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RESEARCH PAPER

Effect of (+)-limonene and 1-methoxy-2-propanol on *Ips typographus* response to pheromone blends

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Abstract: We compared two different strategies to increase the catches of *Ips typographus* (L.), particularly males, in pheromone-baited traps. The first of these strategies, the barrier approach, used alternating pheromone blends, targeting males and females respectively, in closely-spaced traps forming a barrier around forest stands. The second strategy, the single trap approach, used widely-spaced traps that were all baited with the same lure and intended to trap the highest possible numbers of males without compromising trapping of females. In the blend used for the barrier traps targeting primarily males, with a lower percentage of (4*S*)-*cis*-verbenol (cV), the (−)-α-pinene was replaced step wise with (+)-limonene at rates of 0%, 1%, 10%, 35%, 60% and 90%. This replacement had no significant effect on the numbers of responding *I. typographus* males, but there was a slight effect on the percentage of males caught. In the attractant blend for the barrier traps targeting females, with a higher percentage of cV, the 2-methyl-3-buten-2-ol (MB) was replaced with 1-methoxy-2-propanol (MP) in a similar fashion as for the male-specific blends. The replacement did not significantly affect the catch of females. Thus, it is possible to use the MP in the blend with cV and ipsdienol without significant change in catch efficacy. In the blends for single traps, the (–)-α-pinene was replaced with (+)-limonene and MB with MP. The replacement of (–)-α-pinene had only a slight effect on the percentage of males, but the results suggest that replacing MB with MP in the blend will not significantly reduce trapping efficacy.

Keywords: pheromone trap barrier; single traps; (4*S*)-*cis*-verbenol; 2-methyl-3-buten-2-ol; (−)-α-pinene

Introduction

Barriers of pheromone traps are used as a part of Integrated Pest Management against *Ips typographus* (L.). They represent one of the possibilities for the protection of spruce stands (*Picea abies* [L.] Karst.), and it is possible to use them to protect the selected stand by significantly lowering the primary beetle attack rate. The use of pheromone trap barriers for the protection of spruce stands has been discussed along with the method's advantages and risks by several authors (Vité 1989; Niemeyer 1997; Jakuš 1998). *I. typographus* males initiate attack and the colonisation of trees; consequently, the male catch is very important for the overall efficacy of pheromone trap barriers. High concentrations of pheromones attract equally both sexes from long distance (Schlyter et al. 1987a), even though males are reported to be more sensitive to the pheromone (Dickens 1981). In addition, high concentrations of pheromones may result in attacks on trees surrounding the pheromone sources.

Jakuš and Šimko (2000) tried to use the existing commercial pheromone dispensers along with a reduced release rate lure in order to catch more males in pheromone trap barriers. However, they found that the coupling of the common commercial pheromones with the ones with a reduced release rate did not result in either higher numbers or higher proportions of trapped males. Jakuš and Blaženec (2002) suggested using a combination of traps baited with dispensers having two different release levels of (4*S*)-*cis*-verbenol (cV) in the pheromone mixtures in order to achieve higher catches of males.

The relationship between different release levels of cV and 2-methyl-3-buten-2-ol (MB) in the pheromone mixture and the catch of the different sexes of *I. typographus* was well described by Schlyter et al. (1987b). An increase in the cV release rate with constant MB increases the number of beetles caught, however, the proportion of males is reduced when higher doses of cV are used in the pheromone mixtures.

Jakuš and Blaženec (2003) show that the efficacy of pheromone trap barriers with alternating dispensers, having two different release levels of cV in the pheromone blends, can be changed by adding (−)-α-pinene. The number of beetles captured by traps

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baited with a low level release of cV was increased by adding (−)-α-pinene. However, adding (−)-α-pinene did not increase the catches of traps containing high releases of cV.

Further improvement of pheromone trap barriers is possible by using compounds synergistic with the basic *I. typographus* pheromone mixture of cV and MB. Reddemann (1993) and Gossenauer-Marohn (1988) studied the significance of primary attraction of monoterpenes on the spruce bark beetle and their influence on attractiveness of the commercially manufactured pheromones or trap-trees. According to Reddemann (1993), supplementing (−)-α-pinene with (+)-limonene, resulted in a significant increase in trap catch (by 81%) with lures that were one-third filled Pheroprax bait with the following chemical composition: MB, cV, Ipsdienol in a ratio 96.08:3.56:0.36. This approach was further tested in the field by Niemeyer and Watzek (1996), but they could not confirm a statistically significant increase in the catch.

Another possibility for improving the blend is the use of 1-methoxy-2-propanol (MP), which is a chemical solvent used in several commercial pheromones produced in the Czech and Slovak Republics. MP is present neither in beetles nor in spruce, but is relatively cheap and is used in order to decrease the cost of the pheromone dispenser. Previous tests performed during the development of commercial dispensers indicated the potential for replacing MB with MP. The experiment was based on the idea of a pheromone trap barrier with alternating dispensers releasing different pheromone mixtures that affect males and females differently. The aim of the present study was to test the possibility of increasing the efficacy of pheromone trap barriers by replacing (−)-α-pinene with (+)-limonene in the pheromone mixture with low levels of cV (targeting males), and replacing MB with MP in the mixture with high levels of cV (targeting females). To that end, we tested, the efficacy of single pheromone traps by replacing (−)-α-pinene with (+)-limonene together with successive replacement of the MB with MP in the pheromone mixture with intermediate levels of cV.

