THE EFFECT OF SUBLETHAL PESTICIDE EXPOSURE ON TEMPORAL DIVISION OF LABOUR AND LONGEVITY IN THE HONEY BEE (*APIS MELLIFERA* L.).

by

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ABSTRACT

The objective of this study was to evaluate the effects of sublethal pesticide exposure on temporal division of labour and longevity in the honey bee (*Apis mellifera* L.). A number of different variables were examined including dosage, various treatment ages (between 0 and 16 days old), number of exposures (one, two and three times), and type of pesticide (diazinon, carbaryl, or resmethrin). In addition, experiments were repeated within and between years, and two hive types (observation hives and standard field colonies) were used. Data examined for each variable included life span, and the first day and duration of tasks commonly performed by workers.

Both longevity and one aspect of division of labour, foraging, were affected by sublethal exposure to pesticides. The lifespan of newly emerged workers was reduced by a single application of diazinon in the observation hives and by a single application of carbaryl in the standard field colonies. In addition, older workers treated three times with diazinon had shorter lifespans than controls. For foraging age, diazinon-treated, newly emerged workers tended to begin foraging earlier than controls, while workers treated at a later age and with three dosages of diazinon began foraging later. In most cases, treatment with pesticide also reduced the duration of foraging. Few differences were seen in any of the other tasks examined.

Treatment age, pesticide type, and number of treatments also had some effects on these aspects of honey bee behaviour. Newly emerged workers were particularly susceptible, and repeated exposure to pesticides was deleterious to both young and older workers. Of the three pesticides tested, carbaryl caused the greatest reduction in longevity, with resmethrin intermediate and diazinon the

least harmful. To properly evaluate pesticide effects, these factors should be considered in future research.

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A. INTRODUCTION

The honey bee (*Apis mellifera* L.) is an important component of modern agriculture. In the Western World about one-third of man's total diet is dependent, directly and indirectly, on bee pollination (McGregor 1976). The total value per year of commodities and crops that depend in some way on bees for pollination has been estimated at $18.9 billion (U.S.) in the United States (Levin 1984) and $1.2 billion (Canadian) in Canada (Winston and Scott 1984). About 40 major crops (each valued at greater than $1 million (U.S.) per year) in the U.S.A. and 25 major crops in Canada are obligately pollinated by bees. An additional 70 crops in the United States (McGregor 1976), some 105 crops in the tropics (Crane and Walker 1983), and over 135 economically important crops in 38 families worldwide (Free 1970) are known to benefit from bee pollination.

An increased demand for food and fiber world-wide has led to larger-sized farms and increased acreage, mechanization, and pesticide use (Atkins 1975; Metcalf 1980; Wilson *et al.* 1980). The development of modern agriculture has, in turn, led to a greater dependence on managed bees for pollination due to decreases in native pollinators through habitat destruction and pesticide use. Of these managed pollinators, honey bees are the most important because they can be handled in large numbers (Johansen 1984). Unfortunately, honey bees are susceptible to many commonly used pesticides, especially insecticides (Anderson and Atkins 1968; Atkins 1975; Johansen 1977, 1979, 1983; N.R.C.C. 1981). The organophosphorous insecticides, acephate, diazinon, dimethoate, fenitrothion, malathion and parathion; the carbamates, carbaryl and propoxur; and the pyrethoids, permethrin, decamethrin and fenvalerate, are all highly toxic to honeybees (N.R.C.C. 1981). To maintain vigorous pollinator populations while reducing pest populations to acceptable levels, the development of integrated

pest management systems which employ a combination of control methods is essential.

Bee poisoning from pesticides has been a problem since the late 1800's (Johansen 1977). While poisonings are difficult to document and often not proven, some examples of bee kills from pesticides show the extent of the problem. Beekeepers lost an estimated average of 62,500 colonies every year from 1962 to 1973 in California (Atkins 1975). In 1975 the loss of 31,000 colonies was estimated to have cost approximately $966,000 (U.S.) to the Californian beekeepers (Siebert 1979). Pesticides such as carbaryl, malathion and methyl-parathion applied to corn and soybeans killed 440 and damaged another 1370 hives of honey bees in Iowa in 1978 (DeWitt 1979). When carbaryl was applied to asparagus in Washington in 1979 an estimated 2000 colonies were destroyed or severely damaged (Mayer *et al.* 1980). Repeated applications of insecticides to flowering cotton was thought to be the primary reason the number of bee colonies in Arizona were reduced from over 100,000 in 1964 to 53,000 in 1971 (Wilson *et al.* 1980). The annual cost in the United States of bee losses from poisonings has been estimated at $135 (U.S.) million (Pimental *et al.* 1980).

In Canada large bee kills have also occurred. In 1981 the aerial application of propoxur in mosquito control programs in Manitoba resulted in 3725 colonies damaged and $87,455 (Canadian) lost due to both poor honey production and bee kill (Dixon and Fingler 1982). In 1983 the impact of malathion sprays were more severe. Compensation payments of $845,000 (Canadian) for honey losses, the death of 576 colonies, and the subsequent loss of an additional 2000 overwintering colonies were made (Dixon and Fingler 1984). Concern over pesticide poisoning have led Johansen (1977) and Atkins (1975) to rate it as the number one problem facing beekeepers today.

A great deal of research has been focused on the issue of pesticides and honey bees. Most of this work has concentrated on acute mortality studies, both

in the laboratory and the field. When rating pesticide hazard, both types of studies must be considered, since it can be very difficult to relate laboratory studies in controlled conditions to the field (Atkins 1975; Bacilek 1982; Clinch 1981; Erickson *et al.* 1983; N.R.C.C. 1981). Often, conflicting results are found. Some pesticides, rated as highly toxic in the laboratory, have been found to be fairly safe in the field. The synthetic pyrethrins, such as permethrin, have high acute toxicities but are of low hazard in the field (Pike *et al.* 1982; Shires *et al.* 1984). This is due to two factors, low application rates needed to kill pest organisms and a repellent action of the chemical. Laboratory studies rate herbicides and fungicides relatively non-toxic to bees (Atkins 1975; N.R.C.C. 1981), yet, the phenoxy herbicides were found to reduce brood production (Morton and Moffett 1972; Morton *et al.* 1974) and the fungicide mancozeb to cause adult mortality (Buckner *et al.* 1976) in the field.

Field results have been found to depend on a number of factors, including weather, application rate and pesticide formulation. Methomyl, used in the more arid western states, was thought to be safe to use with honey bees. However, in Wisconsin it caused adult mortality and winter loss (Erickson *et al.* 1983). Higher losses of workers occur in hot, sunny weather due to increased foraging under these conditions (Stevenson 1983). Climatic conditions influence crop flowering, which in turn affects worker activity (Bacilek 1982). Formulations are also very important. Carbaryl is highly toxic to honey bees as a wettable powder spray and dust (Johansen and Brown 1972), but is safe for bees and effective against target pests such as the corn earworm in a newly developed formulation of micronized carbaryl suspension with a latex- based sticker (Anon. 1983; Hanny and Harvey 1982). Methyl parathion is very toxic to honey bees. As a microencapsulated formulation it is collected with pollen and has caused severe losses to both adults and larvae (Atkins *et al.* 1978; Rhodes *et al.* 1979; Stoner *et al.* 1979, 1982a). However, application of this formulation to sunflowers

caused little adverse effects (Waller *et al.* 1984a) and higher adult losses have been attributed to the emulsifiable concentrate formulation used in the above study and one other (Moffett *et al.* 1983). The systemic insecticides such as dimethoate and phosphamidon are also of concern as levels sufficient to cause mortality have been found in the nectar of plants treated with these chemicals (Barker *et al.* 1980; Jaycox 1964; Waller *et al.* 1984b). Therefore, formulation, application rates and methods, environmental and meteriological conditions, and the crop maturity must all be taken into consideration when rating pesticide hazard to honey bees.

The above studies suggest, however, that acute toxicity studies alone are not adequate to explain pesticide impact on honey bees. Poor overwintering success, low brood and honey production, and queen loss have all been associated with pesticide spray programmes (Dixon and Fingler 1984; Johansen and Brown 1972; Melksham *et al.* 1985; N.R.C.C. 1981; Robinson and Johansen 1978). Connections between pesticide sprays and other problems such as disease have also been made (Morse 1961, 1965; Wahl and Ulm 1983), and the physiological condition of individual bees has been shown to affect pesticide sensitivity (Wahl and Ulm 1983). Therefore, other areas of pesticide-pollination interactions in addition to acute toxicity must be involved; one of these areas may involve the exposure to low, sublethal dosages of chemicals.

Within the last 10 to 15 years researchers have started to explore this problem. Many studies have focused on whole-colony effects. Chronic feeding of low dosages of pesticides to honey bees, such as acephate (Stoner *et al.* 1985), carbaryl (Winterlin and Walker 1973), parathion, methyl-parathion and methoprene (Barker and Waller 1978a), dimethoate (Barker *et al.* 1980; Stoner *et al.* 1983; Waller and Barker 1979; Waller *et al.* 1979) and carbofuran (Stoner *et al.* 1982b) in sugar syrup, and diflubenzuron in sugar cake and syrup (Barker and Taber 1977; Stoner and Wilson 1982) and in water (Barker and Waller 1978b),

adversely affected such colony characteristics as worker population size, honey production, food consumption and brood rearing, as well as worker survival. Acephate was also shown to reduce queen survival and brood production (Stoner *et al.* 1985). In addition these colonies were unable to rear new queens. Microencapsulated methyl-parathion fed in pollen also reduced worker population and brood production (Stoner and Wilson 1983). Herbicides fed in water were shown to reduce brood production (Morton *et al.* 1974).

A few researchers have examined sublethal effects of pesticides on individual workers. Schricker and Stephen (1970) found methyl parathion impaired the ability to communicate the location of food sources to other workers. Time-trained bees treated with parathion returned to the station at an incorrect time the following day (Schricker 1974a) and when moved to a new area could not relocate a feeding station placed at a similar location (Schricker 1974b). Smirle *et al.* (1984) determined that low dosages of diazinon and malathion could reduce worker longevity. Foraging patterns can be affected as well. Bees treated with permethrin spent more time self-cleaning and less time foraging than did treated controls in a study by Cox and Wilson (1984). Robinson (1985) found methoprene, *albeit* in high dosages, shortened worker life and reduced the age of both orientation and foraging.

One area that has not yet been examined is the effect of pesticides on an important aspect of honey bee colony functioning, temporal division of labour. Temporal division of labour can be defined as an ontogenetic sequence of activities performed throughout a worker's lifetime (Michener 1974), which tends to follow physiological changes in gland development. Generally, young individuals perform hive activities, while older workers undertake the more hazardous outside activities (Michener 1974; Wilson 1971). Young workers perform such tasks as cleaning, comb building and brood care, while older workers guard and forage (Free 1965; Kolmes 1985a; Lindauer 1953; Ribbands 1952;

Sekiguchi and Sakagami 1966; Winston and Punnett 1982). All of these studies stress the great variability and flexibility found in the performance of various tasks. Seeley (1982) delineated four distinct age castes: cell cleaning, broodnest, food storage and forager. The sharpest behavioural demarcation is found in the change from hive to field duties (Kolmes 1985a). However, foragers can return to hive duties if needed (Free 1965; Lindauer 1953).

The factors influencing temporal division of labour in honey bees are poorly understood, but seem to involve internal colony conditions, external factors such as forage availability and weather conditions, and worker age (Nowogrodzki 1984). Colony growth and reproductive rates in honey bees are related to brood area and population age distribution (Winston 1979; Winston *et al.* 1981). Internal colony conditions can affect worker lifespan and task performance. For example, brood rearing was stimulated by the presence of honey stores (Barker 1971; Ribbands 1952) and pollen collection by the presence of brood (Free 1967; Jaycox 1970), a queen (Jaycox 1970), pollen deprivation (Lindauer 1953), and honey stores (Barker 1971; Ribbands 1952). When deprived of wax, workers were more active and built more comb (Kolmes 1985a). In addition, shorter worker lifespans were associated with brood rearing (Maurizio 1950; Neukirch 1982; Villumstad 1977; Woyke 1984) and early foraging (Sekiguchi and Sakagami 1966; Winston and Fergusson 1985; Winston and Katz 1981). It is generally agreed that workers perform tasks in an age-related sequence (Gary 1975; Michener 1974), although foraging age and possibly other tasks can be dependent on colony population and the amount of unsealed brood (Winston and Fergusson 1985). Environmental conditions also can influence task performance. In poor conditions, brood rearing decreased and in good conditions foraging increased (Free 1965), and colony population was correlated with the amount of brood in a poor forage year and longevity in a good one (Woyke 1984). In addition to genetic differences, environmental conditions influenced longevity and foraging age (Winston and Katz

1981, 1982). Thus, temporal division of labour is an interactive process that involves the integration by individual workers of internal colony and external environmental conditions, as well as forage availablity and physiological age.

Pesticides at both the acute and subacute levels have been shown to affect some of the factors involved in determining temporal division of labour, such as longevity, colony population, food collection, and brood rearing. Longevity is an economically important trait that is correlated with honey production (Milne 1980, 1985), and honey production also is related to brood production, colony population and worker longevity (Woyke 1984). The relationship between the effects of pesticides and the stimuli for temporal division of labour has not yet been explored. These areas are important, especially when considering the objective of the beekeeper and the crop producer, to maximize colony population with peak nectar flow, thus producing a maximum honey yield and/or to maximize pollination of crops. If even low dosages of pesticide alter temporal division of labour, this objective may not be met.

Pesticide-pollinator interactions also have not yet been adequately studied. Much of the current literature focuses on acute mortality studies, both the in laboratory and the field, and on determining losses due to pesticides. The lack of information on this topic led the Subcommittee on Pesticides and Industrial Organic Chemicals of the National Research Council of Canada to state that "there is little information on the effects of long-term and repeated exposure to insecticides on bees" (N.R.C.C. 1981). They recommended research into managed pollinators and pesticides be directed into this area. The limited number of studies on sublethal exposures of honey bees to pesticides have shown a number of adverse effects in colonies, such as reduced populations of both brood and adults, reduced honey production, and poor overwintering success, and on individual workers such as lower longevity and foraging success. However, these results pose a new series of questions. For example, is brood and honey

production reduced simply due to a smaller work force, or are there other factors involved? How is overwintering success altered due to pesticide exposure? Are workers that are exposed to pesticides more susceptible to disease and stress? Does pesticide exposure alter the regulation of temporal division of labour? If so, how does this affect the colony as a whole?

