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Natural volatiles impair the response of *Hylobius abietis* adults to synthetic attractants in Norway spruce clear cut areas

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Abstract. For over 20 years it has been known that mature adults of *Hylobius abietis* are attracted by the combination of alpha-pinene and ethanol. However, it is not clear to what extent weevil response to these stimuli is influenced when large quantities of similar volatile substances are present in the environment, and how the response depends on the release rate of volatile substances from traps. Nothing that, in fresh Norway spruce clear-cuttings, the mature weevils were equally attracted to the traps baited with dispencers having different release rates, we assumed that the experiment results were affected by the abundance of natural volatile substances issued from the fresh slash, which masked the differences between olfactory signals released from traps. To verify this hypothesis, the experiment conducted in fresh clear-cuttings was repeated in exactly the same place after almost a year, when the overground slash were old. For seven weeks, at the beginning of growing season 2008, in two experimental areas, 6 different combinations of alpha-pinen and ethanol were tested using the traps buried in the soil. In both experimental area was captured about the same number of weevils and catch dynamics were similar. In the first two weeks of experimentation, when there were the highest captures, but also for the entire period of experimentation, there were significant differences between the tested variants in what concerns the average number of captures, the traps baited with dispenser providing higher release rate of ethanol and alpha-pinene having higher catches. This shows that in the first season of vegetation the weevil response to the attractants was affected by the profusion of similar volatile substances issued from natural sources (fresh cutt stumps, branches, foliage, bark etc.).
Keywords: *Hylobius abietis*, synthetic attractants, alpha-pinene, ethanol, release rates, weevil`s response, fresh/old clear cuttings

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Introduction

Nowadays, *Hylobius abiestis* remains a major threat to coniferous plantation forestry in many countries of Europe (Day & Leather 1997, Langström & Day 2004). Protective measures are necessary in order to forestall the damages that could be caused by this pest, and the development of an integrated pest management (IPM) program is desired (Langström & Day, 2004), but an IPM decision-support system requires much information, including that concerning pest population size (Norris et al. 2003). However, no standardized method for assessing the weevil abundance was available until recently, when Nordlander (1987) submitted one based on the use of synthetic attractants, alpha-pinene and ethanol, whose synergic attraction for weevil was previously proved (Tilles et al., 1986).

Although the synthetic attractants do not have the drawbacks of natural materials used for insect attracting and trapping out (Nordlander 1987), the captures do not reflect accurately the population size, but rather the activity of mature weevils. The pre-reproductive weevils, emerging in the late summer, are not attracted (Nordenhem & Eidmann 1991, Malphettes et al. 1994), and the post-reproductive insects are only sporadically caught when preparing for hibernation, while the reproductive beetles are less caught during intense oviposition (Örlander et al. 1997). Consequently, a good correlation between insect captures and seedling damage was found only in certain situations (Nordlander 1987, Örlander et al. 1997), and the use of weevil traps for monitoring purposes is regarded as complicated (Langström & Day 2004).

Any progress in this direction implies a better understanding of weevil's behaviour, including the reactions to variable stimuli encountered in their habitat. In this sense, we have conducted several experiments aiming at testing the effects of different release rates of alpha-pinene and ethanol on weevil's response. One of them, described by Olenici et al. (2007), was quite similar with that accomplished by Nordlander (1987), and leaded to similar results: mature pine weevils were equally attracted by all combinations of alpha-pinene and ethanol, as long as the release rates of volatile substances were 0.07-0.38 ml/day and 0.08-0.57 ml/day, respectively.

Because the both above-mentioned experiments were conducted in fresh clear-cuttings, we assumed that weevil response/the number of trapped weevils was affected by the presence of natural materials releasing high quantities of terpenes and ethanol. In order to prove this hypothesis, our experiment was repeated at the beginning of the growing season 2008 in one year old clear cut areas, and the results are presented in this paper.

Materials and methods

Experiment location

Taking into account the objective of our research, the experiment was carried out in the same plots where the experiment presented by Olenici et al. (2007) took place. The two clear cut areas were one year old, and we expected the existent pine weevils to be represented exclusively by mature individuals which came during the previous growing season and overwintered on place. They should have been inter-reproductive and reproductive weevils, according to Christiansen (1971). These areas are located in the Nothern part of Eastern Carpathians and belong to the Forest Direction Suceava, Forest District Pojorâta (III, 111A - Valea Putnei experimental area: 47°26'47"N; 25°25'28"E) and Forest District Cârlibaba (V, 31B - Cârlibaba experimental area: 47°36'39"N; 25°02'04"E). They had quite similar site and stand characteristics (Table 1), differences existing only at the slope aspect and

Table 1 The main characteristics of the site and tree stand[1] within the experimental areas

Experimental area	Area (ha)	S.T.[2]	F.T.[3]	Soil type[4]	Altitude (m)	Aspect	Slope (g)	Composition[5]	Age (years)	Canopy cover	Cutting period
V. Putnei	3.0	2333	1111	3301	985-1015	S	25	9Ns+1Sf	110	0.6	autumn 2006
Cârlibaba	1.8	2333	1111	3301	1050-1100	N	30	10 Ns	110	0.5	winter 2006-07

1) Data refer to tree stand existing before the clear cut. 2) Site types: 2333 - Mountain spruce forest with high productivity, brown acid soil or andosol, with big or moderate edaphic volume, with *Oxalis-Dentaria* ± acidophilous plants. 3) Forest type: 1111 - Normal spruce forest with *Oxalis acetosella* (s). 4) Soil type: 3301 - Typical brown acid soil. 5) Ns - Norway spruce (*Picea abies*), Sf - Sylver fir (*Abies alba*).

concerning the soil. In Cârlibaba experimental area, the soil has a very high proportion of big rock fragments and is covered with a thick layer of moss, while at Valea Putnei it has a low skeleton content and it is covered with grassy vegetation.

