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Preference for Coffee varieties and Volatile compounds by Coffee White Stem Borer, *Xylotrechus quadripes*

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ABSTRACT: The study revealed a significant difference in host preference between male and female Coffee white stem borer (CWSB) beetles in response to arabica and robusta coffee varieties. When arabica was positioned in the W-N and E-S directions and robusta in the N-E and S-W directions, both male and female beetles distinctly favored arabica coffee, as indicated by $\chi 2$ values of 21.49 and 22.01, respectively. Altering the spatial orientation of host plants, with arabica in the W-N and N-E direction and robusta in the S-W and E-S direction, led to a significant difference in female preference ($\chi 2$=11.58), while no significant difference was observed in male preference ($\chi 2$=2.56). In Y-tube olfactometer experiments, female CWSB beetles displayed a significantly higher response to Cauvery bark volatile (66.67) compared to the control (33.33) ($\chi 2$=11.11). Additionally, a higher response to (E)-2-hexenal (79.33) compared to (Z)-3-hexenol (17.33) was noted ($\chi 2$=39.76). These findings shed light on the preferences and responses of female CWSB beetles to various volatile compounds, offering insights for pest management strategies. Overall, the results emphasize the stronger inclination of CWSB towards arabica coffee compared to robusta. This preference may contribute to the higher incidence of CWSB infestation in arabica coffee plantations.

Keywords: Behavior, Y-tube olfactometer, Arabica coffee, *Xylotrechus*.

INTRODUCTION

One of the globally cultivated crops of utmost significance is coffee, with India being home to two predominant coffee species: Coffea arabica (commonly referred to as arabica) and *Coffea canephora* (often identified as robusta; Dastagiri, 2017). Remarkably, India stands as the seventh-largest producer of coffee, reaping annual export revenue of $836 million (US) (The Coffee Board of India, 2019). Unlike the prevalent practice of coffee monoculture across the world, India has garnered recognition for its approach to shade-grown coffee. It is noteworthy that approximately 98% of the roughly 250,000 Indian coffee growers, distributed across several states, are small-scale producers (Lee *et al.*, 2007).

In stark contrast to monocultural coffee plantations, the adoption of agroforestry practices for coffee cultivation has been substantiated by numerous studies as advantageous for biodiversity and affords opportunities for conservation initiatives (Robbins *et al.*, 2015; Karanth *et al.*, 2016; Chang *et al.*, 2018). However, this transformation of forests into coffee plantations has also provided a niche for Xy*lotrechus quadripes*, a member of the longhorn beetle family (Cerambycidae), known by the moniker "Coffee White Stem Borer" (CWSB).

Longhorn beetles, comprising over 36,000 recognized species worldwide, are an immensely prevalent insect family within the order Coleoptera (Allison *et al.*, 2004; Wang, 2017). Notably, despite the implementation of a variety of chemical and cultural control strategies, cerambycids are acknowledged for their substantial impact on agricultural crops and forest trees (Robert, 2017; Wang, 2017). The extensive harm inflicted by CWSB is a pivotal factor driving the shift from arabica to robusta coffee cultivation (Gana, 2016). The first documented incidence of CWSB attacking coffee trees dates back to 1838 (Le Pelley, 1968). Significantly, CWSB ranks among the six primary pests of arabica coffee (Venkatesha, 2010), inflicting annual crop losses in the range of $17.5–40 million (Hall *et al.*, 2006; Venkatesha, 2010).

Over the course of the past century, an array of management techniques has been employed to combat CWSB infestations, including mass trapping utilizing pheromone-baited traps, uprooting and incinerating infested plants, adult and early instar insecticide applications, 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). The effectiveness of each of these methods ranges from moderate to high; however, CWSB remains a formidable challenge due to regional climate variations, elevation disparities, sporadic monitoring, and the substantial expenses and labor requirements associated with their implementation.

Comparative research between arabica and robusta coffee varieties has revealed the heightened susceptibility of arabica to severe CWSB infestations (Veeresh, 1995; Venkatesha and Dinesh 2012). Furthermore, it has been established that CWSB exhibits a preference for arabica over robusta (Venkatesha and Dinesh 2012). Notably, laboratory studies have demonstrated CWSB's attraction to stems adorned with coffee sawdust and larval frass (Rhainds *et al.*, 2001). The observed host preference exhibited by CWSB suggests the potential for identifying non-host repellents or potential host attractants from these plant species.

