

SUBLETHAL DOSAGE EFFECTS OF CARBARYL
ON
HONEY BEE (Apis mellifera (L.)) COLONIES

by

Chapman Kemper Loyd

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APPROVED:

R. D. Fell, Chairman

D. G. Cochran

J. E. Roberts, Sr.

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Section I

INTRODUCTION

The value of the honey bee in today's agricultural system often is considered only in terms of honey and beeswax production. While this value is not insignificant--\$130 million in 1980 (Anonymous, 1981)--the true value of honey bees lies in the crop pollination they perform. Although a great variety of insects contribute to crop pollination, bees are considered to be the most dependable and efficient insect pollinators. Among the various species of bees that visit crop plants, honey bees are considered by far to be the most important, being responsible for an estimated 80% of all bee-rendered pollination (Levin, 1971).

Despite the great importance of honey bees to agriculture, they are increasingly endangered by an agricultural practice: the use of pesticides. Damage from pesticides is considered to be the greatest problem facing the beekeeping industry today.

In 1980, there were approximately 4.1 million colonies of honey bees in the United States (Anonymous, 1981), compared with 5.6 million in 1950 (Hannawald, 1971). This reduction of 1.5 million colonies in 30 years was due

largely to the development and use of newer, more bee-toxic pesticides: chlorinated-hydrocarbons, organo-phosphates, and carbamates (McGregor, 1976). In 1967, for example, Levin (1970) reported that 500,000 colonies were killed or damaged by pesticides in the U.S. Annual losses of approximately 10% are considered to be the current level in this country (McGregor, 1976).

Bee losses from pesticides have precipitated a great deal of research on the problem. Many studies (Atkins et al., 1970b, and others) have been performed to determine the toxicities of various compounds to honey bees. Unfortunately, the vast majority of those studies have dealt only with the effects of acute, lethal dosages of pesticides on honey bees; few have dealt with the possible effects of chronic, sublethal dosages. This is an area which requires further research.

A honey bee colony is a complex society, and social order is maintained through complex communication systems and behavior patterns. Disruption of these may result in a breakdown of social order and subsequent deterioration of the colony. Lethal pesticide dosages may cause that breakdown to occur, accompanied by a significant death of colony members. However, the possibility also exists that lower, sublethal dosages may cause a breakdown in social

order, without a significant bee-kill. That possibility was the subject of this research.

The effects of sublethal dosages of an insecticide on five aspects of honey bee colonies were studied: brood production, brood mortality, adult mortality, colony weight change, and resistance to the toxicant. Carbaryl (1-naphthyl N-methylcarbamate) was chosen as the test material for two main reasons. First, it is a very widely-used insecticide. Union Carbide (1978) lists over 50 field crops and over 20 fruits to which carbaryl may be applied. Second, it is a compound that has caused serious honey bee colony loss or damage in the past (Johansen, 1959, 1977; Johansen and Shipman, 1961; Anonymous, 1967; Morse, 1972).

Section II
REVIEW OF LITERATURE

2.1 EXPOSURE TO PESTICIDES

In a field situation, honey bees may be exposed to pesticides in three ways: through contact, ingestion, or fumigation. A given pesticide is not limited to one of those paths, but may act through two or all three.

The most common route of exposure is through contact, since many pesticides act primarily in that manner. The importance of that route is reflected by the large number of toxicity tests that are based on a contact method of application (Anderson and Atkins, 1968).

The major source of pesticide contact by honey bees is through contaminated surfaces. Morse (1964) showed that contamination of interior and exterior hive surfaces with an insecticide (carbaryl) had little effect on bees. Therefore, plant surfaces have the major role in this contact. Several researchers have utilized treated plant foliage/flowers, either in the laboratory or in the field, to expose honey bees to a pesticide for toxicity testing (Anderson and Tuft, 1952; Johansen, 1954; Anderson and Atkins, 1958; Shaw, 1959; Clinch, 1967; Mayland and

Burkhardt, 1970). Attempts to substitute artificial flowers for real flowers (clover) have also been made, but these were not successful (Clinch, 1967).

Another source of pesticide contact is through direct application, which may occur when pesticides are applied to fields in which honey bees are actively foraging (Palmer-Jones and Forster, 1958). Affected bees may die in the field or soon after returning to the hive, often resulting in an accumulation of dead bees in front of the hive (Anderson and Atkins, 1958; Atkins, 1975).

The second route of pesticide exposure is through ingestion. Application of an insecticide to a flowering crop has been shown to directly contaminate nectar (Maurizio and Schenker, 1957). The majority of bees that collect contaminated nectar are killed before they can return to the hive, but occasionally contaminated nectar is returned to the hive and stored (Atkins, 1975). Solov'eva (1971) found that bees will forage on sucrose solutions containing up to 10 ppm carbaryl and 50 ppm BHC. Waller et al. (1979) reported that honey bees will forage on sucrose solutions containing up to 25 ppm dimethoate and will continue to forage until they have collected 20-25 times the oral LD_{50} . They estimated that foragers absorbed 5% of the ingested dimethoate and passed the remaining 95% on to house bees in the hive.

The presence of several insecticides has been demonstrated in honey. Stanger and Winterlin (1975) found carbaryl residues in honey produced by bees that foraged on treated alfalfa. Ogata and Bevenue (1973) and Estep et al. (1977) showed the presence of chlorinated-hydrocarbon insecticides in commercial honeys at concentrations up to 2.60 ppb, and Estep et al. found slightly higher levels of the same compounds in beeswax. Thus far, however, no pesticides have been found in sufficient quantities to be hazardous to humans.

Plant nectar also may be indirectly contaminated by systemic pesticides. Demeton, dimethoate, fosfinon, methylisostox, phorate, phosphamidon, and schradan have been shown to be secreted in the nectar of treated plants (Glynne-Jones and Thomas, 1953; Johansen and Quist, 1955; Maurizio and Schenker, 1957; Lord et al., 1968). Several of those insecticides--dimethoate, fosfinon, methylisostox, phosphamidon, and schradan--have been found in quantities toxic to honey bees (Johnsen, 1953; Maurizio and Schenker, 1957; Jaycox, 1964). Mizuta and Johansen (1972) criticized much of the other work with systemic insecticides because of abnormally-high application rates, plant species tested, and lack of good techniques. They maintained that proper use of systemics will not result in toxic quantities of insecticides in nectar.

In addition to nectar, pollen can be contaminated by pesticide applications to flowering crops. Contaminated pollen may kill bees in the field, but it also may be carried back to the hive and stored. Later, when it is used for brood rearing, toxicity may result.

Insecticides found in stored pollen have included lead arsenate, carbaryl, monocrotophos, and microencapsulated methyl parathion (Dyce, 1947; Stanger and Winterlin, 1975; Burgett and Fisher, 1977). Lead arsenate has remained toxic in stored pollen after several years (Dyce, 1947). Carbaryl-contaminated pollen retains 30% of its toxicity after 8 months of storage (Johansen and Brown, 1972), and microencapsulated methyl parathion has remained toxic in stored pollen after 14.5 months (Rhodes et al., 1979).

The process of storing pollen has been hypothesized to reduce insecticide levels by Stanger and Winterlin (1975), who found higher levels of carbaryl and monocrotophos in entrance-trapped pollen than in stored pollen. However, another explanation is possible. Insecticides are known to cause bees to behave erratically and may decrease their ability to carry pollen through a pollen trap. Also, Atkins (1975) states that seriously affected bees returning to a hive are usually removed without expelling their pollen load. These factors increase the probability that pollen

collected from more highly-contaminated sources will be deposited in entrance traps rather than stored. This increased trap deposition may be responsible for the higher levels of insecticides found there.

Honey bees also may ingest pesticides with water, which can become contaminated through pesticide applications, runoff, or accidents. Morse (1961) reported honey bees collecting water from puddles on which droplets of oil and insecticide were visible following an aerial application of carbaryl. Morton et al. (1974) found that bees would collect water treated with 1000 ppm of 2,4,5-T or paraquat, although they would preferentially collect untreated water if it was available.

The final route of pesticide exposure is through fumigation. Honey bees are unlikely to come into contact with a true fumigant, but some other pesticides may have a fumigant effect. Malathion has been reported to have a fumigant action in warm regions (Anderson et al., 1971), and diazinon has been found to be more toxic to honey bees as a fumigant than as a stomach poison (Palmer-Jones and Forster, 1958). McGregor (1976) states that bees may easily be affected by contact with the gaseous form of nerve-type poisons, such as parathion.

Two factors are known to influence the effect of a pesticide application on honey bees in the field: toxicity of the material and honey bee exposure. Toxicity of the material depends on the toxicant itself, formulation, synergism with other compounds, route of exposure, weather (especially temperature and moisture), and age of bees exposed. Honey bee exposure to a pesticide depends on the formulation, crops treated, application placement, application timing, residual life, colony distance from treated crops, colony strength, weather, and availability of alternate nectar, pollen, and water sources (Johansen, 1977).

2.2 EFFECTS OF PESTICIDES

A great volume of research has been published on the toxicity of pesticides to honey bees, and the material has been reviewed by several authors. The most recent review, published in 1977 by Johansen, covered the period 1967 to 1975 and cited 95 references. That review updated the comprehensive article by Anderson and Atkins (1968) that referenced 198 publications. Earlier reviews were published by Todd and McGregor (1960), Hocking (1950), and Shaw (1941).

Most of the published research has dealt with the lethal effects of pesticides on adult honey bees. However, some researchers have specifically examined non-lethal effects, and others have noted non-lethal effects that occurred during other studies. These non-lethal effects of pesticides on honey bees include reduction in brood rearing, queen supercedure, reduction in foraging activity, disruption of communication, reduction in overwintering ability, increase in disease incidence, and increase in aggressiveness.

One effect that has often been reported is a reduction in brood rearing. This reduction may occur indirectly, as a consequence of increased adult mortality, or directly, as a result of the pesticide itself. The former occurs more frequently than the latter. If the adult population of a honey bee colony is decreased by a pesticide, and the remaining bees cannot care for all of the brood that is present, some of it will die from neglect (McGregor, 1976). Anderson (1964) reported a ring of dead brood around the outer brood-nest margins of colonies that had been exposed to carbaryl. Solov'eva (1971) found decreased brood production in colonies that were fed BHC or carbaryl one year previously. However, that decrease probably was due to the reduced strength of the overwintered test colonies.

Pesticides also may have a direct effect on brood rearing. Applications of herbicides to honey bee colonies were found to prevent brood development and/or reduce or eliminate brood production. Also, eggs were found to be non-viable in colonies that were fed 1000 ppm of phenoxy herbicides (Morton and Moffett, 1972). Robinson and Johansen (1978) reported that forest applications of carbaryl or acephate disrupted honey bee colony brood cycles or caused erratic brood patterns. Disruption was temporary for the carbaryl-exposed colonies and permanent for those exposed to acephate.

When brood rearing is disrupted, queen supercedure may occur. Robinson and Johansen (1978) reported that 2 of 4 colonies exposed to carbaryl superceded the queens. Johansen (1977) states that queenlessness has been associated with arsenicals, dieldrin, carbaryl, malathion, and parathion, and he hypothesizes that it is caused by reduced secretion of queen substance.

A third effect of pesticide exposure is reduction in foraging. Gary (1967) reported that flight activity of honey bee colonies was depressed approximately 40% following insecticide treatments of clover fields in which they were located. This reduction in flight activity was not directly attributable to adult bee mortality. A similar effect was found by Todd and Reed (1969), who reported that pollen

collection by honey bees was reduced 50% following insecticide applications to alfalfa and safflower. This reduction, however, was attributed to forager mortality.

Reduction in foraging may have been caused by a disruption of communication within the affected colonies. Schricker and Stephen (1970) fed foraging honey bees approximately 0.02 ug parathion/bee and studied the effects of the toxicant on the communication dance. They found that treated bees were unable to accurately communicate the direction of a food source to other bees within the hive. Also, the time-sense of the treated bees was impaired.

Solov'eva (1971) found that colonies fed 0.5 ppm BHC or 2.5 ppm carbaryl in the fall did not overwinter as well as untreated colonies. Treated colonies did survive the winter, but they were reduced in strength and produced less brood and honey the following year.

Solov'eva also reported that overwintered, treated colonies had a much higher incidence of Nosema disease. This effect has been reported by other researchers. Morse (1961) and Morse and Gunnison (1967) reported European Foulbrood and Sacbrood, respectively, in colonies that had been exposed to carbaryl. Morse (1978) states that low levels of pesticides cause stressful conditions in which several honey bee pathogens flourish.

An additional effect of pesticides that has been noted is an increased aggressiveness and tendency to sting. This effect has been reported for lindane, BHC, and organo-phosphate compounds (Johansen, 1963, 1977; McGregor, 1976; Robinson and Johansen, 1978), and also for carbaryl (Solov'eva, 1971; Robinson and Johansen, 1978).

2.3 EXPERIMENTAL METHODS

Pesticide applications to honey bee colonies have taken place by two methods: direct application to the colony, and allowing the bees to forage in a treated area. Although the latter method provides more reliable data regarding field applications, the former method allows more accurate control of applied dosages.

Pesticides have been directly applied to honey bee colonies in sucrose (or honey) solutions. This method was used by King (1964), Solov'eva (1971), Morton and Moffett (1972), Winterlin et al. (1973), and Tucker (1980). A second method--spraying pesticide solutions directly into the hives--was used by Keener and Pless (1974).

