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Chapter 10

Chemoecological Methods in Coconut Pest Management

☆ *Kesavan Subaharan*

1. Introduction

The need to produce inexpensive and abundant food supply for a growing population is a great challenge and warrants higher use of inputs like fertilizers and pesticides. Farmers resort to chemical control as their availability and application is done with ease. Though the use of pesticides keeps the pest at check, the experience gained during the recent past indicates that improper use of pesticide has caused undesirable side effects like buildup of residue in the marketable commodity, emergence of insecticide resistance in pests, negative impact on beneficial arthropods *etc.*

Increased dependence on pesticides have negative impact on farmers, consumers, non-target organism and environment. This situation prompts the search for safe alternative for pest control. Crop production and environmental protection can be balanced if emphasis is laid to adopt eco-friendly pest management strategies. In pest management alternative to chemical pesticides is the use of insect behavior modifying chemicals (IBMC). Semiochemicals are defined as chemicals that deliver behavioral messages between organisms (Dusenbury, 1992), they include pheromone (that aid in finding mates, food and habitat resources), allomone (favor the producer) and kairomone (favor the receiver).

In plantation crops the damage by insect pests have profound influence on crop production. Use of chemical pesticides leads to buildup of residues in the commodities that have high commercial value. Major pests that influence the crop yield in coconut are, rhinoceros beetle, *Oryctes rhinoceros*, red weevil, *Rhynchophorus ferrugineus*, black headed caterpillar, *Opisina arenosella*, eriophyid mite, *Aceria* *guerreronis* and white grub, *Leucopholis coneophora.* Safe alternate strategies in coconut pest management include use of biological agents and chemoecological approaches. Among the orders that cause damage to crops, coleopterans form a major share. Considering their long life cycle and their cryptic behavior using the conventional approaches becomes difficult to manage them. Though biocontrol agents are effective, their timely availability in quality and quantity is a question. This necessitates the need to depend on pheromones and kairamonesas they are an effective tool in monitoring and mass trapping.

Understanding the chemoecological approaches using cutting edge technologies involving chemical detectors (GCMS) and electrophysiological tools resulted in developing robust behavioural manipulations in coconut pest management. The compounds identified to be used in behavioral pest management will aid to decline the dependence on xenobiotics.

2. Methods Involved in Isolation and Identification of Semiochemicals

2.1. Sampling and Analysis of Semiochemicals

The increasing scientific interest in the biochemistry, physiology, ecology and atmospheric chemistry of VOCs has led to the development of a variety of systems for the collection and analysis of volatiles (Millar and Haynes, 1998). Volatile analysis has been improved by the design of relatively sensitive bench-top instruments for gas chromatography – mass spectrometry (GC-MS) (Dorothea *et al.*, 2006). Volatiles surrounding the airspace (headspace) around the insects are sampled and concentrated prior to analysis. Headspace sampling is a non-destructive method for collecting volatiles. When compared with solvent extractions of pheromone glands from insects, headspace analysis gives a more realistic picture of the volatile profile emitted by insects. It provides real time information for ecologically relevant applications. Materials like glass, metal and special plastics such as teflon that are inert are to be used for volatile trapping (Dorothea *et al.*, 2006).

An important advance in static headspace analysis is the development of solid phase microextraction (SPME) which is a fast and simple method for collecting volatiles at detection limits in the ppbv (parts per billion by volume) range. Solid phase microextraction is based on ad/absorption and desorption of volatiles from an inert fiber coated with different types of ad/absorbents. The fiber is attached within the needle of a modified syringe and volatiles can be sampled by inserting the needle through a septum of a headspace collection container and pushing the plunger to expose the fiber. Following equilibration between the fiber and the volatile sample, the fiber is retracted into the needle and can be transferred to a gas chromatograph for direct thermal desorption. Solid phase microextraction fibers can be re-used approximately 100 times. Thermal desorption of VOCs from the fiber eliminates the need for solvents that may contain impurities which will interfere with sample analysis (Dorothea *et al.*, 2006).

