Behaviour Modifying Chemistry, Semiochemicals and Insect Chemical Ecology - Principles and Relevance

Chris Moore[1] and Robert Kofi Mensah[2]
[1]Farming Systems Institute, Queensland DPI, c/o Locked Bag 4, Moorooka, Q. 4105
[2]Australian Cotton Research Institute, Locked Bag 1000, Narrabri, NSW 2390

Background

The use of natural (non-synthetic) chemicals to influence insect behaviour is likely to be well known, in one way or another. Those troubled by mosquitoes are likely to have tested the hypothesis that citronella oil-based candles are repellent to mosquitoes, with varying degrees of success. Those who have tried to control fruit-flies may well have tried protein-bait traps whose "scent" lures flies to a pesticide-containing solution, and so to their deaths; the attractant, to which both males and females respond, is a complex mixture of chemicals derived from the breakdown of the protein - commonly brewers yeast. Another "natural" fruitfly attractant is a single chemical, methyl eugenol, a component of many flower fragrances. Only the males of many fruitfly species find it attractive, and it is used in monitoring and control programs.

Catnip, a perennial herb of the mint family, is known to produce a scent that is highly stimulating to cats. Recently, the scent has been shown to be repellent towards cockroaches. Coincidentally, aphids use the same chemical to attract a mate. In this case an insect is using the chemical as a pheromone, a category of chemicals used by insects and other organisms to locate mates, communicate alarm, etc. The study of the sex pheromone chemistry of moths, in particular, has accelerated since the elucidation of the chemical structure of the silkworm moth sex pheromone in 1959.

The use of synthetic (man-made) equivalents of the pheromone of Heliothis/Helicoverpa species, eg. *Helicoverpa armigera*, to attempt to monitor populations and/or disrupt mating is likely to familiar to most people in the cotton production industry. What may not be familiar to most are recent studies of the role of other chemicals in the life of Heliothis, with a view to developing environmentally friendly methods of control. These include studies of the aroma of plants which are attractive to the insects, with a view to using synthetic mixtures in various ways. The lead has been taken by the United States Department of Agriculture, but some research in this area is being undertaken in Australia as well, under the auspices of the Australian Cotton CRC and separately as part of the Supporting Green Industries Initiative of the Queensland Department of Primary Industries.

Other studies related to plant aromas have produced compelling evidence that plants damaged by the larval stages of moths emit mixtures of chemicals whose composition varies depending on the species of insect causing the damage - and that the mixture specific to a particular pest is uniquely attractive towards particular beneficial insects - parasitic wasps. This phenomenon amounts to a distress signal emitted by a plant that uniquely identifies its attacker to wasps that are interested in that pest, and in little else.

The examples above concern volatile chemicals, ie. those which can exist in a vapour form and be carried in the air. They can be classified broadly as **attractants** and **repellents**. In addition to these chemicals, a complex range of chemicals which do not normally exist in a vapour form (non-volatiles) is present in both the surface wax of a leaf and in the internal structure of a plant. They are implicated in feeding and egg-laying behaviour, where the insect senses the presence of such chemicals carrying information about the acceptability or otherwise of the material potentially available as a food source. Some volatile chemicals also appear to be involved at this level, but exert their influence, as in the case of the non-volatiles, through the insects' sense of taste after it samples the leaf surface or the internal tissue.

Chemicals involved in these phenomena can be classified as **stimulants** and **deterrents**. A classic example is seen in the monarch butterfly, whose milkweed host contains particular non-volatile chemicals which the insect must detect before it will lay eggs; which also are important constituents of the adult diet, but which will deter egg-laying if present in too high a concentration. A similar concentration-dependent switch from egg-laying stimulation to deterrence based upon leaf chemicals has been noted in the interaction between Heliothis and pigeonpea.

Studies of the broad range of chemicals that can be classified as stimulants and deterrents are in their infancy. With respect to cotton, the known variations in the susceptibility of different cotton varieties to both egg-laying and larval feeding, suggest that an investigation of leaf-surface chemistry would be a fruitful of area of investigation. To date, such studies have been restricted largely to the effect of the presence of gossypol and related chemicals. The behavioural and chemical studies required are more complex than is the case with volatile chemicals, because the chemicals involved are likely to be more complex in structure than the relatively small-molecule volatile chemicals, and the elements of behaviour more difficult to disentangle.

Studies of the chemically-mediated interaction of insects with their environment - and with each other - falls into the discipline of insect chemical ecology. Because the natural chemicals involved are signals being interpreted by the insects, they are called semiochemicals. The study of insect semiochemistry is technically and intellectually a considerable challenge, involving the full battery of techniques available to the organic chemist. Some aspects of the process are described in the following section.

The Chemistry

The process involved in the determination of chemical structure involves, in general, the isolation of single chemical compounds from all other compounds; the collection of a range of instrumental data which allows deductions about the structure to be made; the purchase or synthesis (manufacture) of likely candidate compounds; and the demonstration that the natural material is identical in all respects with the compound purchased or synthesised.