Conner et al. (1978) demonstrated the importance of sucrose concentration on the toxicity of ingested pesticides. Rate of insecticide penetration through the foregut was found to be inversely proportional to the

sucrose concentration in both in vivo and in vitro studies. Additionally, the oral toxicity of carbaryl showed the same dependence on sucrose concentration in vivo.

Brood areas within colonies have been measured two ways: with a grid system, and by assigning relative values to frames of brood. Anderson and Atkins (1968) utilized a grid of 1-inch mesh hardware cloth to measure brood. A similar method appears to have been used by Solov'eva (1971) and Morton and Moffett (1972), although no specifics are given.

Morse and Gunnison (1967) and Strang et al. (1968) utilized a point system in which a full frame of brood was given a value of 4, a half frame of brood was given a value of 2, etc. Strang et al. used this system to equalize colony brood areas to reduce colony variation.

Morton and Moffett (1972) and Garofalo (1977) utilized a common method to measure brood mortality. A frame with a delineated section of comb containing 100 cells was placed into a queenright colony. After 24 hr, the eggs in the section were counted, and they were then followed through the rest of their development. One problem with this method was that, since the queens had free access to the combs, they frequently replaced eggs that failed to hatch and oviposited in cells that were previously empty.

Measurements of adult mortality are made with dead bee traps. The two most common traps in use are the Todd trap (Atkins et al., 1970a) and the Gary trap (Gary, 1960). Both traps have proved to be 90-95% efficient in the collection of worker bees that die within the colony (Morse and Gunnison, 1967; Atkins et al., 1970a). Less efficient traps have been designed and used by Johansen and Quist (1955), Johansen et al. (1957), Anderson (1964), Bailey (1965), and Rhodes and Wilson (1978).

Methods used in laboratory toxicity testing were reviewed by Anderson and Atkins (1968). Several researchers have investigated the variables present in that testing to determine their effects on test results. Using the topical drop method of application, Graves and Mackenson (1965) found that test results were influenced by bee age, post-treatment confinement (alone or in groups), and post-treatment temperature. This temperature dependence was also demonstrated by Georghiou and Atkins (1964), who found that carbaryl was 3.81 times more toxic to honey bees at 15.6°C (60°F) than at 26.7°C (80°F). Graves and Mackenson (1965) found no difference in test results when the site of drop application was varied from the dorsum of the thorax to the dorsum of the abdomen.

Variation among bees in susceptibility to a toxicant also may affect test results. On a single test day, Tahori et al. (1969) found significant inter-colony variation in the contact LD_{50} values for trichlorfon, ronnel, and DDT. Additionally, significant intra-colony differences in LD_{50} values were found on different testing days for ronnel and DDT.

Other factors reported to influence toxicity test results are pre-treatment care, time of feeding, humidity, and length and type of pre-treatment anaesthesia (Anderson and Atkins, 1968). Standardization of these factors was recommended to increase the accuracy and reproducibility of toxicity test results.

Section III
MATERIALS AND METHODS

3.1 RESEARCH--1980

During the summer of 1980, the possible effects of low-level carbaryl exposure on brood production and adult mortality in honey bee colonies were investigated. The colonies utilized for this study were located at the VPI & SU Price's Fork Research Lab near Blacksburg, Virginia.

3.1.1 Colony Preparation

Twelve honey bee colonies were prepared approximately five weeks prior to initiation of the project. All experimental colonies were hived in single-story, 10-frame Langstroth hives. Each colony was started with a young, mated queen and four frames of bees, brood, and honey taken from a larger colony. An effort was made to start each colony with an equal amount of bees and brood. Five additional frames with comb foundation were added to each colony, and the colonies were fed with a 1.6 M (50% w/w) sucrose solution to stimulate growth until the study began.

The study was started on August 9 and ended on October 7, 1980, a period of 60 days. At the beginning, the test

colonies had brood on an average of 4.3 frames, and bees on an average of 6.0 frames.

Two days prior to carbaryl administration, nine colonies were selected at random and weighed, both with and without bees, to determine the mean hive population. The mean weight of bees was 1.93 kg with a standard deviation of 0.60 kg. Based on 7700 bees/kg (Mitchell, 1970), this corresponded to a mean population of 14,900 bees and a standard deviation of 4600 bees. Carbaryl applications were based on that mean population level.

3.1.2 Carbaryl Administration

The carbaryl treatments were based on daily doses of 1/50 and 1/10 of the oral LD_{50} for honey bees. An oral LD_{50} value of 0.178 ug carbaryl/bee (25% sucrose solution, 24 hr, 32°C) (Alvarez et al., 1970) was used for all calculations. Similar values were reported by Stevenson (1968), Argauer et al. (1972), and Conner et al. (1978). The calculated doses were 0.0036 and 0.0178 ug carbaryl/bee/day for the 1/50 and 1/10 LD_{50} levels, respectively. These two levels were chosen somewhat arbitrarily, since no previous research indicated the levels at which significant changes in brood production might be expected. However, the Oral Dosage-Mortality curve given by Alvarez et al. (1970) indicated

that, for a single feeding, adult mortality of less than 2% could be expected at the chosen toxicant levels.

Four randomly-chosen colonies were used for each of the 1/50 and 1/10 LD₅₀ treatments, in addition to the Control treatments. Carbaryl treatments were applied to the colonies on a daily basis, beginning on Day 27 of the experiment and ending on Day 40. This 14-day feeding period was designed to simulate the residual life of carbaryl in the field (Johansen, 1966). The total potential doses were 0.0504 and 0.2492 ug carbaryl/bee for the 1/50 and 1/10 LD₅₀ treatments, respectively.

Stock solutions were prepared with technical-grade carbaryl(1), acetone, 95% ethanol, and distilled water. A measured amount of carbaryl was dissolved in 10.0 ml of acetone, added to 40.0 ml of 95% ethanol, and taken to a total volume of 100.0 ml with distilled water. A 10.0 ml volume of this solution was added to 200.0 ml of distilled water to make the stock solution. For the 1/50 LD₅₀ treatment, 55.70 mg carbaryl was used to make a stock solution containing 26.52 ug carbaryl/ml. For the 1/10 LD₅₀ treatment, 278.48 mg carbaryl was used to prepare a stock solution containing 132.61 ug carbaryl/ml. In addition, a

1 Lot No. 74215, Assay 100%. Union Carbide Corp., New York, NY 10017.

Control treatment stock solution, containing no carbaryl, was prepared.

The carbaryl was fed to the colonies in a 0.95 M (30% w/w) sucrose solution that approximated the median sugar content of plant nectar (Crane, 1975). The solutions were delivered in pint-size (0.47 l) canning jars, each having about 20 small holes (approximately 1 mm diameter) in the lid. Two jars were inverted over the inner cover of each colony to give the bees ready access to the solutions (Figure 1).

A daily application consisted of 2.0 ml of the correct stock solution mixed in approximately 600 ml (300 ml/jar) of sucrose solution and placed on the colonies. That volume of solution insured that a bee which collected a full honey-stomach load of solution (approximately 40 ul (Combs, 1972)) would not ingest a dosage of carbaryl greater than that specified in the treatment. The carbaryl concentrations of the solutions were 0.00, 0.09, and 0.44 ppm for the Control, 1/50 LD₅₀, and 1/10 LD₅₀ treatments, respectively. These concentrations were well below the oral LC₅₀ for honey bees of 3.8-4.5 ppm carbaryl (60% w/v sucrose solution, 48 hr, 27°C) reported by Winterlin et al. (1973), and a similar value reported by Solov'eva (1971). Concentrations also were below the 2.0 ppm carbaryl that Winterlin et al.



Figure 1: Feeding Method for Carbaryl Solutions

selected as their maximum sublethal concentration. The average time required by the bees to empty the feeder jars was about 12 hrs.

3.1.3 Brood Production

The effect of carbaryl on brood production was followed in each test colony by measuring brood area. Measurements were made at 4-5 day intervals throughout the experimental period. This gave a total of 16 measurements for each colony that survived throughout the experiment: seven measurements pre-exposure, four during exposure, and five post-exposure.

Measurements were made by tracing the brood area of each frame within a given colony. Tracings were made with permanent marking pens on transparent acetate film(2). The total brood area within each colony was traced. At the same time, separate tracings of egg-stage brood were made to differentiate it from brood in later stages.

Brood tracings were measured on a Numonics Model 274-167 Electronic Graphics Calculator (Numonics Corp., Lansdale, PA 19446). Areas of egg-stage brood, larval+pupal brood, and total brood were determined in sq

2 Formula 867, Gage 5.0. Supplied and cut to width by Georgia-Pacific, through XCEL Corporation, Newark, NJ 07105.

cm.

3.1.4 Adult Mortality

Three test colonies in each treatment were chosen at random to study the effect of carbaryl on adult mortality. Estimates of mortality were made by collecting the adult bees that died in each colony with a Gary dead bee trap (Gary, 1960) over a 24-hr period. These traps partially restrict flight activity, making it difficult for bees to carry dead colony members away from the hive. The dead bees accumulate in the traps, where they can be removed and counted.

The traps were not available for use until Day 25 of the experimental period. To provide pre-exposure data, two consecutive 24-hr counts were made at that time. Thereafter, counts were made at intervals of 4-5 days throughout the test period. A total of 10 counts was made for each colony: two pre-exposure, three during exposure, and five post-exposure.

To determine the accuracy of the dead bee traps, 20 dead bees were marked with enamel paint (The Testor Corp., Rockford, IL 61101) and introduced into each test colony at the start of each 24-hr collection period. Following that period, the marked bees retained by a trap were counted, and

the accuracy of that trap computed. The number of unmarked dead bees was then adjusted by that trap accuracy to give a corrected mortality.

3.2 RESEARCH--1981

In 1981, research dealt with possible effects of low-level carbaryl exposure on three other aspects of honey bee colonies: brood mortality, colony weight change, and resistance to carbaryl. In addition, brood production and adult mortality were re-examined with refined techniques.

The above studies, with the exception of the brood production study, were performed with honey bee colonies located at the Price's Fork Research Lab. The colonies utilized for the brood production study were located at the VPI & SU Turkey Research Center, in Blacksburg, Virginia.

3.2.1 Price's Fork Research Lab: Colony Preparation

Fifteen colonies were prepared approximately eight weeks prior to the start of the study. The colonies were set up as discussed previously (Part 3.1.1), except that all colonies were started with sister queens(3), and only four frames with foundation were added to each colony (for a

3 Special order from The Wilbanks Apiaries, Inc., Claxton, GA 30417.

total of eight frames). The project was started on June 28 and ended on October 1, 1981, a period of 96 days.

The adult bee populations and brood areas were adjusted in each colony approximately three weeks before the study began. Each colony was weighed, both with and without bees, to determine the adult bee population of each. In addition, the brood area was estimated using a transparent plastic sheet marked with 25 sq cm (5 cm X 5 cm) grids. Bees and/or brood frames were then added or removed to give each colony a population of approximately 15,000 bees (1.94 kg) and a brood area of 3200 sq cm (128 grids).

3.2.2 Carbaryl Administration

Carbaryl treatments based on daily doses of 1/10 and 1/5 of the oral LD_{50} (Alvarez et al., 1970) were used in this study. The calculated doses were 0.0178 and 0.0356 ug carbaryl/bee/day for the 1/10 and 1/5 LD_{50} levels, respectively. The higher treatment level (1/5 LD_{50}) was substituted for the 1/50 LD_{50} level of the previous year to increase the probability of significant effects. The Oral Dosage-Mortality curve published by Alvarez et al. in 1970 indicated that a single feeding at that higher level would produce about 7% adult mortality.

Six randomly-selected colonies were used for the 1/10 LD₅₀ treatment and three for the 1/5 LD₅₀ treatment. Six colonies were also used for the Control treatment. Treatments were applied daily for 14 days, beginning on Day 25 and ending on Day 38. The total potential doses were 0.2492 and 0.4984 ug carbaryl/bee for the 1/10 and 1/5 LD₅₀ treatments, respectively.

Five colonies were selected at random and weighed four days before treatments were applied to estimate the average hive population. The mean population was determined to be about 19,000 bees (2.46 kg) with a standard deviation of 3000 bees (0.39 kg). That population level was used as the basis for the carbaryl applications.

Stock solutions were prepared in a slightly different manner than the previous year to insure that all of the insecticide remained in solution. A measured amount of carbaryl was dissolved in 20.0 ml acetone and taken to a total volume of 500.0 ml with 95% ethanol. The 1/10 LD₅₀ solution contained 84.80 ug carbaryl/ml, and the 1/5 LD₅₀ solution contained 169.60 ug carbaryl/ml. The Control solution contained no carbaryl.

The feeding method was identical to that described previously (Part 3.1.2), with two exceptions. First, 4.0 ml of stock solution was applied to each colony daily, and

second, the volume of sucrose solution fed was increased to 800 ml daily to adjust for the higher mean population. Carbaryl concentrations for the Control, 1/10 LD₅₀, and 1/5 LD₅₀ treatments were 0.00, 0.42, and 0.84 ppm, respectively.

3.2.3 Brood Mortality Studies

To study brood mortality in response to carbaryl exposure, each test colony was installed in a hive that was divided lengthwise into two equal compartments by a vertical section of queen excluder. Each compartment contained four frames, and the entrance to one compartment was restricted with another section of queen excluder. The queen was confined to that compartment, but the workers moved freely throughout the hive.

To measure brood mortality within each colony, a broodless frame (or one with emerging brood) was moved from the queenless compartment into the compartment with the queen. The queen was allowed to oviposit on the frame for 24 hr. At the end of that period, the frame was removed, a section of comb containing 100-400 eggs was delineated, and the eggs were counted. The frame was then moved back into the queenless compartment where the eggs were allowed to develop normally.

