Field assessment of synthetic attractants and traps for the cerambycid beetles *Tetropium castaneum* (L.) and *Tetropium fuscum* (F.) in the Eastern Carpathians, Romania

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Abstract Tetropium species are significant forest pests in some countries, and their importance is expected to increase with climate change and biological invasions. The research presented in the paper aimed to test the response of adults of Tetropium castaneum (Linnaeus, 1758) and Tetropium fuscum (Fabricius, 1787) to synthetic attractants and to verify the effectiveness of three types of traps in their capture. The experiments were conducted in the northern part of the Eastern Carpathians, Romania. We investigated the beetle's response to fuscumol, both alone and in combination with host tree volatiles (monoterpenes and ethanol) released at different rates. We also tested a combination of (-)-alpha-pinene, ethanol, and aggregative pheromone of Ips typographus (Linnaues, 1758). The effectiveness of capturing Tetropium beetles was evaluated for Crosstrap, Barrier and MultiWit traps. Traps baited with fuscumol or combinations of attractants captured significantly more beetles of both species than unbaited traps. However, the beetles responded significantly more strongly to fuscumol and host volatile combinations than to fuscumol alone. There were no significant differences in the average catches of traps baited only with fuscumol compared to those baited with (-)-alpha-pinene, ethanol, and synthetic *I. typographus* pheromone. Fuscumol baits attracted mainly females, while males responded more strongly to combining fuscumol with host volatiles. The Crosstrap and Barrier traps captured significantly more specimens of T. castaneum and T. fuscum than the MultiWit traps, and the difference between the first two trap types was not statistically significant. For the effective detection and monitoring of *Tetropium* native species, fuscumol lures, in conjunction with host volatile baits, and Crosstrap or Barrier traps are recommended. This approach improves population monitoring and ensures reliable results.

Keywords: black spruce beetle, brown spruce longhorn beetle, fuscumol, (-)-alpha-pinene, monoterpenes, ethanol, *Ips typographus* aggregation pheromone, Crosstrap, Barrier trap, MultiWit trap.

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Introduction

According to data published by Löbl & Smetana (2010), five species of Tetropium Kirby, 1837 (Coleoptera, Cerambycidae, Spondylidinae) are found in continental Europe. These species include: Tetropium aquilonium Plaviltsshikov, 1940; T. castaneum (Linnaeus, 1758); T. fuscum (Fabricius, 1787); T. gabrieli Weise, 1905; and T. gracilicorne Reitter, 1889. Among these, the most widespread species are T. castaneum and T. fuscum, which are also present in Romania (Panin & Săvulescu 1961, Bense 1995). These two species primarily inhabit spruce (Picea) and pine (Pinus) trees, but they can also be found on fir (Abies) trees. They tend to colonise freshly cut logs and trees that have been felled by wind or weakened due to other causes, such as drought, attacks by Armillaria (Fr.) Staude, or infestations by bark beetles (Panin & Săvulescu 1961, Hellrigl 1974, Bense 1995, CABI International 2022).

In the larval stage, all species of *Tetropium* feed on phloem and cambium. However, when prepupal larvae are ready to pupate, they create pupal cells either in or beneath the bark, provided it is sufficiently thick, or in the sapwood, at depths of 2 to 4 cm. This feeding behaviour causes both physiological damage to living trees and technical damage, which refers to the qualitative downgrading of the wood. Specifically, timber from infested trees can experience a 20% reduction in value compared to non-infested trees (Schimitschek 1929, Panin & Săvulescu 1961).

While these species are generally considered secondary pests based on the trees they colonise, *T. fuscum*, accidentally introduced in Canada, has shown an ability to attack living, apparently healthy, trees of *Picea rubens* Sarg., *P. mariana* (Mill.) BSP, and *P. glauca* (Moench) Voss (Smith & Humble 2000, Sweeney et al. 2001). However, research has shown that trees attacked by this species exhibited less vigour before the attack than truly healthy trees (O'Leary et al. 2003).

The significance of these species as forest pests varies by country and is influenced by the development of forest management practices. In the phytosanitary statistics for forests in Romania (Arsenescu et al. 1966, Ștefănescu et al. 1980, Simionescu et al. 2001), only T. castaneum is mentioned. The authors note that these insects primarily target old spruce trees that are either growing poorly, in the process of dying or are found in unpeeled materials in forests or log yards. However, no data is provided on the volume of infested trees. According to Hellrigl (1974) and Schwerdtfeger (1981), T. castaneum and T. fuscum are among the most damaging cerambycids. Although classified as secondary pests, they can kill drought-weakened trees that might otherwise recover if left undisturbed. Evans et al. (2007) also regard Tetropium species as significant pests for similar reasons.

From the data reviewed by Evans et al. (2007), T. castaneum is recognised as a forest pest in only six countries (the Czech Republic, Estonia, Italy, Romania, Slovakia, and Switzerland). In comparison, T. fuscum is identified in two countries (Estonia and Romania), while T. gabrieli is identified in five countries (the Czech Republic, Germany, Hungary, Slovakia, and Switzerland). Based on information from Juutinen (1955), Evans et al. (2007) indicate that T. fuscum and T. castaneum were once considered significant factors contributing to the mortality of senescent Norway spruce trees in the Nordic countries. However, with the advancement of forestry practices, their impact has diminished.

Although there is limited data on the damage caused by these pests, it is evident that the impact can be significant, especially following catastrophic windthrows. If fallen trees are not managed quickly, it allows for the colonisation of these trees by pests and the reproduction of insects. This situation was observed in Romania in the 1990s, where windthrown spruce trees, dispersed over 180,000 hectares and totalling more than 225,000 cubic meters, were infested by these species (Evans et al. 2007).

Climate change is expected to increase the frequency and severity of natural disturbances, particularly in conifer-dominated forests (Seidl et al. 2017, Patacca et al. 2023). As a result, we can anticipate more significant damage caused by native Tetropium species. Furthermore, the intensification of global trade over the last few decades has increased the number of non-native insect species introduced into forest ecosystems where they were previously absent (Hulme 2009, Roques 2010). In this context, there are valid concerns at the European level regarding the spread of T. gracilicorne in Europe (Orlinski 2006). This species originates from Asia and is already found in the central and northern parts of Russia's European territory (Danilevsky 2020). It was first intercepted in Austria in 1998, arriving with larch wood from Siberia (Krehan & Holzschuh 1999). In its native range, T. gracilicorne primarily attacks Larix species but can also affect Picea, Pinus, and Abies (Orlinski 2006). Morphologically, T. gracilicorne is very similar to T. gabrieli (Krehan & Holzschuh 1999; Tuffen 2015). The latter species was intercepted in Sweden in the late 20th century, when it was found in imported wood (Lundberg 1986). It established its first breeding populations after 2005 (Ericson 2010) and is now well established in Sweden, with a wide distribution in the southeast (Lindelöw et al. 2015). Recently, T. gabrieli has also been reported in Lithuania (Lynikienė et al. 2021).

