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



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## 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.

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*I would like to dedicate this thesis to my husband and friend,  
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## 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<sup>2</sup> 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<sub>50</sub> values<sup>1</sup>, of these studies may be inappropriate for the field as captive honey bees behave differently to those from established colonies. The LD<sub>50</sub> 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

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<sup>1</sup> LD<sub>50</sub> 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<sup>2</sup> (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,

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<sup>2</sup> 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 50 $\mu$  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<sup>3</sup>.

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<sup>3</sup> 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.

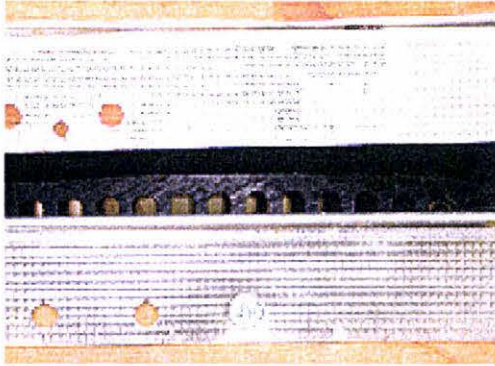


### *Condos*

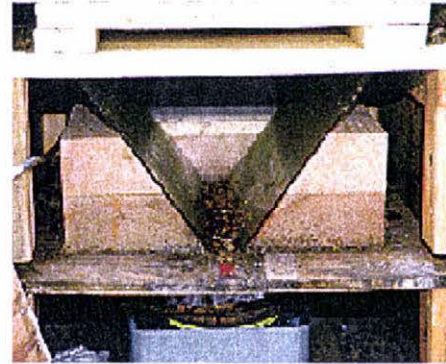
The name “condo” refers to the shell, that surrounds experimental nucs (Fig. 1.2.). The bee counter attached to the front of the condo consists of 14 tunnels that determine the number and direction of the bees entering and exiting the hive by light sensors (Fig. 1.3.). The data are processed at thirty-second 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<sup>4</sup> 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.

<sup>4</sup> 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.

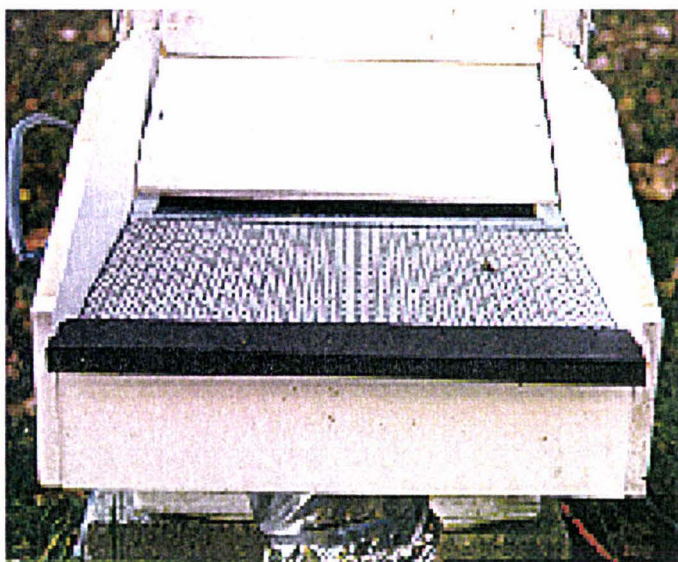


### *Application Porch*

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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## Chapter 2: Can Electronic Systems Detect Toxic Events That Occur In *Apis mellifera* Colonies?

### ABSTRACT

An electronic honey bee counter, designed to record the number of bees entering and leaving the hive, enables toxic events to be detected when they occur in *Apis mellifera* colonies. Classic dead bee counts showed that doses of 100mg, 250mg and 500mg of methyl parathion had an immediate effect on the colony. The flight data collected by the counters supported these results, and also enabled the progression of colony-recovery to be observed in “real time”.

### INTRODUCTION

In the United States of America accidental honey bee mortality was first attributed to pesticides during the 1870s, but remained unproven until fruit trees in bloom were sprayed during 1889 to 1896 (Johansen, 1977). Intentional treatment of blooming crops is now rare but bee poisoning still occurs when weeds on the edges of crops are contaminated whilst in bloom (Barker *et al.* 1979).

Lethal dosages of insecticides commonly cause excessive build-up of dead and dying bees at the hive entrance. Further symptoms include aggressiveness, stupefaction, paralysis, back spinning and abnormal rapid or jerky movements (Johansen 1977 & 1979, Barker *et al.* 1979, Atkins *et al.* 1992). Organophosphate insecticides also induce workers to regurgitate nectar from their honey sacs. This leaves the hives sticky and causes the workers to die with their tongues extended. Ultimately, the queen may cease to lay eggs in response to a declining workforce (Johansen 1977, Barker *et al.* 1979).

Effects of acutely toxic insecticides to pollinators are observed more frequently than are the effects from doses labelled as “sublethal” (Tasei *et al.* 1994). However, under certain circumstances the latter are just as detrimental to honey bees, and have been confirmed to cause death, reduce learning power and pollen collection, cause

fecundity and lifespan to decline, produce abnormal larval and pupae growth, and alter genetic fingerprints (Moriarty 1969, Robert *et al.* 1989, Tasei *et al.* 1994). Studies by Schricker and Stephen (1970, 1974a, 1974b) show that sublethal doses of parathion also cause foragers to make communication mistakes regarding distance and timing for feeding sites.

The hazards of pesticides to bees have been assessed through numerous methods. Anderson *et al.* (1971) used a combination of dead bees at the colony, colony strength, bee visitation, and caged bees. Clinch (1971) collected bees from treated crops with a vacuum, whereas Johansen (1977) exposed laboratory bees to field-weathered residues on foliage samples from treated crops. Smirle *et al.* (1984) proposed a standardised bioassay, from 17 references, that considered worker bee age and the environment, and Atkins (1992) used acute and chronic feeding tests.

All of these assessment methods analyse the effects of pesticides retrospectively and several correlate acute mortality with the presence of pesticide residue ( $LD_{50}$ ) in the laboratory. These methods are ineffective for preventing hive annulment because the analysis occurs after the toxic event has impacted the colony. Applying laboratory results to the field is often inappropriate because sublethal exposures of the pesticide are not accounted for in the laboratory. Secondly, methyl parathion residues, found on dying bees, vary from zero to excess of the lethal dosage within a single experiment (Barker *et al.* 1979, and Melksham *et al.* 1981, Smirle *et al.* 1984). Correlating field trial results with those from the laboratory may also be confounded by the fact that foragers poisoned by fast-acting compounds do not often return to their hive (Johansen 1977).

To identify the effects of pesticides on field colonies, as well as the direct effect on forager bees, we require parameters that can be quantified as these changes occur. MacKenzie and Winston (1989) previously suggested that foraging activity may be of some use, but until now, the number of bees leaving and entering a hive has been impossible to manually record (Pearson 1983). However, the development of the bee counter has enabled 60-80,000 flights that were made by a single nuc, on one summer day, to be recorded (Bromenshenk *pers. comm.*). Based on this class of data, the counter system may be able to actualise the usefulness of a “foraging” parameter.

Flight activity is susceptible to biotic and abiotic factors which complicates the usefulness of this parameter. Weather, light availability, and temperature, influence the foraging activity of bees and also determine the effectiveness of methyl parathion in the field (Crosby 1972, Spencer *et al.* 1973, Johansen 1979, Harper *et al.* 1983, Willis & McDowell 1987). Long, sunny days induce flight activity and maximise pesticide impact. Conversely, short, wet and windy days suppress flight and inhibit pesticide effectiveness. In order to use flight data when determining the effects of pesticides, it is essential for the electronic system to discriminate flight data from daily weather patterns.

The purpose of this study was to determine whether bee counters attached to *Apis mellifera* hives could detect colony response to a toxic event, and whether they could be used to monitor the colony's recovery, with the prospect of preventing hive annulment. Identifying deviations from the basal rate of "real time" flight data was the technique used to assess this.

## **METHODS**

### **Study area and sampling methods**

The experiments were conducted in an apiary at Fort Missoula, Missoula, Montana, United States of America. Approximately 7km east of Forte Missoula, a second apiary at West Campus was used to isolate untreated, and later treated, hives. Missoula is a valley that is 981m above sea level and is surrounded by five mountains. It experiences an average summer high of 28°C, compared with a winter low of -10°C (Microsoft 1994).

Packages of Italian-race honey bee workers weighing 0.9 kg, each with approximately 10,000 workers and a mated queen, were obtained in April 1998 from an apiary in Western Montana, and then established in individual nuc hives.

### Experiment 1

Vapour-exposure experiments by Boelter and Wilson (1984) showed volatile methyl parathion was released from contaminated pollen at both 10mg and 500mg concentrations. Because we were testing the ability of the system to detect acute toxic events, rather than the effect of different pesticide concentrations on the colony, we dosed two colonies with a 250mg concentration of methyl parathion, and two with a 500mg concentration. Seven nucs were selected for this experiment in early July 1998, and randomly placed in the condos at Fort Missoula. Each nuc was queen-right<sup>1</sup>, and contained eggs, larvae and capped brood. Two of the seven colonies swarmed<sup>2</sup> on the 9<sup>th</sup> of July and one did not recover to the required experimental criteria in time so it was rejected from this experiment. Three days prior to the treatment, the colonies were checked to ensure they were still of similar size and composition, and then the queen was caged in the centre frame of the lower hive body, to prevent experimental damage. Two of the six remaining colonies were used as controls, two were dosed with 250mg, and two with 500mg of methyl parathion.

On 20<sup>th</sup> July 1998, 19.03g  $\pm$  0.02g of freshly collected pollen was measured into six treatment beakers, and 0.165ml of ethylene glycol, which is non-toxic to bees at low doses (Standifer 1972, Moffett *et al.* 1973), was added to each as a surfactant. Methanol was used to dissolve the 99% concentrate methyl parathion supplied by Radian International, USA, and 8 ml of methanol containing the specified amounts of methyl parathion were mixed with the pollen and spread onto plastic petri dishes containing drawn comb. The treated pollen was tamped into the cells with individual metal rods and sealed until application at 10am.

The vapour density of methyl parathion is 9.1 times denser than air, 10.8g/l compared with 1.186g/l respectively (Appendix 1). To allow the vapour to filter throughout the hive, without the bees directly contacting the treated pollen the petri dishes were placed upside down on the top screen of their allocated hives. The hive lid was replaced and the dead bees were cleared from the dead bee trap. Subsequent

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<sup>1</sup> Queen-right means the colony has a laying queen.

<sup>2</sup> Swarming is when a group of workers and drones leave their hive, with the queen, to establish a new colony. The remaining workers continue to raise the new queen that has already been developing in a queen-cell.

collections were at 10pm each evening from 17<sup>th</sup> to 29<sup>th</sup> July. The flight data were recorded from 6am to 10pm by the computer system.

The treated pollen was removed from the tops of the hives five days after treatment. On the 29<sup>th</sup> July the status of each colony was determined and then the queens were released once the bees had been moved to the West Campus apiary. Ten hours later, seven new colonies were introduced to the condo hives to ascertain whether a new colony would be affected by pesticide residues in the previously dosed hive.

### **Experiment 2**

A second experiment was designed to evaluate the system's ability to detect and monitor toxic events for various strength colonies. A weak colony (containing six or less frames of bees) and a strong colony (greater than eight frames of bees) were dosed with 100mg of methyl parathion. A second set of colonies was dosed with 250mg, and a third set was used as experimental controls.

On 6<sup>th</sup> August 1998, the same methods were employed as in experiment 1 except: Dead bees were collected but their numbers were not analysed for this study; 16.60g  $\pm$  0.01g of pollen was combined with the methyl parathion, instead of 19.03g  $\pm$  0.02g; the queens were not caged, just restricted to the bottom hive body by a queen excluder; and a second group of colonies were not introduced to the dosed hives. The methyl parathion treatments were removed 15 days after treatment and the hives were taken to the West Campus apiary.

### **Data analysis**

The dead bees from each hive were collected and tabulated. The extent of mortality was categorised using Atkins *et al.* (1970) values that were determined using Todd dead bee traps<sup>3</sup>. Normal mortality for a commercial sized hive is less than 100 bees per day (Atkins *et al.* 1970). A low kill is considered 200-400 dead bees, 500-900 is moderate, and more than 1000 bees per day is a high kill. With these data as a basis, the results from each dose were averaged and the standard errors, where  $SE = \sigma/\sqrt{n}$

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<sup>3</sup> A Todd dead bee trap is a modified wooden hive base. The base has angled slats that the dead bees fall through onto a removable board for their collection.