The purpose of this study was to examine one of these questions, the effect of exposure to low levels of pesticide on temporal division of labour and longevity in the honey bee. The study of temporal division of labour is difficult due to both a lack of overall understanding of the factors regulating it and inherent variablility. In a study of this kind it becomes important to evaluate general trends. Therefore, a number of variables, such as treatment age, different chemicals and number of exposures, were used, and repetition within the same year and over two summers was performed (Table 1). In addition, observation hives and standard field colonies were used. In this manner it was hoped that any effects and/or consistent patterns due to exposure to low dosages of pesticide on honey bee workers would be found.

TABLE 1: Summary of the experiments on the effects of sublethal doses of three insecticides on honey bee longevity and temporal division of labour.

Experiment Number[1]	Date	Hive Type	Number of Hives	Treatment Age (days)[2]	Pesticide	Number of Treatments
1	1983, June and July	Observation	1	0	Diazinon	1
2	1983, May and June	Observation	1	6	Diazinon	1
3	1983, June to Aug.	Observation	1	0,4 & 8	Diazinon	1,2 & 3
4	1985, May to July	Observation	1	0	Diazinon	1
5	1985, May to July	Observation	1	6	Diazinon	1
6	1985, May to July	Observation	1	15	Diazinon	1
7	1985, July to Aug.	Observation	1	0,3, & 6	Diazinon	1,2 &3
8	1985, July to Aug.	Observation	1	10,13 & 16	Diazinon	1,2 & 3
9	1985, July to Sept.	Standard	3[3]	0	Diazinon, Carberyl and Resmethrin	1
10	1985, July to Sept.	Standard	3[3]	14	Diazinon and Carberyl	1

[1]From this point in the text, experiments will be referred to by Experiment Number.

[2]Treatment age is expressed as the number of days after emergence.

[3]The same colonies were used in Exp. 9 and Exp. 10.

B. OBSERVATION HIVE EXPERIMENTS

1. MATERIALS AND METHODS

Chemical

A commercial formulation of the organophosphorous insecticide diazinon, formulated as a 12.5% emulsifiable concentrate (Later's Diazinon, P.C.P. Act No. 11437, Later Chemicals Ltd., Richmond, B.C.), was used. It was diluted in acetone (reagent grade) to the required concentration.

The Colonies

The test colonies were located at Simon Fraser University, Burnaby, B.C. Studies were conducted from April to August 1983 and 1985. Four-frame observation hives made of transparent castable polymethacrylate (Plexiglas®) were used for the experiments. These hives were kept indoors and had Plexiglas®-covered entrance ramps 22 x 10 x 4 cm deep leading from the base of the lowest frame to outside. An angled Plexiglas® slab was glued to the entrance ramp to force incoming marked workers to enter the colonies with their labels in view.

In 1983 three observation hives were used, each with two frames of drawn comb, two frames of foundation, 0.9 kg of worker bees, and a queen. Two of these were "Dial-a-Bee" hives which had a moveable ring with portholes to allow the removal of workers without dismantling the hive (Pickard 1980). In 1985 three observation hives consisting of one frame each of honey, pollen and brood, one empty frame, enough workers to cover the combs, and a laying queen, were used. On 19 June, 1985 the hives used for single exposures at six days and 15 days of age (Exp. 5 and Exp. 6, Table 1) were found to be queenless, and they were

requeened on 20 June. On 19 July 1985 the queen was missing in the hive used for repeated exposure (Exp. 8, Table 1) and the hive was requeened on 20 July. Colonies were manipulated only to treat workers at the desired ages, to introduce test workers, and to feed sugar syrup as necessary to prevent starvation.

Each year workers to be treated were obtained from a single colony (not one of the observation hives) to minimize genetic variation in response. Combs containing emerging workers were placed overnight in an incubator at 34°C and 50-70% R.H. Newly emerged workers were marked on the dorsal surface of the thorax with coloured and numbered plastic labels (Opalithplättchen, Chr. Craze, KG, Endersbach, West Germany), and either treated immediately or introduced into a test hive and removed and treated at the desired age.

Treatments

Honey bee workers were treated with a topical insecticide application on the dorsal surface of their abdomens. An S.M.I. Micro/Pettor-A® was used to dispense 1 µl of treatment solution to each insect. Workers were held by the hind leg with forceps while insecticide was applied. A group of workers was treated with 1 µl acetone as a control in all experiments and some also contained an untreated group of marked workers as a second control. The bees were introduced into the proper hive by placing the treated workers into a jar which was put over the feeding hole located at the base of the lowest frame opposite the entrance ramp.

Acute toxicity tests (LD_{50}) were conducted to choose the dose range for sublethal exposure for newly emerged bees in 1983 and for all treatments in 1985 (Table 2). Between 40 to 50 workers were treated at each of the four to seven doses in the mortality tests. To determine LD_{50} values for the newly emerged workers (0 days), individual bees were treated and placed into holding cages. The two older age groups (7 and 14 days) in 1985 were marked at emergence, placed

TABLE 2: Dosages used and regression line equations from acute toxicity studies for experiments in 1983 and 1985.

Year	Pesticide	Worker Age (days)	Number of Bees per Dosage	Number of dosages	Regression Line[1]	Coefficient of determination (r^2)	Dosage (µg/bee) LD_1	LD_5	LD_{10}	LD_{20}	LD_{25}	LD_{50}
1983	Diazinon	0	50	5	y = 11.7 + 6.4x	0.992	0.040	0.050	0.060	0.068		0.090
	"	Adults[2]					0.050	0.075	0.100	0.125		
1985	"	0	40	5	y = 22.5 + 13.5x	0.959		0.035	0.040		0.045	0.052
	"	7	40	4	y = 17.4 + 12.8x	0.999		0.080	0.086		0.095	0.108
	"	14	40	5	y = 17.9 + 13.7x	0.988		0.085	0.090		0.100	0.115
	Carbaryl	0	50	7	y = 12.6 + 6.1x	0.999		0.023	0.028		0.037	0.055
	"	14	50	5	y = 10.8 + 6.8x	0.964		0.084	0.094		0.115	0.142
	Resmethrin	0	50	6	y = 14.6 + 6.9x	0.996		0.020	0.025		0.032	0.045
	"	14	50	5	y = 12.2 + 6.4x	0.983		0.045	0.050		0.060	0.076

[1]Units of regression line: x = log (dose, µg/bee), y = probit value (Swaroop 1966; Zar 1984).

[2]Approximate dosages from Smirle (1983) on a random sample of adults of unknown age, regression line not available.

into standard colonies, removed when they reached the appropriate ages (grasped by their hind legs with forceps and held in cages), treated and placed into holding cages. Sugar syrup (50%) and water were supplied *ad libitum* via gravity feeding bottles. Carbon dioxide was used to immobilize the older workers before exposure. Mortality counts were taken at 24 h and data analyzed following the World Health Organization method for insecticide susceptibility tests (Swaroop 1966) and by regression analysis (MINITAB) (Zar 1984). The resultant regression lines were used to calculate doses corresponding to the desired mortalites. In 1983 the approximate doses for treatment of older workers were the same as published in Smirle (1983) (Table 2).

For experimental purposes, doses approximating those causing 1%, 5%, 10% and 20% mortality were used in 1983 (the same as those used by Smirle 1983) and 5%, 10% and 25% mortality were used in 1985. In 1983, the number of workers treated at each dosage was between 20 and 30. In order to increase the sample size in 1985, one dose (LD_1) was omitted and at least 50 and up to 65 workers were then treated at each dosage. The highest dosage was changed to LD_{25} in 1985 to increase the likelihood of obtaining adverse effects.

Experiments containing single and repeated exposures were carried out in both years (Table 1). Single applications were given to bees at 0 days of age (Exp. 1 and Exp. 4) and 6 days of age (Exp. 2 and Exp. 5) in 1983 and 1985. An additional age group, 15 days old (Exp. 6), was added in 1985. Workers have been shown to begin foraging about this time (Michener 1974) and would then have the potential to be exposed to pesticides in the field.

Three experiments were performed to examine repeated exposure to pesticide. In 1983 workers were treated at 0, 4 and 8 days of age (Exp 3), and in 1985 one group was treated at 0,3 and 6 days (Exp. 7), while a second was treated at 10, 13 and 16 days of age (Exp. 8). Treatments were closer together in 1985

due to differences in observation schedules. Observations were done every second day in 1983 and every third day in 1985.

Single Exposures (Experiments 1, 2, 4, 5, and 6)

For Exp. 1 thirty newly-emerged workers were treated at each dosage on 8 June in 1983 and for Exp. 4 at least 50 per dose on 20 May in 1985. In Exp. 2 on 17 May in 1983, workers of 6-days old were removed through the portholes in the 'Dial-A-Bee' hive by grasping their hindlegs with forceps, treated, and reintroduced to the hive. Twenty workers were treated per dose. For Exp. 5 (6-day old) and Exp. 6 (15 day old) in 1985 the hives were taken apart, workers removed, treated, and returned to the reassembled hive, with at least 50 workers treated per dose. Treatments were done 26 May and 4 June for Exp. 5 and Exp. 6 respectively.

Repeated Exposures (Experiments 3, 7 and 8)

Three studies of the effect of repeated pesticide exposure were done. There were six treatments groups in each study: workers treated either 1) one, 2) two or 3) three times with the calculated LD_{10}, and workers treated with 1 µl acetone 4) one, 5) two or 6) three times as controls. In Exp. 3 (1983), newly emerged workers were treated before introduction, while the later treatments (4 and 8 days old) workers were removed through the portholes in the 'Dial' of the hive. Treatments began on 30 June with at least 20 bees in each group. Exp. 7 (1985) began 10 July when newly emerged workers were treated. Subsequent treatments were done at 3 and 6 days of age. The bees for Exp. 8 emerged 9 July and treatments were performed at 10, 13 and 16 days of age. At least 20 bees were treated in each group in 1985. The hives were taken apart to treat the older bees, then reassembled and workers returned as before in the single exposure experiments.

Observations

Observations of marked workers began the day after introduction to allow for any initial rejection (usually <5% for untreated bees, this study and Winston and Punnett 1982). Both within-hive and entrance observations were made.

During within-hive observations, frames were scanned and marked workers were recorded as performing one or more of the following tasks:

(1) clean: a worker cleans cells or removes cell cappings, dead larvae, pupae or adults from the hive;

(2) brood: a worker inserts her head into or enters a cell containing larvae or eats pollen;

(3) comb: a worker constructs new cells, repairs old cells or caps cells;

(4) nectar: a worker receives, ripens or eats nectar, or deposits nectar into cells;

(5) groom: a worker uses her mandibles to clean the back, wing articulations or wing of another worker;

(6) fan: a worker stands in the hive and fans her wings;

(7) drone: a worker feeds or grooms a drone;

(8) queen: a worker feeds, antennates or grooms the queen;

(9) dorsoventral abdominal vibrations (DVAV): a worker vibrates the abdomen in a vertical direction directly on the comb or while grasping another worker;

(10) dance: a worker performs the round or waggle communication dance;

(11) forage: a worker packs pollen into a cell, carries pollen or propolis in the corbiculae, or dances;

(12) inspect: a worker moves through the hive at any rate and in any direction or inspects cells for a brief period; and

(13) rest: a worker stands in hive motionless or grooms self.

During the entrance observations the times of marked workers leaving and returning to the hive and activities at the entrance were recorded. The following tasks were noted:

(1) entrance: a worker guards or fans at the hive entrance,

(2) orient: flights lasting less than five minutes whereby young workers leave the colony, fly in circles in the immediate vicinity of the hive and return to the colony (Winston and Katz 1982), and

(3) forage: flights lasting five minutes or longer, during which time workers collect nectar, pollen, propolis or water (Winston and Katz 1982).

Single Exposures (Experiments 1, 2, 4, 5 and 6)

In 1983 the observation hives were observed every second day until the bees were 45 days old. Hives were observed for three hours each day, 1-1/2 hours between 0800 and 1200 and 1-1/2 hours between 1200 and 1700. These periods consisted of 45 minutes of within-hive and 45 minutes of entrance observations. In 1985 observations continued until fewer than 10 bees remained alive (approximately 50 days of age). Observations were made every third day between 1000 and 1700 hours and consisted of two hours, one hour of within-hive and one hour of entrance observations.

Repeated Exposures (Experiments 3, 7 and 8)

Observations were performed as previously described in the single dosage experiments. Observations continued until fewer than 10 marked bees (a total of all of the treatments) remained alive, 35 days of age in 1983 and 50 days of age in 1985.

Data Analyses

Longevity in days (the last day a worker was seen), the total number of tasks performed, and the first day and duration in days (the number of days

between the first and last day a worker was seen performing the task) of a specific task were calculated from the observational data. Longevity, number of tasks and the duration and first day of foraging were analyzed for all experiments. Drone, queen, DVAV and dance are rare tasks and in no cases were there sufficient data ($n \geq 3$) to analyze. Rest and inspect are common throughout a workers's life and therefore were not analyzed for first day or duration. However, these were used to calculate the number of tasks each individual performed. The rest of the tasks, including clean, brood, comb, nectar, groom, fan, entrance, and orient were analyzed only when sufficient data ($n \geq 3$) were collected.

Four experiments in the observation hives contained marked workers which were not treated with either pesticide or acetone. These workers were considered as untreated controls and compared by t-test to acetone-treated controls (Table 3) (SPSSX) (Zar 1984). For further analyses, only treated controls were considered as few differences were seen between the untreated and treated controls in 1983 and no differences in 1985.

Single Exposures (Experiments 1, 2, 4, 5 and 6)

Analyses of longevity, number of tasks and the first day and duration of specific tasks were done by oneway analysis of variance and the SNK multiple comparison test (SPSSX) (Zar 1984). Only those workers seen the second observation day after treatment (72 hours in 1983 and 96 hours in 1985) and beyond were used in the analyses to ensure only sub-acute results were included.

