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Synergistic effect of plant volatiles and pheromone for trapping coffee white stem borer, *Xylotrechus quadripes*

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Abstract
Coffee white stem borer, *Xylotrechus quadripes* primarily targets *Coffea arabica* while showing less preference in *Coffea robusta*. *C. arabica* bark might contain volatiles that attract female beetles for egg deposition. Our study aimed to identify these plant volatiles and assess their efficacy when used in conjunction with pheromones to enhance the performance of sex pheromone traps in the field. In the electroantennography (EAG) investigation, we employed virgin females, mated females, and males of the CWSB to evaluate eight chemicals that had exhibited heightened responses in preliminary assessments. Notably, when presented with (E)-2-Hexenal and (E)-2-Hexenal:(S)-2-hydroxy-3-decanone (1:1 ratio), males and virgin females displayed significantly stronger responses, while mated females did not exhibit a significant response. Experiments conducted in wind tunnels corroborated the findings from the EAG analysis. In comparison to males and mated females, virgin females displayed a more favorable response to these eight treatments. However, in the wind tunnel experiments, the response to (E)-2-Hexenal: (S)-2-hydroxy-3-decanone (1:1) was higher in virgin females, mated females, and males. Subsequently, the field trail results aligned with the outcomes of the EAG and wind tunnel experiments. Most of the test compounds yielded comparable results, suggesting that *X. quadripes* may employ a variety of substances within its habitat and host community. These findings shed light on the complex chemical ecology of the coffee white stem borer and offer insights into potential pest management strategies.

Keywords: *Xylotrechus quadripes*, *Coffea arabica*, pheromone, plant volatiles

Introduction
The primary menace to arabica coffee crops in India, Sri Lanka, China, Vietnam, and Thailand is the Coffee White Stem Borer (CWSB), *Xylotrechus quadripes* Chevrolat (Coleoptera: Cerambycidae) (Le Pelley, 1968; Rhainds *et al.*, 2002) [17, 28]. It's estimated that the relentless attacks of *X. quadripes* annually damage approximately 9 million Indian coffee trees, incurring replacement costs and lost productivity exceeding $40 million (Hall *et al.*, 2006) [12]. This is a significant concern, as the presence of CWSB can curtail agricultural productivity by as much as 20 percent (Veeresh, 1995) [37]. Over the past century, an assortment of management strategies has been employed to combat CWSB infestations. These methods include mass trapping utilizing pheromone-baited traps, uprooting and incineration of infested plants, the application of insecticides to target adult and early instar CWSB, bark scrubbing, stem wrapping to deter oviposition, and shade management to discourage beetles from shaded regions (CCRI, 2003; Hall *et al.*, 2006; Venkatesha and Dinesh, 2012; Manikandan *et al.*, 2019) [5, 12, 40, 19]. Although these techniques have demonstrated varying degrees of efficacy, the persistence of CWSB as a formidable challenge can be attributed to regional climate variations, elevation disparities, irregular monitoring, and the substantial costs and labor associated with their implementation.
Insecticides have also been utilized to control CWSB infestations, but their effectiveness is often limited as CWSB lays its eggs deep within the coffee plant stems, beyond the reach of non-systemic insecticides. Additionally, the use of chemical pesticides may lead to the accumulation of residues on coffee beans (dosReis *et al.*, 2015) [8], potentially impacting non-target insects and natural enemies, which could exacerbate CWSB infestations. Consequently, an ecological approach that considers CWSB's natural history and host preference behavior offers promise in the development of integrated pest management strategies aimed at reducing pesticide use. Studies on CWSB mating behavior have revealed that these beetles mate on different parts of the coffee plant (Seetharama *et al.*, 2004) [32] and employ pheromones for mate attraction, with no reliance on visual cues (Venkatesha *et al.*, 1995) [39].