Six days after oviposition, the frame was re-examined, and the larvae that had hatched from the eggs were counted. The frame was then replaced into the queenless compartment. Similar examinations were made 10 days, 20 days, and 25 days after oviposition to count the cells containing recently-capped brood, brood expected to emerge within one day, and brood that failed to emerge, respectively.

New frames of eggs were started every five days, when possible, beginning on Day 2 and ending on Day 57. After that period, no new counts were begun, but the brood frames on which counts had been started were followed to the end of the development period. A total of 12 sets of eggs were started and followed in each colony during the test period.

Brood mortality was determined from the amount of brood that survived from one stage to the next. Percent survival was computed for the egg stage, larval stage, capped stage, and also for the entire developmental period.

3.2.4 Colony Weight Change

The response of colony weight to carbaryl exposure was measured for all colonies at five-day intervals, beginning on Day 1 and ending on Day 56. Each colony was weighed at or before dawn to insure that all bees were present and included in the measured weight. The colonies were each

weighed a total of 12 times: five times pre-exposure, three times during exposure, and four times post-exposure. Colony weight changes were computed on two different bases: change in weight per five-day period, and net change from original weight.

3.2.5 Resistance to Carbaryl

Two randomly-selected colonies were chosen from each treatment for the carbaryl resistance study. Contact dosage-mortality tests were performed four times during the study to determine if low-level carbaryl exposure altered worker resistance to the insecticide.

Five days prior to each test, a frame with ready-to-emerge bees was removed from each colony, caged, and placed in an incubator set at a temperature of $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Newly-emerged worker bees were collected after 72 hr, placed into separate (by colony) quart-size (0.95 l) cardboard food cartons (Neptune Paper Products, Inc., Long Island, NY), and held for an additional 48 hr at that temperature. Cartons were fitted with screen tops and supplied with three 2-dram vials filled with 0.95 M sucrose solution and stoppered with cotton. At the end of that period, the bees were used for the toxicity tests. Worker ages varied from 48-120 hr.

Contact dosage-mortality tests were performed on Day 29, Day 47, Day 53, and Day 96 of the study. The first test was performed to determine the toxicity of carbaryl before exposure, and to provide a basis for comparison of the later data. The second test was performed on bees that had passed through the entire larval stage and part of the pupal stage while carbaryl treatments were made to the colonies. The bees used in the third test developed from eggs that were deposited approximately three days after carbaryl application began. Those bees not only went through the complete egg and larval stages during the time of carbaryl administration, but the queen herself was exposed before she deposited the eggs. The final test was performed on bees that began development more than five weeks after the carbaryl exposure ended.

The values given for the topical LD_{50} (dorsal thorax, 24 hr, 27°C) in the literature range from 0.06 ug carbaryl/bee (age 0-24 hr) (Tucker, 1980) to 2.3 ug carbaryl/bee (age unknown) (Mullipudi and Fukuto, 1979). Therefore, preliminary tests were run to determine the carbaryl toxicity for the bees used in these studies. A contact LD_{50} value of 0.11 ug carbaryl/bee was obtained. Four doses--0.07, 0.10, 0.15, and 0.20 ug carbaryl/bee--were then chosen to bracket that value. Also, a Control dosage, containing no carbaryl, was used.

The carbaryl was dissolved in acetone, and a 1.00 ul drop of solution was topically applied with a Haydon Model M Microapplicator (Instrumentation Specialities Co., Inc., Lincoln, NE 68507) to the thorax of each bee. Each dose was applied to 60 bees from each colony, using three replicates of 20 bees each. Bees were anaesthetized with carbon dioxide prior to treatment, and, after treatment, each replicate was placed in a separate pint-size (0.47 l) cardboard food carton and held at $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Each carton was fitted with a screen top and supplied with a cotton-plugged 2-dram vial of 0.95 M sucrose solution.

Bee mortality counts were made after 24 hr, and corrected mortalities were computed using Abbott's formula (Abbott, 1925). Probit analysis was performed on the VPI & SU computing system, using the Probit procedure in the Statistical Analysis System(4) package.

3.2.6 Adult Mortality

The materials and methods used in this adult mortality study were essentially identical to those described previously (Part 3.1.4), with the exception of the carbaryl treatments. Counts were taken at five-day intervals from Day 2 to Day 57, giving a total of 12 counts per colony:

five pre-exposure, three during exposure, and four post-exposure.

3.2.7 Turkey Research Center: Colony Preparation

Fifteen colonies were prepared approximately six weeks prior to the study. They were set up as described for the study conducted in 1980 (Part 3.1.1). The project started on September 8 and ended on October 20, 1981, a period of 43 days.

Colonies were equalized one week prior to the start of the study by the method described previously (Part 3.2.1). The adjusted hives had populations of 10,500 bees (1.36 kg) and brood areas of 3125 sq cm (125 grids).

3.2.8 Carbaryl Administration

Carbaryl treatments were applied daily for 14 days, starting on Day 21 and ending on Day 34. Treatment levels (Control, $1/10$ LD₅₀, and $1/5$ LD₅₀) and methods were essentially identical to those previously described in this section (Part 3.2.2). The mean colony population was estimated to be 11,000 bees at the start of the application period, and the dosages fed to colonies were adjusted to that population level.

The carbaryl stock solutions were prepared also as previously described in this section. The 1/10 LD₅₀ solution contained 48.96 ug carbaryl/ml, the 1/5 LD₅₀ solution contained 97.90 ug carbaryl/ml, and the Control solution contained no carbaryl. A 450 ml volume of 0.95 M sucrose solution was used to apply 4.0 ml of the applicable stock solution to each colony. Carbaryl concentrations were 0.00, 0.43, and 0.86 ppm for the Control, 1/10 LD₅₀, and 1/5 LD₅₀ treatments, respectively.

3.2.9 Brood Production

Brood production measurements were made in a manner similar to those of the previous year (Part 3.1.3). Brood area within each colony was measured at 3-4 day intervals throughout the study, for a total of 13 measurements; six pre-exposure, four during exposure, and three post-exposure. In this study, however, only egg-stage brood was measured.

Section IV

RESULTS

The results of the brood production studies in 1980 are presented in Figures 2, 3, and 4 for eggs, larvae+pupae, and total brood, respectively. Egg production for the 1981 study is shown in Figure 5.

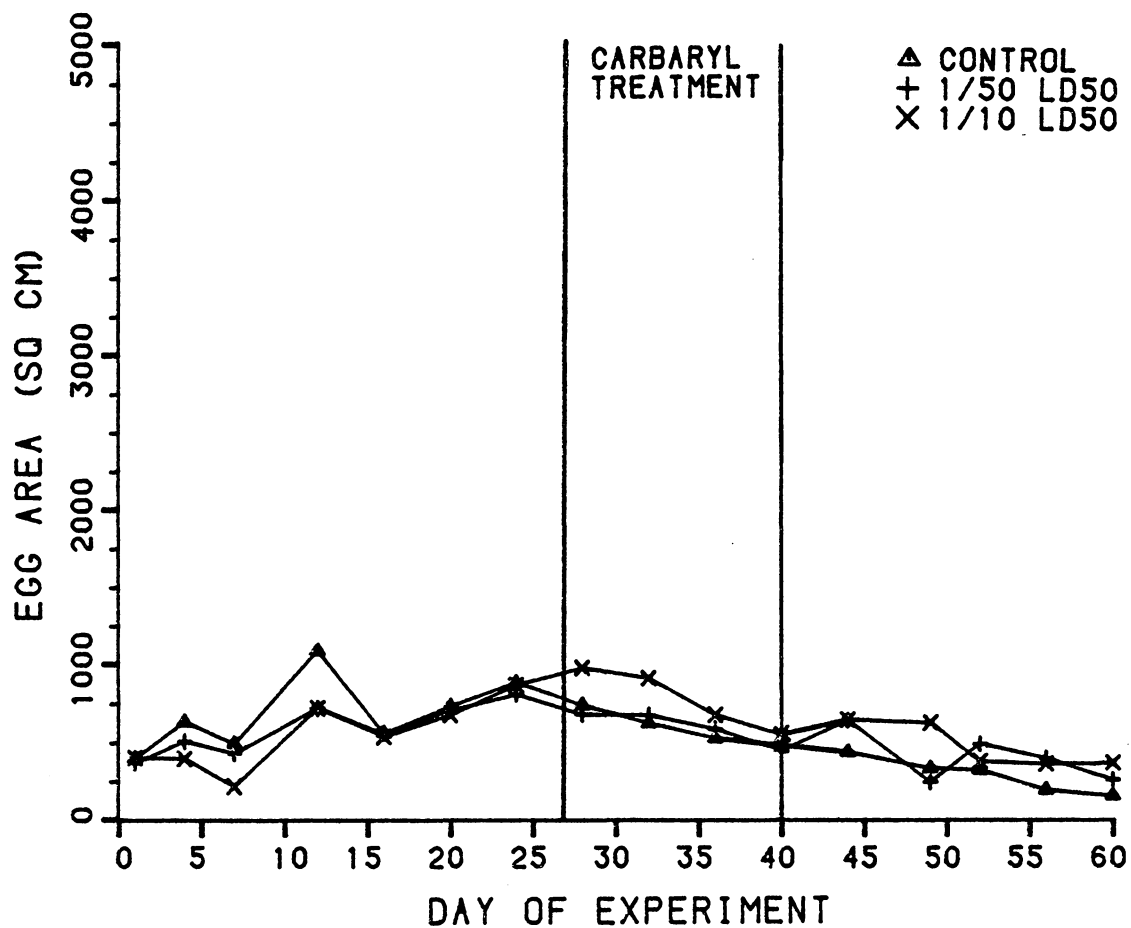
In the 1980 studies, the production of all brood stages showed a general increase until approximately the start of carbaryl application (Figures 2-4). Subsequent to that date, a general decline in production occurred. During that year, only one significant difference ($P \leq 0.05$) in brood production was observed. An analysis of variance indicated that the 1/10 LD₅₀ group had a significantly higher egg production on Day 49 (post-treatment) than either of the other treatment groups (Figure 2).

In the 1981 study, the egg production declined throughout the test period (Figure 5), but that decline was probably seasonal in nature. During that year, no significant differences in egg production were observed during the treatment or post-treatment periods. However, two significant differences were found during the pre-treatment period. The egg production for the 1/5 LD₅₀ group was significantly greater than for either of the other

treatment groups on Day 5, and significantly greater than for the Control group on Day 8.

Mean brood production figures for pre-treatment, treatment, and post-treatment periods were calculated from brood measurements. In 1980, the mean post-treatment egg area was significantly greater for the 1/10 LD₅₀ group than for the Control group (Figure 2, bottom), but the mean pre-treatment larval+pupal and total brood areas were significantly smaller (bottom of Figures 3 and 4). In 1981, the figure for pre-treatment egg area was significantly greater for the 1/5 LD₅₀ group than that for the Control group (Figure 5, bottom). To determine if the effects of carbaryl on brood production were masked by those unequal pre-treatment brood levels, covariate analyses were performed using the pre-treatment values as covariates. Analyses indicated that no significant differences in brood production, other than those previously stated, occurred in either year.

The results from the brood mortality studies are shown in Figures 6-9. Egg survival (Figure 6) fluctuated considerably during the course of the study, but no significant differences ($P \leq 0.05$) were indicated by either analysis of variance or analysis of covariance. Mean egg survival figures, calculated for pre-treatment, treatment,

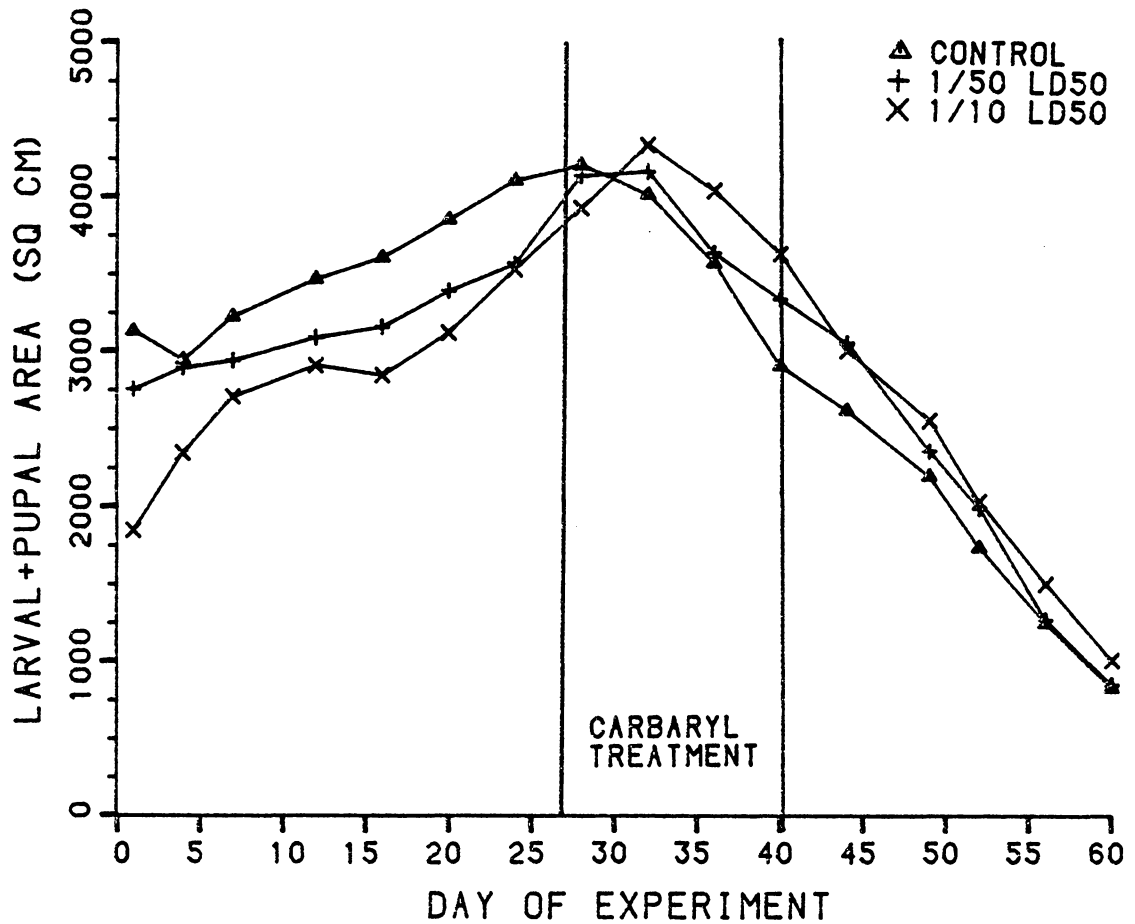


Mean Egg Area (sq cm)

<u>Dosage</u>	<u>Pre-treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	686.4a	594.4a	301.8a
1/50 LD ₅₀	587.3a	609.1a	408.5ab
1/10 LD ₅₀	546.7a	796.6a	477.3b

Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Figure 2: Egg Production--1980.

