The Effects of Methyl Parathion on the Colony Dynamics of *Apis mellifera*

A thesis submitted in partial fulfilment
of the requirements for the degree of

Master of Science in Ecology

At Massey University,
Palmerston North,
New Zealand

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2000

TABLE OF CONTENTS

ABSTRACT

The detrimental effects of pesticides to honey bee colonies were assessed using a combination of electronic and manual sampling techniques. Initial experiments determined that electronic bee counters could be used to identify and monitor toxic events occurring in honey bee colonies, and also identified that 30 minutes after application, the bees did not avoid direct contact with methyl parathion. Dead bee counts, flight activity, percent return of foragers, and determination of colony composition were used to assess the effects of methyl parathion on the colony dynamics of *Apis mellifera*. In particular, the combination of dead bee counts, colony composition analysis, and "real time" data, provided an extensive monitoring system that enabled the progression of colony recovery to be followed, and generated information of use for the application of pesticides in the local environment.

The analysis of colony composition identified that brood declined in response to decreased worker bees, and that colony recovery was dependent on brood and food reserves within the hive.

The foraging activity of honey bee colonies dosed with methyl parathion was lower than that of untreated colonies because their flight activity and percent return rate declined for at least six weeks following methyl parathion application.

Keywords: Honey bees, *Apis mellifera*, Pesticide effects, Methyl parathion, Flight-monitoring

Explanation of Text

These studies were conducted through a Study Abroad Program between Massey University and the University of Montana (UM). This research was conducted under the auspices of Jerry Bromenshenk at the University of Montana, who leads the team that designed the bee counters that I used to study the progress of methyl parathion treated colonies, and follow the colony composition through weekly checks.

Outline of Honeybee research at Montana University

UM assesses areas of environmental interest by analysing the chemicals that honey bees accumulate in their hives. Through identification of these chemicals we have shown that pesticides also accumulate within the hive. UM aims to identify behavioural activity that will flag chemical changes within the environment so that chemical analysis is only conducted when necessary. In an attempt to identify and calibrate this detection system UM has designed an electronic bee counter which records the number of honey bees entering and leaving the hive.

American date notation has been used in sections of chapter 2 and chapter 3 of this thesis to prevent confusion whilst completing these experiments in America. ie. mm/dd/yy.

ACKNOWLEDGEMENTS

I appreciate the time and effort Dr Jerry Bromenshenk, my adviser at Montana University, United States of America, has given to enable the completion of this thesis. Also for the financial assistance provided through his funding, the invaluable advice and experiences he shared, and for believing in me.

I would like to thank my primary supervisor, Professor Brian Springett, for help in editing this manuscript, and for providing guidance and constructive comments throughout this study.

I am especially thankful to my husband and best friend, Byron Taylor for his never ending support, encouragement and selflessness. For taking a year off from teaching to work with me on the bee project and for enduring the inevitable stings.

Thank you to the technical staff of Montana University, past and present, who have provided insight and support as well as making this an enjoyable experience. I would especially like to thank Dr Bruce King and Robert Seccomb for their amazing abilities to design systems and make computers comply. Dr Garon Smith and Dave Jones for their support and chemistry related and advice. Lenny Hahn and the work studies for their reliable bee collecting and counting. Thanks to Dr Colin Henderson for his invaluable guidance and patience in data analysis.

I would like to acknowledge the use of honey bee colonies from the Beltsville Bee Laboratory, Maryland USA. Huge thanks to Dr Jeff Pettis who provided ongoing encouragement and discussion of experimental design and analysis, Dr H. Shimanuki for supplying numerous references, and Mark Feldlaufer for providing the initial contacts.

My parents, Graeme and Jeanette Ward, have been wonderful throughout my studies with encouraging words and letters, as have my new parents, Peter and Margaret Taylor, over this last year, and my four sets of grandparents that have taken great interest in my "study with the bees".

Thanks to a wonderful beekeeper and friend Dave Simmons for the use of his property, his support, advice and friendly hassles over coffee. The Susquehanna Beekeepers Association for providing valuable insights into the beekeeping strategies in Maryland, USA, as well as taking time leave from work to help with the research.

I thank the Massey University Study Abroad Program for enabling the dream to study overseas become a reality. Especially Dr Russell Death for inspiring me to apply. The administration staff of the ecology department at Massey University, Erica Reid and Jodi Matenga have been great for keeping the communication lines open whilst I was in America.

