forests

MDPI

Article

Initial Location Preference Together with Aggregation Pheromones Regulate the Attack Pattern of *Tomicus brevipilosus* (Coleoptera: Curculionidae) on *Pinus kesiya*

Fu Liu, Chengxu Wu, Sufang Zhang, Xiangbo Kong, Zhen Zhang * and Pingyan Wang

Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing 100091, China; liufu2006@163.com (F.L.); muzixuan58@126.com (C.W.); zhangsf@caf.ac.cn (S.Z.); xbkong@sina.com (X.K.); aqwangpy@163.com (P.W.)

* Correspondence: zhangzhen@caf.ac.cn; Tel.: +86-136-7102-2209

Received: 22 January 2019; Accepted: 11 February 2019; Published: 12 February 2019

Abstract: Research Highlights: We found that the initial attack location together with the aggregation pheromones played an important role in mediating the aggressive behavior of *T. brevipilosus* on *P. kesiya*. Background and Objectives: *T. brevipilosus* was identified as an aggressive species, which possesses the ability to kill live, healthy *P. kesiya*. In this scenario, we study the top-down attack pattern of *T. brevipilosus* on *P. kesiya* during the entirety of the reproductive period. Materials and Methods: We investigated the phenology of trunk attack on *P. kesiya* over a period of three years in Pu'er City, China. The hindguts extracts of the females and males *T. brevipilosus* were analyzed by coupled gas chromatography-mass spectrometry (GC-MS). The candidate aggregation pheromone compounds of *T. brevipilosus* were determined through electrophysiology experiments (electroantennographic detection, EAD and electroantennography, EAG), laboratory olfactometer bioassays, and field trapping. Results: we found that the pioneer beetles preferentially infested the crown of *P. kesiya* at the early stage of attack following spring flight with the later arriving beetles selectively attacking the lower area of the trunk to avoid intraspecific competition and better utilize limited resources, which exhibits a top-down attack pattern. During gallery initiation, the beetles release aggregation pheromones to attract conspecifics to conduct a mass attack. The chemical analyses indicated that the hindgut extracts of gallery-initiating beetles contained a larger amount of myrtenol, *cis*-verbenol, *trans*-verbenol, and verbenone. Myrtenol and *trans*-verbenol were identified as candidate aggregation pheromone compounds. In addition, a blend of these two components with *S*-(−)-*α*-pinene and *S*-(−)-*β*-pinene attracted more *T. brevipilosus* individuals in a field bioassay. Conclusions: We concluded that the preference for the initial attack location together with the aggregation pheromones played an important role in mediating the top-down attack pattern of *T. brevipilosus* on *P. kesiya*.

Keywords: *Tomicus brevipilosus*; location preference; aggregation pheromones; attack pattern; aggressiveness

1. Introduction

Species of *Tomicus* are noteworthy for their damage to pine forests in Europe, North America, Asia, and North Africa [1–4]. In Southwestern China, these bark beetles are also the main cause of extensive tree damage [5–9]. They attack the fresh shoots of living trees for maturation feeding, which can weaken the host and curtail growth [10]. During the reproductive phase, they infest the trunk of the host, which directly causes death [11–14].

Tomicus brevipilosus (Eggers) is native to Asia and has been reported from China, India, Japan, Korea, and the Philippines [15,16]. This bark beetle is known to infest several *Pinus* species, including *Pinus yunnanensis* Franch. [17], *Pinus koraiensis* Siebold & Zucc., and *Pinus kesiya* Royle ex Gordon [15]. *T. brevipilosus*, together with *other* two *Tomics* species (*Tomicus yunnanensis* Kirkendall & Faccoli, and *Tomicus minor* (Hartig, 1834)) have caused extensive mortality of *P. yunnanensis* in Southwestern China [5]. The life cycle of *T. brevipilosus* contains two phases including a feeding maturation and a reproduction stage. Extensive shoot feeding by adults can cause growth loss and lower a tree's natural resistance, which facilitates trunk attacks [6,13]. Gallery construction and larval feeding are regarded as the direct causes of *P. yunnanensis* tree mortality, particularly when a mass attack occurs on individual trees. The colonization pattern of *T. brevipilosus* varied dramatically, depending on the other two *Tomicus* species that were already present on the tree trunks or not. On trees that were already infested by the other two *Tomicus* species, *T. brevipilosus* colonized areas of the trunk that were not already occupied. When there were no newly infested by the other two *Tomicus* species, *T. brevipilosus* attacked *P. yunnanensis* by itself, infesting the lower parts of the trunk first, and then infesting progressively upward along the trunk into the crown, which exhibits a down-up attack pattern. The ability of *T. brevipilosus* to adjust its infestation pattern in response to other *Tomicus* species likely decreases interspecific competition as well as better utilizes the limited resource. Thus, *T. brevipilosus* appears to be an aggressive bark beetle since it can successfully attack and colonize vigorous *P. yunnanensis* on its own [17].

T. brevipilosus is an oligophagous pest that could cause significant damage to *P. kesiya* pine forests in Pu'er City of China as well [18,19]. The maturation feeding of adults on shoots normally occurs from March through October, which severely injures *P. kesiya* hosts. After completing their sexual development, the adults attack the tree trunk to initiate their reproductive phase. Their longitudinal galleries disrupt the nutrient flow within the phloem tissue, which gradually leads to the death of the *P. kesiya* trees.

Bark beetle aggregation and aggressive behavior are generally mediated by pheromones released by the beetles themselves or by volatiles derived from the host trees [20–22]. *T. piniperda* used host volatiles to locate trees suitable for brood production, the host monoterpenes were effectively lured for this bark beetle [23]. However, Poland et al. concluded that *trans*-verbenol is an aggregation pheromone component for immigrant North American populations of *T. piniperda* [22,24]. *T. destruens* that was known to be attracted to *P. pinea* shoots and the host extracts [25]. *trans*-verbenol was identified from hindgut extracts of both sexes of *T. minor*, which suggested that this beetle might generate aggregation pheromones [26]. Volatiles present in the phloem of infested *P. yunnanensis* could promote aggregation of *T. yunnanensis* [27,28]. In later work, Wang et al. found that both sexes of *T. brevipilosus* showed stronger attraction to damaged shoots than undamaged ones, but the specific components have so far had little reporting [9].

Strategies to reduce tree mortality caused by bark beetle attacks are becoming urgent. An understanding of ecological and behavioral aspects of pheromone production is critical for the development of semiochemical-based strategies to control bark beetles [29]. Application strategies using aggregation pheromones have been successful in monitoring and controlling pest population in Europe and North America, where commercial products exist for major species, *Ips* and *Dendroctonus* [30]. Push-pull tactic have also been successful against *Dendroctonus ponderosae* (Hopkins, 1902) and *Ips paraconfusus* Lanier, 1970 [31,32].

Nowadays, *T. brevipilosus* remains one of the greatest threats to *P. kesiya*, and there are few effective management tactics to control it. The present studies are designed to reveal (1) the regulatory mechanisms of the top-down attack pattern of *T. brevipilosus* on *P. kesiya* during the reproductive period and (2) the semiochemical components of *T. brevipilosus*. One further aim of this study is to utilize effective chemical lures as a pest management strategy to ensure the health of forests.

