Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Hort Agrobot, 2012, 40(1): xx-xx

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Effects of Volatile Emissions of *Picea abies* Fresh Debris on *Ips duplicatus* Response to Characteristic Synthetic Pheromone

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Abstract

Ips duplicatus is an important pest of Norway spruce (*Picea abies*) planted outside of its natural range. This species uses olfactory signals to identify the spruce trees favourable for colonisation. The knowledge of the beetles' response to these stimuli is important for managing this pest. Therefore, the response of *Ips duplicatus* to a specific synthetic pheromone was investigated under some natural sources of volatile emissions characteristic of Norway spruce. The pheromone traps were installed in two types of forests: one with fresh and large Norway spruce stumps and piles of branches, releasing large amounts of host volatile substances (terpenes and alcohols) and one without such fresh material. The experiment was repeated in three pairs of sites located in plantations out of the natural range of Norway spruce. Finally it was found that *Ips duplicatus* beetles, regardless of sex, have been concentrated in areas where large amounts of fresh material were available, confirming that *Ips duplicatus* beetles are using both natural pheromones and specific host volatiles as olfactory stimuli in searching for food.

Keywords: *Ips duplicatus*, natural host volatiles, spruce, synthetic pheromone

Introduction

The northern bark beetle *Ips duplicatus* (Sahlberg), (Coleoptera: *Curculionidae*, *Scolytinae*) is a common species in the conifer forests from Boreal Eurasia, extending its range during the twentieth century in the Central and South East Europe, where it is considered an invasive species (DAISIE, 2009; Pfeffer, 1995; Sauvard *et al*., 2010; Turcani *et al.,* 2001; Zúbrik *et al*., 2006). In Romania, this species is present in most of the Norway spruce area growing at less than 1000 m altitude a.s.l. (Duduman *et al*., 2011), producing outbreaks only in Norway spruce plantations installed outside the natural range, in the North-Eastern part of the country, where, in 2009, killed about 14,000 spruce trees (between 30 and 50 years old) (Olenici *et al*., 2009, 2011).

Like other bark beetles species, *I. duplicatus* identifies the already attacked trees through a series of stimuli, an important role being played by olfactory sense. Among these stimuli, the aggregative pheromones play an essential role in coordinating the beetle attack against the host tree (Blomquist *et al*., 2010; Rudinsky, 1962; Wood, 1982). Nevertheless the spectrum and the quantity of volatiles released by the hosts (terpenes and alcohols) have a special importance in finding new host trees. This was already demonstrated for *Ips typographus*, where the monotherpenes (with other volatile) released by the Norway spruce trees are important olfactory signals for the beetles, before colonizing new trees (Baier *et al*., 1999; Byers *et al*., 2000; Rudinsky *et al*., 1971). These conclusions were also confirmed in two studies carried out on the behaviour of *Dendroctonus rufipennus* (Moeck, 1978) and *I. typographus* (Austara *et al*., 1986) in areas with fresh stumps and branch piles.

The present study tried to find out if the *I. duplicatus* beetles behave as most bark beetle species do. The initial assumption was that *I. duplicatus* would concentrate more in areas were a great quantity of host volatiles being released by large amounts of fresh wood debris.

Materials and methods

The field tests of *I. duplicatus* response to synthetic aggregative pheromones were carried out in the North-Eastern part of Romania, in three Norway spruce plantations (Zamostea, Calafindeşti and Feteşti) outside of the species natural range. Plantations are under administration of Pătrăuţi and Adâncata forest districts (subunits of the Suceava County Branch of the National Forest Administration Romsilva). The stands with similar characteristics (Tab. 1) have strong populations of this bark beetle species, which attacked and killed many of the spruce trees of the foci developed. In each location two experimental plots were installed: one nearby a stand edge recently created after a clear-cutting in January-March 2011, with large and similar amounts of fresh host material (branches, stumps etc.), and another at an old stand edge, without any fresh spruce debris, so with minimal sources of host

