ALPHA

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INSECT INVADERS AND THE SEDUCTION OF SCENT

the ROYAL SOCIETY of NEW ZEALAND

Since human migration to New Zealand began some 700–800 years ago, enormous numbers of unwanted exotic species have been introduced into the country. Despite tight biosecurity measures at our borders, this continues. Many pests are small in size which makes them difficult to detect. For example pests can arrive here on a piece of fruit, in a shipping container or on a car tyre. Sometimes it is only after damage appears that we become aware of a new invader.

Insect invaders, in a new environment without their normal predators and parasites and with a new range of hosts, can cause enormous damage. Right now they are damaging our honey industry, vegetable and fruit crops, trees and forests. They can take over niches occupied by native insects and starve them out.

New Zealand earnings from exporting primary products such as wood, fruit and vegetable crops, and seeds accounts for almost half our export earnings – $12 billion. These earnings are eroded by the huge costs of controlling introduced pests and lost production. In 1999 this was estimated to be about $840 million, and a more recent analysis suggests that the true cost is much higher.

Red imported fire ant.
MAF Biosecurity Issue 45, 1 August 2003

Fall webworm moth (*Hypathantria cunea*)
From MAF Biosecurity Issue 43 1 May 2003

The Ministry for the Environment, the MAF Biosecurity Authority, and the Department of Conservation have a variety of mechanisms to control insect invaders. They monitor shipping ports and international airports where many potential pests can enter New Zealand. For example, in March 2001, a nest of red imported fire ants (a tiny aggressive ant with a fierce sting) was found at Auckland International Airport. The nest was treated and all the ants killed.

When an insect pest moves from its point of introduction, techniques to control it are usually designed to **eradicate** it. Where eradication is not possible, scientists work to find ways of **suppressing** the pest so their damage level is acceptable. Methods to eradicate pests include aerial spraying, habitat removal, poison traps and other forms of chemical control. Methods to suppress also include trapping, and introducing natural enemies of the pest. Breeding for **host resistance** is another technique used to create an unfavourable environment for pests and control population numbers. Some crops and animals resist insects and similar pests better than others and some are immune to certain pests.

Scientists in New Zealand have worked on a variety of projects for international clients using genetic engineering to build pest resistance into plants. The protein crystal in *Bacillus thuringiensis* (Bt) that is toxic to many caterpillars, for example, has been incorporated into pine trees and other plants making insecticide applications unnecessary.

Horticultural and agricultural workers also take action to suppress pests by spraying when pests exceed an economic threshold. A zero tolerance of live insects for pests on export fruits, vegetables and flowers exists because of international quarantine restrictions. Other tactics, often called cultural controls, include making the local habitat unsuitable for the invaders by **removing** weedy ground cover in orchards, **rotating crops**, planting **trap crops** to attract insects and altering planting and harvest times.

Some of these methods have not been popular with the public, such as the aerial and ground spraying of the bacterial insecticide Btk over suburban areas of Auckland to prevent the spread of the white-spotted tussock moth (*Orgyia thyellina*). The moth was discovered in Auckland's eastern suburbs in April 1996 but after an extensive spraying programme, followed by trapping, was declared eradicated in 1998. The leaf-eating caterpillars of the moth could have caused serious damage to trees and shrubs and scientists believe the moth would have spread throughout the country in 5–10 years if left unchecked.

From MAF Biosecurity Issue 35 1 May 2002

In mid-1999, the painted apple moth, *Teia anartoides*, was discovered in Glendene, West Auckland. Native to Australia, female moths are flightless but male moths can fly and newly hatched caterpillars can 'balloon' a few hundred metres in the wind using silken threads. MAF has now established a vegetation control zone in the area from which people must not move plant material. MAF is also working to eradicate the painted apple moth using aerial spraying, trapping, host plant removal, and release of sterile male moths. This is the first use of the **sterile insect technique** in New Zealand and involves the mass rearing, sterilisation and release of large numbers of insects to reduce or eliminate subsequent generations of feral populations of the pest. More than 40 countries have used this approach for controlling a range of insect pests. The HortResearch team's results suggest that the technique has made a useful contribution to the suppression and possible eradication of painted apple moth, and this approach is proposed in the future.

There is widespread support for the campaign to eradicate painted apple moth from the Department of Conservation, the Royal Forest and Bird Protection Society, Forest Owners' Association, and the Farm Forestry Association.