2.2. Gas Chromatographic Separation and Detection of Plant VOCs

Insect semiochemical adsorbing matrices are analyzed by the standard technique of GC (Handley and Adlard, 2005; Lockwood, 2001; Merfort, 2002). For GC analysis of semiochemicals, samples are either injected as solvent extracts into the heated injector in a split or splitless mode or desorbed from the adsorbent by placing it directly in a thermal desorption tube, heated to 250–300°C. In a two stage thermal desorber, the thermally released volatiles are concentrated by a cold trap (or cryotrap) prior to their injection into the GC column (Dorothea *et al.*, 2006).

For analytical purposes, volatiles are commonly separated on fused silica capillary columns with different stationary phases, such as the non-polar dimethyl polysiloxanes (*e.g.* DB-1, DB-5, CPSil 5), and the more polar polyethylene glycol polymers, including Carbowax-20M, DB-Wax, and HP-20M.

Following separation on a GC column, semiochemicals are analyzed by a variety of detectors. Flame ionization detector (FID) is commonly used for quantitative analysis because of their wide linear dynamic range, their very stable response and their high sensitivity with detection limits of the order of picograms to nanograms per compound. Mass spectrometry (MS) detectors are the most popular type of detector for routine semiochemical GC analysis. In the mass spectrometers of most standard GC-MS benchtop instruments, compounds exiting the GC column are ionized by electron impact (EI) and the resulting positively charged molecules and molecule fragments are selected according to their mass-to-charge (m/z) ratio by entering a quadrupole ion trap or a quadrupole mass filter. Total ion chromatograms are obtained, which provide information on the retention time of each compound and its mass spectrum consisting of a characteristic ion fragmentation pattern. Detection limits of highly sensitive mass spectrometers are in the picogram range for the full scan mode (scanning ions over a wide molecular range) and may be as low as in the femtogram range (in quadrupole mass filters) in the selected ion monitoring (SIM) mode scanning selected ions that are representative of a compound. Identification of compounds in GC-MS analysis is done by using the popular mass spectral libraries such as Wiley and NIST MS databases (Dorothea *et al.*, 2006).The orientation response of an insect to semiochemicals is evaluated by olfactometry and electrophysiological assays.

2.3. Electroantennography to Assess the Physiological Response to Volatile Organic Compounds

Electrophysiological techniques have been employed to study the nervous system of insects since the late 1950s. In 1957, Schneider for the first time announced that it was possible to measure the electrophysiological responses from the antennae of an insect, *Bombyx mori*, using an electroantennogram (EAG). The EAG technique has since been developed for several insect species of various insect orders (Millar and Haynes, 1998). Despite its usefulness, the method is limited in its use. For instance, the sensitivity of the technique is low, a shortcoming often observed in insects whose antennae have *e.g.* a limited number of sensilla (Millar and Haynes, 1998). To overcome this problem, a technique called single sensillum recordings

(SSRs) was developed, which can measure responses of single ORNs (Millar and Haynes, 1998). Single sensillum recordings allow for the analyses of specific types of sensilla and for the determination of the mechanisms by which insects code for different odors (Majid, 2007).

In SSRs, two sharpened tungsten electrodes are used: a ground electrode is in contact with the haemolymph while the recording electrode is inserted near the base or in the shaft of a single sensillum. Voltage differences generated between the electrodes, when amplified, can be viewed on an oscilloscope when placed within the circuit (de Bruyne*et al.*, 2001).

The introduction of gas chromatography (GC) coupled SSRs, first used in moths and aphids allowed for the first detection of active components within a complex blend of compounds. This technique has later been used to identify novel ligands of ORNs in a large number of insect species. At the GC part an extract likely to contain odorants detected by the ORNs is injected onto the GC-column. The column is located in an oven where it is possible to regulate the column temperature. As the temperature of the column is increased the components of the extract are separated while traveling down the column and exit the GC set-up. The separated components of the extracts encounter the single sensillum from which a stable electrical contact is established. Responses of the ORNs housed in a single sensillum to the extract components are recorded. The chemical identity of the response eliciting component(s) can be further identified using mass spectrometry (MS).The olfactory receptors respond to compounds that are attractive and repulsive. Hence the behavioural experiments have to be carried out to ascertain the nature of compounds using olafctometers and wind tunnels.