Identifying and monitoring wood-boring insect populations is becoming increasingly important in this scenario. Traditionally, detection methods relied on assessing the damage these pests caused and observing the insects during their flight periods or in the galleries they created beneath the bark and inside the wood (Mihalciuc 2000). However, these methods have significant drawbacks: they do not facilitate the quick identification of specific species in a given area or the easy monitoring of population changes from year to year. These limitations hinder the effective implementation of protection measures for trees and logs that remain in the forest during the summer. In the case of invasive

species, effective strategies are essential to limit their spread and, when possible, eradicate their populations. Enhancing our detection methods can better protect our forests and manage these harmful species effectively. Over the past few decades, research has focused on identifying attractants that, when used in conjunction with various types of traps, can effectively attract and capture *Tetropium* adults.

Research into detecting *Tetropium* species using traps with attractants began in Canada, Switzerland, and Poland. The primary focus was on *T. fuscum* and *Tetropium cinnamopterum* Kirby, 1837, with additional attention given to *T. castaneum*. In their early investigations, Sweeney et al. (2004, 2006) discovered that a synthetic mixture of monoterpenes, resembling the compounds found in the bark and sapwood of *P. rubens* trees, attracted *T. fuscum* beetles more effectively than a racemic mixture of alpha-pinene, ethanol, or their combination. Furthermore, adding ethanol to the synthetic mixture of terpenes significantly increased the capture rates of both *T. fuscum* and *T. castaneum*.

Sweeney et al. (2004) also noted that various combinations of 5:95 (+):(-)-alpha-pinene, ethanol, and the aggregation pheromone of *Ips typographus* (C. Linnaeus, 1758) failed to attract *T. fuscum*. However, they suggested that the pheromone of *I. typographus* might influence the positive response of *T. castaneum* to a combination of 3:97 (+):(-)-alpha-pinene and ethanol. This finding was corroborated by Weslien & Schroeder (1999), who positioned traps near an *I. typographus*-infested spruce log.

Silk et al. (2007) discovered fuscumol [(E)-6,10-dimethyl-5,9-undecadien-2-ol], the main component of the *Tetropium* beetle's pheromone. The researchers found that fuscumol, when combined with a synthetic mixture of monoterpenes and ethanol, attracted significantly more beetles than the terpene mixture alone. However, only the S-fuscumol enantiomer exhibits a synergistic effect, while the R-fuscumol enantiomer has no impact (Sweeney et al. 2009, 2010).

However, the published research indicates that the responses of *T. fuscum* beetles to tested attractants differ between populations from Canada and those from Poland (Sweeney et al. 2006, 2010). This suggests that populations of the same species can vary based on their geographical origin. Additionally, the findings show that adults of *T. castaneum*, the most common species of *Tetropium* in Europe, respond differently to the tested attractants compared to *T. fuscum*.

This research paper evaluates the response of *Tetropium* adults in the Carpathian Mountains to attractants similar to those tested in other regions. The study also aims to assess the effectiveness of capturing *Tetropium* adults by traps currently used in Romania for bark beetles, comparing their performance against two other trap types.

The working hypotheses are as follows: i) Adults of both *Tetropium* species in Romania exhibit weak attraction to traps that are primed solely with fuscumol; ii) The addition of baits containing monoterpenes and ethanol enhances the beetles' response to fuscumol; iii) The response of *Tetropium* adults to a combination of (-)-alpha-pinene, ethanol, and the aggregation pheromone of *Ips typographus* varies by species.

Materials and Methods

Research areas

The research was conducted in the northern part of the Eastern Carpathians (Fig. 1), specifically in areas that had been freshly logged by clear-cutting of stands primarily composed of Norway spruce (*Picea abies* (L.) H.Karst.), with some mixed stands including European silver fir (*Abies alba* Mill.) and European beech (*Fagus sylvatica* L.). The tree stands were aged between 75 and 125 years and were located at altitudes ranging from 730 to 1200 meters (Table 1).

According to data from the Romanian National Meteorological Administration (ANM) (2015-2017), the mean monthly temperatures recorded during the experiments varied as follows: 8.1-10.0°C in May 2016 at Demacuşa_2 and Ionu, and in May 2017 at Cârlibaba; 10.1-12.0°C in May 2015 at Demacuşa_1 and Ciumârna, and in May 2017 at Frumosu; 12.1-14.0°C in June 2015 at Demacuşa_1 and in June 2017 at Cârlibaba; and 14.1-16.0°C in June 2015 at Ciumârna, June 2016 at both experimental areas, and June 2017 at Frumosu.

Table 1 The main characteristics of the research areas (Olenici & Vasian 2024).

	Exp.	Experimental	l Production		w			-	Previous tree	stand		Completion
	No/	area/ Forest district		(ha)	(N)	Longitude (E)		Exposure/ Slope (g)	Composition	Age (years)		of wood harvesting
1	Exp.1/	Demacuşa_1/ Tomnatic	I/50G	4.3	47°40′12″	25°26′18″	890-1000	NE/20	6Nsp2Esf2Ebe	80	0.4	April 2015
2	2015	Ciumârna/ Vama	III/355A	20.0	47°41′24″	25°35′41″	700-925	W/20	10Nsp	115	0.3	April 2015
3	Exp.2/	Tomnatic	I/98F	23.2	47°43′18″	25°25′14″	830-935	NE/23	5Nsp2Esf3Ebe	: 125	0.3	April 2016
4	2016		II /83B%	15+13	47°36′48″	25°28′18″	1000-1180	SW/24	7Nsp3Esf	120	0.4	Winter 2015-2016
5	Exp.3/	Cârlibaba/ Cârlibaba	VI/113A%	3.0+3.0	47°36′18″	25°11′12″	1100-1300)NE/25	10Nsp	95	0.6	Spring 2017
6	2017	Frumosu/ Vama	I/44A	2.45	47°35′25″	25°34′58″	730	N/15	10Nsp	75	0.6	December 2016
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Note: Nsp - Norway spruce, Esf - European silver fir, Ebe - European beech

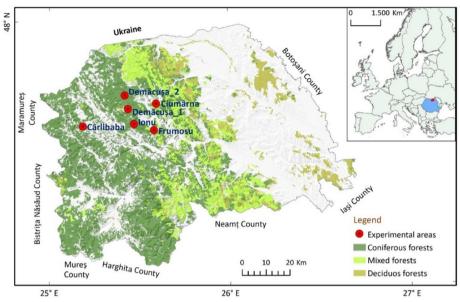


Figure 1 Locations of the research areas.

