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Toxicity of synthetic pyrethroid insecticides to the honey bee (*Apis mellifera* L.)

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TOXICITY OF SYNTHETIC PYRETHROID INSECTICIDES TO THE HONEY BEE (*Apis mellifera* L.)

By

Kevin Stuart Taylor

A Thesis Submitted to the Faculty of the

DEPARTMENT OF ENTOMOLOGY

In Partial Fulfillment of the Requirements
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THE UNIVERSITY OF ARIZONA

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Date

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ABSTRACT

Honey bees (*Apis mellifera* L.) were exposed to six pyrethroid insecticides using four application techniques. Toxicities of the insecticides were compared.

Results of topical and contact tests placed the six pyrethroids in one of three categories based on their relative toxicity to honey bees: highly toxic (cyfluthrin, cypermethrin, and permethrin), moderately toxic (flucythrinate and fenvalerate), and non-toxic (fluvalinate). The contact tests were valuable because they gave results similar to those from the topical tests but required less labor. The residue tests, by contrast, simulated field conditions by using sprayed cotton leaves for exposure. This test showed that both compound and formulation played an important role in determining toxicity.

The conditioning test combined some of the previously used techniques and refined them into a test for detecting behavioral changes to bees following sublethal exposure to pesticides. Insecticide-treated honey bees had a lower learning curve than their respective control group. This indicates that, although bees may survive poisoning from pesticides, certain physiological functions are affected.

INTRODUCTION

Estimates for the value for crops requiring honey bee (*Apis mellifera* L.) pollination range from $4.5 billion in 1982 to over $20 billion in 1980 (Metcalf et al., 1962; Ware, 1973; McGregor, 1980). Besides pollination, the honey bee also provides honey, pollen, and beeswax.

Unfortunately, in the last 100 years the honey bee has been put in jeopardy by the use of agricultural chemicals, most noticeably insecticides. Since the use of Paris green (copper acetoarsenate) over 100 years ago, honey bee kills due to insecticide use have become a serious problem for beekeepers, farmers, and pest control specialists (Todd and McGregor, 1960; Johansen, 1977). In 1986, over 56.4 million kilograms of insecticides were sprayed on crops in the United States. Many of these crops are pollinated by honey bees (Erickson and Erickson, 1983). In the United States alone, the combined annual losses due to honey bee mortality (honey production and pollination) resulting from the use of pesticides is approximately 135 million dollars (Crane and Walker, 1983).

Even in our highly advanced state of agricultural production, we have failed to adequately protect one of our most important beneficial insects, the honey bee. Crane (1983) discussed the impact of pest management on bees and pollination in lesser-developed countries. She asked, "How can we export our pesticide programs when we cannot yet even protect our beneficial insects?" Thus, the present study was initiated to evaluate the toxicity to honey bees of a relatively new class of insecticides, the synthetic pyrethroids.

Honey bees were exposed to six pyrethroid insecticides using several different techniques, viz., topical, contact, residual, and conditioning tests. Relative toxicities of these materials were compared through different techniques, and certain honey bee behavioral responses were studied.

LITERATURE REVIEW

Honey Bee/Pesticide History

After World War II, damage to honey bee colonies from insecticides was reduced owing to the banning of arsenicals and the introduction of DDT. This first commercially available chlorinated hydrocarbon insecticide was relatively safe for honey bees and reportedly solved the bee poisoning problem (Vansell, 1946).

Crop protection economics became a factor when additional synthetic organic compounds (chlorinated hydrocarbons, organophosphates and carbamates) were introduced into the crop-protection scene (Matsumura, 1975). Unfortunately, most of these new compounds were more toxic to honey bees than DDT and caused unprecedented kills in agricultural areas.

Between 1967 and 1978, beekeepers in California, Arizona and Washington received over 50% of all federal indemnity payments for pesticide losses (Economic Reseasrch Service, 1982). This government program was created to reimburse beekeepers for bees lost from pesticide poisonings. The majority of these losses

occurred when honey bees foraged on alfalfa (Medicago sativa), cotton (Gossypium spp.), and sweet corn (Zea mays), and the major insecticides involved in the poisonings were methyl parathion, carbofuran, and carbaryl (Erickson and Erickson, 1983).

McGregor (1978) reported that between 1963 and 1977, the number of honey bee colonies in Arizona dropped from 116,000 to 60,000. It was because of the concern for crop pollination that, in 1949, the United States Department of Agriculture (U.S.D.A.) established a research facility at Tucson to conduct honey bee research, primarily focused on pollination and pesticide studies (M.D. Levin, pers. comm.).

Pyrethroid History

The term pyrethrum generally refers to an extract from the flowers of Chrysanthemum cinerariaefolium. The active ingredients involved are referred to as pyrethrins, made up of two constituents, pyrethrin I and pyrethrin II (Matsumura, 1975). Lhoste (1964) states that the Chinese in the first century AD were acquainted with pyrethrum's insecticidal properties. A U.S.D.A. bulletin by McDonnell et al. (1920[revised 1926]), provides an excellent review of the early history of pyrethrum in Europe and the United States.

Staudinger and Ruzucka (1924) were the first to isolate the active constituents of pyrethrum, which were single isomers derived from two carboxylic acids. This development helped Campbell and Harper (1945) synthesize the first pyrethrin analogue (or pyrethroid) with some practical application in insect control. This compound was named allethrin and proved to be a satisfactory substitute for natural pyrethrins (Elliot, 1954). Although allethrin is not a light-stable compound, and therefore of little use outdoors, it has been widely used in aerosol sprays for control of household pests.

In the early 1970's, chemists at the Sumitomo Chemical Company in Japan experimented with substitutions at the benzylic carbon atom (alpha-substitution) to produce several pyrethroid compounds. By using an alpha-cyano addition, they synthesized the first light-stable pyrethroid called fenpropathrin (Sumitomo, 1973). More experimentation with the alpha-cyano substitution led to the developement of fenvalerate in 1972 (Sumitomo, 1976). Fenvalerate was the first light-stable, highly insecticidal pyrethroid and was introduced into the commercial market in 1976. During the same period of time, the National Research Development Corporation (NRDC) in England developed another compound called

permethrin (NRDC, 1975). This insecticide was shown to be more light stable than their previous experimental pyrethroid compounds and possessed a low mammalian toxicity. Another important compound, cypermethrin, was developed from this series of investigations at the NRDC. Also, as a result of the alpha-cyano additions, permethrin, fenvalerate, and cypermethrin all gained considerable importance as agricultural insecticides and are sometimes called the first generation pyrethroids.

Since the introduction of the first marketable, light-stable pyrethroids, imaginative research by scientists employed by a number of companies have developed several additional marketable pyrethroid insecticides. Pyrethroids are among the most potent chemicals known for insecticidal activity and therefore require low rates of application, thus providing an increased margin of safety to non-target organisms (Smart and Stevenson, 1982). The compounds flucythrinate, fluvalinate, cyfluthrin and others (second generation pyrethroids) have shown effectiveness as broad-spectrum insecticides and some also have shown effectiveness in suppressing phytophagous and parasitic mites (N. Koeniger, pers. comm.).

Currently, newpyrethroidsare being developed and marketed in the United States and throughout the world.

Pyrethroid Use

Pyrethroid insecticides accounted for 25% of the world foliar insecticide market in 1983 (Wood-Mackenzie, 1983) and are generally recognized as having the following main characteristics:

- knockdown effect,
- repellent effect,
- excitation effect,
- relative absence of vapor activity,
- highly lipophilic tendencies,
- absence of systemic effect.

Some of these characteristics may be seenas either negative and/or positive, depending on the situation in which they are used.

Pyrethroids have been used successfully against several insect taxa in the agricultural sector. Their main use is for control of lepidopteran pests, mainly on cotton, but also on some vegetables (Hill, 1981). Effectiveness against coleopteran pests has also been recorded (Burris et al., 1981; and McClanahan, 1981). Contribution to reduction of pathogen transmission by aphids on sugar beets (Beta vulgaris) and potatoes (Solanum tuberosum) has been shown by Bocquet et al. (1983) and Rice et al. (1983).

Non-crop uses for pyrethroids has also been developed. Veterinary applications include the control of several dipteran pests on range animals (Elliot et al., 1978) and as an animal dip for control of certain ectoparisites (Escuret and Scheid, 1982). Fluvalinate is currently being tested as a miticide for control of honey bee pests (N. Koeniger, pers. comm.). Low doses of fluvalinate are being fed to honey bee colonies in hopes of systematically eliminating the Varroa mites (Varroa jacobsoni) from the host's body.

