

Non-target Bark Beetles in *Ips duplicatus* (Sahlberg) Pheromone Traps Baited with Host Volatiles

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Abstract

Response of several non-target bark beetles (Coleoptera: Curculionidae, Scolytinae) to different combinations of the northern spruce bark beetle's synthetic pheromone with the monoterpenes (-)-alpha-pinene and (+)-limonene has been studied in choice experiments in the field with flight barrier traps. The experiments were organized in four Norway spruce stands (40-50 years old) outside its natural area, in the north-east of Romania, where *Ips duplicatus* (Sahlberg) populations had reached an epidemical level. Each experiment had five treatments randomly replicated in six blocks within each experimental plot. Four non-target bark beetle species were captured together with *Ips duplicatus*: *I. typographus* (L.) (2611 beetles), *Pityogenes chalcographus* (L.) (184 beetles), *Hylastes cunicularius* Erichson (107 beetles) and *Dryocoetes autographus* (Ratzeburg) (24 beetles), representing 1.77%, 0.13%, 0.07% and 0.02% respectively of total captures. Beetles of *I. typographus* were attracted by synthetic pheromone blend of *I. duplicatus* and have intensified their response in the presence of (-)-alpha-pinene or a combination between (-)-alpha-pinene and (+)-limonene, but the other species have been captured in the traps accidentally. The positive response of *I. typographus* to the present formulation of *I. duplicatus* pheromone suggests the possibility to use the pheromone dispensers for both species in the same traps when mass-trapping is the main goal, but new studies should clarify the real effects of putting together pheromone dispensers of *I. typographus* or *P. chalcographus* with those of *I. duplicatus*.

Keywords: by-catches, northern spruce bark beetle, synthetic attractants, (-)-alpha-pinene, (+)-limonene

Introduction

Since 1990, the northern spruce bark beetle, *Ips duplicatus* (Sahlberg), has become an important pest of Norway spruce stands not only in the Central Europe (Grodzki, 1997; Holuša *et al.*, 2013), but also in Romania (Olenici *et al.*, 2011). This process has urged researchers to come up with means for monitoring and control of its populations. Pheromone traps have been usually used for monitoring or mass-trapping bark beetles (Gitau *et al.*, 2013), but frequently many non-target species are captured, either accidentally or as response to different pheromone components (Babuder *et al.*, 1996; Valkama *et al.*, 1997; Schmidt *et al.*, 1999). Mainly, the non-target species are bark beetles that share some pheromone components with the target species (Mendel, 1988). Nevertheless, semiochemicals released from traps are also used as kairomones by competitor species, like wood-boring beetles in the genus *Monocharmus* Dejan (Allison *et al.*, 2001, 2004) or by predators, mainly clerid, histerid and nitidulid beetles (Bakke and Kvamme, 1981; Hansen, 1983; Avtzis, 1991). The number of non-target species captured is even higher when host volatiles are associated to pheromone

baits (Miller *et al.*, 2011; Panzavolta *et al.*, 2014), because host volatiles are used by many species as kairomones to find their oviposition substrate or their prey. Consequently, knowing which non-target species respond to different pheromone lures or combinations of pheromone and host volatiles may be relevant to understand the chemical ecology of different insect species, to study the abundance of the bark beetle predators (Williams *et al.*, 2009; Sharon *et al.*, 2012), to selectively remove pests (Aukema *et al.*, 2000; Dahlsten *et al.*, 2003), or to simultaneously attract a variety of target pest species (Hanks *et al.*, 2012).

There are several studies on the pheromones of *I. duplicatus* (Bakke, 1975; Byers *et al.*, 1990; Schlyter *et al.*, 1992; Ivarsson *et al.*, 1993; Ivarsson and Birgersson, 1995) and their use in forest protection (Schlyter *et al.*, 2001), but none considering the non-target species attracted by synthetic pheromones of this species. This is the reason to present in this paper the data on non-target scolytine species caught during field tests concerning the response of *I. duplicatus* to different combinations of synthetic pheromone with (-)-alpha-pinene and (+)-limonene that was presented in a previous paper (Duduman, 2014).