Lure composition and release rates

Two experiments were conducted to test the previously stated hypotheses: the first in 2015 and the second in 2016. In these experiments, we used similar attractants but not identical to those employed in Canada and Poland. Specifically, we utilised pheromonal lures for Tetropium beetles produced by the "Raluca Ripan" Chemical Research Institute in Cluj-Napoca, Romania. The fuscumol was synthesised in the laboratory by reducing geranylacetone with sodium borohydride (NaBH4) in isopropanol. Both geranylacetone and sodium borohydride were commercially sourced from Sigma Aldrich, Germany. We targeted a release rate of 10 mg/day; however, the exact release rate under laboratory conditions (20°C and 50% relative humidity) could not be measured. This was because the fuscumol baits absorbed moisture from the air, increasing their mass. The polyethylene glycol (MW 400) used as a carrier for fuscumol is highly hydrophilic, which exacerbated this issue.

We utilised several volatile substances that serve as kairomones to mimic the host volatiles. These included (-)-alpha-pinene and (+)-alpha-pinene, both with a purity of 98%; (-)-β-pinene and (+)-limonene, with a purity of 97%; and (+)-3-carene, which has a purity of 90%. All of these products were sourced from Sigma Aldrich, Germany. Additionally, we used 96% ethanol (non-food-grade ethyl alcohol) produced by Chemical Company S.A. in Iaşi, Romania.

To prevent the oxidation of the terpenes when exposed to sunlight, we added 3% Butylated Hydroxytoluene (BHT) FCC/KOSHER with a purity of over 99%. Furthermore, we used AtraTyp Plus® pheromone lures, also produced by the "Raluca Ripan" Chemical Research Institute and widely used for capturing *I. typographus* beetles.

The dispensers containing terpenes and ethanol were handcrafted in the Forest Protection Laboratory at the "Marin Drăcea" National Institute for Research and Development in Forestry, located in Câmpulung Moldovenesc, Romania. Their specifications, including the volatile substances used and their release rates at 20°C and 50% relative humidity, are detailed in Table 2. All lures were stored at -20°C until used in the field.

Table 2 Constructive elements of dispensers and release rates of volatile substances from them.

Table 2 Constructive ciem				Constructive			
Dispenser	Dispenser code	release rate	Laboratory release rates s (mg/day)	Recipient	Internal	Dimensions of cellulosic support (mm)	Volume of volatile substances
		(mg/day)	$(\text{mean}{\pm}\text{SD})$	Type	dimensions		(mL)
					(mm)		
[1Fu: 6.5PEG 400]	FUS	10	not determined ¹	Bag of PE film 40 μm	50 x 70	40 x 60 x 2.5	0.4
[94.8MB : 4.6CV : 0.5Id]	IT	-	$31.4\pm0.2^{\scriptscriptstyle 2}$	Bag of PE film 40 μm	50 x 75	40 x 60 x 2.5	
[(-)AP]	AP	500	$472.8\pm2.2^{\scriptscriptstyle 3}$	Bag of PE film 150 μm	50 x 80	45 x 75 x 4	6.5
[2(+)AP : 2(-)AP : 1L]	TE1	500	591.04	Bag of PE film 150 μm	50 x 80	45 x 75 x 4	8.0
[22(+)AP : 22(-)AP : 19(-) BP : 10 C : 9AT : 18L]	TE2	500	548.05	Bag of PE film 150 μm	50 x 80	45 x 75 x 4	8.0
[ET]	ET1	275	227.5 ± 8.2	PE vial; lid's hole Ø 3 mm	0 28 x 43	15 x 50 x 4	20
[E1]	ET2	550	566.8 ± 21.8	PE vial; lid's hole Ø 5 mm	(A 28 x 43	15 x 50 x 4	25

Note: (-)AP, (-)-α-pinene; (+)AP, (+)-α-pinene; AT, α-Terpinolene; (-)BP, (-)-β-pinene; C, (+)-3-carene; CV, (+)-cis-verbenol; Id, ipsdienol; Fu, racemic fuscumol; L, (+)-limonene; MB, 2-methyl-3-buten-2-ol; PE, polyethylene; PEG 400, polyethylene glycol 400; 1) The release rate could not be determined because in the climate chamber the fuscumol baits increased their mass, absorbing water from the air, PEG 400 being strongly hydrophilic. 2) Olenici et al. (2007); 3) Duduman (2014); 4) Value was determined indirectly by comparing the field release rates of AP and TE1 dispensers from both experimental areas from 18.05 to 19.06.2015. 5) Value was determined indirectly by comparing the field release rates of AP and TE2 dispensers from both experimental areas from 18.04 to 28.06.2016.

During the experiments, the ethanol dispensers were refilled during each trap check. The terpene lures (TE1, TE2, AP) were replaced as needed, typically every two weeks, depending on weather conditions. In contrast, the pheromonal lures remained unchanged from the time of installation until the conclusion of the experiments. Before being installed in the traps and after their retrieval from the field, the terpene dispensers were weighed to calculate their release rates under field conditions. The release rate (mg/day) of lures with TE1 ranged from 405.9 to 814.4 at Demacusa 1 and 481.9-1007.3 at Ciumârna. Those with TE2 showed rates between 140.5 and 466.7 at Demacusa 2 and 108.9 to 487.8 at Ionu. The alpha-pinene (AP) lures had the following release rates (mg/ day): 128.3-690.5 at Demacuşa 1, 215.9-775.1 at Ciumârna, 124.1-480.6 at Demacuşa 2, 85.1-493.7 at Ionu, 224.3-409.9 at Cârlibaba, and 233.8-476.6 at Frumosu.

Traps

In Experiments 1 and 2, we utilised "Barrier" traps (Fig. 2a) produced by the "Raluca Ripan" Chemical Research Institute at "Babeş-Bolyai" University in Cluj-Napoca, Romania. These traps are similar to the Intercept Panel Trap (INT PT), a commonly used model for detecting and monitoring scolytine and cerambycid species, particularly in North America (Czokajlo et al. 2001, 2003). In 2015 (Experiment 1), the traps were equipped with dry jars containing a small sponge impregnated with 1% Fastac Forst insecticide to kill the captured insects. In 2016 (Experiment 2), the traps utilised jars filled with liquid.

