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Volatile and Non Volatile Compounds of the Host Plant Affecting Behaviour of the Low Country Live Wood Termite (*Glyptotermes dilatatus* Bugnion and Popoff) of Tea

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ABSTRACT. *Damage by termites is considered to be a major problem especially in low country tea plantations. A study was carried out to identify the chemical constituents, which attract or repel termites to tea clones. It was found that plants already damaged by the Low-country Live-wood termite (Glyptotermes dilatatus), rotted wood and die back wood of susceptible tea clones are the main sources of volatiles that attract the termite G. dilatatus.*

Extracts of rotted wood of termite tolerant clone, TRI 2027 and termite susceptible clone TRI 2023 showed statistically similar attractiveness to termites. Thus, there may be factors other than attractants, which make tea clones tolerant or susceptible. Bioassays using an olfactometer with extracts in Dichloromethane showed that highly susceptible clone TRI 3063 is more attractive than susceptible clone TRI 2023. As these findings are in agreement with field observations on susceptibility of clones, it proves the suitability of the olfactometer in recognising attractants or repellents in plant extracts, in relation to low country live-wood termites. Both non-volatile compounds and volatile compounds are responsible for attractiveness of the tea clones TRI 2023 and TRI 3063.

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INTRODUCTION

Termites are social insects and are weak flyers, incapable of flying long distances and the number of alates (winged form) are rather low. These termites start to damage tea bushes, particularly in vegetatively propagated (VP) tea. Existence of termites in neighbouring old VP clearings become a source of infection to new tea lands (Vitarana, 1986).

The snag which is the "die back" portion of a pruned stem that undergoes rotting (rotted wood) is the most commonly attacked point on a tea bush. It initiates a gallery there and continues its damage and feeds on heartwood. Soft wooded high yielding clone TRI 2023 is susceptible to low country live-wood termite, stem canker and drought effects. As such it has been restricted from planting in the low country tea areas. These factors collectively lead to death of bushes there by reducing the number of bushes in the tea land, which directly cause yield loss in tea.

Termite pests on tea lands may be divided into three groups according to the damage they cause. These are live-wood termites, scavenging termites and dry-wood termites. Of these groups, live-wood termites are directly injurious to the tea plant (Vitarana, 1986). There are several live-wood termite species occurring in the low country tea areas (Jepson, 1929). Among these, the commonly occurring termite species are *Glyptotermes dilatatus* and *Neotermes greeni*, with the former predominating.

Termites are attracted to dead and decaying wood in tea bushes caused by wood rot following attack by shot hole borer, branch canker, sun-scorch, mechanical damage and die back of branches (Ranaweera, 1962).

Although the low country live-wood termite was recognized as a pest on seedling tea, its attack became of widespread importance in Sri Lanka with the introduction of high yielding soft wooded clonal tea (Sivapalan *et al.*, 1981). Susceptibility to die back and wood rot appears to be a key factor affecting termite attack (Sivapalan *et al.*, 1981).

Many insects use airborne volatiles emitted from plants to locate their hosts. The information on volatile chemicals that attract insects to their hosts and stimulate oviposition upon arrival could be of benefit to pest control programs (Cosse *et al.*, 1994). Sharma and Raina (1998), screened some chemical compounds to test toxicity. Linaool, citronellal, and carvone gave promising results on building termites. Reyes *et al.* (1995) studied the antitermitic activity of *Lonchocarpus castilloi* (tropical tree) flavonoids and heart wood extracts. Successive extraction with Hexane, Diethylether, Acetone, Methanol and water reduced its resistance to attack by *Cryptotermes brevis*. These extracts were bioassayed with filter paper discs.

The present study was carried out with the objectives of determining attractant or repellant effects of volatile and non-volatile chemical compounds of termite susceptible, immune tea clones at various stages of infestation by wood rot to termites.

MATERIALS AND METHODS

Collection of wood materials

Wood rotted stems of tea clones of TRI 2023, TRI 3063, TRI 2027, TRI 2016 were collected from the Tea Research Institute Kottawa substation in Galle, during the swarming season in 1997, *i.e.* just prior to the monsoon season of the year. These stems were cut into small pieces using a shredder machine, before extraction.