The use of pyrethroids for protection of food stuffs, wood, and textiles is currently being explored (WHO, 1984). The varied uses for pyrethroids range from conventional crop treatment to the protection of fish during drying in Africa and incorporation in paper or plastic to prevent insects from penetrating storage containers.

In 1984, the World Health Organization (WHO) published the fourth revision of its guide for the use of chemical products against insects important in public health. One of the objectives of this revision was to include new compounds, among them the pyrethroids, which are becoming important in control of malaria, Chagas'

disease and sleeping sickness by controlling their insect vectors.

Pyrethroid Metabolism and Mode of Action

The information available on the metabolism of pyrethoids by insects, though limited, does indicate certain esterases and oxidases do facilitate the breakdown of these chemicals (Shono et al., 1979). Although these metabolisms may proceed at different rates both within and between insect species, insect recovery often occurs after poisoning by pyrethroid insecticides. Recovery may not always be complete, but poisoned insects appear to function normally.

Some toxicologists and physiologists say that, "Type I pyrethroids (e.g. permethrin) cause repetitive firing in the peripheral nervous system while Type II pyrethroids (e.g. fenvalerate and cypermethrin) act on the central nervous system by depolarization" (Gammon et al., 1981). As Gammon points out, however, there are exceptions. Adams and Miller (1980), postulated from their research that the Type II pyrethroids (alpha-cyano) either act on a different site than the Type I pyrethroids or they cause a more subtle poisoning system that is overlooked because of the extreme bursts of nerve activity associated with TypeII

pyrethroid poisoning. The distinction between peripheral versus central nervous system action is important in determining the path of disruption of the insect nervous system related to the poisoning process.

Pyrethroids have a negative temperature coefficient (Sparks et al., 1983); however, temperature dependence is not always predictable because pest species differ in their responses (or response by members of the same species may vary in different environments).

The only completely acceptable theory of pyrethroid mode of action is that they act on the nervous system. Pyrethroids have been shown to be active on some sensory nerve structures, motor nerve structures, presynaptic terminals, and neurosecretory axons. The most general effect of pyrethroids is their interaction with sodium channels (Miller and Salgado, 1985). Although this may be expressed in different ways, type I pyrethroids are believed to cause repetitive firing, while type II pyrethroids depolarize the nerves.

Pyrethroid / Honey Bee Investigations

Topical Tests. Probably the most widely used method of testing the toxicity of insecticides to honey bees (and most other insects) is to topically apply the chemical directly to insects in the laboratory and then record the mortality level at 24, 48, and 72 hours. Stevenson (1968) was one of the first to to use this method to investigate the toxicity of pyrethroids to honey bees. Since that time, other researchers have also used topical tests to obtain mortality data for pyrethoids on honey bees.

Olsson (1978), Wilkaniec (1980), Pederson (1980), Atkins (1981), and Danka et al. (1986) all found permethrin to be highly toxic (usually meaning an LD_{50} of less than one microgram per bee) to honey bees treated topically in the laboratory. Atkins (1981) determined the LD_{50}'s of four pyrethroids in his laboratory tests; flucythrinate, permethrin, and fenvalerate were considered highly toxic and fluvalinate non-toxic. Shires et al. (1983) and Delabie et al. (1985) found cypermethrin to be highly toxic to honey bees when topically treated. Stark (1984) also reported that fenvalerate was toxic to bees when this method was used.

Oral Tests. Other researchers have determined oral toxicity levels of certain pyrethroids to honey bees by feeding these substances in sucrose solution. Brown-Westerdhal and Gary (1983) found permethrin to be more toxic orally than fenvalerate while fluvalinate was the least toxic of the three. Mansour et al. (1984) fed insecticide-treated clover flowers in sucrose solution to caged honey bees and found fenvalerate to be one of the least hazardous chemicals. Stoner et al. (1985) fed honey bee colonies (long-term controlled low levels of fluvalinate and fenvalerate and found no effect except slight adult mortality at 100 ppm with fenvalerate.

Bull and Wilkinson (1982) and Gough and Wilkinson (1984) found that oral LD_{50} values for permethrin were higher than for contact tests. They also reported that, when groups of bees were fed orally, there was variation in the quantity of sucrose solution (e.g., toxin) each bee ingested at first. This distribution became acceptable after a few hours. They found that individual feeding of bees is time-consuming and can cause higher control mortalities because of greater stress and longer handling times.

The conclusion of scientists attending a meeting to standardize procedures for testing pesticide effects on bees included a statement that oral toxicity tests

were the least valid and most difficult to conduct of all procedures (International Commisssion on Bee Botany, 1982).

Residual Tests. Scientists have studied the residual effects of pyrethroids in several different ways. El-Banby and Kansouh (1981) studied the residual effects to honey bees of cypermethrin, fenvalerate and seven other insecticides of other classes using the flowers and leaves of the cotton plant (Gossypium spp.). Johansen et al. (1983), studied the residual effects to honey bees of insecticides sprayed on alfalfa (Medicago sativa). They tested cypermethrin, permethrin, fenvalerate, and fluvalinate and concluded that cypermethrin was the most toxic, while fluvalinate was the least toxic.

Stoner et al. (1985) exposed honey bee colonies to wax foundation sheets impregnated with four levels of fenvalerate. The only negative effects on these colonies was reduction of egg hatch and larval survival at the highest level (1,000 ppm). Honey bees constructed comb from the wax foundation at all levels of exposure.

Debray and Leblanc (1983) found cypermethrin residues in dead bees, live bees and pollen but no residues in honey or wax after bees were allowed to

forage in an alfalfa field sprayed with cypermethrin. They also detected cypermethrin in wax and honey samples after bees foraged in a treated field of oilseed rape (Brassica napus).

Behavioral Tests. Few studies have been done to examine the behavior of individual bees exposed to sub-lethal doses of pyrethroid insecticides. Cox and Wilson (1984) studied the behavior of honey bees exposed to permethrin in a sub-lethal dosage of 0.009 µg/bee. They observed that treated bees spent large amounts of time preening, trembling, abdomen tucking, rotating and cleaning their abdomens, while rubbing their hind legs together. Untreated bees spent more time walking and food-giving and made more foraging trips. Johansen (1984) reported that bees regurgitated proventricular contents and exhibited aggressive behavior following field poisoning by pyrethroid insecticides.

Schricker and Stephen (1970) disrupted honey bee communication dances with a sub-lethal dose of methyl parathion. An insect surviving exposure to permethrin has been shown to have an impairment of its responses to olfactory signals; tests were coducted with Pectinophora gossypiella (Saunders) (Floyd and Crowder, 1981; Haynes

and Baker, 1985) and Pectinophora molesta (Busck) (Linn and Roelofs, 1984).

Field Tests. Although laboratory tests are valuable in determining relative toxicity of a pesticide to a target or non-target insect, they do not always predict how a chemical will affect that insect in the field. Field tests, unfortunately, may be difficult for a number of reasons: interference from pesticides applied to nearby fields, lack of cooperation by nearby farmers, difficulty in replicating with proper experimental designs, effects of weather, relative attractiveness to bees of the target crop versus other nearby crops, and the relatively high cost of labor and materials for conducting field research.

Pike et al. (1982) showed that, at 0.22 kg ai/ha, permethrin did not cause abnormal mortality of honey bees foraging in sweet corn (Zea mays). Pedersen (1980) found that permethrin, however, caused only slightly more honey bee kill than was recorded in control fields of flowering rape (Brassica napus), while Gerig (1979) considered permethrin to be "moderately toxic" in fields of flowering phacelia (Phacelia spp.).

Gerig (1979) found fenvalerate to be "moderately toxic" to bees in the field, while Moffett et al. (1982)

treated flowering alfalfa with fenvalerate and observed no visible damage to honey bee colonies. Atkins (1984) stated that fluvalinate was essentially non-toxic to bees located in a field of blooming alfalfa.

Shires et al. (1983) and Debray and Leblanc (1983) conducted extensive field studies in both alfalfa and flowering rape using cypermethrin; both groups reported that cypermethrin was far less toxic in field studies than in laboratory tests.