I am grateful for the editing my uncle, David Beach, provided and for the way he has gone out of his way to make this thesis happen. Truly amazing.

Great thanks to all the people we had contact with at the Aberdeen Proving Ground post in Edgewood including John Wrobel, Pete Pritchard, Pop Schenk, Pop Taylor, Bruce, Big John, Larry Overbay and Walter Vuncanon.

Most importantly I would like to thank God for the joy, peace, sound advice, and assurance, that He has filled my life with throughout the completion of this thesis.

I would like to dedicate this thesis to my husband and friend,
Byron Taylor.

Chapter 1: General Introduction

Apis mellifera, honey bees, are social insects which play an important role in pollinating much of the world's food supply, simply by foraging. One in every three mouthfuls we swallow is prepared from insect pollinated plants (McGregor 1976, Barker *et al.* 1979). So to protect our crops from the 4% of insects that are pests of economic importance (Heading 1983), pesticide sprays have become an integral part of general crop management (Lyman 1979). Protection of beneficial insects, especially honey bees, from pesticides during pollination and crop growth is critical world-wide (Johansen 1979, Mel'nichenko 1980, Metcalfe 1980, Rhodes *et al.* 1980, Ware 1980, Crane 1981, Field 1981, Melksham *et al.* 1981, Mayer *et al.* 1983, Erickson 1994). Despite this importance, the effects of pesticides on colony dynamics and how this affects pollination remains one of the weakest links in our understanding of agro-ecosystem functioning and the assurance of crop yields.

Honey bees have large workforces in comparison to other *Apis* species and this enables them to forage, and therefore pollinate, more effectively (Jamieson 1950). New Zealand estimates the value of honey bee pollination at over 60 times the value of the products and services they produce (Matheson 1997). A survey by MacFarlane and Ferguson (1984) deemed honey bees as New Zealand's most important kiwifruit pollinators as they were present in 95% of the fifty-four orchards surveyed, and were four times more numerous than the next most common insect group, the bumble bees. Pollination is also critical to the United States of America, where their annual value of crops pollinated by honey bees is around 24 billion dollars, and commercial bee pollination produces an annual profit of around 10 billion dollars (http://www.cyberbee.net/research.htm).

Beekeepers, pollination companies, and scientists alike, are interested in the impact that pesticides have on the entire colony. To understand the immediate and long–term effects that pesticides cause it is important to study the bees' behavioural responses to pesticides in both the laboratory and the field.

An intimate relationship exists between a honey bee colony and the environment because the workforce primarily forages within 2km of its hive, but occasionally forages up to 6km or more (Eckert 1933, Visscher and Seeley 1982, Wenner *et al.* 1991, Oldroyd *et al.* 1993). Consequently, particles from this 12-110 km^2 area, passively adhere to the branched hairs of individual workers and accumulate within the hive. This natural phenomenon of extensive, environmental sampling puts the colonies at risk of pesticide poisoning. Yet, it also centralises the colony's response to these toxic events and allows us to monitor their recovery and possibly identify the effects this may have on the surrounding environment.

To date, research on the effects of pesticides on honey bees is predominantly based on toxicity assays determined using small samples of caged bees (Johansen *et al.* 1990). The experimental end points, LD_{50} values[1], of these studies may be inappropriate for the field as captive honey bees behave differently to those from established colonies. The LD_{50} values do not account for field variables nor inform beekeepers and scientists of the effects that pesticide exposure has on the recovery of the colony, pollination effectiveness, or honey production.

The toxicity of a pesticide to a colony is typically evaluated by counting dead bees. This classical method analyses pesticide residues in relation to mortality (Atkins and Kellum 1978), and is able to retrospectively identify detrimental events for further analysis. Like the toxicity assays, dead bee counts do not provide a "real-time" holistic view of the colony's initial response, or the recovery process, relating to toxic events. The ability to detect the initial stages of colony adversity and hive annulment through "real time" data, increases the accuracy and usefulness of research conducted in the field.

