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Efficiency and Sustainability of *Ips duplicatus* (Coleoptera: Curculionidae) Pheromone Dispensers with Different Designs

Mihai-Leonard Duduman [1,2], Kateřina Beránková [3], Rastislav Jakuš [3,4], Jaromír Hradecký [3] and Anna Jirošová [3,*]

1 Applied Ecology Laboratory, Forestry Faculty, "Ștefan cel Mare" University of Suceava, Universității Street 13, 720229 Suceava, Romania; mduduman@usv.ro
2 Faculty of Forestry and Forest Engineering, "Transilvania" University of Brașov, Sirul Beethoven 1, 500036 Brașov, Romania
3 EXTEMIT-K, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 16500 Prague, Czech Republic; berankovakaterina@outlook.cz (K.B.); jakus@fld.czu.cz (R.J.); hradecky@fld.czu.cz (J.H.)
4 Institute of Forest Ecology, Slovak Academy of Sciences, 96001 Zvolen, Slovakia
* Correspondence: jirosovaa@fld.czu.cz; Tel.: +42-022-438-3903

Abstract: *Ips duplicatus* (Coleoptera: Curculionidae) is a bark beetle that recently invaded southern Europe. It produces intense outbreaks in the spruce stands installed outside their natural area. Pheromone dispensers with different designs are used for monitoring this species. Compounds are released either by wick (ID Ecolure) or polyethene foil (ID RO and Pheagr IDU). We performed a series of laboratory and field experiments to compare the efficiency and sustainability of these dispensers in terms of pheromone release. The first experiment compared the *Ips duplicatus* capture dynamics between the ID Ecolure and ID RO in pheromones traps in three localities for 60 days. Release rates of used dispensers were evaluated in the same period. The second experiment analyzed headspace chemical composition in three dispensers (ID Ecolure, ID RO, Pheagr IDU) and tested their activity in pheromone traps. Active compounds content released by tested dispensers varied. The wick dispensers' release rates (ID Ecolure) were strongly reduced from 365 to 50 mg/day within two weeks and then steadily to 16 mg/day after 60 days. Release rates of the polyethene dispensers (ID RO) remained constant over 60 days (20–24 mg/day). The highest initial emission was in the ID Ecolure. The ratio of pheromone components (ipsdienol/E-myrcenol) was lower for ID Ecolure (5) and ID RO (10) and higher for Pheagr IDU (565). In the field experiments, significantly more *Ips duplicatus* were caught with ID Ecolure than ID RO and Pheagr IDU in the first two weeks. Later, the attractivity of ID RO remained high, but captures by ID Ecolure were significantly reduced. The captures of *Ips typograhus* were also considered. We propose that the wick-type dispensers are a promising tool for controlling the hibernating beetle generation. The foil release dispensers can be used to monitor *Ips duplicatus* flight activity.

Keywords: pheromone dispenser; invasive bark beetle; forest pest; ipsdienol; E-myrcenol; double-spined bark beetle; northern bark beetle; *Picea abies*; *Ips typographus*

Citation: Duduman, M.-L.; Beránková, K.; Jakuš, R.; Hradecký, J.; Jirošová, A. Efficiency and Sustainability of *Ips duplicatus* (Coleoptera: Curculionidae) Pheromone Dispensers with Different Designs. *Forests* **2022**, *13*, 511. https://doi.org/10.3390/f13040511

Academic Editor: Celia K. Boone

Received: 8 February 2022
Accepted: 18 March 2022
Published: 25 March 2022

1. Introduction

The northern spruce bark beetle (double-spined bark beetle) *Ips duplicatus* (Sahlberg, 1836) (Coleoptera: Curculionidae) is native to Eurasian boreal forests [1]. It can develop outbreaks in association with *Ips typographus* (Linnaeus, 1758) (Coleoptera: Curculionidae) and other bark beetle species. It is most often due to the accumulation of large quantities of favourable host material after storm-falling events [2]. *Ips duplicatus* is more abundant in rare cases than *I. typographus* [3]. This bark beetle prefers the spruce species as a host but can successfully colonize other conifer species such as pines [1,4–8]. In the last century, this species has spread to many parts of Europe [9–13], being favoured by the transport of infested wood [9,14,15], and is currently considered invasive [9,16–18]. In the last half-century, mass attacks of *I. duplicatus* have increased, especially in Central Europe. These outbreaks have predominantly developed in artificial Norway spruce (*Picea abies* (Linnaeus) Karsten 1881) (Pinales: Pinaceae) monocultures planted at low altitudes outside of natural areas [9–11,19–21]. This bark beetle has up to 3–4 generations per year [10,22,23], resulting in significant quantities of infested trees [24,25]. The high potential of *I. duplicatus* colonization to develop into an outbreak is favoured by the physiological weakening of spruce trees due to the effects of current climate change [11,26–29], attack by different pests and diseases [24,28,30] and, last but not least, improper management of spruce stands [19,24].

Management of this bark beetle is considerably more difficult because it attacks the trees in the stand in a scattered manner. Differing from the other bark beetle, *I. duplicatus* cannot colonize fallen trap trees and can establish up to three generations during one vegetation season [31,32]. For the efficient control of these bark beetles, therefore it is important to monitor their populations and identify and remove infested trees from the forest before the new generation of beetles emerges [20,33]. Currently, the activity of this species is mainly monitored using a pheromonal trap baited with specific synthetic lures [16,34,35]. However, the seasonality of these lures (ID Ecolure) has been frequently observed, with significantly higher captures recorded in spring, when the overwintering *I. duplicatus* generation emerges from forest litter, than in summer, when offspring generation appears [36]. Further, the trap captures have not fully described the population density of *I. duplicatus*, given that new infestations are also broadly detected in summer [23]. For predicting the summer threat of *I. duplicatus*, correlation coefficients were determined between the first- and second-generation captures.

As with other bark beetle species, *I. duplicatus* use olfactory signals (pheromones and kairomones) for spatial orientation and to identify favourable host trees for colonization [37,38]. The aggregation pheromone of this species is produced by pioneer males who attract conspecifics to aggregate and overcome host tree defences [39]. To date, two important active components have been identified: ipsdienol (Id) and E-myrcenol (EM) [2,40,41]. Id is a major component attracting adults, where EM contributes to its attractivity [40]. The ratio between these two compounds is variable (Id:EM of 1:1–9:1), and most likely depends on the geographical location of *I. duplicatus* populations [42–44]. In addition *I. duplicatus* is sensitive to other compounds, such as 2-methyl-3-buten-2-ol, *cis*-verbenol (aggregation pheromone of cohabitating *I. typographus*) [44], and host tree kairomones (monoterpenes and alcohols). Among monoterpenes, it has been shown that (−)-α-pinene (AP) together with (+)-limonene (L) has a synergic effect on the attractivity of Id+EM (synthetic pheromone for *I. duplicatus*). Thus, adding AP+L to the lure consisting of Id and EM, in the ratio (Id+EM):AP:L from 1:40:40 to 1:1000:1000 has shown synergistic effects, resulting in approximately 100% increased attractivity [45].

Identifying pheromones that mediate aggregation and sexual attraction of beetles and kairomonal components used by *I. duplicatus* beetles for locating host material allowed development of synthetic baits used to monitor this bark beetle. Some manufacturers developed dispensers releasing volatiles through wick (ID Ecolure-Fytofarm, Bratislava, Slovakia) or dispensers with polyethene foil release (Pheagr IDU-SciTech, Prague,
Czech Republic; Duplodor-Poland; Dupliwit -Witashek, Feldkirchen, Austria; AtraDup-Chemical Institute "Raluca Râpan" Cluj-Napoca, Romania). As in the case of baits used for other species of bark beetles [46–48], those used for *I. duplicatus* have been employed in several field tests to verify their attractivity for *I. duplicatus* adults and other non-target species [42,49]. Thus, the six constructive dispenser types (wick dispenser, polyethylene vials, two types of polyethene bags, Fytofarm aluminium bag with wick and Fytofarm ampule) were baited with component mixtures specific to *I. duplicatus*. The activity of dispensers was compared over seven weeks and no statistical differences were found between captures [42]. Additionally, the activity of Ecolure ID, Pheagr IDU, and Duplodor

was compared in a long-term trapping study in the Czech Republic (2000) and Poland (2008) [49], where the baits were replaced after eight weeks. The activity of ID Ecolure was significantly the highest of all three baits. However, the captures were high only in the first flight of *I. duplicatus* at the beginning of the season. They decreased after approximately two weeks, with slight increases in captures observed in the middle of the season, after the baits were replaced, and probably when offspring generation appeared. When the activity of the above listed *I. duplicatus* dispensers was studied, the captures of the *I. typographus* on these lures were also recorded. The suggested reason *I. typographus* are attracted to them is the content of the 2-methyl-3-buten-2-ol, the main component of *I. typographus* aggregation pheromone, and content kairomones with common activity for both species.