(Martin *et al* 1993) were calculated. Any number greater than 100 was considered abnormal. The dead bee data were graphed using version 4 of the program Deltagraph.

The flight data from both experiments were analysed by colour mapping the total number of incoming and outgoing bees. The six bars of the colour map represent the six hives and each is read in two dimensions. Successive days are added to each bar from top to bottom, whereas the duration of the day is represented by the width of the bar, from left to right. The hotter colours denote greater flight activity. These Deltagraph colour maps were used visually to compare the colony response to dosages of methyl parathion in order to determine the usefulness of the bee counters for collecting flight data. Colourmaps from experiment 2 were used to identify any possible effects related to pesticides, that could be attributed to colony size.

## RESULTS

### Experiment 1:

The average dead bee counts obtained from the dosed hives two days prior to colony treatment are presented in Table 2.1. Mortality returned to normal on day 2, but all colonies were affected by the introduction of the treated pollen at  $t_0$  (Fig. 2.1). Six hours later, the four colonies treated with methyl parathion were obviously irritated and those dosed with 500mg hung in a beard formation on the front of the hive for the first two nights. High bee kills were collected from both the 250 and 500mg hives for these first 36 hours post-treatment, and then moderate kills were obtained from the hives up to 156 hours. Low kills were collected from the 250mg set of hives until they were removed from the condos at 228 hours, whereas the 500mg set had low mortality until 204 hours, which reduced to normal by 228 hours.

Flight activity was inversely proportional to dead bee data because as it decreased, bee mortality increased. At the beginning of the experiment all six hives were making between 75 – 90,000 trips per day (Fig.2.2). On dose day, day 3, the flight activity of the four methyl parathion treated hives dropped to less than 60,000 flights per day, and by 84 hours had declined to less than 40,000 flights per day. The first control

colony exhibited more than 90,000 flights per day at the start of the experiment, whereas the second control made 65,000 flights per day. By the end of the experiment the activity of the first control had declined to 65,000 flights per day and the flight activity had increased 90,000 flights for the second control.

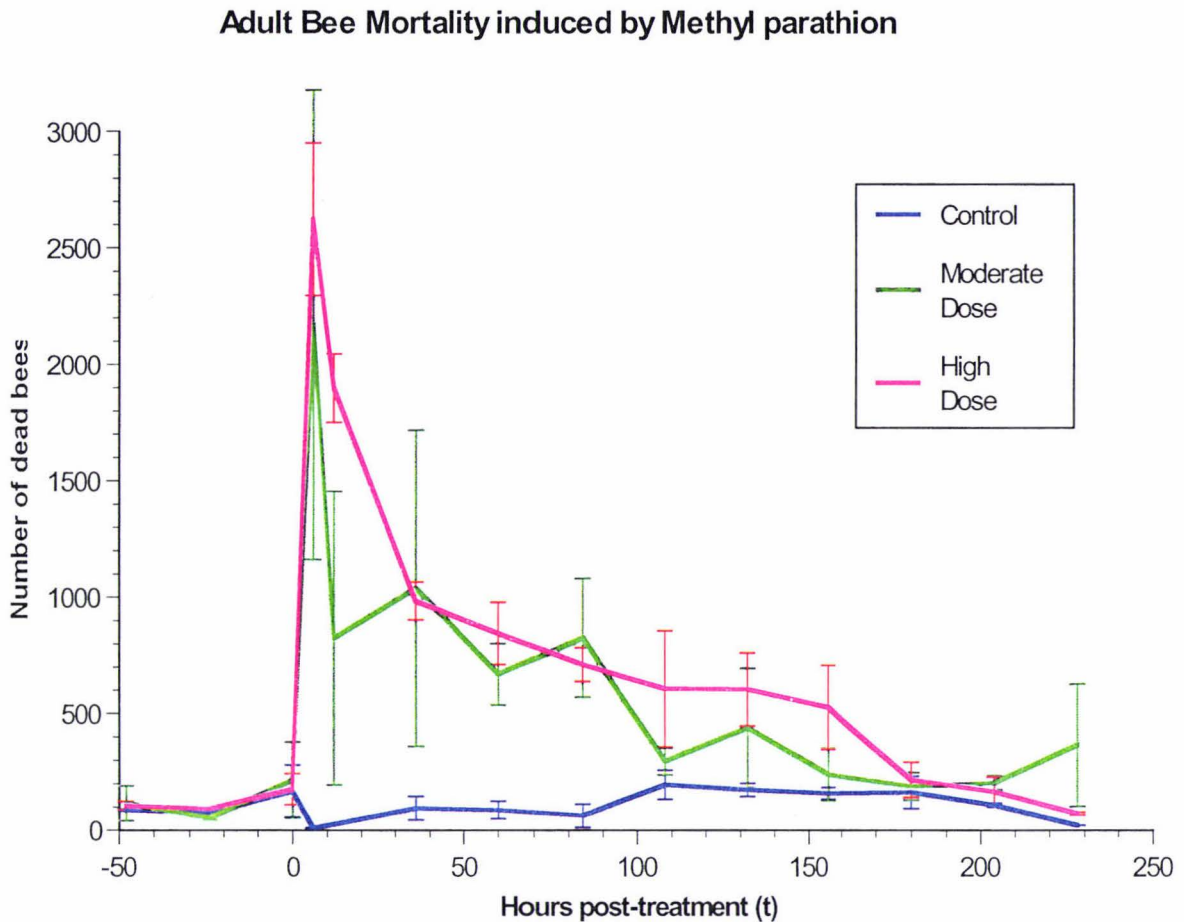
All six queens, surrounded by a small amount of brood, survived experiment 1.

The replacement colonies that were introduced to the control hives, and the hives dosed with 250mg, did not appear to be affected by methyl parathion residues. However, moderate and low dead bee counts were obtained from the colonies that were introduced to the hives previously dosed with 500mg hives (Table 2.1.). Because sample size (n) was only 2, the standard errors of the dead bee data varied from 9 to 1008 for the results from within a colony. Between colony variance ranged from 1.5 to 1008. The results were not analysed further because the standard error suggests the statistical accuracy would be small.

**Table 2.1.** The mean number of dead honey bees collected from colonies that were treated with pollen dosed with 250mg or 500mg methyl parathion, during July 1998 in Missoula, Montana, United States of America. N = 2. SE = Standard Error.

Day	Hours Post-Treatment (t)	Methyl parathion dosage								
		Control		250 mg		500 mg				
			SE		SE		SE			
1	-48	87	n	4	115	L	75	106	L	18
2	-24	75	n	16	51	n	9	94	n	2
3	0	168	L	113	215	L	163	177	L	66
3	6	10	n	1.5	2169	h	1008	2624	h	327
3	12	26	n	1	825	m	630	1899	h	148
4	36	95	n	51	1038	h	679	985	h	81
5	60	88	n	37.5	669	m	132	847	m	134
6	84	64	n	50.5	825	m	255	712	m	73
7	108	197	L	62.5	295	L	59	608	m	250
8	132	175	L	28.5	438	m	256	606	m	157
9	156	159	L	26	238	L	112	529	m	180
10	180	164	L	70	188	L	59.5	218	L	75
11	204	109	L	9	203	L	29.5	167	L	63
12	228	22	n	1	365	L	262	73	n	6
Replacement colonies										
13	262	101	n	15.5	49	n	18	404	m	56
14	286	61	n	9.2	59	n	19.5	204	L	37
15	310	67	n	6	41	n	17	110	L	42
16	334	48	n	20	19	n	4.5	128	L	1.5
n = Normal    L = low kill    m = moderate kill    h = High kill										

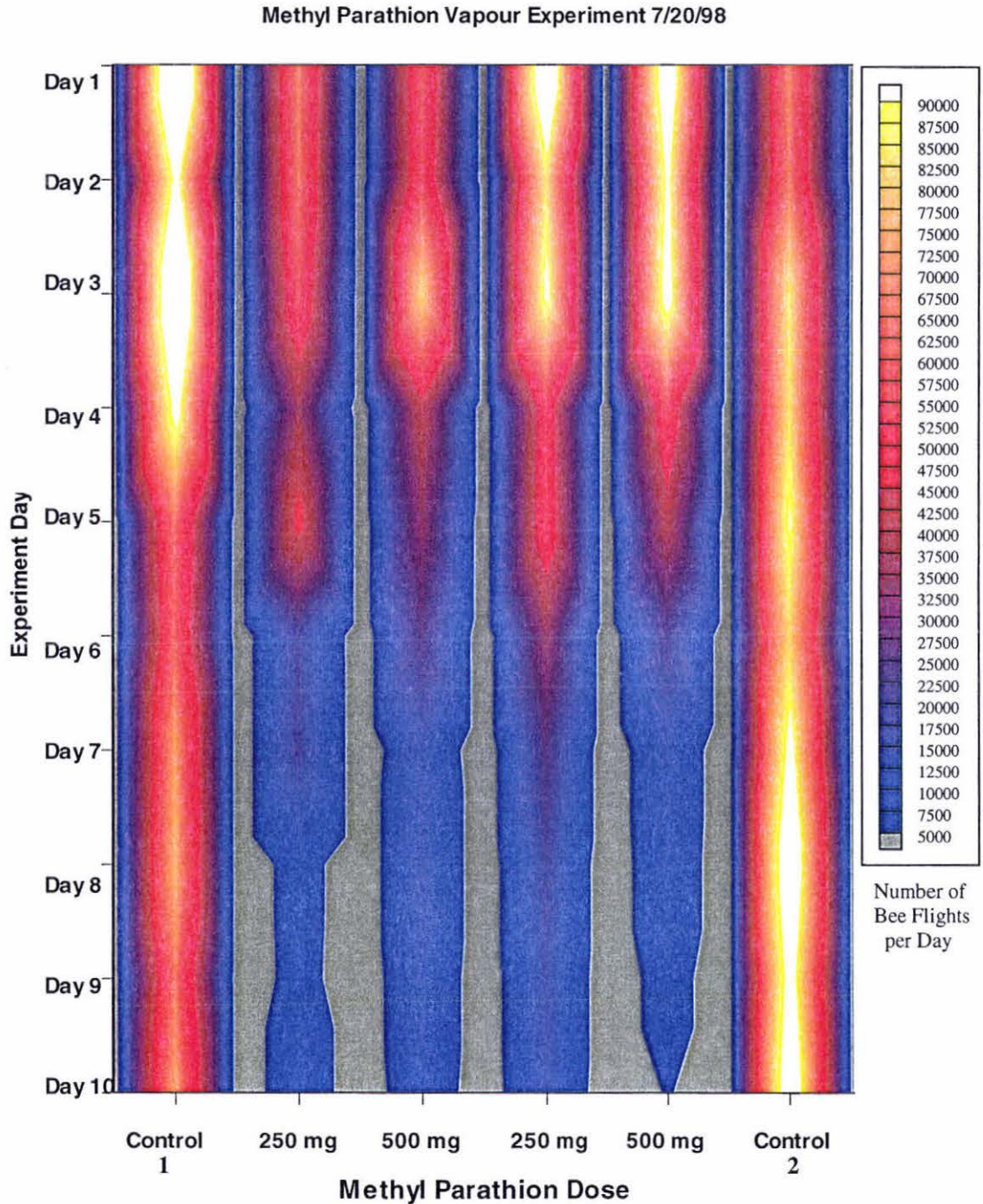
**Figure 2.1.** Average number of dead honey bees collected from the three treatment groups each evening. The colonies were dosed at  $t_0$  where the high dose was 500mg of methyl parathion and the low dose was 250mg.



### Experiment 2:

The control colonies began the experiment making 90,000 and 60,000 flights per day. The larger one declined to 65,000 and the smaller one remained consistent, so at culmination they were both making approximately the same number of flights per day (Fig. 2.3). The two strong hives dramatically reduced their flight activity after 60 hours (8<sup>th</sup> August). The flight activity of the strong colony that was dosed with 250mg declined from 90,000 to 40,000, whereas the strong hive treated with 100mg finished the experiment with the highest activity of 50,000 flights, out of those dosed with methyl parathion. The flight activity of the weak colonies declined from 55,000 for the 100mg dose, and 30,000 for the 250mg dose, to 30,000 and 20,000, respectively.

**Figure 2.2.** Flight activity of six similar sized honey bee colonies recorded by electronic bee counters. Two hives were dosed with 250mg of methyl parathion and two hives were dosed with 500mg of methyl parathion on day 3. The hotter (red and yellow) colours denote greater flight activity.





