*5-10:OH dosage series conducted in these areas at the same time. Thus, at Zuoz the experiments lasted from June 23 to July 13, and at Lingen, from June 1 to 9. Six replications for the same series were tested on each locality. This series consisted of 1 trap baited with pure *Z*5-10:OH alone, 4 traps containing different *Z*5-10:OH/*Z*5-10:Ac mixtures, and 1 blank (control) trap.

At Zuoz, only 639 moths were captured by the 36 traps during the 3 weeks, again demonstrating the very low level of *C. laricella* population in this area. At Lingen, the 36 traps caught a total of 1917 moths within 1 day, June 1 to 2, and another 3187 moths through June 9. Captures are listed for the 3 weeks test period at Zuoz and the 1 day interval at Lingen (Tables VII, VIII).

Table VII. Captures of *C. laricella* males in tetratraps, baited with varying combinations of *Z*5-10:OH and *Z*5-10:Ac, in 6 replicates at Zuoz, June 23 to July 13, 1981

Amount of chemical per trap [µg]		Total catch	[%]
*Z*5-10:OH	*Z*5-10:Ac		
0	0	0	(0)
100	0	553	(86.5)
100	0.3	53	(8.3)
100	1	28	(4.4)
100	3	2	(0.3)
100	10	3	(0.5)

Despite the extreme difference in moth density in the two areas, the relative captures obtained for the 6 different lures evidently followed the same proportion: the traps baited with 100 µg of pure *Z*5-10:OH accounted for 86.5% (Zuoz) and 85.8% (Lingen) of the total catch; those with the 100 + 0.3 µg mixture, for respectively 8.3% and 9.2%; and the 100 + 1 µg mixture, for 4.4% and 2.6%. The captures by the 100 + 3 µg and 100 + 10 µg mixtures were in the order of 1% of the total, in both experiments, thus not differing significantly from blank trap captures. Furthermore, at Lingen, these capture proportions were apparently the same for "lower-density" and "higher-density" replication sites (Table VIII), in contrast to the relationships reported above for the "dosage series" (see also discussion, p. 965).

Summarizing, at different population densities, the responses of *C. laricella* males to sources of 100 µg *Z*5-10:OH seem to be totally abolished by the addition of 3% or more of the acetate analogue; a small portion of the male moth population responds to the mixture with 1% acetate; and a higher portion (corresponding to a tenth of those responding to *Z*5-10:OH alone), to the 0.3% acetate mixture. The "threshold amount" of *Z*5-10:Ac (required to produce a significant reduction of captures in comparison to pure *Z*5-10:OH alone) remained undetermined in this study.

Test of female pheromone extract

In June 1980, pupae of *C. laricella* were collected in the Tinizong area and the emerging moths used for pheromone gland extraction. During the early

Table VIII. Captures of *C. laricella* males in tetratraps, baited with varying combinations of *Z*5-10:OH and *Z*5-10:Ac, in 6 replicates at Lingen, June 1 to 2, 1981.

Amount of chemical [µg/trap]		Replicate No.						Total	[%]
*Z*5-10:OH	*Z*5-10:Ac	1	2	3	4	5	6		
0	0	1	1	2	5	3	0	12	(0.6)
100	0	159	102	435	622	154	172	1644	(85.8)
100	0.3	28	10	52	82	3	2	177	(9.2)
100	1	14	2	10	18	3	2	49	(2.6)
100	3	2	0	14	5	0	0	21	(1.1)
100	10	1	0	4	6	1	2	14	(0.7)
		∑ 205	115	517	738	164	178		

evening hours (the main swarming period in the field) the abdominal tips of 100 unmated females (1–3 days old) were snipped into *n*-hexane and the extracts stored at −18 °C until use.

In EAG measurements on *C. laricella* male antennae, 10 FE of this extract elicited response amplitudes equivalent to 0.1 µg of synthetic *Z*5-10:OH. This same extract was field tested in July 1980 at Tinizong. Four traps, each baited with 10 FE and hung on separate trees, caught between 263 to 395 (mean, 317) male *C. laricella* moths over the 2 weeks of exposure. During the same time (July 15 to 31), 6 traps each baited with 1 µg of synthetic *Z*5-10:OH (the lowest dose tried in the field) caught between 251 and 737 moths (mean, 459; see p. 957). Thus, the attractivity of the 10 FE corresponded to < 1 µg of *Z*5-10:OH, in agreement with the EAG results.