The studies published on the attractivity of different pheromone baits for *I. duplicatus* have described the quantitative aspects, such as the number of bark beetles caught but the qualitative aspects regarding the different types of dispensers' functioning were less addressed. In this context, we developed the hypothesis for this study. Due to the construction design, the *I. duplicatus* dispensers function differently over time, affecting their attractivity and efficiency. In the series of experiments, we compared the attractivity of two design types of *I. duplicatus* pheromone lures in the context of their chemical compositions and the changes in their release rates over time. Dispensers vary according to the material from which the pheromones are released, from either wick or polyethene foil. We aim to evaluate the overall activity of dispensers and their activity peaks in periods which manufacturer recommends for their usage. With this knowledge, we suggest the selecting most effective dispenser type that will actively attract *I. duplicatus* later in the season, in the period when the activity of some dispensers is observed to decrease. Additionally, we propose selecting the dispenser type with the highest activity to catch the mass of beetles in the first flight when the beetles' population needs to be reduced.

2. Materials and Methods

The series of experiments were carried out in two stages. The main experiments were done in spruce stands in Romania (2011). The confirmation experiment was done in the Czech Republic (2017).

In 2011, two designs of pheromone dispensers for *I. duplicatus* were tested. The volatiles were released from the wick in ID Ecolure and through the polyethene film in ID RO. The gravimetric method was used to measure the variations in the dispensers' release rates over sixty days. Subsequently, the bark beetle's attractiveness of two dispenser types was tested in the field in pheromone traps. This experiment was carried out in conditions of high *I. duplicatus* population in three different locations over sixty days. The research continued in 2017 with the confirmation experiment in the Czech Republic. The chemical compositions of the released volatile mixture for three types of dispensers for *I. duplicatus* (ID Ecolure, ID RO and newly added referential Pheagr IDU) were measured using GC×GC–MS. The Pheagr IDU, as an additional design type of polyethene film releasing dispenser, was added to testing to compare the other commonly used commercially dispensers as a reference. Subsequently, the attractivity of these three dispensers for bark beetles' captures was compared in the field study in pheromone traps. *Ips duplicatus* population in this location was low after the population collapsed several years prior the experiment. Dispensers were tested later in the season for thirty days.

2.1. Dispensers Used

ID Ecolure dispensers are produced by Fytofarm Company Ltd., Bratislava, Slovakia. The active substances include Id (1.6%), an alcohol solvent mixture (96.0%), and synergic components (2.4%) [50]. The volatile mixture is released using wick tape in contact with the support material impregnated with the chemical mixture, located inside on an aluminium envelope.

ID RO dispensers are experimentally developed in the Applied Ecology Laboratory (Forestry Faculty, "Ştefan cel Mare" University of Suceava, Romania). These dispensers contain a 3.4 g mixture of Id (2.0%), EM (2.0%), AP (1.0%), L (1.0%), and 2-methyl-3-buten-2-ol as a solvent (94.0%), protected with the antioxidant (butylhydroxytoluene, 2.5 g per 100 mL mixture). The volatile mixture is impregnated on a cellulosic material and is released from a low-density polyethene film envelope (50 µm, 50 mm × 70 mm) [35,45].

Pheagr IDU (SciTech, Prague, Czech Republic) contains Id (3.13%), EM (0.31%), and 2-methyl-3-buten-2-ol (94.04%). The mixture is stabilized using butylhydroxytoluene [51,52]. Volatiles are released from the polyethene film envelope.

2.2. Dispenser Tests in Lab Conditions

2.2.1. Comparison of Release Rates of Dispenser Types

In the first experiment in 2011, release rates of the ID Ecolure and ID RO were gravimetrically established over sixty days (15 February–15 April 2011). The controlled climate required for the tests (20 °C, RH 50%) was obtained in the Conviron G30 climate chamber (with a volume of 400 L). Ten dispensers of each design were weighed before being placed into the climate chamber and subsequently at one-day intervals (in the first nine days of testing), at a two-day interval (between 10th and 30th day of testing), and every 5–7 days starting from the 32nd day of testing. The dispensers were weighed on a Radwag analytical balance (0.1 mg). On the 30th day of testing, the wick of the ID Ecolure dispensers was shortened according to the manufacturer's instructions.

2.2.2. Headspace Analysis of Proportion of Active Compounds in the Tested Dispensers

In the second experiment in 2017, the headspace analyses of the dispensers (ID Ecolure, Pheagr IDU and ID RO) were performed at CULS Prague using solid-phase microextraction (SPME) techniques. Volatiles were collected with SPME fibre (100 µm PDMS; Supelco, USA). A dispenser that had been opened for one day was placed in a round bottom flask (1 L), and the flask was sealed with Teflon septa and placed in a water bath to obtain the required temperature (50 °C, for 15 min). After 15 min, the Teflon septa were penetrated by the fibre, and volatiles were collected for an additional 15 min. Immediately after sorption, VOCs were analyzed using two-dimensional gas chromatograph with a time-of-flight mass spectrometer (GC×GC-TOF-MS; Pegasus 4D, Leco, St. Joseph, MI, USA) GC×GC-TOF-MS. This instrument was used to analyze the samples for its superior separation power and possibility to provide trustworthy mass spectral deconvolution. The GC was equipped with a combination of a nonpolar column ZB-5MS (Phenomenex, Torrance, CA, USA, length 30 m, inner diameter 0.25 mm, film thickness 0.25 µm) and a medium polar column RTX-50 (Restec, Bellefonte, PA, USA, length 1.5 m, inner diameter 0.1 mm, film thickness 0.1 µm). Initial temperature for the primary column was 50 °C (1 min), with a gradual increase of 8 °C/min up to 250 °C (5 min). The temperature program for the secondary column and modulator copied the temperature program in the 1st dimension oven with an offset of 10 °C and 15 °C, respectively. Helium (He) with a constant flow rate of 1 mL/min was used as the carrier gas. The transfer line temperature was set at 260 °C. The data acquisition rate of the MS was set at 100 spectra/s in the mass range 29–400 m/z using electron ionization at 70 eV. Identification of selected GC–MS signals was performed by comparing mass spectra with the NIST 2.3 library (2017). Confirmation was done by comparing measured retention indexes with those listed in the NIST library or/and retention times of commercially available standards. Comparison of the relative abundances of selected compounds, extracted from the dispenser's headspace, was based on Total Ion Current (TIC) record.

2.3. Field Experiments

The differences between attractivity of those two design types of lures (the volatile mixture is released directly from the wick and the volatile mixture is released through the polyethene foil's pores) were tested in field conditions through two different field trapping experiments in 2011 and 2017.

The first field experiment in 2011 was carried out between 18 April and 21 June for sixty days since the ID Ecolure dispensers have been guaranteed to operate in the field for at least eight weeks [51]). The study consisted of three replicated experiments in three locations situated in northeastern Romania (Suceava County), which are under the administration of Pătrăuţi and Adâncata forest districts (Suceava County Branch of the Romanian National Forest Administration). The traps were installed in Norway spruce stands (40–50 years old) planted outside of the natural range of this species. In these stands, *I. duplicatus* has been responsible for severe outbreaks since 2007 [20], and these populations reached an epidemic level in 2011. The experiment was conducted in a fresh clear-cut area (harvested in early 2011) located at Calafindești (47°50′59.57″ N, 26°08′36.87″ E), Zamostea (47°52′52.02″ N, 26°08′32.46″ E), and Fetești (47°43′05.03″ N, 26°19′27.95″ E).