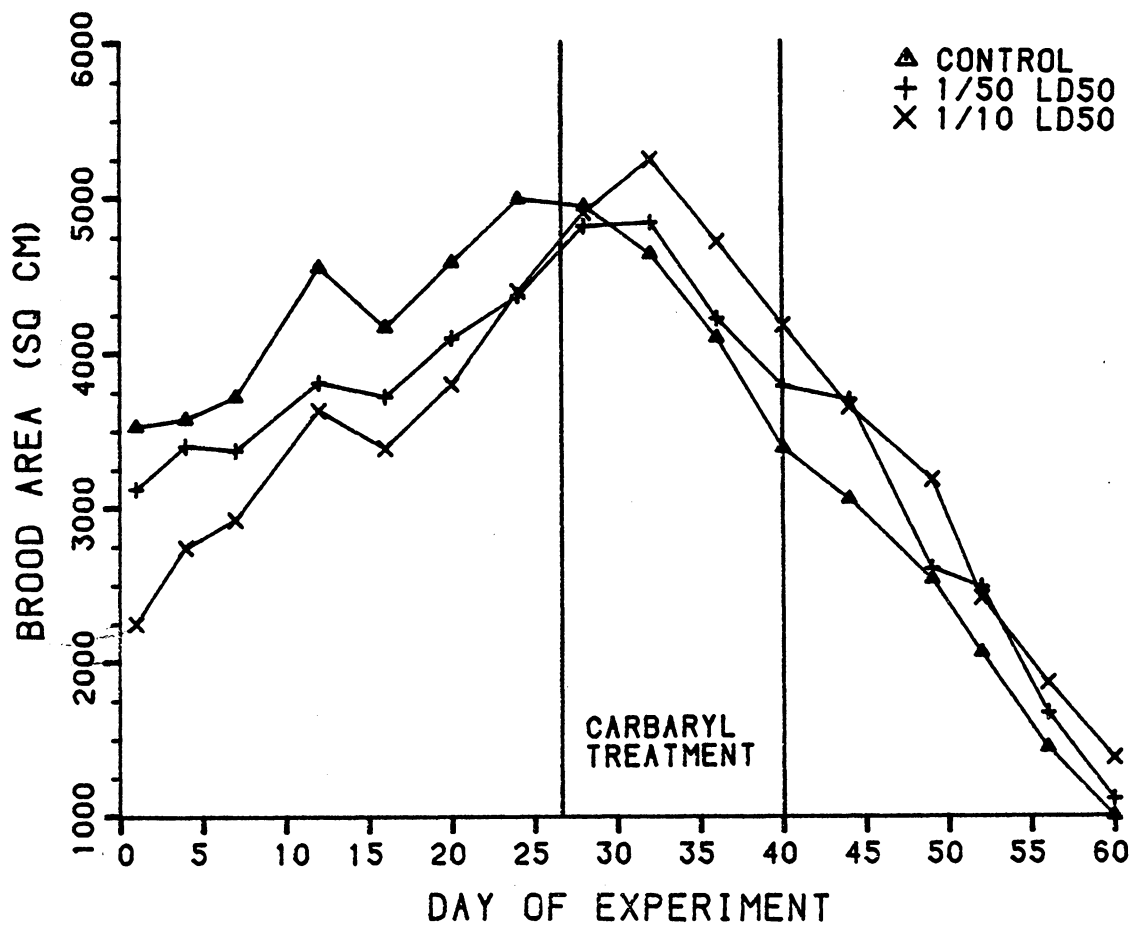


Mean Larval+Pupal Area (sq cm)

<u>Dosage</u>	<u>Pre-Treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	3478.6a	3677.4a	1804.0a
1/50 LD ₅₀	3115.9ab	3854.8a	1906.6a
1/10 LD ₅₀	2760.5b	3982.7a	2022.9a

Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Figure 3: Combined Larval and Pupal Production--1980

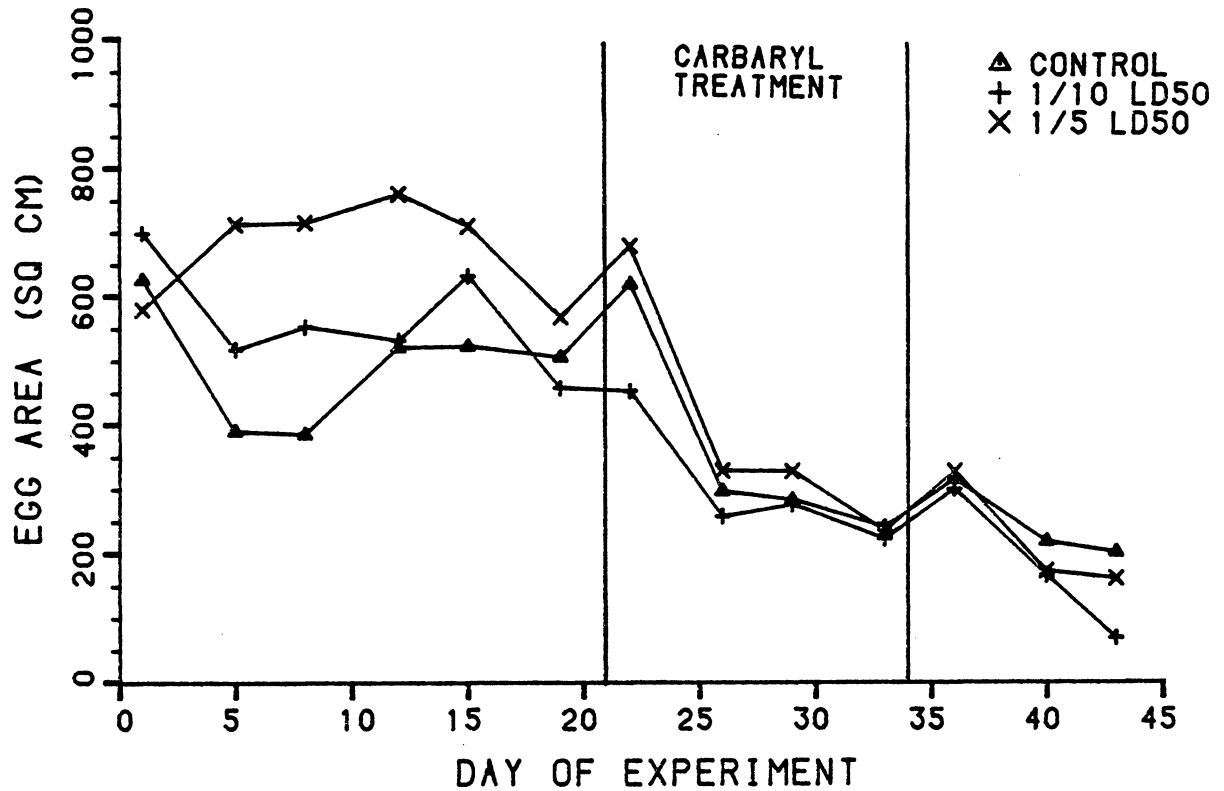


Mean Brood Area (sq cm)

<u>Dosage</u>	<u>Pre-Treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	4165.0a	4271.7a	2105.8a
1/50 LD ₅₀	3703.2ab	4463.9a	2315.0a
1/10 LD ₅₀	3307.2b	4779.2a	2500.2a

Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Figure 4: Total Brood Production--1980



Mean Egg Area (sq cm)

<u>Dosage</u>	<u>Pre-Treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	491.7a	360.2a	245.2a
1/10 LD ₅₀	562.4ab	306.1a	178.5a
1/5 LD ₅₀	679.7b	392.6a	220.3a

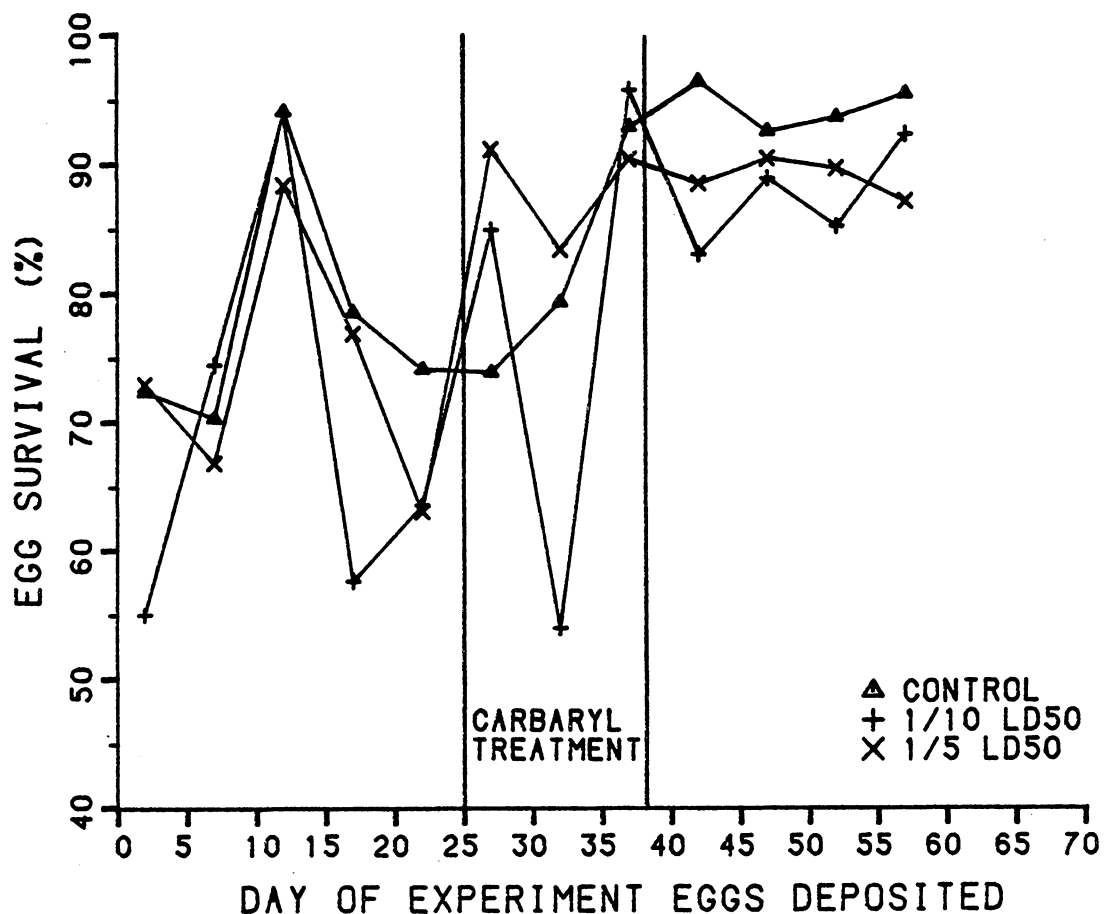
Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Figure 5: Egg Production--1981

and post-treatment periods, indicated one significant difference. Mean post-treatment egg survival was significantly lower for the 1/10 LD₅₀ group than for the Control group (Figure 6, bottom). A general increase in egg survival was observed throughout the study, but the increasing proficiency of the researcher probably accounted for that trend.

Larval and capped brood survivals are presented in Figures 7 and 8, respectively. For these brood stages, the survival fluctuations were reduced considerably from those noted for the egg-stage brood (Figure 6). Neither analysis of variance nor analysis of covariance indicated any significant differences in either larval or capped brood survival, but a trend was visible in Figure 7. There, larval survival throughout the study appeared to be considerably less for the 1/10 LD₅₀ group than for the other treatment groups.