Repellency. Despite low mortality of bees following the application of some pyrethroid insecticides, there is often a near absence of bees foraging on the treated crop. Such inhibition of foraging activity has been attributed to repellency. Formulated permethrin and fenvalerate acted as a repellent by suppressing bee visitation to flowers of Phacelia spp. for about one or two days when Gerig (1979) conducted laboratory experiments with a simulation test using potted flowering plants. Gerig (1981) reported that the duration of the reduced bee visitation in open-air tests depended on interactions of the chemical and further unknown environmental factors.

Atkins (1981) reported that permethrin acted as a repellent and recommended using this material as a

mixture with other insecticides to reduce bee hazard. Permethrin applied at a rate of 0.22 kg/ha eliminated most bees from sweet corn fields (Pike et al., 1982). Brown-Westerdahl and Gary (1983) compared the relative toxicity and repellency of fluvalinate, fenvalerate and permethrin to foraging honey bees and reported that only permethrin acted as a repellent. By contrast, Moffet et al. (1982) stated that honey bee visits to alfalfa flowers were reduced 70% in the afternoon following fenvalerate applications and Stark (1984) concluded that the repellent action of fenvalerate lasted two or three days on turnip (Brassica rapa) and spring rape (Brassica spp.).

Shires et al. (1983) found that the hazard from cypermethrin applied to flowering oilseed rape was much less than might be expected when considering its high laboratory toxicity. They attributed this to a repellent action that markedly reduced foraging immediately after application. Debray and Leblanc (1983) also reported a slight repellent effect following an application of cypermethrin to flowering alfalfa and rape. Shires et al.(1983) concluded that the difference in high toxicity reported in the laboratory and the low toxicity reported in the field is a result of the combined effects of low application rates in the field, repellency that inhibits

foraging behavior and foraging behavior that minimizes contact with the insecticide-treated leaf surfaces.

MATERIALS AND METHODS

Topical Tests

Young bees from a honey bee colony were caught while on the comb and put into a 9-cm diameter plastic petri dish, 20 bees per dish (Figure 1). Each dish had approximately 15-20 holes for ventilation and filter paper on the bottom to absorb feces and excess food.

Bees in each dish were anesthesized with carbon dioxide just prior to a topical treatment. Dry ice was placed in a one-liter jar fitted with plastic tubing, thus allowing the carbon dioxide to be directed into the dishes with bees when the tubing was held against a hole in the lid.

Each bee was given a 1 µl dose of insecticide solution in acetone (treated) or pure acetone (control) on the thoracic dorsum. An automated microapplicator (Instrument Specialties Co., model M) fitted with a tuburculin syringe was used to dispense the insecticide (Figure 2).

Technical grade insecticides were dissolved in acetone for application. A broad range of concentrations was tested for each insecticide. Each concentration was replicated at least four times with a dish of 20 bees serving as one replication. Acetone alone was applied to the control bees. The LC_{50} level was determined for six pyrethroid insecticides. The term LC_{50} here refers to the lethal concentration of insecticide, measured in μg/bee, needed to kill half the test population.

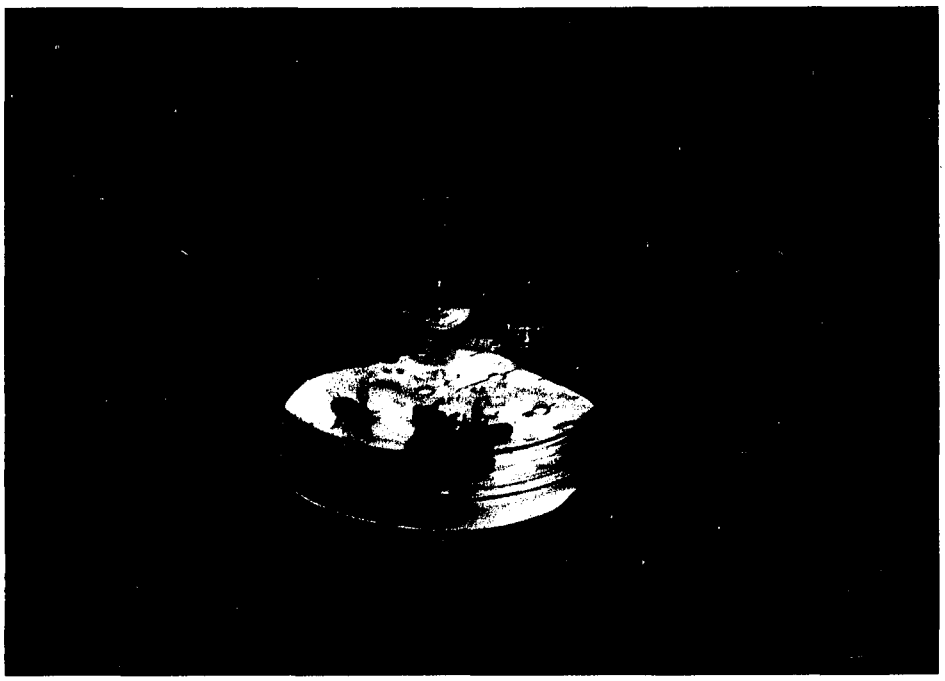

Fig. 1. Petri Dish with Vials of Water and Syrup Vials and Filter Paper Substrate

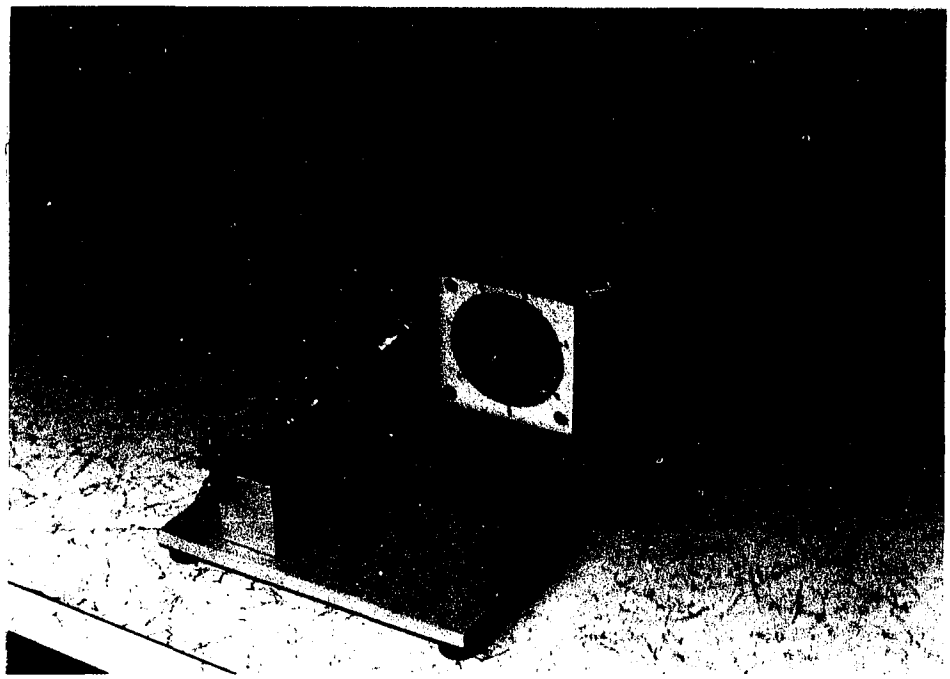

Fig. 2. Automated Microapplicator for Dispensing Insecticides

Upon completion of the applications, bees were kept in dishes and placed in a controlled-temperature cabinet (Environator Mfg. Co., model#E-3448) at 27 $\pm 2^{o}$ C and 50% RH. Bees were fed *ad libitum* a 30% sucrose solution and kept in total darkness. General observations of bees were made after applications of permethrin. Mortality was recorded after 24, 48, and 72-hours. A probit analysis using the 72 hour counts was used to obtain an LC_{50} for each chemical (Finney, 1971). Computor-generated LC_{50}'s and estimates using probit graph paper were compared.

Contact Tests

Filter paper (9 cm diameter) was placed in a glass petri dish and saturated with 1.5 ml solution of an insecticide dissolved in acetone at various concentrations. The concentrations used were calculated as mg of technical grade active ingredient (ai) per 1.5 ml of acetone. A dose of 0.8 mg ai/dish, was used because it represents twice the field rate. The insecticide was pipetted onto the filter paper and allowed to dry for 1 hour.

When the paper was dry, a plastic lid with holes (similar to those used in the topical tests) was used for ventilation and to accommodate vials of food and water.