A synthetic version of the CWSB pheromone is currently in use; however, its lure has not proven highly attractive to the beetles (Rhainds *et al.*, 2001a; Hall *et al.*, 2006, Mangalgikar *et al.*, 2023) [30, 12, 20].

Comparative research has underscored the heightened susceptibility of arabica coffee to severe CWSB infestations, with CWSB exhibiting a pronounced preference for arabica over robusta coffee (Venkatesha and Dinesh, 2012) [40]. Laboratory studies have further revealed CWSB's attraction to coffee stems adorned with sawdust and larval frass (Rhainds *et al.*, 2001b) [29], suggesting the potential for identifying non-host repellents or potential host attractants from these plant species. Given the proclivity of CWSB for specific host plants, exploring potential host attractants or non-host repellents is a promising avenue. Notably, most research in chemical ecology literature related to pest host attraction has focused on temperate, open fields, rather than the unique shade-grown rainforest habitat characteristic of Indian coffee plantations. While previous studies have examined arabica volatiles from green tissues, branches, and stems, the capture of relatively few beetles was achieved (Murphy *et al.*, 2008; Prashant, 2014) [21, 22].

Robusta coffee, in contrast, remains largely untouched by insects, with early research suggesting that volatiles from arabica bark may entice females to deposit their eggs (Rhainds, 2001b; Reddy, 2010; Rajus *et al.*, 2021) [29, 10, 24]. Efforts have been made to identify these plant volatiles and explore their synergistic effects with pheromones, aiming to enhance the effectiveness of sex pheromone traps in the field. This knowledge could prove invaluable in the development of oviposition attractants for the capture of females or for monitoring CWSB emergence.

Methodology

Insects: Infested coffee stems were collected from the field (coordinates: 13.1365° N, 75.6403° E, elevation 970 meters above mean sea level) and stored in a 3 x 3 x 3-meter nylon enclosure within an open area shaded by trees at BCRL, Bengaluru. Upon emergence, the adults were sorted by sex and individually placed in separate 100 ml plastic containers. These containers were provisioned with cotton wads saturated with a 10% honey solution and maintained under controlled laboratory conditions at 23 °C and 70% relative humidity.

Compounds: The test compounds of highest purity were procured from Sigma Aldrich (Table 1). The purity of the compounds was determined by gas chromatography. All compounds were diluted in hexane.

Table 1: Ratio of plant volatiles and CWSB pheromone used in the study

Sl. No.	Particulars of blends	Source	Ratio	Purity %
1	(E) -2- Hexenal:2-hydroxy-3-decanone	BCRL	1:1	98.94
2	Z-3 Hexenol:2-hydroxy-3-decanone	BCRL	1:1	100
3	(E)-2-Hexenol:2-hydroxy-3-decanone	BCRL	3:2	93.4
4	((Z)-3- Hexenol:2-hydroxy-3-decanone	BCRL	3:2	98
5	Z-3 Hexenol: (E)-2-Hexenol:2-hydroxy-3-decanone	BCRL	3:3:2	96
6	Methyl salicylate:2-hydroxy-3-decanone	BCRL	3:2	99
7	2-hydroxy-3-decanone	BCRL	100 parts	>99.5
8	(E) -2- Hexenal	BCRL	100 parts	98

Purity of the compounds determined by gas chromatography

Electroantennogram (EAG): Antennae were excised from 2-day-old males and females, and placed between electrodes with the aid of electroconductivity gel (Signa-gel, Parker Laboratory Fories, Inc., Fairfield, NJ, USA). A constant flow of charcoal-filtered, humidified air at 0.5 L/min was directed over the mounted antennae. Each stimulus generated by the stimulus controller was delivered for 0.5 seconds at 0.2 L/min, with an inter-stimulus interval of at least 90 seconds. Following the loading of stimuli onto a filter paper piece (60 × 5 mm, Whatman #1) inserted into a 14.6 cm Pasteur pipette, a given cartridge was not stimulated more than once. The order of stimulation proceeded from solvent to ascending doses. The signal was pre-amplified using a custom-built amplifier (10X gain, Hanson B-102), high-pass filtered at 0.1 Hz, digitized, and analyzed using Autospike, Syntech, Germany. Only one antenna per beetle was employed, initially stimulated with hexane, followed by volatiles in ascending dose order. In the male-female pheromone synergism experiment (Mangalgikar *et al.*, 2023) [20], a total of five antennae were tested for each sex.