Material and Methods

Pheromone baits

Synthetic pheromone baits from Fytofarm Ltd. (Bratislava, Slovakia) were used. Chemicals, their purities and sources are presented in Table 1.

Six variants of the pheromone mixtures were assigned respectively to increase catch of males (BM), females (BF) and for single traps (SL) (Table 2). In the BM variants, (−)-α-pinene was successively replaced with (+)-limonene; in the BF variants, MB was successively replaced with MP; and in the SL variants, (−)-α-pinene was successively replaced with (+)-limonene together with successive replacement of MB with MP. The replacement ratio was effectuated by 0%, 1%, 10%, 35%, 60%, and 90%.

The control was a commercial dispenser IT Ecolure (Fytofarm Ltd.). According to the original label, IT Ecolure has the following chemical composition: cV ≥ 3.2%, MB ≤85%, plus a mixture of minor compounds with synergistic effect (ethanol, spruce oil, (+)-limonene in a ratio 20:10:1) ≥11.8%.

Special wick - aluminium foil protected dispensers provided by Fytofarm Ltd. (Varkonda 1996) were used. All compounds in a blend were mixed in an individual dispenser, as was the commercial pheromone IT Ecolure (Fytofarm Ltd.). The average release rate of mixtures from all of the dispensers was about 50 mg/day (Fytofarm Ltd.) under field conditions. All dispensers were filled with 3 ml of pheromone mixture.

Table 1. Compounds, purity and source in pheromone baits tested in the experiments

Compound	Purity	Source
(4*S*)-*cis*-verbenol	≥ 98%	Fytofarm Ltd. (Slovakia)
2-methyl-3-buten-2-ol	≥ 98%	FLUKA AG (Switzerland)
(−)-α-pinene	≥ 98%	FLUKA AG (Switzerland)
(+)-limonene	≥ 90%	FLUKA AG (Switzerland)
1-methoxy-2-propanol	≥ 98%	SH-Chem (Slovakia)
(S)-(+)-ipsdienol	≥ 95%	Bedoukian Research, Inc. (USA)

Pheromone traps

Cross type pheromone traps called Ecotrap from Fytofarm Ltd. were used. This universal, selective and omnidirectional pheromone trap is made from black plastic and his effective size is 5 550 cm^2.

Experiment design and locality

The experiments were carried out by setting up two closely-spaced pheromone trap barriers and one group of widely-spaced single standing pheromone traps. The distance between two neighbouring traps for the barrier approach was approximately 12 m, and for the single-trap approach it was 50 m. The distance between the traps and the forest edge was about 15 m.

Barrier M: This barrier was used for improving the catch of males. Pheromone traps used for the rotation of experimental dispensers were alternated with fixed traps baited with lures that had been shown to yield the highest total catch of females in previous experiments (Jakuš and Blaženec 2003; BF-0, Table 2)

Barrier F: This barrier was used for improving the catch of females. Pheromone traps used for the rotation of experimental dispensers were interspaced with fixed traps baited with lures that had been shown to yield the highest catch of males in previous experiments (Jakuš and Blaženec 2003; BM-0, Table 2).

Single traps: These widely spaced traps were baited with pheromone blends aimed at gaining a higher total catch and a reasonable percentage of males in the individual standing traps.

The positions of experimental baits were changed and insects were collected when at least 20 beetles were caught in the trap with the lowest catch. The positions were changed according to the Latin square experimental design (Byers 1991). The experimental baits were rotated 7 times in all of the experiments. Beetles from the fixed traps were collected at the end of the experiment.

Table 2. Chemical composition of pheromone baits used in the experiments

Barrier M bait variant:	BM-0 (%)	BM-0.4 (%)	BM-3.5 (%)	BM-12.3 (%)	BM-21 (%)	BM-31.5 (%)
2-methyl-3-buten-2-ol	64.1	64.1	64.1	64.1	64.1	64.1
(4*S*)-*cis*-verbenol	0.9	0.9	0.9	0.9	0.9	0.9
(−)-α-pinene	35.0	34.6	31.5	22.7	14.0	3.5
(+)-limonene	0.0	0.4	3.5	12.3	21.0	31.5
Barrier F bait variant:	**BF-0 (%)**	**BF-0.9 (%)**	**BF-9 (%)**	**BF-31.5 (%)**	**BF-54.1 (%)**	**BF-81.1 (%)**
2-methyl-3-buten-2-ol	90.1	89.2	81.1	58.6	36.0	9.0
(4*S*)-*cis*-verbenol	9.0	9.0	9.0	9.0	9.0	9.0
(S)-(+)-ipsdienol	0.9	0.9	0.9	0.9	0.9	0.9
1-methoxy-2-propanol	0.0	0.9	9.0	31.5	54.1	81.1
Single traps bait variant:	**SL-0-0 (%)**	**SL-0.1-0.9 (%)**	**SL-1-8.7 (%)**	**SL-3.5-30.4 (%)**	**SL-6-52 (%)**	**SL-9-78 (%)**
2-methyl-3-buten-2-ol	86.7	85.8	78.0	56.3	34.7	8.7
(4*S*)-*cis*-verbenol	3.3	3.3	3.3	3.3	3.3	3.3
(−)-α-pinene	10.0	9.9	9.0	6.5	4.0	1.0
(+)-limonene	0.0	0.1	1.0	3.5	6.0	9.0
1-methoxy-2-propanol	0.0	0.9	8.7	30.4	52.0	78.0