Repeated Exposures (Experiments 3, 7 and 8)

For the repeated exposures in the observation hives, longevity, number of tasks and first day and duration of specific tasks were analyzed. Initially a comparison of diazinon-treated workers with controls (acetone-treated) for each number of treatments (one, two or three) in each experiment was done by t-test (SPSSX) (Zar 1984). Workers seen the second observation day after the specific number of treatments (72 hours in 1983 and 96 hours in 1985) were included in

TABLE 3: Comparison of acetone-treated and untreated control worker honey bees in observation hives (Experiments 2, 4, 5 and 6).

Year	Worker Age (days)	Category[1]		Mean ± Standard Error[2] Treated	Untreated	Significance[3]
				EXPERIMENT 2[4]		
1983	6	Longevity		29.5 ± 2.2	36.8 ± 1.8	0.02
		Number of Tasks		8.7 ± 0.3	8.8 ± 0.4	NS
		Clean	- 1st Day	7.8 ± 0.5	9.7 ± 1.2	NS
			- Duration	7.4 ± 4.4	7.0 ± 2.0	NS
		Brood	- 1st Day	16.7 ± 1.1	15.3 ± 1.2	NS
		Comb	- 1st Day	11.3 ± 1.3	9.1 ± 0.6	NS
			- Duration	4.0 ± 2.3	8.9 ± 1.5	NS
		Nectar	- 1st Day	11.1 ± 1.1	11.6 ± 1.2	NS
			- Duration	15.9 ± 2.2	21.2 ± 2.6	NS
		Groom	- 1st Day	16.3 ± 2.1	21.4 ± 2.0	NS
			- Duration	2.3 ± 1.3	8.3 ± 1.9	0.02
		Fan	- 1st Day	18.0 ± 1.9	15.7 ± 2.0	NS
		Entrance	- 1st Day	18.3 ± 2.8	23.7 ± 3.2	NS
			- Duration	8.5 ± 3.4	5.7 ± 2.5	NS
		Orient	- 1st Day	16.3 ± 1.8	17.2 ± 2.0	NS
		Forage	- 1st Day	23.5 ± 1.7	22.1 ± 1.9	NS
			- Duration	7.5 ± 2.0	15.9 ± 2.7	0.02
				EXPERIMENT 4[4]		
1985	0	Longevity		39.9 ± 1.6	38.8 ± 1.8	NS
		Number of Tasks		6.0 ± 0.2	5.5 ± 0.3	NS
		Clean	- 1st Day	21.4 ± 4.2	15.6 ± 4.3	NS
		Brood	- 1st Day	6.6 ± 1.3	4.9 ± 1.2	NS
			- Duration	4.4 ± 0.9	5.9 ± 1.7	NS
		Comb	- 1st Day	12.5 ± 2.0	15.6 ± 2.5	NS
			- Duration	3.9 ± 0.8	8.3 ± 2.7	NS

(Cont.)

TABLE 3: (Cont.)

Year	Worker Age (days)	Category[1]		Mean ± Standard Error[2] Treated	Untreated	Significance[3]
1985	0 (cont.)	Nectar	- 1st Day	18.8 ± 2.0	14.0 ± 1.9	NS
			-Duration	13.8 ± 2.0	14.6 ± 3.2	NS
		Orient	- 1st Day	16.5 ± 2.2	14.5 ± 2.5	NS
			- Duration	5.8 ± 2.9	6.0 ± 3.3	NS
		Forage	- 1st Day	30.6 ± 1.1	32.4 ± 1.4	NS
			- Duration	10.3 ± 1.1	9.1 ± 1.4	NS
		EXPERIMENT 5[4]				
1985	6	Longevity		31.7 ± 1.7	29.4 ± 3.8	NS
		Number of Tasks		5.4 ± 0.3	5.5 ± 0.8	NS
		Nectar	- 1st Day	17.4 ± 1.6	17.5 ± 3.7	NS
			- Duration	9.3 ± 1.8	11.0 ± 4.6	NS
		Forage	- 1st Day	24.8 ± 1.5	22.9 ± 3.0	NS
			- Duration	10.5 ± 1.8	12.1 ± 3.9	NS
		EXPERIMENT 6[4]				
1985	15	Longevity		32.0 ± 1.1	33.3 ± 1.8	NS
		Number of Tasks		5.7 ± 0.2	5.9 ± 0.2	NS
		Nectar	- 1st Day	23.3 ± 1.2	25.0 ± 2.0	NS
			- Duration	5.5 ± 1.6	4.4 ± 3.0	NS
		Forage	- 1st Day	25.7 ± 0.8	26.8 ± 1.1	NS
			- Duration	7.0 ± 0.9	7.7 ± 2.1	NS

[1]Analysis performed only where the number of observations ≥ 3.

[2]For each category mean includes workers alive 72 hours after exposure in 1983 and 96 hours after exposure in 1985; Longevity expressed in days is the last day a bee was seen, Number of Tasks is the total number of tasks performed, Task - 1st Day is the first day a task was seen performed, and Task - Duration expressed in days is the time between the first and last performance of a task; and the number of observations for longevity and number of tasks ranges between 19 to 27 for Exp. 2, 30 to 49 for Exp 4, 11 to 44 for Exp. 5 and 18 to 46 for Exp 6 and for tasks - 1st day and duration 5 to 19 for Exp. 2, 10 to 40 for Exp. 4, 6 to 33 for Exp. 5 and 8 to 39 for Exp 6.

[3]Probabilities of t-test, NS = Not Significant, $p > 0.05$.

[4]Experiment # from Table 1.

the analyses to ensure only sub-acute results were included. This allowed the inclusion of those workers treated once or twice which began a task or died before the subsequent treatment date. Analyses of all six treatments (pesticide and control) in each experiment were done by two-way analysis of variance (i.e. control or diazinon and number of exposures) and the SNK multiple comparison test (SPSSX) (Zar 1984). Only those workers seen the second observation day after the final treatment (72 hours in 1983 and 96 hours in 1985) were included in this analysis.

2. RESULTS

Single Exposures (Experiments 1, 2, 4, 5 and 6)

One statistically significant difference was found in 1983 (Table 4). In Exp. 1 longevity of bees treated at 0 days was reduced by pesticide treatment.

Three statistically significant differences were found in 1985 (Table 5). For the task "cleaning" in the 0 day treatment (Exp. 4), bees exposed to diazinon began earlier than controls in 2 of the 3 dosages. In this same treatment, foraging began earlier in the lowest dosage compared to the highest dosage. No significant differences were found between controls and any pesticide treatment in the group treated at 6 days of age (Exp. 5). For workers treated when15 days old (Exp. 6), entrance activites tended to begin later in pesticide-treated bees.

Repeated Exposures (Experiments 3, 7 and 8)

When pesticide-treated workers were compared by t-test to controls for each number of treatments, some statistically significant differences were found (Table 6). However, of the 12 differences, eight were caused by single pesticide treatments and two each by pesticide treatments of two and three times.

For Exp. 3, control workers treated once began nectar-handling later and continued it for a shorter period of time than workers treated with diazinon (Table 6). Controls treated three times began foraging earlier than pesticide-treated bees.

In Exp. 7, longevity was increased, the number of tasks was greater, brood care and nectar handling began later, and comb building continued longer in control workers treated once compared to diazinon-treated workers (Table 6). The duration of cleaning was greater in control workers treated twice with

(text cont. p. 31)

TABLE 4: Effects of a single exposure to diazinon on longevity and division of labour tasks of worker honey bees in observation hives in 1983 (Experiments 1 and 2).

Worker Age (days)	Category[1]	Control	Mean ± Standard Error at Specified Dosage[2] LD_1	LD_5	LD_{10}	LD_{20}	Significance[3]
			EXPERIMENT 1[4]				
0	Longevity	41.1 ± 1.9 a[5]	33.6 ± 3.2 ab	33.5 ± 2.9 ab	37.7 ± 2.6 ab	28.1 ± 4.0 b	0.05
	Number of Tasks	8.3 ± 0.4	7.6 ± 0.6	8.0 ± 0.5	8.9 ± 0.4	7.2 ± 0.6	NS
	Clean - 1st Day	5.9 ± 1.6	4.6 ± 1.1	5.0 ± 0.9	5.8 ± 1.3	5.0 ± 1.2	NS
	- Duration	18.4 ± 2.5	14.0 ± 2.5	12.6 ± 2.0	16.5 ± 2.8	13.5 ± 2.7	NS
	Brood - 1st Day	11.8 ± 1.6	12.2 ± 2.0	14.7 ± 1.7	12.1 ± 2.0	12.0 ± 1.9	NS
	- Duration	8.2 ± 2.0	9.3 ± 2.4	5.3 ± 1.8	6.7 ± 1.6	6.4 ± 2.9	NS
	Comb - 1st Day	12.0 ± 2.4	13.4 ± 3.3	9.9 ± 1.7	12.3 ± 2.3	8.0 ± 2.4	NS
	- Duration	2.5 ± 1.2	5.6 ± 2.3	6.4 ± 1.8	4.8 ± 1.3	3.5 ± 1.9	NS
	Nectar - 1st Day	7.0 ± 1.3	7.4 ± 1.7	5.8 ± 0.9	5.3 ± 1.0	5.8 ± 1.3	NS
	- Duration	26.0 ± 3.1	19.7 ± 3.2	22.3 ± 3.2	26.6 ± 3.3	19.1 ± 3.5	NS
	Groom - 1st Day	17.8 ± 3.4	17.4 ± 2.3	20.5 ± 2.6	21.4 ± 3.6	15.3 ± 3.0	NS
	- Duration	7.0 ± 2.9	5.3 ± 2.0	7.5 ± 3.4	7.0 ± 3.0	3.7 ± 2.3	NS
	Fan - 1st Day	13.0 ± 3.6	17.4 ± 4.5	13.9 ± 3.4	15.6 ± 3.3	10.2 ± 2.1	NS
	Entrance - 1st Day	17.9 ± 3.9	15.3 ± 4.2	22.6 ± 5.3	22.8 ± 3.6	19.8 ± 6.6	NS
	- Duration	5.6 ± 1.9	2.0 ± 0.8	2.1 ± 1.1	2.8 ± 0.9	1.8 ± 0.8	NS

(Cont.)

TABLE 4: (cont.)

Worker Age (days)	Category[1]		Control	Mean ± Standard Error at Specified Dosage[2] LD_1	LD_5	LD_{10}	LD_{20}	Significance[3]
0 (cont.)	Orient	-1st Day	26.6 ± 3.6	27.3 ± 2.8	20.2 ± 3.0	22.3 ± 2.9	22.1 ± 3.0	NS
		-Duration	6.8 ± 3.3	6.0 ± 2.4	4.7 ± 1.7	8.1 ± 2.6	6.8 ± 2.3	NS
	Forage	- 1st Day	25.9 ± 3.5	24.3 ± 2.6	19.0 ± 2.9	27.1 ± 3.6	15.4 ± 3.1	NS
		- Duration	16.9 ± 3.5	14.5 ± 2.6	19.8 ± 3.8	14.5 ± 3.4	23.4 ± 3.9	NS
				EXPERIMENT 2[4]				
6	Longevity		29.5 ± 2.2	28.6 ± 2.3	28.5 ± 2.7	31.6 ± 2.9	28.4 ± 2.8	NS
	Number of Tasks		8.7 ±0.3	8.7 ± 0.5	8.6 ± 0.5	8.4 ± 0.5	8.4 ± 0.5	NS
	Brood	-1st Day	16.7 ± 1.1	15.2 ± 0.8	11.8 ± 1.4	14.2 ± 2.1	13.0 ± 2.0	NS
	Comb	-1st Day	11.3 ± 1.3	11.3 ± 1.2	11.3 ± 1.5	9.4 ± 0.9	12.4 ± 2.5	NS
		- Duration	4.0 ± 2.3	7.0 ± 1.2	6.3 ± 2.2	7.9 ± 1.4	7.0 ± 1.9	NS
	Nectar	-1st Day	11.1 ± 1.1	13.3 ± 2.1	9.9 ± 0.7	8.8 ± 0.6	15.0 ± 2.5	NS
		- Duration	15.9 ± 2.2	13.3 ± 2.8	14.1 ± 2.9	23.3 ± 3.4	17.0 ± 3.4	NS
	Groom	-1st Day	16.3 ± 2.1	18.8 ± 2.2	19.4 ± 2.9	15.9 ± 2.6	16.5 ± 1.8	NS
		-Duration	2.3 ± 1.3	3.2 ± 2.2	4.3 ± 1.7	3.9 ± 1.8	6.0 ± 2.2	NS
	Fan	-1st Day	18.0 ± 1.9	16.0 ± 2.6	15.5 ± 2.2	23.0 ± 4.6	19.5 ± 3.6	NS
	Entrance	- 1st Day	18.3 ± 2.8	18.8 ± 1.8	21.3 ± 2.0	23.9 ± 2.8	17.6 ± 3.4	NS
		- Duration	8.5 ± 3.4	3.9 ± 1.5	2.1 ± 0.9	6.1 ± 2.9	3.9 ± 1.6	NS

(Cont.)

TABLE 4: (cont.)

Worker Age (days)	Category[1]		Mean ± Standard Error at Specified Dosage[2] Control	LD_1	LD_5	LD_{10}	LD_{20}	Significance[3]
6 (cont.)	Orient	- 1st Day	16.3 ± 1.8	13.8 ± 2.5	15.0 ± 2.2	13.8 ± 2.2	20.4 ± 4.4	NS
	Forage	- 1st Day	23.5 ± 1.7	17.8 ± 2.1	19.5 ± 2.7	19.6 ± 1.8	18.5 ± 2.5	NS
		- Duration	7.5 ± 2.0	10.9 ± 2.4	9.2 ± 2.2	9.5 ± 2.7	11.4 ± 2.5	NS

[1]Analysis performed only where the number of observations ≥ 3.

[2]For each category mean includes workers alive 72 hours after exposure; Longevity expressed in days is the last day a bee was seen, Number of Tasks is the total number of tasks performed, Task - 1st Day is the first day a task was seen performed, and Task - Duration expressed in days is the time between the first and last performance of a task; and the number of observations for longevity and number of tasks ranges between 18 to 26 for Exp. 1 and 16 to 20 for Exp. 2 and for tasks - 1st day and duration 4 -24 for Exp. 1 and 6 to 15 for Exp 2.