In preliminary studies, we screened 30 different compound combinations against *X. quadripes* at a single concentration (20 µl). Compounds that elicited EAG responses equal to or greater than those elicited by the standard were selected for further investigation. All test compounds were stored in 2 ml screw-cap vials in a refrigerator. Twenty microliters of each sample were pipetted onto a piece of filter paper (6 x 0.5 cm, S & S 5892) and placed in a Pasteur pipette. The pipette was connected to a syringe, with the pipette tip inserted through a small hole in the glass tube to ensure continuous airflow over the excised antenna. The syringe plunger was rapidly depressed to pass 1 ml of air through the pipette into the airstream, with the duration of this air puff fixed at 0.10 seconds. As antennal responses diminished during the experiment, the responses (amplitudes) to the test compounds were expressed as millivolts (mV) of the electroantennogram (EAG) response. The mean of all recorded antennal depolarizations elicited by the test compounds was calculated.

Wind tunnel Studies

The behavior of the Coffee White Stem Borer (CWSB) was investigated in an acrylic wind tunnel measuring 8ft x 2ft x 2ft. The wind tunnel olfactometer was thoroughly cleaned and ventilated for an hour before the insects were released. The insects were then introduced into the tunnel and left for an hour to acclimatize. Following this acclimatization period, the source was introduced inside the wind tunnel, and the chamber housing the beetles was opened to allow them free movement. Each experiment involved the use of six female beetles and was replicated three times. The movement and responses of the beetles towards the source were meticulously recorded. As a control, the same experiment was conducted using the solvent hexane (Prashant, 2014) [22].

Field studies of lures prepared from plant volatiles and CWSB pheromone

The pheromone trap studies were conducted in a farmer's field at Mallenahalli, Chikmagalur, during two distinct periods: April to June 2013 and September 2013 to January 2014.

For both field studies prior to trap installation, the emergence holes were recorded, and the holes were sealed with soap for easy tracing. Following the emergence period, we conducted a recount and calculated the percentage of catches. The treatments, which were diluted in hexane, were dispensed into PVC vials equipped with lids and affixed to sticky cross-vane traps (Fig. 1). A total of 90 traps (n = 10) were randomly positioned, suspended at a canopy height of 1.83 meters within the coffee plantations, with a spacing of 200 meters between adjacent traps. The timing of the experiment was synchronized with the flight activity of the beetles. Traps were regularly inspected and maintained, with revisions made every 10 days, during which captures were meticulously counted and individuals were sexed.

Fig 1: Sticky white cross vane trap

Results

Antennal response in the electroantennography

In comparison to the control group, the mean EAG response of female antennae exhibited a substantial increase in the presence of (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) (0.71±0.14). Notably, no significant differences were observed among the remaining treatments (Table 2). Under consistent conditions, the mean EAG response of male antennae was notably heightened in the presence of (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) (0.50±0.09), with the hexane control group displaying the lowest response (Table 2). While all other treatments elicited comparatively weaker responses, mated female antennae exhibited a heightened mean EAG response in the presence of (Z)-3-hexenol: (E)-2-hexenal: 2-hydroxy-3-decanone (3:3:2) (0.54±0.10) and (E)-2-hexenol: 2-hydroxy-3-decanone (3:2) (0.52±0.08). The hexane control group again exhibited the lowest response (Table 2).

