AP247
Pheromone concentrations during mating disruption of codling moth

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HAL

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Pheromone Concentrations during Mating Disruption of Codling Moth

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Project AP247
Final Report

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INTRODUCTION

Control of moths by disruption of mating using sex pheromones is an environmentally benign control method which can substitute for insecticidal sprays. By releasing a synthetic version of the sex pheromone of the insect into the air from dispensers distributed throughout an orchard mating of the target insect can be disrupted. This lowers the number of progeny and hence reduces future damage to plants and fruit. A very successful implementation of this approach for the control of Oriental Fruit moth was developed by CSIRO and marketed as Isomate-M by Biocontrol Pty. Ltd. Implementation of mating disruption against Codling moth is currently under development as a key element in improved management of this pest.

One recurrent problem associated with the use of pheromones for mating disruption is lack of information about the concentration of pheromone in the air in treated areas. If the amounts are too low then insects will be able to mate and if too high the extra pheromone is wasted and makes the technique less economic. Although the amounts of pheromone released for mating disruption are large compared to those released by the insect they still tax the sensitivity of measuring instruments. In the past it has been necessary to draw air through a retaining filter medium for many hours, between 4 and 8, to collect enough pheromone for measurement using Gas Chromatography and Mass Spectroscopy. This presents many problems with contamination making such measurements a major research undertaking. As a consequence only a small number of such measurements have been made. These measurements are also inevitably longterm averages which provide no information on short-term variations which could be of great importance. There is therefore much uncertainty as to concentrations and distribution of pheromone under the many varied orchard canopies and environmental conditions encountered. The need for techniques to carry out concentration measurements both routinely and quickly has exercised the minds of pheromone researchers for some time.

There is one type of instrumentation, or more accurately a technique, that in principle is sensitive enough to measure pheromone concentrations in the air directly. This technique is an electrophysiological one which is already in common use for rapid screening of libraries of compounds during the pheromone identification process. It is known as an Electroantennogram, or EAG for short. It is relatively simple to set up involving the measurement of the electrical potential across the antenna of a male moth and is not very demanding in terms of equipment to carry out the measurements. Pheromone researchers have considerable experience with the technique in the laboratory and a number of demonstrations of its ability to detect pheromone pulses in the open air have been published (Baker & Haynes 1989).

Laboratory equipment normally used to measure EAGs makes little attempt at miniaturisation but conversion to a field portable machine is a matter of design not of principle. However a field EAG would vary in one very significant respect from laboratory equipment. In the laboratory the antenna to be measured is placed in as pure a supply of air as possible. Compounds to be tested are subsequently injected into the clean air stream. A supply of bottled instrumentation air is often used, filtered through charcoal, humidified, and made to flow past the antenna. Odours carried in ordinary room air, which could elicit their own response from the antenna and affect its sensitivity, are thus excluded. In the field version the requirement would be for just
the opposite. Since orchard air has to be passed over the antenna to sample any pheromone present any unwanted odours in the air would inevitably also reach the antenna. These would include plant, soil and insecticide odours, fumes from equipment, etc. The effect of these external odours on EAG responses from the pheromone is not predictable.

A number of scientific establishments have initiated programs to develop portable EAG instruments with the University of Kaiserslautern in Germany (Sauer 1989, 1991, Sauer et al. 1990) being the first to produce a working instrument. Other instruments have been developed at USDA in the U.S., HortResearch in New Zealand, and the CSIRO. The prototype CSIRO instrument had already been used to evaluate the potential of the technique and to obtain information on how to build a working field instrument prior to this proposal. This instrument operated to expectations in the laboratory and under limited conditions in the open within the CSIRO site but not being fully portable had not been tested under true field conditions. Results emanating from Kaiserslautern indicated high expectations for applicability of the technique in the field.

MATERIALS AND METHODS

It was decided to design an instrument which would measure not one but two antennae simultaneously. Such redundancy would allow a series of measurements to continue if one antennae failed prematurely. This could be important because antennae have limited lifetimes in an EAG context. A maximum working lifetime for an antenna of only 30 to 60 minutes could be expected from laboratory experience. The outputs from the two antennae could also be correlated to minimise biological noise and help identify signals arising from pheromone pulses. This was attractive because EAGs often exhibit sudden changes in potential which arise from the antenna itself rather than as a response to an external stimulus. In laboratory experiments the time of application of the stimulus is known. Internal changes in potential are therefore easily identifiable and discounted. In the field the arrival of pheromone pulses would be asynchronous making it difficult to distinguish between the two. Possible problems arising from responses to extraneous odours might also be solvable by measuring a male and a female antenna together. Female antennae of Codling and Lightbrown apple moths do not respond to pheromone. So if both antennae respond similarly to environmental odours then the pheromone signal could in principle be obtained as the difference in the signal from a male and a female antenna.