In Experiment 3, which aimed to assess the effectiveness of different trap types for capturing *Tetropium* adults, we utilised three types of traps: Barrier traps, Crosstrap® traps manufactured and marketed by Sanidad Agrícola Econex S.L. in Spain, and MultiWit® bark beetle slit traps produced by Witasek PflanzenSchutz GmbH

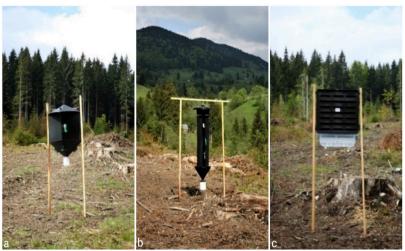


Figure 2 The types of traps used in experiments: a) Barrier; b) Crosstrap; c) MultiWit.

in Austria (Figs. 2b-c). All traps were equipped with a collection vessel filled with liquid. The preservation liquid consisted of water mixed with sodium chloride and a small amount of detergent to reduce the surface tension.

The Barrier trap consists of two intersecting panels, each measuring 72 cm by 50 cm. Each panel features a slot for bait placement, sized at 11 by 7 cm. The trap includes a pyramid-shaped cap and a funnel that is 40 cm square at the top opening and approximately 20 cm deep. Additionally, there is a collecting cup with a diameter of 9 cm and a depth of 13 cm. The total height of the trap is 93 cm, and it has a cross-sectional area of 0.20 m² between the top and bottom funnels. All trap components, except for the collecting vessel, are made from black cellular polypropylene plates with a thickness of 3 mm.

The Crosstrap® trap features a polypropylene lid with a diameter of 34 cm, to which two reinforced PVC vanes are attached. Each vane measures 22 cm by 98 cm and extends above a polypropylene funnel with a top opening diameter of 26.5 cm and a depth of approximately 25 cm. Below the funnel is a collecting cup with a diameter of 10 cm and a depth of 15.5 cm. When fully unfolded, the trap stands 146 cm tall and has a cross-sectional

area of 0.22 m² above the funnel. According to the manufacturer, the PVC vanes, funnel, and collecting cup have been treated with a slippery film to significantly increase captures and prevent pests from escaping.

The MultiWit® trap is a box that stands 50 cm tall, 49 cm wide, and 6.5 cm deep (excluding entry slits). It is constructed from dark brown or black dense plastic featuring a remarkably smooth surface, which prevents bark beetles from gaining a grip. Below the box is a transparent plastic trap tub that measures 16.5 cm by 50 cm by 6.3 cm, making it suitable for wet and dry trapping. The total height of the trap is 67 cm, and it has a cross-sectional area of 0.25 m² between the top and the collecting tub. The combined area of the entry slits is 0.11 m².

In all experiments, the traps were set up on 2-meter-long wooden rulers inserted into the ground, ensuring that the upper part of the traps was approximately 1.75 to 1.8 meters above the ground.

Organisation of experiments

Table 3 presents details regarding the treatments and replication numbers for each experiment. In Experiment 3, the traps were primed with FUS+2AP+ET2.

Table 3 Details regarding the organisation of the experiments.								
Treatment	Experiment 1 - 20	15	Experiment 2 - 201	6	Experiment 3 - 2017			
Treatment	Attractants	Replications	Attractants	Replications	Trap types	Replications		
V1	FUS	5	FUS	6	Barrier	5		
V2	FUS+TE1+ET1	5	FUS+2TE2+ET2	6	Crosstrap	5		
V3	FUS+AP+ET1	5	FUS+2AP+ET2	6	MultiWit	5		
V4	AP+ET1+IT	5	Control	6				
V5	Control	5						

Table 3 Details regarding the organisation of the experiments.

Note: The periods of performing the experiments: 2015 – Demacuşa 1 - 5.05-30.06; Ciumârna 6.05-16.06; 2016 – Demacusa 2 – 18.04-27.06; Ionu – 21.04-28.06; 2017 – Frumosu 19.04- 22.06; Cârlibaba – 3.05-26.06

In the field, the treatments were organised into randomised complete blocks, with traps positioned 20-25 meters apart within each block and at least 25-30 meters apart between blocks. Depending on the specific conditions in the field, the traps were placed either closer to or farther from the edge of the tree stand. For example, in 2015, at Demacuşa_1, the traps were set 5-10 meters from the edge of the mature forest. At Ciumârna, the traps in the first three blocks were placed 10-25 meters from the edge of the stand, while blocks 4 and 5 were positioned parallel to blocks 2 and 3, but more than 30 meters away, inside a logged area where no edge of the stand was available.

In 2016, at Demacuşa_2, the traps were positioned at least 5 to 10 meters from the forest's edge, where mature trees were present. The last block of traps was placed within the clear-cut area, over 50 meters from the forest's edge. At Ionu, traps from four blocks were arranged in three nearly parallel rows along the level curve, with more than 30 meters between each row within a larger area. Additionally, traps from two other blocks were set in a smaller clear-cut area, located approximately 200 meters downstream from the first logged site within the same forest compartment.

In 2017, at Frumosu, most traps were situated between 100 and 150 meters from the edge of the mature tree stand, except for the last block, which was located approximately 10 to 15 meters from the forest edge. Similarly, at Cârlibaba, the traps were placed along the edge approximately 10 to 15 meters from the trees.

In Experiment 1, the positions of the traps were permuted within the blocks to minimise their influence on the catches. This adjustment was not made in Experiments 2 and 3.

Due to the specific working conditions (insufficient personnel, weather conditions, etc.), we were forced to adjust the duration and periods of conducting the experiments while being careful not to compromise the established objectives.

Collection, identification and sexing of Tetropium specimens

The insects captured in the traps were collected at intervals of 7 days (Experiment 1; Experiment 3 - Frumosu) or 12-14 days (Experiment 2; Experiment 3 - Cârlibaba). The collected insects were stored in a freezer until they underwent laboratory analysis. Tetropium species were identified by their morphological characteristics, as described by Bense (1995). The specimens were categorised by sex based on distinct characteristics. In males, the antennae slightly exceed half the length of the elytra, the femora are thicker than those of females, and the last abdominal segment is short. In contrast, females have antennae that do not reach half the length of the elytra, and their last abdominal segment is slightly wider than long (Panin & Săvulescu 1961). In addition, the fifth visible abdominal sternite in females is more elongated than in males (Cherepanov 1988).

The nomenclature of the plants and insects followed the Global Biodiversity Information Facility (GBIF).