A colony that fails to forage food is unable to replenish its reserves and will only last as long as the food is available. This suggests that flight activity is a good indicator of

[1] LD_{50} is the dose (micrograms per bee) per individual honeybee which is expected to kill 50% of a group of bees in a laboratory.

foraging frequency. The University of Montana (UM) has designed an electronic honey bee counter that counts the number of incoming and outgoing bees. The flight activity of each colony is displayed on a graph and is updated in "real time". Studies completed by UM (Bromenshenk pers. comm.) reveal that a nucleus colony, containing ten to fifteen-thousand bees, makes sixty to eighty-thousand flights per day. This flight activity substantiates why pesticide residues that adhere to bee hair is subsequently transported back to the hive and becomes hazardous as it accumulates. It also justifies why the main exposure to pesticides occur when worker bees forage on treated crops (Johansen *et al.* 1990).

My research studies the response of established colonies to contact exposure of the commonly used pesticide, methyl parathion, by quantifying changes in colony composition and flight activity.

Honey bees

The European or black race honey bees, *Apis mellifera* L. (Apidae), were introduced from England to Northland, New Zealand in 1839 and from Australia to Nelson in 1842. Due to the increased use of Italian queens, after their introduction in 1880 and the 1950 ban of bee imports[2] (Matheson 1997), the Italian race is now predominantly used on a commercial basis, for pollination and high honey production in New Zealand. The Italian race of bees is also common in the United States of America and was used in these studies. Throughout this thesis I will refer to honey bees as bees and identify other *Apis sp.* specifically.

Honey bees are vegetarians, foraging mainly on nectar and pollen from plant blooms, sugar syrup and honey-dew. This highly integrated society of social insects is made up of three castes; queens, drones, and workers. Each caste has distinct body characteristics,

[2] Except for quarantined Italian honey bee semen introduced in the early 1990's to improve New Zealand's bee stock (Matheson 1997).

and developmental stages. A queen may live 1 to 3 years and lay up to 1500 fertilised eggs per day. She achieves this by mating with 7-17 different drones until she has stored 5-6 million sperm in her spermatheca (Matheson 1997). Drones are male bees whose primary function is to mate with virgin queens. Sterile female worker bees make up the majority of the hive and perform tasks associated with their age called "division of labour". These include the gathering and processing of food, caring for brood, regulating hive temperature, and defending their colony. The workers live 4 to 6 weeks during summer, 4-8 during autumn and about 20 weeks during winter (Johansen & Mayer 1990, Matheson 1997).

The body of a worker bee is specially adapted to make it an effective pollinator. Branched hairs, antenna cleaners, and pollen baskets (corbiculae) help the workers collect and transfer pollen to the hive to make bee-bread for larvae. Pollen contains protein, minerals, fats, vitamins and trace elements critical for honey bee growth (Matheson 1997). To rear brood, a commercial colony collects between 15 and 55 kg of pollen each year. A typical 15mg load of pollen is obtained by visiting between 1-500 flowers. This means a colony makes at least 1.3 million foraging trips to collect 20kg of pollen (Matheson 1997).

Methyl parathion

Methyl parathion, (0,0-Dimethyl 0-p-nitrophenyl phosphorothioate) was chosen for these studies as it is USA's most widely used organophosphate insecticide (Bennett *et al.* 1990). This organophosphate was developed as a result of World War II nerve-gas research and is a potent neurotoxic agent that kills insects and other animals by disrupting transmitters in their nervous systems (Lowell 1979, Lyman 1979, EWG 1999). It is used as a pesticide in New Zealand and the United States of America to protect agricultural crops such as apples, peaches, pears, rice, wheat, sugar beet, peas, onions, and cotton.

In the United States of America, a law was passed in 1996 for the Environmental Protection Agency (EPA) to reassess the tolerance levels of hundreds of pesticides by

August 1999. Methyl parathion was included in this assessment because of its toxic effect on the human nervous system. The EPA was directed to apply "an additional tenfold margin of safety" for infants and children as the Environmental Working Group estimates that more than 1 million children consume "an unsafe dose" of organophosphates each day. A "restrictive-use" ban was enforced in August 1999, but of the 1.9 million kilograms of methyl parathion that were applied to 2 million hectares in 1998, 75% of the kilograms and hectares produced cotton, corn and wheat, and these remain unaffected by the ban until the completion of further research. (The New York times 1999).