This limited data is in further support of the hypothesis of *Z*5-10:OH being the only active principle of the sexual atractant produced by virgin *C. laricella* moths.

Trap placement study

The mating flight of *C. laricella* takes place during the daytime in the crowns of the host trees where males may be observed "swarming" in bright sunlight (see [1, 2]). In all preceeding test series, traps were accordingly placed on green branches within larch crowns.

In 1981, we also started to investigate the effect of trap placement on moth captures. The significance of such studies is illustrated here by data obtained in June 1981 in the Black Forest near Strittberg (35 km southeast of Freiburg). This area, at an altitude of 850 m a.s., is forested with spruce and European larch, growing in mixed stands of various age classes. A total of 20 traps, baited with the "standard attractant" of 100 µg of *Z*5-10:OH, were set out on June 2. Traps No. 1–6 were placed on the branches of young larch trees, 4–5 m in height and dispersed individually (with full exposure to sun) along a lane through a spruce culture. In contrary, traps No. 7–20 were fixed to the trunks (or dry branches) of older larch trees inside closed stands, using a 17 year old stand (height of trees, 8–9 m) for traps No. 7–10, and a 22 year stand (height, 10–12 m) for No. 11–20. Distances from stand edges were between 20–70 m. All 20 traps were positioned at approx. 1.8 m above ground; accordingly, with the younger trees (No. 1–6) the traps were within the crown regions, whereas with all other trees (No. 7–20) they were several meters beneath the lower crown surface. Trap positions and synthetic lures remained unchanged for the 4 weeks test period but the sticky boards were replaced when counts were made on June 9, 16 and 22.

It was found that only those traps positioned on green branches (*i.e.*, No. 1–6) showed substantial captures, whereas for all traps inside the closed stands (No. 7–20) the captures were consistently poor (Table IX).

When inspected in May 1981, some trees at the periphery of the old stand (used later for positioning traps No. 11–20) had shown definite *C. laricella* infestations, whereas no detectable feeding could be noted on the younger trees (used for placing traps No. 1–6). These observations are seemingly in disagreement with the trapping results. Nonetheless we believe that the captures on traps No. 1–6 did reflect the low and non-damaging local populations

Table IX. Captures of *C. laricella* males in tetratraps, baited with 100 µg *Z*5-10:OH and positioned at eye level. Traps No. 1–6 were in the crowns of younger larch trees, whereas traps No. 7–20 were attached to the trunks of older trees inside closed stands (see text for details). Strittberg, June 2 to 28, 1981.

Trap No.	Catch in period [a]			
	I	II	III	IV
1	127	236	7	9
2	36	138	7	7
3	91	163	7	8
4	56	113	4	4
5	81	217	6	5
6	155	191	0	6
7	1	7	0	1
8	1	1	0	0
9	3	2	0	0
10	2	11	0	0
11	0	8	0	0
12	0	28	1	0
13	0	24	0	0
14	1	8	0	3
15	1	22	1	0
16	0	9	1	0
17	0	25	0	6
18	0	16	0	0
19	0	13	0	0
20	0	3	0	0

[a] Periods were June 2–9, 10–16, 17–22, and 23–28, respectively.

on these trees, and we assume that the moth populations present in the tree canopies in the older stands were unresponsive to the traps positioned on the tree trunks at eye level (the small captures by these traps possibly representing drifts from outside, as these captures gradually decreased with distance from the periphery).

Such data suggest that in *C. laricella* the mating flight occurs only in the canopy habitat context. In comparison, in similar tests with the European fir budworm (*Choristoneura murinana*), the spruce budworm (*C. fumiferana*) or the pine beauty moth (*Panolis flammea*), males were effectively "lured down" from their host crowns to traps placed at eye level (see [15, 19–22, 25–27]).

Discussion and future prospects

The results presented are consistent with the hypothesis of *Z*5-10:OH being a primary component of the yet unidentified sex pheromone of the female larch casebearer moth. In EAG measurements on conspecific males, this chemical was clearly the most stimulatory among the series of olefinic compounds tested; and in field trials, *Z*5-10:OH as a single compound strongly attracted native *C. laricella* males at all study sites. Though *Z*5-10:OH has has not been reported before as an insect pheromone (or attractant) component, related structures are known from some other lepidopteran species; for example, the (*E*)-5 isomer, *E*5-10:OH, is a component of the pheromone blend produced by the female peach twig borer moth (*Anarsia lineatella*) [28], whereas the acetate analogue, *Z*5-10:Ac, is a pheromone or attractant component in several species of Noctuidae [29–36], and also some other moths (including other *Coleophora* spp. [37]).