In each of the three locations, the experimental design was similar. The two treatments (ID Ecolure and ID RO dispenser types) were evaluated within a complete randomized block design. A total of 30 traps were installed in the three locations. Ten traps were placed in each. Five of the traps were baited with Ecolure ID, and five with RO ID (see Figure S1 in Supplementary Materials). The dispersers were ten times permutated in traps during sixty days to reduce the position effect of traps to catches. Consequently, ten collections of beetles were made, resulting in five replications of beetle catches to one tested lure type per one date, per one location. Five replications per one location is a sufficient dataset for statistical processing, which was done separately for each location.

The flight Intercept® traps were installed 12–14 m from the forest edges. The distance between the two traps within a block was 20 m, and the two neighbouring blocks were 20 m apart (Figure S1). In this distance, tested treatments do not interfere with each other, and the neighbour lure does not influence the catches. [45,53]. The traps were placed at 1 m height from the ground (the level of the collecting cup) and baited alternatively with one lure type. The captures were collected at 4–10 days. The increase of the collection period was dependent on weather, which in certain periods led to reduced insect activity (when temperatures below 15 °C or rainy days were registered) (Figure S2).

In 2017, the second field experiment was conducted in Libava (49°37′7.56″ N, 17°33′46.56″ E), in the Czech Republic between 7 July and 15 August in a fresh clear-cut area after harvesting I. typographus infested spruce trees in 2016. The broader region was originally covered by mature planted spruce monocultures outside of the natural range of spruce. There was an *I. duplicatus* outbreak in the area in 2005 [54], and in 2017 was *I. duplicatus* population suppressed by the prevailing *I. typographus* epidemy. At the time of the study, clear-cuts after bark beetles outbreak dominated.

Three commercial dispensers, ID Ecolure, Pheagr IDU, and ID RO were tested. An empty trap was used as control. Four intercept pheromone traps Ecotrap® (Fytofarm, SR, Bratislava, Slovakia) were installed along clear-cut edges 15 m apart and approximatively 30 m from the forest edge (Figure S3). Tested dispensers were placed in the traps, and these lures, including the empty traps, were regularly permutated according to a scheme, creating a complete Latin square (Table S1). In total, 4 × 10 different positions could be obtained without unwanted repetition, resulting in one replication per treatment at one time. The captured beetles were processed as described below.

2.4. Collection and Processing of Trapped Insects

In both experiments, the beetles caught in pheromone traps were collected at each treatment rotation and transported to the laboratory in plastic bottles, either dry (2011) or filled with 95% ethanol (2017). The insects were preserved until laboratory analyses through freezing (2011) or storage in ethanol (2017). Our research was primarily focused on the bark beetle *I. duplicatus*. However, *I. typographus* and other small insects or spiders were also caught in the pheromone traps, the contents of the vials were sorted with using of a binocular microscope. Other captured insect species were not further evaluated. The *I. duplicatus* and *I. typographus* specimens were placed back into their originally labelled vials for sex identification (by dissection). A total of 50 individuals were randomly selected from each vial, and the entire contents of the vials were used when there were fewer than 50 beetles in the vial.

2.5. Data Analysis

The release rates of the dispensers (mg/day) resulted from reporting the differences between two successive weighing and the number of days between the two weighing. The differences between release rates of dispensers were analysed using t-tests. The number of captured beetles was transformed into the number of beetles caught per day for catch dynamic analysis. The homogeneity of variances was tested using Levene's test and the normality of distribution using the Shapiro–Wilk test. When necessary, the data were transformed ($x' = \log_{10}(x + 1)$) to obtain homogeneous data and normal distribution. If the conditions of homogeneity and normality were not met, the differences in the numbers of attracted beetles between each dispenser type were analyzed using a one-way ANOVA test. If the conditions for one-way ANOVA were not met, the nonparametric Kruskal–Wallis test was used. When significant differences were found, Tukey's test for multiple comparations was used to compare means (for data analyzed by ANOVA,), or the Dunn procedure for multiple pairwise comparations (for data analyzed by Kruskal–Wallis) [55]. The differences were considered significant at a 0.05 level of confidence. Data analyses were performed by XLSTAT-PRO (Addinsoft, New York, NY, USA) plugged into EXCEL (Microsoft Corp., Redmond, Washington, WA, USA).

3. Results

3.1. Dispenser Tests in Lab Conditions

3.1.1. Comparison of Release Rates of Dispenser Types

The two types of dispensers tested were differentiated in terms of variations in the volatile mixture release rates. Thus, during the 60 days of testing, the release rates for the ID Ecolure dispensers decreased from 365.8 ± 16.8 to 15.9 ± 1.9 mg/day. The ID Ecolure dispensers released a high quantity of volatiles in the first four days of the tests. The highest release rate was recorded six hours after the initiation of the measurement (365.8 ± 16.8 mg/day). After four days, the release rates of ID Ecolure were reduced by 6.5 times, reaching 56.6 ± 2.6 mg/day. After the fifth day, the release rates did not decrease with the same intensity. After thirty days of the experiment, the ID Ecolure dispensers released 27.5 ± 0.4 mg/day before wick cutting, which the manufacturer recommended. The wick was cut on the thirtieth day of testing, and the subsequent release rates temporarily increased to 80.1 ± 1.1 mg/day (on the thirty-second day of testing). Later, the release rates of the wick dispensers dropped to levels close to the ID RO dispensers. The release rates for the ID RO dispensers varied between 19.7 ± 0.1 and 23.9 ± 0.1 mg/day. Basically, for this dispenser type, the release rates were relatively constant throughout the sixty days of testing, but in the first two weeks, they were significantly lower than the release rates recorded for ID Ecolure. Only on the last day of testing were the release rates of the two types of dispensers relatively close, though the differences were often insignificant (Figure 1).

3.1.2. Headspace Analysis of Proportions of Active Compounds in Tested Dispensers

The abundance of these compounds in the freshly opened dispensers at 50 °C was three times higher in ID Ecolure (sum of TIC peak areas 2×10^9) than in ID RO (sum of TIC peak areas 9×10^8, which corresponded with release rate obtained by weighing), and seven times higher than in Pheagr IDU (sum of TIC peak areas 3×10^8).

A high abundance of 2-methyl-3-buten-2-ol was detected in all analyzed dispensers since it was used as the solvent. Its high concentration did not allow for proper quantification; therefore, this compound was excluded from the relativization of the data. The relative percentages of the active compounds Id and EM (pheromone) counted from the sum of the headspace content of all selected compounds were the lowest in ID Ecolure (Id 0.1%; EM 0.02%), and the highest in ID RO (Id 77.07%; EM 7.7%), followed by Pheagr

IDU (Id 25%; EM 0.04%). The ratio of these two pheromonal components was similar in ID Ecolure (Id/EM 5) and ID RO (Id/EM 10) but differed in Pheagr IDU (Id/EM 565) (Table 1).