## DISCUSSION

The “low” bee mortality seen at the start of experiment 1, was expected as this commonly occurs when hives are relocated, or when hive checks have been made. These results can probably be attributed to the hive checks that were made on the 17<sup>th</sup> July. For future experiments, to allow the colonies to acclimatise to their surroundings, and for colony mortality to return to normal, hive checks and the relocation of hives to condos, should be made approximately three days before the treatment, or as far in advance as possible without decreasing the reliability of the colony status check.

The dead bee collections clearly identified the initial mortality caused by methyl parathion. This supports the literature that deems this parameter as a useful first indicator of colonies exposed to pesticides (Johansen 1977). However, the usefulness of this parameter does not extend beyond the initial identification because the number of dead bees declined each day, post-treatment. This decline of dead bees does not indicate that the colony has recovered but merely that fewer bees were dying. This became obvious when the mortality counts from the colonies that were dosed with 500mg had returned to normal by the conclusion of the experiment, yet only a small population remained in the hive. However, flight activity data supported the hive checks. The flight activity from the treated colonies declined each day post-treatment, indicating that the forager forces had declined and that the hives had not yet recovered. This inversely proportional relationship between flight activity and dead bee counts authenticates flight activity as a more accurate parameter, than the dead bee counts, for indicating the colony status over time. Consequently, dead bee counts were not analysed in experiment 2.

The first control colony exhibited more flight activity at the start of the experiment than the second control, but this declined by the end of the experiment. The flight activity may have been greater simply because it was a more active hive, and not because it was a larger population (Bromenshenk pers. comm.). Contrary to this, the control colony may have robbed energy supplies from the hives that were weakened by the methyl parathion doses and this may have killed some of its foragers.

Throughout the experiment the bees appeared to display selective behaviour. As the flight activity of the treated colonies dwindled, the flight activity of the second control colony appeared to gain workers because the flight activity increased. This control was positioned two meters from the 500mg-treated hive, which may suggest that if bees return to their hive and it is unfavourable, they will endeavour to join a neighbouring hive that is unaffected. This is contrary to the literature which states bees are loyal to their colony (Johansen 1977).

The irritation and clustering behaviour displayed by the colonies that were exposed to methyl parathion are classic responses exhibited by honey bees when the temperature outside their hive rises above 21°C (Atkins *et al.* 1977). It is not known what caused the bees in this experiment to hang on the outside of the hive at night, but there are two possible explanations. Firstly, the concentration of the pesticide may have caused a suffocating effect strong enough to drive the bees out. Secondly, as the bees have tried to remove the vapours from the hive, the increased activity has increased the hive temperature. When hive quality deteriorates by predation, fire, pests, overheating, or pesticides, the expected response is for the colony to abscond (Fletcher 1975-76, Winston *et al.* 1979, Schneider 1990). However, the colony did not abscond because the queen was caged, and even though the majority of the colony hung outside the hive for 36 hours, due to unfavourable conditions, the queen survived. This suggests the colony was able to adjust to the hive atmosphere or manipulate it to become tolerable.

The hives that were initially treated with 500mg doses of methyl parathion contained residues that caused the replacement colonies to experience moderate to low bee mortality. This is thought to be the result of the beeswax comb absorbing methyl parathion vapours (Boelter *et al.* 1984). Residue levels are effected by numerous factors (Melksham *et al.* 1985), so to prevent any unknown contact with methyl parathion, future experimental colonies should be allocated to hives that have not been in contact with pesticides.

Experiment 2 showed that the electronic system could detect differences in hive size and revealed that even though the workforce of large colonies were more seriously affected than those of smaller colonies, larger colonies had a better chance of

recovering from toxic events. This is because they have more foragers to be exposed to the pesticides, and this often results in up to four times more mortality (Johansen 1979, Johansen *et al.* 1990). Once initial mortality occurs the colony suffers no additional loss of adult workers as few or no bees forage until new bees emerge (Johansen *et al.* 1990). To compensate for the decline in the workforce, young bees are then forced to forage earlier than the usual three weeks after development (Sanford 1983). Because the queens in experiment 1 were confined to a small area for 13 days, they were unable to lay sufficient brood to compensate for lost workers. As a result, the colonies had significantly reduced workforces and the hives contained only small amounts of brood at the conclusion of the experiment. Since the experimental methods had altered colony behaviour and prevented the analysis of colony recovery, the queens were not caged during ensuing experiments.

In conclusion, the University of Montana's electronic bee counter system can detect, and suitably monitor, acute toxic events. These "real time" flight data have enabled the progression of field experiments to be observed and for timely modifications to be implemented. Studying changes in the flight activity of bee colonies in "real time" is an important step towards protecting hives from colony poisoning. It is probable that such data will enable techniques to be devised that will prevent severe crop losses due to lack of pollination.

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## Chapter 3: Contact Response of *Apis mellifera* to Methyl Parathion

### ABSTRACT

Experiments were designed to determine whether *Apis mellifera* would accept or avoid direct contact with methyl parathion that has been applied to filter paper. Presence/absence observations were used to identify that bees initially avoided direct contact with methyl parathion, but the least disturbing exposure duration was thirty minutes after treatment as the initial avoidance response had dissipated.

### INTRODUCTION

Application drift, volatilisation and wind erosion of residues often cause humans, animals and plants to become unintentionally exposed to pesticides (Spencer *et al* 1973, Lewis *et al.* 1976, Seiber *et al.* 1980). Pesticides kill honey bees through direct contact, stomach poisoning, and fumigation (Atkins 1992). The vapours are absorbed through the respiratory system, stomach poisons are absorbed through the alimentary canal, and contact poisons are absorbed through the integument.

Pesticide uptake by honey bees, and how they affect the colony, is influenced by meteorological conditions, formulation, chemical properties of pesticides, and application methods (Taylor 1979, Lyman 1979, Seiber *Et al.* 1980).

The exposure-surface of a pesticide needs to be considered when designing the method of application because it alters the effect on honey bees (Mayland & Burkhardt 1970). Numerous surfaces including glass (Way & Synge 1948), grease-proof paper (Jones & Connell 1954), and filter paper (Beran & Neurer 1956, Beran 1958 & 1963), have been used to study these effects. No specific surface material has been recommended for simulation of field conditions in the laboratory since 1970 because bee mortality counts from treated leaf-surfaces are inconsistent and do not allow for comparisons to be made between glass, and plastic surfaces (Mayland & Burkhardt 1970). However, Mayland and Burkhardt (1970) were able to conclude that volatilisation of toxic chemicals was unaffected by the type of absorbing surface.

Based on these studies, experiments were designed to determine whether *Apis mellifera* would accept or avoid direct contact with methyl parathion when applied to filter paper. Presence/absence observations were used to identify whether the bees would walk on the methyl parathion saturated filter paper, as well as determining, after the paper had been treated, the least disturbing exposure duration for the bees.

## METHODS

Mated queens and their associated 0.9kg packages of Italian-race honey bees, were transported in April 1999 from Georgia, United States of America, to Edgewood, Maryland where they were established as nucs.

Only forager bees were used in this experiment because the majority of pesticide poisoning occurs when worker bees forage in treated crops (Johansen *et al.* 1990). A second reason was because older bees are more susceptible to methyl parathion than younger bees due to their lower brain concentrations of acetylcholinesterase (AChE). This enzyme is required to hydrolyse the neurotransmitter, acetylcholine, which is blocked by methyl parathion (Koch 1958 & 1959, Ladas 1972, Mayland *et al.* 1970, Nazer *et al.* 1974).

As the foragers returned to the front of the hive they were collected in a wire cage that was covered to reduce light. A wooden frame (470mm x 376mm x 51mm), covered with wire screen on the base and lid (Fig. 3.1), was used to dose the bees. The enclosure was divided into a 3 x 4 grid, of 120 x 112mm squares. Between the squares was a 4mm diffusion buffer and for each trial a new piece of filter paper was placed on each of the 12 sections.

250mg of 99% pure methyl parathion was dissolved in 25ml methanol and then mixed with 7.825l of water to form a solution equivalent to a field application of 0.56kg/hectare. Six squares were randomly chosen and dosed with 20ml of this solution and the remaining six were dosed with 20ml of methanol.

### **Experiment 1:**

The aim of this experiment was to determine whether honey bees would avoid contact with methyl parathion treated filter paper. 100 forager bees were introduced to the dosing-grid, which was covered to calm the bees. After ten minutes the presence/absence of the bees was recorded for each square. The bees were removed and the filter paper was dosed with methyl parathion. 100 new bees were introduced to the enclosure and the presence/absence of bees in each square was recorded at 10, 30, 50, and 60 minutes post-treatment.

### **Experiment 2:**

To remove the possibility that volatiles in experiment 1 may have effected the behaviour of the bees over time (Taylor 1979), sets of 100 bees were exposed to treated filter paper that had been dosed 15, 30, and 75 minutes prior. The bees were covered for ten minutes after introduction to the enclosure, to let them to settle down before recording the squares of filter paper that contained bees. This also enabled determination of the time duration since the paper had been dosed, that caused least disturbance to the bees.

## **RESULTS**

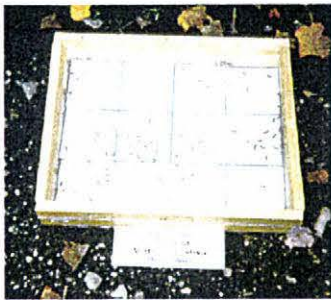
### **Experiment 1:**

The bees that were introduced to the enclosure as soon as the filter paper had been dosed, fanned their wings constantly. Ten minutes after dosing the paper, all the bees were clustered on the left hand side of the screen roof above the treated filter paper (Fig. 3.1.) After 30 minutes the bees had ceased fanning and were seen to be randomly located throughout the entire enclosure as all six treated and untreated squares contained bees (Table 3.1). At 50 minutes the bees were still present in all of the treated squares, as well as five of the six non-treated squares. There appeared to be a preference for the left-hand side, so the enclosure was turned 180 degrees in azimuth. At the end of 60 minutes the bees were dispersed more evenly across all of the squares, which may suggest the left-hand side was preferred. However, the bees

did not appear to be averse to the methyl parathion. This experiment was repeated and similar results were observed.

**Figure 3.1.** Methyl parathion acceptance/avoidance experiment where the same set of bees was observed at -10, 10, 30, and 50-minute intervals after dosing the squares of filter paper with 0.56kg/hectare. The squares are labelled from one to twelve across, and down the page. Squares 1, 4, 5, 7, 9, and 10 were dosed.

10 minutes pre-treatment:



10 minutes post-treatment:



30 minutes post-treatment:



50 minutes post-treatment:



60 minutes post-treatment:



**Table 3.1.** The presence (P) or absence (A) of 100 honey bees in the 12 grid-squares dosed with methyl parathion at -10, 10, 30, 50, and 60 minutes post-treatment. The presence percentage is included.

Time of Dose ( $t_{\min}$ )	Presence of honey bees															
	Dosed Squares						Total	%P	Undosed Squares						Total	%P
	1	4	5	7	9	10			2	3	6	8	11	12		
-10	P	P	P	P	P	P	6/6	100	A	P	P	P	P	P	5/6	83
10	A	A	P	A	A	A	1/6	17	A	A	A	A	A	A	0/6	0
30	P	P	P	P	P	P	6/6	100	P	P	P	P	P	P	6/6	100
50	P	P	P	P	P	P	6/6	100	P	P	P	P	P	A	5/6	83
60	P	P	P	P	P	P	6/6	100	P	P	P	P	P	P	6/6	100

**Experiment 2:**

At 15 minutes post-treatment the bees appeared to be avoiding the squares dosed with methyl parathion as they were present on only 2 of the 6 squares (Fig. 3.2.). At thirty minutes the bees were present on all of the dosed squares whereas the bees introduced 75 minutes after dosing the filter paper were located only on three of them. It was assumed that the bees were randomly located in the enclosure after 30 minutes.

**Figure 3.2.** Methyl parathion delayed introduction acceptance/avoidance experiment. Where three sets of bees were observed at 15, 30, and 75 minutes post-treatment of 0.56kg/hectare of methyl parathion. The squares are labelled from one to twelve across the page, and down. Squares 2, 3, 5, 7, 10 and 12 were dosed.

**15 minutes post-treatment:****30 minutes post-treatment:****75 minutes post-treatment:**

**Table 3.2.** The presence (P) or absence (A) of three sets of 100 honey bees in the 12 grid-squares dosed with methyl parathion introduced 15, 30, and 75 minutes post-treatment. The presence percentage is included.