In Lepidoptera, although multi-chemical pheromone systems are usual, there are examples of apparent single-component attractants. Among European forest Lepidoptera, the pine shoot moth (*Rhyacionia buoliana*), the green oak leafroller (*Tortrix viridana*), and the nun moth (*Lymantria monacha*) seem to follow this kind of pattern [13, 38–42]. The larch case bearer evidently provides a further example of this type of attractant system.

All four test doses of *Z*5-10:OH, from 1 µg to 1000 µg, were effective in capturing male moths. A stepwise increase towards the higher attractant doses occurred in all experiments conducted on lower moth densities; this held throughout all tests at Zuoz, and for the "lower-density" replications and/or test periods at Tinizong and Lingen. Thus, evidently all four doses were appropriate in eliciting male orientation and landing responses such as to lead them into the traps, the greater "active space" of the odour plum emanating from the higher source probably accounting for its greater trapping efficiency.

However, with "higher-density" test replications at Tinizong and Lingen, capture differences for the different lure doses were usually less pronounced; in particular at peak flight, the 10 µg dose of *Z*5-10:OH often revealed the same high catch as 100 µg or 1000 µg, and for 1 µg many counts exceeded 100 moths/trap. As has been pointed out, in such tests the sticky boards in the traps baited with higher attractant doses were frequently at a stage of "saturation", thus not further retaining the arriving moths. With tetratraps, "trap filling" was usually observed to occur at 200–300 captured *C. laricella* moths for fresh males (at begin of the flight period) and approx. 400 moths at peak flight [17]. Evidence has been presented showing that, during maximum flight, the higher-dosage (100 µg, 1000 µg) traps may "saturate" within a few hours. This effect, by allowing continuous captures only for the less effective (lower-dosage) traps, has apparently accounted for the "flattened" dose-capture curves as observed with some test replications at Tinizong and Lingen. It has been suggested that, even in such "high-density" test situations, the initial captures (before saturation) had followed a $>10/1$ relationship for the 100 µg/1 µg doses; and we assume that this relationship would hold throughout test periods if unlimited capture capacity of traps were provided. The dose-capture relationships for pure *Z*5-10:OH at higher moth densities are presently (1982) under study, using types of non-sticky traps with retaining capacities for >10000 *C. laricella* moths/trap.

When comparing different lure doses, possible shifting of the optimum dosis with aging of the lure sources had to be considered. Such a phenomenon has been reported, *e.g.*, from field work on the European fir budworm *Choristoneura murinana* [15, 21]. In the experiments on *C. laricella*, losses in attractivity of lure sources over exposure time were expected to occur, considering the short (approx. 5 day) evaporative half-lives on rubber septa as reported for several other C_{10} alcohols [43, 44]. However, as shown by the field trapping data,

sources of 1 µg of *Z*5-10:OH were still attractive after > 2 weeks of exposure, and no drastic shift in relative effectivity can be established for the other lure doses used, in any test series. Similarly, in work on other moth species using the corresponding acetate (*Z*5-10:Ac), rubber septa loaded with 1 µg of this chemical have shown attractivity in the field for at least 3 weeks [31, 35]. The emission rates of *Z*5-10:OH from the rubber caps remain undetermined but there is experimental evidence [43, 44] that these dispensers release various other olefinic compounds at rates roughly proportional to the amount present.

For the 12 trees used in the test at Tinizong, larval counts had led to population estimates ranging between 200000 to 500000 moths/tree ([24]; see p. 956). The captures obtained on these trees also differed up to three-fold (with one exception) for "lower-density" phases of the two concurrent experiments, and about two-fold when the entire test period (including "trap filling"phases) is considered (see Tables II, VI). At Lingen, where "higher-density" and "lower-density" plots had been selected, the local differences in captures were in the range of up to seven-fold (see 1-day period in Tables V, VIII). These differences roughly correspond to local population estimates as based upon larval and egg counts [6]. Thus, with the experimental arrangement used, capture differences among test replicates may be attributed mainly to differences in local moth densities.

At Tinizong, the capture totals per tree ranged from 2214 to 4532 moths (Tables II, VI). This would represent only 1% of the above population estimate. With such high moth densities, the 4 traps placed on the same tree should have operated independently from each other (not showing "competition" between neighbouring traps as experienced with some other forest Lepidoptera in similar experimental arrangements (see [15, 21, 22]). At Lingen, the trap distances of 10 m (maintained in all test series) should have excluded appreciable trap interference.