Table 1. The treatments used in the experiments [Id – ipsdienole, EM – E-myrcenole, MB – methyl-buthenole, AP – (-)-alpha-pinene, L – (+)-limonene]

Treatment	Specification of dispensers (composition and release rates)	Ration of pheromone to monoterpenes (Id+EM) : AP : L
Experiment 1: May 16 - June 3, 2011		
V1.1	[1Id:1EM:38MB] 20 mg/day	1 : 0 : 0
V1.2	[1Id:1EM:38MB] 20 mg/day + [AP] 40 mg/day	1 : 40 : 0
V1.3	[1Id:1EM:38MB] 20 mg/day + [AP] 200 mg/day	1 : 200 : 0
V1.4	[1Id:1EM:38MB] 20 mg/day + [AP] 1000 mg/day	1 : 1000 : 0
V1.5	control (blank)	0 : 0 : 0
Experiment 2: June 24 - July 10, 2011		
V2.1	[1Id:1EM:38MB] 20 mg/day	1 : 0 : 0
V2.2	[1Id:1EM:38MB] 20 mg/day + [L] 40 mg/day	1 : 0 : 40
V2.3	[1Id:1EM:38MB] 20 mg/day + [L] 200 mg/day	1 : 0 : 200
V2.4	[1Id:1EM:38MB] 20 mg/day + [L] 1000 mg/day	1 : 0 : 1000
V2.5	control (blank)	0 : 0 : 0
Experiment 3: July 16-28, 2011		
V3.1	[1Id:1EM:38MB] 20 mg/day	1 : 0 : 0
V3.2	[1Id:1EM:38MB] 20 mg/day + [AP] 40 mg/day + [L] 40 mg/day	1 : 40 : 40
V3.3	[1Id:1EM:38MB] 20 mg/day + [AP] 200 mg/day + [L] 200 mg/day	1 : 200 : 200
V3.4	[1Id:1EM:38MB] 20 mg/day + [AP] 1000 mg/day + [L] 1000 mg/day	1 : 1000 : 1000
V3.5	control (blank)	0 : 0 : 0
Experiment 4: May 19 – June 19, 2012		
V4.1	[1Id:1EM:38MB] 20 mg/day	1 : 0 : 0
V4.2	[1Id:1EM:18MB] 20 mg/day	2 : 0 : 0
V4.3	[1Id:1EM:38MB:1AP:1L] 20 mg/day	1 : 1 : 1
V4.4	[1Id:1EM:18MB:0,5AP:0,5L] 20 mg/day	2 : 1 : 1
V4.5	[1Id:1EM:18MB:1AP:1L] 20 mg/day	2 : 2 : 2

Materials and Methods

As all details concerning the material and methods were published with the main results of the experiments (Duduman, 2014), the present study has been focused on those elements necessary for understanding the results concerning non-target bark beetles.

Experimental site

The data were produced by four experiments deemed to evaluate the response of *I. duplicatus* to different combinations of synthetic pheromone, alpha-pinene (AP) and limonene (L). These experiments were conducted during the spring and the summer of 2011 and 2012, in four areas with pure Norway spruce stands (40-50 years old) growing outside the natural area of the species, in the north-eastern Romania (Suceava county), where *I. duplicatus* populations have already reached an epidemical level in the previous years. The experiments were installed in clear-cut areas, along the edges of the stands. The first three experiments (Table 1) were conducted in 2011 in the plots Zamostea (47°52'46.31"N; 26°08'33.38"E; 375 m a.s.l.); Calafindești (47°51'05.11"N; 26°08'46.97"E; 490 m a.s.l.) and Fetești (47°43'04.52"N; 26°19'28.88"E; 400 m a.s.l.), while the 4th was conducted in 2012, in the experimental plots Calafindești, Fetești and Mitocaș (47°44'58.70"N; 26°15'16.87"E; 440 m a.s.l.).

Experimental design

The experimental design was the same for all four experiments. Each experiment had five treatments randomly replicated in six blocks within each experimental plot. In order to reduce the influence of the trap position over the insects captures, the treatments were moved by one position within each block observing the four permutations conceived for each experiment.

The synthetic lures used as treatments were installed in flight intercept traps. The traps were placed at 15 m from each other and 12-14 m from the forest edge. The minimum distance between two blocks was 15 m.