Time of Dose ( $t_{min}$ )	Presence of honey bees															
	Dosed Squares						Total	%P	Undosed Squares						Total	%P
	2	3	5	7	10	12			1	4	6	8	9	11		
15	A	P	P	A	A	A	2/6	33	P	P	P	A	P	P	5/6	83
30	P	P	P	P	P	P	6/6	100	P	P	A	P	P	P	5/6	83
75	P	A	P	A	P	A	3/6	50	P	P	P	A	P	A	4/6	67

Appendix 4 discusses experimental observations made when methyl parathion treated pollen was placed inside the hive. However, as they were not directly relevant to this thesis they are included for interest.

## DISCUSSION

A soluble solution of methyl parathion was used in the experiment as it is less hazardous to honey bees than formulations of dust, wettable powders, flowables, emulsifiable concentrates and soluble powders (Johansen *et al.* 1990).

The avoidance of all 12 squares for the first ten minutes post treatment may possibly be in response to the dampness of the filter paper. The constant fanning of the bees wings over the pesticide has also been recorded (Mayland *et al.* 1970) who said this caused small particles to become airborne and taken into the respiratory system which then led to suffocation. Experiment 1 was not run long enough to see the end results, but it appears the fanning may have helped the treatments to dry quickly and the vapours to disperse because all the bees appeared to be randomly located on the base of the enclosure after 30 minutes post-treatment. Experiment 2 supported results from experiment 1 where the bees appeared to be more randomly located after 30 minutes than at the 15 and 75 minute checks.

Residues predominantly decline by volatilisation for both stable pesticides that have been applied to surfaces, and volatile pesticides (Seiber *et al.* 1979). This could suggest that the methyl parathion volatiles may have produced some unknown effects. To remove this possibility, in future experiments, the volatiles should be extracted using an organophosphate filter.

From this we can conclude that if the dosed filter paper is left to dry for 30 minutes then it causes less disturbance to the colony than immediate exposure to the treated filter paper.

Direct contact was the method of application used for this experiment and since the bees did not appear to avoid the treated filter paper after 30 minutes, this method of application should be employed for future studies.

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## Chapter 4: The Effects of Methyl Parathion on the Colony Dynamics of *Apis mellifera*.

### ABSTRACT

This study determines the effects on established *Apis mellifera* colonies of two field doses, and the LD<sub>50</sub> concentration, of methyl parathion. Dead bees, flight activity, percent return of foraging bees, and brood composition were studied to quantify changes in colony behaviour associated with pesticide exposure, and to determine how these changes may impair pollination effectiveness. Flight activity and percent return of *Apis mellifera* declined as a result of methyl parathion, whereas bee mortality increased during treatment and then returned to normal. Brood composition took longer to respond but did eventually decline.

### INTRODUCTION

The fact that pesticides kill honey bees has initiated numerous studies to determine how hazardous pesticides are to bees. Pesticide impact is often quantified by the extent of mortality that occurs in small groups of bees exposed to a specified concentration of a pesticide (Smirle *et al.* 1984). Johansen and Mayer (1990) have extensively reviewed specific bee poisoning data for insecticides. They conclude that for any pesticide considered toxic, there is a dose below which it causes no harm. However, because their results are based on laboratory studies they do not discuss the effect that pesticides have on colony dynamics, or how this ultimately effects pollination effectiveness. Even though a pesticide may appear to cause only a low to moderate kill, this may be enough to interrupt pollination, and even hive annulment in the long-term. Consequently, crop yields diminish and this type of long-term loss far exceeds immediate monetary losses caused by bee poisoning (Johansen *et al.* 1990).

Studies that determine the effects of pesticides on the behavioural dynamics of a colony are limited to those that discuss the effects in terms of the entire colony, rather than changes to individual parameters (Melksham *et al.* 1985). Johansen *et al.* (1990) maintain that it is flower contamination that kills bees, not contaminated beehives. However, to determine the true cause of a colony's ineffectiveness or annulment, it is

necessary to identify how pesticide contamination alters the dynamics of honey bees within a colony.

This study determines the effects on established *Apis mellifera* colonies of two field doses, and the LD<sub>50</sub> concentration, of methyl parathion. Dead bees, flight activity, percent return of foraging bees, and brood composition are the four characteristics studied to quantify changes in colony behaviour associated with pesticide exposure and determine how these changes may impair pollination effectiveness.

## METHODS

In this study the effects of contact exposure to specified field applications of methyl parathion were quantified by two methods: Firstly, dead bees and weekly hive composition were manually recorded. Secondly, flight and temperature data were collected electronically.

Eighty colonies of Italian race honey bees, *Apis mellifera*, each with a 1999 laying queen, were established in a stock-group at Edgewood, Maryland. Thirty queen-right colonies, each in a two-storey hive that contained eggs, larvae, capped brood, four frames of honey, and at least seven frames of bees were chosen to form a stock group. A queen excluder was inserted between the top and bottom hive bodies to limit the queen's laying space to the five bottom frames and enable the workers to store honey in the top frames.

The experiments were limited to seven electronic condos (described in General Introduction), and three of these doubled as the controls for an unrelated project. One of the four colonies to be treated was used to determine the appropriate range for the three concentrations of methyl parathion, and identify the duration of colony recovery after being treated with a medium dose. Because the number of sample hives was limited the experiment was repeated three times.

At least 5 days prior to each trial, four colonies of similar sized populations were randomly chosen from the stock-group and taken 18km, NNE, to the Churchville

study site (Fig. 4.1.). This time enabled the bees to sufficiently acclimatise to their surroundings (Moffett *et al.* 1983) and randomly allocated condo, so the dead bee counts returned to normal and a base line for daily flight data was established.

**Figure 4.1.** Study site set up of six condos and a nuc in Churchville, Maryland, USA. The metallic tubes attached to the porch remove the methyl parathion volatiles through a vacuum system.



The three dose days, “day 4” in the analysis, were August 19, September 10 and September 26. These dates were chosen because nearby crop spraying was complete by late summer/early autumn, and also because the colonies were fully functional in terms of population size, brood production and honey storage.

The LD<sub>50</sub> value for caged honey bees, 0.11 $\mu\text{g ai}^1/\text{bee}$  (Johansen *et al.* 1990), was used to determine the range of methyl parathion doses for the three test colonies. The LD<sub>50</sub> value of a pesticide in micrograms per bee can be converted to kilograms of the chemical per hectare, by multiplying by 1.12 (Atkins 1971). The required concentrations of methyl parathion were calculated by scaling the known field applications from kg/hectare, to the size of the application board (5153mm<sup>2</sup>).

The LD<sub>50</sub> dose was made by mixing 250mg methyl parathion with 1.727l methanol to form a soluble 0.56kg/hectare stock solution, and then adding 70.9ml of methanol to each 20ml of the solution (Appendix 3). The 0.11 $\mu\text{g}/\text{bee}$  dose, or 0.12kg/hectare, appeared to have no response on the colony, so the colony was treated with a typical

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<sup>1</sup> ai means active ingredient in a pesticide. In this case the ai is 99% concentrate methyl parathion.

field application of 0.56kg/hectare. This caused noticeable mortality and a dramatic decline in flight activity. Based on this, the LD<sub>50</sub> dose was designated as the base concentration, the 0.56kg/hectare field application was the medium dose, and a second commercial field application of 1.12kg/hectare was designated as the high dose.

The residual toxicity of methyl parathion is less than 3 days (Johansen *et al* 1990), and most of the pesticide loss occurs during daylight hours, 0600 to 1800 (Willis *et al.* 1992). Residual toxicity in the field is also greatly increased by low temperatures at night, which causes a high kill the following day especially when cold nights follow hot days because of the large build up of condensation on foliage (anon, 1975). So to simulate crop spraying, the allocated concentrations of methyl parathion were applied to the filter paper, using a 20ml pipette, on the fourth day of each trial, left to dry for half an hour and then placed on the application porch at 07:00 each morning, for three days. A behavioural reason for removing the treatments from the application board at 19:00, was because bees do not forage in the dark so the pesticides do not affect them at night.

To ensure the behavioural changes caused by the pesticides were the result of contact exposure, the vapours were extracted using an organophosphate filter attached to the base of the application porch. The trials were run for fourteen days in the condos and then the hives were transferred to the stock-site for the continuation of brood composition monitoring.

The effects of the methyl parathion treatments were assessed by quantifying four parameters associated with colony behaviour:

**Dead bees:** The dead bee traps inside and outside the condo were cleared daily between 16:00 and 20:00. Collecting began 3 days prior to application, until the hives were removed from the condos eleven days later. The dead bee data were normalised using a square-root transformation.

**Flight data:** The electronic bee counters recorded the number of bees entering and leaving the hive each day, for the fourteen days they were in the condos. The flight

activity of each colony was assessed by a three-step method that enabled the recovery rates of each colony to be determined:

- 1) The flight data were coded by adding the lowest flight value to each of the data values. This effectively scaled the values up. No square root transformation was required as there was no decrease in variance.
- 2) The "trial" effect was removed by treating the trials as replications of the experiment.
- 3) The controls were removed from the analysis so that a direct comparison between the treated hives could be made.

**Percent return:** To determine the percentage of foraging bees that were returning to the hive after they had been effected by the pesticide, the incoming flight data collected by the bee counters was calculated as a percentage of the bees leaving the hive  $((\text{beesIn}/\text{beesOut}) \times 100)$ . The data were normalised by a square-root transformation followed by an arcsin transformation.

**Colony composition:** Weekly hive examinations monitored the changes in hive composition. A wire grid (75mm x 75mm, per square) was used to quantify the contents of the wax cells. There were six grid-squares (33750 mm<sup>2</sup>) to each side of a frame, which equates to 1249 cells because there are 3.7 cells for every 100mm<sup>2</sup> (Harbo 1993). The contents of each square was categorised as either, eggs, larvae, capped brood, pollen, honey, empty or undrawn. ie Frame 2, side A = 2 squares of honey (side A on frame 2 contains 33% honey), 1 pollen square (16.5%), 2 squares of capped brood (33%), and 1 square of eggs (16.5%). Only the combined amount of eggs, larvae, and capped brood were analysed after a square-root transformation had normalised the data. Seven weeks of brood data were collected from the colony that was used to establish the methyl parathion doses. These data were graphed to identify changes in eggs, larvae and capped brood composition, in relation to total brood.

### **Data analysis**

Dead bee counts, hive composition and flight data were analysed by a Repeated Measures, Analysis of Variance, conducted using SPSS Base 9.0 (Statistical Package for Social Scientists). For each of the parameters three tests were used to identify

differences between means of the three trials, differences between the means of the four treatments applied to the hives, and variation that occurred within each hive. These tests were 1) Least Significant Difference (LSD), 2) Sidak, and 3) Student-Newman-Keuls (SNK). The LSD tests were more sensitive than Sidak and SNK so were often selected against to try and reduce the Type 1 error of rejecting the null hypothesis ( $H_0$ ) when  $H_0$  is true. Treatment 1 represents 0.11 kg/hectare, and treatments 2, 3 and 4 represent 0.5 kg/hectare, 1 kg/hectare and the control respectively.

The long-term recovery of each colony was monitored by the hive checks and was visually compared.

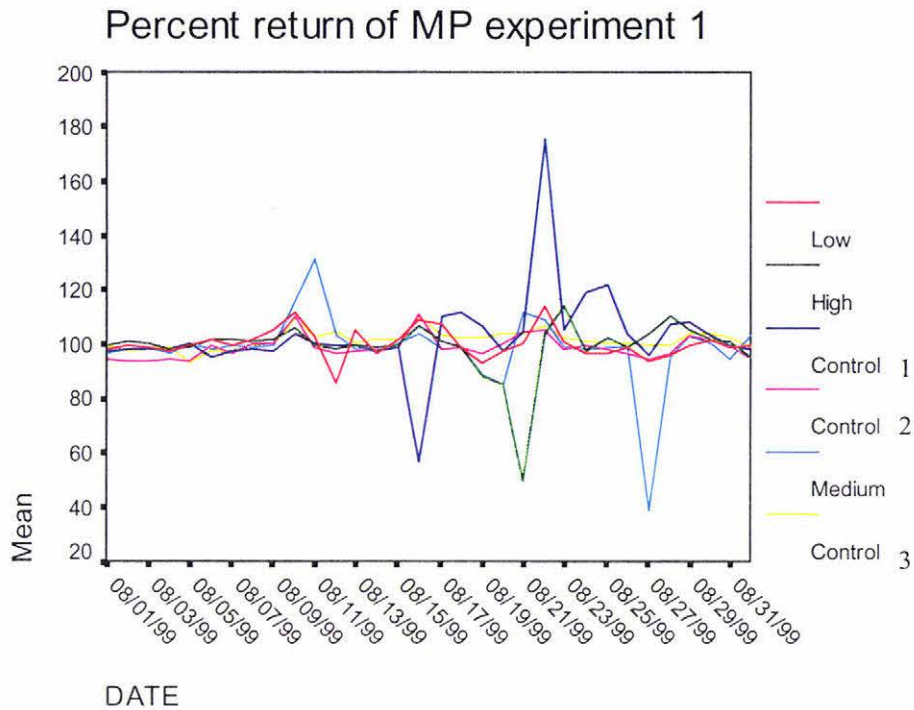
## RESULTS

One of the three control colonies requeened<sup>2</sup> during the first experiment and then absconded. This colony was rejected from analysis because its behaviour was inconsistent with the other controls, which confounded the analysis as seen in the percent return graph (Fig 4.2.). The inconsistent behaviour is the result of no eggs being laid during the time of queen maturation, which causes the brood nest to shrink and the forager force to decline.