Statistical analysis

The cumulative catch data collected throughout each experiment were used in the calculations for each species. During our exploratory analysis, we observed significant variability in the data and some outliers, which we assessed using box-and-whisker plots. These outliers appeared in nearly all data sets. Most of them were mild outliers, with only a few classified as extreme according to the literature criteria (Dunn 2021). These outlier values resulted from natural variability, not from errors in counting or recording. Consequently, we decided against removing them, mainly since the sample size was already relatively small.

We combined the data from both experimental areas for each experiment, considering that a test's power function relies on the sample size. To ensure this combination was appropriate, we analysed the similarity of the datasets.

For datasets that followed a normal distribution (as determined by the Shapiro-Wilk test), we verified the equality of variances using Levene's test. We compared the means using a two-tailed *t*-test for independent samples. In cases where at least one of the datasets did not conform to a normal distribution, we employed the Mann-Whitney two-tailed test, which tests the null hypothesis that the difference in location between the samples is zero. Additionally, we used the Mood's Median test to compare the medians of two independent samples. These analyses were conducted separately for datasets representing males and females, as well as for datasets that pooled specimens of both sexes.

To evaluate whether there were statistical differences in the means of the catches from each treatment, we first assessed the normality of the groups formed by combining the data and analysing their variances using the tests mentioned earlier. If the raw data failed to meet the assumptions of normality and equal variances, we transformed the data using either a logarithmic transformation ($\log(x+1)$) or taking the square root ($\sqrt{x+3/8}$), as recommended in the literature (Zar 2010). We conducted an ANOVA test for normally distributed data, followed by Tukey's post hoc test (HSD). In

cases where the normality assumption was not met and the data could not be normalised through transformation, we applied the Kruskal-Wallis test, followed by the Steel-Dwass-Critchlow-Fligner procedure (Hollander et al. 2014).

After conducting the ANOVA test, we confirmed that the residuals also met the assumptions of normality and homoscedasticity.

In our analysis using ANOVA, we examined whether the experimental area had a significant impact on the size of the catches. We found no significant influence in the first two experiments and processed the pooled data. However, in the third experiment, we observed a substantial effect. To ensure the accuracy of our findings, we conducted a thorough, separate analysis for each experimental area in this case.

We used the Chi-square test to compare the proportions of males in the total catches across different treatments. Then, we applied the Marascuilo procedure (Marascuilo 1966) to assess the significance of the differences in these proportions. The same approach was used to analyse the proportions of the two species in the total catches according to treatment.

To assess whether the two beetle species exhibited different levels of adult response to the attractants being tested, we analysed the statistical significance of the differences between the proportions of catches in traps baited with specific attractants and those in control traps for *T. castaneum* and *T. fuscum*, respectively. We utilised 2x2 contingency tables and applied Fisher's exact test for this analysis.

All data processing, except contingency tables, was conducted using XLSTAT version 2019.1.1 (Addinsoft 2021). Contingency tables were analysed using the PAST program (Hammer et al. 2001).

The main results regarding the number of beetles caught in the traps are displayed using box and whisker plots. These plots illustrate the data distribution across quartiles, highlighting the mean, represented by an "x" on the chart, and any outliers. The boxes include vertical lines known as "whiskers," which indicate variability beyond the upper and lower quartiles. Any points that fall outside these lines are classified as outliers.

Results

Experiment 1

Both *Tetropium* species were captured in Experiment 1, and *T. castaneum* was much more abundant than *T. fuscum* (8,639 specimens and 1,159 specimens, respectively). Males (M) and females (F) were almost equally captured in both species (M/F = 1:1.02 for *T. castaneum* and M/F = 1:1.03 for *T. fuscum*). The mean capture of *T. castaneum* and *T. fuscum* among the five treatments differed significantly (Table 4 and Figs. 3-4).

Catches of *T. castaneum* and *T. fuscum* were 3.51-4.42 and 3.65-5.13 times higher in traps baited with fuscumol (V1) compared to unbaited traps (V5). The differences between V1 and V5 were statistically significant for both species and sexes (males, females, and combined) (Figs. 3-4). Still, the two species did not differ in the intensity of the response to fuscumol (Fisher's exact test p = 0.87375 for M, 0.68231 for F, 0.78287 for M+F captures).

Traps baited with a combination of attractants (AP+ET1+IT, V4) recorded similar catches to those from traps baited with fuscumol (V1) for both *T. castaneum* (Fig. 3) and *T. fuscum* (Fig. 4). All differences between V4 and V5 were statistically significant. Both species reacted equally strongly to the combination of (-)-alpha-pinene, ethanol, and the *I. typographus* aggregative pheromone (Fisher's exact test p = 1 for M., 0.35459 for F, and 0.52834 for M+ F).

Traps that contained FUS+TE1+ET1 (V2) captured 1.74 times more *T. castaneum* and 2.79 times more *T. fuscum* beetles (males and females combined) than traps that were only baited with FUS (V1). The differences were statistically significant for both species and

both sexes. Additionally, traps with fuscumol, terpene, and ethanol caught significantly more males and females from both species than control traps. Adults of *T. fuscum* were more attracted to this combination of attractants than *T. castaneum*. The difference was statistically significant for females and total catches (males and females combined), with Fisher's exact test yielding p-values of 0.14226 for males, 0.00224 for females, and 0.00135 for the combined total.

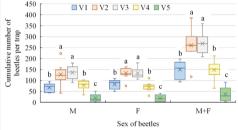


Figure 3 The number of *T. castaneum* caught in traps baited with different attractants within Experiment 1. Treatments: V1 – FUS, V2 – FUS+TE1+ET1, V3 – FUS+AP+ET1, V4 – AP+ET1+IT, V5 – control; M – males, F – females. Different letters at bars indicate significant differences at p < 0.05 (ANOVA test and Tukey (HSD) posthoc test on raw data) between treatments.

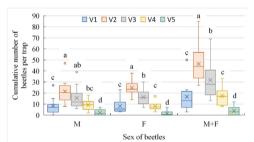


Figure 4 The number of *T. fuscum* caught in traps with different attractants within Experiment 1. Treatments: V1 – FUS, V2 – FUS+TE1+ET1, V3 – FUS+AP+ET1, V4 – AP+ET1+IT, V5 – control; M – males, F – females. Different letters at bars indicate significant differences at p < 0.05 (ANOVA test and Tukey (HSD) post-hoc test on data transformed by sqrt(x+3/8)) between treatments.