The mean survival figures for larvae and capped brood indicated few significant differences. The mean larval survival for the 1/10 LD₅₀ group was significantly lower during both treatment and post-treatment periods than for the other treatment groups (Figure 7, bottom). Mean survival figures for capped brood indicated no significant differences (Figure 8, bottom). A general decrease in

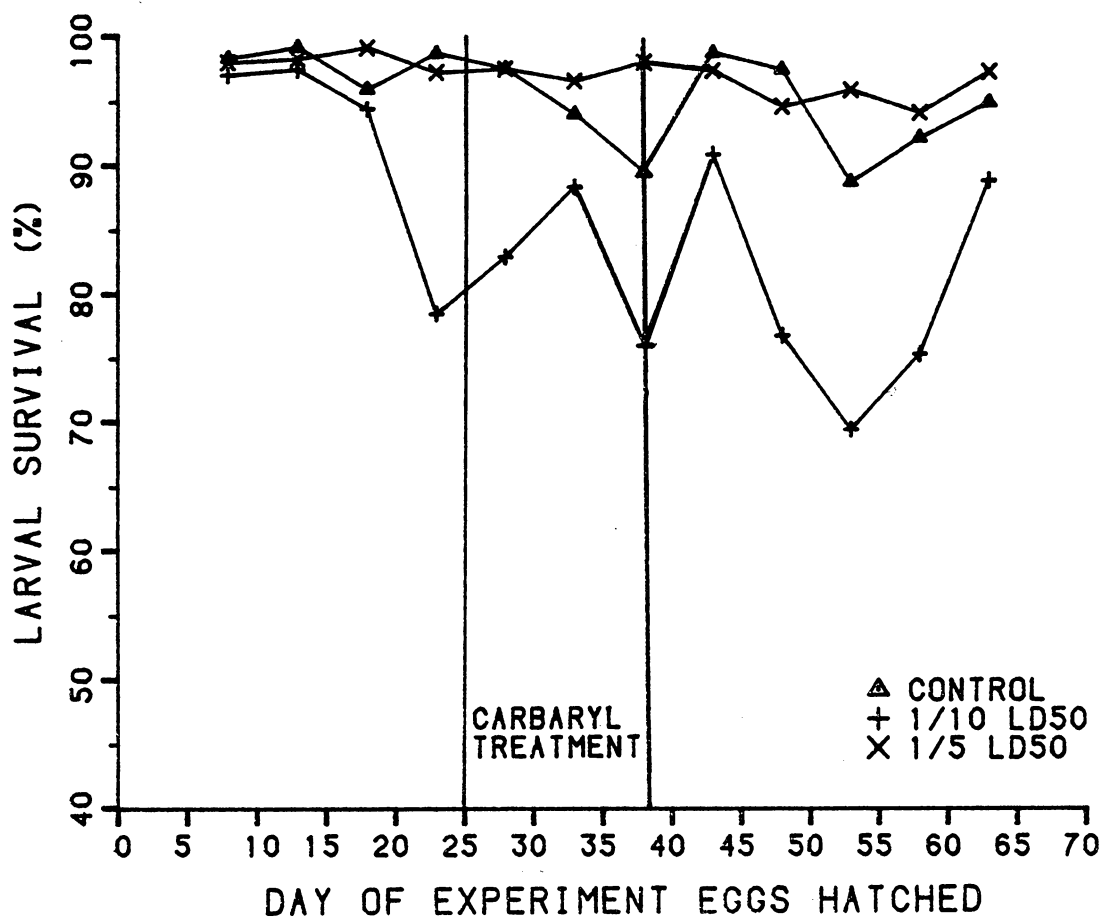


Mean Egg Survival (%)

<u>Dosage</u>	<u>Pre-treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	77.5a	82.3a	94.6a
1/10 LD ₅₀	67.6a	77.7a	87.4b
1/5 LD ₅₀	73.4a	88.3a	89.0ab

Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Figure 6: Egg Survival

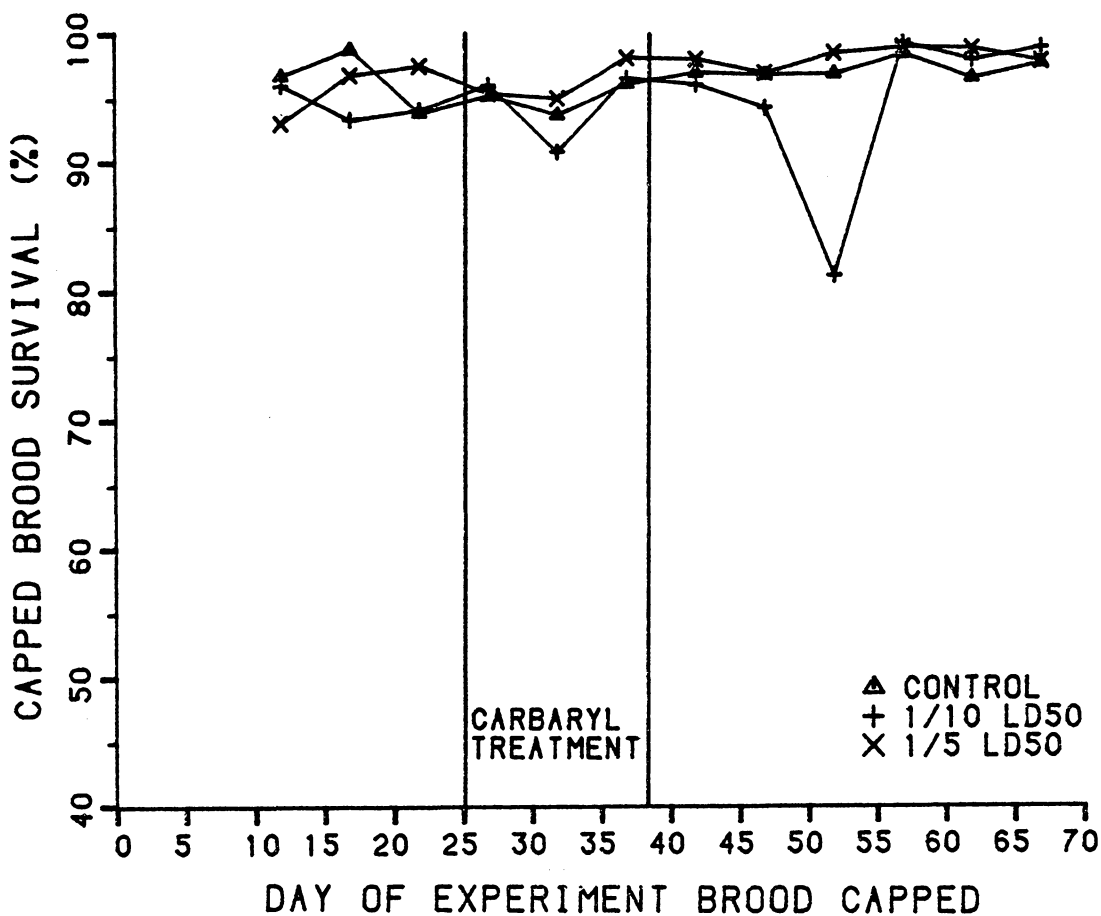


Mean Larval Survival (%)

<u>Dosage</u>	<u>Pre-treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	98.2a	94.0a	94.5a
1/10 LD ₅₀	91.4a	82.8b	79.9b
1/5 LD ₅₀	98.3a	97.5a	96.0a

Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Figure 7: Larval Survival

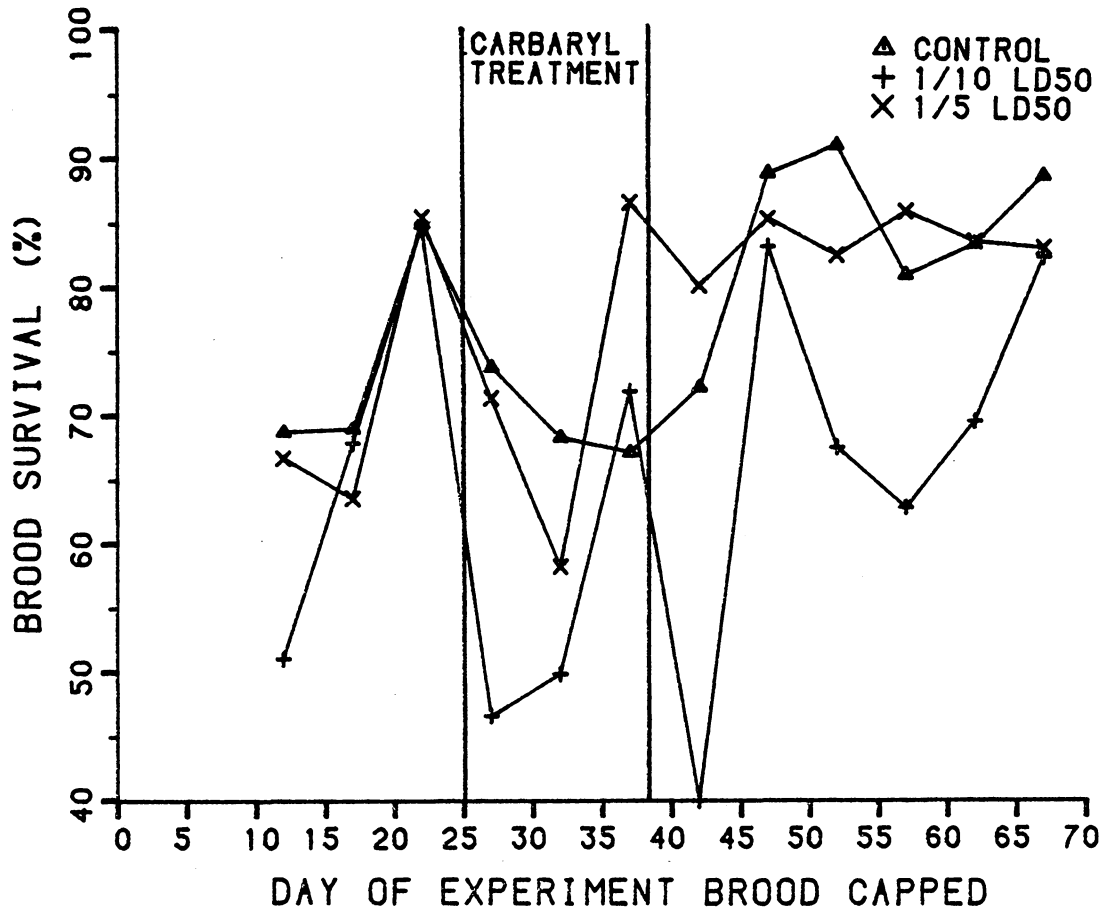


Mean Capped Brood Survival (%)

<u>Dosage</u>	<u>Pre-treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	96.7a	95.0a	97.2a
1/10 LD ₅₀	94.5a	94.5a	94.5a
1/5 LD ₅₀	95.8a	96.0a	98.1a

Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Figure 8: Capped Brood Survival



Mean Brood Survival (%)

<u>Dosage</u>	<u>Pre-treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	73.9a	69.8a	84.6a
1/10 LD ₅₀	66.8a	57.0a	67.1b
1/5 LD ₅₀	72.0a	70.1a	83.5a

Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Figure 9: Total Brood Survival

larval survival was observed throughout the study, but an increase was noted for the capped brood survival.

Survival data for the complete development of honey bee brood are shown in Figure 9. Fluctuations were again observed, probably due to the fluctuations in egg survival (Figure 6). A significant difference in survival was noted on only one date. On Day 42 (post-treatment), the 1/10 LD₅₀ group had a significantly lower brood survival than either of the other treatment groups. Analysis of covariance, with pre-treatment colony means as covariates, indicated no other significant differences. Mean survival figures (Figure 9, bottom) indicated that post-treatment survival was significantly lower for the 1/10 LD₅₀ group than for the other groups. A general increase in survival was observed during the pre-treatment and post-treatment periods, but a decrease was observed during the treatment period.

Gary dead bee traps were utilized to measure adult mortality in 1980 and 1981, and trap accuracy was measured during both years. The mean values are presented in Table 1. Trap accuracy was greater in 1980 than in 1981, and the standard deviation was smaller. During both years, the maximum accuracy observed was 100%, but the minimum accuracy varied from 60% (1981) to 70% (1980).