Test insects

The low country live-wood termite (*Glyptotermes dilatatus*) alates (winged forms) and workers were obtained for bioassays from laboratory cultures maintained for this study at the Tea Research Institute, Ratnapura. Termites (*G. dilatatus*) were collected from infested bushes from Hadaraganga Division at Hapugastenne Estate for bioassay.

Extraction

Extraction of volatiles of wood materials were carried out using Simultaneous Distillation and Extraction (SDE) apparatus using a method described by Yamanishi *et al.* (1989). A sample of 150 g of wood material was used in each SDE and the distillation time was one hour.

A cold extraction method was used to extract both volatiles and non-volatiles of wood material. The wood rot samples (2 kg) were placed in 3 shaker bottles and 2 litres of dichloromethane was added to each. These bottles were shaken at 150 rpm at 26-27°C for 18 h and the solvent was filtered off. The filtrate was concentrated under low pressure in a rotovapour and yielded 3 g of the extract.

Olfactometry

Olfactometers made of Perspex were used in screening experiments to determine attractant and repellent properties of plant extracts (Patterson and Stephansson, 1991) (Figure 1). This Olfactometer has 4 arms and a center hole through which air is drawn by a slow suction pump. End of each arm has an inlet with which an odour stimulus can be applied. The arena is divided into 5 zones, 4 arm zones and 1 indifferent zone in the central part of the arena. The floor of the arena is covered with a piece of black paper, to make the environment gloomy and to facilitate easy movement of the insect. Air is sucked through holes of the 4 arms of the olfactometer while the odour stimulus was applied to one of the specific arms. A termite (alate) was introduced into the central part of the arena and the tube from the suction pump was connected. A positive behaviour of 1 was recorded when the insect was in the arm zone and a negative behaviour of 0 recorded when it was in the middle arena. Observations were made in intervals of 5 minutes continuously for 45 minutes, after an initial settling period of 10 minutes. Solvent used in the treatment was used in the control, after being allowed to evaporate.

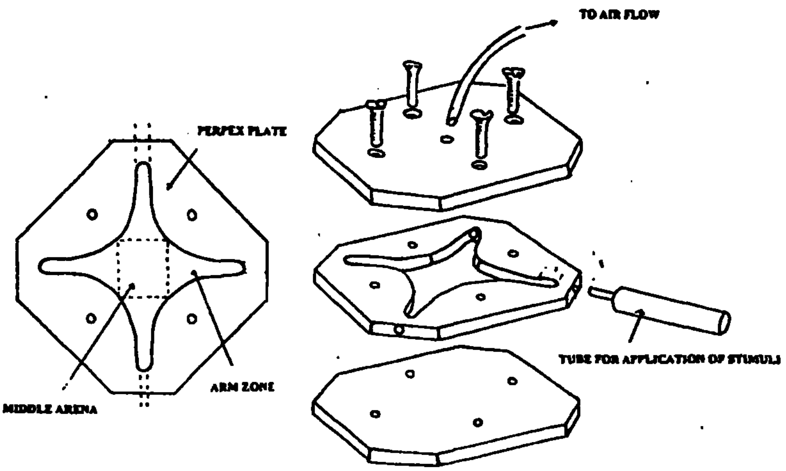

Figure 1. Olfactometer used in the experiment (Patterson and Stephansson, 1991).

Preliminary studies with olfactometer

Though the bioassay work was done with alates of the termites, it was difficult to find alates in the field at times other than during the swarming period. Therefore, for this study 01, 02 and 04 nymphs were used and their behaviour were observed in the olfactometer. The study was continued with alates to see whether the alates could be used instead of nymphs for the test by keeping an alate in the middle of the arena and one alate, two alates and four alates were used as different treatments.

Experiment I

In order to investigate the attractant effect of the stems with the development of wood rot, a bioassay was carried out using Dichloromethane (CH_2Cl_2) extracts of dead ends (die-back) of prune cuts of branches of termite susceptible tea clone TRI 2023 before wood rot set in, after wood rot had set in (rotted wood) and after infestation by termites (infested wood).