2. Materials and Methods

2.1. Study Area

The field study reported in this case was carried out in Pu'er City (N 22°54′09.20″, E 101°15′27.87″) at an elevation of 1400 m, and the samples of *T. brevipilosus* were collected on *P. kesiya*, and there is no coexistence of other *Tomicus* species. The studied host trees were originally planted by aerial seeding in the mid-1980s, which ranged from 10 m to 15 m in height and had breast height diameters from approximately 35 cm to 48 cm.

2.2. Insect Collection

Three to five attacked trees were selected at random and cut down every month during the trunk attack period November through January over three years. The pine trunks colonized by *T. brevipilosus* were divided into 1 m-long sections from the base to the top, and the bark was carefully peeled to reveal the beetle numbers and gallery lengths. According to our statistics, the length of *T. brevipilosus* can reach 90 mm during its reproductive phase. The colonization stages were then sampled by the length of the galleries: Stage I (<10 mm): mainly contained female beetles within tunnels where boring had just started, Stage II (10–20 mm): paired males and females that had started mating, Stage III (20–30 mm): mostly paired males and females that had completed their mating behavior and oviposition had occurred, Stage IV (30–50 mm): mostly females in a spawning state (males had gradually left the mating chamber), and Stage V (>50 mm): only females and eggs that were inside a completed gallery system (males had left entirely).

Based on the gallery length classifications, the beetles were individually placed into 5 mL plastic centrifuge tubes with several holes, returned to the laboratory, and then stored at 4 °C for 24 h before testing. Then, the beetles were separated by species and sex using a stereoscope because of the presence of erect hairs, granules, and punctures on the elytral declivity [16].

2.3. Chemicals

The chemicals tested in the study included *S*-(−)-*α*-pinene (98%), *S*-(−)-*β*-pinene (98.5%), *cis*-verbenol (95%, enantionmeric excesses ≥50%), *trans*-verbenol (95%, enantionmeric excesses ≥98%), verbenone (≥93%), myrtenal (≥97%), myrtenol (≥95%), (−)-camphene (80%), (+)-camphene (80%), styrene (99%), linalool (99%), terpinen-4-ol (97%), and heptyl acetate (98%), all obtained from Sigma-Aldrich Co. (St Louis, MO, USA). And *n*-hexanol (99.9%, Fisher Scientific Co., Waltham, MA, USA), *R*-(+)-*α*-pinene (98%), *β*-phellandrene (85%), (−)-limonene (97%), and (+)-limonene (97%, J&K Scientific Ltd., Beijing, China).

2.4. Hindgut Extracts

The hindguts from the females and males of *T. brevipilosus* were dissected under a microscope (Olympus, SZ61) and placed into 2 mL glass vials containing 200 μL *n*-hexane at 4 °C for 12 h. Then, these extracts were evaporated to 50 μL with nitrogen, transferred into small glass ampoules (100 μL), and stored at −20 °C before testing. Every extract contained five hindguts.

2.5. Coupled Gas Chromatography-Mass Spectrometry (GC-MS)

The component analysis was carried out by using an Agilent gas chromatograph coupled with a mass spectrometry system (TRACE GC 2000). The GC was equipped with a polar HP-5MS column (30 m × 0.25 mm × 0.25 μm) and included an injector temperature set to 230 °C. The oven temperature for the HP-5MS GC column was initially programmed at 45 °C for one minute and then subsequently increased to 105 °C at 2 °C per minute. Lastly, the temperature was increased to 220 °C at 10 °C per minute with helium used as the carrier gas. Heptyl acetate (2 ng/μL *n*-hexane) was then added to the extract as an internal standard. All the samples were analyzed with the same apparatus under

the same conditions. Compound identification was based on a comparison of the retention times to those of the synthetic standards, and the reference mass spectra were taken from the NIST11 library (Scientific Instrument Services, Inc., Ringoes, NJ, USA).

2.6. Gas Chromatography–Electroantennographic Detection (GC-EAD)

The GC-EAD system consisted of an Agilent 7890A gas equipped with a HP-5MS column (30 m × 0.25 mm × 0.25 µm, Agilent Technologies, Wilmington, DE, USA) and an electro-antennogram detector (EAD), a 1:1 effluent splitter that allowed simultaneous flame ionization (FID) and electro-antennographic (EAD) detection of the separated volatile compounds. Hydrogen was used as the carrier gas. The injector and detector temperatures were 220 °C and 230 °C. The oven temperature was initially programmed at 60 °C for one minute and then subsequently increased to 100 °C at 5 °C per minute. Lastly, the temperature was increased to 250 °C at 10 °C per minute. Each antenna was prepared by cutting the basic segment carefully and clearing surface debris slightly, which was then inserted into a glass capillary. The glass capillaries housed 0.39 mm silver wires (Sigmund Cohn Corp, Mt. Vernon, NY, USA) and were filled with 0.9% NaCl saline solution. Electrodes were connected to a combi Probe (PRG-3, Syntech, Buchenbach, Germany). A reference electrode was inserted into the base of the excised beetle. The tip of the recording electrode was removed so its opening matched the diameter of the antennal club, and then one side of the antennal club was laid flat against the opening so its entire surface was in contact with the saline [33]. The purified and humidified supplemental airflow (400 mL/min) was supplied continuously. The FID and EAD signals were processed with Syntech software (GcEad version 4.6). In addition, 20 individuals of *T. brevipilosus* housed for 24 h in 3 cm centrifuge tube. Then all hindguts were dissected under a microscope (Olympus, SZ61) and placed into 2 mL glass vials containing 200 µL *n*-hexane at 4 °C for 12 h.

2.7. Electroantennogram (EAG) Recording

The EAG procedure used in this study was similar to that reported elsewhere [34]. The tested compounds were released from Pasteur pipettes containing a piece of filter paper (0.4 cm × 5 cm) impregnated with 10 µL of each freshly prepared solution of the test compounds (1 µg/µL of each in hexane) and passed over the antennae. In addition to the pipettes containing the test preparations, one Pasteur pipette containing a filter paper impregnated with 10 µL of the hexane solvent was used as a control. The puff containing the test substance was delivered into a continuously humidified and purified air stream moving at 800 mL/min and passing for 0.1 s through the impregnated filter paper in the pipettes. A control stimulation was made at the beginning and end of every four tested compounds. The test compounds were then applied randomly at intervals of 40 s. The EAG amplitudes in response to the synthetic compounds were expressed in relation to the responses to the control because of the large differences in the overall sensitivity between the individual antennae and to compensate for the decline in antennal sensitivity during each measurement session. In this normalization procedure, the responses to the control were defined as 100%. The values obtained between the two controls were corrected, according to the values of the references by linear interpolation. The compounds were tested using 15 antennae of males and 15 antennae of females.

2.8. Olfactometer Bioassays

The response of beetles to odor sources was tested using a modified open-arena olfactometer [9,35]. The synthetic compound was placed on a piece of filter paper (1.2 cm × 1.2 cm) in the odor region. *n*-hexane was dropped on the same filter papers placed on either side of the odor region. One beetle of each sex was released at the center of the larger circle for each trial. The number of times that the beetle entered the odor region within 5 min after release was recorded. Males and females of *T. brevipilosus* were tested for their behavioral attraction response to six relevant synthetic compounds at five concentrations, which ranged from 0.01 ng/µL to 100 ng/µL. For each concentration,

n-hexane was used as the control, and 40 biological replicates were performed. Mean attraction values were calculated as follows: Mean numbers = Total numbers of attraction/Total replicates.