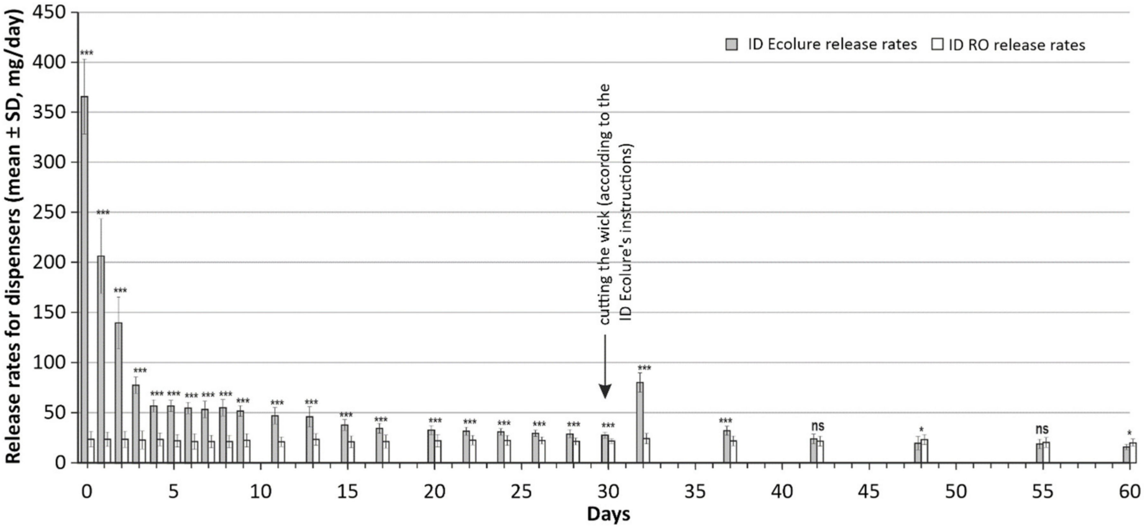

Figure 1. Comparison of release rates of ID Ecolure and ID RO dispenser types, obtained in laboratory conditions (20 °C, RH = 50%). The symbols above the error bars indicate the significance of the differences between the release rates according to *t*-test (ns—not statistically significant ($p > 0.05$); * significant ($p < 0.05$); *** very highly significant ($p < 0.001$)).

Table 1. Relative abundances of selected active compounds, together with the presence of solvent and antioxidant in three types of freshly opened dispensers tested in 2017. Ratios of Id and EM are presented separately.

			% of Main Active Compounds †		
	1D RT (s)	**2D RT (s)**	**ID Ecolure**	**ID RO**	**Pheagr IDU**
ethanol	225	0.97	45.25	0.30	58.39
α-pinene (AP)	537	1.19	42.12	9.14	12.41
limonene (L)	648	1.31	12.51	5.78	4.27
ipsdienol (Id)	777	1.54	0.10	77.07	24.88
E-myrcenol (EM)	885	1.58	0.02	7.71	0.04
Ratio:					
Id/EM			5	10	565
Solvent	255	1.27	MB	MB	MB
Presence of BHT	1146	1.56	traces	Yes	Yes

† SPME collection at 50 °C, 2 days after opening, RT: retention time, MB: 2-methyl-3-buten-2-ol, BHT: butylated hydroxytoluene.

The relative percentages of ethanol, α-pinene, and limonene in the mixture with pheromone (Id, EM) were the highest in ID Ecolure (EtOH 45%, AP 42%, L 12%); Pheagr IDU recorded the highest percentage of EtOH (58%) and relatively lower contents of the other two compounds (AP 12%, L 4%). A low percentage of EtOH (0.3%) was recorded in ID RO, with similar levels of the other two compounds as also recorded in Pheagr IDU (AP 9%, L 6%) (Table 1).

The antioxidant butylated hydroxytoluene BHT was detected in ID RO and Pheagr IDU, while only traces of this compound were recorded in the headspace of Ecolure ID. Other antioxidants were not targeted in this study. The other peaks in the chromatograms (Figure 2), which were not described in this study, were mainly hemi- and monoterpenes.

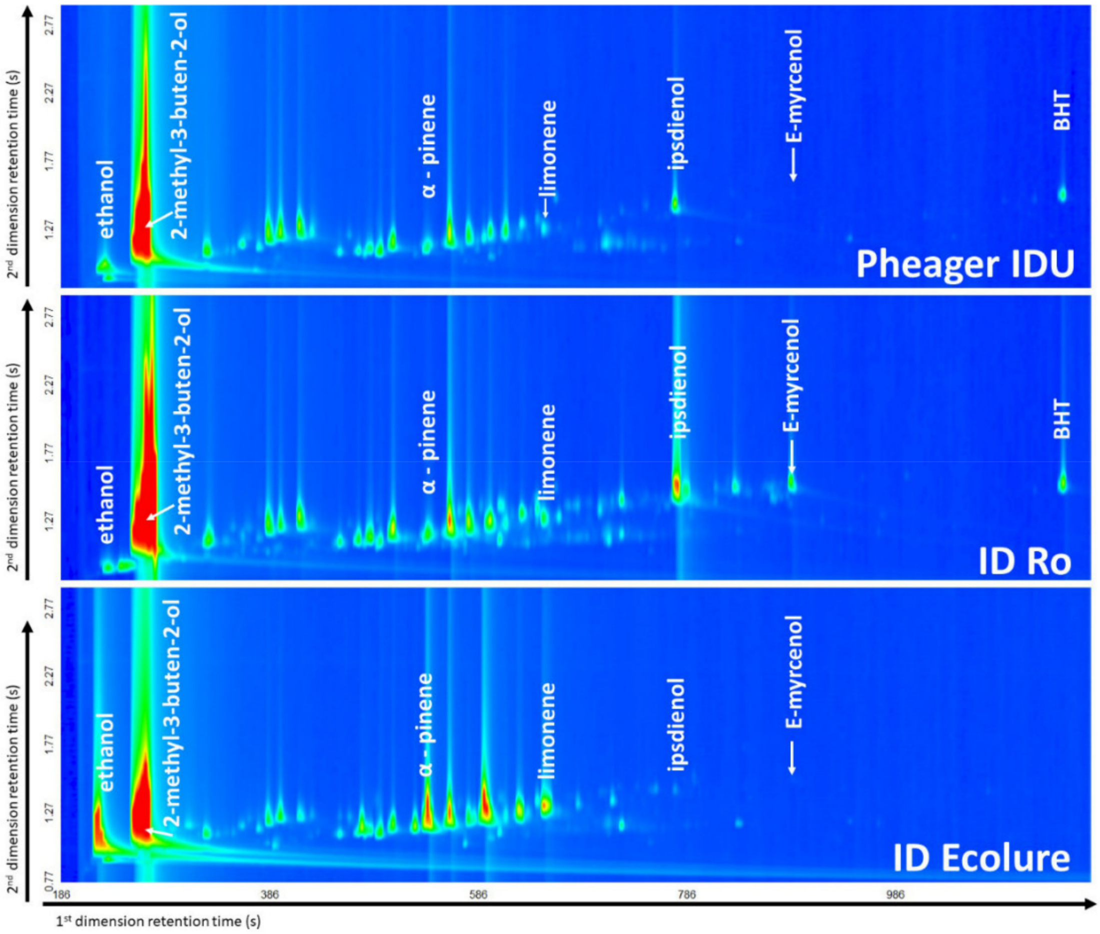

Figure 2. Headspace analysis of active compounds in dispensers tested in 2017. GC×GC chromatograms of volatiles collected to SPME fibres. Volatiles were collected on the second day after dispenser opening. Dispensers were heated to 50 °C. The *x*-axis is retention time on the first-dimensional column, *y*-axis is retention time on the second-dimensional column. Only chromatographic peaks of the active compounds, solvent, and antioxidant are labelled.

3.2. Ips duplicatus Response to Tested Dispensers

3.2.1. Total Captures

The Experiment Conducted in 2011 (18 April–21 June) (Romania)

A total of 68,689 *I. duplicatus* were caught in all three experimental localities in Romania, the ratio between males and females was approximately 1:1. The most insects were caught in Zamostea: 30,214 beetles (51.3% males), while at Calafinești were caught 24,029 beetles (48.1% males) and at Fetești only 14,446 beetles (46.9% males). In all experimental areas, *I. duplicatus* beetles were attracted by all dispenser types (Table 2). Significantly more *I. duplicatus* were caught in the traps baited with ID Ecolure dispensers than those baited with ID RO dispensers. The differences in the captures on these two dispensers varied between 24.9% (Calafindești) and 35.5% (Fetești) (Figure 3). Attractivity to the dispensers did not differ significantly for males and females (Table 3).

The experiment was conducted in 2017 between 7 July and 15 August in the Czech Republic. There were 958 *I. duplicatus* in total (42.5% males) (Table 4) captured in the traps baited with the wicks dispenser (ID Ecolure) and polyethene dispensers (ID RO and Pheagr IDU). Only five beetles were caught in the control traps, which was 0.5% of the total number of beetles caught across all replications, indicating there was no interference in the experimental area. ID Ecolure attracted most beetles. The traps baited with this type of dispenser caught approximately 1.4 and 1.6 times more beetles than the traps baited with

ID RO and Pheagr IDU, respectively, but these differences are not statistically significant (Table 5, Figure 4).

