Research Paper

Volume : 3 | Issue : 2 | February 2014 • ISSN No 2277 - 8179

Can Chemical Traps Manage Teak Defoliator Outbreaks? Indications From Teak Leaf Chemistry

Biology

KEYWORDS : Hyblaea puera, Tectona grandis, leaf volatiles, insect outbreak

Dr. Bindu K. Jose	Research scholar Tree health helpline Kerala forest research institute, Peechi, 680 653, Thrissur
Dr. V. V. Sudheendrakumar	Rtd. Scientist , Forest protection division Kerala forest research institute Peechi, 680 653 , Thrissur
Dr. T. V. Sajeev	Scientist E1 Kerala forest research institute Peechi, 680 653 , Thrissur

ABSTRACT

Teak defoliator (Hyblaea puera Cramer) outbreak happens in teak (Tectona grandis) plantations with tender foliage. Present study identified biochemical and volatile profile of teak tender leaves as opposed to mature leaf in different seasons, so as to identify the cues detected by the female moth to identify tender teak leaves. In biochemical analysis, there was no significant difference in oil, ash, total sugar, reducing sugars and non reducing sugars. Six volatiles compounds were present in the tender foliage; while only two were found in the mature teak leaves. Tender teak leaves analyzed during pre-outbreak, outbreak and post outbreak showed the presence of six, six and one volatile respectively. Through this study it is understood that host plant recognition in Teak defoliator is mediated by the cocktail of volatile compounds. Development of the right combination of the volatiles and behavioral testing using it would open new avenues in controlling incipient outbreak.

Introduction

Insects are a major link between the plants and animals at higher trophic level (Bernays and Chapman, 1994). They have been evolving together for one hundred million years ago, with different kinds of interactions that are studied today in every branch of biology, from biochemistry and genetics to behavior and ecology. Majority of the insect species depend on plant parts and consume ten per centage of plant annual production. Insects exactingly choose their host plants on which they deposit eggs (Schoonhoven, 1968; Schoonhoven, 2006). Usually plants are protected from insect herbivore or pest attack but most insects are highly specialized feeders as to overcome the plant defenses.

Host plant searching is essential for phytophagous insects because eclosion of adults from pupae may occur away from the host plants. To locate a host plant, the insect needs to move towards it and contact it to examine the characters (Beck, 1965; Kennedy ,1977). The physical and chemical properties of plants play major role in the selection, rejection and acceptance of plants by phytophagous insects (Hsiao, 1969). The first chemical that detected by insect from a plant is volatiles. All plants release volatiles through open stomata especially in active growth phase and have little control over this (Bernays, and Chapman, 1994; Gibbs, 1974).

The components of the volatile organic compounds (VOCs) that insects use to locate their host plants are often complex and specific (Schoonhoven, 1968; Schoonhoven, 2006). Detailed study on VOCs features will facilitate to understand the underlying mechanism of insect attraction towards specific plants and in turn can use as an insect trap for pest management. The current study was designed to understand the chemical cues originating from teak (*Tectona grandis*) which would attract phytophagous insects associated with it. The relationship between teak leaf VOCs and its major pest *Hyblaea puera* (Lepidoptera: Hyblaeidae) commonly known as teak defoliator was analyzed, compared and studied.

Materials and methods

Teak leaves of different maturity collected through different seasons were studied for its biochemical and volatile compounds. The method used to detect teak leaf volatiles was GCMS (Head Space) analysis. Fresh leaves of tender and mature teak leaves were collected between 9.30 am and 10 am from lower branch of a single teak tree. Maturity of the leaves was decided according to their position in the branch. The 1st and 5th positioned leaves were treated as tender and mature respectively. The leaves were plucked from the petioles by minimum damage and placed in glass bottles with rubber caps, supplied with the GCMS (head space), and were sealed and transported immediately to the laboratory.

The collected leaf materials were subjected to volatile analysis using Head Space GCMS Varian MS #1. The column used was VF5MS. Following was the program configuration selected for the volatile analysis. Injection mode – GC Head space, syringe – 1ml headspace, syringe temperature - 35^{0}C, sample agitator - agitate and heat, agitator temperature - 50^{0}C, incubation time 0.25 min, incubation rpm – 250, agitator on – 10 sec, agitator off – 2sec, plunger fill speed – 100.000ml/sec, Fill srokes – 3, viscosity delay – 1.00sec, injector- front, preinjection delay – 0.500sec, plunger injection speed – 250.000µl/sec, post injection delay – 0.500 sec, syringe flush time – 30 sec, and GC cycle time – 0.30 min.