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<sup>2</sup> A hive requeens when the present queen has become injured or when her laying capacity has decreased. This occurs either by the development of "supersedure" or "emergency" queen-cells so the hive may go without a queen for up to three weeks.

**Figure 4.2.** Percent return for the six colonies from trial 1. The line of control 1 appears noisy as a result of the colony requeening. The colonies were dosed on 8/19/99. The low hive received 0.11kg/hectare of methyl parathion. The medium dose was 0.56kg/hectare and the high dose was 1.1kg/hectare.



#### Dead Bees:

The ANOVA determined there were no significant “trial” or “trial by treatment” differences, at a 95% confidence level, but that there was a significant difference between the four treatments (Table 4.1.). The Student-Newman-Keuls analysis of treatments indicated a tiered response had occurred where each dose was only associated with its neighbouring treatments. (Table 4.2.). This is where the control counts were similar to treatment 1, the latter was similar to treatment 2, and treatment 2 was similar to treatment 3. The graphs of the daily counts of dead bees (Fig. 4.3.) show that treatment colonies 2 and 3 were immediately affected by the methyl parathion but these high dead bee counts only occurred during days 4 to 6 whilst the methyl parathion was present. This suggests that because the volatiles were being removed by the vacuum system, very little residual methyl parathion remained once the filter paper was removed. Because the number of dead bees was more than 1000 for the medium and high doses, the behaviour of the low and control doses was masked. A second graph without results from the high and medium doses showed that the low dose caused lower mortality than seen in the controls.

**Table 4.1.** ANOVA of the dead bee count data that were normalised using a square-root transformation.

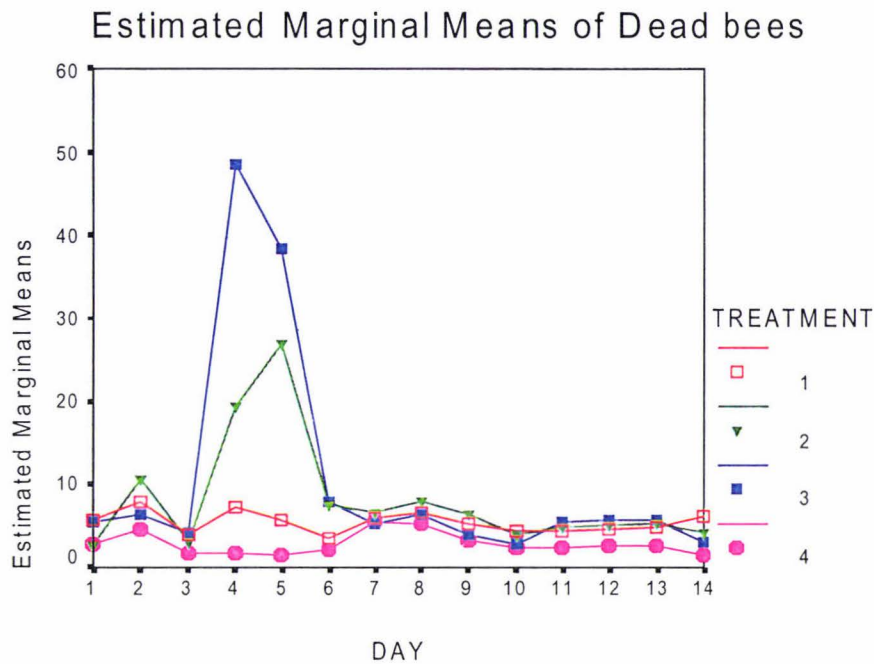
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power <sup>a</sup>
Intercept	9770.278	1	9770.278	468.395	.000	468.395	1.000
Treatment	1340.409	3	446.803	21.420	<b>.016</b>	64.260	.916
Trial	121.622	2	60.811	2.915	<b>.198</b>	5.831	.247
Treatment by trial	46.443	6	7.740	.371	<b>.861</b>	2.227	.076
Error	62.577	3	20.859				

<sup>a</sup> Computed using alpha = .05

**Table 4.2.** Student-Newman-Keuls analysis of dead bee counts that groups the means for treatments in homogenous subsets.

	Treatment	N	Subset		
			1	2	3
Student-Newman-Keuls	4	6	3.9712		
	1	3	5.7014	5.7014	
	2	3		8.6219	8.6219
	3	3			10.2396
	Sig.		0.160	.052	.181

**Figure 4.3.** Dead bee counts from the honey bee colonies that were dosed with methyl parathion throughout the three trials. The colonies were dosed on day 3. Treatment 1 was 0.11kg/hectare of methyl parathion, treatment 2 was 0.56kg/hectare, treatment 3 was 1.1kg/hectare of methyl parathion and treatment 4 was the control.



Treatment	Standard Error of the flight Activity values for each day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.3	.6	1.4	.9	.4	.4	.2	.9	.8	2.8	1.7	1.1	.6	.8
2	1.4	1.7	1.3	2.4	.4	4.0	1.7	1.2	1.5	1.9	1.8	.9	.2	.8
3	.8	.6	.9	8.1	2.3	10.11.2	.8	1.9	1.2	.9	1.3	1.7	.9	
4	1.3	.9	.6	1.1	.8	.6	.7	.9	.5	1.2	.7	.8	.7	.7

#### Flight activity:

The ANOVA for the scaled flight data determined that there was no significant trial or treatment effects (Appendix 5.B.i.). However, the non-significance for the treatment effect was only slight, as the p-value was 0.051 at the 95% confidence level, and from the graph this can be attributed to the inconsistent flight activity displayed by the controls (Appendix 5.B.ii.). To determine the significance of the treatment effects, the trials were removed by treating them as experimental replications. A second ANOVA (Appendix 5.B.iii.) revealed there was a significant difference between the treatment effects because the p-value was 0.014 at the 95% level of confidence. However, the graph was still confounded by the inconsistent control data (Appendix 5.B.iv.), so the

last step was to remove the control and compare the flight activity from each of the treated colonies. Once the trials and controls were removed, the final ANOVA (Table 4.3.) showed the treatments were significantly different with a p-value of 0.005 at the 95% confidence level. The Student-Newman-Keuls analysis (Table 4.4.) supported this and showed that the high treatment effects differed from the low and medium treatment effects which was also seen in the graph (Fig. 4.4.).

**Table 4.3.** ANOVA of the scaled flight data from the 1999 methyl parathion experiments with pooled trial data, but no control data.

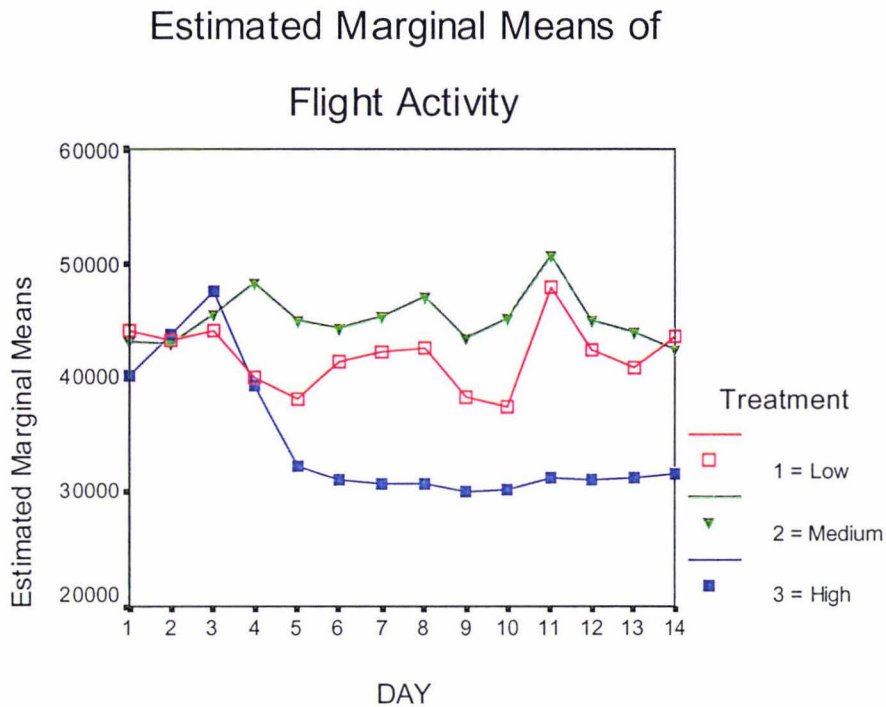
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power <sup>a</sup>
Intercept	2.07x10 <sup>11</sup>	1	2.07x10 <sup>11</sup>	2393.426	.000	2393.426	1.000
Treatment	2.54x10 <sup>9</sup>	2	1.27x10 <sup>9</sup>	14.665	.005	29.330	.962
Error	5.19x10 <sup>8</sup>	6	86579559 (6.8%)				

<sup>a</sup> Computed using alpha = .05

**Table 4.4.** Student-Newman-Keuls analysis of the scaled flight data from the 1999 methyl parathion experiments, excluding trials and controls, that groups the flight data means for treatments in homogenous subsets.

	Treatment	N	Subset	
			1	2
Student-Newman-Keuls	3	3	34489.794	
	1	3		41957.690
	2	3		45214.151
	Sig.		1.000	.160

**Figure 4.4.** Scaled flight activity data from the 1999 methyl parathion experiments, with pooled data trial and no control data. T1 is flight data from the colonies dosed with 0.11kg/hectare of methyl parathion, T2 is the 0.56kg/hectare dose data, and T3 are the 1.1kg/hectare dosed colonies. Dose day was day 4.



Treatment	Standard Error of the flight Activity values for each day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1441	491	1296	789	821	4126	910	1890	5619	6078	4282	1599	2953	7834
2	599	699	1298	2316	1475	2062	1341	2323	714	1896	443	2342	1924	876
3	4318	643	3790	3738	3488	3198	2103	2124	2742	2616	1691	1763	1616	1325

#### Percent Return:

The ANOVA states there is a significant “treatment” effect, a possible “trial” effect and no “treatment by trial” effect (Table 4.5.). The trials were selected to be pooled despite the 0.05 level of significant difference because the confidence intervals slightly overlapped (Appendix 5.C.ii.), and all three trials were grouped separately from controls, by the Student-Newman-Keuls analysis (Table 4.6) and supported by the Sidak test (Appendix 5.C.iii). The barely significant trial effect resulted from a hurricane that occurred during trial 3, seven days post-treatment, which reduced the flight activity of all the colonies. As previously mentioned, LSD is a sensitive test so it identified this difference between trials 1 and 2. The Student-Newman-Keuls

analysis (Table 4.7.), and the LSD test (Appendix 5.C.iv.) both showed treatment 3 was significantly different to treatments 1 and 2, as well as the control.

Forager return declined on dose day 4 for all colonies treated with methyl parathion (Fig 4.5.). The percent return for the low dosed colonies returned to normal by day 5, whereas the medium dose produced an intermediate response. This returned to normal by day 7 but may have weakened the colonies as they were affected by a subcritical perturbation on day 12. The number of returning bees from the high dosed colony dramatically declined, then increased on day 7, but dropped again on day 8. The forager force appeared to weaken as the perturbation on day 12 caused the return rate to decline below that caused by the dose.

