To the Graduate Council:

I am submitting herewith a thesis written by Timothy Edward McCown entitled "A Residue Study of Permethrin on Tomato Plants." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.

John Bloor, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

Thesis
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M.S.

A RESIDUE STUDY OF PERMETHRIN ON TOMATO PLANTS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Timothy Edward McCown

March 1980

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TO BECKY

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ABSTRACT

A study of permethrin residues on tomato plants was undertaken with the use of a gas chromatograph-mass spectrometer. Sixty tomato plants (CV. Walter) were arranged in a randomized complete block replicated four times. Applications evaluated were 0.0 lb per acre, o.1 lb per acre, and 0.2 lb per acre. Leaf samples were collected at various intervals over a two week period. After solvent partitioning and adsorption column cleanup procedures, the samples were analyzed by using a gas chromatograph-mass spectrometer operating in the selected ion monitoring mode.

The results of this study showed that permethrin residues underwent rapid decline during the first two to three days after application, declined at a much slower rate thereafter, and did not seem to be affected by the incidence of rain. This study indicated that increased spray concentrations would not greatly increase the persistence of the insecticide. Permethrin should pose little threat to field workers due to its rapid loss and its low toxicity to mammals. Is has an expected reentry time of approximately 24 hours.

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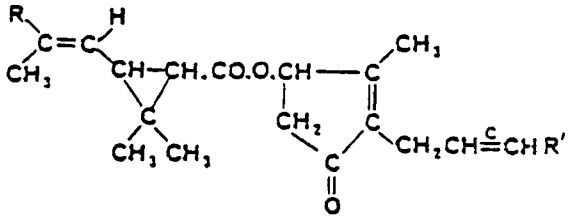
CHAPTER I

INTRODUCTION

A. Historical

In recent years the disadvantages of DDT and many other chlorinated insecticides have become increasingly evident. These circumstances have stimulated research in developing new insecticides which have sufficient insecticidal activity, low mammalian toxicity, and adequate stability.[1]

Pyrethrum, probably the oldest of organic insecticides, fills some of these requirements. Pyrethrum is a mixture of compounds extracted from the flower heads of various species of chrysanthemum. It was first sold in the early 1800's.[2] The compounds were extracted with acetone or methanel and impurities were removed with activated charcoal.[3] Pyrethrum's two main constituents are pyrethrin I and pyrethrin II, and consists of[4]



Pyrethrin I: $R=CH_3$ $R'=CH{=}{=}CH_2$

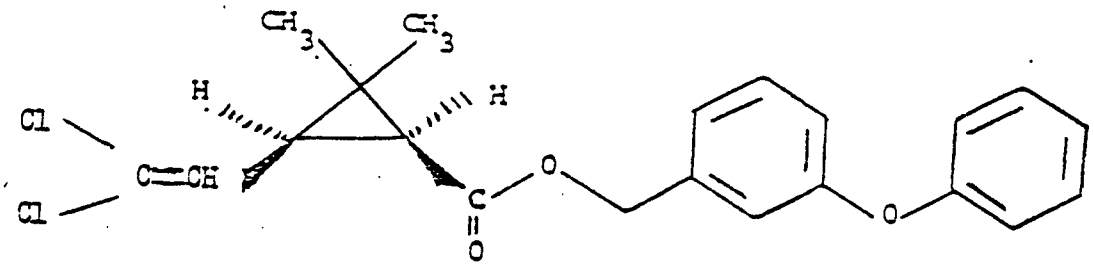
Pyrethrin II: $R=CH_3OCO$ $R'=CH{=}{=}CH_2$.

1

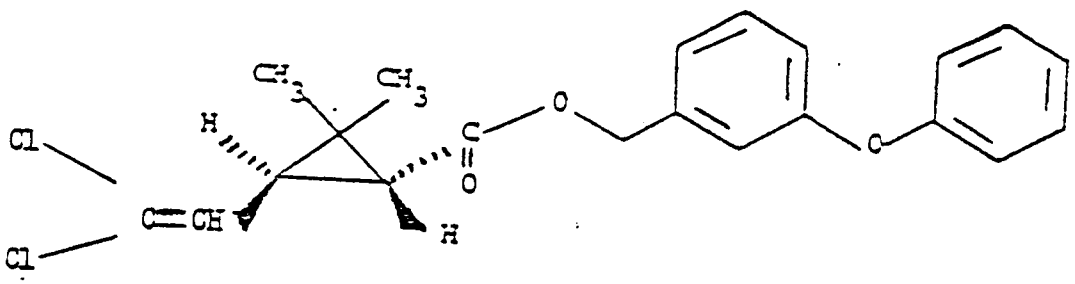
This natural insecticide is of value because, under normal conditions, it has low toxicity for mammals while being highly toxic to a wide

range of insects. These qualities make it useful in homes and barns. However, pyrethrum is rapidly biodegraded and photolabile, which leads to little opportunity for its use as a persisting agent.[5] Even though pyrethrum is not a persistent insecticide, it is in widespread use today.

In recent years, efforts have been made to develop similar synthetic compounds which incorporate pyrethrum's safety with stability comparable to the more dangerous insecticides.[1] These new man-made compounds are generally referred to as synthetic pyrethroids. One of the most promising of the new synthetic pyrethroids was synthesized by Elliott[6, 7] and is called 3-phenoxybenzyl (±)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate. The two structures, as shown below, are commonly called permethrin.