Twenty young bees were then removed from a brood comb and placed in each petri dish. Young-looking bees were selected to reduce mortality resulting from the inclusion of old or stressed bees. Bees were then placed in a temperature cabinet in total darkness and given vials with 30% sucrose solution and tap water. The cabinet was kept at 27± 2° C and approximately 50% RH. Mortality counts were taken at 24, 48, and 72 hours. A probit analysis was done with the 72-hour counts as with the topical tests discussed previously.

In order to be assured that bees were not being intoxicated by pyrethroid vapors, permethrin, cyfluthrin and acetone controls were tested with bees held on a screen one centimeter above the impregnated filter paper. None of the bees died within 72 hours, giving us reason to believe that it was the contact with insecticide-treated filter paper and not toxic vapor that killed the bees in the contact exposure test.

Residue Tests

In August and October of 1984, cotton (Gossiypium hirsutum) plants were sprayed with insecticide to determine the residual toxicity of several pyrethroids to honey bees. Four pyrethroids were tested: Ambush 2EC® (permethrin), Ambush ULV® and Ambush® 25W;

Mavrik 2EC® (fluvalinate), Capture 2EC® (bifenthrin) and Cymbush 3E® (cypermethrin). The latter was included only in the October test. A cotton field (cv. Deltapine 55, short staple) at the University of Arizona Marana Agricultural Center (August tests) was divided into eight plots; each one was sprayed with one of the seven formulations, with the eighth plot serving as an unsprayed control. There was no replication of treated plots.

The rates used were 0.11kg ai/ha for permethrin and 0.067kg ai/ha for fluvalinate and bifenthrin. The October test was done on a privately owned farm and cypermethrin was also tested at a rate of 0.09kg ai/ha. Insecticides were applied using a manually drawn ground applicator at a rate of 55 liters of water per hectacre for the aqueous mixes. The ULV formulations were applied at two liters per hectare using once-refined cottonseed oil as the diluent. The plants were approximately 100 cm tall when treated.

Twenty-five exposed leaves were taken from the upper 30 cm of the crop canopy in each plot at five intervals after the application of insecticide to the field (for both the first and second tests). Leaves were transported in plastic bags and kept on ice until they reached the laboratory. Mean leaf area was 75 cm^2

determined by an electronic planimeter on a representative sample of leaves (n=25).

Four leaves were placed in each wire-screen cage (19 cm x 14 cm x 5 cm), which was then closed securely with thumb tacks (Figure 3.). Exiting foragers were collected from a normal, healthy colony by using a Gary flight cone (Gary, 1967) and a clear polyethylene tube to direct the bees through a hole into the cage. Fifty bees were placed in each cage, which was kept in a temperature cabinet in total darkness at 27 $\pm 2^{o}$ C and approximately 50% relative humidity. Each cage was provided with water and a 30% sucrose solution using inverted plastic vials.

Mortality counts were taken at 24, 48, and 72 hours after placing the bees in cages. Cages were thoroughly washed with water and detergent after each treatment andwere then reused in subsequent tests.

Bee mortality was analyzed for statistical significance using analysis of variance (ANOVA).

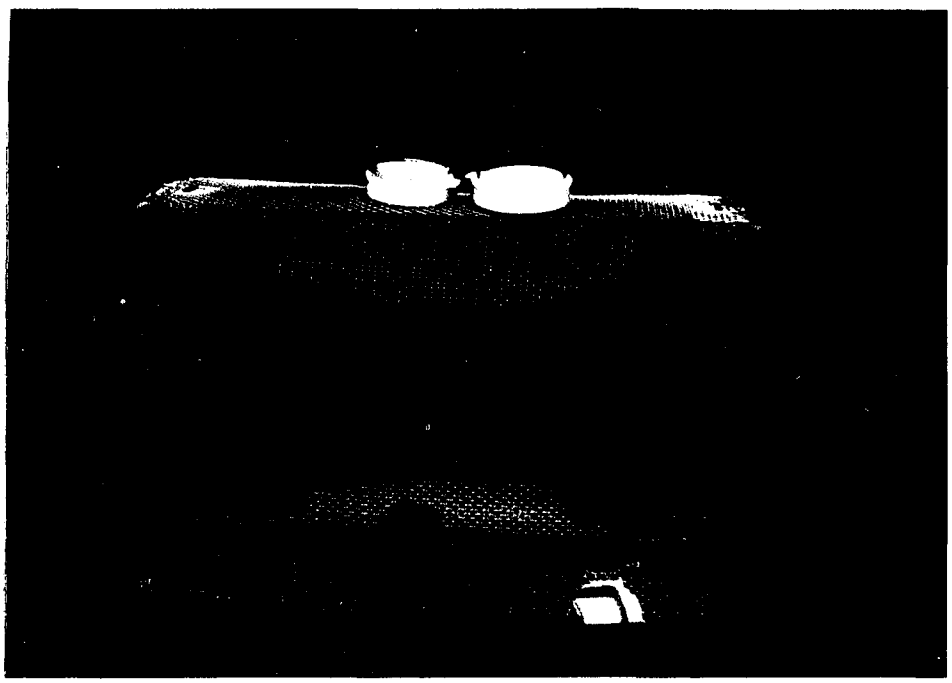

Fig. 3. Wire-Screen Cage used for Cotton Leaf Residue Test

Insecticide/Bee Conditioning Test

Using a Gary flight cone (Gary, 1967), approximately 20 exiting forager bees were directed into a 9-cm diameter glass petri dish (like those used in the contact tests previously described) containing an insecticide-treated filter paper. These bees were maintained for 24 hours in a darkened controlled-environment chamber at 27 $\pm$ 2° C on 30% sucrosesolution and water.

Before the bees were caught, a 9-cm diameter filter paper was placed in each glass petri dish and treated with 1.5 ml of acetone, either with or without an LC_{50} dosage (previously determined in the contact tests) of one of the six pyrethroids tested. The filter paper was allowed to dry at room temperature for one hour prior to installing the honey bees. A plastic lid (with holes for sugar and water bottles and for ventilation) was taped to the glass petri dish base. After a 24-hour exposure period, the surviving honey bees (those appearing to be healthy) were used to compare their learning when conditioning them to an odor.

The methods for restraining, odor training, and testing the learning responses of honey bees were based on earlier work. Takeda (1961) held honey bee workers by

their wings and Frings (1944) demonstrated the proboscis extension response to odor training. Bitterman et al. (1983) perfected this technique with an improved bee-holding device and standardized procedures based on earlier work by Menzel et. al. (1974) and Menzel (1979). Thus, with these techniques one can examine both behavioral and physiological responses in the honey bee (Menzel, 1979; Menzel and Bitterman, 1984).

To facilitate proboscis-extension conditioning, individual bees were restrained in a specially designed bee holder having a small (2 X 0.7 cm) cylindrical tube to hold the bee's body and reduce mobility (Figure 4). To prevent damage to the bees while fastening them into the bee holder, individual bees were put into glass vials which were capped and then covered with ice until the bee ceased movement.

Chilling was used to immobilize the bees (instead of carbon dioxide) because investigations by Ribbands (1950) and Ebadi et al. (1980) indicated that carbon dioxide caused behavioral changes from anoxia. Each chilled bee was then quickly slipped into the metal tube on the bee holder and the stanchion closed around its neck. All bees were fed once after being placed in the bee holder and then held for three hours without additional nourishment to insure that each bee was

sufficiently hungry to give a positive response to the sugar solution when tested, but not so long as to starve them.

Individual bees were positioned in front of an air exhaust duct and thyme-scented air from a sealed container (conditioned stimulus, CS) was blown over the antennae using a small air pump (Figure 5). During the secondhalf of a six-second, scent-conditioning interval, the antennae were touched with 30% sugar solution on a micropipette (unconditioned stimulus, US). After six seconds, the odor stream was removed from the antennae and a pipette dipped in sugar solution was then touched briefly to the extended proboscis (compound-US). When proboscis extension was not induced by either the odor stream or the antennal or proboscis contact with sugar solution, an attempt was made to free the proboscis in hopes that the bee would respond in subsequent training bouts. A bee unable to extend its proboscis after the first three training bouts was categorized as non-functional and was not included.

Each bee was conditioned and tested with the combination of odor and sugar solution eight times with inter-trial intervals of approximately 15 minutes. Erber (1976) reports that a "steady state" of learning is reached 15 minutes after the reward. Specifically, a 3-

second CS-US interval (Menzel and Bitterman, 1983) and 15 minutes between conditioning sequences was employed to facilitate memory consolidation (Menzel, 1984).