Painted apple moth – larvae and adult.
From MAF Biosecurity Issue 41 1 February 2003

Sex could hold the key

In the future, the use of pesticide sprays may be greatly reduced or more specifically targeted. Scientists at HortResearch have been developing special biological controls which target specific species of insects, mainly moths. They are using synthetically produced insect **pheromones**, particularly sex pheromones that make up mating signals, to attract and trap the unwanted pests, or to disrupt their normal reproduction. Such traps have become an important part of prevention monitoring and control of many unwanted insects.

The word 'pheromone' comes from Greek and means 'carrier of excitement'. Pheromones are produced by many insects and insect-like organisms to cause responses in other insects of the same or closely related species. To disrupt normal reproduction of an insect species, synthetic pheromone is released from numerous sources placed throughout an at-risk crop; the males are unable to locate the females and the number of

Male painted apple moths respond to synthetic sex pheromone from the microsprayer.
Photo courtesy HortResearch

Painted apple moth larva.
From MAF Biosecurity Issue 41 1 May 2003

matings and offspring is reduced. Scientists believe that the high levels of pheromone not only confuse male insects but also camouflage a female's pheromone emission and cause some males to tune out all sources of the pheromone.

Pheromones in traps are often used in **integrated pest management** (IPM) monitoring. In 1993, risks posed by imported containers and ships arriving in New Zealand from the Russian Far East led to a nation-wide, early warning gypsy moth trapping system being put in place. So far only one moth has been trapped in Hamilton. Pheromone traps have also been used to monitor codling moth, oriental fruit moth and leafroller in pipfruit and summerfruit orchards. Orchardists now routinely check pheromone traps and use a threshold for deciding whether or not to apply an insect growth regulator against caterpillars. Sprays are only applied if the pest is abundant. The use of pheromone traps and pest thresholds has helped orchardists minimise insecticide use.

Some orchardists, such as organic apple growers, are now using pheromone dispensers for mating disruption. Special dispensers release the pheromone throughout the season, and maintain an invisible and harmless cloud that confuses male moths and reduces the next generation of the pest. This approach is increasingly being used internationally.

A history of pheromone research

In the 1870s, French naturalist Jean-Henri Fabre watched a female great peacock moth emerge from a cocoon in his study. He put her under a wire-gauze bell-jar and left her to spread her wings to dry. Later that evening dozens of male great peacock moths, with wings up to 6 inches across, flew in through the open doors and windows. "Coming from every direction and apprised I know not how," Fabre wrote, "here are forty lovers eager to pay their respects to the marriageable bride born that morning amid the mysteries of my study." Over the following week Fabre caught more than 150 males.

Fabre researched this attraction over several years and concluded that the female moth must release an odour that is powerfully attractive to the male moth. New York entomologist, Joseph A Lintner came to the same conclusion and Lintner took Fabre's research further. He not only concluded that the female releases a chemical substance to which the male is highly attracted; he hypothesised that harnessing such chemicals could provide a control for insect pests.

In the 1930s, German chemist Adolph Butenandt began to study the chemicals involved in silkworm pheromones. He snipped off the abdominal tips of female silkworm moths and ground them up. Then, using analytical chemistry techniques, he separated the moth slurry into various extracts and tested these on male silkworm moths. The male silkworm moth cannot fly but will flutter its wings when excited by a nearby female, and Butenandt managed to produce the same response with one of the extracts.

In 1959, half a million female silkworm moth abdomens later, Butenandt announced his success – the pheromone for the silkworm moth was a kind of alcohol that he named bombykol, after the moth's Latin name, *Bombyx mori*.

Also in 1959, German biochemist Peter Karlson and Swiss entomologist Martin Lüscher were working on identifying the chemicals that maintain the elaborate caste system of termites. They coined the term 'pheromone' to describe a substance that an animal gives off to trigger a specific behavioural or developmental reaction in another of the same species.

In 1957 biologist Dietrich Schneider, knowing that nerve cells responded to stimuli with a small burst of electricity, developed a method to use moth antennae as a test for the presence of pheromones. Schneider removed an antenna from a male silk moth and lodged it between two electrodes. He then exposed the antenna to air that swept past an extract containing bombykol and noted a peak of electrical activity in the antenna corresponding to exposure to the extract. This odour-prompted electrical response of an insect antenna was called an **electroantennogram** (EAG).

Observed known response behaviours (also called behavioural assays), such as the wing-fluttering response used by Butenandt, remained key to identification of pheromones throughout the 1960s. Using behavioural assays, researchers identified the pheromones used as attractants by some beetles and a few other insects.

Progress was slow. Extracts that were highly attractive to male insects when left in their crude form often lost their allure when purified into their various components.