2.9. Field Trapping

According to the results of chemical analyses, electrophysiology experiments, and laboratory olfactometer bioassays, different blends of *R*-(+)-*α*-pinene, *S*-(−)-*α*-pinene, *S*-(−)-*β*-pinene, *cis*-verbenol, *trans*-verbenol, and myrtenol were formulated for field trapping tests inside a plantation of *P. kesiya* from November to December 2016. Then, 10 mL of the blend was placed into a series of polyethylene bottles. Black cross-barrier traps (Pherobio Technology Co., Ltd., Beijing, China) were suspended at a 1.5-m distance from the ground. Randomized block experimental designs were used to evaluate the results of the traps. The traps were separated by at least 15 m from each other within a block. Each treatment block contained seven treatments, and five blocks were spaced at a distance of 1 km from each other. A blank control with an un-baited trap was placed in each treatment block. Seven kinds of lures used for field trapping with a corresponding ratio were listed in Table 1.

Table 1. Seven kinds of lures used for field trapping (*v*/*v*).

Compounds	A	B	C	D	E	F	K
S-(−)-*α*-pinene	10	10	-	-	5	5	-
R-(+)-*α*-pinene	-	-	10	10	5	5	-
S-(−)-*β*-pinene	1	1	1	1	1	1	-
trans-verbenol	0.025	0.0125	0.025	0.0125	0.0125	0.025	-
cis-verbenol	-	0.0125	-	0.0125	0.0125	-	-
myrtenol	0.5	0.5	0.5	0.5	0.5	0.5	-

A–F: The lures of A–F; K: The lure of the control.

2.10. Statistical Analyses

The number of times that walking beetles entered the odor region was used to evaluate the attraction level and inhibition for each treatment group. The SPSS statistical analysis software (version 16.0; IBM Inc., Chicago, IL, USA) was used to process data, and all the figures were drawn using Excel 2010 software. A heat map population distribution of *T. brevipilosus* on *P. kesiya* was constructed based on the mean numbers of beetles found in different stages and the mother gallery lengths, while one-way ANOVA was used to compare the amounts of the chemicals (nanogram) identified from the hindgut extracts of females and males during different trunk-breeding phases. The data from these procedures were analyzed with Tukey's multiple range test ($p < 0.05$) as well as the EAG response data from female and male beetles with respect to each stimulus and different compound concentrations. The mean numbers of the walking beetles versus the different concentrations of the chemicals and field trapping data were then $\log_{10}(x + 1)$ transformed. This resulted in homogeneous variances and data were also analyzed using one-way ANOVA followed by Tukey's multiple range test ($p < 0.05$).

3. Results

3.1. Population Dispersal on Trunk Surfaces

Individual *T. brevipilosus* breeding attacks on *P. kesiya* were observed over three consecutive months during the three years of the study. The results showed that both the timing and duration of the breeding attacks were similar, usually beginning in November and extending through January. The pioneer beetles preferentially attacked the upper crown of the individual hosts first at the early stage of the breeding attacks in November (Figure 1). The subsequent beetles gradually moved downward in the trunk over the course of the next two months (December and January), which exhibits a top-down attack pattern (Figure 1A,B).

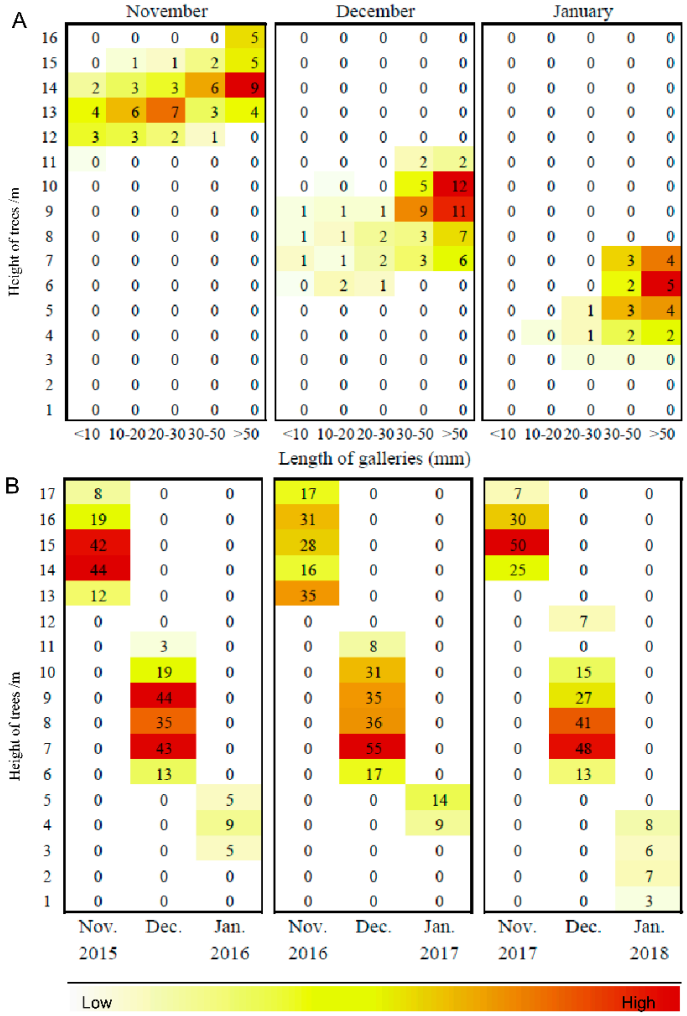

Figure 1. Population distribution heat map for *T. brevipilosus* on *P. kesiya*. (**A**) Length of galleries of *T. brevipilosus* from November to January 2015–2018. (**B**) Distribution of *T. brevipilosus* from November through January 2015–2018.

3.2. Chemical Analysis of Hindgut Extracts

Ten components were identified from the hindgut extracts of the female *T. brevipilosus*, including four terpenes and six oxygenated monoterpenes (Table 2). The amount of myrtenol at stage I was greater and significantly different compared to that of the other stages ($F_{(4,15)} = 4.402$, $p = 0.015$) and declined rapidly from stage I to III. The amount of *cis*-verbenol, *trans*-verbenol, and verbenone decreased from stage I to III, but these were not significant differences ($F_{(2,10)} = 2.121$, $p = 0.160$, $F_{(2,10)} = 0.587$, $p = 0.574$, and $F_{(2,10)} = 4.057$, $p = 0.051$, respectively). The data showed that the amount of *cis*-verbenol and verbenone increased suddenly at stage V and exhibited significant differences at stages III and IV ($F_{(4,15)} = 3.324$, $p = 0.039$, $F_{(4,15)} = 5.367$, $p = 0.007$, respectively). However, this was not the case for stages I and II ($p > 0.05$). In addition, the amounts of terpene and styrene at stage V were significantly lower than those at stage I, but terpinen-4-ol exhibited the opposite pattern. There were no significant differences for β-phellandrene and linalool in the five stages ($p > 0.05$).

For males (Table 2), the data showed that the amounts of *cis*-verbenol, myrtenol, and verbenone significantly decreased from stage I to stage III ($F_{(2,6)} = 5.283$, $p = 0.048$, $F_{(2,6)} = 29.530$, $p = 0.001$, and $F_{(2,6)} = 10.051$, $p = 0.012$, respectively). The amount of *trans*-verbenol decreased from stage I to III, but there were no remarkable differences ($F_{(2,6)} = 3.021$, $p = 0.124$).