3. Chemoecological Approach in Coconut Pest Management

3.1. Coconut Red Palm Weevil, *Rhynchophorus ferrugineus* (Oliver)

The red palm weevil, *Rhynchophorus ferrugineus* (Oliver) is distributed in Asia and Europe. The host range of the weevil includes coconut, oil palm, date palm and sago (Wattanapongsiri, 1966). The adult female oviposit on young, damaged, stressed and healthy palms (Kalshoven, 1950). On hatching the larvae bore into the palm and develop into adult in two months (Giblin Davis *et al.*, 1996). Trapping by baiting with coconut stem tissue was done to reduce the weevil populations in India (Abraham and Kurian, 1975). Evidence of male produced aggregation pheromone was established in laboratory bio assay by Abraham (1987). The aggregation pheromone 4 methyl 5 nonanol (Ferrugineol) was synthesized by Hallet *et al.* (1993). Coconut logs treated with toddy, yeast and acetic acid were effective in trapping *R. ferrugineus* (Kurian *et al.*, 1984).

R. ferrugineus weevils are opportunistic oligophages to early fermentation volatiles like ethanol emanating from wounded host (Gunatilake and Gunawardane,1986), on feeding the palm tissue they produce an aggregation pheromone (4 methyl 5 nonanol) that attract their conspecifics (Giblin-Davis *et al.*, 1996). Food volatiles strongly enhance the attraction of *Rhynchophorus* species to aggregation pheromone (Rochat *et al.*, 1993). The palm esters, ethyl acetate, ethyl propionate, ethyl butyrate and ethyl isobutyrate were identified as kairomones for *R. phoenicis*, *R. palmarum*, *R.cruentatus*, *R.ferrugineus* and *R. vulneratus* (Gries *et al.*, 1994). The American palm weevil, *R. palmarum* adults were attracted to odors of variety of plant tissues (pineapple, banana and coconut) that were used as baits in traps (Jaffe *et al.*, 1993; Oehlschlager *et al.*, 1993). Among the complex pattern of odorants emitted by host plants only a few key compounds were used to locate the host. The identification of natural volatiles emitted by host plants aided in development of synthetic blends (rhyncophorol + host volatiles) to trap *R. palmarum* (Rochat and Avand-Faghih, 2000). Synergy between pheromone and plant volatiles (PVs) was confirmed by analyzing the locomotory responses of *R. palmarum* in a four choice olfactometer (Said *et al.*, 2003).

Racemic nonanoic lactone and 4 hydroxy 3 methoxy styrene from the steam volatiles of coconut bark caused electrophysiological response of *R. ferrugineus* antennae (Gunawardane *et al.*, 1998). Ethyl acetate, ethyl propionate, ethyl butyrate and ethyl isobutyrate synergized attraction of *R. cruentatus* to cruentol. None of the palm esters tested in combination with the pheromone were as attractive as palm or sugarcane tissue (Giblin-Davis *et al.*, 1994). Coconut petioles in traps attracted maximum weevils when they were 2-5 days old, the catch declined thereafter as volatile profile changed due to fermentation (Hallet *et al.*, 1993). The proportional changes in volatile from fermenting palm are attributed to abiotic conditions and microflora present (Nagnan *et al.*, 1992; Samarajeeva *et al.*, 1981). Fermented plant tissues produce a spectrum of odorants that are significantly different from those released by healthy plants (Giblin-Davis *et al.*, 1994; Rochat and Avand-Faghih, 2000). Fermented sap exuding from dead or wounded palms was highly attractive to *R. cruentatus* (Giblin-Davis *et al.*, 1996). Moist fermenting tissue from various palm species, fruits, sugarcane, pineapple and molasses are similarly attractive to palm weevils (Giblin-Davis *et al.*, 1994). The efficiency of the kairomones in attracting insects depends on the odour quality and/or the amount released. Timing of volatile trapping to identify the compounds and their ratios in their matrix is essential to formulate an effective pherosynergestic blend.

Acetoin a volatile product of anaerobic fermentation with ethyl acetate was an effective synergist with aggregation pheromone of *R. palmarum*. Stimulation by plant volatile blends excited three olfactory receptor neurons. Pheromone baited traps containing plant volatile blends attracted twice as many *R. palmarum*as control (Said *et al.*, 2005). Odors from plant tissue along with the aggregation pheromone that attract and orient the weevil from longer distances needs to be explored. No such studies are available for red palm weevil. Indigenously formulated CPCRI lure containing ferrugineol resulted in a weevil catch of 2– 6 weevils/month and it was 50 per cent efficient as compared to imported lures but was cost effective as compared to imported lures.