Each extract was studied for its degree of attraction with 18 replicates using olfactometry.

Experiment II

Effect of crude extract, volatile fraction separated by SDE and non-volatile fraction by SDE of TRI 2023 clonal wood rot were tested using termite alates by the olfactometer method. Each treatment was replicated 15 times.

Experiment III

To differentiate between the attractant effect of susceptible (TRI 2023), tolerant (TRI 2027) and immune (TRI 2016) tea clones, crude extracts of clonal wood rot obtained by cold extraction method were bioassayed using termite alates by the olfactometer method with 15 replicates.

Experiment IV

Volatiles of TRI 2023, TRI 2027, TRI 2016 were extracted using one kilogram rotted material of clonal wood of each clone using SDE and GC chromatograms were taken.

Experiment V

As a preliminary study, cold extracts of two clones, TRI 2023 and TRI 3063 in Hexane, in Dichloromethane (CH_2Cl_2) and in Methanol (Meth.) were bioassayed using the petri-dish couplet method described below to determine the most attractive extract to the termites. Petri-dish couplets were used as test chambers. A filter paper was kept in each petri dish and divided into 4 by drawing 2 cross lines. Four circles were drawn inside each section and an extract was kept inside one circle and the other three circles were used as controls (Figure 2). Four termites of the worker caste were used in each treatment and the experiment was replicated 6 times. Solvent used in the extraction was used in the control and allowed to volatilized before the bioassay was carried out. Termites were allowed to move freely inside the petri dish and their behaviour recorded at 15 min intervals for 3 h. Behaviour was recorded as 1 when a termite was in a particular section. Since the Dichloromethane extract was found to be the most attractive to the termite, this extract was used for further study.

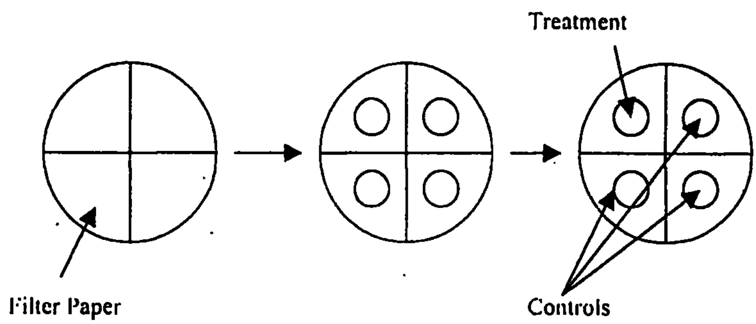

Figure 2. Filter paper arrangement in a petri-dish couplet method of bioassay.

Experiment VI

Since experiment II indicated the presence of attractants in the non-volatile fraction as well, these fractions were further analyzed to identify the active compounds present. Vacuum Liquid Chromatography (VLC) was used to separate the non-volatile fraction and 13 fractions were obtained. Fractions from each clone were bioassayed using alates in olfactometers to determine their degree of attraction. The solvent systems used for the separation of fractions were n-hexane, CH_2Cl_2, n-hexane (1 : 3); CH_2Cl_2, n-hexane (1 : 1); CH_2Cl_2, n-hexane (3 : 1); CH_2Cl_2, MeOH (1 : 99); MeOH, CH_2Cl_2 (1 : 49); MeOH, CH_2Cl_2 (3 : 97); MeOH, CH_2Cl_2 (1 : 24); MeOH, CH_2Cl_2 (1 : 19); MeOH., CH_2Cl_2 (3 : 37); MeOH., CH_2Cl_2 (1 : 10); MeOH.

Each fraction was studied for degree of attraction in 18 replicates using olfactometry and all data were analyzed statistically using the chi-square test for association.

RESULTS AND DISCUSSION

Experiment I

In the study where extracts of rotted wood, termite infested wood and die back wood of tea clone TRI 2023 in CH_2Cl_2 were tested for the degree of attraction or repellence, all the extracts were observed to be attractive to the termites (Table 1).

Table 1. Attractant effect of crude extracts of the tea clone, TRI 2023 at different stages of decay on live wood termites.