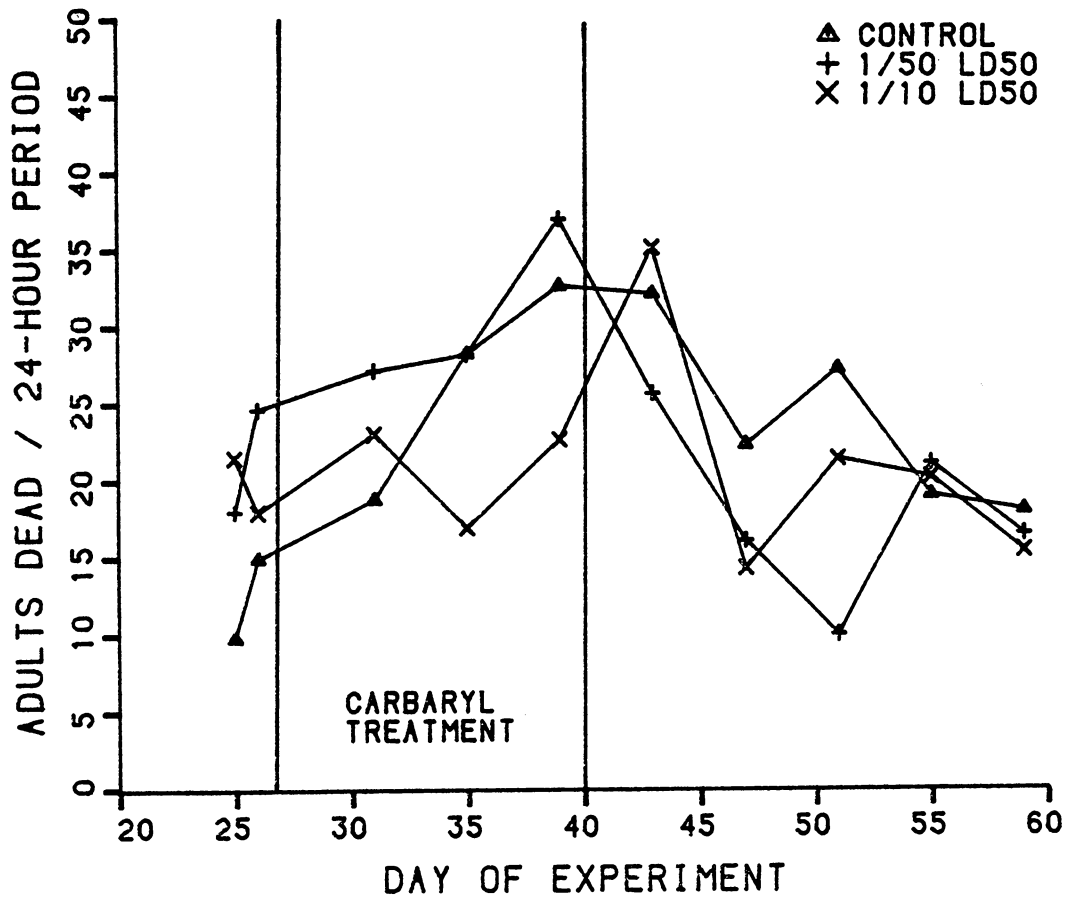
TABLE 1
Accuracy of Gary Dead Bee Traps

<u>Year</u>	<u>N</u>	<u>Mean</u>	<u>Standard Deviation</u>	<u>Minimum Value</u>	<u>Maximum Value</u>
1980	90	95.7%	7.0%	70.0%	100.0%
1981	107	93.9	8.5	60.0	100.0
Total	197	94.7	7.9	60.0	100.0

The observed adult mortalities were corrected for trap accuracy, and the results are shown in Figures 10 and 11 for 1980 and 1981, respectively. In 1981, higher adult mortalities were observed than in 1980, and the uniformity of the data was greater. Neither analysis of variance nor analysis of covariance showed significant differences ($P \leq 0.05$) in adult mortality for either year. In Figure 11, the adult mortality on Day 41 (post-treatment) is considerably greater for the 1/5 LD₅₀ group than for the other treatment groups. However, that greater mortality was caused by a single observation of 1145 dead adults in one 1/5 LD₅₀ colony, which was probably not related to treatment effects.

Mean adult mortality figures, calculated for the pre-treatment, treatment, and post-treatment periods, also showed no significant differences in adult mortality (bottom of Figures 10 and 11). In 1980, a general increase in mortality was observed during the treatment period, but the opposite occurred in 1981.

The colony weight changes based on original weights are presented in Figure 12. Mean colony weights at the start of the study were 21.43 kg, 20.80 kg, and 20.56 kg for the Control, 1/10 LD₅₀, and 1/5 LD₅₀ treatments, respectively. Weight changes calculated on that basis showed greater

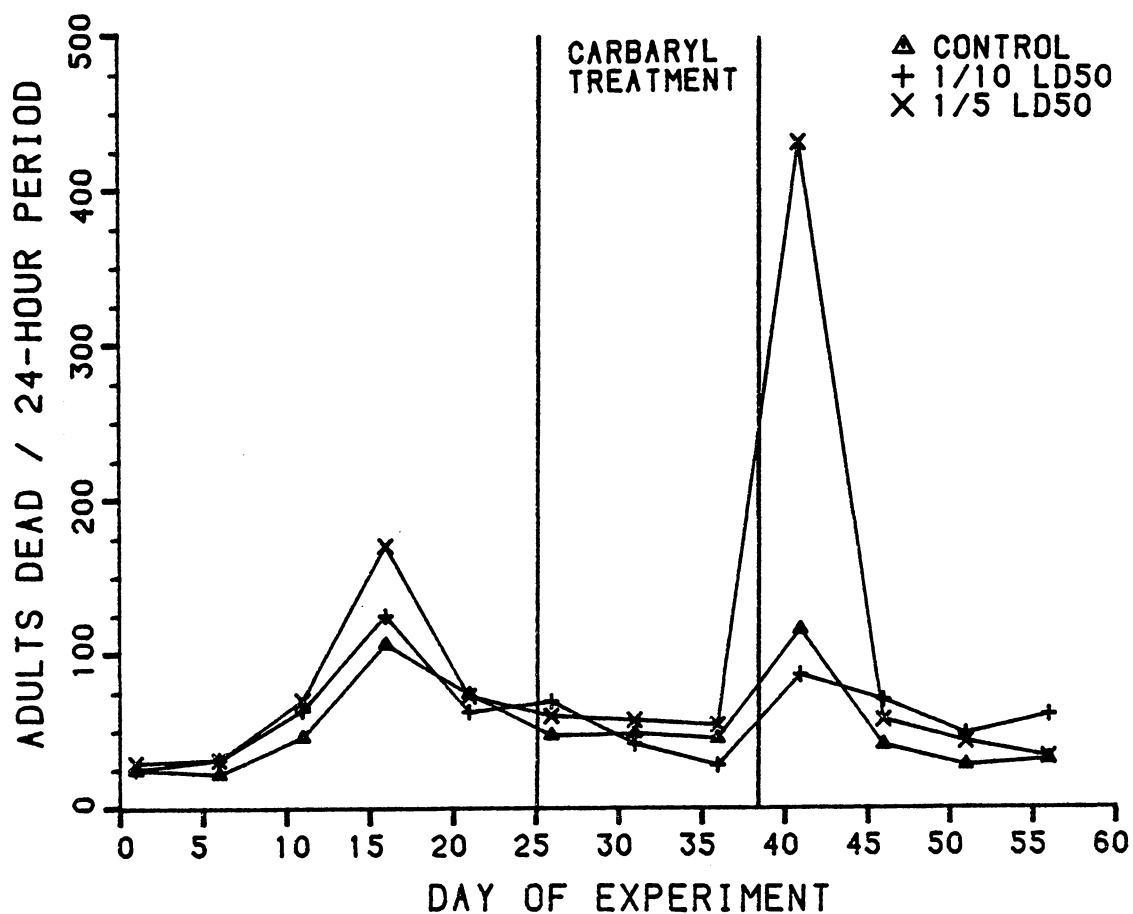


Mean Dead Adult Bees/24 Hr

<u>Dosage</u>	<u>Pre-treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	9.8a	23.7a	23.8a
1/50 LD ₅₀	18.0a	29.3a	17.9a
1/10 LD ₅₀	21.5a	20.2a	21.3a

Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Figure 10: Adult Mortality--1980



Mean Dead Adult Bees/24 Hr

<u>Dosage</u>	<u>Pre-treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	54.8a	47.1a	53.8a
1/10 LD ₅₀	61.4a	46.2a	66.3a
1/5 LD ₅₀	74.8a	57.0a	140.8a

Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Figure 11: Adult Mortality--1981

uniformity than those calculated on the basis of change per five-day period (Figure 13). Mean colony weights were not significantly different ($P \leq 0.05$) at the start of the study, and only one significant difference was observed during the course of the study. For both calculation bases, analysis of variance indicated that the $1/5 LD_{50}$ group showed a significantly greater weight increase on Day 6 than either of the other treatment groups.

Mean weight changes were calculated for pre-treatment, treatment, and post-treatment periods, but no significant differences were observed (Table 2). Mean weight change figures did indicate that all treatment groups lost weight during the pre-treatment and post-treatment periods, but gained weight during the treatment period.

The results of the carbaryl resistance studies are shown in Figures 14, 15, and 16 for the Control, $1/10 LD_{50}$, and $1/5 LD_{50}$ treatments, respectively. For all treatments, calculated Dosage-Mortality curves varied considerably between test dates. Significant differences ($P \leq 0.05$) in LD_{50} values occurred between test dates for all treatments, but the dates of occurrence were not uniform. As indicated in Figures 14 and 16 (bottom), significantly different LD_{50} values occurred during the fourth test (Day 96) for the Control and $1/5 LD_{50}$ groups, respectively. In both cases,