For the analysis of biochemical contents of teak leaves, mature and tender teak leaves were collected from teak plantation. The collected leaves were immediately transferred in to plastic bags, sealed and brought to the laboratory for biochemical analysis. carbohydrate (Anthrone method) (James, 1999; Sadasivam and Manickam, 1996), nitrogen and protein Micro-Kjeldahl method) (James, 1999; Sadasivam and Manickam, 1996), total sugar (Lane and Eynon method)[4], reducing sugar (Lane and Eynon method) (Eynon, and Lane,1923), non reducing sugar (difference between total sugar and reducing sugar) ,total oil (extraction with petroleum ether) (Lehninger *et al* .,1993;) ,crude fiber (acid alkali treatment) (William *et al*., 2005; Sadasivam and Manickam, 1996), total phenols (Folin Ciocalteau reagent) (William *et al*., 2005; Sadasivam and Manickam, 1996), Tannins (Folin-Denis method) (William *et al*., 2005; Sadasivam and Manickam, 1996), lignin (refluxing with acid detergent solution) (William *et al*., 2005; Sadasivam and Manickam, 1996), Sodium and potassium (Atomic Absorption Spectro photometer), moisture content (Toluene distillation method) (William *et al*., 2005; Sadasivam and Manickam, 1996) and pH (using pH meter) were tested.

Results

Volatile analysis

It was observed that the tender leaf holds more volatiles than the mature one**s.** Figure 1,2,and 3 presents the retention time of volatile compounds in tender and mature teak leaves during three different seasons- pre monsoon, monsoon and post monsoon.

The leaves collected during February and July months had more number of volatiles than in those collected in November. In February the leaves had six compounds - ethanol, 2-methoxy, 1R-α pinene, β- phellandrene, sabinene, 1-4 methanol-1-x cyclox and α- thujene. In July, ethanol, 2-methoxy, 1R- α- pinene, β - phellandrene, sabinene and caryophyllene were found in the leaves. Only one volatile compound (caryophyllene) was present in November. The amount of ethanol, 2-methoxy increased to 4.48 per cent, 1R- α- pinene to 93.14 per cent, and sabinene to 0.637 in July. Caryophyllene and α - caryophyllene was newly recorded in the month of July. 1-4 methanol-1-x cyclox and α- thujene were absent in July. Only one compound, caryophyllene was present in the month of November.

In the month of February the mature leaf contained three volatiles ethanol, 2-methoxy-; β- phellandrene and 1-4 methanol-1-x cyclox. In July, the amount of ethanol, 2-methoxy- showed a very high increase to 85.84 per cent and 1R-α-pinene was newly found.

Biochemical contents of tender and mature teak leaves
The biochemical contents were different in mature and tender teak leaves (Table 1). It can be seen that except for oil, sugars, sodium, potassium, phenol and tannin, there was marked difference in the content of all other components. Carbohydrate, protein, ash, nitrogen, lignin and moisture were high in the tender leaves as compared to that in the mature leaves. Fiber content and pH value were more in mature leaves. But there was no significant difference in the biochemical composition of teak leaves, which were collected during two different periods.

Discussion
In this study we exploited insect host finding cues from teak (*Tectona grandis*). Leaf biochemical parameters and volatile organic compounds (VOCs) in different seasons were analyzed and compared. The seasonal variations observed in the combination of VOCs from teak are imperative for pest incidence study in teak plantation. The outbreak of defoliator (*Hyblaea puera* Cramer) a major pest of teak, is seasonal and the infestation starts after the pre monsoon shower when teak tree are in new flushings (Nair, 2007; Nair and Mohandas, 1996). It is evident that some of the VOCs detected through this study are capable of attracting the defoliator moths towards the teak plantation as they had been reported to be insect attractants by earlier workers. This information supports *H.puera* immigration to teak plantation from a distant location (Chandrasekhar *et al*., 2004; Nair, 1986).