Tab. 1. Location and the main characteristics of the experimental plots

No	Experimental plots	Abre-viation	Forest district	P.U.*	m.c.**	Coordinates	Elevation (m)	Age (years)	Fresh host material
1	Zamostea 1	Z1	Adâncata	VIII	3B	47°52'52.02"N 26°08'32.46"E	375	40	Present
2	Zamostea 2	Z2	Adâncata	VIII	4A	47°53'11.04"N 26°08'43.49"E	335	40	Not present
3	Calafindești 3	C3	Pătrăuți	III	22A	47°50'59.57"N 26°08'36.87"E	490	40	Present
4	Calafindești 4	C4	Pătrăuți	III	22A	47°51'03.09"N 26°08'50.16"E	496	40	Not present
5	Fetești 5	F5	Adâncata	VI	36A	47°43'05.03"N 26°19'27.95"E	400	40	Present
6	Fetești 6	F6	Adâncata	VI	61B	47°43'32.74"N 26°21'02.66"E	350	40	Not present

Note: * P.U. - production unit; ** m.c. - management compartment

volatiles, considered control plot . The two types of surface were treated as experimental replicates.

In each experimental plot, five pheromone traps were installed, at 15 m one from another and at 10-12 m from the forest edge. In Z1, Z2, F5 and F6 experimental plots (without fresh host material) the Intercept® wing-type traps were installed. In area C3 and C4 there were used Theysohn type traps. The traps were baited with synthetic pheromone specific for *I. duplicatus*. The experiment was conducted between 16 May and 15 June 2011 and the trap catches were collected periodically, after 6-9 days. In each location the temperature was monitored during the experiment with field sensor data logger (Hobo® U23-001, USA).

The synthetic pheromone used is based on the distinctive components of the natural aggregation pheromone of *I. duplicatus*: ipsdienol (Id) and E-myrcenol (EM) (Bakke, 1975; Byers *et al.*, 1990). Equal shares of these two components are considered optimal for the European populations of this bark beetle (Schlyter *et al.*, 2001). To obtain the synthetic pheromone, these components were dissolved in metylbuthenol (MB), which is olfactory indifferent for *I. duplicatus*. The ratio used for the combination was: 1Id/1EM/33MB. To prevent oxidation of this compound, Buthylated hidroxytoluene (BHT) antioxidant was added: 2.5 g per 100 ml of pheromones, as prescribed by Erbilgin *et al*. (2007). Different producers supplied the chemical components used in the experimental field, as shown in Tab. 2.

Tab. 2. Characteristics of chemical compounds used to synthetic pheromones

Compound	Purity	Supplier
Ipsdienol Racemic (Id)	≥93%	Bedoukian Research inc., USA
E-myrcenol (EM)	≥95%	„Raluca Ripan" Institute for Research Chemistry, Romania
Methilbuthenol (MB)	≥98%	Sigma Aldrich GmbH, Germany
Buthylated hidroxytoluene (BHT)	≥99%	Sigma Aldrich GmbH, Germany

The dispensers (50 × 70 mm envelopes) used to release of the pheromone were made from low-density polyethylene film of 50 μm. Each envelope has contained a 55 × 40 mm cellulose support for the pheromone mixture; both the polyethylene film and the cellulose support were provided by "Raluca Ripan" Institute of Chemistry Cluj Napoca. The dispensers were injected with 3.5 ml of pheromones mixture. The released rate was determined in standard condition (20°C, RH 50%) provided in a climatic chamber (Conviron G30, Canada); after 10 days of testing the average release rate of the pheromonal mixture, for 10 dispensers, was 23.2 ± 0.4 mg/day.

The analysis of the biological captures was done in laboratory conditions; all *I. duplicatus* beetles were sexed when catches were less than 50 beetles/trap. For larger captures, the beetles were sexed in groups of 50 randomly chosen individuals according to the same procedure used by Lobinger (1996), Erbilgin *et al*. (2007) and Blaženec and Jakuš (2009) for *I. typographus.* The sexes were identified by analysing the genital armature of the adult after dissection.