For a given test area, maximum flight was observed to occur synchronously at all replication sites. At Tinizong (1980) peak flight was in period III (July 25–29) on all 12 trees (see Tables II, VI); and at Lingen (1981), in the first days of June, in all replicates of both series. Even at Strittberg (1981), despite the great variations in trap placement, with all traps that revealed substantial captures the maximum was observed on June 16 (Table IX). These findings agree with those by McMillian and Borden [9] who, in their field tests on *C. laricella* in western Canada (using female-baited traps), obtained > 95% of captures for a given area within a 6-day period synchronized by weather conditions. Similarly, in a field study conducted in Upper Bavaria on various species of casebearer and leafminer moths (including other *Coleophora* spp.), the species-specific peaks in trap captures generally occurred within approx. 5-day intervals [37].

We are recommending the 100 µg dose of *Z*5-10:OH to be used as the "standard lure" in all future population studies on *C. laricella.* This formulation has shown excellent attractive properties throughout all phases of the present study. In the tests at Zuoz and Strittberg, this attractant permitted the detection and quantification of extremely low *C. laricella* populations (undiscovered by visual inspections for larval feeding); and further, it allowed the evaluation of possible movements (actively or passively) of the moths from their larch crown habitats. With most future studies of the dispersion dynamics of *C. laricella,* including the yearly spread of infestation boundaries in some New World areas (see [45]), the use of delta-sticky traps should be appropriate. However, for monitoring higher moth densities, sticky traps are impractical because of their limited retaining capacities. At Lingen, correlation of trap captures with results of conventional population estimates has shown [17] that various types of sticky traps all "saturated" (within < 24 h at peak flight) at densities of severalfold below the economic threshold values (as given by approx. 1 larva/spur shoot). Thus, for establishing "warning threshold" values, traps with far higher holding capacities are needed; various types of non-sticky traps are therefore presently being tested, in search for a "standard trap" appropriate for monitoring higher *C. laricella* populations.

The impact of mass trapping on local *C. laricella* populations is also under study. For these tests we are using box traps [46] and other traps of almost unlimited holding capacities (see [21]). Mass trapping procedures will, of course, not be practical on a broader scale but the method might prove valuable for keeping local, isolated infestations below the economic threshold.

The acetate analogue, *Z*5-10:Ac, has shown strong "inhibitory" effects on male attraction to *Z*5-10:OH sources; a 0.3% addition of this ester lowered captures to one tenth of those obtained with pure *Z*5-10:OH alone, and a 1% admixture was sufficient to abolish catches. These relationships held for both low and high moth populations (see Zuoz *vs.* Lingen). Moreover, whereas with the "dosage series" the capture proportions drastically changed following "saturation" of the more attractive traps, no such effect could be observed for the "inhibitor series" (see Table VIII). Such data point to different patterns of male orientation behaviour for sources of pure attractant, as compared to *Z*5-10:OH sources masked with the acetate inhibitor. Current field work therefore includes detailed visual observations of male orientation responses to lure sources of different chemical composition.

With such highly potent inhibitors, practical problems could arise with respect to possible contamination of attractant formulations by the inhibitor. For example, with the cabbage moth *Mamestra brassicae*, trap captures to *Z*11-16:Ac (the primary female pheromone) were strongly reduced when the attractant sources contained $\geqq 0.1\%$ of the corresponding alcohol, *Z*11-16:OH [47], thus demanding great efforts to remove the parent alcohol from the synthetic formulations. However, no such problems should exist with species (as *C. laricella*) using alcohol pheromones and acetate inhibitors, as usual alcohol samples can be considered free of acetate.

The biological function(s) of the acetate inhibitor in *C. laricella* remains unknown. A role in species isolation is suggested by the strong attraction of males of other *Coleophora* spp. to sources of pure *Z*5-10:Ac or specific *Z*5-10:OH/*Z*5-10:Ac mixtures, observed in field screening tests [37]. On the other hand, McMillian and Borden [9] have provided data showing that traps baited with mated *C. laricella* females contained less males than did blank traps. Such observations could suggest the release by the mated female moth of an attraction-masking chemical. If there is a female-released, intraspecific inhibitor in *C. laricella*, the acetate analogue of the attractant alcohol might be a likely structure.

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