The characteristic odor of a plant is produced by a group of chemically related volatiles, such as terpenoides in conifers, sulfides from onion and garlic. The first chemicals that insects detect from the host plant are volatiles (Bernays and Chapman, 1994; Metcalf and Metcalf, 1992; Metcalf, 1987). Through this study six volatiles were identified from the tender teak leaf in preoutbreak period (February). Among them, four (1R- α -pinene, β – phellandrene, sabinene, and α - thujene) are insect attractants and/or oviposition stimulants[6] and low molecular weight (136g/mol) compounds with same molecular formula ($C_{10}H_{16}$) but which are structurally different (http://webbook.nist.gov, 2011). These low molecular weight compounds which can diffuse to long distances would have played a major role in luring defoliator moths towards the teak plantations.

High molecular weight (204.3 g/mol) compounds caryophyllene and α – caryophyllene with a molecular formula $C_{15}H_{24}$ were newly noticed to the VOCs profile of next season -the teak defoliator outbreak period (June/July) . These two compounds in general would contribute to the formation of characteristic plant odors, oviposition stimulants, allomones and kairomones in outbreak period (Bernays and Chapman,1994; Grant et al.,2007). These slightly volatile compounds make aerial bouquet of teak and could influence on the further inhabitance of defoliator pests on tree.

Present study is well-informed that the biochemical contents in teak leaves do not show significant difference in their concentration in accord with seasons, and hence they do not propose the initial base for host selection and defoliator outbreak. Both olfactory and visual elements are important for the attraction of insect towards host plant from a distance. Among, olfactory signals are making insect to take off and move towards the odor from host plant (Bernays and Chapman, 1994). The combination of volatiles from trees is important in insect behavior and forage (Rodriguez *et al*., 2009).Teak produces a mixture of volatiles, those are significantly different according to seasons and leaf maturity. During teak defoliator pre-outbreak, outbreak and post- outbreak periods, teak leaf produces different volatiles in different combinations and concentrations (fig 4).

A large number of insect pests utilize plant volatiles in host location (Metcalf and Metcalf, 1992; Metcalf, 1987). The identified teak leaf volatiles and their relation on teak defoliator behavior could eventually provide tools for managing this pest in future. Present study identified six teak leaf volatiles, amongst four of them are generally act as cue in insect outbreak causation as kairomones and oviposition / feeding stimulants. Further studies on this would open up new avenues in mass trapping of immigrant pest moths towards teak plantation so as to control incipient outbreaks.

Acknowledgement
This work was supported by research grant from Kerala Forest Research Institute (KFRI), Thrissur, Kerala. Cashew Export and Promotion Council (CEPC), Kollam, Kerala provided technical support for this study.

Figures and Pictures

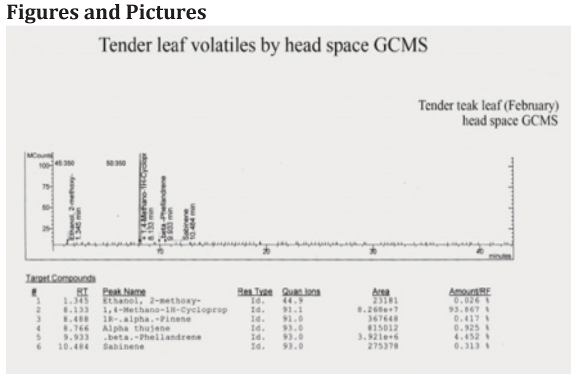

Figure 1: a. Volatiles from tender teak leaf in preout-break period of defoliator

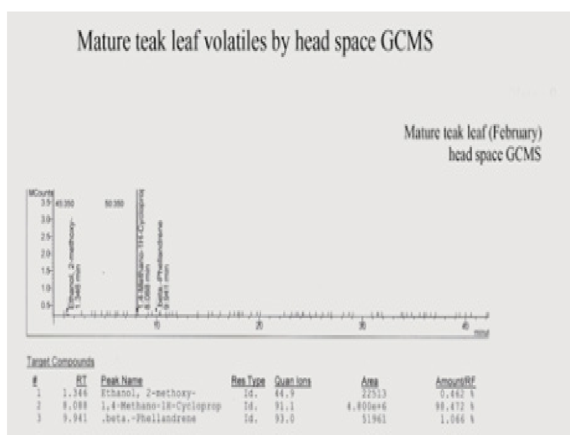

Figure 1: b. Volatiles from mature teak leaf in preoutbreakperiod of defoliator

Figure 1: retention time of teak leaf volatiles in pre-outbreak period of teak defoliator