In May 2008, the experimental plot from Cârlibaba was reforestated by planting of about 4,000 coniferous seedlings (Norway spruce, Scots pine, European larch) per hectare. The seedlings were treated by dipping into synthetic pyrethroid emulsion before planting.

Experiment design

Seven experimental variants were settled (Table 2), distributed in 10 blocks in each experimental areas, each block being a replication. The traps were set exactly in the same positions as in the experiment conducted in 2007, after a linear disposal, with a distance of 4-5 m between them. The minimum distance between the experimental blocs was of 10-15 m and the distance between the trap blocks and the forest edge was of minimum 25 m. The variants were randomly deployed. The check-up of the traps was conducted every 5-7(8) days and, in order to decrease the effect of the trap position over the captures, the variants were moved one step at each control, until each treatment passed through all trap positions. The experiment was conducted between 7th of May and 26th of June at V. Putnei, and between 13th of May and 1st of July at Cârlibaba.

Traps

The traps we used (Fig. 1) resemble those described by Nordlander (1987). They were made of cylindrical pots of transparent polypropylene, with 14.0 cm diameter and 15.5 cm height. Ten holes of 1 cm diameter were drilled equidistantly around the circumference, at 2.5 cm from the top, for weevil acces. Other eight holes of 2 mm diameter were made at 6.5 cm from the bottom of the trap, to drain any excess of water. The traps were buried in the ground so that the entering holes for the insects were tangent to the surface of the ground, and were covered with an opaque plastic lid. In addition, they were covered with fragments of wood from old, dry tree branches, in order to protect the traps from disturbing factors (people, animals etc.). Thus, they were not directly exposed to insolation during sunny days.

The dispensers were suspended vertically, with the openings at the level of insect entry holes. To prevent the condensed water droplets from entering the dispensers, each vial was protected with a plastic cover, fastened at about 1 cm from the dispenser brim. During the functioning period, traps were provided with a preserving solution of water (cca. 250 ml) and salt (NaCl, 15-20 g).

Dispensers

The dispensers with alpha-pinene consisted of glass vials with the opening diameter of 8 and 10 mm respectively, while polyethylene phials were used for dispenseres filled with ethanol.

Table 2 Tested treatments

Experimental treatment	Desired release rate (ml/day)	
	AP – (-)-alpha-pinene	ET – ethanol
V_1	0.1	0.1
V_2	0.3	0.3
V_3	0.1	0.3
V_4	0.3	0.9
V_5	0.2	0.1
V_6	0.6	0.3
V_7- control	-	-

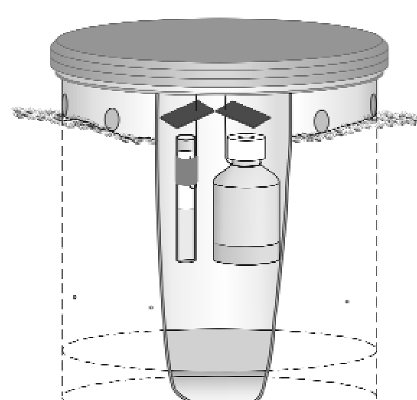

Figure 1 Trap with dispensers used in the experiment

In order to obtain the desired release rates, each dispenser with alpha-pinene was provided with a strip of filter paper, having the upper end at a certain distance below the vial brim. The release rates of ethanol were adjusted by different sizing of the hole practiced in the lid of the bottle (for the rates of 0.1 and 0.3 ml/day) and by adding a strip of filter paper (for a rate of 0.9 ml/day) (Table 3). Dispenser calibration was performed in the laboratory conditions (average temperature of 20°C and relative humidity of 60-65%).

The monoterpene we used, (-)-alpha-pinene, was produced by Sigma-Aldrich GmbH from Germany, and had a chemical purity of 98% and enantiomeric purity of 91%. The ethanol was alimentary cereal alcohol of 95.5°. The strips were made from filter paper type MN 640 m - white box.

The actual release rates in the field conditions were quantified by measuring the consumption of volatile substances. Syringes of 1 ml, amounting to one gradation mark for 0.01 ml, were used for this operation. The measuring was conducted at every second check-up of the traps, when initial quantities of volatile substances in dispensers were restored. When we noticed at the intermediary check-up that some types of dispensers were at risk of getting out of function until the next measuring, those dispensers were supplied with the necessary quantities of volatile substances. Such situations were met only at the alpha-pinene dispensers envisaged to release 0.1 ml/day, which were initially supplied with only 1 ml of terpene, as in the calibration tests.