CIS PERMETHRIN



TRANS PERMETHRIN

B. Previous Work on Permethrin

Whenever a new potential product is developed, especially a chemical to be used in the environment, extensive testing must be performed. Studies involving permethrin have covered such topics as toxicity to plant pests,[8, 9, 10, 11] efficacy comparisons with other insecticides,[12, 13, 14] mode of insecticidal action,[15, 16] photostability,[7] selectivity,[17, 18] degradation in soil,[19] temperature influences,[20] synergism,[21, 22] metabolism,[23, 24, 25] and mammalian toxicity.[7] From these investigations permethrin was found to be a broad spectrum compound that compared favorably with other insecticide types for insect control. Studies have shown that mixtures of the cis and trans isomers of permethrin are repetitive and rapid in nerve stimulation, and that its killing power may be related to this stimulation.[15] Researchers have found that permethrin is more photostable than many of the other pyrethroids, and that its principle photoreactions involve trans/cis-isomerization and ester bond cleavage.[26] In one laboratory study, permethrin has been shown to act against an insect pest while not harming the beneficial predator.[18] However, further field studies need to be performed to substantiate this claim. Permethrin has the favorable characteristic of being degraded in soil very rapidly, with the trans isomer being degraded more rapidly than the cis isomer.[19] Permethrin has a negative temperature coefficient of toxicity which may have important practical applications in controlling insect pests that appear in cool weather and which are difficult to control with current insecticides.[20]

Toxicity of permethrin to mammals is approximately 500-2000 mg per kilogram (oral LD_{50} to rats).[27] A drawback of permethrin is its lack of useful systemic action which might aid in its effectiveness against leaf miners and many species of aphids.[28]

There have been a few studies on permethrin related to tomato plants. Four studies on permethrin have shown it to be effective against tomato fruitworm,[29] green peach aphid,[30] whiteflies,[31] and tomato pinworm[32] when applied to tomato plants. Permethrin provided effective insect control on tomato plants at low rates,[29, 30] and was found to produce little or no phytotoxicity.[31, 32]

C. Previous Permethrin Residue Studies

In the case of insecticides, it is important to gather information on the duration of the chemical in the area on which it is applied. Most of the routine instrumental methods used in residue studies today are in the category of chromatography, i.e. gas-liquid chromatography, thin layer chromatography, and high performance liquid chromatography.[33]

Several analytical procedures have been developed to determine the presence of permethrin in soil and various plant and animal materials.[34, 35, 36, 37] These procedures have been used in only a few actual field residue studies. In two studies by Zitko et al.,[38, 39] gas chromatography and gc-ms were used to detect permethrin levels in salmon and lobster to determine toxicity to these organisms. Zitko et al. found that permethrin was extremely lethal to salmon

and lobster, with the cis isomer being more lethal than the trans isomer.[39]

A study by Simonaitis et al.[40] investigated the persistence of permethrin on woolen products subjected to extended washing, dry cleaning, and other adverse conditions. The purpose of this study was to determine if permethrin was suitable for use in protecting woolen products from moth attack. Permethrin residues were determined by gas-liquid chromatography using flame ionization detection. Their findings indicated that permethrin demonstrated exceptional fastness to the fabrics.

Seven previous actual field studies have involved the use of cabbages, green peas, cotton plants, apple trees, pasture grass, celery, and asparagus. In the residue study by George et al.,[1] permethrin was applied on green peas and cabbage at a rate of 112 g AI per hectare. Quadruplicate samples of cabbage leaves were taken at 0 days, 1 day, and 7 days after application. After cleanup procedures, residues were analyzed by gas chromatography using an electron capture detector. Permethrin residues declined approximately 30% during day one, and 70% within 7 days. The same procedure was also used by George's[1] group on the vine and pod of green peas. They found a 30% decline in residue during the first day, 50% after 3 days, and 80% by 7 days. No residue was detected after 7 days. They increased sensitivity of the molecule to electron capture detection by derivatization of permethrin samples collected to add extra chlorine molecules to its structure. The samples were then

reanalyzed to corroborate the previous results. Halfhill's standard curve for the underivatized molecule ranged from 5 to 50 nanograms. A ten-fold greater sensitivity was achieved by using the two derivatized molecules.

Permethrin was applied to cotton foliage at a rate of 224 g AI per hectare by Ware et al.[41] Triplicate samples were collected by using a leaf punch of 25 mm diameter to cut out leaf discs. The samples were analyzed by the FMC corporation using an unspecified method. The results were a residue increase of 9% during the first day, a decrease of 25% by 2 days, a decrease of 30% by 3 days, and a decrease of 40% by 4 days.

In Hameed and Allen's investigation,[42] apple trees approximately two meters tall were sprayed with permethrin at concentrations of 0.05%. High concentrations were used to insure residue measurements up to 40 days. Surface residues of permethrin were removed by washing the whole apple with a solvent mixture. No cleanup procedures were used on the extracted samples, and quantitation was performed by gas-liquid chromatography using electron capture detection. Results were graphically represented by a plot of the log of ug of permethrin per square cm versus time in days. Error bars around each of the averages used in this graph indicated large deviations in values used to obtain the averages.