Volume : 3 | Issue : 2 | February 2014 • ISSN No 2277 - 8179

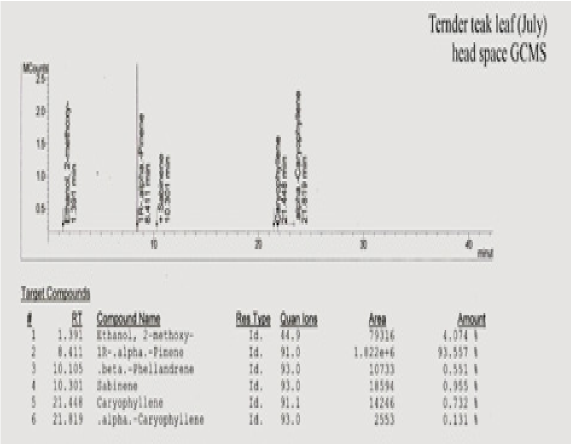

Figure 2: a. Volatiles from tender teak leaf in outbreak period of defoliator

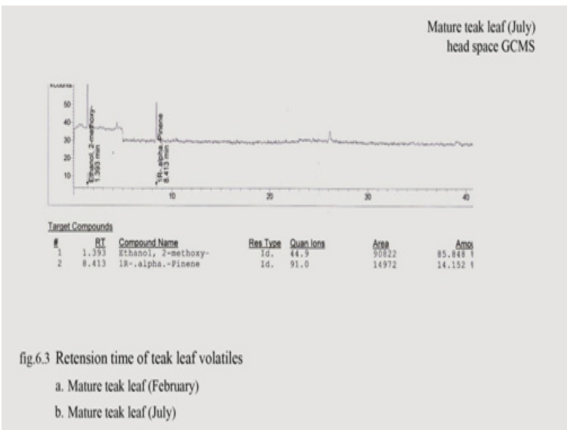

Figure 2: b.Volatiles from mature teak leaf in outbreak period of defoliator

Figure 2: retention time of teak leaf volatiles in outbreak period of teak defoliator

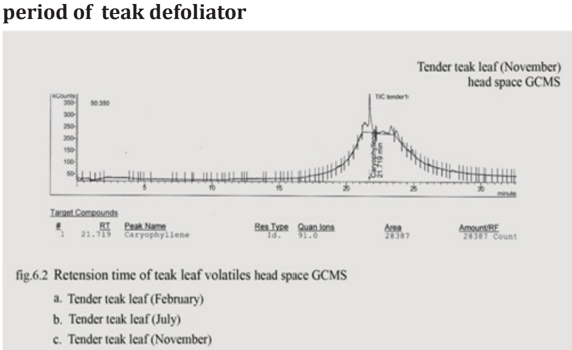

Figure 3: retention time of teak leaf volatiles in post outbreak period of teak defoliator

Figure 4: Teak volatile combinations and defoliator behavior in different seasons

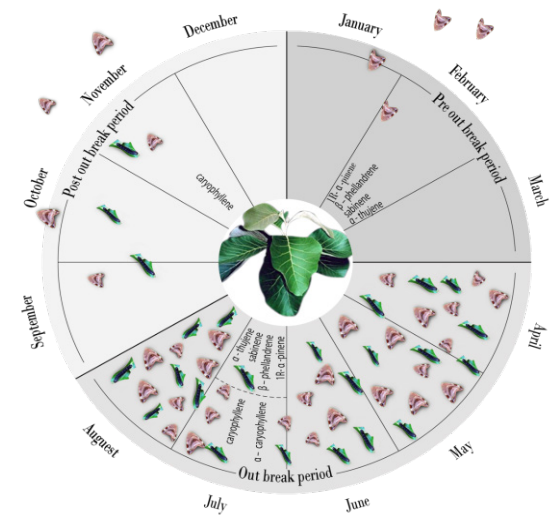

Table: 1. Biochemical contents of tender and mature teak leaves in different seasons