A bee that extended its proboscis during the first three seconds (when no antennal contact with sugar was in progress) was considered to have responded positively to odor training. When a bee extended its proboscis only during sugar solution contact with antennae (second three seconds), it was considered as having shown a negative response (not trained). A bee that extended its proboscis immediately after it had been positioned in front of the exiting air duct (position response), viz. before the training scent was blown over its antennae, was not considered to have shown either positive or negative response for that particular training sequence and was discarded from further testing.

Data for percent positive responses by bees from three test dates with each of six pyrethroids and their associated acetone-treated control bees were subjected to ANOVA procedures. Homogeneity of variances were determined by Bartlett's test (Li, 1964) and it was determined that transformation of the percentage of positive responses was not neccesary. Six separate complex ANOVA's were done for all pyrethroid-treated bees and all control bees after testing sequences one, two,

and three; all pyrethroid-treated bees and all control bees in testing sequences four, five, six and seven, and all pyrethroid-treated and all control bees in testing sequences one through seven. Each of these ANOVA's having a significant treatment effect for sequences was tested by individual degrees of freedom (linear, quadratic, etc.) using orthogonal comparisons in regression (Steele and Torrie, 1980). Seven additional ANOVA's were done, one for each test sequence combining data from both pyrethroid-treated bees and control bees. From each of the latter ANOVA's the Student Newman Kuels (SNK) mean separation technique was used to determine the significance of response differences between bees treated with each pyrethroid and their respective control group of bees (Sokal and Rohlf, 1969).

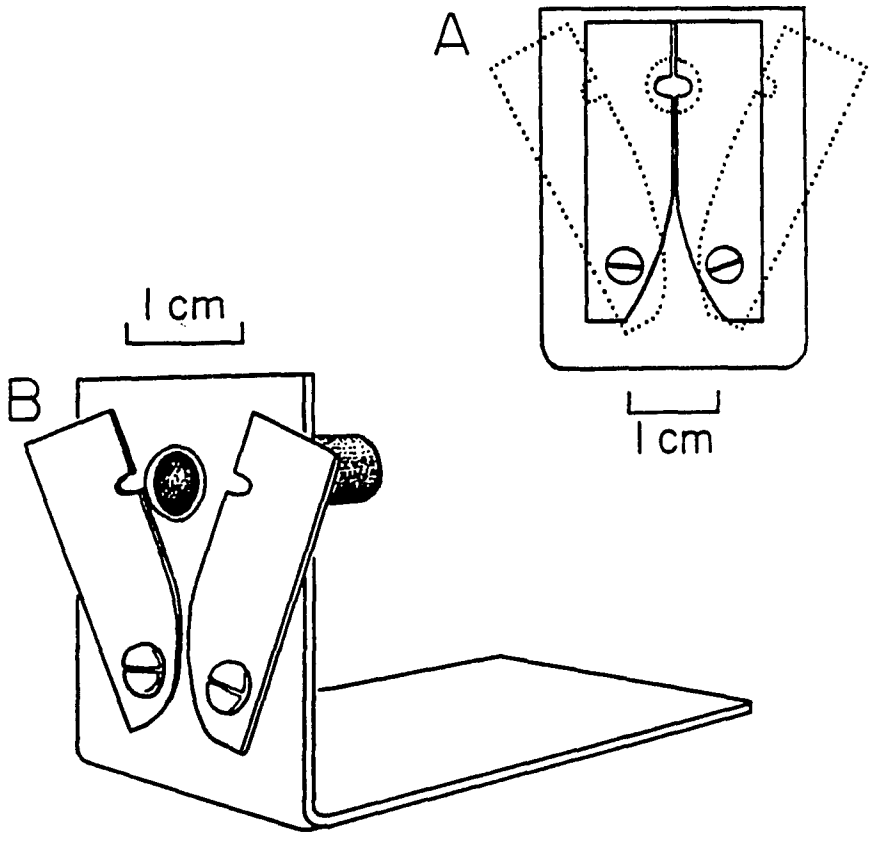

Fig.4. Bee Holder used for Conditioning Tests

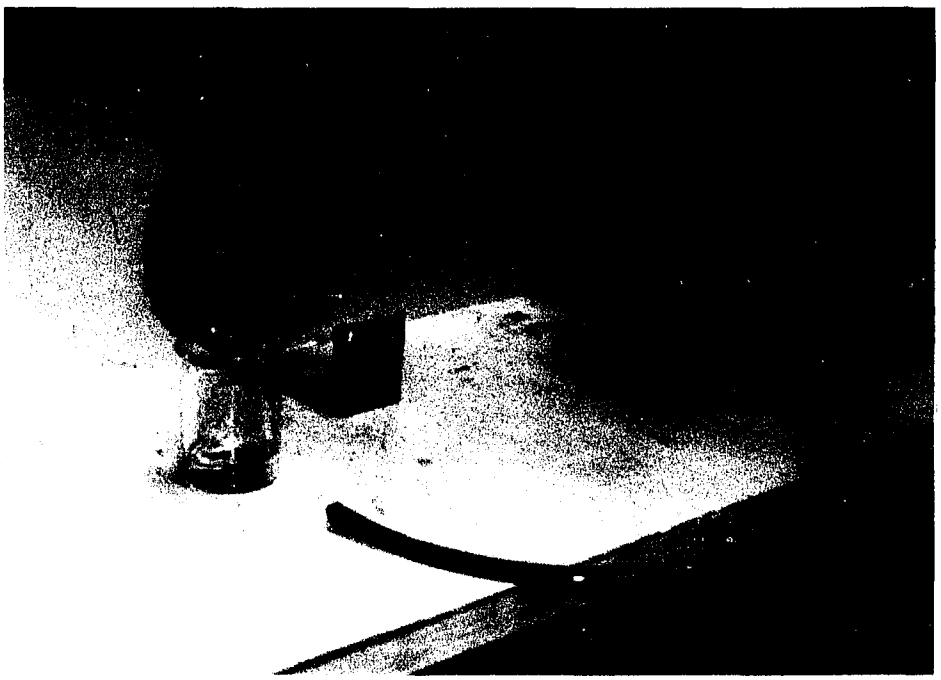

Fig.5. Bee Conditioning Apparatus Showing Scent Source and Air Exhaust Duct

RESULTS

Topical Tests

General behavioral observations were made after applications of permethrin to honey bees. These observations were taken from both this investigation and other related experiments. Ninety percent of the bees that died did so within the first 12 hours after being dosed and 50% within the first four hours. No noticeable paralysis was observed after treatment, but soon after application bees began to scratch their hind legs together and clean their antennae constantly. Later, many poisoned bees began the unnatural behavior of spasmodically rolling on the substrate; approximately 20% of bees showing this behavior recovered and were able to stand upright and move about "normally." The average time of recovery was 9.5 hours, ranging between 1/2 hour and 30 hours after the insecticide applications. There was no noticeable abnormal or unnatural behavior in control bees subjected to identical confinement.

The permethrin-treated bees that were not immobilized immediately by the toxicant showed a considerable propensity to congregate soon after

treatment and did so for up to 10 hours. The opposite was true for the acetone-treated control bees; viz., there was little congregation of bees until the 10th hour, when a noticeable amount of aggregation began to occur.

Results for topical applications are shown in Table 1. Of six pyrethroids tested using this method, fluvalinate had the highest LC_{50} (7.4 µg/bee), more than 10 times higher than fenvalerate which was the next least toxic. Fenvalerate (LC_{50} = 0.513 µg/bee) was slightly safer than the third safest, flucythrinate (LC_{50} = 0.083 µg/bee). After these were permethrin (LC_{50} = 0.023 µg/bee), cypermethrin (LC_{50} = 0.020 µg/bee) and cyfluthrin (LC_{50} = 0.009 µg/bee).

Contact Tests

Permethrin, cypermethrin and cyfluthrin caused a behavioral response similar to that in the topical tests within 15 minutes at most of the concentrations. Fenvalerate and flucythrinate caused the poisoned bees to scratch their hind legs and constantly clean themselves. Few of the fluvalinate treated bees exhibited this behavior.