Applications of permethrin on pasture grass were used by Henzell and Lauren[43] to perform residue studies. Grass samples were collected during a two week period, and the permethrin residues were

analyzed by gas-liquid chromatography using an electron capture detector. Their work indicated permethrin had an effective field half-life of about five days, with rapid residue loss occurring the first two days followed by a slower loss over the next two weeks. They developed an equation to estimate residue levels occurring after the second day. The equation

$$R = R_o \exp(-0.145t) \quad (1)$$

where t is the number of days since application, R is the residue in mg/kg of grass at t days, and R_o is the original concentration calculated from the intercept value at day 0, may be used to calculate residue levels assuming first order decay.

In studies by Harris et al., celery[44] and asparagus[45] were sprayed with permethrin at the rate of 70 g and 140 g AI per hectare. Celery samples were collected at 7, 14, and 21 day intervals. Recovery of permethrin after cleanup procedures was 65%. They found some permethrin residue detectable after 21 days at about 5% of the original level for the 140 g AI per hectare samples and at about 15% of the original level for the 70% g AI per hectare samples. Asparagus samples were collected at 7 and 14 day intervals. Small amounts of permethrin were detected (about 2% for 140 g AI per hectare and 10% for 70 g AI per hectare) after 7 days, while none was detected after 14 days. In both of these studies permethrin residues were analyzed by gas-liquid chromatography using an electron capture detector.

D. Objective of This Investigation

In view of the lack of analytical residue information pertaining to permethrin on tomato plants, a field study using tomato plants was undertaken. The objectives of the residue study were to determine any possible decay trends, to provide information for future use in determining proper spraying intervals, and to provide information relating to possible residue hazard to field workers. The use of a gc-ms as the analyzing instrument was employed to investigate the advantages and any possible disadvantages associated with this system as compared to other gas chromatographic methods.

E. Reasons for the use of the Gas Chromatograph-Mass Spectrometer

In the last decade the use of mass spectrometry has had a phenomenal growth, especially in pesticide analysis. Mass spectrometry has been of great value in identifying compounds in complex mixtures.[46]

The standard methods of residue analysis today generally involve the use of a gas chromatograph equipped with a selective detector. These detectors, especially electron capture, are highly sensitive and are more than adequate to monitor compounds at low concentrations. However, their specificity always covers a whole group of compounds, which means greater care must be taken in cleanup procedures to eliminate substances which could exhibit similar retention times to the compound of interest. Any solvents used in cleanup procedures must be of high purity for the same reason.[47]

The gc-ms system offers the advantages of using the gas chromatograph for separating components, and the mass spectrometer as a highly sensitive detector which can be made almost substance specific to nearly any compound. This specificity helps eliminate the need for concern over extraneous compounds with retention times similar to the compound of interest.[47] The gc-ms system also offers two sets of data to be used in identifying the compound of interest, retention time and a mass spectrum of the compound. These advantages show significant reasons why this system should be used in the study of permethrin residue.

CHAPTER II

APPARATUS AND REAGENTS

A. Apparatus

1. The Gas Chromatograph-Mass Spectrometer

A Hewlett Packard Model 5980A Gas Chromatograph-Mass Spectrometer equipped with a 5934A Data System was used in the analysis of permethrin residue. This model is capable of chemical ionization, as well as electron ionization. However, only electron ionization was utilized in the study of permethrin residue. The mass analyzer on the Model 5980A was of the quadropole type.

The gas chromatograph in this system was a Hewlett Packard Model 5710A equipped with a membrane separator for sample enrichment. This membrane separator had an upper temperature limit of 275° C, which limited the column temperatures used to 275° C and below.

All chromatograms and mass spectra were monitored by a Tektronix cathode ray tube display, and any copies needed were provided by a Tektronix 4631 Hard Copy Unit.

2. The Ultraviolet Spectrophotometer

A Bausch and Lomb Spectronic 505 was used to gather data to aid in calculating the distribution ratio of permethrin in the solvent partitioning step.

3. The Evaporator

A Buchler Model PF-10DN flash-evaporator was used in concentrating samples when solvent evaporation was necessary. The flash-

evaporator was also used in a nonevaporating capacity to rotate leaf samples with solvent for extracting purposes.

4. The Separatory Funnel

A 250-ml modified separatory funnel was used for filtering and solvent extraction purposes. The separatory funnel was modified by cutting away the ground glass stopcock and replacing it with a buret end approximately 1.2 cm in diameter and 8 cm in length. The buret end was equipped with a removable teflon stopcock and glass tip. This modification aided in the filtering process.

5. The Liquid Chromatographic Cleanup Apparatus

A 50-ml Kimax buret with dimensions of 1.2 cm by 60 cm was used in the liquid chromatographic cleanup step. The buret was equipped with a removable teflon stopcock and a removable glass tip.

6. The Gas-Liquid Chromatographic Column

The chromatographic column used on the gc-ms system for this residue study was a 6 foot by 4 mm id glass column pack with 3% OV-1 on 80-100 mesh Chromosorb W-HP.[38] This column was obtained from Hewlett Packard and conditioned at 280° C for 48 hours with a 15 ml per minute flow of helium.

B. Reagents

1. Hexane

Drum grade hexane was redistilled in an all-glass apparatus. This hexane was used whenever hexane was needed.

2. Acetonitrile

Reagent grade acetonitrile was used in solvent partitioning.

3. Sodium Sulfate

Reagent grade anhydrous sodium sulfate was used in filtering and drying solvents.