Table 2. *Ips duplicatus* beetles caught in the field in 2011 experiment.

Location	Number of *I. duplicatus*		
	Total	Traps Baited with	
		ID Ecolure	ID RO
Calafindești	24,029	13,256	10,773
Zamostea	30,214	17,113	13,101
Fetești	14,446	8313	6133

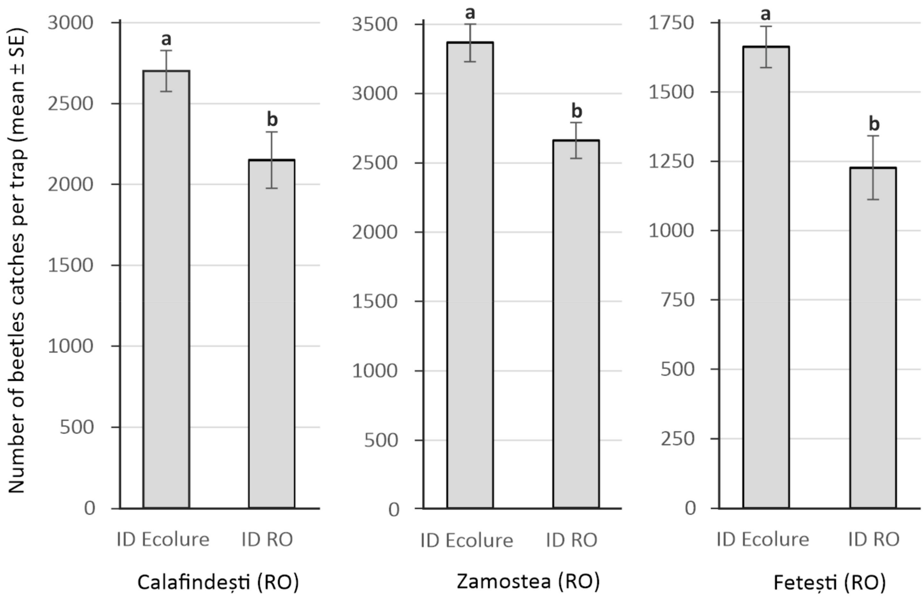

Figure 3. Comparison of the total *Ips duplicatus* captures obtained on the first field experiment for traps baited with ID Ecolure and ID RO dispenser types. Bars with different lowercase letters indicate significant differences according to (Tukey's multiple comparison test: $p < 0.05$).

Table 3. Results of statistical analysis of differences between *Ips duplicatus* captures depending on dispenser type and beetle sex (ANOVA test, $p < 0.05$).

Statistical Value	d.f.	*F*	*p*	d.f.	*F*	*p*	d.f.	*F*	*p*
Location	**Calafindești**			**Zamostea**			**Fetești**		
Dispenser type	1	11.0293	0.0043	1	23.4236	0.0002	1	18.4709	0.0006
Beetle sex	1	1.7082	0.2097	1	1.4547	0.2453	1	3.0260	0.1011
Dispenser type × beetle sex	1	1.9773	0.1788	1	0.1105	0.7439	1	5.8931	0.0574

Table 4. *Ips duplicatus* beetles caught in the field in 2017 experiment.

Location	Number of *I. duplicatus*			
	Total	Traps Baited with		
		ID Ecolure	ID RO	Pheagr IDU
Libava	958	422	300	236

Table 5. Statistical analysis of differences between *Ips duplicatus* captures depending on dispenser type and beetle sex (Kruskal-Wallis: $p < 0.05$).

Statistical Value	d.f.	K	*p*
Location		**Libava**	
Dispenser type	2	2.9206	0.232
Beetle sex	1	0.008	0.929

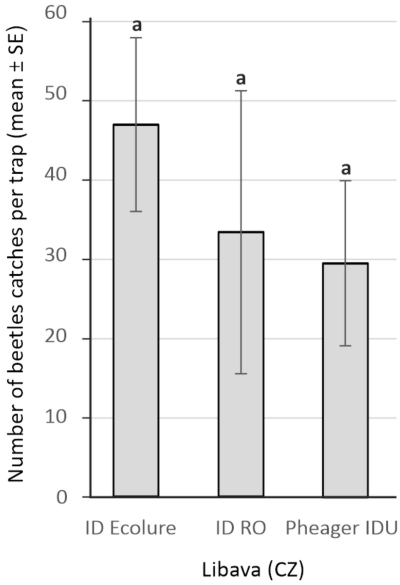

Figure 4. Comparison of *Ips duplicatus* captures obtained on the second field experiment for traps baited with different dispenser types. Bars with the same letters indicate no significant differences (Dunn test: $p < 0.05$).

3.2.2. Capture Dynamics

The Experiment Conducted in 2011 (18 April–21 June) (Romania)

In the first field experiment, the dynamics of *I. duplicatus* captures were strongly influenced by the type of dispenser used and the test location. Regardless of the locality, traps baited with ID Ecolure, had the highest activity in the first two weeks of the test interval (26 April in Zamostea and Fetești and 30 April in Calafindești). Later, the insect response significantly decreased, except for a slight increase recorded after the wick of the ID Ecolure dispensers was shortened according to the manufacturer's instructions (23 May).

In traps baited with ID RO dispensers, the periods of maximum captures varied between experimental localities. For Calafindești, most bark beetles were caught on 30 April, and similar captures were recorded on 21 Jun. At Zamostea, the maximum number of captures was registered on 22 April and 26 April, followed by a sharp reduction in captures until 23 May, when the number of beetles started to increase, exceeding half of the maximum recorded in April. At Feteşti, the maximum number of captures was recorded near the end of the experiment; however, increased captures were obtained in the middle of April and the middle of May.

Regarding the differences in the response of *I. duplicatus* to the two types of dispensers, there were significant differences between captures for most of the experiment. Only at Zamostea on 31 May and at Fetești between 16 May and 6 June did responses not differ significantly. These responses did not manifest themselves differently between males and females, with both sexes being similarly attracted to the two types of tested dispensers (Table 4).

In all locations, the responses of the beetles to the dispensers were significantly more intense for ID Ecolure than ID RO at the beginning of the experimental period, differences that, however, decreased significantly towards the middle of the experimental period. Starting with 6 June, the traps baited with ID RO attracted significantly more beetles than those baited with ID Ecolure. This trend continued until the end of the experiment, given that from 23 May onwards, the proportion of males in captures increased, which indicated an increase in the population of flying adults amid the emergence of new generation individuals (Table 6, Figure 5).

Table 6. Statistical analysis of differences in *Ips duplicatus* captures for between collection sites depending on dispenser type and beetle sex (ANOVA for Calafindești, Zamostea, and Fetești and Kruskal-Wallis for Libava).