Temperature recording

For a better understanding of dispenser functioning under field conditions, and of the weevils' response evolution over the experimental period, a HOBO® Pro v2 datalogger was installed in each experimental area to record temperature and relative humidity every 15 minutes. The device was put in a supplementary unbaited trap, placed in a part of experimental plot considered as representative for site conditions of that area.

Data analysis

The data concerning the weevil`s captures were analyzed by using the XLSTAT program (Addinosoft) which works under Microsoft (TM) Excel. Weevil's response type and intensity was established by comparing the average (cumulated) captures per treatments and by comparison to control trap captures. Variance analysis was performed using ANOVA parametric test, taking into account only the treatment and the block as variation factors. The model which included also the interaction between the above mentioned factors proved not to be adequate. The effect of these factors was analysed both for each observation period, and for the entire experimental period. In order to ensure the homogeneity and the normality of the distributions, the original data were transformed in log (x+1). After the data transformation, all the distributions were normal. As long as the control variant (V_7) had no or very few captures, it was necessary to exclude this variant from the ANOVA in order to have a homo-

Table 3 Constructive characteristics of dispensers

Volatile substance	Desired release rate (ml/day)	Recipient type	Constructive traits: Recipient dimensions	Paper strip: Dimensions (mm)	Paper strip: Level below vial brim (mm)	Initial volume of the volatile substance (ml)
(-)-alpha-pinene	0.1	glass vial	inside: 8 x 54 mm	7.5 x 49	1	1.0
	0.2		inside: 8 x 54 mm	7.5 x 53	4	2.0
	0.3		intside:10 x 54 mm	9.5 x 53	1	3.0
ethanol	0.1	polyethylene phial	outside 55 x 32.5 mm, with the lid's hole diameter of 2 mm	-	-	4.0
	0.3		Outside 55 x 32.5 mm with the lid's hole diameter of 8 mm	-	-	4.0
	0.9		Outside 55 x 32.5 mm with the lid's hole diameter of 9.5 mm	9.5 x 54	1	10.0

geneous system of dispersions. The homogeneity of variances was checked with the Fisher`s or Hartley`s test, and the normality of data distributions with the Shapiro-Wilk`s test. When ANOVA test showed that there were significant differences between treatments, the Tukey`s test was used in order to examine the significance of the differences between the average values at $P < 0.05$.

The proportion of males/females in the captures was calculated only for samples with at least ten weevils, and the comparison of proportions was made using the χ^2 test.

Results

Actual release rates

The release rates of the volatile substances under field conditions were lower than those obtained in the laboratory (Fig. 2), because the dispensers functioned at average temperatures lower than the calibration temperature (Table 4). At Valea Putnei, the actual release rates of alpha-pinene represented only 49-52% from the desired rate for the dispensers that should have 0.1 ml/day, 44-48% for those that should have 0.2 ml/day and 37-44% for those that should have 0.3 and 2 x 0.3 ml/day, respectively. Similar levels (53-58%, 43-47% and 40-43% respectively) were obtained at Cârlibaba. Ethanol release rates recorded at Valea Putnei reached 59-80% from the envisaged rate for the dispensers that should have 0.1 ml/day, 47-59% for those of 0.3 ml/day and 32-40% for those of 0.9 ml/day, while at Cârlibaba they represented 66-86%, 51-55% and 37%.

The release rate of volatiles increased from the first to the last period as the average temperature increased. The changes were more obvious at Valea Putnei, and especially to ethanol dispensers, but also to alpha-pinene dispensers with a higher envisaged rate. Consequently, the ratio between components (alpha-pinene/ethanol) was fluctuating. At the treatments that should assure a ratio of 1:1, there were recorded values between 0.64 and 0.84 at Valea Putnei, and between 0.59 and 0.92 at Cârlibaba

For the treatments with a planned ratio of 1:3, the values from the field were 0.29-0.39 and 0.30-0.37, respectively, and for the ratio of 2:1 there were recorded values of 1.20-1.62 and 1.09-1.65, respectively. In spite of these variations, the average ratios obtained in both experimental areas were still close enough to those resulting from the experimental protocol, especially for V_3 and V_4 treatments (Table 5).

Response of the weevils

During the experimentation period, similar numbers of large pine weevils were caught at Valea Putnei and Cârlibaba (2206 and 2660 respectively) and the dynamics of the *Hylobius abietis* captures in the two areas was quite similar, too. The existing small differences are mainly due to the earlier start of the experiment at Valea Putnei. The highest catches were recorded by 21st of May and the males prevailed over females in that time; thereafter captures gradually declined and there were more females than males in weevil`s samples (Fig. 3).

Variance analysis of cumulated captures (for the whole experimentation period) recorded at the baited traps, show that both the treatment and the block had a significant influence over weevil's response. However, the situation was quite different if the data were analysed apart for each observation period (Table 6). In some periods, both factors had a significant influence, but in other only one factor (mostly the treatment) or no one.