The name of the blends consist of abbreviations for the experiment type and the percentage of added compound ((+)-limonene in the Barrier M experiment, MP in the Barrier F experiment and both in the SL experiment)

The experiments were conducted in 80–90-year-old spruce stands situated in the Poľana Mountains in central Slovakia at altitudes of about 700 m above sea level on the southwest slopes in clear-cuts after salvage cutting. The period of testing was from June to August 2000. This sampling period was used in order to avoid spring flight, which has an especially high proportion of males.

Laboratory processing

Beetles were dissected and separated by sex. The beetle individuals were short parboiled in kalium hydroxide (KOH) to soften their chitinized body. The parts of abdomen were separated and the parts of males chitinized copulatory organs were searched under the binocular magnifying. All *I. typographus* individuals were sexed if trap catches were below 50 and, for trap catches above 50, a random sample of 50 individuals were sexed.

Statistics

Relative catch proved to be the most useful variable for statistical analysis. Conversion of the absolute catches to relative catches enabled us to compensate for the effect of differences in time intervals and weather conditions corresponding to the individual repeated measurements and to determine the differences between the baits. Relative catch for one blend in a particular sample was calculated as a percentage of the total catch in that sample. The relative catch was calculated for males and females, and their sum represents the relative catch of both sexes together. The percentage of males was calculated to emphasize the ratio between the sexes. Absolute catch was used as additional information to the relative catch; particularly we can show the catches of fixed traps in barriers. These were emptied at the end of experiments; hence, they cannot be recalculated as relative catches.

Data were arcsine square root transformed prior to analysis. Assumptions for use of parametric statistics were tested (Shapiro-Wilk test and Levene test; Underwood 2001). For the data that complied with the assumptions, Analysis of Variance (ANOVA) was performed, followed by Fisher's LSD test. For the data that did not comply with the assumptions, the nonparametric Kruskal-Wallis test and then Mann-Whitney U test were used.

All statistical calculations were done using Statistica 5.5 software.

Results

Barrier M

Over the whole experiment, 11 360 specimens of *I. typographus* were caught in the barrier traps, of which 5 520 were caught in traps where tested baits were alternating.

ANOVA results (Table 3) do not allow the rejection of null hypothesis; hence, there is no biological effect in the absolute and relative catch in the tested variables. Differences in the mean absolute and relative catch from all seven replications are presented in Figs. 1 and 2. The absolute catch had nearly a similar pattern to that of the relative catch and the highest catch of females was in the variant BM-21 while the highest catch of males was in the variant BM-3.5. The absolute catch from the fixed traps (bait variant BF-0) was comparable to the catch from the other blends.

On the other hand, the null hypothesis can be rejected in the case of the percentage of males. The Fisher's LSD test show a significant effect in the percentage of males in the variants BM-3.5 and BM-31.5 which were higher than the percentage of males in the variant BM-21 (Table 4). No difference was found between the variants BM-0, BM-0.4, BM-12.3 and the control IT Ecolure.

Table 3. ANOVA, or Kruskal-Wallis test results, following results from Levene's test and Shapiro-Wilk W test.

		Levene test (homog. of variances)		Shapiro-Wilk W test (test of normality)		ANOVA			Kruskal-Wallis test		
	sex	F	P	W	P	F	d.f.	P	H	d.f.	P
Absolute catch											
BM	♀♀	0.5548	0.7634	0.9791	0.5270	0.5931	6	0.7340	–	–	–
	♂♂	0.5473	0.7692	0.9815	0.6312	0.1472	6	0.9886	–	–	–
BF	♀♀	0.8028	0.5734	0.9822	0.6590	1.7544	6	0.1322	–	–	–
	♂♂	1.1684	0.3414	0.9771	0.5191	1.1331	6	0.3601	–	–	–
SL	♀♀	0.1985	0.9754	0.8461	0.0000 **	–	–	–	0.4022	6	0.9988
	♂♂	0.4027	0.8731	0.8624	0.0000 **	–	–	–	1.6280	6	0.9506
relative catch											
BM	♀♀	0.7279	0.6297	0.9796	0.5492	0.8810	6	0.5172	–	–	–
	♂♂	0.8314	0.5525	0.9835	0.7155	0.2888	6	0.9390	–	–	–
BF	♀♀	0.7800	0.5903	0.9804	0.5805	4.3741	6	0.0016 **	–	–	–
	♂♂	0.9033	0.5017	0.9891	0.9277	1.7273	6	0.1384	–	–	–
SL	♀♀	0.0731	0.9983	0.8501	0.0000 **	–	–	–	0.2505	6	0.9997
	♂♂	0.2213	0.9678	0.8718	0.0001 **	–	–	–	2.2120	6	0.8992
% of males											
BM		0.8209	0.5601	0.9668	0.1795	2.2288	6	0.0589	–	–	–
BF		1.9832	0.0911	0.9711	0.2683	0.9404	6	0.4767	–	–	–
SL		1.9930	0.0882	0.8723	0.0001 **	–	–	–	11.7793	6	0.0671