4. Ethyl Acetate

Reagent grade ethyl acetate was used in preparing the eluant for adsorption column cleanup.

5. Silica Gel

Davidson 923 silica gel was used as received for column cleanup.

6. Glass Wool

Presilanised glass wool obtained from chromatography suppliers was used whenever glass wool was needed.

C. The Analytical Standard

The standard used for analysis of the permethrin residues was a gift by James P. Ussary from ICI United States Inc. It contained 91.9% permethrin in the form of 60.2% *trans* isomer and 39.8% *cis* isomer.

CHAPTER III

THE SAMPLING PROCEDURE

A. The Tomato Plot

Ten week old tomato plants (CV. Walter) were transplanted into soil on June 3, 1979. The plants were arranged in a randomized complete block design[30, 31] replicated four times as shown in Figure 1 (p 14). Each plot contained five tomato plants spaced approximately eighteen inches apart, with each plot separated by approximately forty inches, and each replication separated by ten feet. A total of sixty plants was used in this study.

B. Application of Permethrin

Application rates tested were 0.1 lb active ingredient per acre, 0.2 lb per acre, and an untreated check. The permethrin evaluated was Ambush® (EC). Permethrin was applied to plant foliage with a two-gallon pressurized sprayer equipped with a flat fan spray nozzle. Each plant was sprayed until runoff, except the untreated check which was not sprayed at all. No chemical other than Ambush was used on these tomato plants.

Treatments were applied on July 5, 1979 and July 31, 1979. The second spraying was considered necessary since rain during the month of July interferred with sample collection during the first spraying.

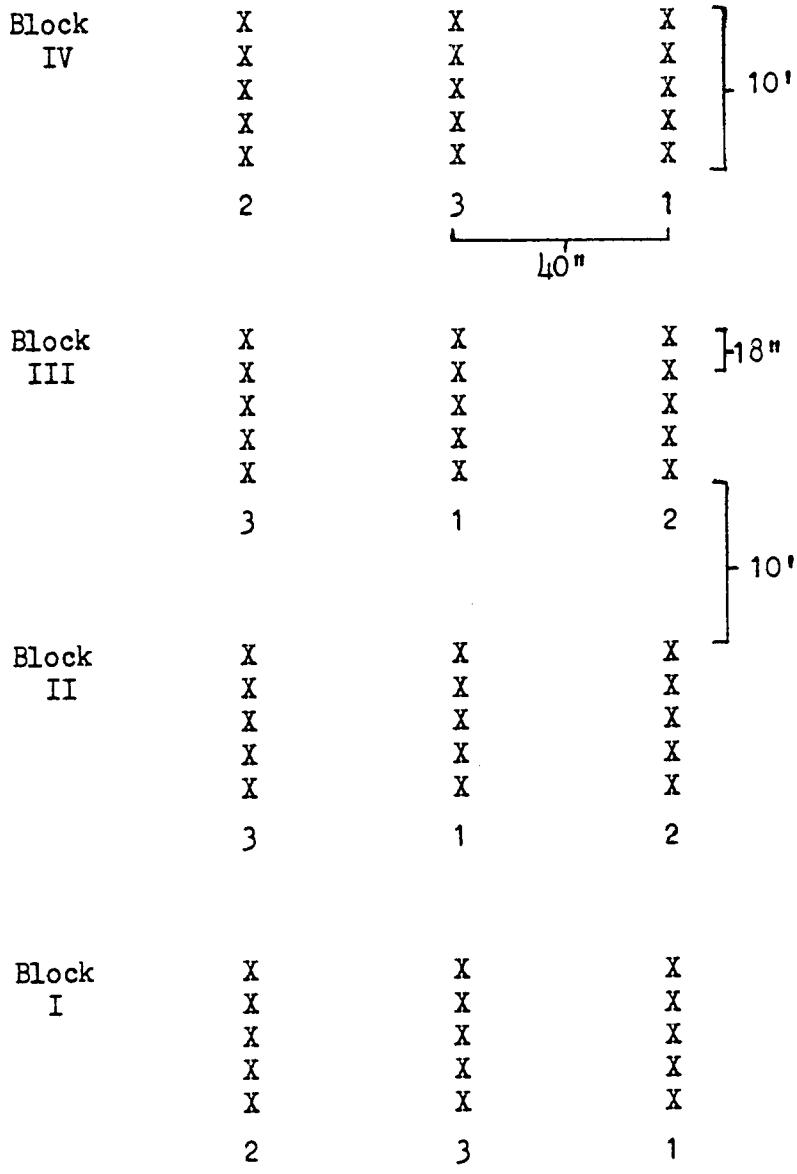


Figure 1. Randomized complete block design.

X: indicates a tomato plant.
1: plants sprayed at rate of 0.1 lb per acre.
2: plants sprayed at rate of 0.2 lb per acre.
3: plants used as an untreated check

C. Sample Collection

Immediately after the spray on the tomato plants had dried, 25-30 leaflets were collected at random from each plant at twenty-four hour intervals. Only the actual leaf material was collected. The leaf material was placed on previously weighed aluminum foil and weighed to be 100.0 grams, ±0.1 gram. Once the sample was weighed, it was wrapped in the aluminum foil, which had been cleaned with hexane, and labelled according to the day, row, and replication from which it was taken. The samples were then refrigerated at 5° C until they could be processed. This collection procedure was also followed during the second spraying, with the exception that only 50.0-gram samples were taken. The reduction in sample weight was necessary to prevent depleting the supply of leaves too early in the experiment.