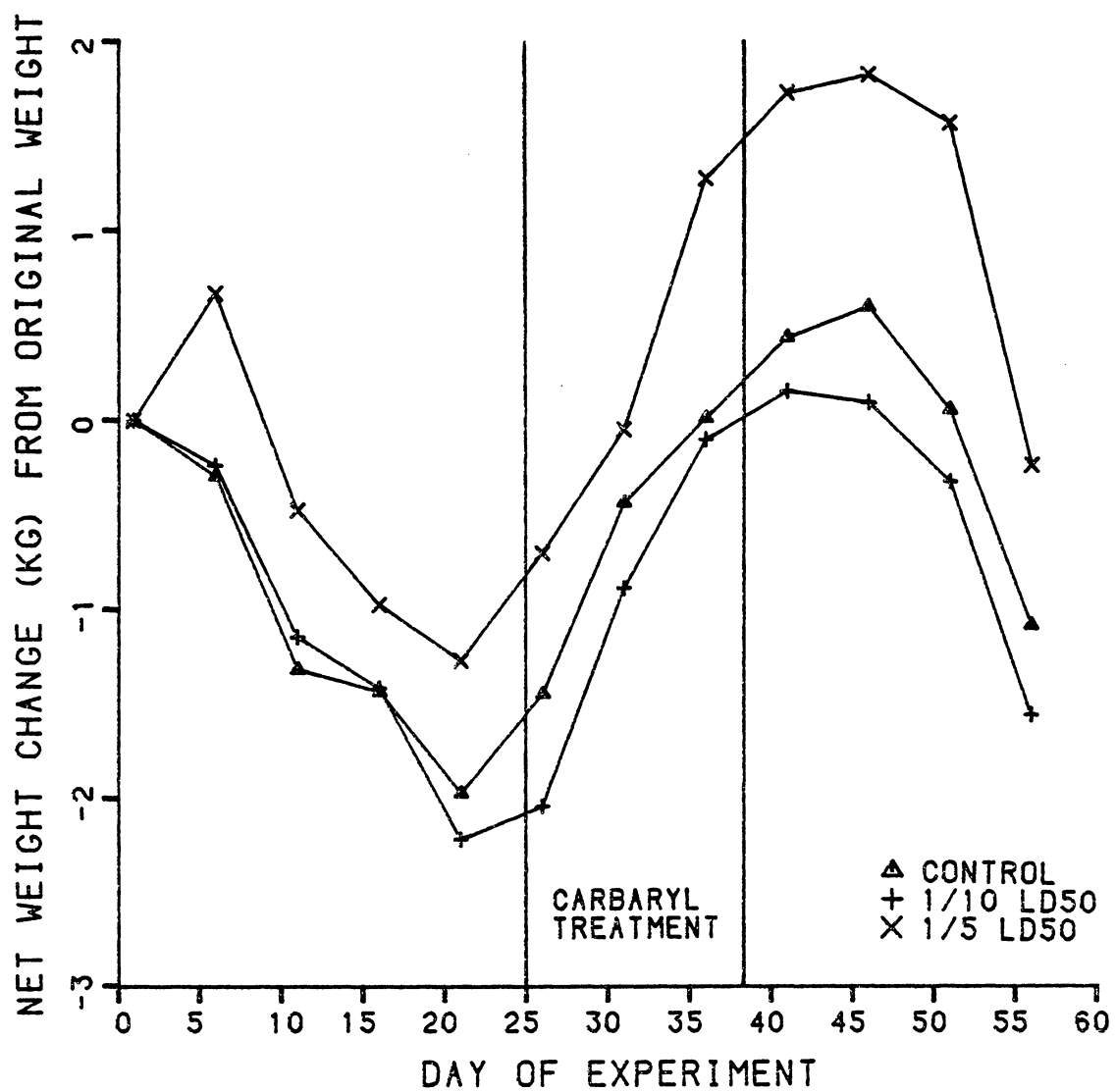


Figure 12: Net Colony Weight Change

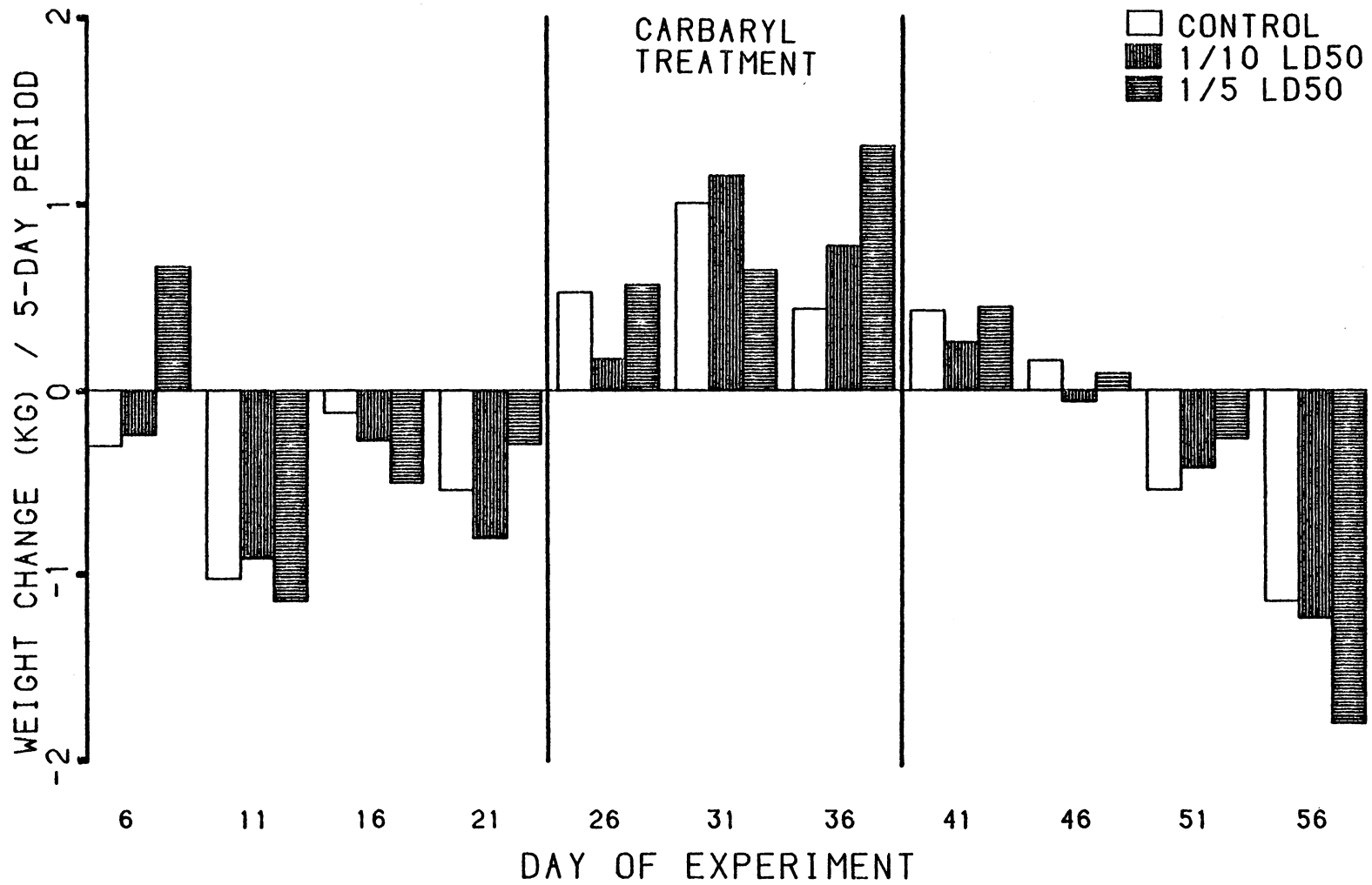


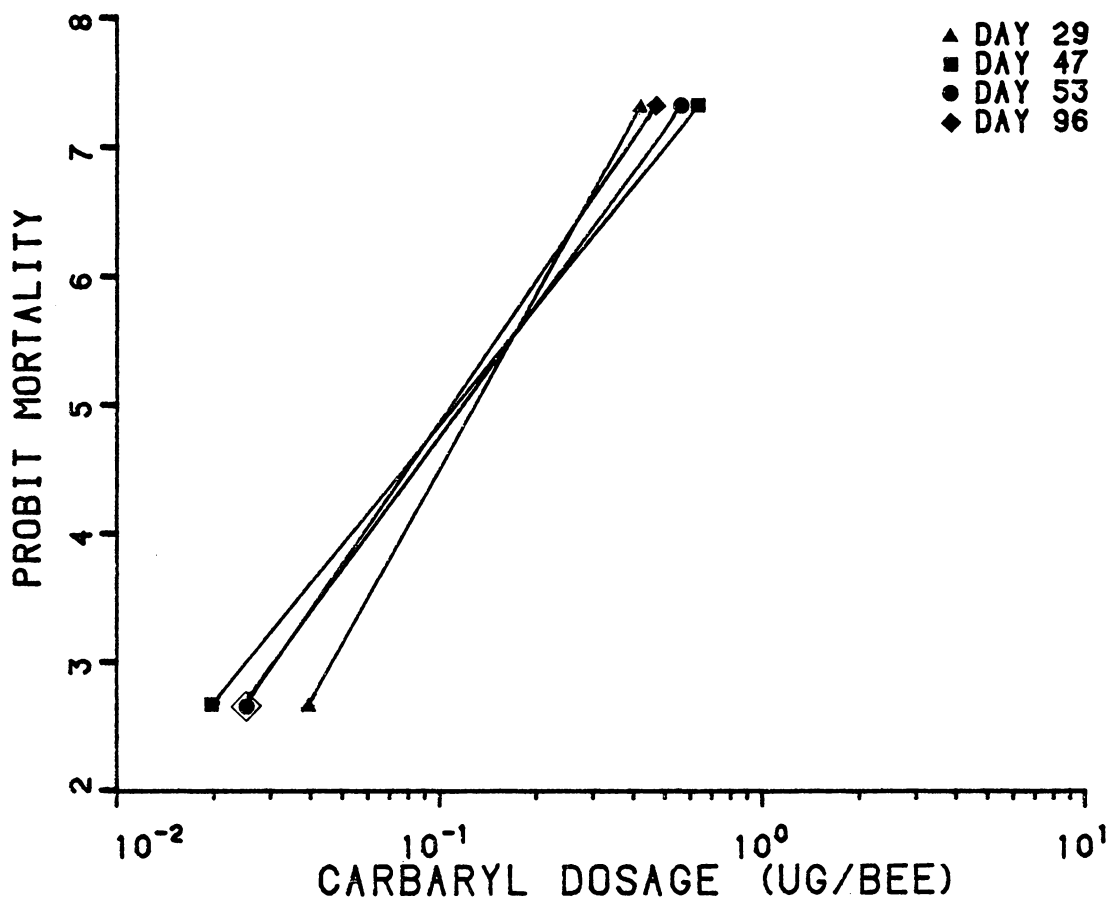
Figure 13: Colony Weight Change/5-Day Period

TABLE 2
Mean Colony Weight Change

<u>Dosage</u>	<u>Mean Change (kg)</u>		
	<u>Pre-treatment</u>	<u>Treatment</u>	<u>Post-Treatment</u>
Control	-1.97a	+1.98a	-1.10a
1/10 LD ₅₀	-2.22a	+2.12a	-1.46a
1/5 LD ₅₀	-1.27a	+2.54a	-1.51a

Column means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

the LD₅₀ values were reduced. The 1/10 LD₅₀ group showed a significantly different LD₅₀ value in the third test (Day 53), but in this case, the LD₅₀ value was increased (Figure 15). In the fourth test (Day 96) of that group, the LD₅₀ value returned to the previous level. No significant differences in Dosage-Mortality curve slopes were observed in any treatment.

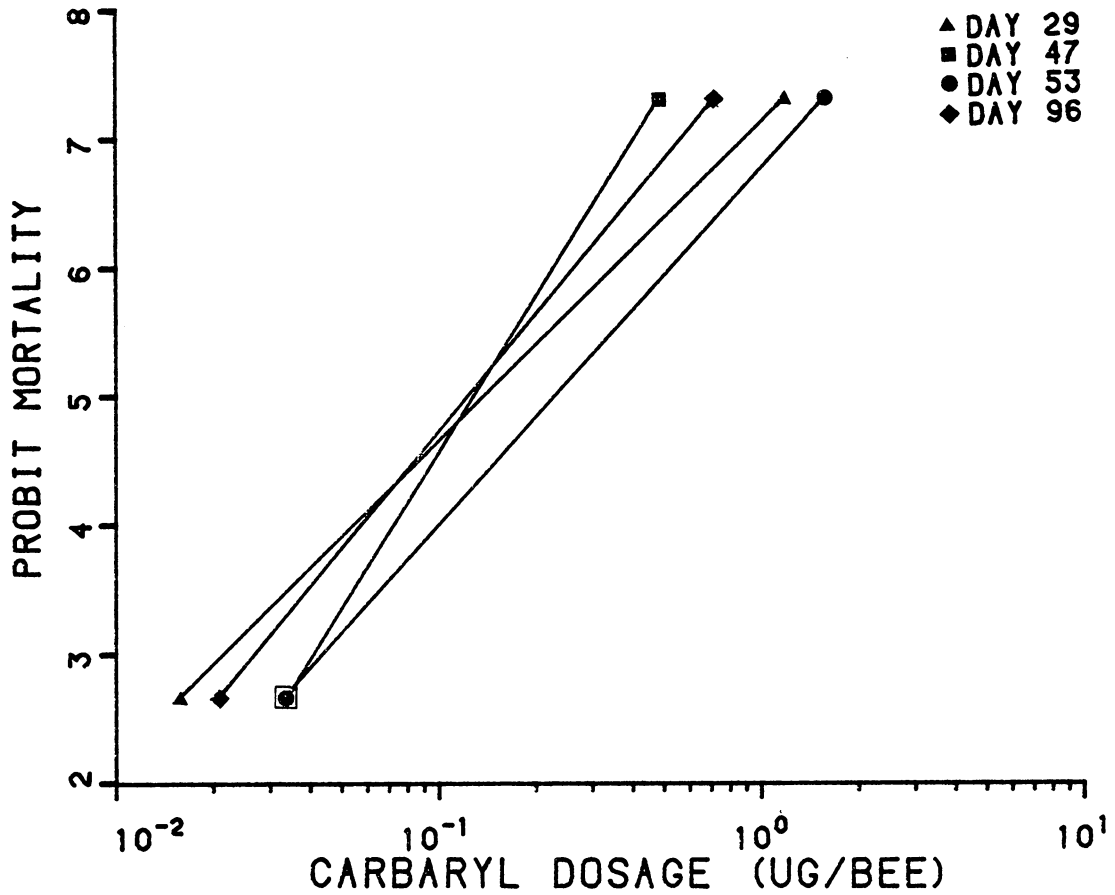


Carbaryl Toxicity

<u>Test</u>	<u>LD₅₀ (ug/bee)</u>	<u>Slope</u>
Day 29	0.128a	4.519a
Day 47	0.111ab	3.084a
Day 53	0.117ab	3.419a
Day 96	0.108b	3.662a

Column values followed by the same letter are not significantly different at the 5% level.

Figure 14: Contact Toxicity to Carbaryl--Control Group

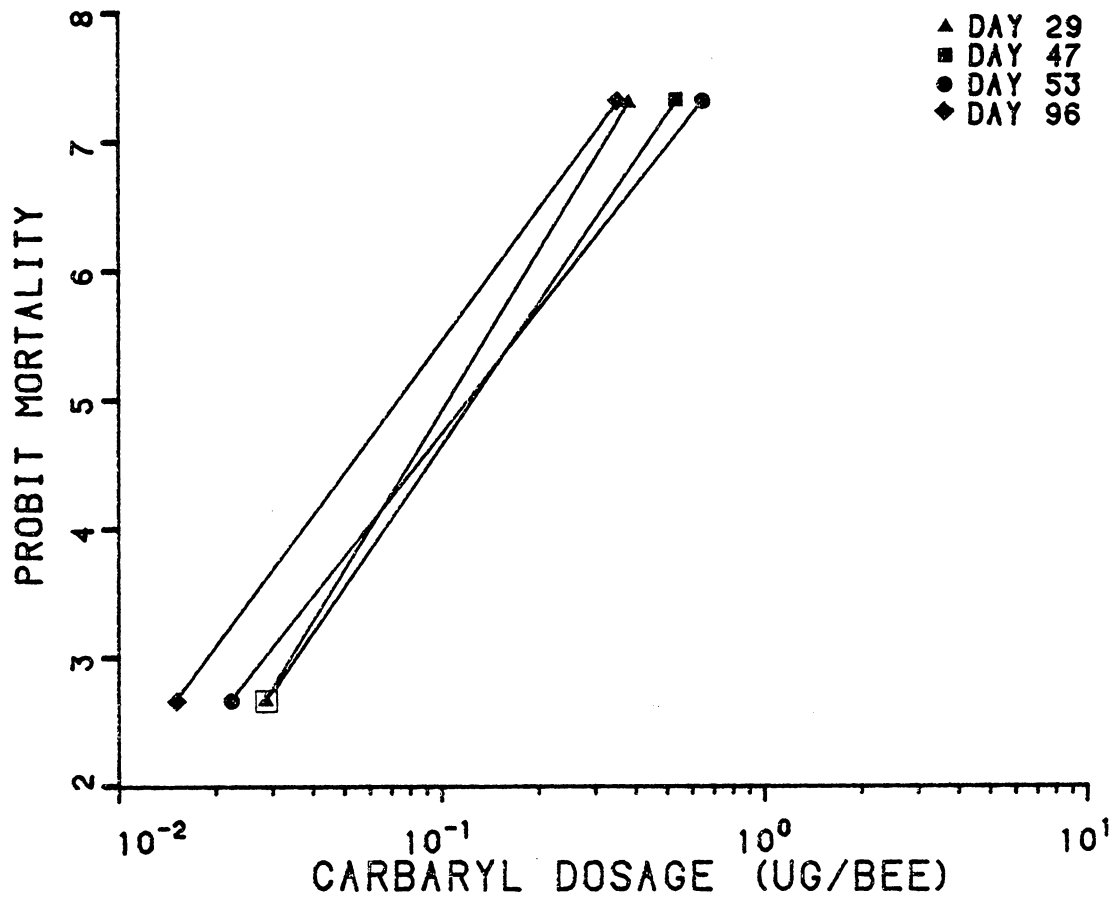


Carbaryl Toxicity

Test	LD_{50} (ug/bee)	Slope
Day 29	0.136a	2.479a
Day 47	0.127a	4.028a
Day 53	0.223b	2.801a
Day 96	0.121a	3.027a

Column values followed by the same letter are not significantly different at the 5% level.