Extract	Observed value		Expected value		Significant level
	Treatment	Control	Treatment	Control	
Wood rot	75	87	40.5	121.5	P<0.005
Wood infested	81	81	40.5	121.5	P<0.005
"Die back"	69	93	40.5	121.5	P<0.005

Though termite infested wood was attractive to the termites as given in Table 1, it is important to further study the attractive effects of die back and wood rot stages of the tea stem before infestation.

Experiment II

Wood rot crude extract, separated volatile fraction and non-volatile fractions from TRI 2023 were attractive to termites (Table 2).

Table 2. The attractant effect of crude extract, volatile fraction and non-volatile fraction of rotted wood of tea clone TRI 2023 on the low country live-wood termite.

Extracts	Observed value		Expected value		Significant level
	Treatment	Control	Treatment	Control	
Crude (volatile + non volatile)	85	103	47.0	141.0	P<0.005
Volatile fraction (SDE)	84	105	48.0	145.5	P<0.005
Non volatile fraction	87	99	46.5	139.5	P<0.005

Low country live-wood termite is significantly attracted to the crude extract, volatile fraction of the crude extract and the non-volatile fraction of the crude extract of wood rotted stems of TRI 2023. This further shows that not only volatile fraction but also non-volatile fraction contains compounds, which attract the termites.

Experiment III

Of the three clones tested, crude extracts from TRI 2023 and TRI 2027 were found to be equally attractive to termites than that from TRI 2016 (Table 3). The statistical similarities of attraction by the extracts, of TRI 2023 and TRI 2027 suggests that there is no relationship between olfactory attraction and tolerance to infestation. At the same time, these results confirmed that the olfactometry could be used for this type of assays.

Table 3. The effect of crude extracts of tea clones, TRI 2023 (Susceptible), TRI 2027 (Tolerant) and TRI 2016 (Immune) on low country live-wood termites.

Extracts	Observed value		Expected value		Significant level
	Treatment	Control	Treatment	Control	
TRI 2023(S)	85	103	47.00	141.00	P<0.005
TRI 2027(T)	70	114	46.00	138.00	P<0.005
TRI 2016(I)	57	128	46.25	138.75	NS

NS - Not Significant

Experiment IV

In gas chromatographic separation of the volatiles of tea clones, TRI 2023 (Susceptible), TRI 2027 (Tolerant) and TRI 2016 (Immune) using SDE, number of volatile peaks on TRI 2023 was found to be greater than the number produced by the other two clones, TRI 2027 and TRI 2016 (Figure 3). Matsumura *et al.* (1969) had carried out similar studies on trail following substances. Isolation and purification from *Reticulitermes virginius* showed that the active principal of the trail following substance from the termite appeared to be identical to that from the wood infested by the fungus *Lenzites trabea.*

Experiment V

Of the three solvent extracts tested on termites by petri dish couplet method, CH_2Cl_2 extract was found to be most attractive to termites. Therefore bioassay directed separation of fractions were carried out with CH_2Cl_2 extracts.