Table 2: EAG response (mV) (Mean ± SE) of CWSB female, male and mated female antennae to different treatments

Treatment No.	Treatments	EAG response (mV) (Mean ± SE)		
		Female	Male	Mated female
T_1	(E) -2- hexenal: 2-hydroxy-3-decanone (1:1)	0.71±0.14^{a}	0.50±0.09^{a}	0.42±0.07^{ab}
T_2	(Z)-3-hexenol: 2-hydroxy-3-decanone (1:1)	0.44±0.06^{abc}	0.37±0.06^{a}	0.48±0.07^{ab}
T_3	(Z)-3- hexenol: (E)-2 - hexenol: 2-hydroxy-3-decanone (3:3:2)	0.45±0.04^{abc}	0.35±0.06^{a}	0.54±0.10^{a}
T_4	(E)-2 - hexenol: 2-hydroxy-3-decanone (3:2)	0.38±0.04^{bc}	0.36±0.05^{a}	0.52±0.08^{a}
T_5	(Z)-3- hexenol: 2-hydroxy-3-decanone (3:2)	0.49±0.07^{abc}	0.31±0.04^{ab}	0.45±0.05^{ab}
T_6	Methyl salicylate: 2-hydroxy-3-decanone (3:2)	0.52±0.09^{abc}	0.36±0.03^{a}	0.50±0.10^{ab}
T_7	2-hydroxy-3-decanone	0.49±0.07^{abc}	0.39±0.08^{a}	0.36±0.08^{ab}
T_8	(E) -2- hexenal	0.44±0.07^{abc}	0.44±0.06^{a}	0.32±0.05^{ab}
T_9	Control (Hexane)	0.20±0.05^{c}	0.09±0.04^{b}	0.20±0.04^{b}
F test		3.23 *	3.48 *	2.19 *
SEM±		0.075	0.060	0.073
CD at 0.01%		0.278	0.224	0.272
p value		0.003	<0.001	0.03

Comparison was made with *post –hoc* test DMRT for log transformed data, values followed by same letters in each column are not significantly different, *=Significant, n=10.

Behavioral synergism in the wind tunnel

In mated females, combination of (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) resulted in a higher response (65.83%) compared to any other treatment. Conversely, treatment with (E)-2-hexenal alone yielded the lowest response (33.34%). These treatments exhibited significant differences from one another. Among females, (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) elicited a higher percentage reaction (85.00%) compared to other treatments. In the case of males, there was no significant difference in their responses to tested (Table 3).

Table 3: Taxis response (%) of CWSB females, males and mated females against different treatments in wind tunnel

Sl. No.	Treatments	Response (%)		
		Mated females	Females	Males
T_1	(E) -2- hexenal: 2-hydroxy-3-decanone (1:1)	65.83 (54.59)[a]	85.00 (67.47)[a]	67.50 (55.58)
T_2	(Z)-3-hexenol: 2-hydroxy-3-decanone (1:1)	63.54 (52.97)[ab]	41.67 (39.40)[abc]	53.33 (46.96)
T_3	(Z)-3- hexenol: (E)-2 - hexenol: 2-hydroxy-3-decanone (3:3:2)	36.46 (36.99)[ab]	77.78 (62.16)[abc]	51.04 (45.60)
T_4	(E)-2 - hexenol: 2-hydroxy-3-decanone (3:2)	38.54 (38.23)[ab]	72.22 (58.44)[abc]	52.08 (50.05)
T_5	(Z)-3- hexenol: 2-hydroxy-3-decanone (3:2)	40.63 (39.42)[ab]	77.78 (62.16)[abc]	34.90 (34.07)
T_6	Methyl salicylate: 2-hydroxy-3-decanone (3:2)	36.67 (36.69)[ab]	83.33 (65.88)[abc]	46.88 (42.93)
T_7	2-hydroxy-3-decanone	61.11 (51.47)[ab]	60.00 (25.66)[c]	27.78 (40.12)
T_8	(E) -2- hexenal	33.34 (34.53)[b]	62.50 (52.28)[abc]	43.75 (41.36)
SEM±		4.45	9.09	9.60
F test		3.48 *	2.97 *	1.07 NS
CD @0.01		17.84	37.45	37.40
p value		0.013	0.033	0.40

Comparison was made with *post –hoc* test DMRT for arcsine transformed data, values followed by same letters in each column are not significantly different, *=Non significant, n=20.