The treatments in experiments 1-3 combined various synthetic pheromone compounds (ipsdienole (Id), E-myrcenole (EM) and methyl-buthenole (MB) in ratio 1Id : 1EM : 38MB) released at constant rate, and terpenes (AP and L, each released at different rates) (Table 1). In the 4th experiment the treatments consisted of mixtures of pheromone and terpenes, released at a constant rate from the same dispenser, the differences between treatments being achieved by changing the ratios of the pheromone components and terpenes (Table 1).

As described by Duduman (2014), the pheromone and alpha-pinene dispensers used in the experiments have been made from polyethylene envelopes with different dimensions. Each envelope contained a cellulosic support impregnated with the mixture of pheromone components or with alpha-pinene. The limonene dispensers consisted of polypropylene bottles, which contained similar cellulosic supports for the active compound.

Collection and processing of captured insects

The insects captured in the traps were collected at every 3-4 days in experiments 1-3 and at 7 days in experiment 4. Afterward the captures were stored in a freezer awaiting laboratory analyses, which consisted in sorting, identifying and counting the bark beetles.

Data analysis

In order to find out the differences between the blocks and the treatments, the data concerning *I. typographus* (L.) were analysed by ANOVA at confidence level of 95%. The very low number of beetle captures from other species precluded further statistical analysis. There were less than 100 beetles cumulated per experiment and area, equivalent to 20 insects per treatment. The homogeneity of variances has been tested using the Hartley test, and, when necessary, the data were log-transformed ($x' = \log(x+1)$) to obtain homogenous variances. When the homogeneity was not confirmed, the heterogeneous population of data that induced inhomogeneity was eliminated from analyses.

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Table 2. Total number of bark beetles captured in each experiment

Location	<i>Ips duplicatus</i>	<i>Ips typographus</i>	<i>Pityogenes chalcographus</i>	<i>Hylastes cunicularius</i>	<i>Dryocoetes autographus</i>
Experiment 1					
Zamostea	4662	230	15	13	2
Calafindești	3105	479	24	47	6
Fetești	1675	194	12	42	1
Experiment 2					
Zamostea	21700	35	22	0	0
Calafindești	27861	121	17	0	0
Fetești	13843	101	9	0	0
Experiment 3					
Zamostea	10085	19	14	0	0
Calafindești	3563	314	7	0	0
Fetești	2000	106	21	0	0
Experiment 4					
Mitocaș	22737	411	3	2	6
Calafindești	17961	586	22	2	7
Fetești	14999	15	18	1	2

Table 3. ANOVA results of the effects of treatments and blocks, as well as their interactions on *I. typographus* captures (DF – degrees of freedom, F – Fisher's test, P – the probability that the null hypothesis is true)

Statistical value	Zamostea/Mitocaș			Calafindești			Fetești		
	DF	F	P	DF	F	P	DF	F	P
Experiment 1									
Treatment	3	21.028	< 0.001	3	4.162	0.045	3	0.571	0.643
Block	5	12.373	< 0.001	5	2.807	0.055	5	5.267	0.005
Treatment x Block	15	1.801	0.099	15	3.128	0.095	15	1.256	0.116
Experiment 2									
Treatment	-	-	-	3	1.447	0.282	3	1.058	0.431
Block	-	-	-	5	7.531	< 0.001	5	9.526	< 0.001
Treatment x Block	-	-	-	15	1.643	0.276	15	1.303	0.108
Experiment 3									
Treatment	-	-	-	3	12.871	< 0.001	3	4.972	0.003
Block	-	-	-	5	8.682	< 0.001	5	3.097	0.031
Treatment x Block	-	-	-	15	1.807	0.090	15	0.824	0.569
Experiment 4									
Treatment	4	0.776	0.554	4	0.419	0.793	-	-	-
Block	5	6.611	0.001	5	22.511	< 0.001	-	-	-
Treatment x Block	20	1.591	0.083	20	1.187	0.245	-	-	-

The normality of the distributions was tested using the Shapiro-Wilk test. When significant differences were found, Tukey's honest significant difference for multiple comparison test was applied for the mean separation. All statistical computations were done using XLSTAT-Pro 2012 software, plugged into MS Excel.