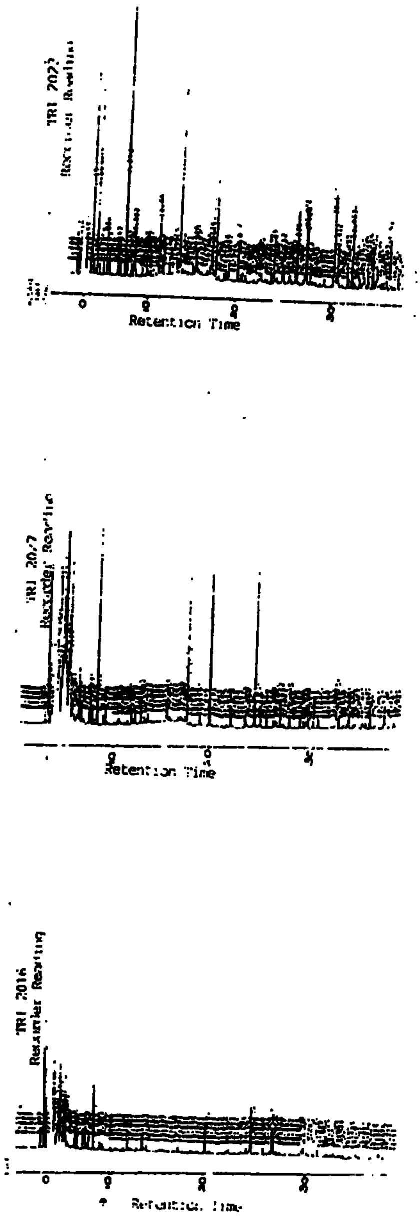

Figure 3. **Gas chromatograms of volatiles of rotted wood of TRI 2023, TRI 2027 and TRI 2016.**

Experiment VI

It was observed that the attractant effect of 5th, 8th, 9th, 10th fractions of both tea clones TRI 2023 and TRI 3063 were significant. Fifth fraction is in 100% Dichloromethane, 8th, 9th, 10th fractions were in 3%, 4%, 5% Methanol in Dichloromethane solvents respectively. 3rd, 13th, fractions of TRI 2023 and 12th, 6th, VLC fractions of TRI 3063 clone also became significantly attractive to termites (Table 4).

Table 4. Attractiveness of 13 Vacuum Liquid Chromatography (VLC) fractions of tea clones, TRI 2023 (Susceptible) and TRI 3063 (Highly Susceptible) to the low country live-wood termite.

Clone	VLC Fraction	Control Mean	Treatment Mean	X^2Value/ Significant	Clone	VLC Fraction	Control Mean	Treatment Mean	X^2Value/ Significant
TRI 2023	1	-	-	-	TRI 3063	1	1.60	1.50	0.03NS
	2	1.50	1.70	0.66NS		2	1.70	1.60	0.02NS
	3	1.87	0.83	9.01**		3	1.77	1.50	0.61NS
	4	1.50	2.00	1.85NS		4	1.49	1.66	0.62NS
	5	1.87	0.75	9.53**		5	1.20	2.66	10.85**
	6	1.50	2.16	2.72NS		6	1.36	2.00	4.11*
	7	1.59	1.88	1.42NS		7	1.46	2.11	3.49NS
	8	1.33	2.66	14.93**		8	1.27	2.94	22.13**
	9	1.35	2.20	6.52*		9	1.30	2.11	5.34*
	10	1.12	2.38	14.82**		10	1.11	2.40	13.2**
	11	1.64	1.05	3.16NS		11	1.35	1.17	0.05NS
	12	1.50	1.60	0.15NS		12	1.70	0.80	6.19*
	13	1.38	2.16	5.16*		13	1.60	1.60	0.011NS

* - Significant at 1% level (highly attractive)
* - Significant at 5% level (attractive),
NS - Not Significant (not attractive)

As both these clones are susceptible to termites and most similar fractions are attractive to termites it could be inferred that similar compounds present in these fractions are responsible for their susceptibility. But according to thin layer chromatographic analysis, the compounds in similar fractions were not shown to be the same compounds. It was observed that TRI 3063 wood rot extracts were highly attractive compared to TRI 2023. This is in agreement with the field observations made by Vitarana, (1997) where it was recorded that tea clone TRI 3063 is more susceptible to low country live-wood termite than the clone TRI 2023. Thus, these results confirm the field observations on susceptibility of tea clone to low country live-wood termites.

CONCLUSIONS

The olfactometer studies showed statistically similar degree of attraction of rotted wood of both tolerant clone (TRI 2027) and susceptible clone (TRI 2023). Thus there may be factors other than attractants making the TRI 2023 clone more susceptible and TRI 2027 clone more tolerant. Results showed the significantly higher attractant effect of highly susceptible clone (TRI 3063) than the susceptible clone (TRI 2023). It was decided to further separate the active fractions of these two clones (TRI 2023 and TRI 3063) for identification of active compounds. Identification of these active compounds has to be finally coupled with Electroantenogram studies using winged form or alates of the low country live wood termites. Then these compounds can be tested in the field with a view to using them as bait traps or to confuse colony founders during swarming periods. The study resulted in a fast (short-cut) method of screening tea clones in relation to their degree of attraction to the low country live-wood termite.

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