** significant at $P < 0.01$

Table 4. Mean percentages and SEs of *Ips typographus* males responding to different lure blends tested in pheromone trap barriers and in single traps

Barrier M bait variants						Control	Fixed traps bait
BM-0	BM-0.4	BM-3.5	BM-12.3	BM-21	BM-31.5	IT	BF-0
30.8 ± 2.8 ^{a,b}	32.8 ± 2.7 ^{a,b}	37.1 ± 2.6 ^{b}	31.4 ± 5,7 ^{a,b}	23.1 ± 3,7 ^{a}	39.8 ± 4,1 ^{b}	32.2 ± 2,4 ^{a,b}	17.3 ± 1.4
Barrier F bait variants						**Control**	**Fixed traps bait**
BF-0	BF-0.9	BF-9	BF-31.5	BF-54.1	BF-81.1	IT	BM-0
15.6 ± 1.6	21.4 ± 2.1	17.3 ± 2.0	22.9 ± 3.2	17.1 ± 2.1	16.4 ± 3.2	20.6 ± 4.5	29.5 ± 2.8
Single traps bait variants						**Control**	
SL-0-0	SL-0.1-0.9	SL-1-8.7	SL-3.5-30.4	SL-6-52	SL-9-78	IT	
23.5 ± 2.8 ^{a}	28.9 ± 6.7 ^{a}	18.6 ± 2.0 ^{a,b}	15.4 ± 0.8 ^{b}	18.4 ± 2.3 ^{a,b}	22.2 ± 3.3 ^{a}	17.2 ± 1.9 ^{a,b}	

Means with the same letter are not different according to the Fisher’s LSD test (Barrier M) and Mann-Whitney U test (Single traps).

Barrier F

The variants of BF baits that we tested and the control IT Ecolure trapped 6050 specimens from the total of 9110 specimens of *I. typographus* caught in the barrier.

According to the ANOVA results (Table 3) for absolute numbers of beetles (Fig. 1), the null hypothesis can be rejected only in the case of the absolute numbers of females. The Fisher’s LSD test shows a significant effect between the variants BF-0, BF-9, BF-54.1, BF-81.1, and the control IT Ecolure. The highest absolute catch of females was in the variant BF-0, and the highest absolute catch of males was in the variant BF-81.1. The catch from the fixed traps (bait variant BM-0) was comparable to the catch from the control IT Ecolure.

The relative catch had a similar pattern to that of the absolute catch. According to the ANOVA results (Table 3) the null hypothesis cannot be rejected in the case of the relative catch of males and the percentage of males, whereas it can be rejected in the case of the relative catch of females. The Fisher’s LSD test shows a significant effect in the relative catch of females and males between the tested variants and the control IT Ecolure (Fig. 2).

The highest relative catch of females was in the variant BF-0, and the highest relative catch of males was in the variant BF-31.5. The highest percentage of males was also in the variant BF-31.5. The lowest relative catch of males and also the lowest percentage of males were in the variant BF-0.

Single traps

In the traps used for testing the variants of the blends SL and IT Ecolure, the number of beetles caught throughout the experiment totalled 7 576 specimens of *I. typographus*.

According to results from the Shapiro-Wilk test (Table 3), the data from this experiment does not comply with the assumptions for the use of parametric statistics; hence, nonparametric statistics were used. According to the Mann-Whitney U test, there was no significant effect on the absolute or relative catch of females and males (Figs. 1 and 2); whereas, there was a significant effect on the percentage of males (Table 4).