Results

Four non-target bark beetle species were captured together with *I. duplicatus*: *I. typographus* (n=2611 beetles), *Pityogenes chalcographus* (L.) (n=184 beetles), *Hylastes cunicularius* Erichson (n=107 beetles) and *Dryocoetes autographus* (Ratzeburg) (n=24 beetles) (Table 2), representing 1.77%, 0.13%, 0.07% and 0.02% respectively of total captures.

Most *I. typographus* beetles have been captured at Calafindești (all experiments) and the least at Zamostea (experiments 2-3) and Fetești (experiments 1 and 4), reflecting the variations of population level between the three locations.

P. chalcographus was captured in all experimental areas, and in all experiments. In general the differences between captures in experimental areas or experiments are small. *H. cunicularius* and *D. autographus* have been captured in all experimental areas, but only in experiments 1 and 4. Most of individuals of the first and the second aforementioned species were found on the experiment 1 and 4, respectively (Table 2).

Analyses concerning the influence of the different factors (treatment and experimental block) on the responses of bark beetles associated with *I. duplicatus* were conducted only for *I. typographus*, when the number of captures exceeded 100 insects. The block position significantly influenced *I. typographus* response in almost all situations (except for the 1st experiment at Calafindești). A statistically significant influence of the treatments on the *I. typographus* response was found only for experiment 1, at Zamostea and Calafindești, and in experiment 3 at Calafindești and Fetești (Table 3).

The treatments generated different responses in associated bark beetles species. In the 1st experiment, the beetles responded intensely to the treatments with AP (V1.2, V1.3) within the experimental areas Zamostea and Calafindești. These treatments attracted more beetles than the treatment without AP (V1.1) or the treatment with high release rate of AP (V1.4). The presence of L alongside the pheromone lures in the treatments V2.2, V2.3 and V2.4 (experiment 2) did not lead to a different response of *I. typographus* beetles compared with the one from pheromone lure (V2.1), and only the blank traps have captured significantly fewer beetles. Adding both AP and L to the pheromone (experiment 3) has increased the captures of *I. typographus*. The number of captures has also increased, as the terpene released rates has increased from 40 to 1000 mg/day (expected rates). A significant increase of attractiveness has been observed only by increasing the release rates of monoterpenes to at least 200 mg/day