Table 2. Chemical constituents identified from the extract of female and male hindguts of *T. brevipilosus* collected from galleries of different lengths.

Retention Time	Component	Gender	Amount of Chemicals from Beetles Collected from Galleries of Different Length (in Nanograms)				
			<10 mm †	10–20 mm ‡	20–30 mm §	30–50 mm ¶	>50 mm ⊥
7:53	styrene	female	2.73 ± 0.49 a	2.45 ± 0.62 a	0.18 ± 0.06 b	0.13 ± 0.04 b	0.52 ± 0.18 b
		male	5.10 ± 0.18 a	1.86 ± 0.53 a	0.20 ± 0.13 a	0.40	0.27
9:36	*α*-pinene	female	2.47 ± 1.25 b	2.12 ± 0.50 b	20.78 ± 5.58 a	23.17 ± 4.90 a	17.77 ± 6.03 a
		male	6.68 ± 4.46 a	8.37 ± 7.39 a	14.10 ± 4.94 a	12.10	9.54
11:42	*S*-(−)-*β*-pinene	female	0.00 ± 0.00 b	0.00 ± 0.00 b	1.71 ± 1.01 ab	2.49 ± 1.09 a	0.00 ± 0.00 b
		male	0.00 ± 0.00 a	0.00 ± 0.00 a	1.53 ± 0.99 a	0.00	0.00
14:33	*β*-phellandrene	female	0.94 ± 0.77 a	0.21 ± 0.07 a	3.46 ± 2.03 a	5.77 ± 2.29 a	3.69 ± 1.53 a
		male	2.24 ± 2.07 a	0.50 ± 0.41 a	3.23 ± 2.20 a	0.68	0.45
18:94	linalool	female	0.76 ± 0.57 a	0.17 ± 0.08 a	0.23 ± 0.13 a	0.56 ± 0.28 a	1.09 ± 0.37 a
		male	0.26 ± 0.22 a	1.08 ± 0.83 a	0.88 ± 0.16 a	0.95	0.90
21:63	*cis*-verbenol	female	6.82 ± 3.69 ab	2.23 ± 0.52 ab	0.65 ± 0.29 b	0.69 ± 0.26 b	19.77 ± 10.99 a
		male	4.61 ± 2.46 a	3.30 ± 1.09 a	0.43 ± 0.25 b	1.27	2.24
21:87	*trans*-verbenol	female	0.87 ± 0.61 a	0.66 ± 0.18 a	0.27 ± 0.05 a	1.39 ± 0.95 a	0.55 ± 0.23 a
		male	0.76 ± 0.24 a	0.39 ± 0.15 a	0.20 ± 0.09 a	0.12	0.20
24:29	terpinen-4-ol	female	0.35 ± 0.35 b	0.13 ± 0.13 b	0.37 ± 0.37 b	0.31 ± 0.31 b	11.61 ± 5.62 a
		male	0.07 ± 0.07 a	0.05 ± 0.05 a	0.18 ± 0.18 a	0.20	0.56
24:99	myrtenol	female	102.24 ± 59.32 a	40.29 ± 15.55 ab	4.11 ± 1.40 b	12.81 ± 5.23 b	21.87±2.64 ab
		male	88.56 ± 40.22 a	30.27 ± 10.84 a	1.39 ± 0.34 b	4.55	7.32
25:75	verbenone	female	4.63 ± 2.21 ab	3.35 ± 0.94 ab	0.49 ± 0.03 b	1.33 ± 0.33 b	10.41 ± 4.43 a
		male	9.35 ± 1.89 a	5.61 ± 1.88 a	1.12 ± 0.37 b	0.52	2.59

† Four replicates for females and three replicates for males. ‡ Six replicates for females and three replicates for males. § Three replicates for females and three replicates for males. ¶ Four replicates for females and one replicate for males. ⊥ Three replicates for females and one replicate for males. Lowercase letters in the same row indicate significant differences (p <0.05) in mean quantities of potential semiochemicals.

Similarly, there were no significant differences for oxygenated monoterpenes (*cis*-verbenol, *trans*-verbenol, myrtenol, and verbenone) between the females and males of *T. brevipilosus* collected from galleries of the same length ($p > 0.05$).

3.3. GC-EAD Analyses

In analyses of solvent extracts of female *T. brevipilosus* by GC-EAD, up to five compounds elicited responses from the antennae of female *T. brevipilosus*, with component 1 (Retention time: 4.72 min), component 2 (Rt: 5:60 min), component 3 (Rt: 9:69 min), component 4 (Rt: 10:75 min), and component 5 (Rt: 10:87 min). By comparing the GC-EAD retention times with those synthetic standards, antennae responded to *S*-(−)-*α*-pinene (1), *S*-(−)-*β*-pinene (2), *trans*-verbenol (3), myrtenol (4), and verbenone (5) (Figure 2). Among these chemicals, *trans*-verbenol and verbenone (peaks 3 and 5) elicited a strong response from both sexes. *S*-(−)-*β*-pinene (peak 2) only elicited a response from females of *T. brevipilosus*.

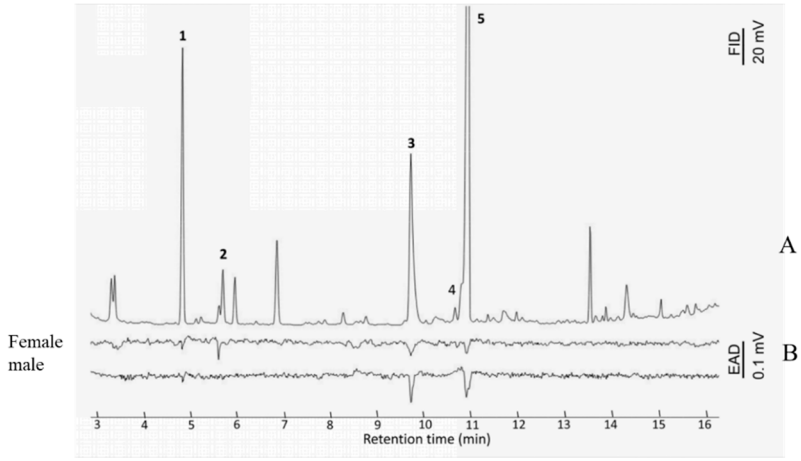

Figure 2. Simultaneously recorded responses from flame ionization detector (FID, **A**) and electroantennographic detection (EAD, **B**, sensing element: female and male *T. brevipilosus* antenna) gas chromatography-electroantennographic detection (GC-EAD) to the volatiles from hindgut of female *T. brevipilosus* ($n = 20$). Peak 1: *S*-(−)-*α*-pinene, 2: *S*-(−)-*β*-pinene, 3: *trans*-verbenol, 4: myrtenol, 5: verbenone.

3.4. Electroantennography (EAG)

Six compounds identified from the hindguts and another six host volatiles were tested in the EAG experiment. The results revealed that five compounds elicited strong stimulation of the antennae of the females and males, specifically *cis*-verbenol, *trans*-verbenol, myrtenol, *S*-(−)-*α*-pinene, and *R*-(+)-*α*-pinene (Figure 3). However, there were no significant differences between females and males for the same substance.