CHAPTER IV

SAMPLE PROCESSING

A. Extraction

The leaf samples were transferred from the aluminum foil with tweezers to a 500-ml round bottom flask. Approximately 50 ml of hexane was used to rinse any permethrin remaining on the tweezers and aluminum foil into the flask. When processing the 100.0-gram samples collected from the first spraying, an additional 150 ml of hexane was added to bring the total volume of hexane in the round bottom flask to 200 ml. For processing the 50.0-gram samples collected from the second spraying, only 100 ml of hexane was added to the washings to bring the total volume of hexane to 150 ml. The purpose of this change in the volume of hexane used during the extraction process was to facilitate the mixing action of the sample and hexane during the rotation of the flask. The flask was rotated for 10 minutes by the flash-evaporator operating in a nonevaporating capacity. The hexane containing the leaf extract was drained into a modified 250-ml separatory funnel containing a glass wool plug and 5 grams of anhydrous sodium sulfate. The extract was allowed to filter through the sodium sulfate and glass wool plug in order to remove excess water and any large suspended particles. A 500-ml round bottom flask was placed under the separatory funnel to collect the extract as it filtered through. In the meantime, hexane was again added to the flask containing the leaf samples, and the flask was returned to

the flash-evaporator to be rotated for another 5 minutes. The amounts of hexane added for this re-extraction step were 100 ml for the 100.0-gram samples and 75 ml for the 50.0-gram samples. After the 5 minute re-extraction time had expired, the hexane was drained into the same separatory funnel that had been used for filtering the first extract. The filtering proceeded in the same manner as the first extract, and was collected in the same 500-ml round bottom flask. After washing the separatory funnel with 10 ml of hexane, the total volume of extract collected was approximately 280 ml for the 100.0-gram samples and 210 ml for the 50.0-gram samples. The sample was then concentrated by evaporating excess hexane using the flash-evaporator and rotating the 500-ml round bottom flask in a water bath heated to 50° C. After concentrating the extract to a volume of about 5 to 10 ml, it was ready for the solvent partitioning cleanup step.

B. Solvent Partitioning Cleanup

The concentrated extract was transferred to a 250-ml separatory funnel which contained 25 ml of acetonitrile saturated with hexane. The separatory funnel was calibrated to indicate a volume of 40 ml. After the extract was transferred, the volume in the separatory funnel was adjusted to the 40-ml mark by adding hexane. This adjustment provided for a volume of 25 ml of acetonitrile and 15 ml of leaf extract in hexane. The separatory funnel was then tightly capped with a teflon stopper, and was gently rocked to allow the two immiscible phases to contact each other. The large separatory

funnel allowed the phases to spread out when in the horizontal position, and provided a large area of contact between the phases, which aided in the speed of the partitioning. The partitioning was allowed to take place without any of the solvents coming in contact with the teflon cap and becoming trapped or lost due to leakage. After gently rocking for three minutes, the separatory funnel was placed on a ring stand to allow the phases to separate for one minute. The bottom phase, the acetonitrile, was drained into a 250-ml round bottom flask. The hexane phase containing the leaf extract remained in the separatory funnel, and 25 ml of fresh hexane-saturated acetonitrile was added. The volume was adjusted to 40 ml with hexane, if necessary, and the solvent partitioning procedure was repeated. Three partitions were performed, making a total of 75 ml of acetonitrile collected in the 250-ml round bottom flask. The hexane phase left in the separatory funnel was then discarded, and the acetonitrile portion was evaporated almost to dryness by means of the flash-evaporator and a hot water bath at 50° C. Great care was taken to insure that, during the evaporation step, the extract was not allowed to evaporate to dryness and thereby lose part of the residue to be analyzed.

At the beginning of the solvent partitioning step, the extract was a yellow-green color. After the solvent partitioning, the acetonitrile was green and the hexane left in the separatory funnel was a definite yellow.

C. Column Chromatographic Cleanup

In order to perform this step of the extract processing, a silica gel adsorption column had to be prepared. The column contained a glass wool plug and 6.0 grams of Davison 923 silica gel. The silica gel was used without further activation, and was packed by pouring it into a glass buret which contained 20 ml of hexane. The sides of the buret were gently tapped to help the silica gel settle evenly to the bottom. An additional glass wool plug placed at the top of the layer of silica gel prevented it from being disturbed when solvents were poured in from the top of the buret. Approximately 20 ml of hexane flowed through before any extract was transferred to the column.

After evaporating the solvent partitioned extract almost to dryness, it was transferred to the silica gel adsorption column and washed with hexane. In transferring the extract, the total volume placed at the top of the column was approximately 10 ml of extract diluted with hexane. The stopcock was opened, and the hexane was allowed to drain until it reached the level of the silica gel, depositing the extract at the top of the silica gel layer. The sides of the buret were then washed with an additional 3 ml of hexane, and this, too, was allowed to drain. The hexane collected so far from the draining buret was discarded, and elution of the sample began. The solvent used to elute the permethrin through the column was a mixture of 3% ethyl acetate in hexane. This solvent mixture had been shaken with anhydrous sodium sulfate to absorb water, which could

change the elution characteristic of the column. The solvent mixture was allowed to flow through the column at a rate of 4 ml per minute, and after 15 ml had passed through, it was discarded. A 250-ml round bottom flask was used to collect the next 75 ml in which the permethrin was eluted. The permethrin was then concentrated using the flash-evaporator and a hot water bath at 50° C. The concentrated permethrin was then transferred to a 5-ml volumetric flask, and was diluted to the calibration with hexane. The sample was then a colorless liquid suitable for analysis by the gc-ms.