Field trap catches to plant volatiles and 2-hydroxy-3-decanone

Traps baited with (E)-2-hexenal captured a higher number of females (2.86±0.77) followed by traps baited with methyl salicylate: 2-hydroxy-3-decanone (3:2) (2.71±1.10) and (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) (1.71±0.78). Catches in all other treatments were lower than those in the pheromone and control treatment. Increased male captures were noted in traps baited with (E)-2-hexenal (1.14±0.55) and methyl salicylate: 2-hydroxy-3-decanone (3:2) (1.14±0.51). Nevertheless, there was no notable difference in trap catches among any of the tested combinations, including the control treatment. (Table 4). The per cent catches of beetles was more in traps baited with (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) 84.37% and all remaining trap catches were less than the control trap (Table 4).

Table 4: Number of male and female coffee white stem borer beetle in traps baited with lures of plant volatiles and synthetic pheromone (April-June 2013)

Treatment No.	Treatments	Mean (±SEM) CWSB beetle caught/10 traps		Catches (%)
		Female	Male	
T_1	(E) -2- Hexenal: (S)-2-hydroxy-3-decanone (1:1)	1.71±0.78	0.86±0.46	84.37
T_2	(Z)-3-Hexenol:CWSB pheromone(1:1)	0.57±0.30	0.14±0.14	47.37
T_3	(Z)-3- Hexenol: (E) 2 - Hexenol: (S)-2-hydroxy-3-decanone (3:3:2)	1.00±0.53	0.86±0.46	38.30
T_4	(E) 2 - Hexenol: (S)-2-hydroxy-3-decanone (3:2)	2.00±0.75	0.57±0.30	48.15
T_5	(Z)-3- Hexenol: (S)-2-hydroxy-3-decanone (3:2)	0.71±0.28	0.29±0.28	35.71
T_6	Methyl salicylate: (S)-2-hydroxy-3-decanone (3:2)	2.71±1.10	1.14±0.51	46.66
T_7	(S)-2-hydroxy-3-decanone	1.29±0.75	0.00±0.00	52.94
T_8	(E) -2- Hexenal	2.86±0.77	1.14±0.55	43.75
T_9	Control	1.29±0.61	0.86±0.34	60.00

Field trial of pheromone traps baited with plant volatiles and pheromone (September 2013-January 2014)

The mean catches of CWSB female was more in the traps baited with (E)- 2-hexenal: 2-hydroxy-3-decanone (1:1) (3.38±0.92), followed by (E)-2-hexenal alone (1.88±0.79), methyl salicylate:2-hydroxy-3-decanone (3:2) (1.88±0.58), 2-hydroxy-3-decanone alone (1.75±0.95), (Z)-3-hexenol:2-hydroxy- 3-decanone (3:2) (1.38±0.70) and in all other remaining treatments beetle caught were less than the control traps (1.25±0.82) (Table 5). The mean catches of CWSB males was more in the control traps (2.00±0.53), whereas in all other treatments the male catches were less (Table 5). The per cent catches of beetles were maximum in (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) (87.80%) traps followed by Methyl salicylate: 2-hydroxy-3-decanone (3:2) (76.00%) compared to other remaining treatments (Table 5).