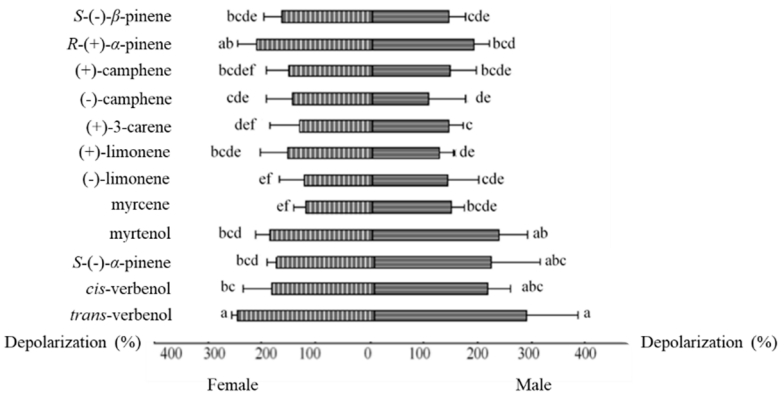

Figure 3. Mean values (± Standard deviation) of the electroantennography response of *T. brevipilosus* females and males to a series of compounds. Mean values marked with the same letter are not significantly different at $p < 0.05$ based on the least significant difference test. Lowercase letters indicate the differences in EAG responses of the same sex bark beetles to different compounds. No significant differences between females and males for the same substance were found.

3.5. Olfactometer Bioassays

Five compounds were tested at five concentrations using a modified open-arena olfactometer (Figure 4). These results revealed that, compared with *n*-hexane, both sexes of *T. brevipilosus* were significantly attracted by *cis*-verbenol and *trans*-verbenol at a 1 ng/μL concentration (Figure 4A,B). Furthermore, a 0.1 ng/μL concentration of *S*-(−)-*α*-pinene was strongly attractive for both genders (Figure 4E). The mean numbers of the females attracted to myrtenol were significantly higher at 1 ng/μL and 10 ng/μL concentrations than at the other concentrations, which was the case at a 1 ng/μL concentration for males (Figure 4C). Both sexes of *T. brevipilosus* displayed weak attractive tendencies to verbenone at the five concentrations, but there were no significant differences between them (Figure 4D).

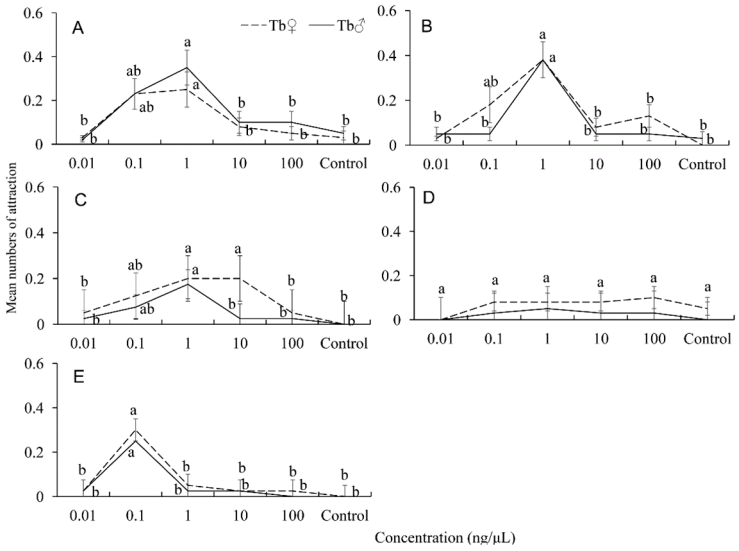

Figure 4. The mean numbers of attracted *T. brevipilosus* to five synthetic compounds at five concentrations (i.e., 0.01 ng/μL, 0.1 ng/μL, 1 ng/μL, 10 ng/μL, and 100 ng/μL) with an *n*-hexane control. Mean numbers = Total numbers of attraction/Total replicates. Lowercase letters indicate significant differences ($p < 0.05$) between the same treatments. The error bars were a standard error. (**A**) *cis*-verbenol. (**B**) *trans*-verbenol. (**C**) myrtenol. (**D**) verbenone. (**E**) *S*-(−)-*α*-pinene.

3.6. Field Trapping

In the field trapping experiment, traps baited with blends of *S*-(−)-*α*-pinene, *S*-(−)-*β*-pinene, *trans*-verbenol, and myrtenol led to the capture of more *T. brevipilosus* individuals within each replicate. The capture by treatment B, baited with blends of *S*-(−)-*α*-pinene, *S*-(−)-*β*-pinene, *cis*-verbenol, *trans*-verbenol, and myrtenol, was less than that by treatment A (Figure 5). However, there was no significant difference between the treatments. Replacing *S*-(−)-*α*-pinene with its enantiomer *R*-(+)-*α*-pinene significantly reduced the catch numbers. While a mix of the three monoterpenes (i.e., *S*-(−)-*α*-pinene, *R*-(+)-*α*-pinene, and *S*-(−)-*β*-pinene, in a 1:1:0.2 ratio, *v*/*v*) along with oxygenated monoterpenes could enhance the catch numbers, the differences were not significant. Fewer numbers of beetles were caught than expected. One possible reason for this is that the mean height of the host, *P. kesiya*, can be more than 10 meters since our traps were suspended 1.5 m above the ground. Another reason is that the top-down attack pattern during the breeding period may have possibly reduced the number of flights and decreased the probability of trap captures.

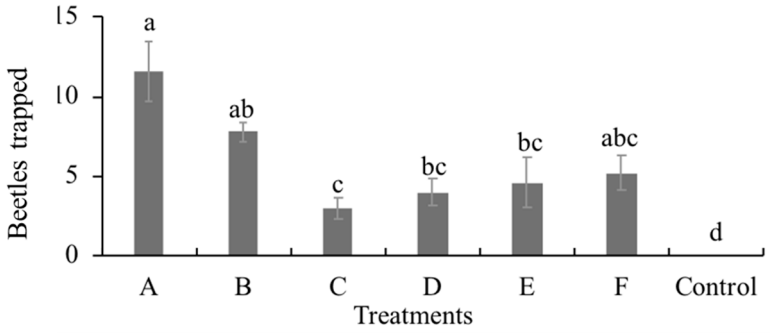

Figure 5. Mean numbers of *T. brevipilosus* caught per trap with seven different baits (between 20 November 2016 and 3 December 2016). Lowercase letters indicate significant differences between trap baits at $p < 0.05$ (one-way ANOVA followed by Tukey's tests). The error bars were standard error. The sample contains *R*-(+)-*α*-pinene (*R*α*p*), *S*-(−)-*α*-pinene (S*α*p), *S*-(−)-*β*-pinene (S*β*p), *cis*-verbenol (cV), *trans*-verbenol (tV), and myrtenol (Mol). (**A**) S*α*p:S*β*p:tV:Mol, (**B**) S*α*p:S*β*p:cV:tV:Mol, (**C**) R*α*p:S*β*p:tV:Mol, (**D**) R*α*p:S*β*p:cV:tV:Mol, (**E**) S*α*p:R*α*p:S*β*p:cV:tV:Mol, (**F**) S*α*p:R*α*p:S*β*p:tV:Mol, and control: unbaited.