Statistical Value	d.f.	*F*	*p*	d.f.	*F*	*p*	d.f.	*F*	*p*
Location	**Calafindești (RO)**			**Zamostea (RO)**			**Fetești (RO)**		
22 April									
Dispenser type	1	69.0437	<0.0001	1	37.7785	<0.0001	1	75.8235	<0.0001
Beetle sex	1	4.2102	0.0469	1	72.3232	<0.0001	1	4.5443	0.0489
Dispenser type × beetle sex	1	0.0340	0.8559	1	1.0838	0.3133	1	0.0408	0.8424
26 April									
Dispenser type	1	25.7831	0.0001	1	68.7199	<0.0001	1	67.2558	<0.0001
Beetle sex	1	14.1755	0.0017	1	43.5801	<0.0001	1	12.5650	0.0027
Dispenser type × beetle sex	1	0.1582	0.6960	1	1.7629	0.2029	1	2.0212	0.1743
30 April									
Dispenser type	1	69.2300	<0.0001	1	121.8809	<0.0001	1	59.6891	<0.0001
Beetle sex	1	0.0115	0.9159	1	2.1285	0.1639	1	2.7178	0.1187
Dispenser type × beetle sex	1	2.1788	0.1593	1	0.0666	0.7996	1	0.0103	0.9203
6 May									
Dispenser type	1	9.8867	0.0063	1	35.7539	<0.0001	1	34.9406	<0.0001
Beetle sex	1	8.7071	0.0094	1	0.0339	0.8562	1	2.2629	0.1520
Dispenser type × beetle sex	1	1.9649	0.1801	1	1.7492	0.2046	1	0.4829	0.4971
16 May									
Dispenser type	1	25.3884	0.0001	1	9.5027	0.0071	1	1.0001	0.3322
Beetle sex	1	31.1495	<0.0001	1	18.8525	0.0005	1	6.2528	0.0236
Dispenser type × beetle sex	1	2.0631	0.1702	1	3.1467	0.0951	1	0.1537	0.7002
23 May									
Dispenser type	1	5.3610	0.0342	1	32.5663	<0.0001	1	0.9217	0.3513
Beetle sex	1	0.5275	0.4781	1	2.3288	0.1465	1	1.8497	0.1927
Dispenser type × beetle sex	1	0.0532	0.8205	1	0.0300	0.8647	1	0.0096	0.9232
31 May									
Dispenser type	1	13.3639	0.0021	1	0.0113	0.9168	1	0.0849	0.7745
Beetle sex	1	51.6891	<0.0001	1	29.1692	<0.0001	1	3.5784	0.0768
Dispenser type × beetle sex	1	0.0010	0.9753	1	0.2519	0.6226	1	0.0248	0.8770
6 June									
Dispenser type	1	66.2809	<0.0001	1	30.9442	<0.0001	1	0.0237	0.8797
Beetle sex	1	19.1216	0.0005	1	4.0924	0.0601	1	1.8133	0.1969
Dispenser type × beetle sex	1	0.6887	0.4188	1	1.2568	0.2788	1	0.0363	0.8514
15 June									
Dispenser type	1	226.3705	<0.0001	1	87.3031	<0.0001	1	59.5073	<0.0001
Beetle sex	1	21.5181	0.0003	1	8.7165	0.0094	1	33.8311	<0.0001
Dispenser type × beetle sex	1	2.1902	0.1583	1	0.8955	0.3581	1	1.8328	0.1129
21 June									
Dispenser type	1	330.1461	<0.0001	1	235.7735	<0.0001	1	64.1967	<0.0001
Beetle sex	1	24.1568	0.0002	1	21.6867	0.0003	1	64.0733	<0.0001
Dispenser type × beetle sex	1	0.3564	0.5589	1	1.2823	0.2753	1	0.2729	0.6086

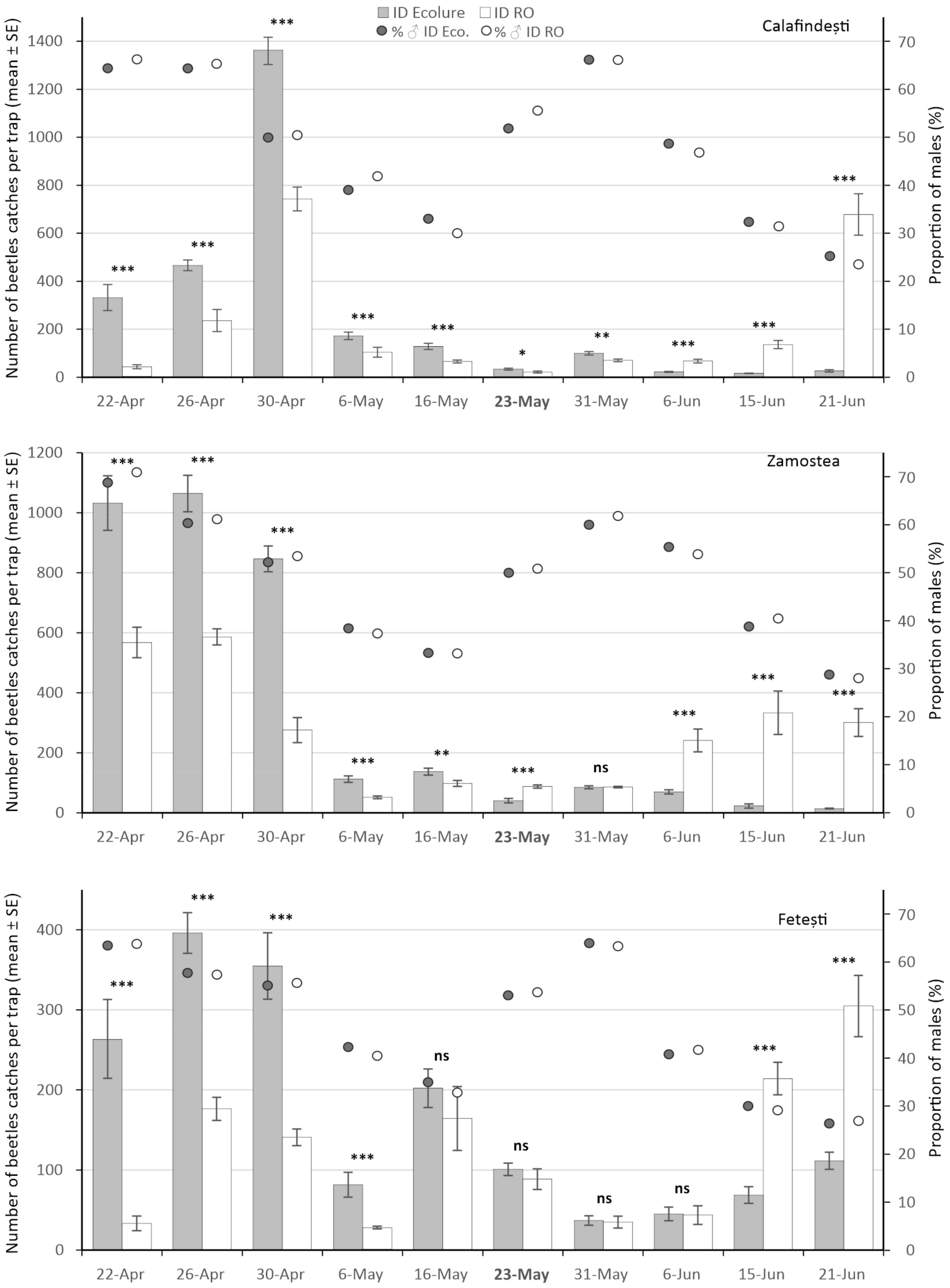

Figure 5. Dynamics of *Ips duplicatus* captures obtained during first field experiment (2011—Romania) at traps baited with ID Ecolure and ID RO dispenser types (mean captures: columns, reported at left *Y* axes, the proportion of males: dots, reported at right *Y* axes). The symbols above the error bars indicate the significance of the differences between the release rates according to *t*-test [ns—not statistically significant ($p > 0.05$); *—significant ($p < 0.05$); **—highly significant ($p < 0.01$); ***—very highly significant ($p < 0.001$)].

The Experiment Conducted in 2017 (7 July–15 August) (The Czech Republic)

In the second field experiment, the trend of the dynamic of the *I. duplicatus* response to ID Ecolure and ID RO dispenses followed capturing dynamic recorded in the first field experiment (2011). At the beginning of the experimental period (in July), more beetles were caught in traps baited with ID Ecolure. In the second part of the experiment (mid-August), most insects were attracted to ID RO dispensers. The *I. duplicatus* responses to PheagrIDU dispensers were intermediate between responses to ID Ecolure and ID RO dispensers. The variability of data is high. Further, the proportion of males varies significantly from one collection date to another, between 46.0% and 76.6% (Ecolure ID); between 38.5% and 83.3% (RO ID) and between 13.7% and 72.2% (PheagrIDU) (Figure 6). These results cannot be supported by the statistical analyses, because only one repetition was collected at a time.