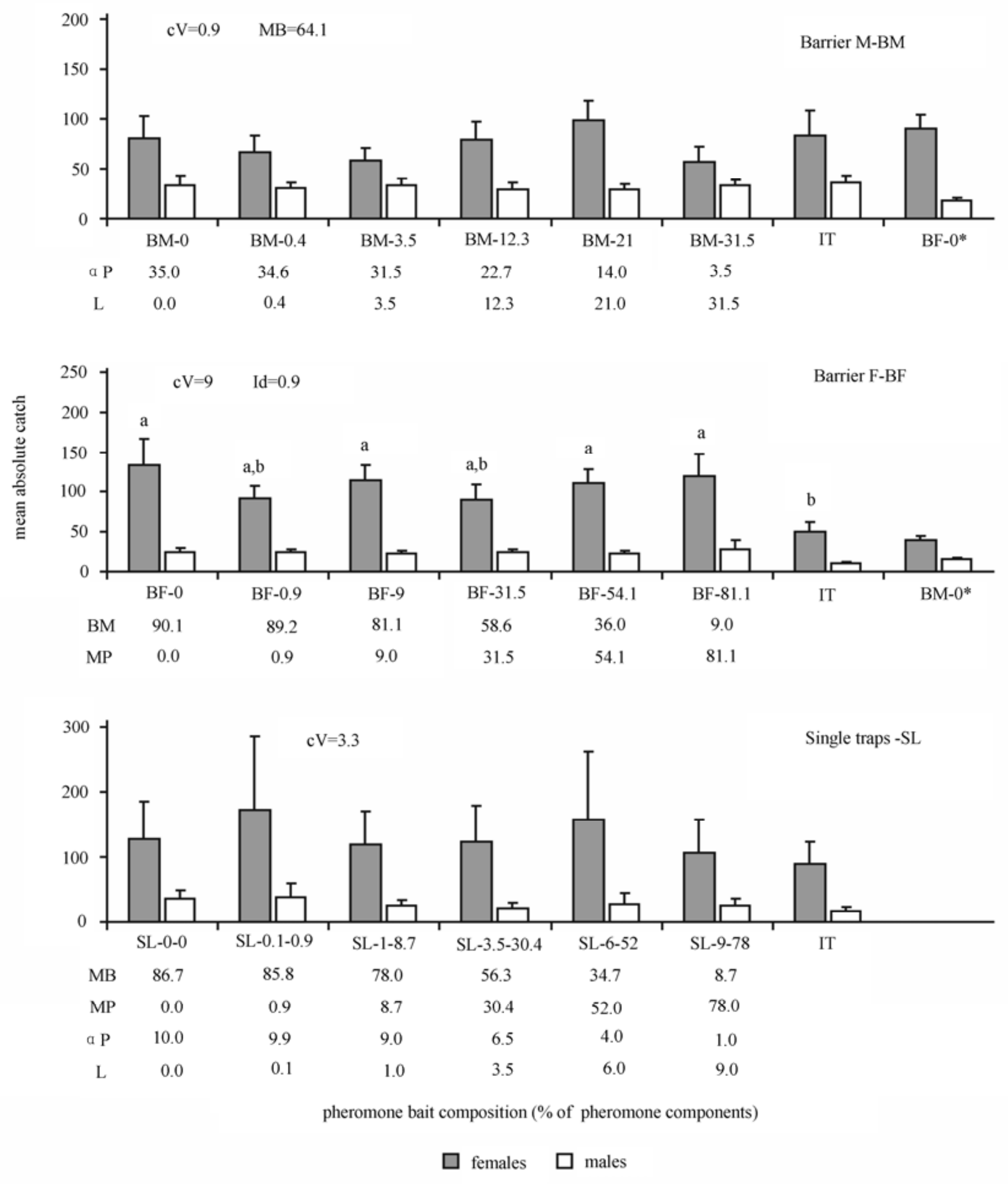

Fig. 1 Mean absolute catch and SEs of *Ips typographus* in different variants tested in pheromone trap barriers and in single traps. Bars with the same letter are not different according to the Fisher's LSD test. Females and males are considered separately. * The catch is calculated as a mean from all fixed traps in the barrier and divided by 7 (repeated measures) to be comparable with the means of tested variants.

The highest absolute catch in all the tested variables was in the blend SL-0.1-0.9; and similar to the relative catch, the absolute catch was the lowest in the control IT Ecolure.

The highest relative catch of females was in the variant SL-0.1-0.9; however, the relative catch in the other variants except the IT Ecolure (control) were similar. The highest relative catch of males was in the variant SL-0-0. The lowest relative catch of females and males was in the IT Ecolure (control).

The highest percentage of males was in the variant SL-0.1-0.9, which was in a homogeneous group with the variants SL-0-0 and SL-9-78. The lowest percentage of males was in the variant SL-3.5-30.4.