The data collected from field catches were analysed by ANOVA, looking for the differences between the experimental variants and trying to detect the interaction between experimental trials and the sex of insects. The hypothesis of normal distribution, required by the ANOVA tests, was checked for each distribution, using the Shapiro-Wilk test. The significance of differences between average values was tested using the Tukey test (Zar, 2010); all statistical computations were done using XLSTAT-Pro 7.5 software, plugged into MS Excel.

Results

Throughout the field experiment, recorded daily maximum air temperatures in all locations (Fig. 1) were frequently over 16,5°C, value considered as the minimum threshold for flight of *I. typographus* (Lobinger, 1994). Therefore, as seasonal activity of both beetle species is

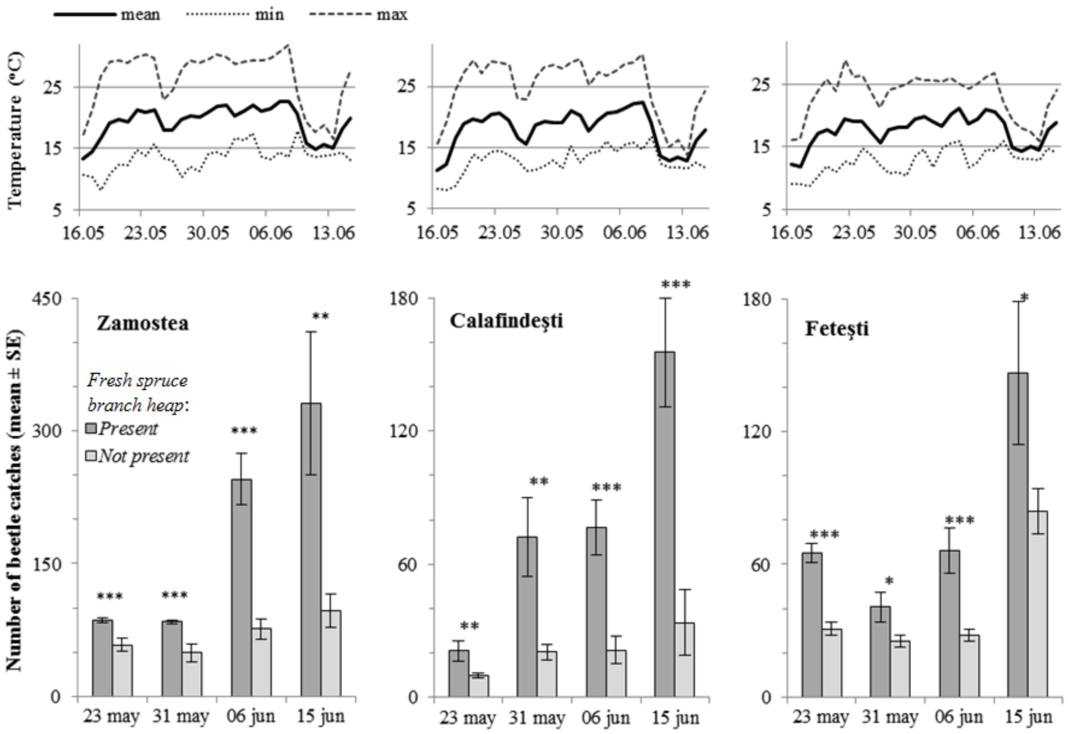

Fig. 1. The response of the *I. duplicatus* beetles to the specific synthetic pheromone in areas with fresh host material comparing to the areas without host material. The air temperature conditions throughout the duration of the experiment

Note: On the top of bars pairs the stars symbolize the significance of differences between averages for the three exceeding likelihood p <0.05 *) significant; p<0.01**) distinct significant; p<0.001***) very significant

identical (Holuša *et al.,* 2003), the recorded air temperatures could be considered favourable for *I. duplicatus* flight activity.