Because each pheromone affects only one specific group of insects, their use usually poses little risk to other organisms, including people. However, few synthetic pheromones are available compared to the number of insect pests because it is costly to discover, produce, and market a chemical that controls only one pest species. As a result, synthetic pheromones are mainly used on high-value crops.

Gum leaf skeletoniser larvae
From MAF Biosecurity Issue 44 1 June 2003

Recently, scientists at HortResearch have been developing pheromone traps for the painted apple moth, the Australian guava moth and the gum leaf skeletoniser. The gum leaf skeletoniser moth, *Uraba lugens,* was first noticed in New Zealand in 1997. More than 50,000 ha of plantation eucalyptus and a large number of trees in urban forests and parks were at risk from the caterpillars which partly or completely skeletonise leaves, slowing tree growth or killing young trees.

For each of the above three species, New Zealand scientists have identified the signal chemicals that regulate insect behaviour, particularly the sex pheromones that make up the mating signals. With the right blend of compounds in the right quantities the traps will fool a male moth into thinking it is on the track of a female.

When scientists have created a new scent for a particular species they test it using a wind tunnel. This is usually a clear plastic device two to three metres

Synthetic compounds that passed the pheromone test in the lab often failed to attract male moths in the field.

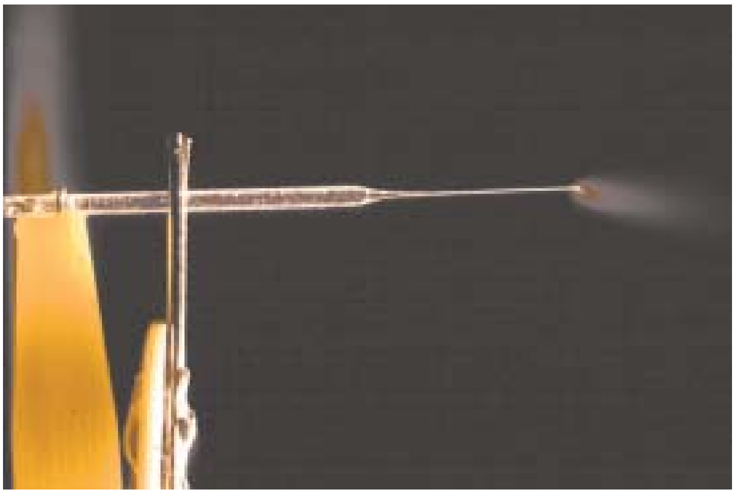
Although testing every fraction of a mixture in combination with every other fraction made pheromone research more complex, it also helped to explain many failures of the past. During the 1970s, several scientists reanalysed the pheromones that had fared well in the laboratory yet failed in the field. Often they discovered that the addition of one or two more components to these single compounds improved field test results. This demonstrates that scientific progress is seldom linear.

In the next few decades technical improvements quickened the pace of pheromone research. Paramount was the use of three techniques known as **gas chromatography**, **mass spectrometry**, and **nuclear magnetic resonance**. These techniques were used in combination with the EAG.

All these techniques, and others, were used in various combinations. Gas chromatography was linked to mass spectrometry so researchers could both separate and identify the pheromone components in their mixtures. By coupling gas chromatography to the EAG, researchers could detect which components in their insect preparations prompted an electrical response. The development of capillary gas chromatography allowed researchers to separate compounds that could not be resolved by previous methods. The development of the EAG took another step forward when it began to be used in the field. In New Zealand the HortResearch team made major progress in the 1990s using field EAGs to develop a better understanding of pheromone plume structures present in orchards treated with mating disruption. While technically challenging, the researchers were able to determine the actual concentrations needed to disrupt insect behaviour for the first time. Pheromones are delivering benefits to growers and the country in a range of new and exciting ways.

Close-up of the electroantennagram
Photo courtesy HortResearch

long, with scent released at one end which is then blown through the tunnel by a fan. If an insect is stimulated to fly upwind in the tunnel toward the chemical scent, scientists conclude that the scent being tested is indeed a pheromone. A wind tunnel also allows researchers to test various mixtures of chemicals at different release rates to find the optimum lure for field traps. The release rate of the odours is very important.