The pyrethroids tested fell into three levels of toxicity: highlytoxic (under 1.00 mg/dish), moderately

Table 1. LC_{50} Values (ug/bee) with Confidence Limits for 6 Pyrethroid Insecticides Applied Topically to Honey Bees, Tucson, AZ. 1983. 1/

Synthetic Pyrethroid	Doses tested (no.)	Range tested (µg/bee)	LC50 (µg/bee)	95% CI for LC_{50} 2/
Cyfluthrin	6	0.0028 - 0.057	0.009	0.004 - 0.024
Cypermethrin	8	0.013 - 0.130	0.020	0.014 - 0.028
Permethrin	8	0.008 - 0.530	0.023	0.016 - 0.067
Flucythrinate	6	0.040 - 0.800	0.083	0.051 - 0.118
Fenvalerate	6	0.075 - 1.520	0.513	0.344 - 0.642
Fluvalinate	8	1.200 - 25.25	7.446	5.017 - 10.427

1/ Four replications were analyzed using 20 bees per replication at each dosage.

2/ LC_{50} and confidence intervals obtained by probit analysis (Finney, 1971).

toxic (between 1.00 and 10.00 mg/dish)and non-toxic (over 10.00mg/dish). Permethrin (LC_{50} = 0.070ug/ul), cyfluthrin(LC_{50} = 0.084mg/disk) and cypermethrin (LC_{50} = 0.097mg/dish) were highly toxic. Flucythrinate (LC_{50} = 1.10mg/dish) and fenvalerate (LC_{50} = 1.70mg/dish) were moderately toxic while fluvalinate (LC_{50} = 11.62mg/dish) was non-toxic compared with the others. These results are summarized in Table 2.

Residue Tests

Both permethrin and bifenthrin caused nearly complete mortality when honey bees were confined with newly treated leaves in Test 1 (August) (Table 3.). The EC and WP formulations of permethrin were equally toxic two days later, but the ULV formulation of permethrin and both ULV and EC formulations of bifenthrin killed only 47%and 52%, respectively. Fluvalinate was almost non-toxic on day zero and day two and was not tested thereafter. Mortalities for all formulations of permethrin dropped rapidly for day three, day seven, and day 10, at which time bee mortality tests were discontinued.

Mortality of honey bees from residues of permethrin and bifenthrin EC and WP formulations were only slightly lower in October (Table 4.) than in August,

Table 2. LC_{50} Values(mg/dish)with Confidence Limits for 6 Pyrethroid Insecticides after Exposure to Honey Bees using the ContactTechnique for Determining Toxicity. Tucson, AZ. 1983.

Synthetic Pyrethroid	Doses tested (no.)	Range tested 1/ (mg/dish)	LC50 2/ (ug/bee)	95% CI for LC_{50} 2/
Permethrin	5	0.05 - 0.20	0.070	0.059 - 0.071
Cyfluthrin	4	0.05 - 0.10	0.084	0.055 - 0.100
Cypermethrin	6	0.05 - 0.40	0.097	0.067 - 0.151
Flucythrinate	10	0.05 - 6.00	1.100	0.401 - 3.550
Fenvalerate	10	0.05 - 6.00	1.700	0.906 - 3.582
Fluvalinate	10	0.05 - 20.00	11.620	7.953 - 16.258

1/ Four replications were analyzed using 20 bees per replication at each dosage.

2/ LC_{50} and confidence intervals obtained by probit

but the ULV formulations of these compounds caused relatively fewer bee deaths in October. Also, the EC formulations were killing more bees over a longer period in October. Cypermethrin caused bee mortalities similar to those from permethrin or bifenthrin up through day five and then was more toxic on day nine and day 13. Fluvalinate was again shown to be nearly non-toxic to the honey bee.

Statistically, the honey bee mortality presented a difficult analysis because there was nearly complete mortality from permethrin, bifenthrin and cypermethrin and nearly no mortality from fluvalinate and the control. Therefore, the latter were omitted from our analysis to reduce heterogeous variances as was the WP formulation. The ANOVA showed for our August test (Table 5) that the compound and formulation effects were only significant on day two. The ANOVA for the October test (Table 6) showed that for the three compounds compared there was no significant difference in bee mortalities, but that the EC formulation was more toxic than the ULV formulation on day zero and day two. The 95% confidence intervals showed that the EC formulations were more toxic than the ULV formulations on day five; however, both cypermethrin and permethrin showed on day nine and cypermethrin alone on day 13 showed this formulation effect.

Table 3. Percent HoneyBee Mortality Following 24 Hours of Confinement in a Cage with Treated Cotton Leaves. Marana, Arizona. August 1984.

Formulation	Permethrin	Fluvalinate	Bifenthrin	Control
		Day 0		
ULV	89	0.4	94	0.0
EC	96	5.3	95	
WP	98			
		Day 2		
ULV	58	0.0	47	0.4
EC	96	0.4	52	
WP	95			
		Day 3		
ULV	40	--	15	0.4
EC	14	--	6.3	
WP	34			
		Day 7		
ULV	6.6	--	5.4	0.8
EC	11.0	--	4.5	
WP	9.1			
		Day 10		
ULV	5.9	--	0.8	1.5
EC	0.8	--	1.2	
WP	0.2			

Table 4. Percent Honey Bee Mortality Following 24 Hours of Confinement in a Cage with Treated Cotton Leaves. Marana, Arizona. October 1984

Formulation	Perm.	Fluval.	Cyperm.	Bifen.	Control
			Day 0		
ULV	72	4	37	63	3
EC	93	0	92	80	
WP	92				
			Day 2		
ULV	17	4	50	8	<1
EC	95	8	71	92	
WP	87				
			Day 5		
ULV	6	0	<1	3	<1
EC	58	0	62	57	
WP	19				
			Day 9		
ULV	3	---	3	5	<1
EC	28	---	60	9	
WP	4				
			Day 13		
ULV	2	---	2	2	0
EC	7	---	19	2	
WP	<1				

Table 5. Analysis of Variance Results (F Value and Significance for Compounds (fluvalinate and control omitted) and Formulations (ULV and EConly, WP omitted) from Honey Bee Mortality

August bee mortality tests (Experiment 1)

SV	Day 0	2	3	7	10 1/
Compound	0.02 ns	7.16 *	1.70 ns	0.01 ns	---
Formulation	1.58 ns	5.06 *	3.23 ns	1.10 ns	---
Interaction	0.40 ns	2.31 ns	0.83 ns	0.46 ns	---

1/ No ANOVA was attempted because some treatments had means near zero, viz. non homogeneous variances.

* = F value significant ($P \leq 0.05$)

ns = F value nonsignificant ($P > 0.05$)

Table 6. Analysis of Variance Results (F value and significance) for Compounds (fluvalinate and control omitted) and Formulations (ULV and EC only, WP omitted) from Honey Bee Mortality

	October bee mortality tests (Experiment 2)				
	Day				
SV	0	2	5	9	13
Compound	0.8 ns	0.2 ns	(95% confidence intervals)		
Formulation	8.4 **	75.8 **	20.7	20.8	6.7
Interaction	0.7 ns	4.5 *	(no ANOVA done)		

1/ No ANOVA was attempted because some treatments had means near zero, viz. non homogeneous variances.

** = F value significant ($P \leq 0.01$)

* = F values significant ($P \leq 0.05$)

ns = F value nonsignificant ($P > 0.05$)

Conditioning Tests

Bees not exposed to a sub-lethal dose of any pyrethroid gave about 60% positive responses after a single training bout. This increased approximately 10% following each of the next three training bouts to achieve approximately 70, 80, and 90% positive responses after two, three, and four training bouts, respectively (Table 7). An ANOVA of the proportion of control bees exhibiting positive responses after only three training bouts showed a significant linear relationship to sequence but a non-significant quadratic relationship (Table 8). Training bouts four through seven resulted in no further increase in positive responses by control bees (non-significant "compound" effect).

Bees surviving exposure to a pyrethroid had means of only approximately 30% positive responses after a single training bout, with a range of 3% to 42% (Table 7). Percent positive response by treated bees showed a statistically significant effect for compounds when either training bouts one to three or training bouts one to seven were tested (Table 8). There was no significant effect when training bouts four to seven were tested. The mean positive response by pyrethroid survivors (all six treatments) also increased by approximately 10% after

the second and third training bouts to approximately 40 and 50%, respectively (Table 7). Pyrethroid-treated bees increased their positive responses slightly after training bout four but reached a mean of only 63% after the seventh training bout. Thus, there was a significant sequence effect ($P \leq 0.05$) (linear only) on positive responses when training bouts four through seven were examined using ANOVA (Table 8). The significant compound by sequence interaction for treated bees in training bouts one through three is a result of different learning response slopes for cyfluthrin and flucythrinate than for the other four pyrethroids.