Sl. No	Compound	Outbreak period		Post out break	
		Tender	Mature	tender	Mature
1	Carbohydrate (per cent /wt)	8.9	2.9	9	2.3
2	Protein (per cent /wt)	1.7	0.9	1.67	0.91
3	Oil (per cent / wt)	0.5	0.47	0.43	0.51
4	Ash (per cent /wt)	1	0.8	0.83	0.84
5	Crude fiber (per cent /wt)	9	17.8	8.8	20.6
6	Tot sugar (per cent /wt)	0.8	0.7	0.67	0.62
7	Reducing sugar (per cent /wt)	0.1	0.1	0.1	0.11
8	Non reducing sugar (per cent /wt)	0.7	0.6	0.57	0.51
9	Sodium (mg/kg)	249	211	221	207
10	Potassium (mg/ kg)	810	794	791	758
11	Nitrogen (per cent /wt)	0.27	0.14	0.27	0.13
12	Phenol (per cent /wt)	2.92	2.44	2.44	2.56
13	Tannin (per cent /wt)	1.55	1.1	0.9	1.42
14	Lignin(per cent /wt)	3.26	2.96	3.1	1.94
15	Moisture (per cent /wt)	82	52	78.6	49
16	pH	5.1	4.3	5.1	4.4

REFERENCE

1. Beck, S.D. 1965. Resistance of plants to insects. A. Rev. Ent. 10: 207-232. | 2. Bernays, E. A. and Chapman, R. F. 1994. Host - Plant Selection by Phytophagous Insects. Chapman and Hall, New York, 305 pp. | 3. Chandrasekhar N., Sudheendrakumar, V. V., Moinak Banerjee (2004). A study on the population dynamics of the Teak defoliator in Nilambur Teak plantation: A novel approach using RAGEP. Proceedings of sixteenth Kerala Science Congress, 412-417pp | 4. Eynon, L. and Lane, J. H. 1923. Determination of reducing sugars by means of Fehlings solution with methylene blue as internal indicator. J. Soc. Chem. Ind. Trans., 42: 32 - 36. | | 5. Gibbs, R.D. 1974. The Chemotaxonomy of Flowering Plants. McGill - Queen's University Press, Montreal. | 6. Grant, G., Jian, G., Linda, D. and Melanie, D. C. 2007. Oviposition response of spruce budworm (Lepidoptera: Tortricidae) to host terpenes and green-leaf volatiles. The Canadian Entomologist, 139(4): 564 - 575. | 7. Hsiao, T.H. 1969.Chemical basis of host selection and plant resistance in oligophagous insects. Ent. Exp. and appl. 12: 777-788. | 8. James, C. S. 1999. Analytical chemistry of foods. A S P E N publication, New York, 175 pp. | 9. Kennedy J.S. 1977. Olfactory responses to distant plants and other odour sources. In: Chemical control of insect behaviour: Theory and application, eds: H. H. SHOREY and J. J. MCKELVEY Jr. New York, John Wiley and Sons. | 10. Lehninger, A. L., Nelson, D. L. and Cox, M. M. 1993. Principles of biochemistry. Worth, New York, 1013 pp. | 11. Metcalf, R. and. Metcalf, L. 1992. Plant Kairomones in Insect Ecology and Control. Chapman & Hall, New York, 168 pp. | 12. Metcalf, R. L. 1987. Plant volatiles as insect attractants. CRC Rev.Plant Science 5: 251-301. | 13. Nair, K. S. S. 1986. The teak defoliator, H. puera: Defoliation dynamics and evidences for short range migration of moths. Proc. Indian Acad. Sci. (Anim. Sci): 7-21. | 14. Nair, K. S. S. 2007. Tropical forest insect pest: ecology, impact and management. Cambridge University Press, Cambridge, England, 422 pp. | 15. Nair, K.S.S. and Mohandas (1996). Early events in the out break of the teak caterpillar, Hyblaea puera. International Journal of Ecology and Environmental Sciences, 22, 271-279 pp. | 16. National Institute of Standards and Technology. 2011. http://webbook.nist.gov | 17. Rodriguez - Saona C., Zsofia, S. 2009. A meta-analysis of insect pest behavioral manipulation with plant volatiles. Entomologia Experimentalis et Applicata, 134(3): 201 - 210. | 18. Sadasivam, S. and Manickam, A. 1996. Biochemical methods. New Age International, New Delhi, India, 251 pp. | 19. Schoonhoven, L. M. 1968. Chemosensory bases of host selection. A. Rev. Ent. 13: 115-136. | 20. Schoonhoven, L. M. 2006. Some aspects of host selection and feeding, 557 – 566: In Insect and mite nutrition. J. G. Rodriguez, North Holland, Amsterdam. | 21. William H. and George W. L. 2005. Official methods of analysis of AOAC International (18th edn.). AOAC International suite 500. North Frederick Avenue, 246 pp. |