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Pheromone Lures: Easy Way to Detect *Trypodendron* Species (Coleoptera: Curculionidae)

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ABSTRACT

Numbers of *Trypodendron* species captured with Trypowit® and Lineatin Kombi® lures were compared in 70 to 100 year old Norway spruce (*Picea abies*) stands at two localities (Kostelec and Tábor) in the Czech Republic. At each locality, five pairs of black, Theysohn window-slot traps were deployed; one trap of each pair contained Trypowit® lure and the other contained Lineatin Kombi® lure, and each trap of the pair was separated by 10 m. Among the *Trypodendron* species captured, *T. lineatum* was the most abundant, followed by *T. domesticum* and *T. laeve*. In most cases, the two kinds of lures caught similar numbers of beetles. *T. lineatum* males were more abundant than females at both localities in traps with Trypowit lures and in traps with Lineatin Kombi lures at Tábor. Males as well as females of *T. domesticum* were more abundant in traps with Lineatin Kombi lures than with Trypowit lures at Tábor but not at Kostelec. Both lures, which contain lineatin and attract all *Trypodendron* species including *T. laeve*, can be used to monitor the abundance of these bark beetle species. The Lineatin Kombi lure, however, contains more alcohol and is slightly more effective than the Trypowit lure for monitoring *Trypodendron* species on broadleaf trees.

Key words: Bark beetles, central Europe, flight activity, pheromones, sex ratio.

INTRODUCTION

Of the four *Trypodendron* species that occur in Central Europe, two prefer coniferous trees and two prefer broadleaf trees. The striped ambrosia beetle, *Trypodendron lineatum* (Olivier, 1795), is a serious pest of conifers in the Palearctic region and in North America (Wood, 1982). *Trypodendron laeve* Eggers, 1939 is widespread in Europe and also attacks conifers (Muona, 1994; Martikainen *et al*., 1996; 1999; Holzschuh, 1990a, 1990b, 1995; Krehan and Holzschuh, 1999; Martikainen, 2000; DAISIE, 2009; Kirkendall and Faccoli, 2010; Lukášová *et al*., 2012; Lukášová and Holuša, 2014). The European hardwood ambrosia beetle (*Trypodendron domesticum* (Linnaeus, 1758)) is a pest of broadleaf, hardwood trees (Schwenke, 1974; Schwerdtfeger, 1981). *Trypodendron signatum* (Fabricius, 1787) occurs in oak forests in lowland and hills and is generally the least abundant of the four species (Pfeffer, 1989; Lukášová *et al*., 2012) but can be very abundant (Gaubicher *et al*., 2003).

The most important method for control of *Trypodendron* species is the logging and removal of all stored wood from forests before *T. lineatum* swarms each year.

Trypodendron species can not infest wood from the end of May until February of the following year because the species are inactive at this time. Wood to be harvested should not be left in areas where *Trypodendron* species were abundant in the previous year, because the beetles will overwinter in the litter or wood of such trees. From a practical perspective, it is not necessary to distinguish wood attacked by *T. lineatum* and *T. laeve*, because the two species are very similar in terms of bionomics, damage, and control (Bussler and Schmidt, 2008).

Lures containing lineatin (the pheromone produced by *Trypodendron* females) are useful for monitoring the abundance of all *Trypodendron* species but are probably ineffective for mass trapping. The population density of *T. lineatum*, for example, was not be substantially influenced by continuous mass trapping with lineatin-baited traps (Dimitri *et al*., 1992). A cost/benefit analysis also indicated that the cost of mass trapping exceeded the benefit (König, 1992). Still, pheromone lures could be used for determining whether a site is suitable for storing wood. If more than 500 *T. lineatum* beetles are captured during the flight period, the risk of stored wood infestation is very high (Zahradník, 2002).

The use of pheromone-based lures to attract *Trypodendron* species facilitates the determination of dates when swarming by these pests begins and ends. Data from traps containing pheromone-based lures enable foresters to take early preventive measures that will reduce the damage resulting from bark beetle infestation. It is also much easier to determine which species are present by examining specimens in traps containing pheromone than by cutting specimens from wood or by capturing specimens that emerge from wood in emergence traps.