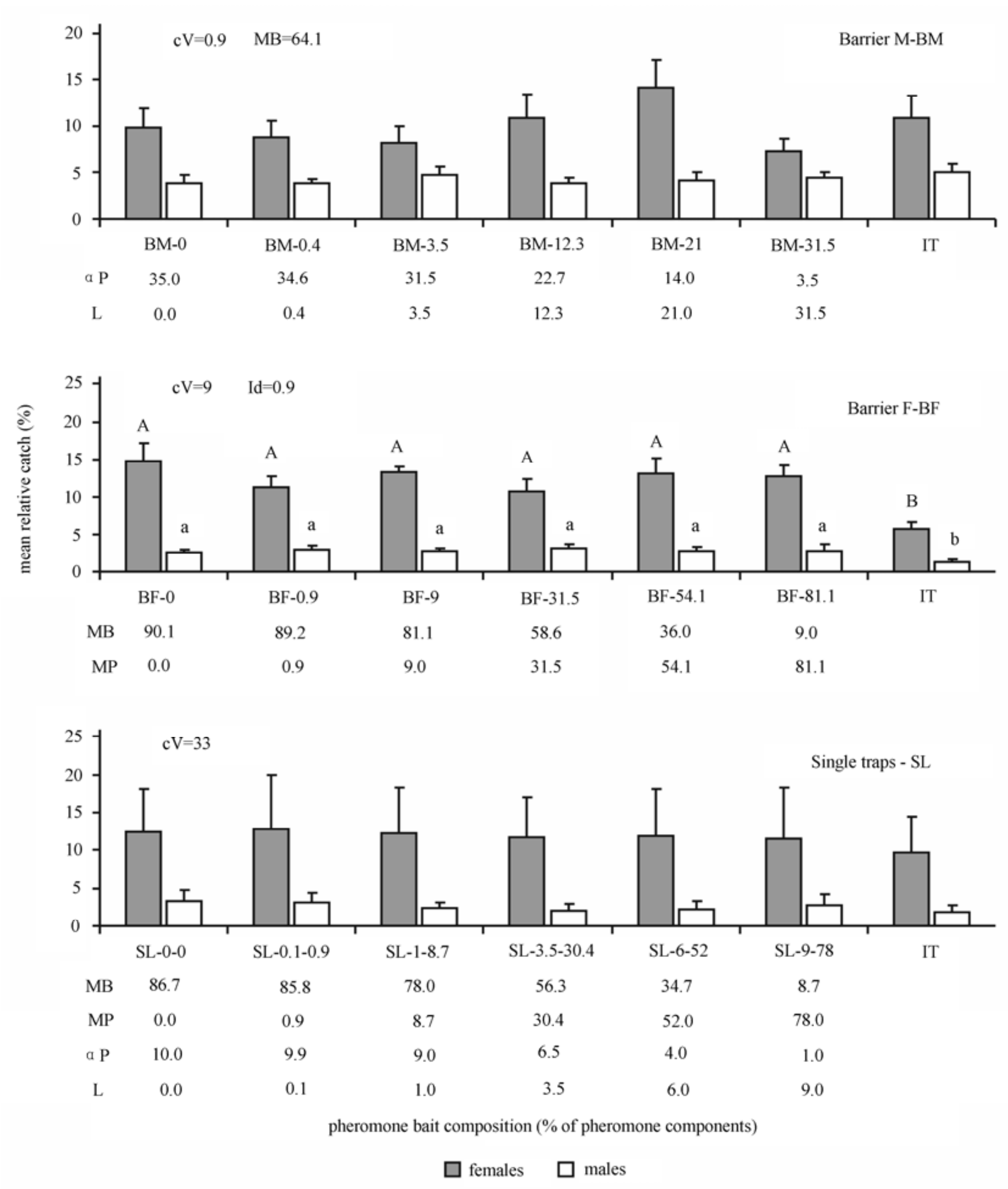

Fig. 2 Mean relative catch and SEs of *Ips typographus* in different variants tested in pheromone trap barriers and in single traps. Bars with the same letter are not different according to the Fisher's LSD test. Females and males are considered separately.

Discussion

The blends with greater attractiveness for males (BM) and greater attractiveness for females (BF) when alternated in the barriers influenced the catches (Jakuš and Blaženec 2003). Evaluating the improvement of the mixtures in Barrier M, according to Reddemann (1993) and Reddemann and Schopf (1996), by supplementing (−)-α-pinene and (+)-limonene separately to one-third filled Pheroprax bait, there was no influence on the amount of bark beetles caught in the traps. On the other hand by adding both monoterpenes in equal amounts (1:1), the catch increased significantly by 81%. The closest to this monoterpene ratio was our variant BM-21 with a ratio of (−)-α-pinene to (+)-limonene of 1:1.5. This variant had the highest catches of both sexes together and of females, which is in accord with observations by Reddemann (1993) and Reddemann and Schopf (1996). There was, however, no significant difference among this blend and the others, including the control IT Ecolure. At the same time, the percentage of the males caught in the variant BM-21 was the lowest, and there was a significant difference from the variants BM-3.5 and BM-31.5, which contained the highest percentages of males caught. Hence, the variant BM-21 was not eligible for improvement of mixtures with higher attractiveness for males. These findings are the same as those of Niemeyer and Watzek (1996), according to whom the supplementing of monoterpenes did not cause any statistically significant increase in the catch.

Absolute catch of females for the mixture BF-0, which was used in the fixed traps of the Barrier M experiment, was comparable with the catch of the mixture variants BM and the control IT Ecolure. The same mixture (BF-0), however, when used in the Barrier F experiment resulted in about 2.5 times higher catches of females compared to the control IT Ecolure. Lower catch efficiency of this mixture in fixed traps was probably caused by carrion odours of decomposing bark beetles (Kretschmer 1990) because, unlike in the case of improved mixtures, the beetles were not removed at the time of individual sampling, but were removed only at the end of the study period. Zhang et al. (2003) sought to identify, using gas chromatography coupled with electroantennogram detection (GC-EAD), which compounds were released from the decomposing beetles that caused the sensitivity in the living bark beetles. From the analysis, it was found that both sexes were the most sensitive to 1-hexanol and verbenone; on the other hand, they did not respond to the typical strong smell of the decomposition that is perceived very intensely by humans. It was not possible to decide unambiguously whether 1-hexanol and verbenone are produced by dead beetles or by microorganisms causing the decomposition. However, both compounds have strong synergistic inhibitory effects on the attraction of the spruce bark beetles (Schlyter et al. 1995).

