# A SOLID SAMPLE INJECTION DEVICE FOR THE ANALYSIS OF *SHOREA ROBUSTA* (DIPTEROCARPACEAE) BAST VOLATILES

**A. Jain**

*Tropical Forest Research Institute, PO RFRC, Mandla Road, Jabalpur 482021, MP, India. E-mail: jainavi@yahoo.com*

*Shorea robusta,* commonly known as sal, is an important resiniferous tree of India belonging to the family Dipterocarpaceae. It ranks with deodar (*Cedrus deodara*) and teak (*Tectona grandis*) as one of the best sleeper woods. Sal forests occupy 16.7% of the total forest area of the country (Chadha 1972). Although sal forests in the country have been managed well, these forests have often been damaged by the heartwood borer, *Hoplocerambyx spinicornis* (Coleoptera: Cerambycidae). Madhya Pradesh is the state with the second largest area under sal forests. The recent sal borer epidemic of 1997 has devastated a large area of forests. Although the trap tree method and other silvicultural practices have been used to manage this insect in the past, these measures need to be substituted with economic and ecological methods. Integrated Pest Management system should be applied to manage this insect pest. The trap tree method utilises volatile chemicals which are released upon beating the bast (portion between the bark and sapwood) of the felled logs. Insects get attracted to these volatiles from a quarter of a mile away and are trapped and killed. Realising the potential of semiochemicals in monitoring and controlling sal borers, it is worthwhile to identify the volatiles released from the bast of *S. robusta.* The identified attractants will form a basis for the development of suitable technology using kairomones to protect the sal forests.

Many semiochemicals derived from plants or insects are often multicomponent mixtures, present in nanogram or picogram amounts. Therefore, their analysis and structure elucidation require a separation technique capable of high resolution and sensitivity (Jones & Oldham 1999). Gas Chromatography-Mass Spectroscopy (GC-MS) provides a powerful technique for the analysis of such complex mixtures. However, because of their relatively low cost compact design quadrupole (Ferguson *et al.* 1965) and ion trap (Todd 1991), mass spectrometers have been most widely used in semiochemical analysis.

This study involved the solventless injection, i.e. Solid Sampling Injection (SSI) of the bast. This technique has been used to extract and identify semiochemicals from insect parts, glands, secretions and nest materials (Keegans *et al.* 1993, Dani *et al.* 1995, 1998). In this technique, the sample was sealed inside a soft glass capillary, which was then crushed inside the hot injector port (operating in a splitless mode) of a GC system in order to release the volatiles immediately into the column. The sample was sealed by carefully melting the ends of the capillary tube forming a shorter tube of about 2 cm length, taking care not to scorch the sample and to seal the tube with a minimum amount of air. Sealed air can cause the pyrolysis of sugars forming furans, pyrans and pyrrols and cause oxidation of the volatiles.

The solid sample injector (called Morgan injector) consists of a crushing plunger and a perforated sample chamber containing the encapsulated sample. This is secured inside

*Received May 2001*

the injector by a large-holed septum-retaining nut. The technique enables the analysis of nanogram quantities from a single biological source without inherent problems like contaminants in solvents such as plasticisers, analyte losses during evaporative concentration and masking the solvent peaks in GC chromatograms of very volatile compounds. This technique also alleviates the problem of non-reproducible retention times by allowing time for volatiles to equilibrate from the sample into the column space and gives sharp peak shapes from the narrow band of extract on the column.

Three logs of *S. robusta* were collected from a wild population of sal forests. The bast was removed from the logs, cut into small pieces and analysed by GC-MS using SSI. The GC-MS was equipped with a fused silica capillary column (SPB-5, 30 m × 0.32 mm, 0.25 μM phase thickness). One mg of the sample was sealed in a soda glass capillary tube and placed in the Morgan injector which was fitted into the GC injector at ambient temperatures. After heating to 300 °C, the capillary was broken by pressing the plunger. Chromatographic conditions were as follows: helium carrier gas at a flow rate of 1–2 ml $min^{-1}$ and a head pressure of 10 psi, oven programmed at 30 °C for the first 5 min, 8 °C $min^{-1}$ to 300 °C and then held for 10 min. The column was coupled directly to the quadrupole mass spectrometer via an interface at 280 °C. The instrument was operated in EI (+) mode at 70 eV. Mass spectra (m/z 33-650) were accumulated at the rate of one/second with an acquisition time of 0.9 s and an interscan time of 0.1 s. The initial solvent delay was 3 min. Data were collected and analysed using LAB BASE software working under Quarterdeck extended memory management on a computer. Spectra were matched with those from the Nightingale Bamford School library using the eight most abundant peaks and ranked according to reverse fit.

The identification of the constituents was made on the basis of kovats standard co-injection under similar experimental conditions and Kovats Retention Indices (KRI) were determined. A solution for Kovats standard was obtained by plotting a graph between Retention Time (RT) v/s Kovats and vice versa and the following two equations were generated for determination of Kovats for bast constituents.

$$y = 1.0998x^2 + 6.316\,x + 666.21 \quad (R^2=1) \qquad (1)$$
$$y = -3E\text{-}06\,x^2 + 0.0265x - 7.72 \quad (R^2=0.9996) \qquad (2)$$

By substituting the values of x (RT) in equation 1, y (Kovats) were determined and matched with those reported in the literature. Identification was further confirmed by comparing the mass spectra of the constituents with corresponding compounds reported in the literature.

