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Response of *Xyleborus Fornicatus* Eichhoff to Some Volatile Compounds Identified From Tea Bark

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ABSTRACT

Some of the volatile compounds present in the bark of tea (*Camellia sinensis* Kuntze) were identified, and examined for their attractant or repellent properties on the adult beetles of *Xyleborus fornicatus* Eichhoff, both under laboratory and field conditions. The selected volatile compounds were used individually and in combination in an olfactometer in the laboratory, and in vertical sticky traps in the field. Field experiments were carried out at selected locations in three geographically-different zones in the tea-growing areas of Sri Lanka, namely the mid-elevation wet zone (Hantane Estate), the mid-elevation dry zone (Attampitiya Estate), and the up-country (St. Coombs Estate).

The olfactometer studies revealed that crude extracts, obtained from the uninfested bark of the tea cultivars, TRI 2025 and TRI 2023, attracted higher numbers of beetles than the numbers attracted by the infested bark. *X. fornicatus* beetles were attracted to the volatile compounds, such as phenyl acetaldehyde, methyl salicylate and linalool, while they did not show any response to geraniol and trans-2 hexenal in the laboratory, or in the field at any of the three field locations. Different combinations (two compounds at a time) of linalool, methyl salicylate and phenyl acetaldehyde attracted more beetles than the compounds individually.

INTRODUCTION

Xyleborus fornicatus Eichhoff (Coleoptera: Scolytidae) is an important insect pest of tea (*Camellia sinensis* Kuntze). Its damage is serious in Sri Lanka at elevations between 600 m and 1200 m. The beetle is a day-flyer, and only females are capable of flying. On encountering a suitable host plant, they construct galleries in the young stems to raise their brood.

Current methods of controlling this pest include use of *X. fornicatus*-tolerant tea cultivars (Anon., 1997) and synthetic insecticides. However, the use of insecticides against this pest is limited owing to the fact that tea is a beverage, and also usage of insecticides leads to many health and environmental hazards. More often the insecticides have little effect, as this pest is minute and occupies the galleries inside the stems. Vast differences

between certain tea cultivars, in relation to *X. fornicatus* infestation, indicate that there can be differences in the chemical composition of these cultivars that influence the insect to differentiate between them in selecting a host.

The laboratory studies have also shown that tea stems with intact bark are more attractive to the female beetles than stems stripped off bark (Sivapalan, 1974). This indicates that attraction to the beetles is possibly regulated by a chemical compound or compounds found in the bark.

The present work demonstrates the response of *X. fornicatus* to several volatile compounds found in tea bark, both in laboratory bioassay studies and in the field.

MATERIALS AND METHODS

1. Extraction and analysis of volatile compounds from tea bark

Branches, of about 1 cm in thickness, of the cultivars, TRI 2025 (susceptible to *X. fornicatus*) and TRI 2023 (resistant to *X. fornicatus*), were collected from two locations, one in the north-east region of the mid-country (Attampitiya Estate, Bandarawela), and the other in the up-country (St. Coombs Estate, Talawakelle). Sampling at either place was carried out during dry season. During sampling, uninfested branches were separated from infested branches, labeled separately, and brought into the laboratory. About 100 g of bark were stripped off the stems and used for analysis.

a. Simultaneous distillation and extraction procedure (SDE)

One hundred grammes of tea bark were placed in a 500-ml round bottom flask and covered with 250 ml of distilled water heated to 60°C. This was placed in a heated mantle and linked to one arm of the SDE apparatus, while a 50-ml flask containing 20 ml of methylene chloride, in a water bath at 40°C, was connected to the other arm of the apparatus. Ten millilitres of methylene chloride were added simultaneously into the U-tube located in the middle of the apparatus. Distillation was limited to 30 min. The condensate of methylene chloride was dried with 2 g of anhydrous sodium sulphate for 15 min, and concentrated to a known volume. This was used as the crude extract. One micro-liter of this concentrate was injected into a pre-programmed Gas Chromatograph, coupled to a MS, for the identification and confirmation of volatile compounds.

Once the volatile compounds were identified and confirmed, analytical-grade samples of the compounds identified (hereafter, referred to as the "standard chemicals") were used in olfactometry and field trials.