Table 4 Treatment and block effect on T. castaneum and T. fuscum captures in Experiment 1 T. fuscum T. castaneum Species Statistical values d.f. d.f. Males (M) 55.904 < 0.0001 < 0.0001 Treatment 4 4 26.731 Block 9 6.149 < 0.0001 7.028 < 0.0001 Females (F) < 0.0001 < 0.0001 Treatment 4 106.750 4 108.302 9 < 0.0001 < 0.0001 9 Block 6.266 12.469 M+F 4 95.044 < 0.0001 34.829 < 0.0001 Treatment 4 9 9 Block 7.771 < 0.0001 5.823 < 0.0001

A similar trend was observed in traps baited with FUS+AP+ET1 (V3), which captured 1.79 times more specimens (M+F) of *T. castaneum* and 1.91 times more *T. fuscum* than those primed with FUS (V1). All differences between captures in V1 and V3 were statistically significant for both species. However, statistically significant differences between V2 and V3 were only found for female and combined catches of *T. fuscum*. Both species responded similarly strongly to the combination of fuscumol, (-)-alpha-pinene, and ethanol (Fisher's exact test p = 0.81087 for M., 0.2268 for F, and 0.49406 for M+F).

were statistically significant differences between treatments regarding the proportion of males in the total captures of T. castaneum (Chi-square = 19.6923; DF = 4; p = 0.0006), but not in the case of T. fuscum (Chisquare = 4.304; DF = 4; p = 0.366). The traps baited with FUS (V1) caught significantly fewer males of *T. castaneum* than those baited with FUS+AP+ET1 (V3) or AP+ET1+IT (V4) (Table 5). Additionally, statistically significant differences were observed in the proportions of T. fuscum participation in the total catches per treatment (Chi-square = 46.714, DF = 4, p < 0.0001). The proportion recorded at V2 (15.1%) was significantly higher than that in the control group (9.4%) and the other treatments (10.0-10.6%). This observation is due to the stronger response of this species to the combination of attractants used in V2.

Table 5 *Tetropium* male proportion according to treatment (Experiment 1)

(Experiment 1):						
Treatment	Males in total catches (%) Tetropium castaneum Tetropium fuscum					
V1						
V 1	45.1 ^b	50.6a				
V2	48.8ab	46.4a				
V3	50.7^{a}	48.9ª				
V4	52.6a	53.4ª				
V5	50.8^{ab}	59.0°				

Note: Values followed by the same letter do not differ significantly at p = 0.05 ($\chi 2$ test, followed by the Marascuilo procedure).

Experiment 2

In Experiment 2, 10,466 T. castaneum and 1,041 specimens of T. fuscum were captured. As in Experiment 1, both sexes were approximately equally represented in each species (M/F = 1:1.17 and 1.06:1 for T. castaneum and T. fuscum,

respectively).

The number of insects captured in both species was significantly influenced by treatment (Figs. 5-6). As the chemical composition of the tested lures was similar to that of Experiment 1, the ranking of the treatments in terms of the number of catches was almost identical to that seen before in Figs. 2-3. This time, catches of T. castaneum and T. fuscum were 4.72-8.86 and 5.22–6.43 times higher in traps baited with fuscumol (V1) than in unbaited traps (V5), and the differences between means were also significant in both species (Figs. 5-6). In this experiment, the strength of the adult response to fuscumol was also found to be independent of species, with Fisher's exact test yielding p-values of 0.57417 for males, 0.15413 for females, and 0.66488 for both sexes combined.

Incorporating TE2 and ET2 lures with those of FUS in V2 led to increased captures of T. castaneum by 1.61 times and T. fuscum by 5.51 times. Therefore, V2 significantly differs from V1 in both sexes and species. Even higher catches of *T. castaneum* were observed in traps baited with FUS+AP+ET2 (V3). However, the differences between V2 and V3 were not statistically significant for either T. castaneum or T. fuscum (Figs. 5-6). Beetles of T. fuscum showed a stronger attraction to the combinations of volatile substances used in V2 and V3 than those of T. castaneum. However. significant differences statistically observed only among males (Fisher's exact test V2: p = 1.6272E-06 for M, 0.55124 for F, 1.1426E-06 for M+F; V3: p = 0.01383 for M, 0.43895 for F, 0.01387 for M+F).

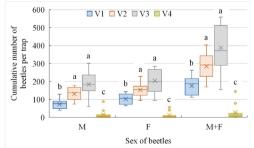


Figure 5 The number of *T. castaneum* caught in traps baited with different attractants within Experiment 2. Treatments: V1 – FUS, V2 – FUS+TE2+ET2, V3 – FUS+AP+ET2, V4 – control; M – males, F - females. Different letters at bars indicate significant differences between treatments at p < 0.05 (Kruskal-Wallis test and the Steel-Dwass-Critchlow-Fligner procedure on data transformed by sqrt(x+3/8)).

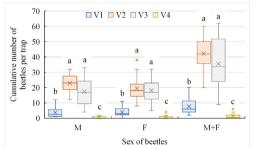


Figure 6 The number of T. fuscum caught in traps baited with different attractants within Experiment 2. Treatments: V1 - FUS, V2 - FUS+TE2+ET2, V3 -FUS+AP+ET2, V4 – control; M – males, F - females. Different letters at bars indicate significant differences between treatments at p < 0.05 (Kruskal-Wallis test and the Steel-Dwass-Critchlow-Fligner procedure on data transformed by sqrt(x+3/8)).

In the case of T. castaneum catches, the proportion of males across different treatments ranged from 41.8% to 57.4%, with significant differences observed. The highest proportion of males was found in the control variant, while the lowest was in V1 (FUS). In contrast, for T. fuscum beetles, the differences between the variants were not statistically significant (Table 6). However, the proportion of males varied slightly less than in *T. castaneum*, ranging from 43.8% to 54.0%.

Table 6 Male proportion in *Tetropium catches* according to

treatment (Experiment 2).

	Males in total	catches (%)
Treatment	Tetropium	Tetropium
	castaneum	fuscum
V1	41.8°	48.9a
V2	46.0^{b}	54.0a
V3	47.5 ^b	49.1a
_V4	57.4a	43.8a

Note: Values followed by the same letter do not differ significantly at p = 0.05 (χ 2 test, followed by the Marascuilo procedure).

The proportions of the two species in the total catch varied significantly according to the treatment (Chi-square = 146.760; DF = 3; p < 0.0001). Thus, T. fuscum was present in a proportion of only 4.7% in V4 (control) and 4.2% in V1, compared to 8.4% in V3 and 12.9% in V2. As a result, V1 does not differ from the control, but V2 and V3 differ from each other and the other two variants.