**Table 4.5.** ANOVA of the percent return data that were normalised by an arcsine-square root transformation.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power <sup>a</sup>
Intercept	346.266	1	346.266	3937.954	.000	3937.954	1.000
Treatment	4.991	3	1.664	18.920	.019	56.760	.885
Trial#	1.668	2	.834	9.484	.050	18.967	.608
Treatment by trial#	4.204	6	.701	7.969	.058	47.814	.594
Error	.264	3	8.793E-02				

<sup>a</sup> Computed using alpha = .05

**Table 4.6.** Student-Newman-Keuls analysis that groups the Percent Return means for trials in homogenous subsets.

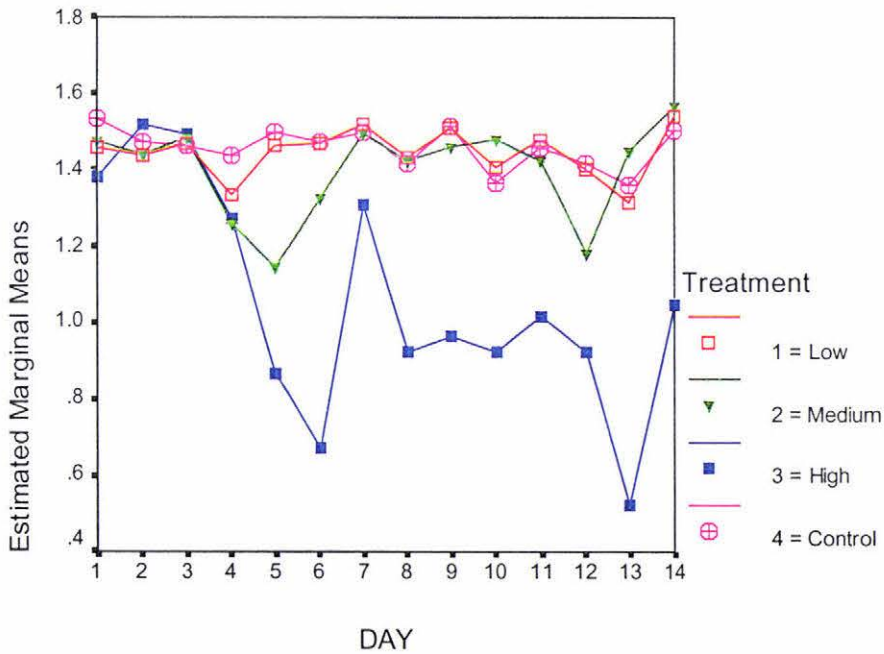
	Trial:	N	Subset 1
Student-Newman-Keuls	2	5	1.2699
	2	5	1.3663
	1	5	1.4632
	Sig		.061

**Table 4.7.** Student-Newman-Keuls analysis of percent return data that groups the Percent Return means, for treatments, in homogenous subsets.

	Treatment	N	Subset	
			1	2
Student-Newman-Keuls	3	3	1.0612	
	2	3		1.4020
	1	3		1.4480
	4	6		1.4606
	Sig.		1.000	.642

**Figure 4.5.** Percent return data from the colonies dosed with 0.11, 0.56 and 1.1 kg/hectare of methyl parathion. Dose day for the three trials was day 4. The perturbation at days 12-13, in trial 2, was due to hurricane Floyd.

Estimated Marginal Means of Percent return



Treatment	Standard Error of the Percent Return values for each day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 = 0.11	.11	.07	.06	.01	.05	.06	.05	.07	.06	.09	.05	.08	.04	.03
2 = 0.56	.10	.00	.04	.03	.01	.12	.07	.10	.06	.05	.02	.25	.06	.00
3 = 1.1	.18	.06	.04	.03	.18	.15	.26	.47	.48	.47	.51	.47	.52	.52
4 = Control	.02	.04	.04	.04	.04	.05	.04	.05	.03	.06	.04	.03	.06	.04

**Colony composition:**

The composition of the colonies were recorded for at least three weeks for each trial. Only changes in brood data were analysed which revealed that the trials were not significantly different at a 95% confidence level (Appendix 5.D.i). However, because the queens from trial 3 had ceased laying in preparation for winter, data collection was limited to three weeks. This did not produce extensive enough data for appropriate analysis (Appendix 5.D.ii.), so trial 3 was disregarded which enabled only five weeks of post-treatment brood data from trial 1 and trial 2 to be analysed.

The ANOVA and Student-Newman-Keuls analysis of pooled data from trials 1 and 2 determined there was no significant difference between treatments (Table 4.8. & Table 4.9.). However, the brood composition graph (Fig. 4.6.), infers that treatments 1 and 4 colonies may differ from treatment 2 and 3 colonies. This is observed from the decline of brood numbers from treatment 2 and 3 colonies after week 2, whereas the amount of brood in the treatment 1 and 4 colonies was fairly consistent.

The brood composition analysis of the test-colony used to determine the doses of methyl parathion, revealed that the total amount of brood declined one week post-treatment (Fig. 4.7.). This response to a 0.56kg/hectare dose of methyl parathion was caused by a combined decline of capped brood and larvae. Conversely, the amount of eggs slowly increased during weeks one to five.

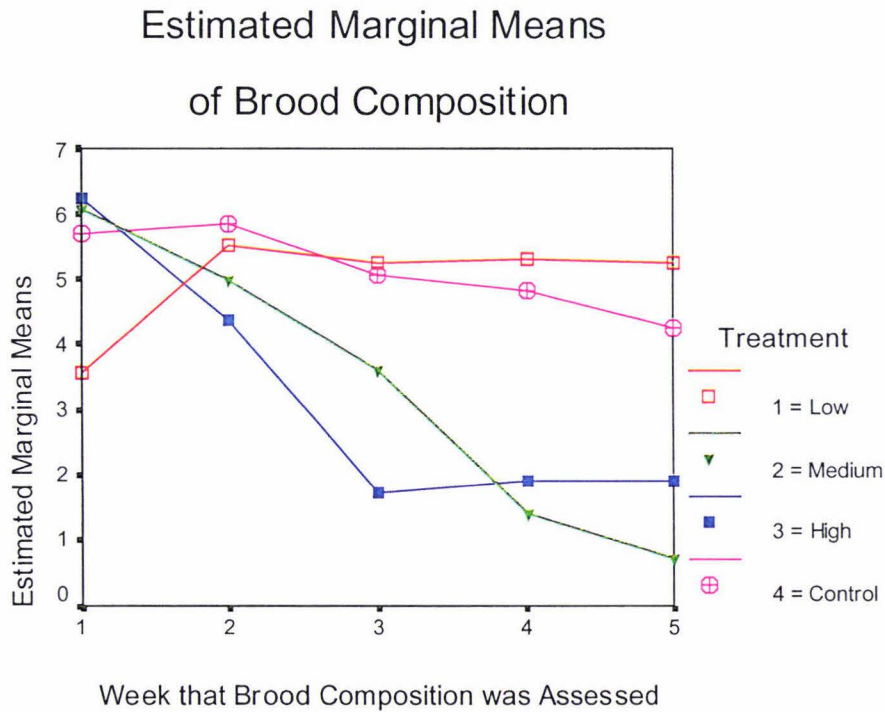
**Table 4.8.** ANOVA for five weeks brood data from trials 1 and 2.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power <sup>a</sup>
Intercept	801.413	1	801.413	162.637	.000	162.637	1.000
Treatment	39.065	3	13.022	2.643	.144	7.928	.381
Error	29.566	6	4.928 (37.8%)				

<sup>a</sup> Computed using alpha = .05

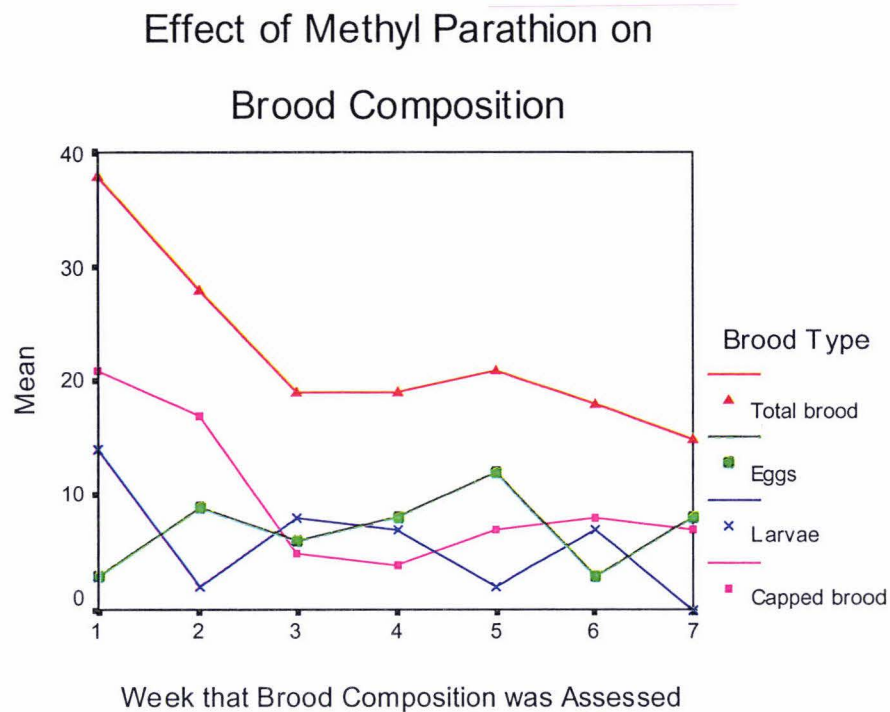
**Table 4.9.** SNK analysis of five weeks brood data from trials 1 and 2.

	Treatment	N	Subset 1
Student-Newman-Keuls	3	2	3.2444
	2	2	3.3584
	1	2	4.9905
	4	4	5.1547
	Sig.		.267

**Figure 4.6.** Brood composition data from trials 1 and 2. The colonies were dosed between weeks one and two with 0.11, 0.56 and 1.1 kg/hectare of methyl parathion..

Treatment	Standard Error of the Brood for each week				
	1	2	3	4	5
1	2.58	0.05	0.90	1.08	1.14
2	0.08	1.55	1.73	1.94	1.94
3	0.12	0.11	0.19	0.52	0.89
4	0.41	0.1	0.14	1.42	0.71

**Figure 4.7.** Brood composition data from the colony that was used to establish the range of methyl parathion to be used for this experiment. The colony was dosed between weeks 1 and week 2 with 0.56 kg/hectare of methyl parathion.



### General results:

The colony treated with 0.56kg/hectare of methyl parathion in trial 1 and the colony treated with 1.1kg/hectare in trial 2, absconded as a result of treatment.

## DISCUSSION

### Dead bee counts:

The tiered dead bee response supports the use of dead bee counts to indicate an immediate effect of methyl parathion on a colony. However, it suggests that the usefulness of this parameter is reduced once the treatments were removed from the colonies as the extraction of methyl parathion volatiles prevented the residues from accumulating within the hive.

Moffett *et al.* (1983) and Estes *et al.* (1992) observed bee mortality that was 100 times greater than pre-treatment counts during the three days post-treatment, when the methyl parathion in the field degrades to <5% of the initial deposit (Bennett *et al.* 1990). Their results were similar to what occurred in this study immediately after treating the colonies with high and medium doses of methyl parathion, where the dead bee counts were high and then decreased to normal once the methyl parathion had been removed.

Collecting dead bees from the front of the hive was of little value because it was only a small proportion of the number of dead bees collected from within the hive. This was expected as Atkins *et al.* (1977) also found them to be of little value. This is why the number of dead bees collected from each hive were combined for the analysis.

**Flight activity:**

The counts of incoming and outgoing bees were a useful indicator of flight activity and enabled the progression of colony recovery to be monitored. Flight activity of the colonies dosed with methyl parathion was significantly reduced and did not return to pre-dose levels throughout the duration of this experiment. This indicates that the colonies did not fully recover within the two weeks. Insecticides are known to reduce pollen gathering and crop visitation (Todd & Reed 1969, Johansen *et al.* 1990), but until now the duration of effects has not been quantified. These data identify that foraging was reduced for two weeks post-treatment. However, further analysis by this system would enable a more accurate quantification to be made.

These results indicate that the effect of methyl parathion on the colony lasts longer than the duration of exposure. This is because the colony has to recover from the impact of losing a large percentage of the foraging force. The “real time” component of this parameter enables a daily pattern to be established and can identify the effect of a toxic event within an hour. This is important for future research because geographic regions predominantly have varying climates, which alters flight activity (Johansen 1977, Bromenshenk pers.comm.). By identifying a colony’s usual flight activity, safe times to spray can be determined (Johansen *et al.* 1990). “Real time”

data enables this sort of information to be gathered and used so as to reduce the effects of pesticides on honey bee foraging, which ultimately impacts pollination.