It is pertinent to highlight that the unique, shade-grown rainforest habitat characteristic of Indian coffee plantations departs significantly from the predominant locales where chemical ecology research on pest host attraction has been conducted primarily in temperate, open fields. The coffee white stem borer commonly feeds on arabica coffee than the robusta coffee, to know the reason this study was conducted with the objective to investigate CWSB's proclivity for various coffee varieties and their associated volatile compounds.

MATERIALS AND METHODS

A. Behavioral preference test

(i) Arabica and robusta in opposite corners. The preference tests were conducted within a 10 × 10 × 10 ft (length × width × height) cage (Fig. 1). In each of the four corners of the cage, identical coffee plant varieties or sources were strategically positioned, and a total of fifty female Coffee White Stem Borer (CWSB) beetles were introduced into the central area of the cage. Over the course of a single day, continuous observations were made to monitor the hourly interactions between the insects and the respective plant sources. Statistical analysis of the data was performed using the chi-square test. It is worth noting that male CWSB beetles were also employed in identical experimental setups.

(ii) Arabica in one side corners and robusta in another side corners. Fifty female insects were introduced into the central area of the mesh enclosure. One hour subsequent to their release, meticulous observations were initiated. Over the course of a single day, the progression of insects toward the attractant sources was recorded at hourly intervals. To derive statistically significant insights from the collected data, a chi-square test was employed for rigorous analysis.

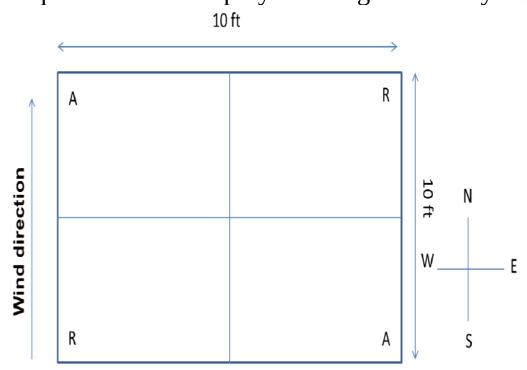

Fig. 1. Illustrative outline of cage in CWSB beetles preference study. A-arabica, R-robusta.

B. Y-tube Olfactometer Assays

This investigation focused on assessing the behavioral responses of female *X. quadripes* to a spectrum of volatile compounds extracted from coffee bark and branches, both in isolation and in combination. A Y-tube olfactometer, crafted from Perspex acrylic (sourced from BCRL, Bangalore), was used (Fig. 2). Hexane served as the solvent for the formulation of solutions for each compound under investigation. The olfactometer, characterized by a 30 cm-long central stem and two arms, each spanning 30 cm, was utilized as the testing apparatus. Specimens were judiciously positioned within two muslin cloth folds, which were employed to seal the olfactometer arms. A diminutive battery-operated fan, positioned 5 cm from the muslin cloth on the exterior, was configured to deliver a controlled airflow rate of 260 ml per minute.

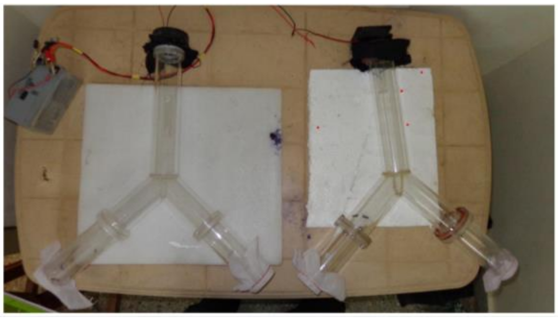

Fig. 2. Y-tube olfactometer.

An exhaust fan was symmetrically positioned at the opposing end of each olfactometer arm, facilitating the confluence of air streams from both branches. The experiments transpired within a dedicated chamber located at the extremity of the olfactometer's stem, in which the beetles were released. Following a thirty-minute period for acclimatization, the fans were set in motion, and the beetle barrier was carefully removed. The experimental trials were systematically carried out during the hours of 10:00 and 17:00, aligning with previous observations indicating peak female activity in the field during this time frame (Reddy, 2010).