Table 4. Response of the non-target bark beetles to the tested treatments

Treatment	Number of captured bark beetles/trap (mean ± SEM)											
	<i>Ips typographus</i>			<i>Pityogenes chalcographus</i>			<i>Hylastes cunicularius</i>			<i>Dryocoetes autographus</i>		
	Zamostea/ Mitocaş	Calafindeşti	Feteşti	Zamostea/ Mitocaş	Calafindeşti	Feteşti	Zamostea/ Mitocaş	Calafin- deşti	Feteşti	Zamostea/ Mitocaş	Calafin- deşti	Feteşti
Experiment 1												
V1.1	3.9±1.1 ^b	16.8±5.2 ^b	7.2±6.4 ^a	0.7±0.6	0.4±0.2	0.3±0.1	0.2±0.1	1.5±0.9	2.0±1.5	-	0.1±0.1	-
V1.2	17.5±4.9 ^a	27.0±4.1 ^a	9.3±6.9 ^a	0.4±0.3	0.9±0.7	0.1±0.1	0.2±0.1	1.4±1.2	1.2±0.8	-	0.2±0.1	-
V1.3	15.3±4.0 ^a	27.8±6.5 ^a	8.3±3.7 ^a	0.2±0.1	1.0±0.8	0.4±0.3	0.5±0.3	0.9±0.6	1.9±1.2	-	0.3±0.2	-
V1.4	4.5±2.1 ^b	8.17±1.9 ^b	7.5±4.1 ^a	0.8±0.6	0.4±0.3	0.6±0.4	0.7±0.5	3.8±1.9	1.5±1.1	0.2±0.1	-	0.2±0.2
V1.5	0.3±0.2 ^c	0.8±0.5 ^c	0.2±0.1 ^b	0.2±0.2	0.8±0.6	0.5±0.4	0.6±0.5	0.4±0.4	1.2±0.8	-	0.2±0.2	-
Experiment 2												
V2.1	1.8±1.2	7.3±2.7 ^a	7.5±4.7 ^a	1.2±0.7	0.2±0.1	0.4±0.3	-	-	-	-	-	-
V2.2	1.7±1.1	4.5±1.8 ^a	4.3±2.6 ^a	0.2±0.1	0.9±0.7	0.1±0.1	-	-	-	-	-	-
V2.3	1.5±0.6	3.0±1.3 ^a	2.8±1.5 ^a	0.6±0.4	0.2±0.2	0.5±0.3	-	-	-	-	-	-
V2.4	0.7±0.6	2.1±0.8 ^a	1.5±1.3 ^a	0.7±0.3	1.1±0.8	0.1±0.1	-	-	-	-	-	-
V2.5	0.2±0.2	0.1±0.1 ^b	0.3±0.2 ^b	0.5±0.4	0.5±0.4	0.1±0.1	-	-	-	-	-	-
Experiment 3												
V3.1	0.2±0.1	4.2±1.8 ^b	1.0±0.5 ^b	0.4±0.3	0.2±0.1	0.9±0.7	-	-	-	-	-	-
V3.2	0.3±0.2	4.2±2.4 ^b	2.8±1.3 ^b	0.6±0.4	0.5±0.3	0.6±0.5	-	-	-	-	-	-
V3.3	1.2±0.3	20.7±7.8 ^a	6.7±2.6 ^a	0.3±0.2	0.1±0.1	1.0±0.8	-	-	-	-	-	-
V3.4	1.7±0.4	23.3±7.3 ^a	7.2±3.3 ^a	0.5±0.4	0.4±0.2	0.8±0.5	-	-	-	-	-	-
V3.5	0.1±0.0	0.1±0.1 ^c	0.2±0.1 ^c	0.3±0.3	0.1±0.1	0.2±0.1	-	-	-	-	-	-
Experiment 4												
V4.1	13.6±3.9 ^a	17.7±7.0 ^a	0.5±0.3	0.0±0.0	0.5±0.4	0.9±0.8	-	0.2±0.1	-	0.3±0.2	0.2±0.1	-
V4.2	10.0±1.8 ^a	23.0±11.4 ^a	0.2±0.2	0.1±0.1	1.1±0.8	0.3±0.2	0.2±0.1	-	-	0.2±0.2	0.5±0.5	-
V4.3	19.5±6.3 ^a	15.7±6.9 ^a	0.5±0.4	0.0±0.0	0.7±0.5	0.5±0.4	-	0.1±0.1	-	0.2±0.1	0.4±0.3	-
V4.4	15.6±4.1 ^a	14.7±6.5 ^a	1.0±0.5	0.3±0.2	1.0±0.9	0.2±0.2	0.1±0.1	-	0.1±0.1	0.1±0.1	0.2±0.1	-
V4.5	10.0±2.7 ^a	26.7±10.7 ^a	0.3±0.2	0.2±0.1	0.3±0.2	0.9±0.7	-	0.1±0.1	-	0.2±0.2	-	-

Note: For each experiment, location and species, the values in the columns followed by the same letters do not differ significantly at $P < 0.05$ (Tukey's multiple comparison test). Values that are not followed by letters were not subjected to statistical analysis.

(V3.3 and V3.4) (Calafindeşti and Feteşti). Also, significantly fewer beetles have been captured in blank traps (V3.5) than in baited ones. In experiment 4, the response of *I. typographus* beetles has not been modified by doubling the release rates of pheromone components and adding small quantities of AP and L.

As for *P. chalcographus*, the captures were low regardless of the experiment, treatment or experimental area, and the blank traps captured similar numbers of beetles as did the baited traps. *H. cunicularius* has also been scantily captured in all treatments tested in experiment 1 and in all experimental areas, without any preference. In the experiment 4, the small captures of *H. cunicularius* were quite irregularly distributed between treatments and experimental areas, not indicating any tendency of beetle response to treatments. *D. autographus* beetles were caught in experiment 1 (mainly at Calafindeşti) and experiment 4 (Zamostea and Calafindeşti), and the distribution of captures between the treatments does not indicate a preference of this species for any tested volatile mixtures (Table 4).