Figure 15: Contact Toxicity to Carbaryl--1/10 LD_{50} Group



Carbaryl Toxicity

Test	LD ₅₀ (ug/bee)	Slope
Day 29	0.104a	4.107a
Day 47	0.124a	3.668a
Day 53	0.120a	3.180a
Day 96	0.073b	3.393a

Column values followed by the same letter are not significantly different at the 5% level.

Figure 16: Contact Toxicity to Carbaryl--1/5 LD₅₀ Group

Section V

DISCUSSION

The adult mortality studies in 1980 and 1981 indicate that the carbaryl dosages utilized in this project were below the lethal range for honey bees. No significant differences in adult mortality between treatments were found in either year, and no trends toward increased mortality in the carbaryl-fed colonies are evident in either Figure 10 or 11.

The carbaryl dosages were specifically selected to fall within the sublethal range for honey bees. In a similar manner, the feeding technique was designed to prevent any bee from consuming more than the calculated dosage. The highest concentration administered, 0.86 ppm carbaryl, was less than 50% of the maximum sublethal concentration (2.0 ppm carbaryl) utilized by Winterlin et al. (1973). Those researchers found no significant differences in adult mortality between Control colonies and colonies that were fed 2.0 ppm carbaryl (60% w/v sucrose solution) for 56 days.

Despite the published studies, the lack of significant differences in adult mortality was unexpected. As stated previously, the Oral Dosage-Mortality curve for carbaryl (Alvarez et al., 1970) indicated that a single feeding at

the 1/5 LD₅₀ level (0.0356 ug/bee, 25% sucrose solution) would cause approximately 7% adult mortality. The present research utilized a 14-day feeding at that level, but no significant increase occurred. Second, Winterlin et al. (1973) reported up to 60% mortality of caged honey bees after a 9 hr feeding of 60% (w/v) sucrose solution containing 0.5 ppm carbaryl. The higher carbaryl concentration (0.86 ppm) and the lower sucrose concentration (30% w/w) used in the present research should have increased the toxicity (Conner et al., 1978).

Several possibilities could account for the lack of observable differences in adult mortality between treatments. First, the house bees may have transferred the carbaryl-containing solutions to the combs very rapidly, allowing insufficient time in the honey stomach for a lethal dosage of the insecticide to be absorbed. Additionally, if the honeyflow was sufficiently strong, the carbaryl-contaminated honey might have become diluted or gone unused during the remainder of the experimental period. Rinderer and Baxter (1978) observed that caged honey bees will store up to 0.188 ml of sucrose solution/bee/day if storage space is available. Based on a honey-stomach load of 40 ul (Combs, 1972), that rate requires fewer than five collection trips/bee/day. Second, the honey bees utilized in this

research might have been more tolerant or resistant to carbaryl than those used in the cited publications. Lastly, intra-treatment colony variation may have been great enough to mask inter-treatment effects of the insecticide. Each of these possibilities is discussed in the following sections.

No significant differences in inter-treatment colony weight changes were found during or following the carbaryl administration period, but a trend is suggested. All colonies were essentially equal (in bees, brood, and weight) before the study began, but the colonies fed the highest dosage of carbaryl seemed to be the most productive (least non-productive) during the study (Figure 12). Although the $1/5$ LD₅₀ group had the greatest post-treatment weight loss, it had the greatest weight gain during treatment (Table 2) and weighed the most at the conclusion of the study. However, since that group was also the most productive during the pre-treatment period (least weight loss), the productivity cannot be attributed to the carbaryl treatment.

The results of the weight study help to discount the idea of rapid storage and subsequent non-use of the carbaryl-contaminated solutions. During the study, all treatment groups had a net loss of weight, ranging from 0.24-1.56 kg, at a time of year when honey bee colony populations are usually expanding (Days 42-55 in Figures 12

and 13 correspond to Days 1-14 in Figures 2, 3, and 4). Additionally, bees often showed an active interest in robbing, indicating that natural food was in short supply. Therefore, it is probable that the carbaryl-contaminated solutions were concentrated during the nectar-ripening process, rather than diluted with natural nectar. Additionally, the net weight loss of all treatment groups indicated that stored foods within the test colonies were consumed during the test period.

Winterlin et al. (1973) state that carbaryl is quite stable in stored honey, and Solov'eva (1971) has reported a residual life in honey of 7-9 months. Since brood rearing is a major use of stored honey during the summer months, exposure of the brood to carbaryl in the treated colonies should have occurred.

As previously stated, pesticides have been shown to not only decrease honey bee brood production, but also brood survival (Morton and Moffett, 1972; Robinson and Johansen, 1978). In the present study, a significant difference in brood production during or following the period of carbaryl application was found to occur in only one instance. That occurrence was an increase in post-treatment egg production for the 1/10 LD₅₀ group on Day 49 (Figure 2). Figures 3 and 4 also show a slight trend of that treatment toward

increased larval+pupal and total brood production, respectively. In Figure 5, the 1/5 LD₅₀ group also shows a trend toward increased egg production, but that increase starts in the pre-treatment period.

Stimulation of brood production by carbaryl has not been demonstrated in honey bees, but it has been observed in another insect. Ball and Su (1979) found that topically-applied sublethal dosages of carbaryl significantly increased the oviposition of western corn rootworm females. However, carbaryl was found to have no effect on the fecundity of a Hemipteran (Walker and Turnipseed, 1976), and a detrimental effect on the fecundity of the migratory grasshopper (Kreasky and Mazuranich, 1971).

Survival figures obtained in the brood mortality studies were similar to those obtained by Garofalo (1977) for comparably-sized hives, although the figure for larval survival was higher than that reported. Significant differences in survival between treatments were not found in data calculated on a daily basis. However, the mean values calculated over the treatment periods indicate that carbaryl exposure at the 1/10 LD₅₀ level caused a significant decrease in larval survival during the treatment period, and in egg, larval, and total brood survivals during the post-treatment period. Also, from the graphs in Figures 6, 8, 9,

and particularly Figure 7, a trend toward reduced egg, capped brood, total brood, and larval survivals, respectively, can be seen for the 1/10 LD₅₀ treatment. However, because these reductions did not occur at the higher dosage rate (1/5 LD₅₀), they may have been due to colony variation, rather than to treatment effects.

The nature of the honey bee microsomal oxidase system could be responsible for the apparent susceptibility of honey bee brood to carbaryl dosages that do not significantly affect honey bee adults. Gilbert and Wilkinson (1974) demonstrated that worker adults and drone larvae have an efficient microsomal oxidase system. This system remains active in in vitro homogenates of drone larvae, but activity is lost in in vitro homogenates of worker adults. In 1975, Gilbert and Wilkinson isolated a microsomal oxidation inhibitor from gut-tissue homogenates of worker and drone adults, and whole-body homogenates of worker and drone larvae. The inhibitor isolated from the worker gut tissue was observed to be high in potency. When released through tissue breakdown, this inhibitor is responsible for the loss of microsomal oxidase activity in worker adult homogenates. In a similar manner, microsomal oxidase activity is retained in the drone larvae homogenates because of the low potency of the inhibitor found there.

However, a different situation was observed in the worker larvae. The microsomal oxidation inhibitor isolated from worker larvae was found to be low in potency, and yet, worker larvae homogenates show no microsomal oxidase activity. Therefore, either microsomal oxidase activity in worker larvae homogenates is inhibited by other factors, or worker larvae lack an efficient microsomal oxidase system. If the latter were true, worker larvae would have a greater susceptibility to toxicants than worker adults.

The honey bees used in this project were not found to have a high resistance or tolerance to carbaryl. The pre-treatment topical LD₅₀ (dorsal thorax, 24 hr, 27°C) averaged 0.122 ug carbaryl/bee. This value was greater than the LD₅₀ values reported for newly emerged bees, but less than those reported for bees of mixed ages (Tucker, 1980). Graves and Mackenson (1965) reported a higher LD₅₀ (0.78 ug/bee) for bees of comparable age.

Tucker (1980) reported a 40% increase in vigor tolerance to carbaryl in newly-emerged worker bees following nine generations of selective breeding. In the present research, a 73% increase in LD₅₀ was obtained in the third test (Day 53) of the 1/10 LD₅₀ group, but the LD₅₀ value returned to normal in the fourth test (Day 96). On the other hand, a 39% decrease in LD₅₀ was obtained in the

fourth test (Day 96) of the $1/5$ LD₅₀ group. The Control group also showed variability in the LD₅₀, with a 16% decrease between test one (Day 29) and test four (Day 96). Variations of this nature are not unusual and have been found to occur for several other insecticides (Tahori et al., 1969). Generally, increases of less than 500% are not considered to be biologically significant(5).

The results of this research showed that application of carbaryl to honey bee colonies at the indicated dosages had no biologically-significant effects on brood production, brood mortality, adult mortality, colony weight change, or development of resistance. Statistically-significant results were observed in some cases, but the variability of those results precluded the formation of definitive conclusions.

The results of this research indicate that a major obstacle to this type of study is the great variation between honey bee colonies. In 1981, honey bee colonies were equalized before the brood production study began, yet, within two weeks, the $1/5$ LD₅₀ group was producing significantly more eggs than the other treatment groups. While the use of sister queens may have reduced intra-

5 Personal communication, Dr. D. G. Cochran, Professor, Dept. of Entomology, VPI & SU.

treatment variation in the earlier studies, the reduction often was not sufficient to bring out treatment effects, even where a trend was quite obvious (Figure 7).

In 1980, the Environmental Protection Agency(6) invited grant requests for "research on the effects of exposure of honey bees to selected insecticides." One of the stated objectives of the research was:

Determination of the low level effects of insecticides (e.g., Carbaryl) on the life stages of honey bees (chronic toxicity, behavior modifications, reproductive effects, biological, physical, and physiological modifications).

Protocol for the research called for a 60-day study period and not less than five honey bee colonies per treatment level.

The variability inherent in honey bee colonies makes their use in the proposed research impractical. Treatment effects may be effectively masked by intra-treatment colony variation, especially when those effects are minor. Efforts to reduce intra-treatment variation, through the equalization of test colony populations and brood areas and the use of sister queens, seemed to have little effect. The variation also could not be removed statistically, through the use of covariate analysis. Use of a larger sample size

6 Dr. Norman R. Glass, Nov. 10, 1980, Commerce Business Daily, Dept. of Commerce, U.S.A.

may effectively reduce this variation, but the time and effort required would make such a study unmanageable, and the associated costs would be prohibitive. This would particularly be true when large numbers of compounds require rapid screening.

Section VI

CONCLUSIONS

1. Based on adult mortality, carbaryl dosages approximating 0.0036, 0.0178, and 0.0356 ug/bee/day were found to fall within the sublethal range when fed to honey bee colonies during a 14-day period. Those treatments were based on daily doses of 1/50, 1/10, and 1/5 of the oral LD₅₀ of carbaryl for honey bees (0.178 ug/bee by Alvarez et al., 1970). The carbaryl was fed in a 0.95 M (30% w/w) sucrose solution, and the carbaryl concentrations were approximately 0.09, 0.44, and 0.86 ppm, respectively.
2. Carbaryl applications at the 1/10 LD₅₀ level caused statistically-significant (P≤0.05) increases in brood production, brood mortality, and worker resistance to carbaryl. These increases were not felt to be biologically significant, however, because of the considerable intra-treatment colony variation.
3. Carbaryl applications at the 1/50 and 1/5 LD₅₀ levels had no significant effects (statistical or biological) on brood production or brood mortality (1/5 LD₅₀ only). A statistically-significant decrease in worker resistance to carbaryl was

observed for the $1/5$ LD_{50} treatment, but that decrease also occurred for the Control treatment.

4. Carbaryl applications at the $1/10$ and $1/5$ LD_{50} levels had no significant effect on colony weight when determined on the bases of net change from original weight or change/5-day period.
5. Variation between honey bee colonies was found to limit their usefulness in this type of research. This variation was not removed through equalization of the test colony populations or brood areas, or through the use of sister queens.

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SUBLETHAL DOSAGE EFFECTS OF CARBARYL
ON
HONEY BEE (Apis mellifera (L.)) COLONIES
by
Chapman Kemper Loyd
(ABSTRACT)

The sublethal dosage effects of carbaryl were studied in honey bee (Apis mellifera L.) colonies. Carbaryl was fed to colonies during a 14-day period in a 0.95 M sucrose solution. Carbaryl dosages were based on 0.0036, 0.0178, and 0.0356 ug/bee/day, and concentrations approximated 0.09, 0.44, and 0.86 ppm, respectively. Five potential areas of sublethal effect were examined: brood production, brood mortality, adult mortality, colony weight change, and worker resistance to the toxicant.

The carbaryl dosages appeared to fall within the sublethal range. No significant differences ($P \leq 0.05$) in adult mortality were found between Control and carbaryl treatments during or following carbaryl application. Statistically-significant increases in brood production and brood mortality were observed at the median dosage, but no significant differences were found at the highest dosage. The lowest dosage also caused no significant differences in

brood production. A significant increase in worker resistance to carbaryl was observed at the median dosage, but a significant decrease occurred at the highest dosage and the Control. No significant differences in colony weight change were observed during the treatment or post-treatment periods at any dosage.

Although statistically-significant differences were observed, they were not felt to be biologically significant. Intra-treatment colony variation was considered to be partly responsible for the differences observed. This variation is great enough to make honey bee colonies unsuitable for general insecticide screening, although they may be useful when prior evidence of sublethal effects exists.