Table 7 Percentage of Honey Bees Responding Positively to Conditioned Odor Responses Following Training Bouts One Through Seven - Bees Either Survived an LC_{50} Dose of the Indicated Pyrethroid or were Acetone-Treated Controls.

	LC_{50} Dosage (mg/dish)	Training bout 1	2	3	4	5	6	7
Fluvalinate	10.00	38.5[1]	52.6ns	58.8*	62.2*	65.7ns	67.7ns	72.7ns
Fenvalerate	1.00	26.7**	41.2**	53.1*	55.9**	66.7ns	59.4*	69.7ns
Permethrin	0.06	41.7ns	47.5*	65.8ns	59.5**	61.1*	61.1*	67.7ns
Cypermethrin	0.10	34.2ns	40.0*	25.8ns	58.3*	67.7ns	57.6*	62.9ns
Cyfluthrin	0.10	02.9**	25.0**	33.0**	53.1**	60.0**	59.4*	60.0ns
Flucythrinate	1.00	24.2*	32.4**	36.1**	44.0**	36.1**	42.9**	47.2**
Acetone Cont. (mean)	----	62.9	76.4	83.9	90.3	91.7	90.5	89.6

1/ Statistical significance of treated bees versus their respective control as determined by Student Newman Kuels mean separation technique

** = $P \leq 0.01$, * = $P \leq 0.05$, ns = $P > 0.05$

Table 8. Analysis of Variance F Values and Significance for Responses of Honey Bee to Odor-Conditioning Tests After Exposure to One of Six Pyrethroids in Acetone or to Acetone Alone.

	Control bees			Treated bees		
Training bout:	1-3 only	4-7 only	1-7	1-3 only	4-7 only	1-7
Source of variation						
Compound 1/	2.4ns 2/	0.7ns	0.7ns	3.2*	2.6ns	5.1*
Sequence	24.5**	0.7ns	37.2**	49.0**	3.1*	14.8**
Linear	47.4**	---	66.3**	96.9**	7.7*	75.5**
Quadratic	1.2ns	---	59.9**	0.0ns	0.4ns	7.4**
Comp. X Seq.	0.6ns	1.2ns	1.0ns	3.7*	0.7ns	0.2ns

1/ Control bees were done at the same time as bees exposed to one of six pyrethroids.

2/ Statistical significance of treatment effect:
** = $P \leq 0.01$, * = $P \leq 0.05$, ns = $P > 0.05$

DISCUSSION

Topical Tests

Olsson (1978), Wilkaniec (1980) and Atkins (1981) found permethrin to be highly toxic to honey bees when using the topical method of testing. My investigations also grouped permethrin in the highly toxic group, along with cyfluthrin and cypermethrin. In addition, Shires et al. (1983) found cypermethrin to be highly toxic when bees were treated topically. I found flucythrinate and fenvalerate to be moderately toxic while fluvalinate was considered essentially non-toxic. Johanson (1980) and Atkins (1981) also found fluvalinate to be relativeley innocuous to bees in laboratory tests.

Pedersen (1980) determined the LD_{50} of permethrin to be between 0.05 and 0.10 µg/bee, while Danka et al. (1986) found it to be 0.015 µg/bee. Gough and Wilkinson (1984) determined it to be 0.025 µg/bee. My investigations produced a toxicity for permethrin of 0.023 µg/bee -- just outside Pederson's range and between that of the other two authors. Gough and Wilkinson (1984) and Delabie et al. (1985) found the LD_{50} for cypermethrin to be 0.025 µg/bee; this is also not very

different from the 0.020 µg/bee I obtained in the topical tests. Other LD_{50}'s determined by Atkins (1981) were for flucythrinate (0.078 µg/bee) and for fluvalinate (65.85 µg/bee); these numbers correspond well with my values (0.083 µg/bee and 7.45 µg/bee, for flucythrinate and fluvalinate respectively). Although my value for fluvalinate varied from that of Atkins by almost ten times, both studies show much less toxicity for fluvalinate than for any other pyrethroids tested.

Investigators have used the topical technique to compare the toxicity of several pyrethroids against each other in one test, such as I have done. Atkins (1981) determined permethrin to be more toxic than fenvalerate, which in turn was more toxic than fluvalinate. Smart and Stevenson (1982) and Bos and Masson (1983) found cypermethrin to be more toxic than permethrin; fenvalerate and flucythrinate were also significantly less toxic than either cypermethrin or permethrin. Brown-Westerdhal and Gary (1983) concluded that permethrin was more toxic than fenvalerate and that fluvalinate was the least toxic of the three. These comparisons show agreement with the information obtained from my investigations.

Contact Tests

There are no other studies using the contact technique to evaluate effects of pyrethroid insecticides on honey bees. Mayland and Burkhardt (1970) exposed bees to several organophosphate, organochlorine and carbamate insecticides via several different surfaces and found that mortality from the treated filter paper was consistently low when compared with plastic, glass, leaves or soil. In my investigations all compounds tested had lower toxicities (higher LC_{50}'s) in the contact method, compared to the topical method of applying the insecticides.

This technique was valuable because it gave results very similar to the topical method without being as labor-intensive, thus, allowing more replications in a shorter period. The six chemicals were again classified into three categories with this method. The three highly-toxic materials were permethrin, cyfluthrin and cypermethrin, (although not in the same orderas in the topical tests). Of the remaining three, flucythrinate and fenvalerate were moderately toxic and fluvalinate had low toxicity to bees. Not only was this method more time saving, but it also offered the advantage of acting as a residual-type test with the filter paper resembling

insecticide-treated substrate, e.g., leaves or flowers, to which bees would likely be exposed.

Residue Tests

These tests confirm that both compounds and formulations contribute to the varying persistence of toxic residues following pyrethroid-treatment (Appendix A). The ULV formulation in cottonseed oil resulted in significantly lower initial insecticide deposits (except for bifenthrin in August) than did the either the EC or WP formulations at the end of the two-week test period. The lower initial deposits of ULV in vegetable oil formulations and their relatively greater persistence during the October test are consistent with results of previous residue studies on Arizona cotton by Ware et al. (1981).

In October, when the weather was cooler and there was no precipitation to dislodge the insecticide residues from the plant surface, the ULV formulations were consistently more persistent (Appendix B). According to Johansen (1983), temperature has a significant modifying effect on residual action. He stated that cold nights followed by hot summer days may cause condensation of dew on foliage, thus increasing the

residual action of insecticides and killing more honey bees the following day.

It is also noted that with many pyrethroids and certain insects a negative temperature coefficient (Miller and Salgado, 1985) is exhibited. This may explain higher death rates during the October experiment.

With the exception of fluvalinate, which is almost totally harmless to honey bees, the bioassay results reflected very well the levels of toxicant that were present. Johansen and Kleinschmidt (1972) compared formulations of DDT and concluded that "...greater sorption of emulsifiable formulations than of powder formulations on and in plant tissues..." resulted in greater bee safety from emulsifiable formulations. My comparison of EC and WP permethrin in August showed only slighty higher residues for the EC formulation, but in October the EC formulation resulted in both higher residues on leaves and higher toxicity to bees.

Johansen et al. (1983) studied the toxicity of field-weathered residues to the honey bee using alfalfa leaves. Cypermethrin had the longest-lasting residual activity, followed by permethrin, fenvalerate, and fluvalinate. In my studies, permethrin and cypermethrin in the EC formulation lasted longer (concerning bee mortality) than did bifenthrin. El-Banby and Kansouh

(1981) found that cypermethrin had a longer residual activity than the other pyrethroids studied. I also found that cypermethrin (EC only) had relatively long-lived residual toxicty to bees.

Conditioning Tests

The conditioning tests combined some previously used techniques and refined them into a test for detecting behavioral changes in bees following sub-lethal exposure to pesticides. This was valuble because it allowed the work to be done on individual bees in the laboratory without the added variables of beehives and outside field conditions. These tests showed that, although bees may survive poisoning from pesticides, certain physiological functions may be affected. It is important to note that comparisons between the pyrethroids tested must be made with caution, because their toxicity (and consequent dosage in these tests) varied widely, and because the procedures required three days to complete the experimentation needed to test each pyrethroid.

The honey bees seemed to respond normally to the sugar solution when touched to the antennae, (e.g., extension of the proboscis). I believe the observed slow rate of learning comes from a failure to integrate

(or associate) the scent and the sugar solution reward. It was shown by Schricker (1974) and Brandes (1984) that bees, following exposure to sub-lethal doses of methyl parathion (an organophosphate insecticide), had difficulty communicating with other bees inside the colony. It should not be surprising to learn that a conditioned response to odor by the honey bee is also affected.