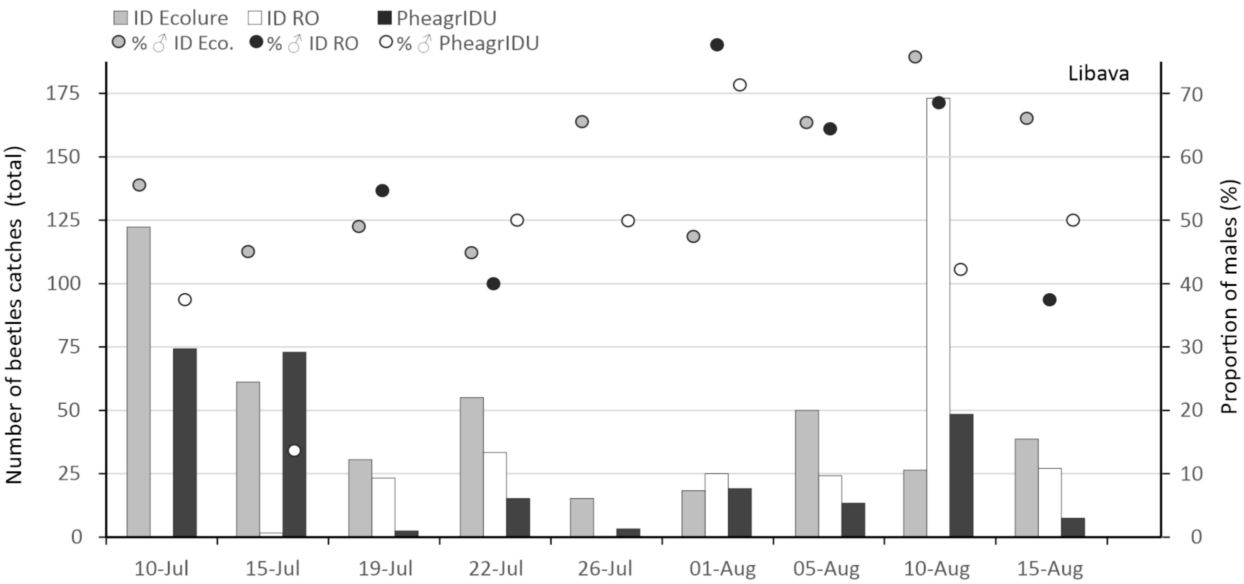

Figure 6. Trends in the dynamics of *Ips duplicatus* captures obtained at second field experiment (2017—Czech Republic) at traps baited with ID Ecolure, ID RO and Pheagr IDU dispenser types (mean captures: columns, reported at left Y axe, the proportion of males: dots, reported at right Y axe).

3.3. *Ips Typographus Response of Tested Dispensers*

As expected, *I. typographus* beetles were also attracted to the baits developed for *I. duplicatus*. Thus, a total of 3903 *Ips typographus* beetles were caught: 2217 individuals in 2011 and 1686 individuals in 2017. Most beetles were recorded at Calafindești and at Libava. For captures collected in 2011, *I. typographus* males represented between 24.4% and 29.8% (Table 7). In 2017 *I. typographus* were not sorted by sex.

Table 7. *Ips typographus* beetles caught in the field.

Location	Number of *I. typographus*	of Which Males	
		N	%
2011 (18 April–21 June)			
Calafindești	1848	451	24.4
Zamostea	131	39	29.7
Fetești	238	74	29.8
2017 (7 July–15 August)			
Libava	1686	-	-

In the experiments conducted in 2011 in Romania, the captures of all *I. typographus* recorded in traps baited with ID Ecolure and ID RO were similar. However, the dispensers affected the responses of males differently than females in two localities: significantly more females were caught at Calafindești and Zamostea, a phenomenon that was not observed at Fetești. In 2017, at Libava, the most *I. typographus* beetles were caught in traps baited with Pheagr IDU, with much lower captures in traps with ID Ecolure and ID RO. However, these differences are not statistically different (Tables 8 and 9).

Table 8. Statistical analysis of differences between *Ips typographus* captures depending on dispenser type and beetle sex (ANOVA for Calafindești, Zamostea and Fetești, and Kruskal-Wallis for Libava).

Statistical Value	d.f.	*F*	*p*	d.f.	*F*	*p*	d.f.	*F*	*p*	d.f.	K	*p*
Location	**Calafindești (RO)**			**Zamostea (RO)**			**Fetești (RO)**			**Libava (CZ)**		
Dispenser type	1	2.1756	0.1596	1	0.8612	0.3672	1	0.5204	0.4811	3	3.7752	0.0203
Beetle sex	1	99.2762	<0.0001	1	19.9929	0.0004	1	1.7809	0.2007	-	-	-
Dispenser type × beetle sex	1	0.0814	0.7791	1	3.1388	0.0955	1	0.0117	0.9152	-	-	-

Table 9. Comparison of *Ips typographus* captures obtained at traps baited with different dispenser types.

Location	Captures (Mean ± SE)							
	ID Ecolure			ID RO			Pheagr IDU	Blank
	Total	Male	Female	Total	Male	Female	Total	Total
Calafindești	170.8 ± 15.7 [A]	36.8 ± 3.4 [b]	134.08 ± 12.4 [a]	198.8 ± 14.4 [A]	53.5 ± 5.2 [b]	145.3 ± 9.8 [a]	-	-
Zamostea	12.0 ± 1.7 [A]	4.4 ± 0.5 [b]	7.6 ± 1.5 [a]	14.2 ± 1.2 [A]	3.4 ± 0.5 [b]	10.8 ± 1.4 [a]	-	-
Fetești	22.2 ± 4.4 [A]	9.5 ± 1.9 [b]	12.4 ± 2.4 [a]	25.4 ± 3.3 [A]	11.3 ± 1.7 [b]	14.1 ± 1.8 [a]	-	-
Libava	48.7 ± 11.2 [A]	-	-	26.6 ± 7.5 [A]	-	-	124.4 ± 49.4 [A]	1.6 ± 0.4 [B]

Note: For every location, the values accompanied by different capital letters indicate significant differences between total catches, and these accompanied by different lowercase letters indicate significant differences between means of males and females. (Tukey's multiple comparison test: $p < 0.05$) in Calafindești, Zamostea, and Fetești; (Dunn test: $p < 0.05$) in Libava.

4. Discussions

4.1. Dispenser Tests in Lab Conditions

The laboratory experiments for the release rates of the ID Ecolure and ID RO dispensers showed significant differences in the functioning of the two types of dispensers. The volatile mixture release rate was the highest for ID Ecolure dispensers at the beginning of the experiment, but over the next five days, the rate dropped strongly to about 20% of the maximum value. The release rate gradually decreased, stabilizing at about 25–30 mg/day after approximately 40 days (when it reached a release rate close to the ID RO dispenser). A similar pattern was found with other types of wick dispensers, in which high rates of volatile release were recorded in the first days of the tests, but later the rates stabilized at significantly lower values, depending on the substance released. [56]. On the other hand, the constant rate recorded for the release of the volatile mixture over a long period from the polyethene envelope dispensers (ID RO) is similar to results from other studies that analyzed this type of dispenser but using other volatile substances [57–59]. The differences between the release rates of the two types of dispensers are primarily due to their construction (in ID Ecolure, the volatile mixture is released directly from the wick, while in ID RO it must pass through the pores of the polyethene foil) but also due to the properties of the components of the mixture (size of molecules, the proportion of components, etc.) [60].

The quantity of compounds listed by manufacturers [35,45,50–52] cannot be directly compared with the dispensers' headspace monitored using SPME, primarily due to SPME selectivity and the different physical features of compounds, such as boiling points, vapour pressure, viscosity or reactivity with membrane material, etc. Nevertheless, this approach allows the comparison of headspace profiles of compounds collected from dispensers. It is worth noting that different dispenser construction plays a role in the evaporation of the compounds, as seen in Figure 1. There were important differences between the dispensers tested regarding the proportion of active components in the mixture. ID RO had a higher percentage of Id and EM than the kairomones α-pinene and limonene (and in terms of absolute abundances, there were also more of these compounds in the dispensers in the beginning). However, interestingly, ID Ecolure had the highest abundance of kairomones and ethanol, and the Id/EM ratio was slightly lower than that of ID RO. The Pheagr IDU dispensers' analyses also revealed the presence of kairomones and ethanol. BHT was detected in the ID RO and Pheagr IDU dispensers, which mainly stabilized the very unstable Id and prolonged its activity [61].