**Percent return:**

Percent return is able to indicate the immediate effect of methyl parathion on a colony because it determines the number of bees that do not return during the day. It does not enable the decline in forager force to be seen in relation to time. However, when return rates are coupled with flight data the forager loss can be quantified, and when combined with the classic dead bee counts, the total loss of bees from the colony can be calculated per day. It is a useful parameter to quantify damage that may have occurred to a colony as the result of pesticide spraying.

**Colony composition:**

Changes in colony composition occurred too slowly to indicate the immediate effects of pesticides on the colony. However, it is useful for determining whether the colony would be able to recover. In this study the queens egg-laying inertia partially masked brood mortality by compensating for forager losses. This is consistent with previous research (Sanford 1983), as was the reduction in the size of the brood nest, which occurred a couple of days after treatment in response to the decline of nurse bees that had replaced the dead foragers.

Colonies that have large reserves of capped brood and recently emerged bees are able to replace the lost foragers quickly by using these young bees to forage before the usual three weeks of age (Sanford 1983). Individual bees can change their tasks according to the needs of the hive and this appears to have little or no effect on the colony (MacKenzie *et al.* 1989). However, if the queen has reduced her laying rate so there is only a small reserve of brood in the hive, as in trial 3, it may take more than six weeks to recover, if at all, depending on the season.

A colony with abundant food reserves will also survive poisoning better than one without reserves. The reserves within the hive can sustain the colony during this crucial period, rather than the bees being forced to use up energy by collecting food,

which may not be easily available. Colonies from trial 3 were treated in autumn after the main nectar-flow had subsided. This resulted in the treated colonies using up their food supplies and subsequently being unable to replenish them. The colonies in trial could not sustain themselves through winter, which resulted in hive annulment. This suggests that if a colony has been exposed to pesticides, it is important to ensure there are ample food supplies for them to raise replacement brood. Harbo (1993a) determined that the weight of brood was 75% of the weight of the honey it took to produce it, and that 121g of honey was required to produce 1000 cells of mixed-aged brood.

The size of a colony alters its dynamics because bee density effects honey loss, adult survival, and brood production (Johansen *et al.* 1990, Harbo 1993b). Colonies with large populations may have an increased chance of survival from pesticides, as long as there is space to produce more brood to compensate for loss of foragers. If this is the case, then it may be inappropriate to extrapolate results obtained from a nuc containing 15,000 bees to full-size colonies (45,000-60,000 bees). However, results from the smaller colonies do provide data which are testable in the larger colonies.

#### **General Comments:**

The two colonies that absconded during trial 1 and 2 exhibited typical behaviour of disturbed colonies. Absconding is primarily characteristic of tropical *Apis* races (Fletcher 1978), but occurs both seasonally and when the quality of the hive deteriorates by predation, fire, pests, over-heating, or pesticides (Fletcher 1975, Winston *et al.* 1979, Schneider 1990). Brood rearing in absconding colonies is greatly reduced (Fletcher 1975, Woyke 1976, Winston *et al.* 1979, Thoenes *et al.* 1992), but it is unknown whether this is due to the diminished laying activity of queens, or brood cannibalism by workers. This study recorded brood composition up until the colonies absconded and supports the idea that cannibalism reduces the amount of brood. This is seen from brood decreasing from approximately four to two sides of a frame per colony. Absconding increases the chance of survival for a disturbed colony but the associated time and energy required to find a new location, make the flight, and then produce wax to build combs, is costly (Otis *et al.* 1981, Hepburn 1988, Schmidt 1995).

The position of our hives may have had an effect on colony recovery because tree coverage caused some hives to receive less light than others. The position of hives has an unknown effect on the accumulation and impact of pesticides and their foraging behaviour.

In 1981, Hoopingarner *et al.* were the first to visually quantify pesticide contamination from dead bees. The result of these experiments are the first to quantify flight activity that occurs as a result of pesticide contamination.

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## SYNTHESIS

This thesis supports the comprehensive pesticide research that has been conducted by Johansen and Mayer (1990) regarding the effects of pesticides on honey bees, and extends it by using “real time” flight activity and forager return rates to quantify these effects. When “real time” flight data are coupled with dead bee counts and colony composition, the short and medium term effects of toxic events on honey bee colonies can be locally established and used to determine when pesticides should be applied, as well as enabling the impact upon pollination to be extrapolated.

Data from chapter 2 and chapter 4 of this thesis support Johansen (1977) who suggests that the climate of a geographical location alters the daily flight pattern of honey bees, which consequently determines the safest time of day to spray (Johansen & Mayer 1990). The “real time” component of the electronic system used in this thesis, enables timely data to be gathered for the reduction of pesticide effects on foraging honey bees, and accurately monitors colony recovery. Monitoring a colony after it has been exposed to pesticides is beneficial because the state of a colony determines whether it can continue to be used for bio-monitoring, pollination, and honey production.

Although the immediate effects of pesticides are of concern, long term effects can be devastating as these impact pollination, and ultimately crop yield. Because science is often regarded as a means to increase the economy, it is the application of this “real time” system that will aid future research. However, to achieve this result, it will be necessary to increase the accuracy of the flight data gained from honey bee colonies by decreasing the large standard error seen in these experiments. This can be achieved by gathering data from colonies that are of commercial size and from a greater number of colonies.

### **Future research:**

Irrespective of the hazard of insecticides to beneficial insects, the use of pesticides in crop management will invariably be supported (Pendergrass *et al.* 1992) because the

economic return of a crop is of primary importance. Further research into both bee behaviour and pesticide application methods will therefore be essential in order to reduce the risk to honey bees.

Electronic data collection has been demonstrated in this thesis to support measurement of the effects of pesticides on the hive dynamics of honey bees. It also provides a way for scientists and beekeepers to identify their colonies flight activity and determine which colonies would be effective for pollination, or of similarly matched activity for research purposes. An example of this would be the ability of Hort Research in New Zealand to use flight activity to determine which honey bee colonies would be effective vectors to spread the suggested biological pathogen to control fireblight (*Erwinia amylovora*) (Hort Research 1998). The recorded flight activity may also lead to an increased understanding of how fast, or otherwise, the spread of this pathogen may occur.

The response of house bees to pesticides in the hive is another behavioural dynamic that is of research interest. Chapter 3 showed that forager bees did not avoid contact with methyl parathion 30 minutes after exposure. To determine whether house bees display similar avoidance behaviour or if they cluster around the contamination, the spatial distribution of a colony within a hive could be reviewed. The University of Montana has designed a bee hive with 96 temperature probes that may be able to identify the distribution of those bees affected by pesticides, by the localised heat emitted from the bees.

The usefulness of a honey bee monitoring system is also relevant to commercial beekeepers because they require the status of their colonies to be monitored on a large scale basis. The electronic collection of colony flight data significantly reduces the time needed to assess a colony's status because the colony does not need to be physically checked, and it also reduces the consequences that result from "working"<sup>1</sup> a colony, such as dead bees.

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<sup>1</sup> Physical inspection of a hive to establish colony status. Achieved by removal and inspection of individual frames.

Further research that would also benefit a commercial beekeeping operation would be the development of a device, associated with the current monitoring system, that could detect the queens location and health, because a weak queen reduces the productivity of a colony, which in turn reduces crop yield. Because workers tend the queen it is possible that the development of miniaturised thermistor transmitters may aid this detection<sup>2</sup>.

An area associated with pesticide use that is in need of review is pesticide labelling. Pesticide labels provide application instructions that suggest that early morning or late evening are the best times of day to apply in order to avoid periods of bee foraging. However, it is questionable whether this actually protects honey bees because, as previously mentioned, flight activity varies between regions: In Missoula, Montana, United States of America, a honey bee colony is active from 6am to 10:30pm in summer where the flight activity peaks around 1pm, whereas bee colonies in Maryland, USA, have two flight peaks, one around 10:30am and one around 2:30pm (Bromenshenk pers. comm.). This suggests that pesticide labelling is too general and that targeted application instructions need to be developed for specific locations depending on the flight patterns of that area. Knowledge is a key resource, whether employed now or in the future, so by collecting flight data from different regions further research can start to identify pesticide effects on the colony dynamics and compare regional honey bee flight patterns in order to integrate them into crop and pest management.

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<sup>2</sup> I proposed this idea during my work with Dr. J. Bromenshenk at the University of Montana, who is currently investigating the use of miniature transmitters for forager bee tracking.

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## APPENDICES

### Appendix 1)

#### Vapour Density of Methyl parathion

Density of air as a reference of 1.

At room temperature 1 mol occupies 24.4L

$$\text{Air: } \frac{28.96\text{g}}{\text{Mol(air)}} \times \frac{1\text{mol}}{24.4\text{l}} = \underline{1.18\text{g/l}}$$

$$\begin{aligned} \text{Where } \text{N}_2 \text{ 78\% (28g)} &+ \text{O}_2 \text{ 21\% (32g)} &+ \text{Argon 1\% (39.9g)} \\ 0.78 \times 28 &+ 0.21 \times 32 &+ 0.01 \times 39.9 \end{aligned}$$

$$\begin{aligned} \text{Methyl parathion: } 11.87 \times 9.1 &= 10800.7\text{g/m}^3 \\ &= \underline{10.8\text{g/l}} \end{aligned}$$

**Ratio 1.186g air : 10.8g methyl parathion**

Density of methyl parathion in relation to air is 9 times ie: 1 : 9.1

## Appendix 2)

**Beetox** – Computer program that details the effects of pesticides on honey bees.

Mortality Rate	Pesticide concentration
%	( $\mu\text{g}/\text{bee}$ )
2	<u>0.14</u>
10	0.18
15	0.19
17	0.20
20	0.21
25	0.22
30	0.24
46	0.28
50	0.29
54	0.30
65	0.34
75	0.38
81	0.40
86	0.44
91	0.48
92	0.50
95	<u>0.54</u>
97	0.58
98	0.60
100	0.66



## Appendix 4)

### Observations made from experiments that dosed honey bees with methyl parathion, at Montana University, 1998.

#### **Experiment 1: Effects of methyl parathion consumption by honey bees.**

Methyl parathion was mixed with methanol (50mg/1ml) and 100mg aliquots were put into an 11g ball of Crisco and sugar mixture (1.25:1 ratio) that was inside a folded packet of unbleached filter (dimensions height 5cm, top width 12.7cm, base 5cm. Each packet contained 3 slits in the side and the top was stapled over. The packets were hung on a wire that was placed over a nail on top of the brood nest frames in the bottom hive body.

When methyl parathion was positioned between the brood frames, a stinging response was stimulated. On removal of the "100 mg" Crisco patties, 10-15 stingers were found attached to them. This suggests high levels of pesticide may kill in two ways, by expected fumigation, and secondly, by eliciting a stinging response which results in their death. This may suggest that it is not just suffocation from the pesticide that causes the death but the honeybees response to a foreign vapour.

When the methyl parathion was placed in the hive a large proportion of bees evacuated and hung in a cluster on the door of the condo. The counters showed the unusual behaviour of movement out of the hive after dark, during the residue tests. This suggests that honey bees display behavioural flexibility.

#### **Experiment 2: Does methyl parathion elicit a stinging response by honey bees?**

Analysis of previous experiment 1 revealed bees had stung the filter packets used to hold the methyl parathion, and left their stingers in the paper.

Two manifold systems, each consisting of three single Plexiglass frames in succession, were set up, and the bees response to contact with methyl parathion was recorded over a three hour period.

The first run was conducted on 4<sup>th</sup> December 1998. There was a control manifold, and a treatment manifold that dosed all three hives with 10mg methyl parathion. The dose was mixed with 160 $\mu$ l of methanol to scale it down from 250mg : 4000 $\mu$ l.

A 20.0g filter packet of 1.25 : 1 ratio of sugar and Crisco was placed in the middle of each frame in both manifold systems, for three hours. The experiment was conducted inside a plastic room because the bee colonies had shut down for the winter. The methyl parathion vapours were dispersed through the frames using the CPVC airflow system, which pulled the air through the manifold across the lowest to the highest dose respectively, and then out through an organophosphate/ pesticide filter into a vacuum.

After three hours, the number of dead bees and the number of stings in each packet were counted and the mortality was used as a final measure of the effects that methyl parathion has on honey bees.

The second run used one manifold as the control and dosed the second manifold with packets containing 10, 20 and 50mg of methyl parathion.

The results were unable to confirm the observations first made in experiment 1 but it would be interesting to redo these experiments when the bees are still physiologically active in the field.