2. Laboratory bioassay experiments

a. Olfactometric bioassays

An olfactometer, made according to the design described by Patterson and Stephansson (1961), was used for the laboratory bioassays (Fig. 1). For every bioassay, a single *X. fornicatus* female beetle was introduced through the center hole of the olfactometer. At the same time, a test volatile compound, or the crude extract, was incorporated into a small wick made out of cotton wool and pegged in chamber 1 of the olfactometer. The beetle's response was measured by its attraction to the test chemical or the crude extract, after a given time period.

All the olfactometric studies were carried out in a dark chamber. A second olfactometer was designed by modifying the model described by Patterson and Stephansson (1961) (Fig. 2), for use in comparative studies.

b. Bioassay with crude extracts

Beetle responses to 4 µl of the crude volatile extracts obtained from the bark (both infested and uninfested) of the cultivars, TRI 2023 and TRI 2025, were compared 1 and 5 minutes after introduction of the beetles. This experiment was replicated 10 times.

c. Olfactometric bioassays with selected standard volatile compounds

Beetle responses to 10 µl of the selected volatile compounds at different concentrations, 200, 500, 1000 and 5000 ppm, were examined one minute after the introduction of the beetles. This was repeated 10 times for selected standards: the volatile compounds, linalool, methyl salicylate, phenyl acetaldehyde, geraniol and t-2 hexenal. A piece of cotton wool, without any chemicals, was used as the control.

d. Modified olfactometric bioassays with selected combinations of standard volatile compounds

The following combinations of the volatile compounds were selected for comparative studies, based on the results of the bioassay with standard volatile compounds.

1. Linalool and phenyl acetaldehyde
2. Linalool and methyl salicylate
3. Phenyl acetaldehyde and. methyl salicylate by concentration

Ten microlitres of the first compound (1000 ppm) of the test combination were incorporated into two pieces of cotton wool, and each was pegged in chambers 1 and 3, while the second compound of the given combination was pegged in chambers 2 and 4.

Paths "a", "b", "d" and "g" were kept open, while "c", "e", "f" and "h" were closed (Fig. 2). By means of this arrangement, chamber I received the odour of the combination of both compounds; chamber II received the odour of the first compound of a given pair; and chamber IV received the odour of the second compound of a pair. Chamber III acted as control.

One beetle was introduced through the centre hole of the olfactometer. A beetle's response was recorded at the entry of the beetle into each chamber. The total number of beetles entering each chamber was recorded after 1, 5 and 10 minutes. This experiment was replicated 10 times.

3. Field experiments

Field experiments were carried out at three geographically-different locations, namely St Coombs Estate, Talawakelle (up-country), Hantane Estate, Kandy (mid-elevation wet zone), and Attampitiya Estate, Bandarawela (mid-elevation dry zone). All of these fields consisted of mature tea (cultivar TRI 2025) infested with *X. fornicatus*. Vertical sticky traps, as described below, were used in the tea fields. The experiment was set up around 10 a.m. Sticky traps, 10 m apart and facing against the wind, were placed at a height of 1.5 m above ground level. The beetle catch in each trap was recorded around 3 p.m. the same day.

Vertical sticky traps

Cardboard sheets (25 cm in diameter and 15 cm in height) were used to trap the beetles. One side of the cardboard was pasted with red, blue, yellow or green coloured varnish paper to give a specific colour for beetle attraction. The glue, "Bird Tangle Foot", was applied to the surface of the coloured side to form a sticky surface for trapping the beetles. Ordinary transparent sheets were used as colourless traps.

a. Response of *X. fornicatus* to varying concentrations of volatile compounds in the field

A concentration range of 200 ppm - 50,000 ppm was prepared using selected volatile compounds, namely linalool, methyl salicylate and phenyl acetaldehyde, for use in the vertical sticky trap experiments. A piece of cotton wool was wetted with 1 ml of the diluted compound, and pegged inside a partially-opened vial which was then placed on the sticky surface of the trap.

The concentration that attracted the highest number of beetles was determined. The experiment was replicated 10 times at each location.

b. Response of *X. fornicatus* to combinations of volatile compounds

Volatile compounds in different combinations (two compounds at a time) were tested in the field. Two pieces of cotton wool were impregnated with 1 ml of a diluted test compound (5000 ppm). The cotton wool was pegged inside a small, partially-opened carton and pasted onto the sticky surface of the trap. Separate traps were used for each combination.

The beetle catch per trap per day was recorded. The experiment was repeated in all three locations and replicated 10 times.

Statistical analysis

Data on the olfactometric bioassay were analysed using the Chi Square test. Analysis of variance was carried out on the responses of the beetles to the test compounds in the field.