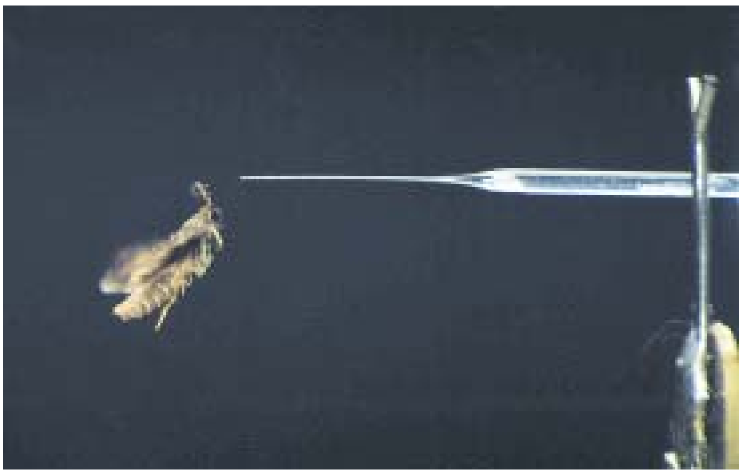


The microsprayer in action
Photo courtesy of HortResearch

Dr Ashraf El-Sayed, now with the HortResearch Chemical Ecology team at Lincoln, invented a new release system while in Switzerland. He used a **piezo-electric disk** (a crystal combined with **square wave high frequency sound**) and set up a microsprayer that pushes the synthetic pheromone through micro tubing to a fine glass capillary vibrated by the piezo disk. This sends out a spray of the scent in much the same way as a female moth would do. This combination of technology and inventiveness allows precise control for delivery of the specific blend, making wind tunnel research more accurate and faster.

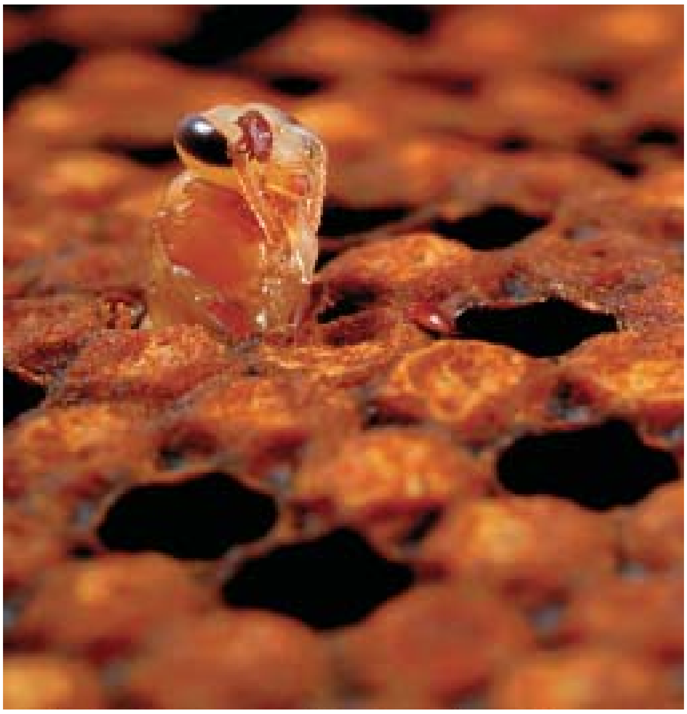
New applications of pheromones are also emerging. For example, the identification of pheromones for biological control agents of both weed and insect pests is an exciting new development. Landcare Research is working on introducing new biocontrol agents against gorse. More than 30,000 caterpillars of one species had been released, but it wasn't until the team at HortResearch identified the biocontrol pheromone that the insect was proven to have established itself. Confirming the presence of the biocontrol population took less than a week after setting up the right blend of pheromones in a trap! Pheromones for natural enemies of pests such as a wasp parasitoid of codling moth, are also being used to see how biological control could be improved.

Future research will help expand the successful use of pheromones in pest management. Scientists at HortResearch and other international research institutions continue to study how insects produce pheromones, how they trigger a response, and what influences that response. They are beginning to uncover the **hormones** that trigger pheromone production as well as the binding **proteins** that bring the pheromones to their receptors. Scientists are also discovering the **neurological pathways** the pheromones stimulate in a responding insect, and the **enzymes** the insects use to break down the pheromone so as to shut off its signalling. This research could lead to better and cheaper design of synthetic pheromones, as well as more efficient ways to use pheromones to manage insect pests. Improving pheromone dispensers in the field so that the chemicals are longer acting, more potent, and easier to release is another area of ongoing research as is research on insect ecology and population dynamics to assess how many pheromone traps are needed and their most effective geographical distribution.