Pheromone lures for *T. lineatum* have been used for a relatively long time, and all of them contain lineatin and also other substances in some cases (Klimetzek *et al*., 1981; Schurig *et al*., 1982; King *et al*., 1983; Lukášová and Holuša, 2014). New lures that are attractive for other *Trypodendron* species have been produced only recently. The Austrian company Witasek Pflanzenschutz GmBH provides the pheromone lure Trypowit® for capturing *T. lineatum* and the pheromone lure Lineatin Kombi® for capturing other *Trypodendron* species including *T. domesticum* and *T. signatum* (http://www.witasek.com/). The Lineatin Kombi lure was developed at the CHEMIPAN Research and Development Laboratories (www.sciencedaily.com/releases/2011/09/110915083651.htm). Field tests with the Lineatin Kombi lure have been carried out by researchers from the Technical University of Dresden in forests near Leipzig, Dresden, and Wermsdorf. The forests consisted of common beeches, oaks, and ashes. The researchers reported that high numbers of *T. domesticum* and *T. lineatum* were captured in the pheromone traps; *T. signatum* specimens were also trapped (www.sciencedaily.com/releases/2011/09/110915083651.htm).

The main aim of study was to compare numbers of *Trypodendron* species captured in traps containing Trypowit® and Lineatin Kombi® lures in spruce forests in the Czech Republic. A second aim was to determine whether the Lineatin Kombi lure attracts *T. laeve*, which was recently detected the Czech Republic, Poland, and Romania (Lukášová *et al*., 2012; Olenici *et al*., 2014).

MATERIALS AND METHODS

Trapping experiments were conducted in 70 to 100 year old Norway spruce (*Picea abies* Karsten) stands at two localities in the Czech Republic (Kostelec nad Černými lesy; 49°58'N, 14°49'E and Tábor; 49°31'N 14°33'E) from March to July 2014.

At each locality, five pairs of pheromone-baited, black window-slot traps (Theysohn, Germany) were deployed. Each trap had a total active surface area of 4,284 cm^2 (42 x 51 cm on two sides). The traps in each pair were 10 m apart, 10-15 m from the nearest forest edge, and 1.5-2.0 m above the soil surface. Adjacent pairs of traps at each locality were 50-100 m apart.

Each pair of trap was baited with a standard synthetic pheromone lure. One trap of the pair was baited with Trypowit® and the other with Lineatin Kombi® (http://www.witasek.com/). The main active chemicals in the Trypowit lure are alfa-pinen (2,6,6-trimethyl-bicyclo-[3,1,1]hept-3-en) and lineatin (3,3,7-trimethyl-2,9-dioxatricyclo-[3.3.10 4,7] nonane). The main active chemicals in the Lineatin Kombi lure are lineatin (3,3,7-trimethyl-2, 9-dioxatricyclo-[3.3.10 4,7] nonane), qublaiacol (2-methoxyphenol), nonyl aldehyde, and 3-hydroxy-2-methyl-2-butanone.

Once either kind of lure is removed from its hermetically sealed, three layered aluminium-plastic laminated pouch, the aggregation pheromone contained in a cellulose plate is gradually released through a permeable polyethylene film. The traps with aggregation pheromone were deployed in mid-March of 2014 and were monitored until the time when two consecutive collections were negative, which occurred in early June/July 2014. Beetles were collected every 7 days and were stored in 2.5-ml Eppendorf microtubes at <−5°C.

Individual beetles were identified to species according to Pfeffer (1989) and Bussler and Schmidt (2008). Sex was determined according to secondary sex characteristics (Pfeffer, 1989).

Because the data were not normally distributed (as indicated by the Shapiro Wilk test), the number of beetles per trap in traps with the two kinds of lures was compared using Wilcoxon signed-rank test, and the number of beetles per trap across lure types was compared between the two localities using a Mann-Whitney U Test. All tests were performed with STATISTICA 12.0 software.

RESULTS

Data concerning the numbers of *Trypodendron* species trapped as affected by lure type, beetle species, beetle sex and locality are presented in Table 1. A total of 10, 822 beetles were captured and three species of *Trypodendron* were recorded. *T. lineatum* was the most abundant (n=8,451), followed by *T. domesticum* (n=2,238) and *T. laeve* (n=133).

Numbers were more than several times higher for *T. lineatum* ($Z=3.43$; $p<0.001$) and *T. laeve* ($Z=3.78$; $p<0.001$) at Kostelec than at Tábor. For *T. domesticum*, numbers were only slightly higher at Kostelec than at Tábor ($Z=2.38$; $p<0.05$).