RESULTS AND DISCUSSION

Analysis of tea bark volatiles

Six major volatile compounds, linalool, linalool oxide, methyl salicylate, phenyl acetaldehyde, geraniol and t-2 hexenal, were identified from the bark of *X. fornicatus*-susceptible (TRI 2025) and *X. fornicatus* -tolerant (TRI 2023) tea cultivars (Tables 1 and 2). These compounds were present in both SHB-infested and -uninfested samples collected from Attampittiya and St Coombs Estates. However, the composition of these compounds varied with the cultivar, the level of infestation, and with the location. For example, linalool oxide II levels are high in the susceptible cultivar (TRI 2025) at both locations when compared with the tolerant cultivar (TRI 2023). The levels of linalool oxide II (cis-furanoid) increased with beetle-infestation on both susceptible and tolerant cultivars. The cis-furanoid of linalool oxide II has two optical isomers (3R,6S and 3S,6R) and their odour impressions are quite different.. Optical isomer 3R,6S has a leafy or earthy odour, whereas isomer 3S,6R has a sweet or floral odour. Therefore in order to get an idea of overall change of the odour impression on *X. fornicatus*, a detailed study on the optical isomers of linalool oxide II is necessary.

Laboratory bioassay experiments

a. Response of *X fornicatus* to the crude extracts obtained from the tea bark, using olfactometric bioassays

It was observed that significantly higher numbers of beetles were attracted to the crude extract obtained from uninfested bark of the cultivar, TRI 2025, (6 ± 0.6) compared with the numbers attracted to the extracts obtained from infested bark (2 ± 0.1). The response to un-infested TRI 2023 was also higher (3 ± 0.2) than the response to infested TRI 2023 (1 ± 0.1), but the overall beetle response was low when compared with TRI 2025. This confirmed that *X. fornicatus* has some partiality towards the healthy bark of either cultivar, as reported by Sivapalan (1974). Changes of linalool oxide II concentration upon infestation is one of the key observations made in the chemical analysis of tea bark. It may be possible that upon infestation, the composition of the optical isomers of linalool oxide II alters in such a way as to create an odour impression that does not attract SHB, thus preventing further infestation.

b. Response of *X. fornicatus* to different concentrations of standard volatile compounds, using olfactometric bioassays

The mean numbers of beetles attracted to different concentrations of the volatile compounds, after 1 min of exposure, are given in Table 2.

Beetle attraction was highest at 1000 ppm level, and this concentration was used for further laboratory bioassays. Out of the five volatile compounds tested, linalool, methyl salicylate and phenyl acetaldehyde, attracted higher numbers of beetles. The attraction to these three compounds was reduced after the first minute of exposure.

The beetles were not attracted to geraniol and t-2 hexenal at any of the concentrations tested. A large number of beetles moved towards the opposite chamber of the olfactometer, which indicates that these two compounds are not attractive.

c. Response of *X. fornicatus* to combinations of the volatile compounds using the modified olfactometric bioassay

The numbers of beetles attracted to each combination of volatile compounds are given in Table 3.

A higher number were attracted to combinations of the volatile compounds than to the individual volatile compounds. The combination of phenyl acetaldehyde and methyl salicylate attracted more beetles than the other two combinations tested. Methyl salicylate appears to be a good SHB attractant, either by itself or in combination with other compounds.

Field experiments

The maximum number of beetles were trapped between 11 a.m. and 2 p.m., indicating that peak flight takes place during this period and that the true flight temperature was 26° C.

These observations are in agreement with those of Calnaido (1965). Field trapping was limited to days with bright sunshine and an average temperature of 26° C.

d. Response of *X. fornicatus* to different concentrations of the volatile compounds

The number of beetles attracted to the different concentrations of the volatile compounds, at St Coombs Estate, is given in Table 4.

With all the volatile compounds tested, the highest number of *X. fornicatus* was attracted at the concentration of 5000 ppm. It is likely that, at lower concentrations, the odour of the solvent used to dissolve the volatile chemical was stronger than that of the volatile compound itself. This could be one of the reasons for poor beetle response at low concentrations. The response of the beetles to a very high concentration of volatile compounds (50,000 ppm) was poor.

e. Response of *X. fornicatus* to the individual volatile compounds and their combinations, at different locations

In all the three locations, a higher numbers of beetles were attracted to the combinations of volatiles, rather than to the individual volatile compounds (Table 5). For example, at St Coombs, the highest number (4.2) were attracted to the combination of methyl salysilate and phenyl acetaldehyde, while at Hantana and Attampitiya the combination of phenyl acetaldehyde and linalool attracted the highest number of beetles (2.1 and 3.5, respectively). It is interesting to note that phenyl acetaldehyde is common in all three combinations. Trans-2-hexenal and geraniol did not have beetle-attractant properties when tested alone, or in combination with the other volatile compounds, at any of the three locations.