The beetles were obviously attracted by all combinations of attractants, but the response to different volatile combinations changed during the experimentation. This fact is very obvious at Valea Putnei, where - during the observation

Table 4 Temperatures recorded in the field during the experiment

Experimental area	Working period	Temperature (°C)		
		Min.	Max.	Mean
Valea Putnei	07.05-21.05	1.6	18.6	8.6
	21.05-10.06	5.7	22.4	12.4
	10.06-23.06	7.0	20.9	13.4
Cârlibaba	13.05-26.05	2.9	18.3	11.2
	26.05-09.06	4.7	19.9	12.8
	09.06-01.07	6.7	19.3	13.5

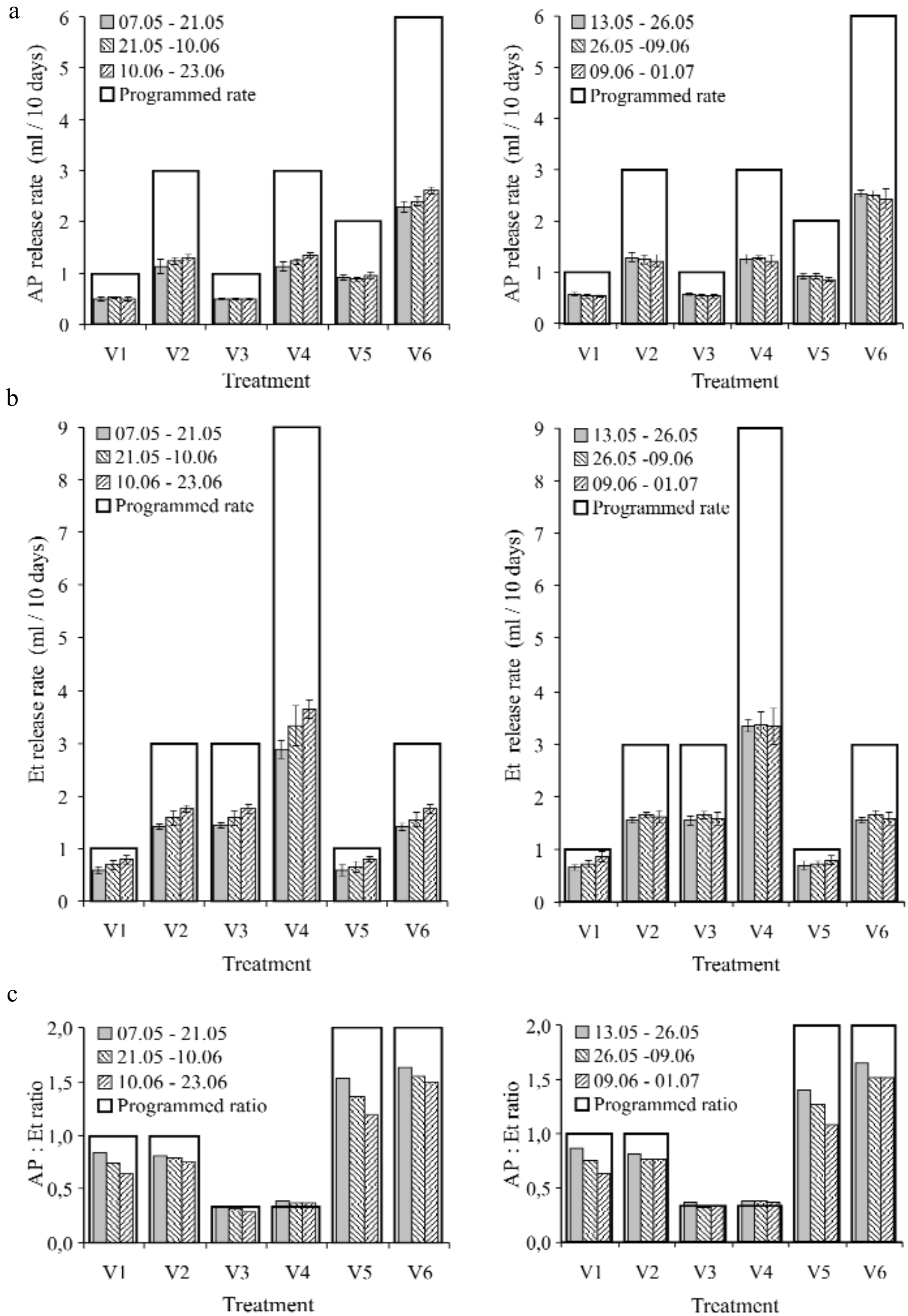

Figure 2 Average release rates of volatiles within the experimental area Valea Putnei (left) and Cârlibaba (right): a) alpha-pinene dispensers; b) ethanol dispensers; c) components ratio

Table 5 Average ratios between the volumes of (-)alpha-pinene and ethanol evaporated in the field conditions

Experimental area	Real values obtained to the treatments with an envisaged ratio AP/ET of ...		
	1:1	1:3	2:1
Valea Putnei	0.76 ± 0.07	0.35 ± 0.04	1.44 ± 0.18
Cârlibaba	0.76 ± 0.12	0.34 ± 0.03	1.45 ± 0.19