In the individual variants of the Barrier F mixtures, the MB was replaced with MP in differing amounts. In combinations with cV and ipsdienol, a rather effective substitution of MB was obtained with all the variants of BF in the case of the relative catches and most variants in the case of the absolute catches being represented by one homogeneous group. According to Tomescu et al. (1979), MB is the limiting compound in the pheromone mixtures for the spruce bark beetles, and the absence of this component lowered the attractiveness of the mixture. Schlyter et al. (1987b) also pointed out that lowering of the MB content in the mixture lowered the attractiveness of the pheromone mixtures.

According to our results, this limiting component of the pheromone mixture can be successfully substituted with MP without significant differences in trapping efficacy.

Experiments with replacing MB by other compounds have been done before. Eidmann et al. (1986, 1987) successfully replaced MB with 2-methyl-3-butyn-2-ol without significant differences in catch efficacy. However Bakke and Vité (1987) disagreed with the results from Eidmann et al. (1986, 1987), and according to their results, the species-specific MB was twice as effective as 2-methyl-3-butyn-2-ol.

MP is an organic solvent that is less toxic alternative to the short-chained ethylene glycol ethers. The principal health effect of MP is irritancy, with studies showing low toxicity for animals (Jones et al. 1997).

The absolute catch of females and males in the case of the mixture BM-0, which was used in the fixed traps of the Barrier F experiment, was considerably lower than the catch in the improved mixtures of BF. The percentage of males caught was, on the other hand, higher than all the variants or the control. In comparison with the results obtained by Jakuš and Blaženec (2003), there is evidence of a considerable difference. The percentage of males in the experiment performed by these authors and designed on the same background was the second lowest for the mixture used in the fixed traps. However, these authors used a mixture without (–)-α-pinene. In spite of lowering the absolute catch to one half in comparison to the same mixture (BM-0) in the Barrier M, for the same reasons as in the case of their filling (BF-0), the expected ratio between the sexes was not influenced or lowered, probably because of the presence of (−)-α-pinene in the mixture.

Regarding the single traps, the highest catch was recorded in the variant SL-0.1-0.9. A similar situation was found in the relative catches in the case of the females. Relative catches of the SL variants were well equilibrated and higher than in the IT Ecolure. However, neither in the absolute nor in the relative catches were significant differences found among the results.

The results show that the effect of substituting (+)-limonene for (−)-α-pinene is far from obvious, and it is not possible to compare it with the effect found for the BM mixtures. The difference may be a result of the total content of monoterpenes in the pheromone mixture. Monoterpenes in the BM mixtures represent 35% of the total amount, while the SL mixtures contain only 10%. According to the research conducted by Jakuš and Blaženec (2003), the optimum amount of (−)-α-pinene in mixtures for separately standing traps is 10%. For such an amount, it is not possible to achieve great efficacy even through addition of active monoterpenes.

At the same time, the results show that it is possible to replace MB with MP without significant changes in catch efficacy. The highest percentage of males was reached in the variant SL-0.1-0.9, which belonged to a homogeneous group with variants SL-0-0 and SL-9-78, and they were statistically different from the variant SL-3.5-30.4. Considering that the pheromone mixture was released at the same time from one dispenser, it is difficult to draw any conclusions about which chemical had more influence on the male percentage. This is not an indicator of any distinct tendency because the results of the individual variants fluctuate considerably. However with the exception of the variant SL-3.5-30.4, the percentage of males caught was higher than the control, IT Ecolure.

Conclusions

Replacement of (−)-α-pinene with (+)-limonene in the blends to increase the catch of males (BM) actually had no significant effect on the absolute numbers of *I. typographus* males trapped, but a slight effect was found in the percentage of males trapped.

The highest catch of females was not significantly affected by the replacement of 2-methyl-3-buten-2-ol with 1-methoxy-2-

propanol. The catch was similar in all the tested blends; consequently, it is possible to use the 1-methoxy-2-propanol in the mixture with (4*S*)-*cis*-verbenol and ipsdienol without a significant change in the catch efficacy.

Using single traps, the results suggest the possibility of replacement of the 2-methyl-3-buten-2-ol with 1-methoxy-2-propanol in the mixture without a significant change in the catch efficacy. Replacement of (−)-α-pinene with (+)-limonene in the mixtures had only a slight effect in the percentage of males trapped.

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References

Bakke A, Vité JP. 1987. Methylbutynol No Efficient Replacement for the Natural Pheromone Component in *Ips typographus*. *Naturwissenschaften*, **74**: 292–293.

Byers JA. 1991. Basic algorithms for random sampling and treatment randomization. *Comput Biol Med*, **21**: 69–77.

Dickens JC. 1981. Behavioural and electrophysiological responses of the bark beetle, *Ips typographus*, to potential pheromone components. *Physiol Entomol*, **6**: 251–261.

Eidmann HH, Weslien J, Harding S, Baeckstrom P, Norin T. 1986. A Compound Replacing a Natural Pheromone Component of the Spruce Bark Beetle. *Naturwissenschaften*, **73**: 629–630.

Eidmann HH, Baeckstrom P, Harding S, Norin T, Vrkoč J, Weslien J. 1987. Methylbutynol Effectively Replaces Methylbutenol, a Pheromone Component of *Ips typographus* (L.) (Coleoptera, Scolytidae). *J Chem Ecol*, **13**: 1555–1560.