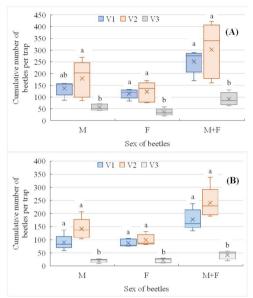


Figure 7 The number of T. castaneum caught in traps baited with FU+2AP+ET2 within Cîrlibaba (A) and Frumosu (B) experimental areas. Treatments: V1 – Barrier, V2 – Crosstrap, V3 – MultiWit; M – males, F - females. Different letters at bars indicated to the content of t significant differences between treatments at p < 0.05(Carlibaba: ANOVA and Tukey (HSD) tests on raw data, but using Levene's median test (Brown-Forsythe) to assess homoscedasticity; Frumosu: ANOVA and Tukey (HSD) tests on raw data of F and M+F, but logtransformed data for M).

Experiment 3

In Experiment 3, 6,361 *Tetropium* specimens were collected, including 5,528 T. castaneum and 833 T. fuscum. In the Cârlibaba experimental area, 3,232 T. castaneum and 451 T. fuscum specimens were captured. Meanwhile, in Frumosu, 2,296 T. castaneum and 382 T. fuscum specimens were collected. In both experimental areas, for both beetle species, males were more prevalent, with maleto-female ratios of 1.36:1 and 1.21:1 for T. castaneum, and 1.55:1 and 1.40:1 for T. fuscum in Cârlibaba and Frumosu, respectively.

Catch size was significantly influenced by treatment (trap type) in both experimental areas and species (Figs. 7-8). Regardless of the species and the experimental area, Crosstraps (V2) caught the most insects. Barrier traps (V1) were in second place, and MultiWit traps (V3) were in third (Figs. 7-8).

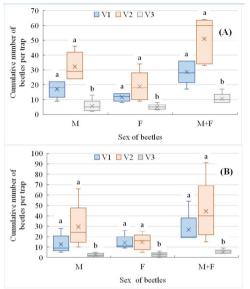


Figure 8 The number of *T. fuscum* caught in traps baited with FU+2AP+ET2 within Cîrlibaba (A) and Frumosu (B) experimental areas. Treatments: V1 – Barrier, V2 – Crosstrap, V3 – MultiWit; M – males, F - females. Different letters at bars indicate significant differences between treatments at p < 0.05 (ÅNOVA and Tukey (HSD) tests for M and F data, but the Kruskal-Wallis test and Steel-Dwass-Critchlow-Fligner procedure for M+F data in both areas; all tests were applied on log-transformed data).

Table 7 Male proportion in *Tetropium* catches according to treatment (Experiment 3).

	Males in total catches (%)					
Treatment	Tetropium o	castaneum	Tetropium fuscum			
	Cârlibaba	Frumosu	Cârlibaba	Frumosu		
V1	54.4 ^b	50.7 ^b	59.4ª	47.0 ^b		
V2	59.2a	58.9a	63.1a	66.7a		
V3	60.9^{a}	48.3 ^b	52.8a	46.2ab		

Note: Values followed by the same letters do not differ significantly at p=0.05 ($\chi 2$ test, followed by the Marascuilo procedure).

Catch differences between V1 and V2 variants were insignificant, but those between V1-V2 and V3 were significant. However, the Crosstrap model captured 20-36.1% and 65.7-78.3% more specimens of *T. castaneum* and *T. fuscum* than the Barrier traps. The Barrier traps performed 63.5-74.4% and 62.9-80.6% better than MultiWit traps in capturing *T. castaneum* and *T. fuscum*, respectively.

There were also differences between the three types of traps regarding the share of males in the total catches. Apart from *T. castaneum* from Cârlibaba, the highest weights were observed at

Crosstrap (V2) and the lowest at MultiWit (V3) (Table 7). However, statistically significant differences existed mainly between V2 and V1, except for *T. fuscum* from Cârlibaba.

Discussion

Due to weather conditions during the three-year experimentation period, intense flight typically began after mid-May. As a result, although the experiments were set up much earlier in the field, the actual duration of insect capture was, in all cases, shorter than 45–50 days. Nevertheless, in all six experimental areas, many specimens of both species of *Tetropium* were captured: 2,296 to 5,436 of *T. castaneum* and 382 to 621 specimens of *T. fuscum*. This was accomplished with only 15 to 20 baited traps in each experimental area (see Table 3).

The captures we observed were much higher than those reported in previous experiments conducted in Białowieża, Poland, from May 20 to July 8, 2003 (Sweeney et al. 2006), and from May 7 to July 2, 2008 (Sweeney et al. 2010). Our lure's release rates of volatile substances were comparable to, or even lower than, those used by Sweeney et al. (2006, 2010). Therefore, it can be inferred that the population densities in our experimental areas were higher than those in the earlier studies, particularly for T. castaneum. The increase in insect density was not caused by large volumes of infested wood in the area from previous years. Instead, it was due to the concentration of insects in locations with fresh logging debris, where it is assumed that the fresh stumps served as a substrate for their oviposition (Schroeder et al. 1999, Skrzecz & Bulka 2010, Skrzecz et al. 2016). This confirms the presence of high levels of volatile substances, such as terpenes and ethanol, released from stumps and other logging debris around the traps used in the experiments.

In this context, traps exclusively primed with racemic fuscumol (V1) captured significantly more specimens of both *T. castaneum* and

T. fuscum than unprimed traps (Figs. 3-6). Our results contrast with those from Canada and Poland (Silk et al. 2007, Sweeney et al. 2010) and appear to be influenced by the environment surrounding the traps, which had a high concentration of natural host volatiles. However, in experiments carried out in Sweden, traps baited with racemic E-fuscumol caught significantly more specimens of both species than those baited only with host volatiles (alpha-pinene and ethanol). The latter caught significantly more specimens of T. castaneum than the unbaited ones (Schroeder et al. 2021), although these experiments did not occur in freshly clear-cut areas.

This suggests differences in beetle responses based on geographic region, as indicated by varying responses of T. fuscum to pure (S)fuscumol in Canada and Poland (Sweeney et al. 2010). Another possibility could be the higher release rates of fuscumol in the Swedish experiments (0.5-2.0 mg/day) and our own, compared to the maximum rate of 0.8 mg/day quantified in studies by Silk et al. (2007) and Sweeney et al. (2010). However, Sweeney et al. (2010) found that in traps with host volatiles and racemic fuscumol, mean catches of Tetropium species were unaffected by fuscumol release rates ranging from 1 to 32 mg/d. Similar bait-and-trap experiments should be conducted in environments with normal concentrations of host volatiles and environments with high concentrations of these substances, as well as in different geographic regions, to verify further the pattern observed in our experiments.