[3]Probabilities of ANOVA F-test, NS = Not Significant, $p > 0.05$.

[4]Experiment * from Table 1.

[5]Different letters in a row indicate significant differences in means by the SNK multiple comparison test.

TABLE 5: Effects of a single exposure to diazinon on longevity and division of labour tasks of worker honey bees in observation hives in 1985 (Experiments 4, 5 and 6).

Worker Age (days)	Category[1]	Mean ± Standard Error at a Specified Dosage[2]. Control	LD_5	LD_{10}	LD_{25}	Significance[3]
		EXPERIMENT 4[4]				
0	Longevity	39.9 ± 1.6	38.0 ± 1.8	39.5 ± 1.6	39.3 ± 1.4	NS
	Number Of Tasks	6.0 ± 0.2	5.9 ± 0.2	5.8 ± 0.2	6.0 ± 0.2	NS
	Clean - 1st Day	21.4 ± 4.2 a[5]	6.3 ± 2.3 b	14.5 ± 3.0 ab	11.2 ± 1.9 b	0.01
	Brood - 1st Day	6.6 ± 1.3	5.8 ± 0.9	7.7 ± 1.0	6.2 ± 1.0	NS
	- Duration	4.4 ± 0.9	6.1 ± 1.1	4.9 ± 1.2	4.7 ± 1.0	NS
	Comb - 1st Day	12.5 ± 2.0	14.7 ± 2.5	14.1 ± 2.3	12.3 ± 1.8	NS
	- Duration	3.9 ± 0.8	5.5 ± 1.6	7.4 ± 2.3	5.3 ± 1.8	NS
	Nectar - 1st Day	18.8 ± 2.0	20.3 ± 2.1	19.0 ± 2.0	19.2 ± 1.7	NS
	- Duration	13.8 ± 2.0	12.3 ± 2.1	12.1 ± 1.8	12.4 ± 1.8	NS
	Entrance - 1st Day	34.8 ± 4.8	31.6 ± 3.1	31.4 ± 3.8	33.7 ± 4.2	NS
	- Duration	4.8 ± 2.8	2.2 ± 0.7	1.8 ± 0.8	2.0 ± 0.7	NS
	Orient - 1st Day	16.5 ± 2.2	20.3 ± 2.3	20.3 ± 2.5	20.1 ± 2.0	NS
	- Duration	5.8 ± 2.9	1.4 ± 0.4	2.3 ± 1.1	2.1 ± 0.8	NS
	Forage - 1st Day	30.6 ± 1.0 ab	27.5 ± 1.0 a	31.2 ± 1.2 ab	31.7 ± 1.1 b	0.03
	- Duration	10.4 ± 1.1	13.7 ± 1.4	10.4 ± 1.1	11.5 ± 1.2	NS

(Cont.)

TABLE 5: (cont.)

Worker Age (days)	Category[1]	Mean ± Standard Error at a Specified Dosage[2]. Control	LD_5	LD_{10}	LD_{25}	Significance[3]
		EXPERIMENT 5[4]				
6	Longevity	31.7 ± 1.7	33.9 ± 1.7	29.4 ± 1.8	31.5 ± 1.4	NS
	Number of Tasks	5.4 ± 0.3	5.7 ± 0.2	5.3 ± 0.2	5.9 ± 0.2	NS
	Clean - 1st Day	17.7 ± 4.2	23.3 ± 4.3	12.3 ± 1.8	12.3 ± 2.6	NS
	Brood - 1st Day	16.3 ± 2.8	11.4 ± 1.3	14.0 ± 1.7	11.9 ± 1.3	NS
	- Duration	3.0 ± 1.4	2.2 ± 0.6	2.3 ± 1.0	3.7 ± 1.9	NS
	Comb - 1st Day	16.8 ± 1.9	18.8 ± 2.4	12.5 ± 2.1	16.3 ± 1.5	NS
	- Duration	3.3 ± 1.8	4.7 ± 1.9	2.5 ± 1.0	2.0 ± 0.5	NS
	Nectar - 1st Day	17.4 ± 1.6	17.0 ± 1.3	16.5 ± 1.2	16.7 ± 1.2	NS
	- Duration	9.3 ± 1.8	13.7 ± 2.4	10.4 ± 1.9	7.5 ± 1.7	NS
	Entrance - 1st Day	19.3 ± 2.0	25.3 ± 2.6	18.5 ± 2.5	23.3 ± 2.1	NS
	Orient - 1st Day	16.0 ± 2.0	22.0 ± 3.4	15.0 ± 1.9	18.4 ± 2.0	NS
	Forage - 1st Day	24.8 ± 1.5	29.3 ± 1.7	24.6 ± 1.8	24.3 ± 1.0	NS
	- Duration	10.5 ± 1.8	10.2 ± 1.7	7.8 ± 1.7	9.1 ± 1.4	NS
		EXPERIMENT 6[4]				
15	Longevity	31.8 ± 1.1	31.4 ± 1.2	33.0 ± 1.3	31.0 ± 1.0	NS
	Number of Tasks	5.7 ± 0.2	5.9 ± 0.2	5.9 ± 0.3	5.9 ± 0.2	NS

(Cont.)

TABLE 5: (cont.)

Worker Age (days)	Category[1]		Mean ± Standard Error at a Specified Dosage[2] Control	LD_5	LD_{10}	LD_{25}	Significance[3]
15 (cont.)	Comb	- 1st Day	18.1 ± 0.9	20.9 ± 1.1	20.8 ± 1.0	21.0 ± 1.1	NS
	Nectar	- 1st Day	23.3 ± 1.2	22.5 ± 1.1	25.8 2.1	24.0 ± 1.0	NS
		- Duration	5.5 ± 1.6	3.3 ± 1.2	3.8 ± 2.0	5.3 ± 1.2	NS
	Entrance	- 1st Day	22.5 ± 1.2 a	31.0 ± 2.7 b	29.8 ± 1.8 b	26.7 ± 1.1 ab	0.01
	Orient	- 1st Day	22.0 ± 0.8	26.8 ± 2.7	24.4 ± 1.5	23.9 ± 1.1	NS
	Forage	- 1st Day	25.7 ± 0.8	25.9 ± 0.7	28.2 ± 0.9	26.8 ± 0.5	NS
		- Duration	7.0 ± 0.9	7.5 ± 1.3	10.5 ± 1.2	7.0 ± 1.0	NS

[1]Analysis performed only where the number of observations ≥ 3.

[2]For each category mean includes workers alive 96 hours after exposure; Longevity expressed in days is the last day a bee was seen, Number of Tasks is the total number of tasks performed, Task - 1st Day is the first day a task was seen performed, and Task - Duration expressed in days is the time between the first and last performance of a task; and the number of observations for longevity and number of tasks ranges between 49 and 62 for Exp. 4, 42 and 54 for Exp. 5, and 44 and 58 for Exp. 6, and for tasks - 1st day and duration, 4 and 54 for Exp. 4, 9 and 43 for Exp. 5 and 5 and 42 for Exp. 6.

[3]Probabilities of ANOVA F-test, NS = Not Significant, $p > 0.05$.

[4]Experiment # from Table 1.

[5]Different letters in a row indicate significant differences in means by the SNK multiple comparison test.

Table 6: A comparison of control (acetone) and pesticide (diazinon - LD_{10}) treatments of one, two or three exposures on longevity and division of labour tasks of worker honey bees in observation hives (Experiments 3, 7 and 8).

Experiment[1]	Category[2]		Number of Treatments	Mean ± Standard Error[3] Control	Diazinon	Significance[4]
3	Longevity		1	20.4 ± 2.1	18.9 ± 2.5	NS
			2	25.8 ± 1.2	26.4 ± 1.6	NS
			3	25.6 ± 1.2	24.0 ± 1.7	NS
	Number of Tasks		1	5.9 ± 0.4	4.7 ± 0.4	NS
			2	7.0 ± 0.3	6.3 ± 0.4	NS
			3	6.2 ± 0.4	6.3 ± 0.4	NS
	Clean	- 1st Day	1	7.9 ± 2.0	7.9 ± 1.7	NS
			2	12.9 ± 1.1	10.4 ± 1.0	NS
		- Duration	1	4.1 ± 1.6	3.0 ± 1.1	NS
			2	3.0 ± 1.4	1.5 ± 0.5	NS
	Brood	- 1st Day	1	5.9 ± 1.9	3.0 ± 1.2	NS
			2	9.0 ± 2.4	8.0 ± 3.0	NS
	Comb	- 1st Day	1	3.5 ± 0.9	4.3 ± 0.8	NS
	Nectar	- 1st Day	1	8.1 ± 1.3	4.5 ± 0.8	0.02
			2	8.6 ± 0.7	11.6 ± 1.4	NS
			3	13.0 ± 2.1	13.0 ± 1.5	NS
		- Duration	1	9.6 ± 1.7	19.4 ± 2.8	0.01
			2	11.6 ± 1.8	14.0 ± 2.2	NS
			3	10.1 ± 3.1	7.0 ± 3.7	NS
	Groom	- 1st Day	1	11.6 ± 3.0	16.6 ± 2.3	NS
			2	15.4 ± 2.0	14.3 ± 2.7	NS
		-Duration	1	6.1 ± 3.3	2.6 ± 1.6	NS
			2	3.7 ± 1.8	9.4 ± 2.8	NS
	Entrance	- 1st Day	2	17.2 ± 2.2	17.0 ± 3.1	NS

(Cont.)

Table 6: (Cont.)

Experiment[1]	Category[2]		Number of Treatments	Mean ± Standard Error[3] Control	Diazinon	Significance[4]
3 (cont.)	Forage	- 1st Day	1	19.3 ± 1.2	19.4 ± 1.1	NS
			2	17.2 ± 0.5	18.8 ± 0.7	NS
			3	16.7 ± 0.5	18.4 ± 0.7	0.04
		- Duration	1	8.6 ± 1.4	8.5 ± 1.6	NS
			2	9.1 ± 1.1	9.4 ± 1.1	NS
			3	7.9 ± 1.2	5.9 ± 1.2	NS
7	Longevity		1	31.6 ± 2.1	22.7 ± 2.0	0.01
			2	28.1 ± 1.6	23.8 ± 1.6	NS
			3	24.5 ± 1.4	23.3 ± 1.3	NS
	Number of Tasks		1	6.1 ± 0.4	5.0 ± 0.3	0.02
			2	5.8 ± 0.3	5.5 ± 0.2	NS
			3	5.5 ± 0.2	5.5 ± 0.2	NS
	Clean	- 1st Day	1	14.8 ± 2.1	10.9 ± 2.0	NS
			2	10.5 ± 1.2	12.7 ± 1.5	NS
			3	16.6 ± 3.2	11.0 ± 1.2	NS
		- Duration	1	7.6 ± 2.3	6.1 ± 2.6	NS
			2	13.3 ± 3.8	3.7 ± 1.0	0.03
			3	1.9 ± 0.5	3.4 ± 1.2	NS
	Brood	- 1st Day	1	6.6 ± 1.3	3.9 ± 0.6	0.05
			2	7.5 ± 0.9	6.7 ± 0.5	NS
		- Duration	1	3.6 ± 0.9	6.7 ± 1.5	NS
			2	4.5 ± 1.5	4.5 ± 1.2	NS
	Comb	- 1st Day	1	7.0 ± 1.6	9.6 ± 2.3	NS
			2	12.3 ± 1.7	11.1 ± 1.7	NS
			3	10.9 ± 1.6	9.8 ± 0.8	NS
		- Duration	1	12.5 ± 2.0	5.4 ± 1.8	0.01
			2	5.7 ± 1.6	4.1 ± 1.2	NS
			3	4.9 ± 1.7	4.3 ± 1.5	NS
	Nectar	- 1st Day	1	15.8 ± 2.2	10.3 ± 1.6	0.05
			2	14.5 ± 2.6	13.8 ± 1.7	NS
			3	12.2 ± 1.4	15.1 ± 2.1	NS
		- Duration	1	11.4 ± 2.8	7.9 ± 2.5	NS
			2	6.8 ± 2.5	6.5 ± 1.8	NS
			3	6.6 ± 1.8	5.1 ± 1.8	NS

(Cont.)

Table 6: (Cont.)

Experiment[1]	Category[2]		Number of Treatments	Mean ± Standard Error[3] Control	Diazinon	Significance[4]
7 (cont.)	Forage	- 1st Day	1	27.6 ± 1.9	23.1 ± 1.9	NS
			2	25.7 ± 1.8	22.2 ± 1.8	NS
			3	18.8 ± 1.3	18.8 ± 1.2	NS
		- Duration	1	10.3 ± 2.0	7.4 ± 1.6	NS
			2	5.0 ± 1.0	5.0 ± 0.9	NS
			3	5.9 ± 1.0	6.2 ± 1.1	NS
8	Longevity		1	26.0 ± 1.8	23.1 ± 1.7	NS
			2	24.0 ± 0.9	24.2 ± 1.1	NS
			3	28.6 ± 1.1	25.6 ± 1.1	0.05
	Number of Tasks		1	5.8 ± 0.3	5.1 ± 0.3	NS
			2	5.8 ± 0.2	5.2 ± 0.2	NS
			3	6.2 ± 0.2	5.9 ± 0.2	NS
	Clean	- 1st Day	1	17.0 ± 2.0	23.0 ± 0.7	0.02
		- Duration	1	2.1 ± 1.1	1.9 ± 0.9	NS
	Nectar	- 1st Day	1	16.5 ± 2.2	22.0 ± 3.6	NS
			2	19.1 ± 1.7	26.0 ± 2.3	0.03
			3	24.1 ± 3.3	23.7 ± 1.9	NS
	Forage	- 1st Day	1	23.0 ± 1.4	23.6 ± 1.5	NS
			2	23.7 ± 1.1	24.2 ± 1.1	NS
			3	25.4 ± 1.2	24.2 ± 1.1	NS
		-Duration	1	5.1 ± 1.4	4.9 ± 2.1	NS
			2	3.5 ± 0.8	3.2 ± 0.9	NS
			3	4.9 ± 1.5	2.2 ± 0.5	NS

[1]Experiment # from Table 1; treatment schedule as follows: Exp. 3 (1983) - at 0,4 and 8 days old; Exp. 7 (1985) - at 0,3 and 6 days old; and Exp. 8 (1985) - at 10, 13 and 16 days old.