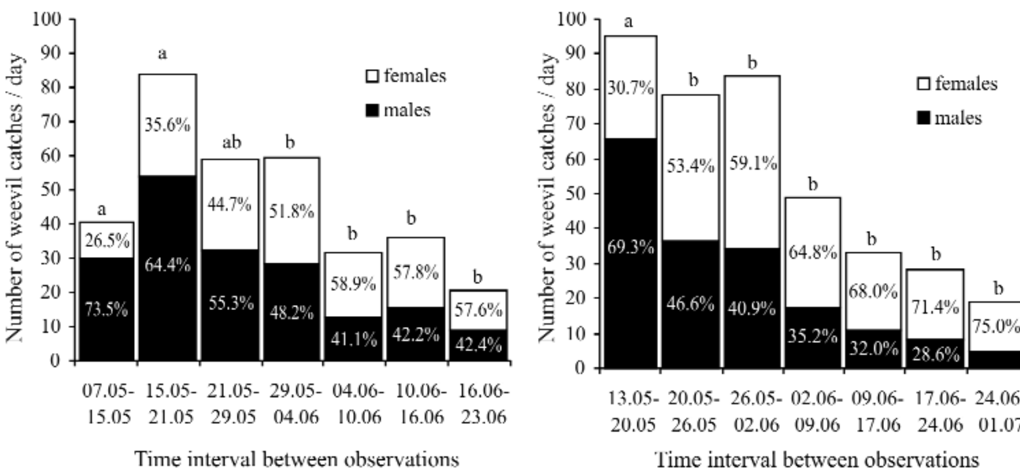

Figure 3 Dynamics of cumulated captures and of sex proportions at Valea Putnei (left) and Cârlibaba (right). (The same superscript on columns denotes that female proportion differences are not significant at $P < 0.05$)

Table 6 ANOVA results concerning the effect of the treatment and the block on the weevil captures

Time period	Factor	D.F.	*F*	*P*	Time period	Factor	D.F.	*F*	*P*
	Valea Putnei					Cârlibaba			
07 - 15.05	Treatment	5	4.92	**0.0011**	13 - 20.05	Treatment	5	4.71	**0.0016**
	Block	9	0.58	0.8019		Block	9	2.83	**0.0101**
15 - 21.05	Treatment	5	5.79	**0.0003**	20 - 26.05	Treatment	5	1.23	0.3100
	Block	9	1.65	0.1309		Block	9	0.78	0.6308
21 - 29.05	Treatment	5	1.85	0.1207	26.05 - 02.06	Treatment	5	1.06	0.3951
	Block	9	4.06	**0.0007**		Block	9	1.74	0.1082
29.05 - 04.06	Treatment	5	6.55	**0.0001**	02 - 09.06	Treatment	5	2.52	**0.0432**
	Block	9	2.12	**0.0473**		Block	9	3.20	**0.0048**
04 - 10.06	Treatment	5	2.08	0.0850	09 - 17.06	Treatment	5	2.18	0.0730
	Block	9	1.31	0.2610		Block	9	0.68	0.7179
10 - 16.06	Treatment	5	2.39	0.0520	17 - 24.06	Treatment	5	0.88	0.5031
	Block	9	1.11	0.3780		Block	9	0.76	0.6573
16 - 23.06	Treatment	5	4.09	**0.0040**	24.06 - 01.07	Treatment	5	1.02	0.4186
	Block	9	0.83	0.5930		Block	9	1.50	0.1797
Total	Treatment	5	13.33	**<0.0001**	Total	Treatment	5	7.33	**<0.0001**
	Block	9	5.87	**<0.0001**		Block	9	9.10	**<0.0001**

periods 7[th]-15[th] of May and 15[th]-21[st] of May - the beetles responded much more to the treatment V_4 than to the others (Table 7). After the 21[st] of May, the differences between the captures recorded in the traps baited with attractants decreased, and they were only sometimes statistically assured.

At Cârlibaba, differences between the captures recorded at the baited treatments were statistically significant only in the first observation period, and - surprisingly - the best weevil`s response was to the treatment V_3, not to V_4.

Analyzing the data for the entire experimentation period (that means cumulated captures), we observe that there were two combinations of attractants (V_1 and V_5) that within both experimental areas attracted less beetles than the other baited treatments (Fig. 4). The two treatments are caracterized by lower release rates of the ethanol (0.061-0.080 ml/day) than the remaining treatments which had rates ranging from 0.14 ml/day to 0.40 ml/day. In the same time, the two treatments had the lowest release rates of the monoterpene (0.051-0.054 ml/day and 0.087-0.095 ml/day, respectively).