For *T. lineatum*, males were more abundant than females in traps with the Trypowit lure at both localities and in traps with the Lineatin Kombi lure at Tábor. Males as well as females of *T. domesticum* were more abundant with the Lineatin Kombi lure than with the Trypowit lure at Tábor.

Averaged across all *Trypodendron* species, the numbers of males and females captured were usually similar with both kinds of lures but were significantly different in several cases. Males of *T. lineatum* were slightly but significantly more abundant with the Trypowit lure than with the Lineatin Kombi lure at Kostelec. Males as well as females of *T. domesticum* were slightly but significantly more abundant with the Lineatin Kombi lure than with the Trypowit lure at Tábor (Table 1).

DISCUSSION

In the current study, the most abundant species of *Trypodendron* was *T. lineatum*, followed by *T. domesticum* and *T. laeve*. The results confirm earlier observations that *Trypodendron* species are attracted by lineatin (Klimetzek *et al*., 1981; Schurig *et al*., 1982; King *et al*., 1983; Paiva and Kiesel, 1985; Kvamme, 1986; Krehan and Holzschuh, 1999; Martikainen, 2000; Lukášová *et al*., 2012; Holuša and Lukášová, 2014). The failure to trap *T. signatum*, which also occurs in the Czech Republic, can be explained by the low numbers of its host trees in the study areas (Lukášová *et al*., 2012). The pheromone traps in the current study were located in Norway spruce forests, and Norway spruce is not a host of *T. signatum*.

We tested two lures in spruce areas with different population densities of *Trypondendron* species *T. lineatum* has been abundant in forests surrounding the town of Kostelec nad Černými lesy for a long time. The numbers of *T. lineatum* caught by the different lures were similar in most cases. Males but not females of *T. lineatum* were more abundant in traps containing the Trypowit lure rather than the Lineatin Kombi lure at Kostelec, while both males and females of *T. domesticum* were more abundant in traps containing the Lineatin Kombi lure rather than the Trypowit lure at Tábor. That both lures generally attracted similar numbers *Trypondendron* species can be explained by the fact that both lures contain lineatin.

In addition to lineatin, the Trypowit lure also contains α-pinen, which attracts *T. lineatum* but repels the two other *Trypondendron* species that attack broadleaf trees (Paiva, 1982; Paiva and Kiesel, 1985). Lures that included α-pinene with the pheromone, either with or without ethanol, attracted significantly more *T. lineatum* than those with the pheromone alone (Shore and Lindgren, 1996). Exposure of *T. lineatum* males to ethanol increased their frequency of steady, upwind flight; however, only lineatin was effective in inducing them to land on and enter traps (Salom and McLean, 1990). Ethanol at a release rate of 1 g/day inhibited the response of North American *T. lineatum* to lineatin, whereas ethanol at 310 and 500 mg/day acted synergistically with lineatin for European *T. lineatum*. α-pinene was synergistic with lineatin for the capture of European *T. lineatum* in traps that simulated tree trunks (Borden *et al*., 1982).

Table 1. Numbers *Trypodendron lineatum*, *T. domesticum*, and *T. laeve* males and females caught in traps and sex ratios as affected by lure type.