[2]Analysis performed only where the number of observations ≥ 3.

[3]For each category mean includes workers alive 72 hours after treatment in 1983 and 96 hours after treatment in 1985 for each number of treatments; Longevity expressed in days is the last day a bee was seen, Number of Tasks is the total number of tasks performed, Task - 1st Day is the first day a task was seen performed, and Task - Duration expressed in days is the time between the first and last performance of a task; and the number of observations for longevity and number of tasks ranges between 14 and 34 for Exp. 3, 27 and 59 for Exp. 7 and 16 and 40 for Exp. 8, and for tasks - 1st day and duration, 13 and 32 for Exp 3, 5 and 38 for Exp. 7 and 9 and 19 for Exp. 8.

[4]Probabilities of t-test, NS = Not Significant, $p > 0.05$.

acetone than those treated twice with diazinon.

Longevity was lower in workers treated three times with diazinon as compared to controls in Exp. 8 (Table 6). Control workers treated once began cleaning earlier, and control workers treated twice began nectar handling earlier than workers treated with diazinon in both cases.

When all treatment groups (diazinon- and acetone-treated once, twice and three times) were analyzed by ANOVA, some statistically significant differences were found for Exp. 7 and Exp. 8, but not for Exp. 3 (Table 7). In Exp. 7, statistically significant results were obtained in four cases. The number of treatments affected longevity. Bees treated three times with either acetone or diazinon lived shorter lives than those treated once or twice with either chemical. Controls treated once lived the longest. Comb building showed a similar pattern, with workers treated three times begining this task earlier. Foraging was significantly different in both initiation and duration. Workers treated once with acetone began foraging later and continued longer than all other groups. Again bees treated three times with either acetone or diazinon began foraging earlier than all other groups. In Exp. 8, the number of tasks performed was higher in controls than in pesticide-treated workers.

TABLE 7: Effects of one, two or three exposures to diazinon at LD_{10} or to acetone on longevity and division of labour tasks of worker honey bees in observation hives (Experiments 3, 7 and 8).

Experiment[1]	Category[2]	Mean ± Standard Error at Specified Number of Treatments[3]						Significance[4]	
		Control (Acetone-Treated)			Diazinon-Treated			Treatment	Times
		One	Two	Three	One	Two	Three		
3	Longevity	26.7 ± 1.3	26.8 ± 1.0	25.6 ± 1.2	27.8 ± 1.7	28.8 ± 1.1	24.0 ± 1.7	NS	NS
	Number of Tasks	6.9 ± 0.4	7.2 ± 0.2	6.2 ± 0.4	6.1 ± 0.3	6.6 ± 0.3	6.3 ± 0.4	NS	NS
	Forage - 1st Day	19.3 ± 1.2	17.5 ± 0.5	16.7 ± 0.5	19.4 ± 1.1	18.8 ± 0.7	18.4 ± 0.7	NS	NS
	- Duration	8.6 ± 1.4	9.1 ± 1.1	7.9 ± 1.2	8.5 ± 1.6	9.4 ± 1.1	5.9 ± 1.2	NS	NS
7	Longevity	33.1 ± 2.0 a[5]	28.1 ± 1.6 b	24.5 ± 1.4 c	26.7 ± 1.6 b	27.0 ± 1.4 b	23.3 ± 1.3 c	0.05	0.01
	Number of Tasks	6.3 ± 0.3	5.8 ± 0.3	5.5 ± 0.2	5.6 ± 0.2	5.9 ± 0.2	5.5 ± 0.2	NS	NS
	Clean - 1st Day	18.4 ± 1.9	12.6 ± 1.2	17.7 ± 3.4	15.4 ± 2.7	15.4 ± 1.8	13.5 ± 1.6	NS	NS
	- Duration	6.2 ± 2.2	7.0 ± 3.9	1.7 ± 0.4	4.6 ± 3.6	4.6 ± 1.4	1.5 ± 0.3	NS	NS
	Comb - 1st Day	18.6 ± 3.4 b	18.4 ± 1.9 b	14.5 ± 2.5 d	19.8 ± 3.2 a	16.9 ± 2.6 c	11.3 ± 1.0 e	NS	0.03
	- Duration	6.6 ± 2.2	4.4 ± 1.7	4.3 ± 2.0	2.5 ± 1.1	4.2 ± 1.6	5.3 ± 2.1	NS	NS
	Nectar - 1st Day	21.3 ± 2.1	18.1 ± 2.8	15.6 ± 1.8	15.2 ± 2.2	18.2 ± 1.8	18.6 ± 2.5	NS	NS
	- Duration	7.2 ± 2.5	5.2 ± 2.2	4.6 ± 1.6	2.6 ± 1.4	7.6 ± 2.5	4.2 ± 1.9	NS	NS
	Forage - 1st Day	27.6 ± 1.9 a	25.7 ± 1.8 b	18.8 ± 1.3 d	23.1 ± 1.9 c	22.2 ± 1.8 c	18.8 ± 1.2 d	NS	0.01
	- Duration	10.3 ± 2.0 a	5.0 ± 1.0 b	5.9 ± 1.0 b	7.4 ± 1.6 b	5.0 ± 0.9 b	6.2 ± 1.1 b	NS	0.01

(Cont.)

TABLE 7: (cont.)

Experiment[1]	Category[2]	Mean ± Standard Error at Specified Number of Treatment[3]						Significance[4]	
		Control (Acetone-Treated)			Diazinon-Treated			Treatment	Times
		One	Two	Three	One	Two	Three		
8	Longevity	29.2 ± 1.3	26.8 ± 0.7	29.2 ± 1.1	27.9 ± 1.0	27.0 ± 0.9	26.8 ± 1.1	NS	NS
	Number of Tasks	6.2 ± 0.2	6.2 ± 0.2	6.3 ± 0.2	5.4 ± 0.4	5.5 ± 0.3	6.1 ± 0.2	0.03	NS
	Forage -1st Day	24.9 ± 1.0	25.2 ± 1.0	26.8 ± 1.1	24.7 ± 1.2	24.7 ± 1.1	24.2 ± 1.1	NS	NS
	-Duration	5.1 ± 1.1	2.9 ± 0.8	3.3 ± 0.7	3.0 ± 1.0	3.4 ± 1.0	2.2 ± 0.5	NS	NS

[1]Experiment # from Table 1; treatment Schedule as follows: Exp. 3 (1983) - at 0,4 and 8 days of age; Exp. 7 (1985) - at 0, 3 and 6 days of age; and Exp. 8 (1985) - at 10, 13 and 16 days of age.

[2]Analysis performed only where the number of observation ≥ 3.

[3]For each category mean includes workers alive 72 hours after last treatment in 1983 and 96 hours after last treatment in 1985 (last treatment = third treatment); Longevity expressed in days is the last day a bee was seen, Number of Tasks is the total number of tasks performed, Task - 1st Day is the first day a task was seen performed, and Task - Duration expressed in days is the time between the first and last performance of a task; and the number of observations for longevity and number of tasks ranges between 14 and 34 for Exp. 3, 27 and 59 for Exp. 7 and 16 and 40 for Exp. 8, and for tasks - 1st day and duration, 13 and 32 for Exp. 3, 5 and 38 for Exp. 7 and 9 and 19 for Exp. 8.

[4]Probabilities of ANOVA F-test, NS = Not Significant, $p > 0.05$; Treatment = acetone or diazinon, Times = number of times treated, Interaction (Treatment by Times) never significant.

[5]Different letters in a row indicate significant differences in means by the SNK multiple comparison test.

C. STANDARD HIVES (Experiments 9 and 10)

1. MATERIALS AND METHODS

Chemicals

Commercial formulations of insecticides were used. The organophosphorous insecticide diazinon was formulated as a 12.5% emulsifiable concentrate (Later's Diazinon, P.C.P. Act No. 11437, Later Chemicals Ltd., Richmond, B.C.), the carbamate carbaryl as a 22.5% concentrate (Wilson Liquid Sevin® Carbaryl Insecticide, P.C.P. Act No. 17971, Wilson Laboratories Inc., Dundas, Ont.) and the pyrethroid resmethrin as a 0.25% solution (House Plant Insect Killer, P.C.P. Act No. 16219, Later Chemicals Ltd., Richmond, B.C.). The formulations were diluted in acetone (reagent grade) to the required concentrations. Carbaryl was first diluted (1:10) with distilled water to facilitate further mixing with acetone.

The Colonies

Test colonies were located at Simon Fraser University, Burnaby, B.C. Studies were conducted from June to September 1985. Workers used in the experiments were obtained from a single colony (not one of the experimental hives) and treated as previously described in the observation hive studies.

Three colonies in standard Langstroth deep equipment were used. These colonies consisted of two boxes (supers) with 10 frames each, enough workers to cover most of the frames, and a healthy laying queen. A third super was added 10 July to alleviate colony crowding. This super was removed 16 Aug. for honey extraction. Experimentation began 18 June and continued until 5 Sept. for Hives 1 and 3, and 1 Aug. for Hive 2. Queen problems occurred in Hives 1 and 3. Hive 1 was found to be queenless 10 Aug. and was requeened 16 Aug. Hive 2 remained queenright throughout the experiment. A virgin queen was found and removed

from Hive 3 on 27 June. Subsequently, the queen appeared to be laying normally, but was superseded sometime before 18 July.

Treatments

The insecticides were applied as before. Acute toxicity studies (LD_{50}) as described previously were done with the three pesticides used in these experiments to determine the dosage range for sublethal exposure (Table 2). Doses causing approximately 5%, 10% and 25% mortality were used for each pesticide.

For Exp. 9 newly emerged bees (0-day group) were treated on 18 to 20 June with the desired dosage of pesticide and placed in the hive through the feeding hole in the top board. For this age group all three chemicals were used. Treatments for Exp. 10 were done on 3 July for Hives 1 and 3, and 4 July for Hive 2. The hives were taken apart and frames examined one by one. Marked workers were picked up by the hind leg, treated, held in a cage until all treatments for that hive were completed, and reintroduced through the feeding hole as above. The number of workers treated per dose ranged from 50 to 65. Workers, treated with acetone only, were the controls for both Exp. 9 and Exp. 10. Only diazinon and carbaryl were used in Exp. 10, because of normal worker death over the two week period and excessive time necessary to find and treat workers. This left a number of untreated workers as a second control group, exposed to neither acetone or pesticide, in each of the three hives.

Observations

Entrance observations began on 22 June and continued every third day until most of the bees were dead. Entrances were blocked with wire screen for 15 minutes, preventing any workers from leaving or entering, then the numbers of marked workers at the entrances were recorded for another 15 minutes. All

returning workers were considered to be foragers. Every 10 to 14 days the hives were checked for surviving workers by removing and examining carefully each frame and recording all marked workers.

Data Analyses

Longevity and the first day and duration of foraging were analyzed. Longevity was determined as the mid-point between the last day seen and the subsequent day survivorship was examined. Foraging was considered to commence on the first day a worker was seen outside the hive in the entrance observations. Duration of foraging was the number of days between the first and last time a worker was seen outside the hive.

Colony conditions were different for the three hives. Queen supersedure occurred within the first month in Hive 3; and a virgin queen was reared by the colony. Usually supersedure occurs when the queen is old or injured, laying poorly and producing an insufficient amount of queen substance (Butler 1975; Michener 1974). Queen loss has been shown to result in brood loss (Punnett and Winston 1983; Winston 1979) and lower foraging and hive activities (Genrikh 1957). Longevity was reduced by increased brood care (Maurizio 1950; Woyke 1984). Hive 1 also had queen problems. The queen was lost in the last three weeks of the experiment. At this time most of the marked workers in this colony had died. However, this may have caused a small delay in activities and increase in lifespan. To evaluate the possible effects of the differences in colony conditions, the marked, untreated workers in the hives were compared by oneway analysis of variance and SNK multiple comparison test (SPSSX) (Zar 1984) for each of the three categories examined. The three hives were significantly different in longevity and the first day of foraging (Table 8). Hives 1 and 2 were similar in duration of foraging but statistically different from Hive 3. Therefore further analyses were done separately for the three hives.

TABLE 8: Comparison of longevity and foraging of marked, untreated worker honey bees in the three standard hives.

Category	Mean ± Standard Error[1]			Significance[2]
	Hive 1	Hive 2	Hive 3	
Longevity	22.2 ± 0.7 a[3]	19.5 ± 0.4 b	39.6 ± 1.2 c	0.01
Forage - 1st Day	23.5 ± 0.7 a	20.6 ± 0.6 b	43.1 ± 1.2 c	0.01
- Duration	2.8 ± 0.3 a	2.4 ± 0.3 a	4.2 ± 0.6 b	0.01

[1]For each category mean includes workers seen at least 2 days after treatment; Longevity expressed in days is the mid-point between the last day seen and the subsequent day survivorship was examined, Foraging - 1st Day is the first day foraging was performed, and Foraging - Duration expressed in days is the time between the first and last day foraging was observed; and the number of observations for longevity ranged from 238 to 249 and for foraging - 1st day and duration, 91 to 118.

[2]Probabilities of ANOVA F-test.

[3]Different letters in a row indicate significant differences in means by the SNK multiple comparison test.

For each hive, longevity and age of first foraging and duration of foraging were analyzed. The treated controls and the three dosages of each pesticide in each age group initially were compared by oneway ANOVA. Significant results were analyzed by Duncan's multiple range test (SPSSX) (Zar 1984). Then, to examine the effects of different pesticides, all pesticide treatments in one age group were analyzed by twoway ANOVA (i.e. pesticide and dosage) and SNK multiple comparison tests in the three hives (SPSSX) (Zar 1984).

2. RESULTS

Some statistically significant differences were seen in the three hives when controls were compared to pesticide treatments (Tables 9, 10, 11). For Exp. 9 (newly emerged bees) there were statistically significant differences in all 3 hives. In Hive 1, carbaryl treatment reduced longevity (Table 9). Workers treated with carbaryl at LD_5 began foraging later than the controls and those treated with LD_{25}.