A special situation is represented by the V_3 treatment which was characterized by a release rate of the alpha-pinene comparable to that of

Table 7 Variation of weekly average captures according to the treatments during the experimentation period

Time period	Number of captures (mean ± SD) at the treatment …						
	V1	V2	V3	V4	V5	V6	V7
				Valea Putnei			
07-15.05	3.5 ± 2.5^{a}	4.6 ± 3.5^{a}	3.3 ± 2.4^{a}	8.8 ± 4.0^{b}	4.3 ± 2.9^{a}	3.6 ± 1.5^{a}	0.0
15-21.05	7.1 ± 3.1^{a}	11.2 ± 4.3^{ab}	9.6 ± 3.9^{ab}	14.7 ± 5.8^{b}	8.1 ± 4.1^{a}	6.0 ± 3.7^{a}	0.1 ± 0.4
21-29.05	5.3 ± 2.8^{a}	7.4 ± 3.6^{a}	5.9 ± 2.8^{a}	8.1 ± 3.9^{a}	6.0 ± 2.8^{a}	8.5 ± 5.8^{a}	0.1 ± 0.3
29.05-04.06	3.8 ± 2.3^{a}	10.5 ± 4.3^{b}	3.9 ± 2.8^{a}	7.6 ± 4.4^{abc}	4.8 ± 3.0^{ac}	9.1 ± 4.9^{abc}	0.5 ± 0.6
04-10.06	2.0 ± 2.0^{a}	5.3 ± 3.9^{a}	3.6 ± 2.2^{a}	3.4 ± 2.2^{a}	2.6 ± 2.0^{a}	4.2 ± 2.9^{a}	0.0
10-16.06	2.5 ± 1.6^{a}	4.6 ± 1.5^{a}	3.7 ± 2.4^{a}	5.3 ± 2.6^{a}	3.8 ± 2.7^{a}	5.1 ± 1.7^{a}	0.0
16-23.06	1.9 ± 2.0^{ab}	3.3 ± 1.7^{a}	0.7 ± 1.1^{b}	2.7 ± 1.4^{ab}	1.9 ± 1.4^{ab}	3.5 ± 1.8^{a}	0.4 ± 0.7
				Cârlibaba			
13-20.05	8.2 ± 2.8^{a}	10.6 ± 4.4^{ab}	17.0 ± 8.1^{b}	11.1 ± 6.0^{ab}	7.0 ± 5.8^{a}	10.2 ± 6.3^{ab}	0.0
20-26.05	8.8 ± 3.2^{a}	7.4 ± 4.0^{a}	6.7 ± 3.9^{a}	11.3 ± 5.3^{a}	7.8 ± 3.4^{a}	9.3 ± 5.5^{a}	1.3 ± 1.1
26.05-02.06	9.5 ± 7.7^{a}	11.9 ± 6.0^{a}	10.7 ± 4.0^{a}	7.2 ± 3.3^{a}	8.8 ± 2.8^{a}	8.5 ± 4.7^{a}	0.1 ± 0.3
02-09.06	5.4 ± 3.1^{a}	5.9 ± 2.5^{a}	6.7 ± 2.9^{a}	6.5 ± 2.6^{a}	4.1 ± 2.3^{a}	4.0 ± 2.7^{a}	0.1 ± 0.3
09-17.06	2.6 ± 1.9^{a}	3.2 ± 2.4^{a}	4.6 ± 2.6^{a}	5.2 ± 3.1^{a}	2.1 ± 2.0^{a}	4.2 ± 2.5^{a}	0.2 ± 0.6
17-24.06	2.0 ± 1.2^{a}	5.1 ± 3.9^{a}	3.5 ± 2.5^{a}	3.5 ± 2.7^{a}	2.4 ± 1.8^{a}	2.6 ± 1.9^{a}	0.0
24.06-01.07	1.1 ± 0.9^{a}	1.8 ± 1.4^{a}	1.4 ± 1.5^{a}	2.4 ± 3.5^{a}	2.6 ± 2.5^{a}	2.7 ± 1.5^{a}	0.0

Means in the same line, followed by the same letter, do not differ statistically at $P < 0.05$.

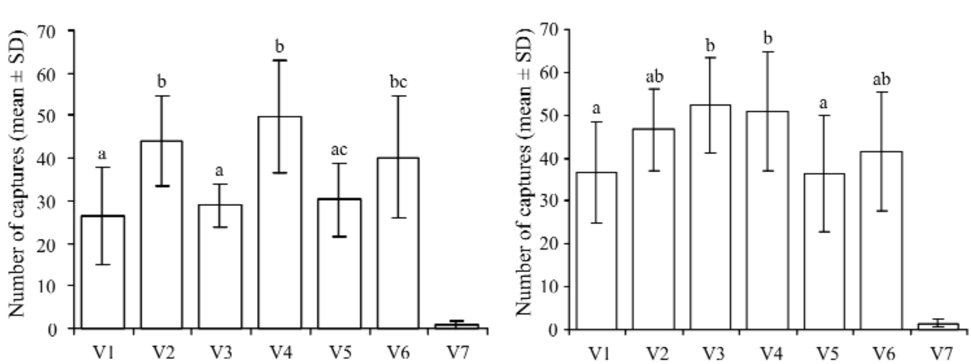

Figure 4 Variation of pine weevil captures according to the treatment at Valea Putnei (left) and Cârlibaba (right) (The same superscript on columns denotes that the average values are not statistically different at $P < 0.05$)

V_1 treatment, but the release rate of the ethanol was higher than that of the V_1 and V_5 treatments. Traps baited with this attractant combination captured almost the same number of weevils as those with V_1 and V_5 treatments, at Valea Putnei, but at Cârlibaba equalized with the V_2 and V_4 treatments.