Conde-Boytel (1985) found that honey bees have chemoreceptors on their tarsi, which may be important because of the method in which the pyrethroids were introduced into their bodies. It is not clear how these chemoreceptors tie into other receptor sites.

The mode of action of pyrethroids on insects is unknown, except for general agreement that the symptoms indicate an attack on the nervous system (Miller and Salgado, 1985). It is not known whether the toxicant has the peripheral or central nervous system (CNS) as its prime target. Impaired response to the scent stimulus and/or sugar water may be the result of peripheral recepter, interneuron, or site-specific CNS blockage. It is suggested that further laboratory and field research is needed to determine the fate and subsequent behavior of honey bees that survive exposure to pyrethroids.

CONCLUSIONS

When tested in the laboratory, pyrethroid insecticides are generally considered highly toxic to bees, however, in the field observable kills are less than expected, indicating that laboratory and field studies are not well correlated. I found, that under laboratory conditions the pyrethroids, with the exception of fluvalinate, were acutely toxic honey bees. Only when I began working with the formulated pyrethroids (Residue Tests) in a field-type situation did the toxicity to bees for individual pyrethroids show differences owing to formulation and climatic conditions.

Conditioning tests suggest that mortality studies may notbe the most important area of study when dealing with honey bee/toxicological matters. Behavioral aberations by intoxicated bees affect colony functions to the point that colonies are less effective as pollinators and honey producers.

Not only the conditions external to the honey bee may cause variation in test results, many factors, including the physical and physiological condition of the bees may influence their sensitivity to pesticides in both lab and field tests. Wahl and Ulm (1983) and

Johansen (1983) review some factors they found to affect the results of honey bee toxicity tests.

Another conclusion from my research experiences is the importance of standardizing methods for testing the toxicity of pesticides to honey bees. Researchers in this field have used many different methods, making comparisons of available literature difficult. It is noteworthy that the International Commission for Bee Botany has sponsored three meetings on the "harmonisation of methods for testing the toxicity of pesticides to bees." These symposia brought together many world experts in the field of pesticide/bee research to discuss the terminology used and different types of tests viz., feeding, contact (topical), cage, field and oral exposure. They also reviewed other factors, such as the source and age of bees used, the season in which they were collected, and the differences between races. Other discussions included the type of anaesthetization used, kinds of cages, and temperatures at which bees are maintained in cages after treatment. This information (and much more) is valuable to people interested in this field of study and should be reviewed before commencing this type of research.

My final conclusion is that there is a large amount of literature available to improve safety to honey

bee during field applications of insecticides. The surest way to protect honey bees is to show care in pesticide selection and application when bee colonies are in the area. Excellent recommendations are available by Atkins (1981), Johansen (1980) and others, giving guidelines whereby honey bee losses might be reduced.

APPENDIX A

INSECTICIDE RESIDUES IN $\mu G/CM^2$ AND PERCENT OF INITIAL DEPOSIT (IN PARENTHESES) COLLECTED IMMEDIATELY AFTER TREATMENT AND ON FOUR LATER DATES AS INDICATED MARANA, AZ, AUGUST 1984

FORMULATION	PERMETHRIN (CIS)	(TRANS)	FLUVALINATE	BIFENTHRIN
		DAY 0		
ULV	.21	.20	.39	.47
EC	.39	.43	.47	.47
WP	.33	.41		
		DAY 1		
ULV	.20(94)	.19(97)	.32(81)	.40(85)
EC	.29(74)	.29(67)	.45(96)	.29(62)
WP	.28(84)	.32(79)		
		DAY 3		
ULV	.073(35)	.068(34)	.11(28)	.16(33)
EC	.12(31)	.10(23)	.15(31)	.09(19)
WP	.095(29)	.099(24)		
		DAY 10		
ULV	.009(04)	.006(03)	.021(05)	.018(04)
EC	.068(18	.042(10)	.017(04)	.026(06)
WP	.037(11)	.025(06)		
		DAY 14		
ULV	.007(03)	.003(02)	.009(02)	.012(03)
EC	.049(13)	.025(06)	.011(02)	.018(04)
WP	.032(10)	.019(05)		

1/ Application rate (kg/ha) 0.11 for permethrin and 0.067 for fluvalinate and bifenthrin.

INSECTICIDE RESIDUES IN μG/CM^2 AND PERCENT OF INITIAL DEPOSIT (IN PARENTHESES) COLLECTED IMMEDIATELY AFTER TREATMENT AND ON FOUR LATER DATES AS INDICATED, MARANA, AZ, OCTOBER 1984.1/

FORM	PERMETHRIN (CIS)	PERMETHRIN (TRANS)	FLUVALINATE	CYPERMETHRIN	BIFENTHRIN
			DAY 0		
ULV	.15	.13	.19	.22	.15
EC	.42	.41	.46	.67	.45
WP	.23	.29			
			DAY 2		
ULV	.14(93)	.13(98)	.19(102)	.24(106)	.13(83)
EC	.31(73)	.31(75)	.29(63)	.57(84)	.37(81)
WP	.16(69)	.21(73)			
			DAY 5		
ULV	.11(78)	.11(82)	.14(77)	.18(81)	.13(89)
EC	.22(53)	.24(57)	.21(46)	.49(73)	.21(46)
WP	.12(52)	.17(59)			
			DAY 9		
ULV	.091(62)	.084(65)	.12(66)	.15(67)	.12(81)
EC	.14(33)	.14(34)	.13(28)	.34(51)	.14(31)
WP	.065(28)	.083(29)			
			DAY 13		
ULV	.078(53)	.078(60)	.11(59)	.12(54)	.10(65)
EC	.10(24)	.11(25)	.062(13)	.19(28)	.10(22)
WP	.043(19)	.053(18)			

1/ Application rate (kg/ha) 0.11 for permethrin, 0.067 for fluvalinate and bifenthrin and 0.09 for cypermethrin.

APPENDIX B

MARANA TEMPERATURES AND PRECIPITATION DURING PYRETHROID RESIDUE / HONEY BEE EXPERIMENT

DATE	DAY	HIGH	LOW	PRECIP
8/6	0	108	72	-
8/7	1	108	73	-
8/9	3	95	72	0.02"
8/13	7	99	68	0.05"
8/14	8	-	-	1.10"
8/15	9	-	-	0.06"
8/16	10	93	74	0.08"
10/10	0	97	59	-
10/12	2	90	61	-
10/15	5	82	61	-
10/19	9	75	43	-
10/23	13	81	45	-

APPENDIX C

ODOR CONDITIONING SUCCESSES COMPARING HONEY BEE SURVIVORS OF LC_{50} DOSAGE OF A PYRETHROID WITH ACETONE-TREATED CONTROL HONEY BEES

CHEMICAL	LC_{50} 1/ DOSAGE UG/BEE	TOTAL BEES	SEQUENCE 1	2	3	4	5	6	7	8
FLUVALINATE	10.00	39	0/37	15/39	20/38	20/34	23/37	25/35	25/37	24/33
ACETONE		41	0/37	25/41	29/40	34/40	33/39	33/37	33/39	30/35
FENVALERATE	1.00	34	1/25	8/30	14/34	17/32	19/34	22/33	19/32	23/33
ACETONE		39	0/37	27/35	29/39	32/38	32/36	35/38	33/36	35/37
PERMETHRIN	0.06	40	1/33	15/36	19/40	25/38	22/37	22/36	22/36	23/34
ACETONE		41	0/41	26/40	34/41	36/41	33/36	36/40	38/41	35/38
CYPERMETHRIN	0.10	38	0/33	13/38	14/35	19/36	21/36	21/36	19/33	22/35
ACETONE		42	0/39	23/39	31/42	30/39	36/40	37/39	32/36	31/36
CYFLUTHRIN	0.10	36	0/33	1/34	8/32	13/36	17/32	18/30	19/32	18/30
ACETONE		42	0/40	22/35	33/42	36/41	37/39	37/39	33/37	35/40
FLUCYTHRINATE	1.00	37	2/30	8/33	12/37	18/36	16/36	13/36	15/35	17/36
ACETONE		38	0/35	18/34	29/38	30/37	33/36	33/37	32/33	32/35

1/ The LC_{50} Values used were determined by the Contact Tests in this Paper.

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