Thus, during the whole experimental trials 9613 beetles of *I. duplicatus* were captured (3615 males and 5998 females). Out of the total, 6947 beetles were captured in experimental plots with fresh spruce material and 2666 beetles were captured in pairwise control plots. The proportion of males was 37.6% on average, ranging from 35.8% to 42.2%. In addition to *I. duplicatus*, some other beetles were captured, as well: *I. typographus* (55 indiv.), *Pityogenes chalcographus* (142 indiv.), *Polygraphus poligraphus* (7 indiv.), *Dryocoetes* sp. (18 indiv.), *Hylastes* sp. (23 indiv.), *Rhagium* sp. (9 indiv.).

Tab. 3. Total of *Ips duplicatus* captures collected in experimental plots

Location	Exp. plot	Number of beetles	Of which males	
			No. of beetles	%
Zamostea	Z1	3732	1335	35,8
	Z2	1400	519	37,1
Calafindeşti	C3	1626	686	42,2
	C4	427	165	38,7
Feteşti	C5	1589	603	37,9
	C6	839	307	36,6

Analyzing the pairs of pilot areas, the most *I. duplicatus* pieces were captured at Zamostea (5132 beetles), followed by Feteşti (2428 beetles) and Calafindeşti (2053 beetles) (Tab. 3). The catches recorded was double at Zamostea comparing to other locations and this situation is justified by the large population of *I. duplicatus* already present there, reaching epidemic levels (Duduman *et al*., 2011).

Concerning the response of *I. duplicatus* to synthetic pheromones in the two experimental plots, it was found that, regardless of location and time, significantly more beetles were captured in traps installed in areas with wooden debris (Fig. 1), while the interaction between experimental plots and the sex of captured beetles was non-significant (Tab. 4). The catches recorded in the area with wooden debris were much higher: from 1.5 times higher (Zamostea, May 16-23) up to cca. 4.6 times (Calafindeşti, June 6-15) comparing to the plots without fresh wooden debris (i.e. the control plots).

The lowest differences between the catches in the two experimental plots were registered in Feteşti, where the wooden debris contribution to larger catches ranged from 1.6 to 2.3 times. These figures do not hold for the other two areas where the differences ranged from 1.5 to 3.4 times at Zamostea and 2.1 to 4.6 times at Calafindeşti.

Analysing the captures in terms of male proportion, large differences in all plots were found among different

Tab. 4. Outcome of controlled factor and combination of controlled factor and sex on the *Ips duplicatus* captures

Experimental period	Statistical value	Experimental area					
		Zamostea		Calafindeşti		Feteşti	
		Fisher's F	P	Fisher's F	P	Fisher's F	P
16-23 may	Controlled factor	17,514	0,001	9,748	0,007	40,001	< 0,001
	Controlled factor x sex	0,018	0,894	0,119	0,734	0,747	0,400
23-31 may	Controlled factor	21,808	< 0,001	13,917	0,002	7,780	0,013
	Controlled factor x sex	0,629	0,439	2,443	0,138	0,018	0,896
31 may-06 iun	Controlled factor	56,306	< 0,001	28,830	< 0,001	21,138	< 0,001
	Controlled factor x sex	2,265	0,152	2,674	0,122	2,948	0,105
06-15 iun	Controlled factor	11,834	0,003	28,745	< 0,001	6,628	0,020
	Controlled factor x sex	2,114	0,165	2,678	0,121	0,871	0,365

Tab. 5. The proportion of males from captures

Location	Experimental plot	Experimental period / (%) (mean ± SE)			
		16-23 may	23-31 may	31 may-06 iun	06-15 iun
Zamostea	Z1	55.2±2.7	51.1±1.5	39.3±0.7	24.4±3.4
	Z2	55.4±1.3	51.7±1.0	38.6±1.0	25.5±4.4
Calafindeşti	C3	54.0±3.6	47.7±3.1	30.4±2.0	25.7±4.2
	C4	50.4±3.3	50.9±1.9	28.8±1.4	26.3±1.1
Feteşti	F5	54.9±3.7	45.8±1.4	33.6±1.2	26.8±2.0
	F6	55.4±2.9	46.6±3.2	33.5±1.9	27.2±2.7

periods along the season, (Tab. 5). However, no significant differences between the male proportions from the captures obtained from the same period in the two experimental variants were observed.