4. Discussion

Aggressive bark beetle species are able to attack and kill living and sometimes quite healthy trees [36]. Epidemics caused by these "aggressive" species may dramatically alter the state and function of forest ecosystems over large areas [37]. Typical examples of aggressive bark beetle species are *Ips typographus* (Linnaeus, 1758) in Europe [38], and *Dendroctonus frontalis* Zimmermann, 1868 and *D. ponderosae* in North America [39,40]. In China, *T. yunnanensis* is regarded as one of the most aggressive species of *Tomicus* [8,9,16]. Chen et al. regarded the aggressiveness of *T. brevipilosus*, which is a sympatric species, as similar to that of *T. yunnanensis* [9]. In our study, *T. brevipilosus* had the ability to breed in healthy *P. kesiya* and killed trees on its own by mass attack, which could explain why *P. kesiya* has suffered high levels of tree mortality in recent years. Accordingly, *T. brevipilosus* should be regarded as one of the most aggressive species of the *Tomicus* genus.

Typically, the location preferences of individual species are related to the place where the pioneer beetles initiate the attack, and these locations vary among *Tomicus* species [12,41]. Earlier research showed that *T. piniperda* tends to initiate attacks along the lower trunks of *P. sylvestris*, and *T. minor*, which flies later, tends to colonize the upper portions of the trunks [2]. In contrast, the initial attacks by *T. yunnanensis* tend to be along the upper parts of *P. yunnanensis*, and the *T. minor* attacks are more concentrated along the lower trunk [9,12]. Chen et al. revealed that *T. brevipilosus*, *T. yunnanensis*, and *T. minor* coexist together on *P. yunnanensis*, and *T. brevipilosus* had to adjust its infestation location in response to the other two *Tomicus* species, which likely decreased interspecific competition and resulted

in two general patterns of infestation [9]. In the first pattern, *T. brevipilosus* breeds preferentially within the middle and lower trunk regions were not already occupied by the other two *Tomicus* species. In the second pattern, *T. brevipilosus* attacks alone, infests the lower parts of the trunk first, and then progressively moves upwards along the trunk into the crown, which exhibits a bottom-up attack pattern. However, our study showed that the pioneer beetles preferentially attack the crown of *P. kesiya* first and gradually move down the trunk, which shows a top-down attack pattern. One possible explanation for *T. brevipilosus* attacking the crowns first before attacking the trunks of *P. kesiya* is that it is the dominant species on *P. kesiya*, and the location of the initial attack was not affected by the presence of other *Tomicus* species. Without competition, it is easy for this beetle to preferentially infest the crown of hosts first during the spring flight.

Aggregation pheromones are used to signal a mass attack by the beetles on pines and allows the insects to coordinate feeding and mating in time and space [42,43]. Two oxygenated monoterpenes, *cis*-verbenol and *trans*-verbenol, have been suggested to be major candidate aggregation pheromone compounds of *T. minor* [26]. In earlier work, Poland et al. concluded that *trans*-verbenol acts as an aggregation pheromone component that influences migrant North American *T. piniperda* populations [22]. In our study, both *cis*-verbenol and *trans*-verbenol were detected in the hindguts of *T. brevipilosus* initially breeding on trunks. In addition, the emergence time for verbenol agreed with previous reports that suggested this aggregation pheromone is generally produced in bark beetles [42,44]. One hypothesis to explain this shared signal is that *Tomicus* species individuals utilize the same pheromones to take full advantage of available resources [45]. Accordingly, the results of the GC-EAD, EAG, and dose-concentration experiments clearly indicated that both *cis*-verbenol and *trans*-verbenol are candidate components of the aggregation pheromone.

Lanne et al. revealed that myrtenol is the major component of the volatile compounds in the hindguts of both sexes of *T. piniperda*, which gave an intermediate response in electroantennography tests [26]. However, the compound demonstrated low entrapment. Our research found that a larger amount of myrtenol was present in the hindguts of the females and males of *T. brevipilosus* at stages I and II when the beetles were in contact with the resin-containing phloem and xylem. Moreover, the antennae from beetles of these stages were characterized by a very strong EAG response. At concentrations of 1 ng/μL and 10 ng/μL, females showed a higher walking response than that observed at other concentrations, and this was the case at the 1 ng/μL concentration for males. Further work is needed to prove that this component is a key constituent of aggregation pheromones of this species.

S-(−)-*α*-pinene and *S*-(−)-*β*-pinene can be considered to be an active kairomone or a coattractant aggregation pheromone for *T. brevipilosus*, since the beetles displayed strong attraction in the EAD analysis and behavioral tests. In early research, *S*-(−)-*α*-pinene mixed with *trans*-verbenol resulted in more trap catches for *T. piniperda* [22,23,46]. In addition, *T. destruens* was clearly attracted by the blend of the host volatiles and ethanol, which acted synergistically. The maximum response was observed at release rates of 300 and 900 mg/day, respectively [47]. Lastly, some bark beetle aggregation pheromones arise through oxygenation of *α*-pinene by oxidases from beetles or their associated microorganisms [48–51].

Clearly, the amounts of the oxygenated monoterpenes, *cis*-verbenol, *trans*-verbenol, and myrtenol, reached a maximum at stage I when the pioneer beetles bored through the outer bark and contacted the resin-containing phloem and xylem of the host. These amounts subsequently declined from stage I to III. The variation found in this study is in agreement with previous reports that the production of major pheromones by beetles exhibits large quantitative variation, i.e., increasing during gallery initiation and then terminating or declining rapidly afterwards [42]. An abnormal phenomenon was that the amounts of oxygenated monoterpenes, *cis*-verbenol, and verbenone rose suddenly at stage V. One possible reason was that the higher concentration of oxygenated monoterpenes inhibited the attraction between the *Tomicus* at the later stage of trunk breeding. Additionally, our earlier study suggested that *trans*-verbenol and verbenone at higher concentrations inhibited the attraction

of *T. minor* and *T. yunnanensis*. Consequently, further studies need to be conducted to clarify this phenomenon.

5. Conclusions

In summary, it is reasonable to assume that the initial location preference along with the aggregation pheromones regulate the top-down attack pattern. *T. brevipilosus* pioneer beetles preferentially attacked the crown of the hosts first at the early stage of spring flight and release aggregation pheromones (i.e., myrtenol, *cis*-verbenol, and *trans*-verbenol) immediately to initiate a mass attack. Then, in the later phase of gallery excavation, the amount of *cis*-verbenol and verbenone increases suddenly, and the high concentration of oxygenated monoterpenes inhibits the attraction of conspecifics. To avoid intraspecific competition and to better utilize limited resources, the subsequent beetles selectively attack the lower area of the trunk to avoid overcrowding within the attack zones. Therefore, the preference of the initial attack zones together with the aggregation pheromones play an important role in mediating the top-down attack pattern of *T. brevipilosus* on *P. kesiya*. However, considering that aggregation pheromones are one of the major factors that regulate the aggressive behavior of *T. brevipilosus* during the reproduction phase, it is still unclear which of these components play an important role in guiding this beetle to infest the host trees. Thus, further research to provide critical information for monitoring and/or mass trapping of this economically important forest pest insect still needs to be conducted.

Author Contributions: F.L. and C.W. contributed equally to the work. F.L. and C.W. contributed equally to the conceptualization. F.L. Writing—review & editing: C.W. Writing—original draft: S.Z. Data curation: X.K. Visualization: Z.Z. Validation: F.L., C.W., and P.W. Investigation: all the authors approved the version to be published.