A small fan was used to draw air from the environment past the antennae, Fig. 1. A charcoal filter fitted to the top of the air-inlet was used to remove all odours from the air thus establishing a baseline for the measurements. Three side-ports allowed calibration of the instrument using a graded concentration series of synthetic pheromone sources. Contact with the ends of the insect antennae was made through small wells filled with saline gel and connected to the input of the amplifier with chloridised silver wires, Fig. 2. Each antenna was connected to its own high impedance amplifier, Fig. 3, the outputs from which were connected via a cable to a box of electronics which further amplified and filtered the signal, Figs. 4 & 5. An Analogue-to-Digital convertor card, plugged into the bus of a portable IBM compatible PC, was then used to digitise the data. Software running under MSDOS
was written to digitise the data, display it on the screen in real-time and store it for later analysis. Communication between the EAG instrument and the rest of the equipment was through a single multiple conductor cable which carried power, analogue and digital signals.

The operation of the charcoal filter was controlled remotely from the PC. Motor-driven vertical movement of the filter was part of the design of the instrument from Kaiserslautern University but their filter remained suspended only a few centimetres above the input orifice. This was considered undesirable so a second motor was added to swing the filter away from the instrument when in the raised position.

RESULTS

Measurements using the portable EAG equipment were carried out at the CSIRO experimental station at Gininderra and at a commercial orchard in Pialligo, both suburbs of Canberra. Two moth species were used for these experiments, the Lightbrown apple moth, *Epiphyas postvittana*, and the Codling moth, *Cydia pomonella*. Preliminary testing of the equipment by hanging a single pheromone dispenser from a tree demonstrated the ability to detect pheromone pulses, Fig. 6.

When the instrument was moved into the orchards it was discovered that antennae responded very strongly to environmental odours in the orchards. Although some response to plant and other environmental odours was expected, the insects do have receptors for food odours, the size of the effect was not. Results from Kaiserslautern using the moth *Lobesia botrana* (Sauer 1991) had not indicated an effect as large as we observed. When the charcoal filter was removed allowing orchard air to reach the antenna, but with no pheromone dispensers deployed, there was a large change in the signal baseline, Fig. 7. Responses to synthetic pheromone sources, required to calibrate the instrument, were sharply defined and highly reproducible when the charcoal filter was installed to remove environmental odours, Figs 8(a) and 9(a). A calibration signal is essential if the concentration of pheromone is to be measured because antennal sensitivity varies greatly between individual antennae. A disturbing effect was immediately apparent when the calibration signal was applied with the filter removed and the antennae exposed to clean, i.e. without any pheromone present, orchard air. The depth of the calibration signal was reduced and its recovery period increased. The calibration signal was restored to its original level when the filter was replaced, Figs. 8(b) and 9(b) but not when the flow of orchard air was maintained, Figs. 8(c) and 9(c). The effect on the EAG response to the calibration stimulus was highly variable. Although all antennae tested were affected to some extent by environmental odours some reacted more strongly than others. Responses also varied with position in the orchards and with temperature. These observations indicated that to measure in absolute terms the concentration of pheromone in the orchard air might prove very difficult.

Various attempts were made at overcoming the effect of the extraneous odours. One of these involved the anticipated possibility of subtracting responses from a male and a female antenna measured together. Measurements carried out in the absence of pheromone, rather surprisingly, showed very little correlation in the signal from the two antennae. It looks as though the populations of sensilla in the two sexes detect
different odours. The poor correlation meant that this technique could not be used to recover the pheromone signal from the male composite response. Other attempts using detailed calibration methods involving a range of source concentrations foundered due to the time required for such measurements and aging and adaptation effects on the antenna and an inability to identify compounds which would reproduce accurately the antennal response to the unwanted odours.

**DISCUSSION**

The interaction between environmental odours and pheromone is not additive in an arithmetical sense and is influenced by many external factors (Rumbo et al. 1994). Thus the strength of both signals is affect by air movements within the orchard especially wind gusts which mix the air under the canopy with air from above. Together with the non-linear response (approximately exponential) to pheromone concentration this makes it very difficult to separate the two. Theoretically it is possible to carry out detailed calibration measurements on each antenna for a range of concentrations but in practice problems arise from aging and adaptation of the antenna. Thus by the time calibration measurements are complete the response of the antenna has invariably changed to the extent that the calibrations no longer apply. The conclusion has to be that using EAGs for routine measurements of absolute pheromone concentrations in an orchard, using either Codling or Lightbrown apple moth antennae, is impracticable. A similar conclusion has been reached independently by Mayer & Mitchell (1996) although they do not specify which insects they were working on. All except one publication using the technique have reported only relative levels of pheromone concentration between different sites and conditions. The exception is Witzgall et al. (1996) working with *Cydia nigricana* who have claimed the ability to measure pheromone concentrations using that insect. They mention that in their case environmental odours had only a very small effect on the antenna. We may therefore have been unlucky in that the insects of interest to us exhibited a contrary response. It is not known what proportion of insects falls into each category. Although measurement of absolute concentrations may prove elusive for some insects it may still be possible to make measurements relevant to mating disruption. Thus Suckling & Angerilli (1996) have reported that the RMS variation in the EAG signal is dependent on dispenser distribution, and Karg & Sauer (1995) have published very useful measurements of relative concentrations in vineyards.