With particular reference to plant chemistry, the process of isolation involves the trapping of volatile chemicals, the extraction of non-volatile chemicals, and the refining (separation) of the complex mixtures so obtained in some way so as to progressively home in on the sub-set of relevant chemicals. This process must involve the regular presentation of the natural material as it is refined to the insect under study to observe its behaviour. This bioassay-directed fractionation directs the course that the analytical process takes.

Separation and Isolation

The basic separation technique used by the organic chemists who are best suited to this kind of research is chromatography - and it takes several forms depending very broadly on whether the chemicals being studied are volatile or non-volatile, and in the latter case water-soluble or not. The analytical instruments involved are called chromatographs. Thus, the analytical instrumentation will include Gas Chromatographs (GC) for the resolution and detection of volatile and semivolatile chemicals (though strictly speaking, the compounds being analysed are rarely gases), and High Performance Liquid Chromatographs (HPLC) for the similar treatment of non volatile compounds. A variety of other related techniques can be applied.

Instrumental Data

The actual chemical structure determination of pure chemical compounds is not a trivial task, particularly if the compounds are unknown to science. Such compounds are often active towards insects at the level of a thousand-millionth of a gram, so the instruments which yield data that can be used to hypothesise structures are remarkably sensitive. In a sense, the scientist is hoping to bring a level of sensitivity to detection which is equivalent to the sensory apparatus of the insect itself. In fact, the insect antenna - in a process called electroantennography - can be used to help identify those chemicals in a complex mixture of volatiles which are most likely to be influencing the insect's behaviour.

Instruments used include a variety of devices which fall within the categories of spectrometers and spectrophotometers. Modern instruments are sophisticated enough so that much information - often enough to lead to an ultimately correct hypothesis as to chemical structure - can be derived from a single instrument which combines the separation/isolation stage with the spectrometric stage. Not surprisingly, such instruments are quite expensive, costing in the region of several hundred thousand dollars.

Synthetic chemistry

The determination of the chemical structure of the kinds of compounds which are the subject of this review will often involve various chemical manipulations of the natural material itself, as well as the actual synthesis of the target compound from synthetic or natural precursors. The demonstration of exact equivalence of the synthetic and natural product in all ways, from properties revealed by a range of instruments to properties demonstrated towards, in this case, insects, is part of the scientific process which must be followed.

Some Findings

A few examples gleaned from the scientific literature in relation to *Helicoverpa* species gives some indication of the range of findings. With respect to plant surface chemicals, particular compounds that stimulate *Helicoverpa zea*, a close relative of *Helicoverpa armigera*, to lay eggs, have been isolated from corn leaves. A group of chemicals of a different type have been isolated from tobacco, and similarly influence *Helicoverpa virescens*. Chemicals obtained from pigeon pea by the same process used to obtain tea-tree oil from Melaleuca species (steam distillation) have been shown to stimulate feeding by *H. armigera* larvae, and to show both attractant and repellent effects towards female adults in a concentration-dependent manner.

It has also been demonstrated that chemicals extracted from non-host plants, then sprayed onto host plants, can exhibit varying degrees of deterrence towards female moths, though *Helicoverpa* species have not been studied in this way. Differences between the susceptibility of cabbage varieties to larval feeding by the diamondback moth have been related to the deterrent effects of certain leaf-surface wax components. The complexity of the phenomenon is illustrated by the fact that similar chemicals have the opposite effect on newly-hatched silkworms when added on an appropriate substrate.

Potential Applications

The potential use of repellent chemicals, for example as spray-on formulations, is fairly self-evident. Attract(lure)-and-kill devices are well known. Stimulant chemicals, again as spray-on formulations, could be used to enhance egg-laying on refuge or trap crops, thereby reducing the size of the population developing in the primary crop or resource. The direction of development of new synthetic pesticides increasingly requires ingestion for effective operation, and the potential role of feeding stimulants is obvious, as is their influence on the uptake of biological pesticides such as viruses. Dispersal of viruses and fungi using the insects themselves, after attracting them to a site where they would become contaminated, is conceivable as well, as is the attraction of beneficial insects to sites of high pest activity.

Conclusions

The behaviour modifying chemicals which have been the subject of this short review can properly be defined as natural chemicals. An additional perspective may follow from the illuminating view of biotechnology offered by the Office of Technology Assessment of the U.S. Congress, which defines biotechnology as "any technique that uses living organisms or their products to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses." Adoption of this useful definition will serve to combat the side-effects of an unfortunate association of the term "biotechnology" exclusively with the genetic modification of organisms.

At a time when "clean and green" methods of insect pest control are being investigated with increasing urgency, and when "biotechnology", particularly in its broadest sense as defined above, is reasonably considered part of the way of the future, it is timely to ensure the dedication of resources to this important new area of research.