Funding: This work was supported by the Special Fund for Basic Scientific Research of Central Public Research Institutes (CAFYBB2016QA008), the Institute Special Fund for Basic Research, Institute of Forest Ecology, Environment, and Protection, Chinese Academy of Forestry (CAFYBB2016SY017), and The National Natural Science Foundation of China, (31370655) and (31770693).

Acknowledgments: We thank the staff of Mengxian Forest Station in Yunnan Province for collecting *T. brevipilosus* individuals.

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

References

1. Bakke, A. Ecological studies on bark beetles (Coleoptera: Scolytidae) associated with Scots pine (*Pinus sylvestris* L.) in Norway, with particular reference to the influence of temperature. *Medd. Nor. Skogforsøksves.* **1968**, *21*, 441–602.
2. Långström, B. Life cycles and shoot feeding of the pine shoot beetles. *Stud. For. Suec.* **1983**, *163*, 1–29.
3. Långström, B.; Hellqvist, C. Induced and spontaneous attacks by pine shoot beetles on young Scots pine trees: Tree mortality and beetle performance. *J. Appl. Entomol.* **1993**, *115*, 25–36. [CrossRef]
4. Peverieri, G.S.; Faggi, M.; Marziali, L.; Panzavolta, T.; Bonuomo, L.; Tiberi, R. Use of attractant and repellent substances to control *Tomicus destruens* (Coleoptera: Scolytidae) in *Pinus pinea* and *P. pinaster* pine forests of Tuscany. *Entomologica* **2016**, *38*, 91–102.
5. Ye, H. Approach to the reasons of *Tomicus piniperda* (L.) population epidemics. *J. Yunnan Univ. (Nat. Sci.)* **1992**, *14*, 211–216.
6. Ye, H.; Lieutier, F. Shoot aggregation by *Tomicus piniperda* L. (Col: Scolytidae) in Yunnan, southwestern China. *Ann. For. Sci.* **1997**, *54*, 635–641.
7. Långström, B.; Li, L.S.; Hongpin, L.; Peng, C.; Haoran, L.; Hellqvist, C.; Lieutier, F. Shoot feeding ecology of *Tomicus piniperda* and *T. minor* (Col., Scolytidae) in southern China. *J. Appl. Entomol.* **2002**, *126*, 333–342. [CrossRef]
8. Lu, J.; Zhao, T.; Ye, H. The shoot-feeding ecology of three *Tomicus* species in Yunnan province, southwestern China. *J. Insect Sci.* **2014**, *14*, 37. [CrossRef]

9. Wang, J.H.; Zhang, Z.; Kong, X.B.; Wang, H.B.; Zhang, S.F. Intraspecific and interspecific attraction of three *Tomicus* beetle species during the shoot-feeding phase. *Bull. Entomol. Res.* **2015**, *105*, 225–233. [CrossRef]
10. Byers, J.A. Attraction of bark beetles, *Tomicus piniperda*, *Hylurgops palliates*, and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes. *J. Chem. Ecol.* **1992**, *18*, 2385–2402. [CrossRef]
11. Ye, H.; Li, L.S. The distribution of *Tomicus piniperda* (L) population in the crown of Yunnan pine during the shoot feeding period. *Acta Entomol. Sin.* **1994**, *37*, 311–316.
12. Ye, H.; Ding, X.S. Impacts of *Tomicus minor* on distribution and reproduction of *Tomicus piniperda* (Col., Scolytidae) on the trunk of the living *Pinus yunnanensis* trees. *J. Appl. Entomol.* **1999**, *123*, 329–333.
13. Lieutier, F.; Ye, H.; Yart, A. Shoot damage by *Tomicus* sp. (Coleoptera: Scolytidae) and effect on *Pinus yunnanensis* resistance to subsequent reproductive attacks in the stem. *Agric. For. Entomol.* **2003**, *5*, 227–233. [CrossRef]
14. Ji, M.; Dong, X.Q.; Liu, H.P.; Li, L.S.; Xu, H.; Yang, X.P.; Li, H.R.; Ze, S.Z. Preliminary study on remote sensing detection of Yunnan pine forest damaged by *Tomicus piniperda*. *J. West China For. Sci.* **2007**, *36*, 87–90.
15. Browne, F.G. *Pests and Diseases of Forest Plantation Trees. An Annotated List of the Principal Species Occurring in the British Commonwealth*; The Clarendon: Oxford, UK, 1968.
16. Kirkendall, L.R.; Faccoli, M.; Ye, H. Description of the yunnan shoot borer, *Tomicus yunnanensis* Kirkendall & Faccoli sp. n. (Curculionidae, Scolytinae), an unusually aggressive pine shoot beetle from southern China, with a key to the species of *Tomicus*. *Zootaxa* **2008**, *1819*, 25–39.
17. Chen, P.; Lu, J.; Haack, R.A.; Ye, H. Attack pattern and reproductive ecology of *Tomicus brevipilosus* (Coleoptera: Curculionidae) on *Pinus yunnanensis* in southwestern china. *J. Insect Sci.* **2015**. [CrossRef] [PubMed]
18. Jia, P.; Li, J.Z.; Tong, Q.; Dai, Y.J.; Wan, L.M.; Liu, Y. The occurrence and distribution patterns of *Tomicus brevipilosus* adult in treetops (in Chinese). *J. Southwest For. Univ.* **2014**, *6*, 70–74.
19. Wan, L.M.; Luo, F.M.; Tong, Q.; Dai, Y.J.; Jia, P.; Liu, Y. The spatial distribution patterns of *Tomicus brevipilosus* (Eggers) adults in trunk. *J. Sichuan For. Sci. Tech.* **2014**, *35*, 49–53.
20. Byers, J.A.; Lanne, B.S.; Löfqvist, J.; Schlyter, F.; Bergström, G. Olfactory recognition of host-tree susceptibility. *Naturwissenschaften* **1985**, *72*, 324–326. [CrossRef]
21. Byers, J.A. Chemical ecology of bark beetle. *Experientia* **1989**, *45*, 271–283. [CrossRef]
22. Poland, T.M.; Groot, P.D.; Burke, S.; Wakarchuk, D.; Haack, R.A.; Nott, R.; Scarr, T. Development of an improved attractive lure for the pine shoot beetle, *Tomicus piniperda* (Coleoptera: Scolytidae). *Agric. For. Entomol.* **2003**, *5*, 293–300. [CrossRef]
23. Schroeder, L.M. Attraction of the bark beetle *Tomicus piniperda* and some other bark- and wood-living beetles to the host volatiles a-pinene and ethanol. *Entomol. Exp. Appl.* **1988**, *46*, 203–210. [CrossRef]
24. Poland, T.M.; De Groot, P.; Haack, R.A.; Czokajlo, D. Evaluation of semiochemicals potentially synergistic to a-pinene for trapping the larger European pine shoot beetle, *Tomicus piniperda* (Col., Scolytidae). *J. Appl. Entomol.* **2004**, *128*, 639–644. [CrossRef]
25. Faccoli, M.; Anfora, G.; Tasin, M. Responses of the mediterranean pine shoot beetle *Tomicus destruens* (Wollaston) to pine shoot and bark volatiles. *J. Chem. Ecol.* **2008**, *34*, 1162–1169. [CrossRef] [PubMed]
26. Lanne, B.S.; Schlyter, F.; Byers, J.A.; Löfqvist, J.; Leufvén, A.; Bergström, G.; Van Der Pers, J.N.C.; Unelius, R.; Baeckstrom, P.; Norin, T. Differences in attraction to semiochemicals present in sympatric pine shoot beetles, *Tomicus minor*, and *T. piniperda*. *J. Chem. Ecol.* **1987**, *13*, 1045–1067. [CrossRef] [PubMed]
27. Zhao, T.; Li, L.S.; Zhou, N. The attraction of Yunnan pine to pine shoot beetle and tree volatile compositions. *J. Northeast For. Univ.* **2002**, *30*, 47–49.
28. Sun, J.H.; Clarke, S.R.; Kang, L.; Wang, H.B. Field trials of potential attractants and inhibitors for pine shoot beetles in the Yunnan province, China. *Ann. For. Sci.* **2005**, *62*, 9–12. [CrossRef]
29. Borden, J.H. Disruption of Semiochemical-Mediated Aggregation in Bark Beetles. In *Insect Pheromone Resea: New Direction*; Carde, R.T., Minks, A.K., Eds.; Chapman and Hall: New York, NY, USA, 1997; pp. 421–438.
30. Schlyter, F. Semiochemical diversity in practice: Antiattractant semiochemicals reduce bark beetle attacks on standing trees-a first meta-analysis. *Psyche J. Entomol.* **2012**. [CrossRef]
31. Lindgren, B.S.; Borden, J.H. Displacement and aggregation of mountain pine beetles, *Dendroctonus ponderosae* (Coleoptera: Scolytidae), in response to their antiaggregation and aggregation pheromones. *Can. J. For. Res.* **1993**, *23*, 286–290. [CrossRef]