The electroantennogram assay on the adult *R. ferrugineus* showed higher antennal response to ethyl acetate (a major component in pineapple odor). A comparative study of the odorant binding protein genes and isolation of the partial OBP gene form *R. ferrugineus* was attempted. Amplification of putative OBP genes of *R. ferrugineus* yielded two genomic fragments. On sequencing they had homology with OBP of other insects (Rajesh *et al.*, 2008; Sree Smitha *et al.*, 2008). Commercial lures are loaded in polymembrane dispensers and they have a release rate ranging from 2-20 mg/day. In an attempt to reduce the loss of the chemistry smart delivery devices were developed at CPCRI. The smart delivery aids in controlled release of pheromone for over six months as compared to commercial lures loaded in polymer membrane that are exhausted in 3 months.

3.2. Coconut Rhinoceros Beetle, *Oryctes rhinoceros*

Thirty-nine species of *Oryctes* have been registered but only few of them have an impact on coconut production. They are distributed throughout South East Asia and South Pacific Islands. Apart from coconut, they also infest Palmyrah, date palm, wild date, areca, sago palm, pandanus, pineapple, colocasia, banana, oil palm and sugar cane. The pest occurs round the year with a spike in their population occurring during June to September, the period when the adults visit the crowns. The black colored beetle bores holes and feeds on the unopened fronds and spathe. On opening, the damaged leaves show geometric cuts (V shaped) on leaf lets. If the damage is severe, several cuts can be seen one above the other. The beetles cause damage to seedlings, young and adult palms. Damage when done to the leaves, reduces the photosynthetic area and renders them unsuitable for thatching purpose, but when damage is done to spathe it causes direct crop loss upto 10 per cent (Nair, 1986). Ramachandran *et al.* (1963) reported an yield loss of 5.5 – 9.1 per cent. The beetles also cause death of the seedling/young palms by destroying the growing points. Apart from feeding damage they serve as predisposers for red weevil attack and bud rot. Repeated damage done to the meristem may be lethal.

Male coconut rhinoceros beetles, *Oryctes rhinoceros* (L.), produce sex-specific compounds, ethyl 4-methylheptanoate, and 4-methyloctanoic acid, the first of which is an aggregation pheromone (Hallet *et al.*, 1995). Ethyl 4-methyloctanoate was synthesized by ChemTica and marketed as Oryctalure in the India. Buckets of 18 lit. capacity with black painted metal vanes were used as trap. The pheromone sachet was hung on the diamond shaped hole made in the vane. The trap was hung on a pole at 3-4 cm above the ground level. Coconut petioles were placed in the bucket trap for better attraction. A nonomatrix has been developed at CPCRI for the delivery of rhinoceros beetle pheromone

3.3. Chemoreception in White Grubs

Holotrichia is a genus of the melolonthine scarabs that occurs across the Indian subcontinent and through southeast and east Asia (Ward *et al.*, 2002). The chafer beetle, *Holotrichia serrata* F. (Coleoptera :Scarabaeidae) in its larval stage is a serious pest of coconut, (*Cocos nucifera*L.), sugarcane, (*Saccharum officinarum* L.), groundnut, (*Arachis hypogaea* L.) and vegetables in parts of Western and peninsular India (Ganeshaiah and Kumar,1993).The grubs cause damage by feeding on the roots and the adults emerge with the arrival of monsoon or heavy pre - monsoon showers (Yadav and Sharma, 1995). On emergence at dusk, they aggregate on plants like neem, (*Azadirachta indica* A. Juss), gulmohar, (*Delonix regia* L.), tamarind, (*Tamarindus indica* L.), mahagony (*Swietenia mahagony* L.), drumstick, (*Moringa oleifera* Lam.)