Comparing the variants which have the same release rate of the alpha-pinene but a variable release rate of ethanol, that is V_1 with V_3 and V_2 with V_4, we notice only a small increase of the captures along with the increase of the release rate of ethanol. Consequently, the differences between the means are not statistically ensured, except for the pair V_1-V_3 from Cârlibaba.

On the other hand, if we compare the treatments that have the same release rate of the ethanol but a variable release rate of the pinene, that is V_1 with V_5, and V_2 with V_3 and V_6, we notice the existence of some different tendencies. In the case of the V_1-V_5 pair, a doubling of the pinene release rate resulted in only a small, not significant, increase of the captures and that happened only at Valea Putnei.

In the case of V_2-V_3-V_6 triplet, the different "behaviour" of the V_3 treatment in the two experimental areas makes difficult to understand the impact of increasing the release rate of the pinene from 0.047-0.053 ml/day (values corresponding to V_3) to 0.100-0.134 ml/day (values corresponding to V_2). A further increase of the pinene release rate up to 0.236-0.259 ml/day (values corresponding to V_6), when the release rate of ethanol is maintained at the level of 0.142-0.168 ml/day seems to induce an inhibition of the weevil`s response, materialized in the decrease of the captures at V_6 comparatively with V_2 (Fig. 4).

Finally, if someone looks at the groups of treatments with the same ratio AP/Et, that means V_1-V_2, V_3-V_4 and V_5-V_6, can see that the response of the beetles was stronger at the treatments with the high release rates of the ethanol and monoterpene. The only exception is V_3 which, at Cârlibaba, attracted almost the same number of beetles as V_4 treatment.

Even though the differences between the variants included in the combinations analyzed before, were not statistically ensured in several cases, they denote the fact that the response of the beetles was affected by the release rates of the volatile substances. Considering the experimental results as a whole (Fig. 5), someone can see a clear trend of increasing catches along with the increase of volatile substances release rates. However, only at Valea Putnei both volatiles significantly affected the captures by increasing of release rates (AP: df = 1, $F = 11.390$, $P = 0.001$; Et: df = 1, $F = 15.159$, $P < 0.001$), while at Cârlibaba increasing the release rate of alpha-pinene had no significant effect (AP: d.f. = 1, $F = 0.096$, $P = 0.758$; Et: d.f. = 1, $F = 7.846$, $P < 0.007$).

Increasing of weevil captures by the release rate augmentation of the volatile substances did not affect the proportion of female or male

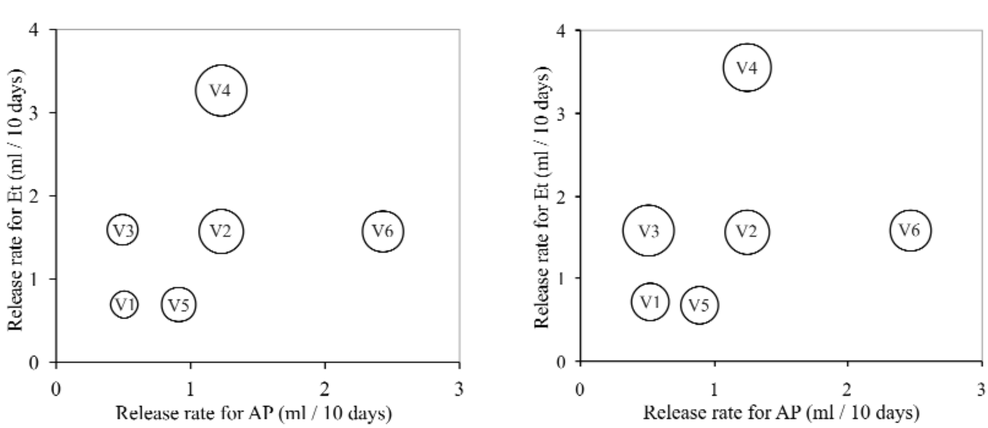

Figure 5 Combined effect of alpha-pinene release rate and ethanol release rate on weevil captures at Valea Putnei (left) and Cârlibaba (right)

weevils in the captures, male and female weevils responding to a stronger signal in the same maner as to weaker one. Thus, the female proportion in weevil captures did not differ significantly from one treatment to another (Table 8).

Discussion

Functioning of dispensers

The actual release rates of volatiles under field conditions were lower than those from our similar experiment conducted in 2007 (Olenici et al. 2007). However, the experiment allows a comparison with the results from the previous test concerning the weevil's response at different release rates of volatiles. In addition, recording both temperature and the release rates of volatile substances, the results allow a good understanding of what happens in different conditions, when using different dispenser types, facilitating the choose of dispensers and of calibration temperature for future experiments.