The study enlisted female *X. quadripes* specimens, aged between 2 to 4 days, sourced from infested stems. Non-responsive individuals were designated as those who failed to make a selection within the allocated 60-

minute timeframe. Six female beetles were used in each experiment, and the procedure was repeated four times, each time with a distinct set of female beetles, culminating in a total of 24 females used for each sample and odor source. The application of odor sources was systematically reversed between the two arms of the olfactometer during successive tests. Furthermore, to eliminate any potential volatile contaminants, the Y-tubes were cleaned thorough with a soap solution after each experimental run, followed by acetone and drying in an oven set at 100°C for a minimum duration of 30 minutes.

Twenty microliters of extract was loaded onto a 0.5×5 cmfilter paper strip. Subsequently, the solvent was permitted to evaporate for 60 seconds before the strip was carefully positioned within the chamber housing the odor source. As a control measure, a 0.5 × 5 cm paper strip, loaded with 20 µl of the solvent, was placed in the control arm. To scrutinize the responses of the beetles, chi-square test was employed (Jaramillo *et al.*, 2013).

RESULTS

A. Behavioral preference of CWSB females and males to different coffee varieties

A marked disparity in host preference was observed between male and female CWSB beetles concerning arabica and robusta coffee varieties. When arabica was positioned along the W-N and E-S directions, and robusta along the N-E and S-W directions, both male and female CWSB beetles exhibited a distinct preference for arabica coffee, as indicated by $\chi 2$ values of 21.49 and 22.01, respectively (Table 1).Upon altering the spatial orientation of these host plants, with arabica in the W-N and N-E direction, and robusta in the S-W and E-S direction, a significant difference in female preference was observed ($\chi 2$=11.58). In contrast, no statistically significant difference in male preference was detected ($\chi 2$=2.56) (Table 1).

Subsequently, when the positions of the host plants were interchanged, with arabica in the S-W and E-S direction, and robusta in the W-N and N-E direction, the preference of both male and female CWSB beetles did not exhibit statistical significance ($\chi 2$=1.22 and 2.02, respectively) (Table 1).

B. Taxis response of female CWSB beetles in Y-tube olfactometer

The findings from the Y-tube olfactometer experiments assessing the response of female Coffee White Stem Borer (CWSB) beetles to different volatile compounds resulted in notable observations (Table 2) that include: a) a significantly higher mean percentage response of female beetles to Cauvery bark volatile (66.67) compared to the control (33.33) ($\chi 2$=11.11); b) a non-significant difference in mean percentage response between robusta bark volatile (33.33) and the control (33.33) ($\chi 2$=0.0); c) a significantly elevated response of female beetles to Cauvery bark volatile (80.00) compared to robusta bark volatile (20.00) ($\chi 2$=36.00); d) a non-significant difference in mean percentage response between 2-hydroxy-3-decanone (30.13) and (E)-2-hexenal (21.60) ($\chi 2$=1.41); e) no significant difference in mean percentage response between (Z)-3-hexenol (56.65) and (E)-2-hexenal (43.35) ($\chi 2$=1.77); f) a significantly higher response of female beetles to (E)-2-hexenal (79.33) compared to (Z)-3-hexenol (17.33) ($\chi 2$=39.76); g) a significantly elevated response of female beetles to (E)-2-hexenol (67.13) compared to 2-hydroxy-3-decanone (32.87) ($\chi 2$=11.74); h) a significantly higher response of female beetles to (E)-2-hexenol+2-hydroxy-3-decanone (53.57) compared to 2-hydroxy-3-decanone (29.75) ($\chi 2$=6.81); i) a significantly lower response of female beetles to (Z)-3-hexenol + 2-hydroxy-3-decanone (28.05) compared to (E)-2-hexenol+2-hydroxy-3-decanone (71.95) ($\chi 2$=19.27); j) a significantly lower response of female beetles to methyl salicylate (20.56) compared to ethyl benzoate (77.75) ($\chi 2$=33.26); and k) no significant difference in mean percentage response between females (5No.) (54.00) and males (5No.) (46.00) ($\chi 2$=0.62). These results provide valuable insights into the preferences and responses of female CWSB beetles to various volatile compounds, shedding light on potential avenues for pest management strategies.

DISCUSSION

Adult Coffee White Stem Borer (CWSB) individuals exhibited a pronounced preference for arabica coffee over robusta coffee. Notably, a statistically significant preference was observed in female CWSB in response to changes in the direction of the host; however, there was no significant difference in the response of male CWSB individuals to changes in host direction. Previous studies have consistently reported a stronger inclination of CWSB towards arabica coffee as opposed to robusta coffee (Kunhi Kannan, 1925; Le Pelley, 1968; Rhainds *et al.*, 2002; Reddy, 2010; Rajus *et al.*, 2021; Venkatesha and Dinesh 2012).