Locality	Species	Sex	Lure	Numbers of beetles per trap (mean±SE)[a]	Statistical comparisons[b]		
					Effect of lure type on numbers of beetles per trap[1]	Sex ratio in traps with Lineatin Kombi lures[2]	Sex ratio in traps with Trypowit lures[3]
Kostelec	*T. lineatum*	Male	Lineatin Kombi	266.6±61.2	**2.02 ***	**2.02 ***	**2.02 ***
	T. lineatum	Male	Trypowit	905.6±256.9			
	T. lineatum	Female	Lineatin Kombi	166.2±39.2	1.21 ns		
	T. lineatum	Female	Trypowit	214.2±53.3			
	T. domesticum	Male	Lineatin Kombi	67.4±17.6	0.94 ns	0.27 ns	1.46 ns
	T. domesticum	Male	Trypowit	91.8±29.7			
	T. domesticum	Female	Lineatin Kombi	67.8±15.1	0.67 ns		
	T. domesticum	Female	Trypowit	78.4±25.0			
	T. laeve	Male	Lineatin Kombi	6.2±1.9	0.53 ns	0.18 ns	1.21 ns
	T. laeve	Male	Trypowit	8.4±4.0			
	T. laeve	Female	Lineatin Kombi	5.4±2.3	0.26 ns		
	T. laeve	Female	Trypowit	5.8±2.7			
Tábor	*T. lineatum*	Male	Lineatin Kombi	34.0±14.2	0.67 ns	0.67 ns	**2.02 ***
	T. lineatum	Male	Trypowit	50.2±26.3			
	T. lineatum	Female	Lineatin Kombi	31.2±12.8	0.67 ns		
	T. lineatum	Female	Trypowit	22.2±13.6			
	T. domesticum	Male	Lineatin Kombi	66.8±27.6	**2.02 ***	**2.02 ***	**2.02 ***
	T. domesticum	Male	Trypowit	21.6±8.4			
	T. domesticum	Female	Lineatin Kombi	44.8±19.2	**2.02 ***		
	T. domesticum	Female	Trypowit	9.0±3.9			
	T. laeve	Male	Lineatin Kombi	-	-	-	1.60 ns
	T. laeve	Male	Trypowit	-			
	T. laeve	Female	Lineatin Kombi	-	1.60 ns		
	T. laeve	Female	Trypowit	0.8±0.4			

[a] Across the entire sampling period in 2014.

[b] ns = nonsignificant, and * = significant at $p<0.05$ according to Wilcoxon signed-rank tests. For sex ratio, a significant effect indicates that the ratio was different from 1:1).

[1] Number of beetles per trap with the two types of lures comparing using Wilcoxon signed-rank test,

[2] Comparison of males and females abundance per trap with Lineatin Kombi lures using Wilcoxon signed-rank test,

[3] Comparison of males and females abundance per trap with Trypowit lures using Wilcoxon signed-rank test.

In addition to lineatin, the Lineatin Kombi lure contains quaiacol, nonyl aldehyde, and 3-hydroxy-2-methyl-2-butanone. These other substances are added because isomers of 2-methoxy-phenol and methyl-butanol can attract *T. domesticum* (Holighaus and Schütz, 2006). The Lineatin Kombi lure does not contain ethanol, although both *T. domesticum* (Petercord, 2006) and *T. signatum* (Galko *et al*., 2013) are attracted by lures that contain ethanol.

The producer's literature for the Trypowit and Lineatin Kombi lures (http://www.witasek.com/) does not refer to *T. laeve*, and the pheromones of *T. laeve* have not been studied. *T. laeve* was previously caught in traps with lures containing lineatin in alcohol (Martikainen, 2000; Grégoire *et al*., 2001; Lukášová and Holuša, 2014).

The sex ratio of *T. lineatum* was significantly shifted toward males in three of four cases (two lures × two localities) while the sex ratio of *T. domesticum* was significantly shifted toward males with both kinds of lures at the Tábor locality but with neither kind of lure at the Kostelec locality. Pheromone traps should catch more males than females because the trapped females produce additional sex pheromones that attract males (Schwenke, 1974; Schwerdtfeger, 1981). In a previous study using lures containing lineatin and ethanol or α-pinene, the sex ratio of captured bark beetles was more male biased for *T. domesticum* than for *T. lineatum* or *T. signatum* (Paiva, 1982). The proportion of females trapped depends on ethanol and/or α-pinene, i.e., significantly more female beetles were trapped when lures contained linatin plus ethanol and/or α-pinene rather than lineatin alone (Shore and Lindgren, 1996).

That both Trypowit and Lineatin Kombi lures (http://www.witasek.com/) contain lineatin probably explains why the lures attracted all species of *Trypodendron* in the two study localities (*T. lineatum*, *T. domesticum*, and *T. laeve*) and why differences in the numbers trapped were not greatly affected by kind of lure. The Lineatin Kombi lure may attract more *T. domesticum* than the Trypowit lure because the Lineatin Kombi lure also contains alcohols. This indicates that the Lineatin Kombi lure may be especially useful for monitoring *Trypodendron* species on broadleaf trees. The sex ratio of *Trypodendron* species in traps containing Trypowit and Lineatin Kombi lures were male biased probably because the lures contain lineatin, which is a female sex pheromone that attracts males. The results are consistent with the view that *T. laeve* is widespread throughout Europe but is less abundant than *T. lineatum*.

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