D. Solvent Partitioning Evaluation

In evaluating the extent or efficiency of the acetonitrile-hexane partitioning cleanup step, an experimental value for the distribution ratio D between these two organic phases was helpful. Approximate D values were obtained by placing a known amount of permethrin standard in a 100-ml volumetric flask and diluting with acetonitrile-saturated hexane. Three 25-ml portions of the permethrin solution were then placed in contact with 25-ml portions of hexane-saturated acetonitrile. The permethrin was allowed to equilibrate between the phases in 125-ml separatory funnels. The equilibration was aided by ten minutes of gentle rocking, followed by 5 minutes of standing in contact. The concentrations of each hexane phase was determined by measuring the UV absorption at 272 nm. A Bausch and Lomb Spectronic 505 was used to measure the required absorbance. Once the permethrin concentrations in the hexane phases had been

calculated, these values were subtracted from the known amounts that had been originally added to the system. These remainders were equal to the concentrations of permethrin in the acetonitrile phases. The D values could then be calculated by the equation:[48]

$$D = \frac{\text{conc. of permethrin in the acetonitrile phase}}{\text{conc. of permethrin in the hexane phase}} . \qquad (2)$$

In this way D values were obtained for permethrin concentrations of 50 ppm, 150 ppm, and 350 ppm. These values were 4.0, 4.2, and 4.4 respectively. In Figure 2 (p 22) a plot of the log of D versus the log of the permethrin is shown. The slope of this plot is 0.049. The small value for the slope would seem to indicate little significant change in the distribution ratio as the permethrin concentration increased, at least in the 50 to 350 ppm concentration range. Apparently little dimerization was occurring.

Using the experimental D values, calculations can be made to determine the extent or completeness of extraction. The concentration C_n of permethrin left in the hexane phase can be calculated by [48]

$$C_n = C_o \; V_h / (DV_a + V_h)^{\;n} . \qquad (3)$$

In this equation C_o is the original concentration of permethrin, V_h is the volume of hexane phase, V_a is the volume of acetonitrile phase, n is the number of extractions, and D is the distribution ratio. If the appropriate values from the hexane-acetonitrile solvent partitioning cleanup step are substituted for variables in the previous equation, the concentration left after the three extractions can be calculated. The concentration not extracted from a 150 ppm sample

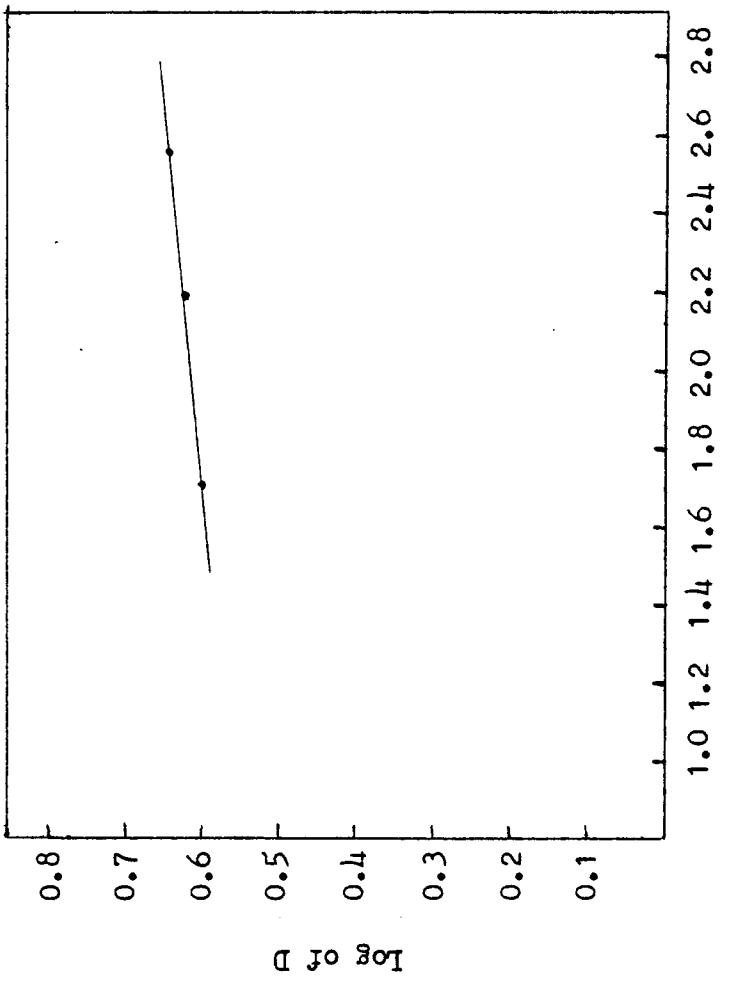


Figure 2. Permethrin solvent extraction plot.

was approximately 0.3 ppm. In other words the extent of extraction was about 99.8% complete. This percentage of completeness was more than adequate.