Response of the weevils

As expected, the weevils responded to all combinations of alpha-pinene and ethanol and about 99.5% of total captures were caught in the baited traps. Because the weevils are active at daily mean temperature above 5°C (Örlander et al. 1997), the first captures at Valea Putnei were recorded even in the first week, characterized by low temperatures (minimum of 1.6°C and a mean of 6.9°C). Rising of temperature induced an enhancement in weevil`s activity and the number of captures increased in the second week of experimentation. In that time (before the 20th of May), males were more active than females and they prevailed in insect samples. A further increase of temperature (daily mean over 10°C and maximum over 18°C) ensured adequate conditions for weevil`s migration (Solbreck & Gyldberg 1979, Örlander et al. 1997) and this caused a decreasing of captures after the 20th of May, but also a changing in male/female proportion. Because the males are the insects which start the migration towards new clear cut areas (Solbreck & Gyldberg 1979), thereafter female weevils became prevalent in insect samples from traps, especially at Cârlibaba. In the last case we suspect that the reduction of male proportion was partly caused by planting of conifer seedlings treated with insecticide. Although, the old weevils (males and females) are equally sensitive to some insecticides as DDT (Eidmann & Novák 1970), it is posible that males were more affected than females by the insecticide on plants, because they were more active during the planting and immediately after.

During the experimentation period, changes occured in weevil`s response to combinations of alpha-pinene and ethanol, and the pattern was quite different in the two experimental areas. At Valea Putnei, in the first week (7-17.05), when temperatures were quite low, the

Table 8 Female proportion (%) in weevil`s captures depending on the treatment

Time interval between observations	Treatment						Time interval between observations	Treatment					
	V_1	V_2	V_3	V_4	V_5	V_6		V_1	V_2	V_3	V_4	V_5	V_6
	Valea Putnei							Cârlibaba					
07-15.05	27.5	30.2	13.2	25.7	30.0	31.0	13-20.05	26.8	29.2	31.2	27.9	38.3	30.4
15-21.05	32.8	34.4	30.5	36.5	33.3	47.1	20-26.05	49.3	50.8	53.2	60.8	46.3	52.5
21-29.05	45.0	42.9	46.3	46.2	46.4	42.3	26.05-02.06	57.9	63.0	60.7	53.3	61.4	56.5
29.05-04.06	56.8	50.0	45.5	43.1	63.4	53.8	02-09.05	55.6	64.4	71.6	62.0	65.9	70.0
04-10.05	58.8	48.9	61.3	66.7	54.5	63.9	09-17.05	63.3	66.7	67.3	67.8	58.3	79.2
10-16.05	54.5	57.5	46.9	54.3	66.7	63.6	17-24.05	65.0	66.7	71.4	80.0	79.2	67.6
16-23.05	57.9	48.5	n.d.	51.9	52.6	60.0	24.06-01.07	81.8	77.8	71.4	77.8	69.2	74.1
Total period	**44.1**	**43.3**	**40.3**	**41.9**	**46.2**	**50.0**	**Total period**	**50.4**	**55.2**	**53.1**	**55.8**	**54.7**	**55.3**

most intense response was at the combination with the highest release rate of ethanol (V_4). This is true also for the second week, but the V_4 treatment was no more significantly different from V_2 and V_3. In the same period (13-20.05), at Cârlibaba, the most intense response was at V_3, which did not significantly differed from V_2, V_4 and V_6. Because the males were predominant in insect samples before the 20th of May, we could infer - from the above mentioned results - that they were more responsive to higher release rates of ethanol. However, the analyses showed that no significant differences between treatments in the female proportion in total captures in any observation period. Consequently, we conclude that the noticed differences in mean captures between treatments, at the beginning of experimentation, are due to the possibility of weevils to perceive the existence of volatile sources with different release rates. Later, when temperature increased and the daily maxima was in the optimum range for feeding, oviposition and migration (Christiansen & Bakke 1968, Solbreck & Gyldberg 1979; Örlander et al. 1997), the differences between treatments vanished almost completely. This fact can be a result of complex weevil's behavior changes in connection with their emigration, as well as a consequence of the population depletion that occurred due to migration and also due to weevil catching. On the other hand, the reduction of capture differences could also be a result of a more intense weevil's activity at higher temperature that would increase the chance of weevils to encounter the traps with a relatively lower release rate of volatiles.

Comparing the treatments on the basis of cumulated captures, it clearly appears that the weevils were more attracted by the traps having dispensers with higher release rates of volatiles, especially ethanol. Such a differentiation of treatments wasn't observed in the experiment conducted in the same areas in 2007, when abundant fresh slash was present. Because the experimentation in 2007 took place much later (27.06-7.08), we do not exclude the possibility that the lack of differences between treatments was a consequence of higher temperatures and higher release rates of volatiles, respectively. However, someone can see that in 2008, even in June (a period with high temperatures) the captures at V_4 were slightly higher than those of V_1 and V_5, although not statistically significant, in both experimental areas. Consequently, we can conclude that abundant natural volatiles affected the response of weevils to baited traps in the experiment conducted in fresh clear-cuttings, in 2007

Conclusion

Conducting the experiment in two consecutive years at the same places allowed to establish that abundant natural volatiles in fresh clear-cut areas affects the response of the large pine weevils to the traps baited with alpha-pinene and ethanol. In such conditions, the captures are similar regardless of the release rates of volatiles from traps, while the traps with higher release rates of volatiles have higher captures in the second season after the cutting.

When the natural volatiles are scarce, captures depend on the release rates of both volatiles, but mainly of that of ethanol. Weevils of both sexes respond similarly to different release rates of volatiles, but male weevils are caught in a higher proportion at the beginning of the season, before their migration. After the beginning of migration, the captures decline and female are prevalent.

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