Table 5: Number of male and female coffee white stem borer beetle in traps baited with lures of plant volatiles and synthetic pheromone (Sept-Dec 2013) at Mallenahalli

Treatment No.	Treatments	Mean(±SEM) CWSB beetle caught/10 traps		Catches (%)
		Females	Males	
T_1	(E)-2-Hexenal: 2-hydroxy-3-decanone (1:1)	3.38±0.92	1.13±0.58	87.80
T_2	(Z)-3- Hexenol: 2-hydroxy-3-decanone (1:1)	0.88±0.61	0.75±0.41	18.84
T_3	(Z)-3-Hexenol: (E)-2-Hexenol: 2-hydroxy-3-decanone(3:3:2)	1.00±0.50	0.63±0.32	52.00
T_4	(E)-2-Hexenol: 2-hydroxy-3-decanone (3:2)	1.00±0.73	0.75±0.49	25.45
T_5	(Z)-3-Hexenol: 2-hydroxy-3-decanone (3:2)	1.38±0.70	0.63±0.38	11.03
T_6	Methyl salicylate: 2-hydroxy-3-decanone (3:2)	1.88±0.58	0.50±0.27	76.00
T_7	2-hydroxy-3-decanone	1.75±0.95	0.75±0.49	71.43
T_8	(E)-2-Hexenal	1.88±0.79	1.13±0.30	28.57
T_9	Control	1.25±0.82	2.00±0.53	45.61

Discussion

Our findings suggest that within these limitations, *X. quadripes* can detect the majority of the volatiles we employed, and a variety of compounds could be used to generate sexual differences. The solvent extracts of the bark from two varieties of coffee exhibited a substantial difference in the EAG response of male and female CWSB antennae. When exposed to individual chemicals, female responses differed significantly. Male and female antennae responded differently to the (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) treatment when exposed to a mixture of pheromone and plant volatiles compared to the control. The mated female's reaction to the (Z)-3-hexenol: (E)-2-hexenol: 2-hydroxy-3-decanone (3:3:2) treatment was considerably different from the control. Most test chemicals produced comparable results, indicating that *X. quadripes* may combine the usage of many substances in its habitat and/or host community.

Dinotiscus dendroctoni did not elicit responses to specific chemicals compared to the typical mixture of oxygenated monoterpenes, as reported by Salom *et al.* (1991) [31]. Males and females responded to doses in a comparable way, except for females having lower thresholds for frontalin, terpinen-4-ol, E, Z-chalcogram, and exo-brevicomin than males. Ipsdienol and the aggregation inhibitor ipsenol, according to Angst and Lanier (1979) [3], caused the Ips pini's EAG amplitude to reach its maximum. Responses to odorants produced by beetles (linalool, verbenone, and trans-verbenol) were generally higher than those to host terpenes (octanol, α-pinene) among the other chemicals studied.

The monoterpenes (α- and β-phellandrene, 3-thujene, γ-terpenene, myrcene, and β-pinene) found in the headspaces of Douglas cones induced lower EAG responses than fatty acid derivatives associated with green odors. According to Thiery and Marrion-Poll (1998), the EAG response profile of males significantly differed from that of females. However, in other cases *Rhynchaenus quercus* (Kozlowaski and Visser, 1981) [16], *Yponomeuta* sp. (Van der Pers, 1981) [36], *Anthonomus grandis* (Dickens, 1984) [7], *Psila rosae* (Guerin and Visser, 1980) [11], *Rhynchaenus spermotrophus* (Thiery and Marrion-Poll, 1998), and *Leptinotarsa decemlineata* (Visser, 1979) [38].

Male *M. carolinensis* and *M. titillator* release monochamol, and both species' antennae can detect it, according to studies of extracts of volatiles collected from both sexes using coupled gas chromatography-mass spectrometry and gas chromatography electroantennogram detection (Allison *et al.*, 2012) [2]. The natural solvent extracts of coffee, specifically (E)-2-hexenal, (Z)-3-hexenol, and (E)-2-hexenol, as well as synthetic compounds containing (E)-2-hexenal and (E)-2-hexenol, demonstrated a response peak in the female antennae of CWSB in this investigation. Arabica coffee had higher concentrations of these two than robusta coffee did.