The beetles showed a similar attraction towards linalool both at St. Coombs and Hantana Estates. However, at Attampitiya Estate, the beetles were significantly more attracted to linalool than to the other compounds.

The preliminary field tests of volatile compounds were only partially successful, owing to the small number of beetles captured in the traps during the study period. It was also observed that the tea volatile compounds not only attracted *X. fornicatus* but also some other Coleoptera, as well as insects belonging to the orders Diptera and Hymenoptera.

CONCLUSIONS

Six major volatile compounds, linalool, linalool oxide, methyl salicylate, phenyl acetaldehyde, geraniol and t-2 hexenal, were identified from the bark of *X. fornicatus*-tolerant and -susceptible tea cultivars. The composition of these compounds vary with the cultivar, level of infestation and location. The concentration of linalool oxide II (cis-furanoid) increased significantly upon infestation, in the bark of both susceptible and resistant cultivars collected from St. Coombs Estate.

Crude extracts obtained from uninfested bark of TRI 2025 and TRI 2023 attracted more beetles than the numbers attracted to the infested bark extracts.

The olfactometry studies revealed that linalool, methyl salicylate and phenyl acetaldehyde have higher SHB beetle-attracting properties. It was also revealed that combinations of these compounds attracted more beetles than the individual compounds, both under laboratory and field conditions.

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Table 1 (a & b). Tea bark volatile compounds and their concentrations (ppm).

1a. Location - St. Coombs Estate

Compound	TRI 2025 (UI)	TRI 2025 (I)	TRI 2023 (UI)	TRI 2023 (I)
Linalool	4.95	3.55	3.47	7.59
Methyl salicylate	6.32	3.02	4.05	3.58
Phenyl acetaldehyde	0.97	0.85	0.61	2.53
Linalool oxide II	5.13	9.9	2.73	8.24
Geraniol	0.15	0.26	0.04	0.16
T2 –Hexenal	0.37	0.42	0.28	0.23

(UI) - Uninfested (I) – Infested

1b. Location - Attampitiya Estate

Compound	TRI 2025 (UI)	TRI 2025 (I)	TRI 2023 (UI)	TRI 2023 (I)
Linalool	2.41	1.68	5.01	3.3
Methyl salicylate	2.64	1.6	4.38	4.99
Phenyl acetaldehyde	1.44	1.2	0.32	0.31
Linalool oxide II	7.13	8.83	3.3	4.43
Geraniol	0.21	0.13	0.16	0.01

Table 2. Mean number of *X. fornicatus* beetles attracted to different concentrations of volatile compounds (n=10).

Compound	200 ppm	500 ppm	1000 ppm	5000 ppm	50000 ppm	Control
P/acetaldehyde	0	0.2	0.4	0.2	0	0
Linalool	0	0.1	0.5	0.3	0.3	0
M/salicylate	0	0.1	0.3	0.2	0	0
Geraniol	0	0	0	0	0	0
t-2 Hexenal	0	0	0	0	0	0

Table 3. Mean number of *X. fornicatus* beetles attracted to combinations of volatile compounds after 5 minutes (n=10)

Combination	Chamber I	Chamber II	Chamber III	Chamber IV
Linalool and P/acetaldehyde	0.4	0	0.1	0.1
Linalool and M/salicylate	0.4	0	0	0.3
P/acetaldehyde and M/salicylate	0.6	.0.1	0	0

Chamber I received the combined odours of both compounds.

Chamber II received the odour of the first compound in the combination.

Chamber IV received the odour of the second compound in the combination.
Chamber III was the control (no odour).

Table 4. Mean number of *X.fornicatus* beetles captured at different concentrations of individual volatile compounds at St. Coombs Estate (n=10).