A total of 12 peaks were found in the spectrum and identified. Table 1 shows the compounds detected in quantifiable amounts from the bast of *S. robusta* by SSI device. Phenols were found to be the major volatile components contributing about 53.0% of the total compounds. Among the different phenols, 2,6-dimethoxyphenol was most abundant (29.8%), followed by 2-methoxyphenol (11.8%). Phenols have been found to act as semiochemicals in some species (Sonenshine 1985).

To avoid some inherent problems like contaminants in solvents such as plasticisers and analyte losses, we looked for a direct loading technique (Senanayake & Wijesekera 1968) which should be applicable to most gas chromatographs without requiring any modification. Thus, we worked with solid sample injectors. Solid sample injection technique has been used by several researchers with regard to kairomone and pheromone research (Attygalle & Morgan 1988).

**Table 1** Volatile components detected in the bast of *Shorea robusta* by solid sample injection device

| Peak No. | Compound | RT (min) | KRI | Area (%) | Formula | Mol. wt. |
|---|---|---|---|---|---|---|
| 1 | 2,5-dimethylfuran | 5.02 | 726 | 4.87 | $C_6H_8O$ | 96 |
| 2 | 2-methyl-2-cyclopenten-1-one | 11.68 | 890 | 2.41 | $C_6H_8O$ | 96 |
| 3 | 2-(2'-furanyl) ethanone | 11.82 | 895 | 1.59 | $C_6H_6O_2$ | 110 |
| 4 | 2-methyl-1,2,3,4-tetrahydrofuran | 12.02 | 901 | 0.49 | $C_5H_{10}O$ | 86 |
| 5 | Phenol | 13.77 | 962 | 10.10 | $C_6H_6O$ | 94 |
| 6 | 2-methoxyphenol | 16.27 | 1060 | 11.76 | $C_7H_8O_2$ | 124 |
| 7 | 2-methoxy-4-methylphenol | 18.45 | 1157 | 1.39 | $C_8H_{10}O_2$ | 138 |
| 8 | 2,6-dimethoxyphenol | 21.48 | 1309 | 29.75 | $C_8H_{10}O_3$ | 154 |
| 9 | 1,2,3-trimethoxybenzene | 23.15 | 1402 | 1.82 | $C_9H_{12}O_3$ | 168 |
| 10 | 2,6-dihydroxy-4-methoxyphenyl-1-ethanone | 24.48 | 1480 | 2.79 | $C_9H_{10}O_4$ | 182 |
| 11 | 2-(1,2,3-trimethoxyphenyl)-ethane | 25.83 | 1563 | 2.89 | $C_{11}H_{16}O_3$ | 196 |
| 12 | 1,1-dimethyl-2,4-bis(1-methylethenyl)-cyclohexane | 41.2 | 2793 | 10.19 | $C_{14}H_{24}$ | 192 |

RT = Retention time
KRI = Kovats Retention Index

## References


ATTYGALLE, A. B. & MORGAN, E. D. 1988. Identification of pheromones in nanogram quantities. *Angewandte Chemie International Edition in English* 27: 460–478.

CHADHA, Y. R. (Ed.). 1972. Pp. 313–322 in *The Wealth of India: Raw Materials.* CSIR Publications, New Delhi.

DANI, F. R., MORGAN, E. D. & TURILLAZZI, S. 1995. Chemical analysis of sternal gland secretion of paper wasp, *Polistes dominulus* (Christ) and its social parasite *Polistes sulcifer* (Zimmermann) (Hymenoptera: Vespidae). *Journal of Chemical Ecology* 21(11): 1709–1718.

DANI, F. R., MORGAN, E. D., JONES, G. R., TURILLAZZI, S., CERVO, R. & FRANKIE, W. 1998. Species specific volatile substances in the venom sac of hover wasps (Hymenoptera: Vespidae: Stenogastrinae). *Journal of Chemical Ecology* 24: 1091–1104.

FERGUSON, R., MC KULLOH, K. E. & ROSENSTOCK, H. M. 1965. Mass spectroscopy. *Journal of Chemical Physics* 42: 100.

JONES, R. G. & OLDHAM, J. N. 1999. Pheromone analysis using capillary gas chromatographic techniques. *Journal of Chromatography* 843: 199–236.

KEEGANS, S. J., MORGAN, E. D., TURILLAZZI, S., JACKSON, B. D. & BILLEN, J. 1993. The Dufor gland and the secretion placed on eggs of two species of social wasps, *Liostenogaster flavolineata* and *Parischnogaster jacobsoni* (Vespidae: Stenogastrinae). *Journal of Chemical Ecology* 19: 279–290.

SENANAYAKE, U. M. & WIJESEKERA, R. O. 1968. A rapid micro-method for the separation and estimation of the purine bases: caffeine, theobromine and theophylline. *Journal of Chromatography* 32(1): 75–86.

SONENSHINE, D. E. 1985. Pheromones and other semiochemicals of the acari. *Annual Reviews of Entomology* 30: 1–28.

TODD, J. F. J. 1991. Ion trap mass-spectrometer: past, present and future. *Mass Spectrometry Reviews* 10(1): 3–52.