The addition of host tree volatiles baits to those with fuscumol led to a significant increase in catches of both *T. castaneum* and *T. fuscum* in both experiments, results consistent with those published by Silk et al. (2007) and Sweeney et al. (2010). When combined with ethanol, the two monoterpene mixtures tested in experiments 1 and 2 were not significantly more attractive than (-)-alpha-pinene mixed with ethanol. The only exception was *T.*

fuscum (F, F+M) in Experiment 1, where the mixture was significantly simplified compared to the spruce blend used in previous studies by Sweeney et al. (2004, 2010) and Silk et al. (2007), as well as in our second experiment. However, compared to the other treatments, the significantly higher proportion of *T. fuscum* in the total catches from V2 indicates that this species responds more strongly than T. castaneum to the addition of other monoterpenes besides (-)-alpha-pinene. It is important to note that when (+)-limonene is added to racemic alpha-pinene (V2, Experiment 1), T. fuscum females exhibit a stronger response. At the same time, males respond more strongly to the complete bouquet of terpenes (V2, Experiment 2). This suggests that creating a combination of lures that optimally appeals to both species and sexes is impossible.

An interesting observation is the notable increase in catches of *T. fuscum* in traps baited with fuscumol, (-)-alpha-pinene, and ethanol compared to traps baited only with fuscumol. This effect was not observed in the Swedish experiments conducted by Schroeder et al. (2021). The discrepancy may be due to variations in the ethanol and alpha-pinene release rates of these experiments.

Since in experiments 1 and 2, the adults of the two Tetropium species were significantly attracted to racemic fuscumol (V1) baits, the differences between the V1 treatment, on the one hand, and the treatments in which the fuscumol baits were combined with host tree volatiles (V2, V3) have been much lower than in the experiments performed by Silk et al. (2007) and Sweeney et al. (2010). Specifically, at V2 and V3, the recorded catches were only 1.6 to 2.2 times higher than at V1 for T. castaneum and 1.9 to 5.5 times higher for *T. fuscum*. In contrast, previous studies have shown that catches of T. fuscum in Canada were 12 to 15 times higher, while in Poland, the increase for both species was approximately 25 to 28 times higher.

Considering that the release rate of host volatiles strongly influences the beetle response

(Sweeney et al., 2006), the differences between the results could also be because we used lures with monoterpene release rates 2-4 times lower than those used in the experiments performed by Silk et al. (2007) and Sweeney et al. (2010). However, comparing the results from V2-V3 of experiments 1 and 2, it is clear that the increased release rate of host tree volatiles in the second experiment significantly boosted the catches of *T. fuscum* but did not have the same effect on *T. castaneum*. Presumably, in an environment rich in host volatiles, *T. castaneum* adults did not detect the increased release rates of volatiles from baits, while *T. fuscum* did.

Adults of *T. cinnamopterum* show a strong response to the combination of fuscumol and spruce blend, as established by Sweeney et al. (2004), along with ethanol (Silk et al. 2007, Sweeney et al. 2010). Similarly, *T. gabrieli* responds well to a mix of fuscumol, (-)-alphapinene, and ethanol (Schroeder et al. 2021).

Sweeney et al. (2004) noted that *T. fuscum* was not attracted to various combinations of alpha-pinene with ethanol and the aggregation pheromone of *I. typographus*. However, in Experiment 1, both *Tetropium* species responded to this combination (AP+ET1+IT, V4). The catches were comparable to those obtained from traps baited with fuscumol alone.

The beetles' responses to the tested attractants varied not only by species but also by sex. Although significant differences between the treatments from this point of view were noted only in the case of *T. castaneum* (Tables 5-6), it was found that in the traps primed with fuscumol or fuscumol in combination with a mixture of host tree volatiles, females predominated in most cases, which is in agreement with the observations from experiments done by Silk et al. (2007) and Sweeney et al. (2010), while in traps baited with alpha-pinene, ethanol and aggregation pheromone of *I. typographus*, the males predominated.

One notable aspect of our experiments is the significantly higher abundance of *T. castaneum* compared to *T. fuscum*, which contrasts with

findings from Białowieża, Poland (Sweeney et al. 2006, 2010). The lower abundance of *T. fuscum* in our study area aligns with its known distribution, primarily found in Central and Northern Europe. However, even within those regions, *T. fuscum* is much rarer than *T. castaneum* (Hellrigl 1974, Klimetzek and Vite 1989, Schroeder et al. 2021). Furthermore, reports of *T. fuscum* are scarce in Romania (Panin & Săvulescu 1961, Serafim 2007, Maican et al. 2019).

In our testing of various traps, the Crosstrap type proved to be the most effective, followed closely by the Barrier type. There were no statistically significant differences in their average catches. The MultiWit traps, typically used for capturing bark beetles, came in last, recording the lowest number of catches. A possible explanation for the greater effectiveness of the Crosstrap and Barrier traps could be the silhouette effect; however, this is unlikely since these species are crepuscular or nocturnal (Sláma 1998). The Crosstrap traps, in particular, might have had a more significant catch due to their PVC vanes being treated with a slippery film, which enhances their effectiveness compared to the Barrier traps.

In a similar experiment, Sweeney et al. (2006) compared three types of traps, the Colossus and IPM-Intercept traps, similar to the Crosstrap and Barrier traps used in our experiment. The cross-vane Colossus trap caught about twice as many beetles as the IPM-Intercept trap, but the mean catch did not differ significantly.

Conclusions

Under the conditions in which they were tested, both fuscumol baits alone and in combination with host tree volatiles were significantly attractive to beetles of both *Tetropium* species in Romania. The addition of monoterpenes and ethanol lures had a synergistic effect on the beetles' attraction to fuscumol. This effect was more pronounced with *T. fuscum* than with *T. castaneum*. The combination of (-)-alpha-

pinene, ethanol and aggregative pheromone of *Ips typographus* was as attractive to the beetles as the fuscumol lures. Lures that feature fuscumol predominantly draw in female insects; however, incorporating monoterpenes and ethanol enhances the lure's effectiveness and slightly boosts the attraction of males.

The results indicate that *Tetropium* populations from different geographical areas may respond differently to fuscumol and its combinations with host volatiles. However, further research is needed to substantiate this conclusion. At the same time, the results indicate that simultaneously optimising lure combinations for two or more species is challenging.

Crosstraps demonstrated the highest performance among the tested traps, while Barrier traps showed no significant difference in mean catches compared to Crosstraps.

To effectively detect and monitor *Teropium* species, it is highly recommended that fuscumol lures be utilised in combination with baits containing host volatiles and Crosstrap or Barrier traps. This approach enhances population monitoring and ensures accurate results.

Conflict of interest

The authors declare no financial or personal interests could influence the work presented in this paper.

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