and subabul, (*Leucaena leucocephala* Lam.) for feeding and mating (A.R.V. Kumar Personal communication and Yadav and Sharma, 1995). Attraction of adult males by females has been documented in *H. serrata* (Ganeshaiah and Kumar, 1993). The pheromone of *H. consanguinea* and *H. reynaudi*, has been isolated and identified as anisole (Leal *et al.*, 1996; Ward *et al.*, 2002). Chemical insecticides are widely used by farmers to manage the grubs of *Holotrichia* (Anitha *et al.*, 2006) and they result in varying degree of success in grub management. Indiscriminate use of insecticides results in buildup of residues and cause negative impact on non target organisms. Hence, it is imperative to search for alternative pest control methods. One such option is to exploit the behavioral features of the insect. Ethological control has been successfully applied in the management of scarabs (Leal *et al.*, 1996; Ruther *et al.*, 2000).Though *H. serrata* is an important pest, there are no reports on its olfactory response till date. As a primary step to identify physiologically active compounds causing antennal responses, the electroantennography was used.

The non-aromatic esters, ethyl acetate and propyl acetate elicited a higher response in both male (-1.40 and -1.35 mV respectively) and female (-0.81 and -1.31 mV respectively) beetles while the hydrocarbon alkanes *viz.*, tridecane and nonane elicited the lowest responses. Non-aromatic esters – saturated open chain compounds were found to elicit significant EAG response from both males and females of *H. serrata*. The amplitude of the responses varied with the carbon chain length of the compound. Ethyl acetate a C4 elicited higher response in males. Female antenna was more responsive to propyl acetate. In either case the response decreased with increase in carbon chain length. In general the antennae of both male and female beetles were more responsive to esters followed by alcohol and hydrocarbon alkane.

Among the sex, the response of males to all the compounds tested was higher as compared to the females. Extract of pheromone glands in diethyl ether (-4.43 mV) when exposed to male antennae elicited a significantly higher response as compared to the extracts made in dichloromethane or hexane which were at par (-3.40 and -3.31 mV respectively). Among the solvents used to obtain extracts from the pheromone gland, diethyl ether, dichloromethane and hexane caused maximum EAG amplitude in the male antennae of *H. serrata*. Extraction of abdominal glands of *H. consanguinea* beetles in dichloromethane and ether yielded a clean profile of anisole and indole; the compounds responsible for attraction of *H. consanguinea* males (Leal *et al.*, 1996).

Both adult male and female antennae responded to host volatiles. But there was a sexual dimorphism in the olfactory perception of the host volatiles by *H. serrata*. The male antenna is more sensitive to host extracts. Perhaps, this indicates the necessity of finding the host first for their mate location, apart from using the female produced pheromone as the cue. The use of green leaf volatiles as a sexual kairamone has been observed in *M. melolantha* and *H. consanguinea* males (Reinecke *et al.*, 2005; Yadav and Yadav, 2004). A combination of the synthetic sex attractant (R,Z)-5-(Idecenyl) dihydro-2(3H)-furanone with a 3:7 mixture of phenethyl propionate (PEP) and eugenol caught significantly more *Popillia japonica* (Klein *et al.*, 1981).

Semiochemicals are a vital tool for monitoring and mass trapping in plantation crops. Being perennial crops the pest occurs round the year. To depend on pesticides is a difficult task in terms of application in the target area and also the frequency at which they have to be taken up. Hence exploiting the ethology is an effective method to monitor and trap the pest population in plantation crops. Though the adoption rate of semiochemicals in pest management is at a lower level as compared to pesticides, considering the shift in the policy by Government to scale down the use of pesticides due to health and environmental concerns there is a scope to increase the adoption rate. A note of caution is that semiochemicals may also face the same fate related issues raised over toxicity issues, as very little effort has been made to test the chemicals that are used in behaviour manipulation.

Another problem is that natural enemies use the cues used by the insect pest to identify its host and if the cues from the host plant to altered to bring in desirable pest control it would have a negative impact on natural enemies as its devoid of the cue to orient itself to its host. Hence a proper understanding of the volatiles role in various trophic levels have to be understood prior to attempting a pest management method. The demand for environmentally safe alternatives to broad-spectrum is on rise. The adoption of behavioral manipulation techniques can help to meet this demand, since the amount of chemicals released into the environment by behavioral manipulation is relatively small and are relatively nontoxic to vertebrates and are selective to the target pest species.

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