In Hive 2, the carbaryl-treated bees again showed significant differences (Table 10). Workers treated with the lower doses of carbaryl lived shorter lives than the controls and those treated with LD_{25}, and the initiation of foraging was earliest in bees treated with carbaryl at LD_5 and LD_{10}.

In Hive 3, significant differences were found for two of the three pesticides (Table 11). For diazinon, the duration of foraging was greatest in the LD_{25} dosage. Bees treated with a dosage of resmethrin at LD_{25} lived a longer life than those treated with either LD_5 or LD_{10}, but were similar to the controls.

For Exp. 10 (14-day old), only one difference was found in any of the three characteristics for any dosage of either diazinon or carbaryl in any hive (Tables 9, 10, 11). In Hive 2, foraging duration was significantly greater in controls than those treated with carbaryl.

Statistically significant differences were also found when treatments of all three pesticides were compared (Tables 12, 13). In Exp. 9 (newly emerged bees) longevity was affected (Table 12). Carbaryl-treated bees lived significantly shorter lives than either diazinon or resmethrin-treated bees in Hive 1; resmethrin-treated were intermediate and diazinon-treated the longest lived. In Hive 2, carbaryl, although only at dosages LD_5 and LD_{10}, had the lowest lifespan. Diazinon- and resmethrin-treated bees had similar lifespans. The

(text cont. p. 47)

TABLE 9: Longevity and foraging of worker honey bees exposed to different dosages of either diazinon, carbaryl or resmethrin or acetone control in Hive 1 (Experiments 9 and 10).

Experiment Number[1]	Pesticide	Category	Mean ± Standard Error at specified Dosage[2] Control	LD_5	LD_{10}	LD_{25}	Significance[3]
9	Diazinon	Longevity	27.2 ± 1.5	25.2 ± 1.6	25.8 ± 1.3	26.0 ± 2.4	NS
		Forage - 1st Day	23.1 ± 0.9	22.5 ± 1.3	23.0 ± 1.1	24.0 ± 2.5	NS
		- Duration	3.9 ± 1.0	1.8 ± 0.3	3.6 ± 0.8	4.6 ± 1.6	NS
	Carbaryl	Longevity	27.2 ± 1.5 a[4]	20.6 ± 5.0 ab	20.8 ± 3.4 ab	20.1 ± 1.8 b	0.05
		Forage - 1st Day	23.1 ± 0.9 a	34.4 ± 8.2 b	24.5 ± 4.4 ab	21.3 ± 1.6 a	0.02
		- Duration	3.9 ± 1.0	2.1 ± 1.1	2.2 ± 0.8	3.3 ± 0.8	NS
	Resmethrin	Longevity	27.2 ± 1.5	25.4 ± 1.1	24.5 ± 1.2	23.4 ± 1.2	NS
		Forage - 1st Day	23.1 ± 0.9	22.2 ± 1.0	22.2 ± 1.2	20.8 ± 1.1	NS
		- Duration	3.9 ± 1.0	3.9 ± 0.7	3.8 ± 0.8	3.2 ± 0.6	NS
10	Diazinon	Longevity	28.4 ± 1.3	26.0 ± 0.8	28.3 ± 1.2	28.6 ± 1.4	NS
		Forage - 1st Day	24.7 ± 0.9	23.0 ± 1.1	23.9 ± 0.9	23.4 ± 0.9	NS
		-Duration	3.7 ± 0.6	4.0 ± 0.7	3.7 ± 0.7	3.9 ± 0.7	NS
	Carbaryl	Longevity	28.4 ± 1.3	28.8 ± 1.3	28.4 ± 1.2	26.7 ± 1.0	NS
		Forage - 1st Day	24.7 ± 0.9	22.1 ± 1.2	25.0 ± 1.0	23.3 ± 1.3	NS
		-Duration	3.7 ± 0.6	4.9 ± 1.0	4.8 ± 0.9	4.9 ± 1.4	NS

[1]Experiment # from Table 1; Treatments for Exp. 9 at emergence and for Exp. 10 at 14 days of age.

[2]For each category mean includes workers seen at least 2 days after treatment; Longevity expressed in days is the mid-point between the last day seen and the subsequent day survivorship was examined, Foraging - 1st Day is the first day foraging was performed, and Foraging - Duration expressed in days is the time between the first and last day foraging was observed; and the number of observations for longevity ranged from 17 to 58 and for foraging - 1 st day and duration, 6 to 36.

[3]Probabilities of ANOVA F-test, NS = Not Significant, $p > 0.05$.

[4]Different letters in a row indicate significant differences in means by Duncan's multiple range test.

TABLE 10: Longevity and foraging of worker honey bees exposed to different dosages of either diazinon, carbaryl or resmethrin or acetone control in Hive 2 (Experiments 9 and 10).

Experiment Number[1]	Pesticide	Category	Mean ± Standard Error at Specified Dosage[2] Control	LD_5	LD_{10}	LD_{25}	Significance[3]
9	Diazinon	Longevity	20.7 ± 0.9	20.8 ± 1.1	22.0 ± 0.8	19.1 ± 2.1	NS
		Forage - 1st Day	20.5 ± 0.6	22.3 ± 1.2	21.3 ± 1.1	22.0 ± 2.3	NS
		- Duration	2.6 ± 0.5	2.2 ± 0.8	3.1 ± 0.7	1.4 ± 0.4	NS
	Carbaryl	Longevity	20.7 ± 0.9 a[4]	14.2 ± 1.6 b	14.0 ± 1.8 b	20.2 ± 1.4 a	0.01
		Forage - 1st Day	20.5 ± 0.6 a	17.0 ± 1.0 b	17.3 ± 0.9 b	21.7 ± 1.6 a	0.02
		- Duration	2.6 ± 0.5	1.8 ± 0.8	2.4 ± 0.9	2.0 ± 0.7	NS
	Resmethrin	Longevity	20.7 ± 0.9	19.6 ± 1.1	20.5 ± 1.0	19.7 ± 0.8	NS
		Forage - 1st Day	20.5 ± 0.6	21.9 ± 1.3	21.1 ± 1.0	20.9 ± 1.0	NS
		- Duration	2.6 ± 0.5	1.6 ± 0.4	2.8 ± 0.8	1.9 ± 0.4	NS
10	Diazinon	Longevity	24.2 ± 0.5	23.7 ± 0.4	23.0 ± 0.2	23.6 ± 0.4	NS
		Forage - 1st Day	21.5 ± 1.1	21.6 ± 0.8	20.7 ± 0.9	19.9 ± 0.8	NS
		-Duration	3.8 ± 0.9	2.6 ± 0.6	1.9 ± 0.4	2.2 ± 0.5	NS
	Carbaryl	Longevity	24.2 ± 0.5	23.0 ± 0.2	23.3 ± 0.3	23.3 ± 0.3	NS
		Forage - 1st Day	21.5 ± 1.1	19.6 ± 0.8	20.2 ± 0.6	19.8 ± 0.6	NS
		-Duration	3.8 ± 0.9 a	1.7 ± 0.5 b	2.2 ± 0.5 b	1.8 ± 0.3 b	0.03

[1]Experiment # from Table 1; Treatments for Exp. 9 at emergence and for Exp. 10 at 14 days of age.

[2]For each category mean includes workers seen at least 2 days after treatment; Longevity expressed in days is the mid-point between the last day seen and the subsequent day survivorship was examined, Foraging - 1st Day is the first day foraging was performed, and Foraging - Duration expressed in days is the time between the first and last day foraging was observed; and the number of observations for longevity ranged from 13 to 63 and for foraging - 1st day, 5 to 32.

[3]Probabilities of ANOVA F-test, NS = Not Significant, $p > 0.05$.

[4]Different letters in a row indicate significant differences in means by Duncan's multiple range test.

TABLE 11: Longevity and foraging of worker honey bees exposed to different dosages of either diazinon, carbaryl or resmethrin or acetone control in Hive 3 (Experiments 9 and 10).

Experiment Number[1]	Pesticide	Category	Mean ± Standard Error at Specified Dosage[2] Control	LD_5	LD_{10}	LD_{25}	Significance[3]
9	Diazinon	Longevity	35.1 ± 3.0	37.8 ± 3.1	38.1 ± 2.1	33.0 ± 4.4	NS
		Forage - 1st Day	37.0 ± 2.7	40.4 ± 3.0	35.7 ± 2.6	39.6 ± 5.3	NS
		- Duration	3.6 ± 1.0 a[4]	1.5 ± 0.4 a	4.2 ± 1.4 a	9.8 ± 4.9 b	0.05
	Carbaryl	Longevity	35.1 ± 3.0	23.0 ± 3.5	30.0 ± 3.8	35.2 ± 3.5	NS
		Forage - 1st Day	37.0 ± 2.7	32.0 ± 4.9	40.3 ± 4.6	37.0 ± 6.4	NS
		- Duration	4.3 ± 1.0	3.0 ± 1.6	1.4 ± 0.3	1.5 ± 0.3	NS
	Resmethrin	Longevity	35.1 ± 3.0 ab	27.8 ± 3.1 b	28.1 ± 2.9 b	38.4 ± 2.4 a	0.01
		Forage - 1st Day	37.0 ± 2.7	43.6 ± 2.5	38.4 ± 2.7	41.3 ± 2.3	NS
		- Duration	3.6 ± 1.0	3.2 ± 1.4	2.7 ± 0.8	5.5 ± 1.5	NS
10	Diazinon	Longevity	44.8 ± 2.0	40.7 ± 1.8	45.0 ± 2.0	45.5 ± 1.4	NS
		Forage - 1st Day	41.1 ± 2.6	37.1 ± 2.1	42.6 ± 1.9	43.3 ± 1.8	NS
		-Duration	5.4 ± 1.3	5.4 ± 1.2	4.5 ± 0.9	4.5 ± 0.9	NS
	Carbaryl	Longevity	44.8 ± 2.0	45.6 ± 1.7	45.6 ± 1.5	46.8 ± 1.5	NS
		Forage - 1st Day	41.1 ± 2.6	46.0 ± 1.8	41.1 ± 2.1	43.1 ± 1.9	NS
		-Duration	5.4 ± 1.3	4.1 ± 1.0	6.2 ± 1.3	4.4 ± 0.9	NS

[1]Experiment # from Table 1; Treatments for Exp. 9 at emergence and for Exp. 10 at 14 days of age.

[2]For each category mean includes workers seen at least 2 days after treatment; Longevity expressed in days is the mid-point between the last day seen and the subsequent day survivorship was examined, Foraging - 1st Day is the first day foraging was performed, and Foraging - Duration expressed in days is the time between the first and last day foraging was observed; and the number of observations for longevity ranged from 23 to 61 and for foraging - 1st day and duration, 8 to 32.

[3]Probabilities of ANOVA F-test, NS = Not Significant, $p > 0.05$.

[4]Different letters in a row indicate significant differences in means by Duncan's multiple range test.

TABLE 12: Longevity and foraging of worker honey bees exposed to different dosages of diazinon, carbaryl or resmethrin at 0 days of age in all three standard hives (Experiment 9).

Category	Hive	Pesticide	Mean ± Standard Error of Specified Dosage[1] LD_5	LD_{10}	LD_{25}	Significance[2] Pesticide	Dosage	Interaction
Longevity	1	Diazinon	25.2 ± 1.6 bc[3]	25.8 ± 1.3 c	26.0 ± 2.4 c			
		Carbaryl	20.6 ± 5.0 a	20.8 ± 3.4 a	20.1 ± 1.8 a	0.01	NS	NS
		Resmethrin	25.4 ± 1.1 c	24.5 ± 1.2 bc	23.4 ± 1.2 b			
	2	Diazinon	20.8 ± 1.1 bc	22.0 ± 0.8 c	19.1 ± 2.1 b			
		Carbaryl	14.2 ± 1.6 a	14.0 ± 1.8 a	20.2 ± 1.4 bc	0.01	NS	0.04
		Resmethrin	19.6 ± 1.1 bc	20.5 ± 1.0 bc	19.7 ± 0.8 bc			
	3	Diazinon	37.8 ± 3.1 f	38.1 ± 2.1 f	33.0 ± 4.4 d			
		Carbaryl	23.0 ± 3.5 a	30.0 ± 3.8 c	35.2 ± 3.5 e	0.01	0.01	NS
		Resmethrin	27.8 ± 3.1 b	28.1 ± 2.9 b	38.4 ± 2.4 f			
Forage - 1st Day	1	Diazinon	22.5 ± 1.3 ab	23.0 ± 1.1 ab	24.0 ± 2.5 b			
		Carbaryl	34.4 ± 8.2 d	24.5 ± 4.4 c	21.3 ± 1.6 a	0.05	NS	0.02
		Resmethrin	22.2 ± 1.0 ab	22.2 ± 1.2 ab	20.8 ± 1.1 a			
	2	Diazinon	22.3 ± 1.2	21.3 ± 1.1	22.0 ± 2.3			
		Carbaryl	17.0 ± 1.0	17.3 ± 0.9	21.7 ± 1.6	NS	NS	NS
		Resmethrin	21.9 ± 1.3	21.1 ± 1.0	20.9 ± 1.0			
	3	Diazinon	40.4 ± 3.0	35.7 ± 2.6	39.6 ± 5.3			
		Carbaryl	32.0 ± 4.9	40.3 ± 4.6	37.0 ± 6.4	NS	NS	NS
		Resmethrin	43.6 ± 2.5	38.4 ± 2.7	41.3 ± 2.3			

(Cont.)

TABLE 12: (Cont.)