### **Experiment 3: Fight or flight response of honey bees to methyl parathion**

Four frames of bees were placed in a sealed Plexiglas hive and left to acclimatise. A clear, 60cm tube was attached to the hive entrance and joined to a second empty Plexiglas

hive. A 20.0g packet of 1 : 1.25 ratio of Crisco : sugar, was combined with 50mg methyl parathion mixed in 1.6ml methanol. The packet was placed in the middle of the hive for 3hrs whilst the bees behaviour was observed and then the number of dead bees in each section were counted. After dosing the colony the entire hive was dead within three hours and only a few were situated in the plastic tube. The bees had not moved in to the undosed plexiglass hive but this may be because they did not have long enough to acclimatise and consequently did not realise they could escape from the hive. Repeating this experiment with colonies that were acclimatised to the Plexiglas hives may lead to more extensive results.

## Appendix 5)

### Analysis made for the four parameters of Chapter 4: The effects of methyl parathion on the colony dynamics of *Apis mellifera*

A) Dead Bee ANOVA:

Multiple Comparisons

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
<b>LSD</b>	1	2	-2.9205	.9966	.061	-6.0923	.2512
		3	-4.5382	.9966	<b>.020</b>	-7.7099	-1.3664
		4	1.7302	.8631	.139	-1.0166	4.4770
	2	1	-2.9205	.9966	.061	-.2512	6.0923
		3	-1.6177	.9966	.203	-4.7894	1.5541
		4	4.6507*	.8631	<b>.013</b>	1.9039	7.3975
	3	1	4.5382*	.9966	<b>.020</b>	1.3664	7.7099
		2	1.6177	.9966	.203	-1.5541	4.7894
		4	6.2684*	.8631	<b>.005</b>	3.5216	9.0152
	4	1	-1.7302	.8631	.139	-4.4770	1.0166
		2	-4.6507	.8631	<b>.013</b>	-7.3975	-1.9039
		3	-6.2684	.8631	<b>.005</b>	-9.0152	-3.5216
<b>Sidak</b>	1	2	-2.9205	.9966	.314	-9.0845	3.2435
		3	-4.5382	.9966	.113	-10.7022	1.6258
		4	1.7302	.8631	.592	-3.6080	7.0684
	2	1	2.9205	.9966	.314	-3.2435	9.0845
		3	-1.6177	.9966	.744	-7.7817	4.5463
		4	4.6507	.8631	.073	-.6875	9.9889
	3	1	4.5382	.9966	.113	-1.6258	10.7022
		2	1.6177	.9966	.744	-4.5463	7.7817
		4	6.2684*	.8631	<b>.032</b>	.9302	11.6066
	4	1	-1.7302	.8631	.592	-7.0684	3.6080
		2	-4.6507	.8631	.073	-9.9889	.6875
		3	-6.2684	.8631	<b>.032</b>	-11.6066	-.9302

Based on observed means.

\* The mean difference is significant at the .05 level.

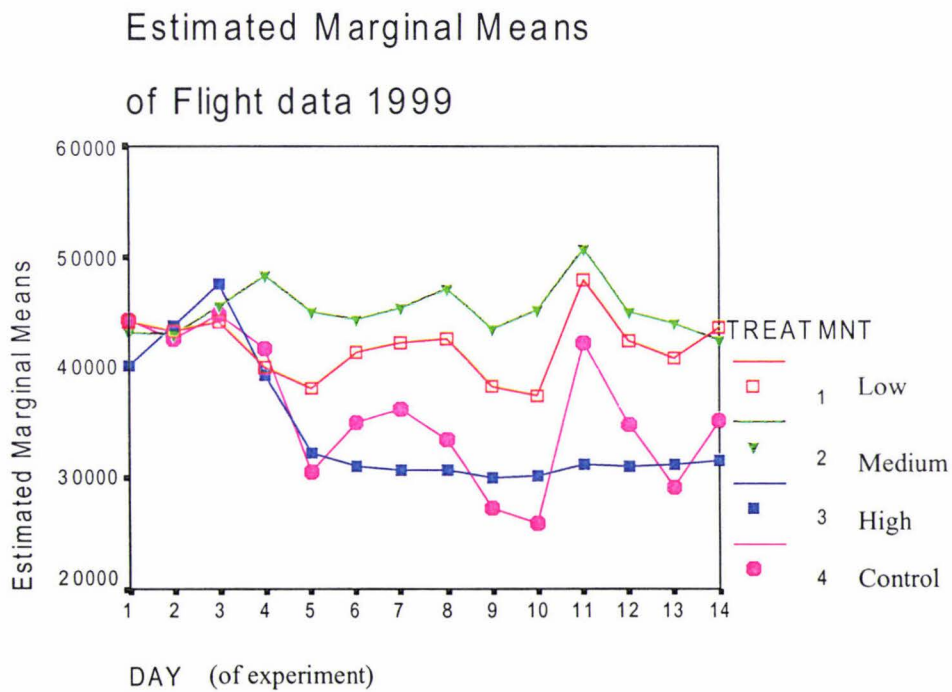
**B) Flight data**

i. ANOVA of the scaled 1999 flight data.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power <sup>a</sup>
Intercept	2.98x10 <sup>11</sup>	1	2.98x10 <sup>11</sup>	2285.495	.000	2285.495	1.000
Treatment	3.57x10 <sup>9</sup>	3	1.19 x10 <sup>9</sup>	9.115	.051	27.344	.618
Trial	1.13x10 <sup>8</sup>	2	56327252	.431	.684	.863	.078
Treatment by trial	1.56x10 <sup>9</sup>	6	2.6x10 <sup>8</sup>	1.994	.305	11.965	.200
Error	3.92x10 <sup>8</sup>	3	1.31x10 <sup>8</sup>				

<sup>a</sup> Computed using alpha = .05

ii Flight data from the 1999 methyl parathion Experiments. T1 = Low dose, T2 = Medium dose, T3 = High dose, T4 = Control



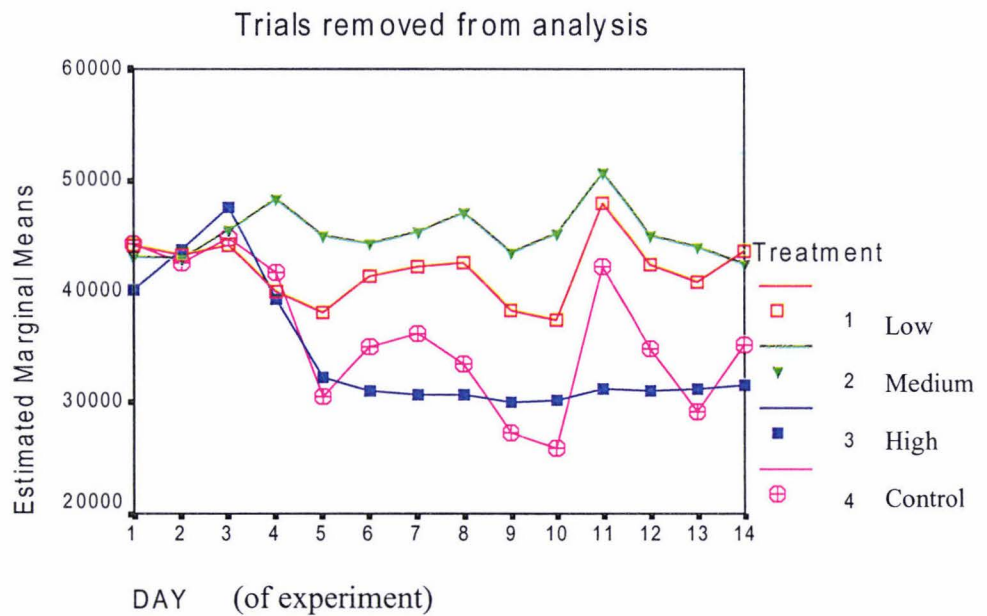
iii ANOVA of the 1999 scaled flight data where the trials are pooled.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power <sup>a</sup>
Intercept	2.98x10 <sup>11</sup>	1	2.98x10 <sup>11</sup>	1408.454	.000	1408.454	1.000
Treatment	3.57x10 <sup>9</sup>	3	1.19x10 <sup>9</sup>	5.617	.014	16.851	.831
Error	2.33x10 <sup>9</sup>	11	2.12x10 <sup>8</sup>				

<sup>a</sup> Computed using alpha = .05

iv Flight data from the 1999 methyl parathion experiments with trials pooled for analysis. The colonies were dosed on day 3.

Estimated Marginal Means of Flight Data



**C) Percent return Analysis:**

**i) Confidence Intervals for treatment analysis**

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	1.448	.046	1.302	1.594
2	1.402	.046	1.256	1.548
3	1.061	.046	.916	1.207
4	1.461	.032	1.358	1.564

**ii) Confidence Intervals for trial analysis**

TRIAL#	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	1.450	.037	1.332	1.568
2	1.223	.037	1.105	1.341
3	1.356	.037	1.238	1.474

iii) ANOVA comparing trial differences.

Multiple Comparisons

	(I) TRIAL#	(J) TRIAL#	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	.1933	5.012E-02	.031	3.382E-02	.3528
		3	9.692E-02	5.012E-02	.149	-6.2589E-02	.2564
	2	1	-.1933	5.012E-02	.031	-.3528	-3.3822E-02
		3	-9.6411E-02	5.012E-02	.150	-.2559	6.310E-02
	3	1	-9.6924E-02	5.012E-02	.149	-.2564	6.259E-02
		2	9.641E-02	5.012E-02	.150	-6.3102E-02	.2559
Sidak	1	2	.1933	5.012E-02	.090	-4.8581E-02	.4353
		3	9.692E-02	5.012E-02	.383	-.1450	.3388
	2	1	-.1933	5.012E-02	.090	-.4353	4.858E-02
		3	-9.6411E-02	5.012E-02	.386	-.3383	.1455
	3	1	-9.6924E-02	5.012E-02	.383	-.3388	.1450
		2	9.641E-02	5.012E-02	.386	-.1455	.3383

Based on observed means.

\* The mean difference is significant at the .05 level.

iv) ANOVA comparing treatment differences.

Multiple Comparisons

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
						<b>LSD</b>	1
		3	.3869	6.471E-02	.009	.1809	.5928
		4	-1.2541E-02	5.604E-02	.837	-.1909	.1658
	2	1	-4.6059E-02	6.471E-02	.528	-.2520	.1599
		3	.3408	6.471E-02	.013	.1349	.5467
		4	-5.8599E-02	5.604E-02	.373	-.2369	.1197
	3	1	-.3869	6.471E-02	.009	-.5928	-.1809
		2	-.3408	6.471E-02	.013	-.5467	-.1349
		4	-.3994	5.604E-02	.006	-.5777	-.2211
	4	1	1.254E-02	5.604E-02	.837	-.1658	.1909
		2	5.860E-02	5.604E-02	.373	-.1197	.2369
		3	.3994	5.604E-02	.006	.2211	.5777
<b>Sidak</b>	1	2	4.606E-02	6.471E-02	.989	-.3542	.4463
		3	.3869	6.471E-02	.055	-1.3348E-02	.7871
		4	-1.2541E-02	5.604E-02	1.000	-.3591	.3341
	2	1	-4.6059E-02	6.471E-02	.989	-.4463	.3542
		3	.3408	6.471E-02	.077	-5.9407E-02	.7410
		4	-5.8599E-02	5.604E-02	.939	-.4052	.2880
	3	1	-.3869	6.471E-02	.055	-.7871	1.335E-02
		2	-.3408	6.471E-02	.077	-.7410	5.941E-02
		4	-.3994	5.604E-02	<b>.034</b>	-.7460	5.2810E-02
	4	1	1.254E-02	5.604E-02	1.000	-.3341	.3591
		2	5.860E-02	5.604E-02	.939	-.2880	.4052
		3	.3994	5.604E-02	.034	5.281E-02	.7460

Based on observed means.

\* The mean difference is significant at the .05 level.

**D) Brood composition Analysis:**

i) ANOVA of the 1999 brood composition data.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power <sup>a</sup>
Intercept	856.026	1	859.026	631.748	.000	631.748	1.000
Treatment	6.344	3	2.115	1.561	.362	4.682	.160
Trial	6.592	2	3.296	2.432	.236	4.865	.214
Treatment by trial	12.289	6	2.048	1.511	.395	9.069	.162
Error	4.065	3	1.355				

<sup>a</sup> Computed using alpha = .05

ii) Graph of brood composition from three weeks of data collected from the colonies dosed with 0.11, 0.56, and 1.1 kg/hectare of methyl parathion, and the control.