The Y-tube bioassay results elucidate a statistically significant difference in the preference of female CWSB between arabica and robusta coffee bark volatiles, arabica bark volatiles, and the control, while no significant difference was observed between robusta bark volatiles and the control. This indicates a substantial preference of female CWSB for arabica coffee in contrast to robusta coffee. This preference may well account for the higher incidence of CWSB infestation on arabica coffee within coffee plantations.

Table 1: Behavioral preference of CWSB female and male beetles to coffee varieties in cage studies.

Beetles	Varieties	Direction	Observed No.	Expected No.	Expected ratio	Chi. Sq. value @ 1%	Table chi.sq.value
Males	Arabica	W-N	20.1	8.9	1:1:1:1	21.49*	11.34
	Robusta	N-E	8.4	8.9			
	Robusta	S-W	1.5	8.9			
	Arabica	E-S	5.6	8.9			
Females	Arabica	W-N	23.6	11.3	1:1:1:1	22.01*	11.34
	Robusta	N-E	11.4	11.3			
	Robusta	S-W	1.9	11.3			
	Arabica	E-S	8.3	11.3			
Males	Arabica	W-N	2.2	4.2	1:1:1:1	2.56	11.34
	Arabica	N-E	3	4.2			
	Robusta	S-W	6.1	4.2			
	Robusta	E-S	5.5	4.2			
Females	Arabica	W-N	14.2	9.35	1:1:1:1	11.58*	11.34
	Arabica	N-E	14.8	9.35			
	Robusta	S-W	3.2	9.35			
	Robusta	E-S	5.2	9.35			
Males	Robusta	W-N	10.6	10.2	1:1:1:1	1.22	11.34
	Robusta	N-E	9.4	10.2			
	Arabica	S-W	12.8	10.2			
	Arabica	E-S	8	10.2			
Females	Robusta	W-N	5.69	6.6	1:1:1:1	2.02	11.34
	Robusta	N-E	4.77	6.6			
	Arabica	S-W	9.62	6.6			
	Arabica	E-S	6.31	6.6			

***-Significance**

Table 2: Taxis response (%) of female CWSB beetles to different volatiles in Y-tube olfactometer assay.

Sr. No.	Treatments	Mean Response (%)		Chi. Sq. value	Chi table value @1 %	
		Source 1	Source 2			
1.	Cauvery bark volatiles vs blank	66.67	33.33	11.11	6.63	S
2.	Robusta bark volatiles vs blank	33.33	33.33	00.00	6.63	NS
3.	Cauvery bark volatiles vs Robusta bark volatiles	80.00	20.00	36.00	6.63	S
4.	2-hydroxy-3-decanone vs E-2-Hexenal	30.13	21.60	01.41	6.63	NS
5.	Z-3-Hexenol vs E-2-Hexenol	56.65	43.35	01.77	6.63	NS
6.	E-2-Hexenal vs Z-3-Hexenol	79.33	17.33	39.76	6.63	S
7.	E-2-Hexenol vs 2-Hydroxy-3-decanone	67.13	32.87	11.74	6.63	S
8.	2-hydroxy-3-decanone vs E-2-Hexenol+2-hydroxy-3-decanone	29.75	53.57	06.81	6.63	S
9.	Z-3-Hexenol + 2-hydroxy-3-decanone vs E-2-Hexenol + 2-Hydroxy-3-decanone	28.05	71.95	19.27	6.63	S
10.	Methyl salicylate vs Ethyl benzoate	20.56	77.75	33.26	6.63	S
11.	5 females vs 5 males	54.00	46.00	00.62	6.63	NS

S-significance; NS-Non significance

CONCLUSIONS

Female CWSB beetles exhibited a substantial preference for arabica coffee, while males displayed a non-significant preference. In the Y-tube bioassay, a notable distinction in preference was evident in female CWSB beetles when comparing arabica and robusta coffee bark volatiles, arabica bark volatiles, and the control. However, no significant difference was observed between robusta bark volatiles and the control. These findings align with the established understanding of CWSB behavior, highlighting the nuanced host preferences exhibited by females, particularly in response to coffee bark volatiles. The differential responses between male and female CWSB beetles emphasize the importance of considering gender-specific behaviors in the context of pest management strategies, and they provide valuable insights into the chemical ecology of these insects.

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Conflict of Interest. None.

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