Although the original expectations of this portable EAG were not realized, in that measurements of absolute pheromone concentrations during Codling moth disruption trials proved impossible, this was not due to any inherent defect in the equipment or the technique. The instrument can detect pheromone levels in treated orchards and the problem encountered are due to biological properties of the antennae of the insects of interest. The first phase of this research venture, the development of the instruments, can therefore be considered over. The next phase will be extensive experimentation to evaluate the true potential of this type of instrument. This is now under way in a number of laboratories and the EAG technique may yet provide us with useful information once its true capabilities and best mode of application are determined. However continued development of the instrument’s capabilities will require concentrated effort such as can be expected from Ph.D. students who can dedicate
their time fully to such projects. We will therefore be relying mostly on information from external sources to find how best to apply our instrument. We do propose however to make a contribution in conjunction with projected pheromone disruption trials against Diamondback moth, \textit{Plutella xylostella}, an important pest of brassicas. It may turn out that complications from odours other than pheromone may not be so severe in this very different growing environment.

**ACKNOWLEDGMENTS**

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**REFERENCES**


INDUSTRY SUMMARY

Mating disruption with sex pheromones is a technique which can be used to control insects such as moths by dispensing synthetic amounts of the natural pheromone throughout the growing area. Implementation of the technique has been complicated by an inability to readily measure the amounts of synthetic pheromone present in the air for which conventional chemical technology does not possess the desired sensitivity. An alternative method based on the ability of the insect antenna to detect the pheromone has been proposed and instruments based on the technique, known as an electroantennogram or EAG, have been developed at various laboratories. The CSIRO had built a prototype of this type of instrument which showed promise and which justified building a portable version which could be used in orchards.

The portable instrument was successfully completed and under laboratory conditions performed well. Problems however arose when the instrument was moved into apple growing orchards. Antennae from the insects of interest, Codling moth and Lightbrown apple moth, were found to react strongly to orchard odours other than pheromone. The response to pheromone in the presence of the other odours was seriously affected. This made it impossible to measure the amount of pheromone in the air in absolute terms, the original object of the exercise. It has since been reported overseas that other insect species may not react so strongly to non-pheromone odours whilst another report has confirmed our findings but without mention of which species were involved. It therefore appears that the technique may only be usefully applied with certain species of moths and it is a matter of luck whether such a moth is the pest species under investigation.

TECHNICAL ABSTRACT

A portable electroantennogram (EAG) instrument was built to allow measurements of the synthetic pheromone content of air in orchards treated with pheromone dispensers to control either Codling or Lightbrown apple moths. The design incorporated two antennae which were measured simultaneously and all functions including placement and removal of a charcoal filter on the inlet could be controlled remotely from a PC. The instrument was able to detect pheromone pulses emanating from single dispensers hanging from a tree at a distance of up to five metres. However antennae from both moth species also responded strongly to orchard odours other than pheromone. The responses to these environmental odours and pheromone were found not to be independent of each other and non-additive. In practice this meant that it was not possible to measure the pheromone response in terms of absolute quantities of pheromone in the air. Fluctuations in the response to the environmental background also made it difficult to unambiguously identify individual pheromone pulses. Attempts to overcome this difficulties by comparing the responses from a male and a female antenna failed because there was poor correlation between the responses from the two sexes to the environmental odours.
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Fig. 1

CHARCOAL FILTER

TWO ANTENNAE

PHEROMONE SOURCE

AMPLIFIER

PUMP

'SNIFFER' APPARATUS
Fig. 2

- Sintered Chloridised Electrode
- Air Gap
- Sliding Block
- Saline wells
- Antenna
- Saline well (Ground)

Adjust for Antenna length
EAG High Impedance Pre-amplifier - one of two

Fig. 3

LM324

100K

TLC272

Input protect short

Antenna

10K

22K
Fig. 4

Amplifier

From Preamp

LM324

220K

To A on Filter

Balance and drift correction

100K

10K

8K2

100nF

33K

10K

*Change to suit A/D range

33K

From PC D to A
RELATIVE RESPONSE

Fig. 6

PORTABLE EAG FIELD TEST
One LBAM Dispenser, 2 metres away
Fig. 7

Response to Environmental Odours

Charcoal Filter On
Fig. 8

(a) Responses from Cydia pomonella

(b) Charcoal Filter On

(c) Charcoal Filter On
Fig. 9

Responses from *Epiphyas postvittana*

(a) Charcoal Filter On

(b) Charcoal Filter On

(c) Charcoal Filter On