A moth moves in – fooled by the synthetic pheromone
Photo courtesy of HortResearch

Glossary



Varroa destructor – bee mite on emerging bee adult.
HortResearch –The hothouse, November 2002, Issue no.7

biosecurity policy, monitoring and control measures which are put in place to protect borders from unwanted organisms.
electroantennogram (EAG) a device consisting of an insect antennae and an oscilloscope which records an insect's antennal response to insect and plant produced volatiles, usually pheromones. Insect antenna are morphologically and physiologically highly specialised organs for odour perception.
enzymes biological catalysts produced in cells, and capable of speeding up chemical reactions by converting one molecule into another.
eradication a process of completely eliminating an introduced pest.
gas chromatography a technique for separating components in a vapour based on how quickly they travel through a column containing an absorbent material.
hormones substances secreted in minute quantities by a plant or animal.
host resistance the deliberate selection of crops, animals, and structures resistant to particular pests. This selection process creates an unfavourable environment for pests and helps to suppress their population numbers.
hosts animals or plants which carry parasites.
integrated pest management (IPM) an ecosystem-based strategy that focuses on long-term prevention of pests or their damage through a combination of techniques such as biological control, habitat manipulation, modification of cultural practices, and use of resistant varieties. Pesticides are used only after monitoring indicates they are needed to remove the target organism. Control materials are selected and applied in a manner that minimises risks.
mass spectrometry a process where positive ions of a substance are separated by an electromagnetic system which allows the precise measurement of the relative concentrations of the various ionic masses present in a substance.
neurological pathways routes within a nerve system along which electrochemical messages are transmitted.
nuclear magnetic resonance based on the principle that atomic nuclei in a strong magnetic field can be made to give off electromagnetic radiation, the characteristics of which depend on the environment of the nuclei.

Working to keep the bee industry humming
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parasites organisms which live in or on another and draw nutriments directly from their host.
pheromones chemical signals, usually odours, which are emitted by an animal to attract a mate normally of the same species.
piezo-electric disks crystals, commonly quartz, which can develop an electromotive force or voltage across opposite faces when subjected to a mechanical strain. They are used as frequency standards for such tasks as replacing balance wheels in watches, and for producing ultrasound.
predators animals which prey naturally upon or exploit another species of animal.
proteins complex, biologically important substances composed of amino acids joined by peptide bonds.
rotating crops a process where different crops are grown in succession on the same patch of ground. This has the effect of limiting pest numbers and not exhausting the soil by nutrient draw-off.
square wave high frequency sound produced when a number of sine wave 'pure' sounds of specific frequencies are overlapped or added to produce a square wave rather than a smooth sine wave. For example, if you take the 1000 Hz sine wave and add its odd multiples, such as 3000 Hz, 5000 Hz, and 7000 Hz to it, it becomes a square wave. Both the original sine wave and the resulting square wave have the same amplitude and frequency, and differ only in their shape, however the sound produced is quite different. High frequency sound is produced by reducing the period of the sine wave or square wave.

The shorter the period, the higher the frequency of the sound. Frequency is defined as how many times the wave goes from zero to maximum, through zero to its minimum, and back to zero – in one second, and is expressed in a unit called a Hertz.

sterile insect technique the mass rearing, sterilisation and release of large numbers of insects to reduce or eliminate subsequent generations of the target pest.

suppression where insects or other pest population numbers are controlled to a point where the damage from their existence is at an acceptable level.

trap crops crops which are planted as a sacrifice for insects pests. The pests find the trap crop preferable to the main crop and are lured away from the valuable target, where they may be safely sprayed with pesticide or the trap crop can be destroyed along with the pest.

Internet references

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Environmental Risk Management Authority: http://www.ermanz.govt.nz
HortResearch: http://www.hortresearch.co.nz
MAF Biosecurity Authority: http://www.maf.govt.nz/biosecurity/
The New Zealand Plant Protection Society: www.hortnet.co.nz\publications\nzpps

Technical papers on the latest results in insect pest management, including biosecurity.

United States National Academy of Sciences' Office on Public Understanding of Science: http://www.beyonddiscovery.org/

A collection of articles on pheromone science as part of its *Beyond Discovery* series. Specifically: http://www.beyonddiscovery.org/content/view.article.asp?a=2702, for a very detailed history of pheromone discovery and science.

For information on specific insect pests in New Zealand see:

http://www.maf.govt.nz/biosecurity/pests-diseases/animals/fire-ants/index.htm
http://www.maf.govt.nz/biosecurity/pests-diseases/forests/gypsy-moth/index.htm
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Recommended further reading

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Top, Fall webworm (*Hypathantria cunea*) communal web. From MAF Biosecurity Issue 43 1 May 2003
Bottom, Australian guava moth larva (*Coscioptycha improbana*). From HortResearch Report 2000/01
Right, Painted apple moth. From Biosecurity Issue 41 1 February 2003

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