Category	Hive	Pesticide	Mean ± Standard Error of Specified Dosage[1] LD_5	LD_{10}	LD_{25}	Significance[2] Pesticide	Dosage	Interaction
Forage -Duration	1	Diazinon	1.8 ± 0.3	3.6 ± 0.8	4.6 ± 1.6			
		Carbaryl	2.1 ± 1.1	2.2 ± 0.8	3.3 ± 0.8	NS	NS	NS
		Resmethrin	3.9 ± 0.7	3.8 ± 0.8	3.2 ± 0.6			
	2	Diazinon	2.2 ± 0.8	3.1 ± 0.7	1.4 ± 0.4			
		Carbaryl	1.8 ± 0.8	2.4 ± 0.9	2.0 ± 0.7	NS	NS	NS
		Resmethrin	1.6 ± 0.4	2.8 ± 0.8	1.9 ± 0.4			
	3	Diazinon	1.5 ± 0.4	4.2 ± 1.4	9.8 ± 4.9			
		Carbaryl	3.0 ± 1.5	1.4 ± 0.3	1.5 ± 0.3	NS	NS	NS
		Resmethrin	3.2 ± 1.4	2.7 ± 0.8	5.5 ± 1.5			

[1]For each category mean includes workers seen at least 2 days after treatment; Longevity expressed in days is the mid-point between the last day seen and the subsequent day survivorship was examined, Foraging - 1st Day is the first day foraging was performed, and Foraging - Duration expressed in days is the time between the first and last day foraging was observed; and the number of observations for longevity ranged from 13 to 63 and for foraging, 5 to 35.

[2]Probabilities of ANOVA F-test, NS = Not Significant, $p > 0.05$.

[3]Different letters in both rows and columns for each hive indicate significant differences in means by the SNK multiple comparison test.

TABLE 13: Longevity and foraging of worker honey bees exposed to different dosages of either diazinon or carbaryl at 14 days of age in all three standard hives (Experiment 10).

Category	Hive	Pesticide	Mean ± Standard Error of Specified Dosage[1] LD_5	LD_{10}	LD_{25}	Significance[2] Pesticide	Dosage	Interaction
Longevity	1	Diazinon	26.0 ± 0.8	28.3 ± 1.2	28.6 ± 1.4			
		Carbaryl	28.8 ± 1.3	28.4 ± 1.2	26.7 ± 1.0	NS	NS	NS
	2	Diazinon	23.7 ± 0.4	23.0 ± 0.2	23.6 ± 0.4			
		Carbaryl	23.0 ± 0.2	23.3 ± 0.3	23.3 ± 0.3	NS	NS	NS
	3	Diazinon	40.7 ± 1.8	45.0 ± 2.0	45.5 ± 1.4			
		Carbaryl	45.6 ± 1.7	45.6 ± 1.5	46.8 ± 1.5	NS	NS	NS
Forage - 1st Day	1	Diazinon	23.0 ± 1.1	23.9 ± 0.9	23.4 ± 0.9			
		Carbaryl	22.1 ± 1.2	25.0 ± 1.0	23.3 ± 1.3	NS	NS	NS
	2	Diazinon	21.6 ± 0.8	20.7 ± 0.9	19.9 ± 0.8			
		Carbaryl	19.6 ± 0.8	20.2 ± 0.6	19.8 ± 0.6	NS	NS	NS
	3	Diazinon	37.1 ± 2.1 a[3]	42.6 ± 1.9 c	43.3 ± 1.8 c			
		Carbaryl	46.0 ± 1.8 d	41.1 ± 2.1 b	43.1 ± 1.9 c	NS	NS	0.02

(Cont.)

TABLE 13: (Cont.)

Category	Hive	Pesticide	Mean ± Standard Error of Specified Dosage[1] LD_5	LD_{10}	LD_{25}	Significance[2] Pesticide	Dosage	Interaction
Forage - Duration	1	Diazinon	4.0 ± 0.7	3.7 ± 0.7	3.9 ± 0.7			
		Carbaryl	4.9 ± 1.0	4.8 ± 0.9	4.9 ± 1.4	NS	NS	NS
	2	Diazinon	2.6 ± 0.6	1.9 ± 0.4	2.2 ± 0.5			
		Carbaryl	1.7 ± 0.5	2.2 ± 0.5	1.8 ± 0.3	NS	NS	NS
	3	Diazinon	5.4 ± 1.2	4.5 ± 0.9	4.5 ± 0.9			
		Carbaryl	4.1 ± 1.0	6.2 ± 1.3	4.4 ± 0.9	NS	NS	NS

[1]For each category mean includes workers seen at least 2 days after treatment; Longevity expressed in days is the mid-point between the last day seen and the subsequent day survivorship was examined, Foraging - 1st Day is the first day foraging was performed, and Foraging - Duration expressed in days is the time between the first and last day foraging was observed; and the number of observations for longevity ranged from 45 to 61 and for foraging 1st day and duration, 14 to 36.

[2]Probabilities of ANOVA F-test, NS = Not Significant, $p > 0.05$.

[3]Different letters in both rows and columns for each hive indicate significant differences in means by the SNK multiple comparison test.

results for Hive 3 were not as clear. The shortest longevity was found in carbaryl LD_5. Carbaryl-treated at LD_{10} and LD_{25} and resmethrin-treated bees were intermediate, while diazinon-treated workers once again had the longest lifespans.

One statistically significant difference was found in foraging (Table 12). In Hive 1 carbaryl-treated bees tended to forage later than bees treated with the other two pesticides.

For Exp. 10 (14 day old), only one statistically significant difference was found (Table 13). In Hive 3, workers treated with diazinon at LD_5 began foraging earlier than any other treatment, while those treated with carbaryl at LD_5 began the latest.

Survivorship was calculated for each treatment group in each hive. Workers were counted as surviving if seen at least once in either entrance observations or survivorship examinations. Poor survivorship was found for groups treated when newly emerged with carbaryl at all three dosages and diazinon at LD_{25} (Table 14). These were all treated on the afternoon of 18 June. Newly emerged workers treated in the morning of 18 June (acetone and diazinon LD_5 and LD_{10}) and 19 June (resmethrin, all dosages) had better survival. Two week old workers were found to have very good survivorship after treatment.

TABLE 14: Survivorship of acetone and pesticide treated worker honey bees in the three standard colonies.

Worker Age (days)	Treatment		% Survival[1] Hive 1	Hive 2	Hive 3
0	Acetone		98.0	90.0	87.0
	Diazinon	- LD_5	73.1	63.5	63.5
		- LD_{10}	94.5	100.0	87.0
		- LD_{25}	35.5	20.6	37.1
	Carbaryl	- LD_5	32.7	38.5	54.0
		- LD_{10}	35.3	42.5	61.1
		- LD_{25}	54.8	41.9	52.5
	Resmethrin	- LD_5	98.1	92.3	86.5
		- LD_{10}	98.1	89.1	90.9
		- LD_{25}	92.1	100.0	96.8
14	Acetone		91.7	92.0	96.0
	Diazinon	- LD_5	95.9	95.9	91.8
		- LD_{10}	94.3	92.6	96.4
		- LD_{25}	93.0	89.8	100.0
	Carbaryl	- LD_5	92.0	89.1	100.0
		- LD_{10}	92.4	89.1	100.0
		- LD_{25}	94.8	88.3	100.0

[1]Workers were counted as surviving if seen at least once in either entrance observations or survivorship examinations.

D. DISCUSSION

The objective of this study was to examine the effects of sublethal exposure to pesticides on temporal division of labour and longevity in the honey bee. Experiments were conducted with variables including treatment age, number of treatments, and pesticide type. The results indicate that exposure to low dosages of topically applied pesticide can reduce longevity and disrupt one aspect of temporal division of labour, foraging (Table 15). Other division of labour tasks were affected occasionally, suggesting that these tasks are less sensitive indicators of pesticide-induced stress.

Longevity and Foraging

Longevity was the most consistently affected trait of any of the categories studied (Table 15). It was reduced by both single and repeated diazinon applications in the observation hives and by carbaryl in the standard field colonies. Newly emerged workers were more sensitive to pesticide treatment, with six of the seven statistically significant differences seen in this age group (Table 15).

The fact that effects on longevity were not seen in all cases is not surprising, considering the number of factors which influence worker lifespan. Smirle *et al.* (1984) studied the effects of sublethal doses of malathion and diazinon on the longevity of newly emerged and and two week old workers in both laboratory cages and standard field colonies. Longevity was reduced in workers treated with diazinon at emergence in field colonies and workers in cages treated at two weeks of age with malathion or with diazinon. Experimental environment, treatment age, and type of pesticide were all partly responsible for the reduction in longevity.

Table 15: Summary of statistically significant results from experiments on effects of low dosages of pesticides on temporal division of labour and longevity in the honey bee.

Category		Experiment Number[1]	Description of Variables	Results
Longevity		1	Single Dose, Diazinon, 0 day, observation hive	Diazinon reduced longevity
		7	Single Dose, Diazinon, 0 day, observation hive	Diazinon reduced longevity
		8	Repeated Exposure, Diazinon, 10,13 & 16 day, observation hive	Diazinon reduced longevity
		7	Repeated Exposure, Diazinon, 0,3 & 6 day, observation hive	Longest lifespan in control treated 1 time, control and pesticide treated 3 times shortest lifespans
		9	Single Dose, Carbaryl, 0 day, 2 standard hives	Carbaryl reduced longevity
		9	Single Dose, Resmethrin, 0 day, 1 standard hive	Resmethrin LD_{25} longer lifespan than other treated group
		9	Single Dose, Carbaryl, Diazinon and Resmethrin, 0 day, 3 standard hives	Carbaryl lowest longevity in all 3 hives, Diazinon longest in 2 hives and similar to Resmethrin in third hive
Number of Tasks		7	Single Dose, Diazinon, 0 day, observation hive	Diazinon reduced number of tasks
		8	Repeated Exposure, Diazinon, 10, 13 & 16 day, observation hive	Diazinon reduced number of tasks
Brood Care	- 1st day	7	Single Dose, Diazinon, 0 day, observation hive	Diazinon-treated began earlier
Cleaning	- 1st day	4	Single Dose, Diazinon, 0 day, observation hive	Diazinon-treated began earlier, LD_5 earliest
		8	Single Dose, Diazinon, 10 day, observation hive	Diazinon-treated began later
	- duration	7	Repeated Exposure, Diazinon, 0 & 3 day, observation hive	Diazinon-treated shorter duration

(Cont.)

Table 15: (Cont.)

Category		Experiment Number[1]	Description of Variables	Results
Comb building	- 1st day	7	Repeated Exposure, Diazinon, 0, 3 & 6 day, observation hive	Both diazinon and number of treatments reduced the age this task began
	- duration	7	Single Dose, Diazinon, 0 day, observation hive	Diazinon reduced duration
Entrance	- 1st day	6	Single Dose, Diazinon, 15 day, observation hive	Diazinon-treated began later
Nectar Handling	- 1st day	3	Single Dose, Diazinon, 0 day, observation hive	Diazinon-treated began earlier
		7	Single Dose, Diazinon, 0 day, observation hive	Diazinon-treated began earlier
		8	Repeated, Diazinon, 10 & 13 day, observation hive	Diazinon-treated began later
	- duration	3	Single Dose, Diazinon, 0 day, observation hive	Diazinon treated continued longer
Foraging	- 1st day	4	Single Dose, Diazinon, 0 day, observation hive	LD_5 began before LD_{25}
		3	Repeated Exposure, Diazinon, 0,4 & 8 days, observation hive	Diazinon-treated began later
		7	Repeated Exposure, Diazinon, 0, 3 & 6 days, observation hive	Diazinon and number of treatments reduced the beginning of this task
		9	Single Dose, Carbaryl, 0 day, 3 standard hives	Carbaryl reduced age of foraging in one hive, LD_5 latest in one hive, no effect in third hive.
		9	Single Dose, Diazinon, Carbaryl, and Resmethrin, 0 day, 1 standard hive	Carbaryl-treated began later, Diazinon and Resmethrin-treated similar
		10	Single Dose, Diazinon and Carbaryl, 14 day, 1 standard hive	Diazinon LD_5 began earliest, Carbaryl LD_5 the latest

(Cont.)

Table 15: (Cont.)

Category	Experiment Number[1]	Description of Variables	Results
- duration	7	Repeated Exposure, Diazinon, 0, 3 & 6 days, observation hive	Controls treated once longer duration than any other group
	9	Single Dose, Diazinon, 0 day, 1 standard hive	LD_{25} longest duration
	10	Single Dose, Carbaryl, 14 day, 1 standard hive	Control longer duration than any treated group

[1]Experiment number from Table 1.

Longevity in worker honey bees can be influenced by other factors. Brood care (Maurizio 1950; Woyke 1984) and foraging early in life (Winston and Fergusson 1985; Winston and Katz 1981) were both shown to reduce longevity. Seasonal differences in lifespan may also be related to worker activity, with short-lived summer bees being more active than long-lived winter bees (Sekiguchi and Sakagami 1966; Tustain and Faulke 1979). In addition, anaesthetics such as carbon dioxide have been shown to reduce longevity (Austin 1955; Simpson 1954; Tustain and Faulke 1979). Longevity appears to be a sensitive trait in honey bees that, under controlled experimental conditions, can be used to measure the effects of stress in many forms including pesticide sensitivity.

Alterations in foraging age and duration were caused by the application of low dosages of pesticide in 11 cases. However, results were somewhat inconsistent (Table 15). The first day of foraging began later in one case and earlier in another after diazinon treatment. Carbaryl treatments reduced foraging age in one case and increased it in another, and diazinon-treated workers tended to forage for a shorter period of time than controls. In three cases, the highest dosages of pesticide resulted in the longest foraging duration.

Longevity and foraging are related. The transition to field activities is a critical stage in a worker's life and foraging is generally thought to be a more hazardous occupation than hive activity (Lindauer 1953). The age at first foraging was important in determining longevity of two honey bee races (Winston and Katz 1981, 1982). When a large proportion of the work force was removed from colonies, both the age at first foraging and longevity were reduced (Winston and Fergusson 1985). Increased worker activity has been related to shorter lifespan in other studies as well (Free and Spencer-Booth 1959; Sekiguchi and Sakagami 1966). Therefore, these two characteristics hold the most potential for evaluating the effect of sublethal exposure to pesticides in the honey bee.

Other Division of Labour Tasks

Statistically significant effects on other tasks were rarely seen (Table 15). This suggests that these tasks are not as sensitive to pesticide-induced stress as longevity and foraging are and that they cannot be quantified as easily. Nectar handling began earlier twice and later once. Cleaning began earlier once and later once. Other tasks such as entrance activities, grooming and brood care showed only one statistically significant difference in all the experiments. In two experiments, the number of tasks performed was reduced by pesticide treatment.