32. Shea, P.J.; Neustein, M. Protection of a Rare Stand of Torrey Pine from *Ips paraconfusus*. In *Application of Semiochemicals for Management of Bark Beetle Infestations*; Salom, S.M., Hobson, K.R., Eds.; Plenum Press: Ogden, UT, USA, 1995; pp. 39–43.
33. Zhang, Z.; Bian, L.; Sun, X.; Luo, Z.; Xin, Z.; Luo, F.; Chen, Z. Electrophysiological and behavioural responses of the tea geometrid *Ectropis obliqua* (Lepidoptera: Geometridae) to volatiles from a non-host plant, rosemary, *Rosmarinus officinalis* (Lamiaceae). *Pest Manag. Sci.* **2015**, *71*, 96–104. [CrossRef]
34. Batista-Pereira, L.G.; Santangelo, E.M.; Stein, K.; Unelius, C.R.; Eiras, A.E.; Corrêa, A.G. Electrophysiological studies and identification of possible sex pheromone components of Brazilian populations of the sugarcane borer, *Diatraea saccharalis*. *Z. Für Nat. C* **2002**, *57*, 753–758. [CrossRef]
35. Byers, J.A.; Birgersson, G.; Francke, W. Aggregation pheromones of bark beetles, *Pityogenes quadridens* and *P. bidentatus*, colonizing scotch pine: Olfactory avoidance of interspecific mating and competition. *Chemoecology* **2013**, *23*, 251–261. [CrossRef]
36. Christiansen, E.; Waring, R.H.; Berryman, A.A. Resistance of conifers to bark beetle attack: Searching for general relationships. *For. Ecol. Manag.* **1987**, *22*, 89–106. [CrossRef]
37. Cedervind, J.; Pettersson, M.; Långström, B. Attack dynamics of the pine shoot beetle, *Tomicus piniperda* (Col.; Scolytinae) in Scots pine stands defoliated by *Bupalus piniaria* (Lep.; Geometridae). *Agric. For. Entomol.* **2003**, *5*, 253–261. [CrossRef]
38. Christiansen, E.; Bakke, A.; Christiansen, E.; Bakke, A. The Spruce Bark Beetle in Eurasia. In *Dynamics of Forest Insects Populations*; Berryman, A.A., Ed.; Plenum Press: New York, NY, USA, 1988; pp. 479–503.
39. Flamm, R.O.; Coulson, R.N.; Payne, T.L. The Southern Pine Beetle. In *Dynamics of Forest Insects Populations*; Berryman, A.A., Ed.; Plenum Press: New York, NY, USA, 1988; pp. 531–553.
40. Raffa, K.F.; Berryman, A.A. Interacting selective pressures in conifer-bark beetle systems: A basis for reciprocal adaptations? *Am. Nat.* **1987**, *129*, 234–262. [CrossRef]
41. Rudinsky, J.A. Ecology of Scolytidae. *Annu. Rev. Entomol.* **1962**, *7*, 327–348. [CrossRef]
42. Wood, D.L. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *Annu. Rev. Entomol.* **1982**, *27*, 411–446. [CrossRef]
43. Seybold, S.J.; Bohlmann, J.; Raffa, K.F. Biosynthesis of coniferophagous bark beetle pheromones and conifer isoprenoids: Evolutionary perspective and synthesis. *Can. Entomol.* **2000**, *132*, 697–753. [CrossRef]
44. Cano-Ramírez, C.; Armendáriz-Toledano, F.; Macías-Sámano, J.E.; Sullivan, B.T.; Zúñiga, G. Electrophysiological and behavioral responses of the bark beetle *Dendroctonus rhizophagus* to volatiles from host pines and conspecifics. *J. Chem. Ecol.* **2012**, *38*, 512–524. [CrossRef]
45. Ayres, B.D.; Ayres, M.P.; Abrahamson, M.D.; Teale, S.A. Resource partitioning and overlap in three sympatric species of *Ips* bark beetles (Coleoptera: Scolytidae). *Oecologia* **2001**, *128*, 443–453. [CrossRef]
46. Song, L.W.; Ren, B.Z.; Sun, S.H.; Zhang, X.J.; Zhang, K.P.; Gao, C.Q. Field trapping test on semiochemicals of pine shoot beetle *Tomicus piniperda* L. *J. Northeast For. Univ.* **2005**, *33*, 38–40.
47. Gallego, D.; Galián, J.; Diez, J.J.; Pajares, J.A. Kairomonal responses of *Tomicus destruens* (Col., Scolytidae) to host volatiles *α*-pinene and ethanol. *J. Appl. Entomol.* **2008**, *132*, 654–662. [CrossRef]
48. Farooq, A.; Tahara, S.; Choudhary, M.I.; Attaur, R.; Ahmed, Z.; Başer, K.H.C.; Demirci, F. Biotransformation of (−)-*α*-pinene by *Botrytis cinerea*. *Z. Für Nat. C* **2002**, *57*, 303–306. [CrossRef]
49. Divyashree, M.S.; George, J.; Agrawal, R. Biotransformation of terpenic substrates by resting cells of *Aspergillus niger* and *Pseudomonas* putida isolates. *J. Food Sci. Tech.* **2006**, *43*, 73–76.
50. Seybold, S.J.; Huber, D.P.W.; Lee, J.C.; Graves, A.D.; Bohlmann, J. Pine monoterpenes and pine bark beetles: A marriage of convenience for defense and chemical communication. *Phytochem. Rev.* **2006**, *5*, 143–178. [CrossRef]
51. Shi, Z.H.; Sun, J.H. Quantitative variation and biosynthesis of hindgut volatiles associated with the red turpentine beetle, *Dendroctonus valens* LeConte, at different attack phases. *Bull. Entomol. Res.* **2010**, *100*, 273–277. [CrossRef]