Discussion

The significantly larger number of *I. duplicatus* beetles captured in areas with fresh host material confirms that the presence of a larger quantity of natural volatile substances characteristically to the spruce (released by the fresh host material) contributes to the increase of the beetles' population in these areas due to insect attraction by these volatiles. This aspect was demonstrated for *I. typographus* by Bakke (1985), who collected larger captures at traps baited with synthetic pheromone installed along the fresh edge of Norway spruce stands, than those collected at the traps installed on edges of three years old. One year later Austara *et al.* (1986) captured about 1.7 more beetles at the pheromonal traps installed on the fresh host materials than the beetles captured in plots installed on areas without such fresh materials.

Larger differences within the three pairs of plots from one experimental period to the next one are probably explained by a larger spectrum of the natural volatile substances, released by the host material. It is known that for *I. typographus*, the terpene with a high concentration (especially of alpha pinene) can be a good repellent (Chararas, 1959; Martin *et al.*, 2002), even when these are combined with specific synthetic pheromones (Olenici *et al.,* 2007). Such situation can be always found after harvesting a large number of trees, when the emissions of terpene are very high, but the alcohols are effectively inexistent (Hietz *et al.*, 2005). On the other hand, once the fresh host material has been decomposed, it releases a great deal of ethanol produced by fermentation process (Hietz *et al.*, 2005) and alcohol vapours have the same synergetic effect as natural terpenes on alluring different bark beetles species (Moeck, 1970; Phillips *et al.*, 1988; Schroeder and Lindelöw, 1989). Yet, these larger amplitudes of the differences between beetles captures couldn't be caused by air temperature, which was quite constant (Fig. 1).

As expected, the *I. duplicatus* bark beetles are directed not only by aggregative pheromones but also by natural volatiles to identify their next host. The spectrum of volatiles released by fresh Norway spruce debris is somewhat similar to spectrum of volatile substances released by debilitated trees, which are actually chosen for colonization. This explains also the interest in studying these species, more and more frequent in forest areas where large quantities of natural host volatiles might occur, due to various reasons.

Equal proportions of males and females in the catches from the two types of experimental plots show that both females and males of *I. duplicatus* are equally attracted by the natural host volatiles. The reduction of *I. duplicatus* males number catches as July is approaching is in line with similar observations reported about the captured dynamics of other species of *Ips* (*I. typographus*), where the maximum percentage of male specimens are caught at the beginning of flight, in May (Faccoli and Buffo, 2004). This aspect highlights the process of decreasing the number of males in flight, because the males are initiating the early stages of tree colonisation (in the case of *I. duplicatus* males are the ones who initiate the attack), when many of them are drowned in the resin released by the tree. The male number can grow up again in the second half of June when the new generation occurs.

Conclusions

Catches of *I. duplicatus* bark beetle into traps baited with synthetic specific aggregative pheromone are significantly higher in experimental plots where fresh spruce debris are on the site, than the number of catches found in the control plots, without wooden debris. It can be concluded therefore that *I. duplicatus* in addition of pheromones is using the volatile substances characteristics to spruce trees to locate areas where trees are predisposed to attack.

Acknowledgement

This work was financed by „The National Authority for Scientific Research" CNCSIS-UEFISCSU, project number PN II-RU 576/2010, contract number 204/2010 and MOTIVE Project–Models for Adaptive Forest Management, funded by 7th EU Framework Programme. The support in the field from the personnel of Suceava County Branch of the National Forest Administration Romsilva (Forest Districts Pătrăuți and Adâncata) is greatly appreciated. We also thank to dr. Marian Drăgoi and dr. Tudor Stăncioiu for the English proofreading of the manuscript.

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