Regulation of temporal division of labour in honey bee colonies is very complex. Many authors have attempted to understand what determines the sequencing and timing of tasks, as well as the intiation of new behaviours. Ontogenetic changes seem to follow changes in gland developement (Michener 1974). However, what is emphasized by repeated studies is the great flexibility in age of task performance. Stimuli such as colony population, worker age distribution, worker longevity, amount of brood rearing, amount of pollen and honey stores, availability of nectar and pollen in the field and general activity levels as well as previous colony history have all been suggested as possible influences on foraging and longevity (Free 1965; Lindauer 1953; Maurizio 1950; Winston and Katz 1981, 1982; Winston and Punnett 1982). All these influences must be integrated by the individual worker and adaptations made depending on them. While age is involved, colony requirements seem to be more important in determining temporal division of labour in the honey bee.

Environmental conditions are also important especially during adverse weather (Free 1965; Free and Spencer-Booth 1961; Woyke 1984). In general, bee activity including brood rearing and foraging is reduced under poor weather conditions. In addition, behavioural transitions are delayed in a year of poor resources (Kolmes 1985a). Seasonal differences in honey bee longevity and foraging are also known.

The highest activity of bees was found to be in spring (Sekiguchi and Sakagami 1966) and during nectar flows (Kolmes 1985a).

Weather conditions may have had an effect in this study (Table 16). Early spring in 1983 was warm and dry, while much of June and July was wet and cold. Summer conditions were much warmer and drier in 1985, with less than one mm of rainfall in July and only 60% of normal rainfall in June. It is somewhat difficult to compare results from 1983 and 1985 due to differences in experimental setup and timing (Table 1). However, in 1983 workers of Exp. 2 (six-day old) had shorter lifespans than those of Exp. 1 (newly emerged) (Table 4). But a confounding factor was that Exp. 2 began earlier in the spring when conditions were more favourable. The very short lifespan and early foraging age in the repeated dosage study in 1983 (Table 7) may be related to colony needs. This study was set up late in June following extremely wet and cold weather. Some warmer weather may have resulted in increased activity of these workers at this time. The single dosage studies in observation hives in 1985 were done at the same time and differences in division of labour characteristics were probably more related to differences in colony conditions for the three hives (Table 5). Results in the standard hives may have been affected by weather conditions (Table 8). Rain in August may have prolonged life and foraging in Hives 1 and 3, while in Hive 2 workers had already died. Therefore, replication of experiments both within and between years can be very important.

The task of foraging appears to be more sensitive to outside influences than any other division of labour category. Foraging is normally the last task a worker undertakes, and only in unusual circumstances, such as altered colony age structure, will a forager return to hive duties (Free 1965; Lindauer 1953). In addition, specialization appears to be common in foragers (Seeley 1983; Sekiguchi and Sakagami 1966; Winston and Punnett 1982), while lacking in hive workers (Kolmes 1985b). Hive workers often undertake a number of different tasks in one

TABLE 16: Mean monthly precipitation and temperature for the study periods in 1983 and 1985 at Simon Fraser University, Burnaby Mountain, Burnaby, B.C.[1]

Month	Precipitation, mm			Temperature, °C		
	1983	1985	Normal[2]	1983	1985	Normal[2]
May	53.5	68.5	67.1	12.7	16.3	11.3
June	139.0	50.4	59.2	13.1	19.5	13.6
July	188.7	0.3	83.0	14.8	13.7	16.8
August	39.6	68.0	90.4	16.7	11.5	16.8

[1]From Monthly Recordings of Meterological Observations in Western Canada, 1983 and 1985, Can. Dept. Environ., Atmospheric Environ. Service.
[2]Normals based on the years 1951 to 1980.

day and appear to perform work as they find it often leaving a task unfinished (Lindauer 1953). While the switch from task to task in most cases is hard to delineate, the change to foraging from hive activities has a distinct demarcation (Kolmes 1985a). For this reason it may be easier to see alterations in foraging age than other tasks.

In addition, changes in foraging activity are often associated with changes in longevity. Both longevity and foraging age were reduced in colonies which had reduced worker populations (Winston and Fergusson 1985). Workers injected with juvenile hormone switched from brood rearing to foraging at an earlier age and also had shorter lifespans (Jaycox 1976; Jaycox *et al.* 1974; Robinson 1985). Exposure to carbon dioxide also reduced both foraging age and longevity in honey bees (Austin 1955; Ebadi *et al.* 1980; Ribbands 1950). In this study, also, both foraging age and longevity were reduced by exposure to low doses of pesticide. It appears that these two categories are sensitive to changes in both internal and external influences and are useful measures of stress and activity in the honey bee worker.

Treatment Age

Age differences in susceptibilities to pesticides in insects is well known. In most cases, early larval instars are more susceptible than later ones, larvae are most sensitive just after a moult and adults most susceptible at emergence (Busvine 1971). The three insecticides used in this study were more acutely toxic to newly emerged worker than older workers (Table 2). Previous studies on acute toxicity have shown newly emerged workers tended to be more susceptable to various pesticides such as toxaphene and DDT (Koch 1958/1959) and malathion and diazinon (Mayland and Burkhardt 1970; Smirle 1983). This may be due to a number of factors, most importantly differences in enzyme activities and the absorptivity of the cuticle. However, Wahl and Ulm (1983) found older workers

were more susceptible to some herbicides and fungicides, especially overwintered honey bees.

Pesticide susceptibility can be affected by diet. In general, diets poor in nutritive content produce undernourished individuals that are more susceptable to pesticides (Busvine 1971). In the honey bee, physiological condition and pesticide sensitivity were found to be related. Different pollens and pollen substitutes vary in their nutritive content. Workers fed poor quality pollen showed increased susceptibility to pesticides (Wahl and Ulm 1983) and decreased longevity (Maurizio 1950). This effect was found in studies with individual workers as well as whole colonies. The workers most sensitive to pesticides are overwintered bees which had cared for brood in the spring. It is thought that inadequate pollen consumption causes protein deficiency which affects the enzyme systems which decompose certain pesticides. In some cases activity may affect pesticide sensitivity. Brood care was shown to increase susceptibility in both honey bees (Wahl and Ulm 1983) and leafcutter bees (Johansen *et al.* 1983).

Effects of low doses of the three insecticides on longevity and temporal division of labour were more pronounced in workers treated when newly emerged than for those treated as older workers. The effect of pesticide on task performance may also have been different for the different age groups. Tasks tended to begin earlier in newly emerged workers treated with pesticide compared to controls, while older workers tended to begin tasks later when treated with pesticide.

Stress in many forms has been shown to reduce longevity and alter behaviour in the honey bee. Narcosis is often used in research on honey bees. The application of carbon dioxide has been shown to reduce longevity and lower orientation flights, hoarding behaviour, pollen collection and foraging age (Austin 1955; Beckman 1974; Ebadi *et al.* 1980; Mardan and Rinderer 1980; Ribbands 1950). Low temperature exposure was also shown to reduce hoarding behaviour

(Mardan and Rinderer 1981). Newly emerged workers may be more sensitive to any stress, including pesticide exposure, than older workers. Smirle (1983) found in his study that younger workers always had shorter lifespans than older workers regardless of the conditions. He believed the stress of handling was more deleterious to newly emerged workers. The stress of handling may have been a factor in this study as well. Poor survivorship of one group of workers treated at emergence in Exp. 9 (Table 14) may have been a result of a handling problem. Newly emerged workers are just beginning their hive activities and the integration of factors involved in temporal division of labour. Stress, of many kinds including pesticide exposure, may be more harmful at this time.

Exposure to cool temperatures, carbon dioxide and the stress of captivity was shown to adversely affect neural function (Beckmann 1974), as was parathion (Stephen and Schricker 1970). It is possible that these neural changes may affect hormones which are involved in the regulation of temporal division of labour such as the juvenile hormones (Jaycox 1976; Jaycox *et al.* 1976; Robinson 1985). In addition adverse effects on older workers may be more difficult to detect. Their response may be more variable as past activity such as brood care and the consumption of poor quality pollen have been shown to increase pesticide sensitivity (Wahl and Ulm 1983).

Repeated Exposures

This appears to be the first time the effect of repeated topical applications of insecticides to individual workers has been studied. This aspect of pesticide-pollinator interactions in rarely mentioned in review articles on pesticide hazards (Anderson and Atkins 1968; Atkins 1975; Johansen 1977, 1979, 1983; N.R.C.C. 1981), although recurrent exposure can be expected to be more common than single episodes in crop-growing regions. In the production of cotton as many as 12 applications of insecticides can be made over one summer. Heavy bee

losses have been attributed to this type of spray program (Moffet *et al.* 1979, 1981; Wilson *et al.* 1980).

When analyses compared pesticide-treated workers with controls by the number of treatments, those treated as tenerals were more likely to be adversely affected (Table 6). This may be due to some of the factors previously discussed such as greater sensitivity to stress. However, other factors may be involved as well. Enzyme systems that metabolize foreign compounds including pesticides are found in most organisms (O'Brien 1967). Induction of these enzymes by zenobiotics are known to occur and may be one reason why few differences were found in the older age groups. In addition, enzyme levels in different ages may also vary. For example, foragers were found to have lower acetylcholinesterase concentration than young workers involved in brood care (Nazer *et al.* 1974) and therefore, lower susceptability to diazinon.

When all treatments are compared, both the number of treatments and pesticide were found to adversely affect longevity and some temporal division of labour tasks (Table 7). For Exp. 7 (treatments at 0, 3 and 6 days of age), controls treated once began foraging the latest, continued foraging the longest and lived the longest. However, both the controls and diazinon-treated workers exposed three times had the lowest values. This suggests that repeated handling also caused adverse effects. These results are an important consideration in the design of future experiments and the development of a standardized bioassay. If repeated exposure is to be investigated, the associated controls must be used to evaluate the effects of handling on workers. Only through this type of approach can the effects of pesticide exposure be determined.

Repeated pesticide application, in combination with other factors, did affect longevity and temporal division of labour. This could have deleterious effects on the whole colony. Foragers which contact repeated field applications are more likely to die and also may collect less food. Other sublethal effects in

the colony may occur, especially if contaminated foragers return to the hive, exposing young workers to sublethal amounts of pesticide which would effect their subsequent activity.

Pesticide

Pesticides were found to vary in their acute toxicities and sublethal effects on colony characteristics in this study. Resmethrin was the most acutely toxic chemical used to both newly emerged and older workers. Carbaryl was the least toxic to older workers, while close to resmethrin for the newly emerged workers (Table 2). Previous work shows similar relationships of the three chemicals to older workers (N.R.C.C. 1981).

There appears to be very little dose dependent relationships with any of the three insecticides used. Often LD_5 and LD_{10} were the most severly affected and LD_{25} was similar to the control in a number of cases (Table 15). These results are difficult to explain but may be related to the method of determining the doses used. In order to achieve a straight-line relationship, probit analysis is used (Swaroop 1966). This transformation tends to spread out the mortalities at the upper and lower ends. In addition, the confidence intervals are larger in these areas. For this reason it is difficult to determine accurately the concentrations necessary for the sublethal doses. Thus, there may be considerable uncertainty of the calulated lethal dose concentrations, which is reflected in the inconsistent results due to dosages.

Longevity of workers treated at emergence was the most affected by pesticide type. Carbaryl reduced longevity the most, with resmethrin intermediate and diazinon least harmful to newly emerged workers (Table 12). The sublethal effects of these three chemicals are, therefore, somewhat different than their acute toxicities as discussed above. However, survivorship was very low in bees treated with carbaryl at all three dosages and those treated with

diazinon at a dosage of LD_{25} in newly emerged bee (Table 14). These groups were treated at a different time than the others, and the results may reflect a handling problem. In a previous study the type of insecticide was also found to be important. Malathion was found to be less acutely toxic and less likely to reduce longevity than diazinon (Smirle *et al.* 1984).

Different pesticides in low dosages can affect honey bee lifespan and behaviour. Data of this kind, including both lethal and sublethal tests, should be considered in the choice of pesticides for application to flowering crops or areas near to honey bees. For example, carbaryl, which is the least acutely toxic to older workers in this study, has been found to be a serious beekeeping problem. High mortality to field colonies occurred in a number of cases (Anderson and Glowa 1984; Erickson and Erickson 1983; Johansen and Brown 1972; Melksham *et al.* 1985). As well, carbaryl was shown to have the longest residual effect of the ten insecticides tested and was rated as highly toxic to honey bees (Mansour and Al-Jalili 1985; Mansour *et al.* 1984).

The results of this study should be of interest to beekeepers. Beekeepers strive to maximumize colony populations during heavy nectar flows and, therefore, increase honey production. In most agricultural areas, workers would probably contact pesticides while foraging. If pesticides, even at low doses, alter temporal division of labour and reduce longevity, sustaining colony population and good honey production will be difficult. One encouraging result is the higher susceptability of newly emerged workers. Bees of this age are less likely to contact pesticides as foraging does not normally begin until approximately two weeks of age. Older workers showed few effects due to low dosages of pesticides.

This work suggests that topically applied sublethal dosages of an insecticide can cause statistically significant effects on longevity and at least one task, foraging, in temporal division of labour in the honey bee. Other

important components such as colony requirements, environmental influences, worker age when exposed to a pesticide, and pesticide type may also influence the regulation of division of labour and worker longevity. The interaction of these factors is not yet understood. Future work should try to determine the relationship between pesticide effects and the regulation of temporal division of labour.

E. CONCLUSIONS

The conclusions drawn from these experiments on the effects of sublethal pesticide exposure on temporal division on labour and longevity in the honey bee are as follows:

1. Single topical applications of low doses of insecticide may reduce longevity and adversely alter foraging. Other tasks of temporal division of labour were not consistently affected.

2. Newly emerged worker honey bees are more sensitive to pesticide exposure than older workers.

3. Repeated exposure to a sublethal pesticide concentration may also reduce longevity and alter foraging.

4. Of the three insecticides used, carbaryl was the most deleterious in sublethal doses, with resmethrin intermediate and diazinon the least hazardous.

5. Longevity and foraging are both sensitive indicators of stress and hold the most potential for use in evaluating the effects of stress, including pesticides, on honey bee workers.

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