Discussion

Even though *I. typographus* captures were proportionally small, this species was clearly attracted by pheromone lures in the first three experiments, and this response was expected due to the presence of MB and Id in the lure composition. Both substances are pheromone components of the European spruce bark beetle (Bakke, 1976; Bakke *et al.*, 1977). On the other hand, the small captures of this species reflect not only the lower level of *I. typographus* populations comparing with *I. duplicatus*, but also the inhibitory effect of EM at high release rates of other pheromone components, as already noted Schlyter *et al.* (1992).

Adding the terpenes to the pheromone lures has increased *I. typographus* captures. The intensification of this species response to the pheromone in the presence of AP was showed by Erbilgin *et al.* (2007), who used as pheromone components only MB and cis-

verbenol. However, in the presence of high release rates of AP with (945.5 mg/day), in our experiments, *I. typographus* responded to the same extent as to the pheromone alone. The high concentration of terpenes did not induce any reaction on bark beetles. A higher rate of AP might have induced a reduced response, as noted in other research (Olenici *et al.*, 2007). Likewise *I. duplicatus* (Duduman, 2014), the L presence has not affected *I. typographus* response to pheromones, confirming the results obtained by Reddeman and Schopf (1996). The intensification of *I. typographus* response to pheromone in the presence of both monoterpenes (AP+L) is supported by the results of previous researches (Reddeman and Schopf, 1996; Hulcr *et al.*, 2006), which revealed a similar behaviour when the specific pheromone for this species was used.

The small number of *P. chalcographus* captured and the similarity of its response to all tested treatments (including the blank traps) show that the individuals of this species might have entered accidentally into the traps, without being attracted by the volatile combinations. Moreover, in other experiments it was found that the presence of AP (released with approx. 170 mg/day) did not lead to changes of *P. chalcographus* response to a specific pheromone (Niemayer and Watzek, 1996).

H. cunicularius and *D. autographus* were captured in small numbers and only in the experiments where no treatments with L were used. From previous studies it is known that the first species is not attracted by AP alone, but by a mixture of terpene and ethanol (Schroeder and Lindelöw, 1989; Lindelöw *et al.*, 1993), while *D. autographus* is attracted by a mixture of AP and pheromone component ex-brevicommin (Gandhi *et al.*, 2009). Also, both species are attracted by host volatiles released by the stored spruce material (Lindelöw and Risberg, 1992; Tunset *et al.*, 1993), especially from tree roots (Eidmann *et al.*, 1991).

The lack of *H. cunicularius* captures in the 2nd and the 3rd experiment could also be a result of the differences between the flight patterns of this species and the other ones, *H. cunicularius*

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flying earlier, especially at low altitudes, mainly in April (Postner, 1974), while *I. typographus*, *I. duplicatus*, *P. chalcographus* and *D. autographus* are flying in midsummer and all of them, excepting the last one, have a quite similar pattern of flight activity (Wegensteiner and Führer, 1991; Holuša *et al.*, 2012).

Given our results and considering that the synthetic pheromone of *I. typographus* attracts many *I. duplicatus* beetles (Valkama *et al.*, 1997), it would be possible to set in the same traps the pheromone dispensers of both species without affecting the captures of either species, thereby reducing the costs of control when these are the main pests. However, new tests should be conducted to clarify what happens with each species, because high release rates of EM inhibit the response of *I. typographus* males (Schlyter *et al.*, 1992). Also, new studies are necessary to determine what happens if synthetic pheromone of *I. duplicatus* is used together with that of *P. chalcographus*.

Conclusions

Among the bark beetle species associated with *I. duplicatus*, which have been captured in traps baited with tested treatments, only *I. typographus* has been attracted by synthetic pheromone of *I. duplicatus* and has intensified its response in the presence of (-)-alpha-pinene or a combination between (-)-alpha-pinene and (+)-limonene. The other species (*P. chalcographus*, *H. cumicularius*, *D. autographus*) have been captured in the traps almost accidentally.

The positive response of *I. typographus* to the present formulation of *I. duplicatus* synthetic pheromone suggests the possibility of using pheromone dispensers for both species in the same traps, to reduce the costs of mass-trapping these pests.

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