Gossenauer-Marohn, H. 1998. Untersuchungen zum Nachweis der Bedeutung primärer Lockstoffe für die Anlockung des Buchdruckers *Ips typographus* L. (Coleoptera, Scolytidae). Dissertation zur Erlangung des Doktorgrades des Forstwissenschaftlichen Fachbereichs der Goerg-August-Universität zu Göttingen.

Jakuš R. 1998. A method for the protection of spruce stands against Ips typographus by the use of barriers of pheromone traps in North-eastern Slovakia. *Anz Schadlingskd Pfl*, **71**: 152–158.

Jakuš R, Blaženec M. 2002. Influence of proportion of (4*S*)-*cis*-verbenol in pheromone bait on *Ips typographus* (Col., Scolytidae) catch in pheromone trap barrier and in single traps. *J Appl Entomol*, **126**: 306–311.

Jakuš R, Blaženec M. 2003. Influence of the proportion of (−)-*α*-pinene in pheromone bait on *Ips typographus* (Col., Scolytidae) catch in pheromone trap barriers and in single traps. *J Appl Entomol*, **127**: 91–95.

Jakuš R, Šimko J. 2000. The use of dispensers with different release rates at pheromone trap barriers for *Ips typographus*. *Anz Schadl – J Pest Sc*, **73**(2): 33–36.

Jones K, Dyne D, Cocker J, Wilson HK. 1997. A biological monitoring study of 1-methoxy-2-propanol: analytical method development and a human volunteer study. *Sci Total Environ*, **199**: 23–30.

Kretschmer K. 1990. Zur Wirkung von Aasgeruch auf die Fangleistung von Buchdruckerfallen. *Anz Schadlingskd Pfl*, **63**(3): 46–48.

Niemeyer H. 1997. Integrated bark beetle control: experiences and problems in Northern Germany. In: Gregoire, J.C., Liebhold, A.M., Stephen, F.M., Day, K.R., Salom S.M. (eds), *Integrated cultural tactics into the management of bark beetle and reforestation pests*. USDA Forest Service General Technical Report NE - 236, pp. 80–86.

Niemeyer H, Watzek G. 1996. Test von Monoterpenen als Zusatz zu Pheroprax bzw. Chalcoprax in Pheromonfallen zum Fang des Buchdruckers, *Ips typographus* L., bzw des Kupferstechers, *Pityogenes chalcographus* L. (Col., Scolytidae). *Anz Schadlingskd Pfl*, **69**: 109–110.

Reddemann J. 1993. Monoterpenkohlenwasserstoffe in der Aggregation von *Ips typographus* L. (Coleoptera, Scolytidae). Inaugural - Dissertation zur erlangung der Doktorwürde der Forstwissenschaftlichen Fakultät der Ludwig- Maximilians-Universität zu München, 134 pp.

Reddemann J, Schopf R. 1996. The importance of monoterpenes in the aggregation of the spruce bark beetle *Ips typographus* (Coleoptera: Scolytidae: Ipinae). *Entomol Gen*, **21**(1-2): 69–80.

Schlyter F, Byers JA, Löfqvist J. 1987a. Attraction to pheromone sources of different quantity, quality and spacing: Density regulation mechanisms in bark beetle *Ips typographus*. *J Chem Ecol*, **13**(6): 1503–1523.

Schlyter F, Löfqvist J, Byers JA. 1987b. Behavioural sequence in the attraction of the bark beetle *Ips typographus* to pheromone sources. *Physiol Entomol*, **12**: 185–196.

Schlyter F, Löfqvist J, Jakuš R. 1995. Green leaf volatiles and verbenone modify attraction of European *Tomicus, Hylurgops*, and *Ips* bark beetles. In: Hain, F.P., Salom, S.M., Ravlin, W.F., Payne, T.L., Raffa, K.F. (eds), *Behavior, Population Dynamics, and Control of Forest Insects*. Proceedings of a Joint IUFRO Working Party Conference – February 1994, Ohio State Univ., OARDC, Wooster, pp. 29–44.

Tomescu N, Dusa L, Stan Gh, Opreanu I, Hodosan F, Tautan L. 1979. Response of *Ips typographus* L. (Coleoptera, Scolytidae) to aggregation pheromone in mixture with alpha pinene. *Revue Roumaine de Biologie, Serie de biologie animale,* **24**(2): 177–181.

Underwood AJ. 2001. Experiments in ecology. Their logical design and interpretation using analysis of variance. Cambridge University Press, 500 pp.

Varkonda Š. 1996. Pheromone dispenser, Industrial pattern 5720, Office of industrial ownership of the Czech Republic.

Vité JP. 1989. The European struggle to control *Ips typographus* – past, present and future. *Holarctic Ecol*, **12**: 520–525.

Zhang Q-H, Jakuš R, Schlyter F, Birgersson G. 2003. Can *Ips typographus* (L.) (Col.: Scolytidae) smell the carrion odours of the dead beetles in pheromone traps? Electrophysiological analysis. *J Appl Entomol*, **127**: 185–188.