Two forms of methyl parathion are used to spray crops, emulsifiable concentrate (EC) and a microencapsulated (ME) form, often referred to as Penncap-M. ME was introduced for commercial use in 1974 (Lowell 1979) and was found to reduce the handling risk for applicators because dissipation of the pesticide was slowed by the polymeric capsules, approximately 30 to 50μ in diameter (Barker *et al.* 1979). This increased the residual activity to >4 days in the field, at 0.56 kg/hectare, compared with <1-3 days for the same EC dose (Johansen *et al.* 1990). Numerous studies confirm that residual action determines whether a pesticide can be safely used on blooming crops because as the residual activity increases, so does the risk to honey bees (Johansen 1979). Anything less than 8hrs is of minimal concern as it can be applied at night, whereas pesticides with residual times longer than 8hrs are not safe to use (Johansen *et al.* 1990). The capsules, similar in size to pollen grains have been proven to adhere to branched bee hairs, transported back to the hive in the corbiculae, and stored in the pollen reserves for up to 7 to 14 months (Burgett & Fisher (1977), Stoner *et al.* (1978), Lowell 1979, Willis 1992). Delayed breaks in brood cycles were seen from season to season as the bees that ate this contaminated pollen died.

Despite the hazard of ME methyl parathion to bees, it continues to be used because the benefit of lowering the acute toxicity to humans, without lowering its effectiveness, has increased the benefit-risk ratio (Lyman 1979, Lowell 1979). Laboratory studies by Atkins and Kellum (1978) showed that a dusting of the EC formulation was twice as toxic to honey bee workers than the encapsulated formulation, but the residue of ME persisted

four times longer. This supported field observations where workers foraging ME were able to make double the number of trips, than those foraging EC, before they accumulated a lethal dosage that consequently affected the entire colony. It is now confirmed that when bees forage sprayed areas, colonies are readily destroyed or damaged by encapsulated methyl parathion and that it is too hazardous to apply to any area at any time when bees are within 1.6km of the treated area (Barker *et al.* 1979). For this reason, and the fact that results could be observed immediately, a soluble solution of 99% methyl parathion mixed in methanol was used in these studies.

The hives used in this thesis differ from standard bee keeping equipment to enable flight data recording as well as effective brood nest sampling, quick identification of queen presence, and easier hive relocation. The following explanations describe this equipment and the terminology used to define it.

Nucs

A nucleus colony, or "nuc", is a small colony that occupies less than a standard hive box, 505 x 405mm. A nuc hive is a small box used to house a nucleus colony (Matheson 1997). In this thesis the term "nuc" refers to a colony consisting of 10-15,000 honey bees which is approximately twenty-five percent of a commercial sized colony. A nuc hive, (fig. 1.1.), is a stack of two hive bodies, each 230mm x 270mm x 240mm, containing five, half-sized frames (205mm x 190mm) of drawn comb[3].

[3] The honey bees have formed hexagon-shaped wax cells on a synthetically produced wax foundation that is fitted inside each frame.

Figure 1.1. A two-storey nuc hive that consists of five frames of wax comb. The metal tool on the top of one hive-body is used to pry the frames apart.

Figure 1.2. A condo containing a nuc hive. The bee counter is positioned on the front of the condo and is marked with a black circle.

Condos

The name "condo" refers to the shell, that surrounds experimental nucs (Fig. 1.2.). The bee counter attached to the front of the condo consists of 14 tunnels that determine the number and direction of the bees entering and exiting the hive by light sensors (Fig. 1.3.). The data are processed at thirty-second intervals by computer software designed by the UM team. Two temperature probes were placed between the hive bodies nearest the brood nest, to record temperature data that were processed at five-minute intervals. The front doors of the condo open and the top portion hinges back to reveal a stack of three plastic boards supporting a nuc hive that is positioned toward the back. The nuc covers a large hole beneath which a trough-shaped dead-bee trap is situated. The front, back and side walls are made of glass, and the base is part wood, part screen (Fig. 1.4.). Below the screen is a dish to collect pollen when a pollen-excluder[4] is inserted between the hive and the top plastic board. The front section of the middle plastic board is non-existent as this creates a passage that leads to the 14 tunnels of the bee counter.

[4] A pollen excluder is a sheet with holes that are the same size as a forager bees body. When the worker returns from foraging and goes through these holes, the pollen is knocked out of the corbiculae.

Figure 1.3. The condo entrance consisting of 14 tunnels.