While previous studies (Francardi *et al.*, 2009; Ibeas *et al.*, 2007) [9, 15] indicated that traps baited solely with kairomones yielded high catch levels, our experiment demonstrated that *X. quadripes* was more effectively trapped when using a combination of pheromones and kairomonal lure, specifically (E)-2-hexenal:2-hydroxy-3-decanone (1:1). These results affirm the increased efficacy of a specific pheromone paired with kairomonal lures, consistent with observations in *M. galloprovincialis* (Rassati *et al.*, 2012) [25] and a study in Spain (Pajares *et al.*, 2010) [23]. Notably, compared to either the sex pheromone or the monoterpene mixture alone, the combination of (3R)-ketol + 1-butanol or (+/-)-3-ketol + 1-butanol with monoterpenes led to significantly higher captures of females (Reddy *et al.*, 2005) [26].

According to Ibeas *et al.* (2007) [15], ipsenol emerged as the most potent kairomonal signal, generating a 95-fold synergistic reaction between M. *galloprovincialis* and α-pinene. The inclusion of methyl butenol doubled the number of males and females trapped in this mixture. *M. carolinensis* showed attraction to monochamol both in the presence and absence of α-pinene, while *M. titillator* was exclusively drawn to the combination of these two substances. Both *M. carolinensis* and *M. titillator* were attracted to -2, 3-hexanediol, but only when α-pinene was present (Allison *et al.*, 2012: Allison *et al.*, 2001; Macias-Samano *et al.*, 2012) [2, 1, 18]. As reported by Clarke (2007) [6], the combination of endo-brevicomin with frontalin and turpentine significantly increased the capture of southern pine beetles, *Dendroctonus frontalis*, in the southern United States. Moreover, it appears to be a potent attractant for *Dendroctonus* bark beetles in Chiapas.

Certain hardwood specialist species were more attracted to the host plant due to its volatile α-pinene, while conifer specialists exhibited a greater attraction to ethanol (Hanks and Millar, 2013; Hanks *et al.*, 2012) [13, 2]. In Y-tube olfactometer bioassays, both male and female individuals of three species—*Xylotrechus colonus, Megacyllene caryae, and Neoclytus mucronatus mucronatus*—responded to volatiles emitted by hickory logs (Ginzel and Hanks 2005) [10]. *Tetropium fuscum* and *T. castaneum* were considerably more likely to be captured when an ethanol lure was added to spruce blend-baited traps (Sweeney *et al.*, 2004) [34].

Conclusion

The EAG responses of female and male CWSB beetles were analyzed under various treatments, revealing significant variations. The combination of (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) notably increased the mean EAG response in female antennae, while male antennae exhibited heightened responses to the same combination. Mated female antennae, on the other hand, responded more strongly to (Z)-3-hexenol: (E)-2-hexenal: 2-hydroxy-3-decanone (3:3:2) and (E)-2-hexenol: 2-hydroxy-3-decanone (3:2). Behavioral synergism in wind tunnel experiments further highlighted the efficacy of (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) in mated females. Field trap catches demonstrated that traps baited with (E)-2-hexenal captured a higher number of females, with (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) exhibiting the highest percentage catches. In contrast, trap catches of males showed no notable differences among the tested combinations. The field trial of pheromone traps, conducted from September 2013 to January 2014, reinforced the attractiveness of (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) to CWSB females, leading to higher mean catches and percentage catches compared to other treatments. However, CWSB males exhibited higher catches in control traps. Our findings suggest the potential efficacy of (E)-2-hexenal: 2-hydroxy-3-decanone (1:1) in attracting and capturing female CWSB beetles, providing valuable insights for the development of targeted pest management strategies. Further research and field trials could explore the practical implications of these findings in the context of integrated pest management practices.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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