E. Evaluation of the Cleanup Step Process

In order to see that significant amounts of the permethrin analyte were not lost during the cleanup processes, tests using known concentrations of permethrin were performed to evaluate the recovery. Permethrin concentrations of 15 ppm, 50 ppm, and 150 ppm in total volumes of 5 ml each were evaluated. The samples underwent all clean-up procedures beginning with the solvent partitioning step under the same conditions that had been applied to the actual crop samples. Each test sample underwent the three solvent partitions, evaporation, transfer to the silica gel column, further evaporation, and a final dilution to 5 ml with hexane. Analysis of the processed samples was performed by the gc-ms system operated under selected ion monitoring (SIM) conditions. Average percentage values for the recoveries were 95% for the 150 ppm concentration, 93% for the 50 ppm concentration, and 89% for the 15 ppm concentration. A plot of permethrin concentration before processing versus permethrin concentration after processing is shown in Figure 3 (p 24). The slope of the line is 1.04 and the extended line passes very near the origin. If all recoveries had been complete, the slope would have been 1.00 and the line would have passed directly through the origin.

By observing the plot some characteristics of the cleanup

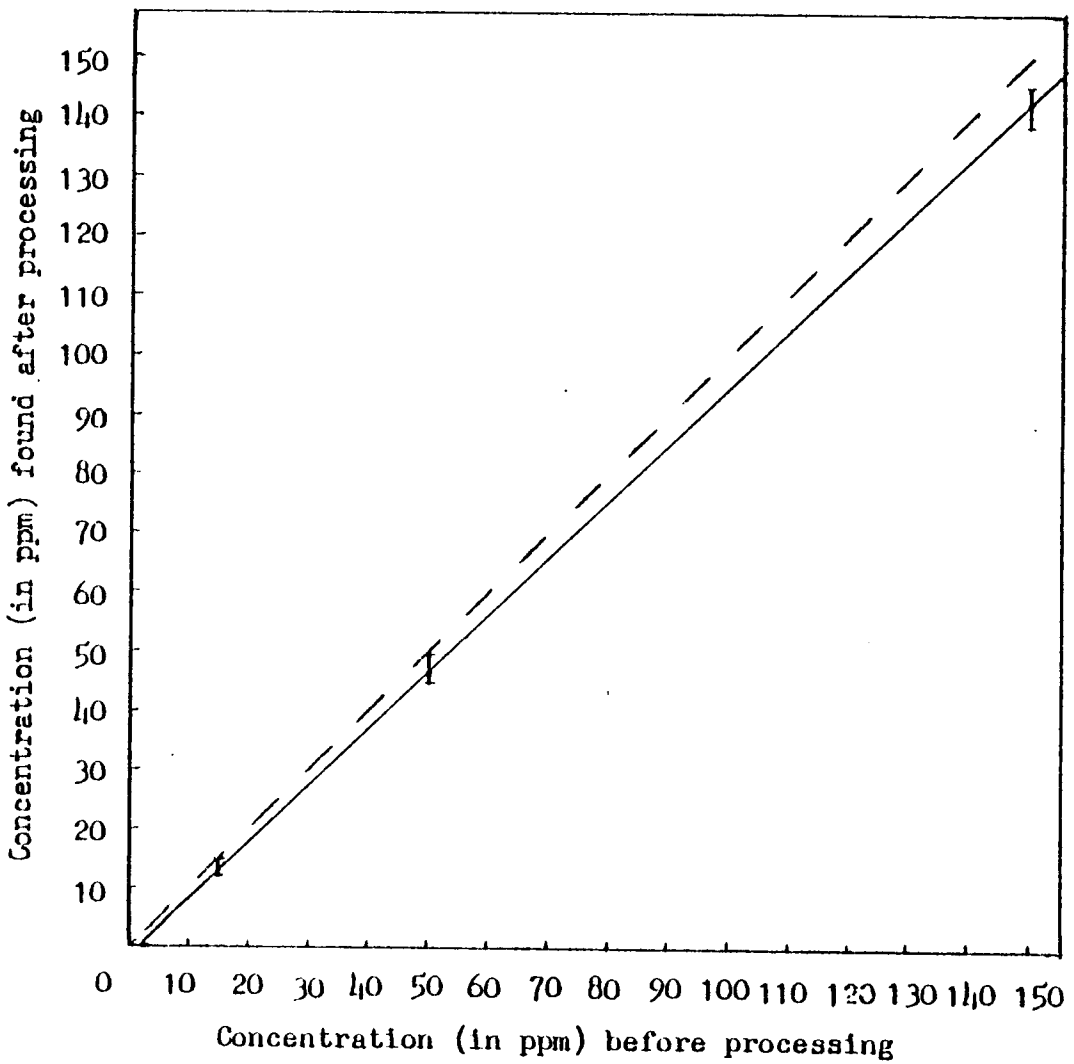


Figure 3. Recovery of permethrin after cleanup processing.
Dashed line: indicates perfect recovery.
Solid line: indicates actual recovery.

process may be discerned. Part of the process must be causing the loss of a constant amount of permethrin since the line does not pass through the origin. However, the constant amount being lost must be small because the line does pass very near the origin. The cleanup step which would more likely cause this type of small constant loss would be the silica gel column. Small amounts of permethrin could become very tightly adsorbed on the silica gel and not be removed by the solvent mixture used in elution. In addition to the constant error, there appeared to be a small percentage error indicated by the plot on the graph which does not run parallel to the ideal (dashed) line drawn above it. This percentage error causes differences which manifest themselves as small numerical errors at low concentrations and larger numerical errors at higher concentrations. Causes for this type of error would more likely involve losses in the transferring and evaporation of the samples. Also losses incurred during the solvent partitioning step would add to the error, although the previous evaluation showed only a very small percentage of permethrin did not extract. Some sample loss due to the gc-ms membrane separator is to be expected, but these losses are difficult to quantitate.