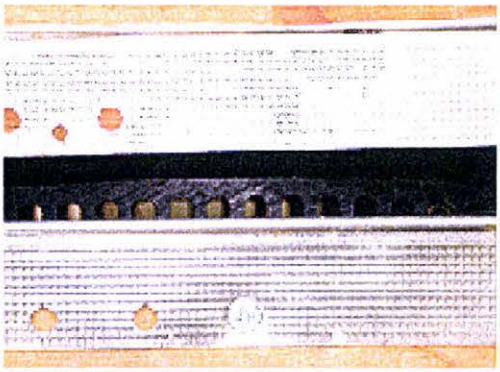
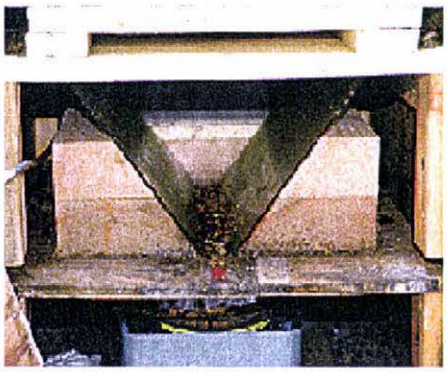


Figure 1.4. The plastic entrance at the front of the condo situated above dead bee trap.

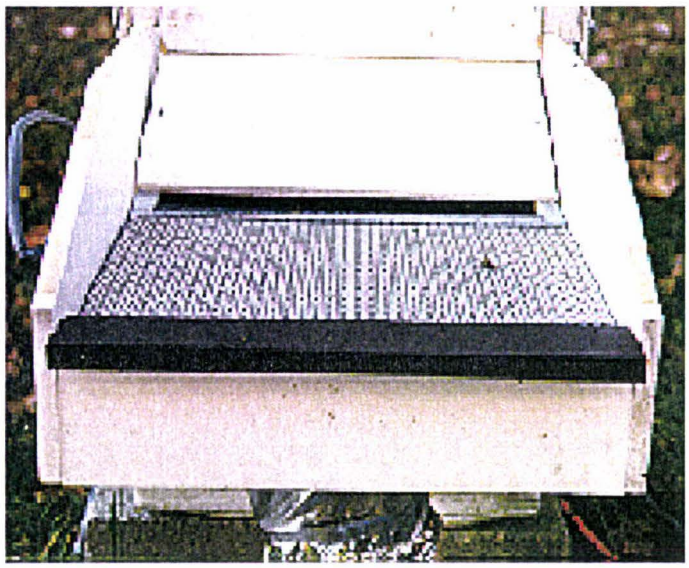


Application Porch

To quantify the effects of contact exposure to methyl parathion on the hive dynamics of *Apis mellifera* (Chapter 4), I designed an application porch that would simulate contact exposure to crop spraying. The porch (380mm x 290mm) was fitted to the front of the counter and contained a shallow well (268mm x 192mm x 20mm) with grooves in the base to create air currents. A perforated metal-screen with folded sides fitted into the well and sat flush with the porch (Fig. 1.5.). The treated filter paper was placed on this screen and the methyl parathion volatiles were extracted by a vacuum system of metallic stretch pipes containing an organophosphate filter that was attached to the base of the well. This ensured only bees outside the bee counter made direct contact with methyl parathion and those inside the hive were indirectly exposed through them. The porches were covered to minimise the effects of rain, wind, temperature, sunlight and relative humidity that all affect the rate of pesticide disappearance (McDowell *et al.* 1987).

A plastic sheet was placed on the ground beneath the porch to collect the dead bees that had been removed from the hive or may have died before entering.

Figure 1.5. The application porch that attaches to the bee counter on the front of the condo. The treated filter paper was placed on the perforated metal screen that the bees were required to walk over to get into the hive.



Methyl parathion is known to kill bees but the quantitative effect that it has on colonies and ultimately pollination is unknown. The impact of pesticide spraying near *Apis mellifera* colonies is embedded in the time of application and associated with the flight dynamics specific to a location. The aims of this thesis were: 1) To identify and quantify changes that occur in honey bee colonies as a result of an application of methyl parathion. 2) To determine if and how the colony returns to a status quo, and whether the hive can continue to be used for bio-monitoring, pollination and honey production once they have been exposed to methyl parathion. The experiments were based on the hypothesis that honey bee colonies dosed with methyl parathion become an ineffective foraging force that consequently decreases pollination.

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