Compound	200 ppm	500 ppm	1000 pppm	5000 ppm	50000 ppm	Control
Linalool	0.16	0.33	1.16	1.8	0.5	0.16
M/salicylate	0.16	0.33	0.16	1.5	0.33	0.16
P/acetaldehyde	0.16	0.16	0.66	2.0	0.33	0.16

Table 5. Mean number of *X. fornicatus* beetles captured using individual volatile compounds and their combinations in the field at three locations (n=10)

Individual compounds and their combinations	St.Coombs	Hantane	Attampitiya
Linalool	1.1	1	2.6
M/salicylate	1.3	0.7	1.3
P/acetaldehyde	2.3	0.3	0.9
Geraniol	0.1	0	0
t-2 Hexenal	0.1	0	0
M/salicylate and P/acetaldehyde	4.2	1.0	1.6
M/salicylate and Linalool	2.2	1.4	1.6
P/acetaldehyde and Linalool	3.2	3.1	3.5
t-2 Hexenal and Geraniol	0	0	0
Linalool and Geraniol	1.4	1.5	2.0
P/acetaldehyde and Geraniol	1.0	0.7	0.3
M/salicylate and Geraniol	0.4	1.1	0.5
Control	0	0	0
LSD (pe"0.05)	1.02	0.09	1.14

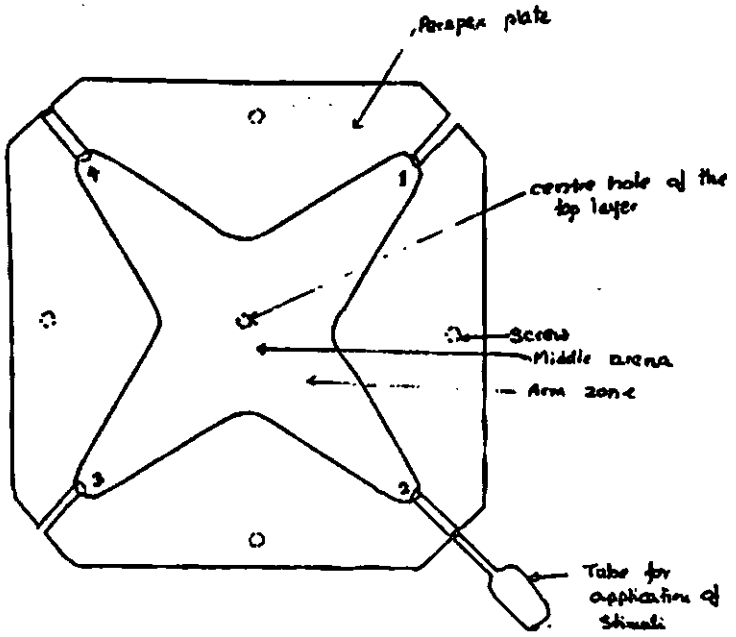

Figure 1. Surface view of olfactometer used for testing individual volatile compounds in the laboratory (From Patterson & Stephansson, 1961)

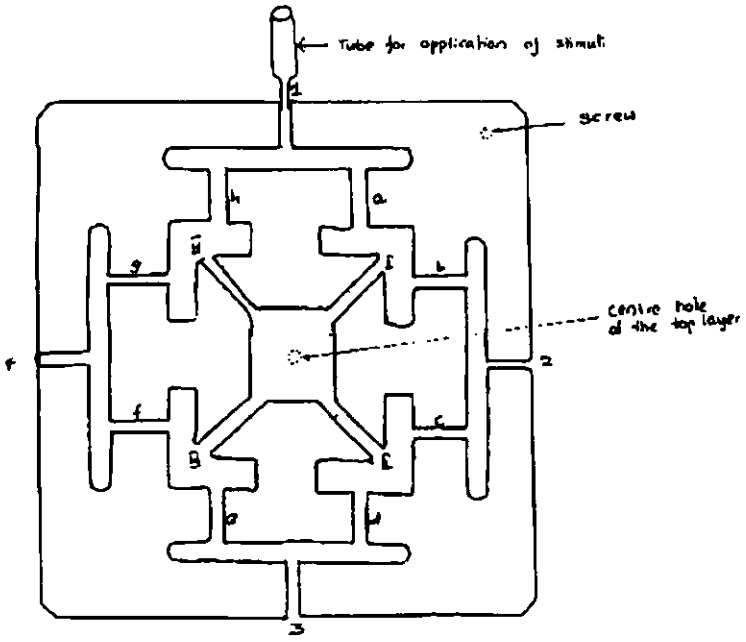

Figure 2. Modified olfactometer used to test various combination of volatile compounds in the laboratory.