4.2. Ips duplicatus Response to the Tested Dispensers

The highest captures of *I. duplicatus* beetles were recorded in 2011 when the experiments took place in spring in the first part of the insect activity season. Most insects caught in this experiment likely belonged to the overwintering generation and first generations. In 2017, when tests took place in July–August, lower captures were recorded. Beetles caught in this experiment belonged mainly to the first and second generations. As the studies describing the seasonal activity of *I. duplicatus* showed, most insects were caught in the spring's first flight. [36,49,62,63].

Regarding the functioning of the dispenser types, it was found in both field experiments that the wick dispensers (ID Ecolure) attracted, in total, more *I. duplicatus* beetles than the polyethene foil dispensers (ID RO and Pheagr IDU), similarly to tests performed by Holuša et al. [49]. These results are most likely due to differences in the release rate of volatile substances. The attractivity of dispensers is considered directly proportional to their release rate [64–67]. On the other hand, the results can be explained to some extent, by the differences between the volatile mixtures specific to each type of dispenser. The headspace analysis (Table 1) showed that the differences in ID:EM ratio between the ID Ecolure and ID RO dispensers was slightly lower; the higher attractivity of ID Ecolure may have been due to the higher proportion of terpene kairomones, since they have a synergistic effect on the attractivity of the pheromone components ID and EM as their proportions increase [45,68]. In the case of Pheagr IDU, the lower ratio of EM (ID:EM ratio of 565:1) may have been one of the reasons for the lower immediate attractivity of this dispenser, as it is known that European populations of *I. duplicatus* best respond to an ID:EM ratio that varies between 1:1 and 10:1 [43].

Notably, during the test period of both field experiments in 2011 and 2017 conducted in different localities and a different part of season, significant changes in the dynamic of attractivity of the wick dispensers were recorded compared to those dispensers with polyethene foil. Thus, most *I. duplicatus* beetles were caught in ID Ecolure dispensers at the beginning of the period. By contrast, ID RO dispensers were significantly more attractive in the second half of the period. The significantly stronger attractivity of ID Ecolure in the first days was most likely due to both the much higher rates of volatile mixture release and the higher proportion of kairomonal terpenes and ethanol in the mixture in combination with the higher *I. duplicatus* population in spring. The reduction in the attractivity of ID Ecolure dispensers was likely due to the decrease in release rates, but also probably because of the change over time in the spectrum of the volatile compounds due to the preferential release of the most volatile components (MB, terpenes). We plan to verify this important hypothesis in future research. A reduction in the attractivity of wick dispensers after the first days of operation has also been described by other researchers [49]. By comparing the attractivity of ID Ecolure dispensers and Duplodor dispensers (polyethene foil, ID:EM ratio of 5:1–10:1), a very strong attractivity of wick release dispensers was found in the first ten days of the experiment. Then, *I. duplicatus* catches decreased and saw similar levels in traps baited with polyethene foil dispenser. Similar results were obtained by Nakládal and Sova [69], who compared the attractivity of Ecolure Classic wick dispensers with Ecolure Tubus plastic tube dispensers for *I. typographus*. The continuous increase in the number of beetles attracted by ID RO dispensers during the second part of the test period was most likely due to the ability of the polyethene foil dispensers to maintain their attractive characteristics together with increase in the number of beetles in flight due to the population entry of the new generation of adults, an aspect highlighted by the increase in the proportion of males caught after May 23.

4.3. Ips Typographus Response to the Tested Dispensers

According to previous observations [70], many *I. typographus* beetles were caught in traps baited with lures specific to *I. duplicatus*, likely due to the presence of *I. typographus* pheromone components (mainly 2-methyl-3-buten-2-ol and Id) in addition to kairomones AP and L in the tested dispensers. Norway spruce also produces 2-methyl-3-butenol-2-ol, but its amount is below the threshold to attract bark beetles [71]. On the other hand, it is evident that the *I. typographus* captures depended on the local population density. Half of the *I. typographus* number was caught in Romania in 2011 compared to the Czech Republic in 2017. However, the responses of the *I. typographus* beetles were not significantly influenced by the dispenser types.

4.4. Limitation of the Study

Our primary experimental setup and trap spacing are similar to designs used in published studies testing *I. duplicatus* lures [34,36,45]. There are two basic approaches to testing pheromone dispensers for bark beetles in the field. When commercial dispensers were compared for their activity and durability, whole season testing was chosen. It was conducted, if possible, in different localities and repeated in several years [47,48,69]. The long-term studies were also used to test lures for monitoring bark beetles [36,72]. When the newly developed dispensers were tested to optimize their composition and construction, the experiments with randomization of several tested baits in traps took place. These experiments had a shorter duration since the qualitative comparison was sufficient when the particular number of randomizations was achieved [34,45,65,67,73,74].

However, we are aware of the limitations of methodology in not repeating the experiment with a uniform design for two seasons and in the testing length. We decided to terminate the studies after the above described periods since the *I. duplicatus* catches at the end of the season can be very low [36] and may cause problems in the statistical analysis of data in qualitative oriented studies. We assume that combining two-month dispensers testing in Romania, including the first beetles swarming and midseason data from the Czech Republic, may partially compensate for those limitations.

5. Conclusions

In a series of experiments, we compared the activity of three types of pheromone dispensers, with two different constructions, for attracting *I. duplicatus* during a season and evaluated the compositions of the active compounds in the headspace of these dispensers when freshly opened.

The foil release dispenser, with a high proportion of pheromone components compared to kairomones, had a constant release rate for over 60 days. In the first two weeks of use, when the generation of hibernating beetles began to emerge, the number of beetles caught by the foil release dispenser was significantly lower than the number caught by the dispenser with wick release. However, after 45 days, when the offspring generation appeared, the activity of the foil release dispenser type increased not only in comparison to the second dispenser type but also in absolute terms.

The wick release dispenser was found to have a lower proportion of pheromonal components than kairomone and emitted higher amount of volatiles when freshly opened. The release rate decreased after three days of use and slowly decreased over the remaining sixty days of the experiment (except for the increase after reopening). The numbers of *I. duplicatus* caught using this dispenser type were two times higher in the first two weeks of the experiment than using the foil dispenser, but from then onwards, it was less active for the generation of offspring beetles.

We propose that the foil release dispensers can be used for the successful monitoring of *I. duplicatus* flying activity, not only in the first stage of swarming for the first generation but also for the following offspring generation.

On the other hand, the wick type dispensers are more efficient for capturing beetles in the first two weeks of use. Therefore, when used for controlling the most aggressive first beetle generation, they can be considered for controlling *I. duplicatus* population density near the spruce forest edges exposed to a possible attack of this pest.

The use of pheromone traps as part of forest management of *I. duplicatus* could be improved by using trap barriers alternately primed with the two types of dispensers according to when they are most effective.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13040511/s1, Figure S1: Layout of experimental blocks for field experiments conducted in 2011—Romania; Figure S2: Minimum, average and maximum daily temperatures as well as rainy days recorded during the field experiments developed in 2011—Romania, Figure S3: Layout of field experiment conducted in the Czech Republic in 2017; Table S1: Field experiment in 2017 in the Czech Republic. Randomization scheme of tested dispensers in pheromone traps.

Author Contributions: Conceptualization, M.-L.D. and A.J.; methodology, M.-L.D. and A.J.; validation, M.-L.D. and A.J.; investigation, M.-L.D., K.B., R.J., J.H. and A.J.; data curation, M.-L.D., K.B. and A.J.; writing—original draft preparation, M.-L.D. and A.J.; writing—review and editing, R.J. and J.H. All authors have read and agreed to the published version of the manuscript.

Funding: M.L.D. was supported by a grant of the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2020-2696, within PNCDI III. A.J.: K.B., R.J., J.H. were supported by Ministry of Education, Youth and Sport, Operational Programme Research, Development and Education, Extemit K, CZ.02.1.01/0.0/0.0/15_003/0000433.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

Id—ipsdienol; EM—E-myrcenol; AP—α-pinene; L—limonene; SPME—solid phase microextraction; BHT—butylated hydroxytoluene.

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