CHAPTER V

INSTRUMENTAL CONDITIONS

A. Gas Chromatograph Conditions

Before analyzing the leaf extracts for permethrin content, an investigation was undertaken to ascertain the most desirable instrumental conditions for this analysis. Several samples of the analytical standard permethrin were injected into the gc-ms and a chromatogram was obtained by scanning the mass range 0 to 400 amu. From the mass spectra obtained, the cis and trans isomers of permethrin were identified to be two overlapping peaks in the chromatogram. After identifying the two permethrin peaks, the gas chromatographic conditions were adjusted in an attempt to separate the isomers. However, the isomers remained as two partially resolved peaks. Since the isomers would not separate, the gas chromatographic conditions were adjusted to reduce the retention time to a minimum. The oven temperature was set at 255° C, just below the maximum temperature (275° C) that the membrane separator would allow. The helium carrier gas flow rate was set at the maximum which the column allowed, 20 ml per minute. A mass spectrum of permethrin can be seen in TABLE I (p 27).

B. Selected Ion Monitoring

In this permethrin residue study, the mass spectrometer was used as a very sensitive selective detector for the gas chromatograph. To obtain the sensitivity and specificity needed for analyzing the

TABLE I

MASS SPECTRUM OF PERMETHRIN

MASS	ABUND	MASS	ABUND	MASS	ABUND	MASS	ABUND	MASS	ABUND	MASS	ABUND
26	.3	75	.8	105	.4	140	.4	181	4.2	222	.3
29	1.6			107	.5	141	1.3	182	3.5	223	.7
		77	6.2	108	.7	142	.3	183	100.0	227	1.1
39	1.0	78	.9	109	2.2	143	.6	184	15.8		
41	5.4	79	.9	110	.4			185	1.4	236	.4
42	.7	80	.4	111	1.9	147	.8			239	.4
43	4.2	81	1.8	112	.5	148	.3	191	.5		
44	.6	82	1.0	113	1.3	149	.7	193	.6	255	2.4
45	2.0	83	2.4	114	.2	151	1.1	194	.6	267	.7
		84	1.3	115	3.8	152	3.0	197	.6		
51	1.3	85	4.9	116	.4	153	5.7	198	.8	265	2.0
53	.5	86	1.2	117	.5	154	3.4	199	7.9	265	1.2
54	.5	87	3.9			155	5.4	200	1.2	268	.4
55	5.2	89	4.8	119	.5			201	.5		
56	4.0			121	.2	163	23.3			281	1.7
57	10.3	90	1.7	123	.9	164	2.4	202	.3	282	.4
58	.9	91	7.0	124	.7	165	21.4	205	.3	283	.5
		92	.6	125	.8	166	2.5	207	4.2		
63	.7	93	.9	127	10.0	167	8.1	208	.7	290	.3
65	1.7	94	.2	128	3.9	168	7.1	209	.8		
67	1.5	95	1.5	129	3.4	169	2.7	210	.7	310	.5
68	.7	96	1.0	130	.5	170	1.2	211	2.2	312	1.0
69	3.0	97	1.7			171	10.5	212	.4		
70	.8	98	2.1	133	.3	172	1.1			356	.9
71	1.8	99	4.1	135	.8	173	.5	218	.5	356	.3
72	.6	100	2.4	137	.8			220	.4		
73	2.7	101	1.3	139	.7	180	.4	221	2.1	391	.4

leaf extracts, the mass spectrometer was operated in what is called the selected ion monitoring (SIM) mode. This technique, also known as mass fragmentography,[47] is the sequential detection of one or more selected masses. In the Hewlett Packard 5980A system, SIM is performed by a computer program in which the operator enters from one to four masses, along with the length of time each mass is to be monitored. Each mass can be monitored for different lengths of time ranging from 50 to 32,767 milliseconds; however, this user found that the program would operate with the time no longer than 9000 milliseconds. In addition to the individual mass monitoring times, the operator also enters a delay time which allows the mass spectrometer to operate and collect data only at specified times which encompass the peaks of interest, thereby saving valuable computer disc space. The SIM program will accomodate up to five groups, a group being one to four masses. These groups are monitored at times selected by the user, and allow the user to gather quantitative and qualitative data on individual peaks or groups of peaks in the chromatogram.

A comparison of the sensitivity of the scanning and the SIM modes of operation is of interest. In Figure 4 (p 29) a chromatogram of an actual processed leaf extract containing permethrin is shown. Three µl of the extract was injected into the column and the mass spectrometer was programmed to scan the eluting compounds in the mass range of 0 to 400 amu. The resulting chromatogram was disappointing. In the region of 10.5 to 12 minutes the chromatogram should contain two partially resolved peaks of permethrin. However,

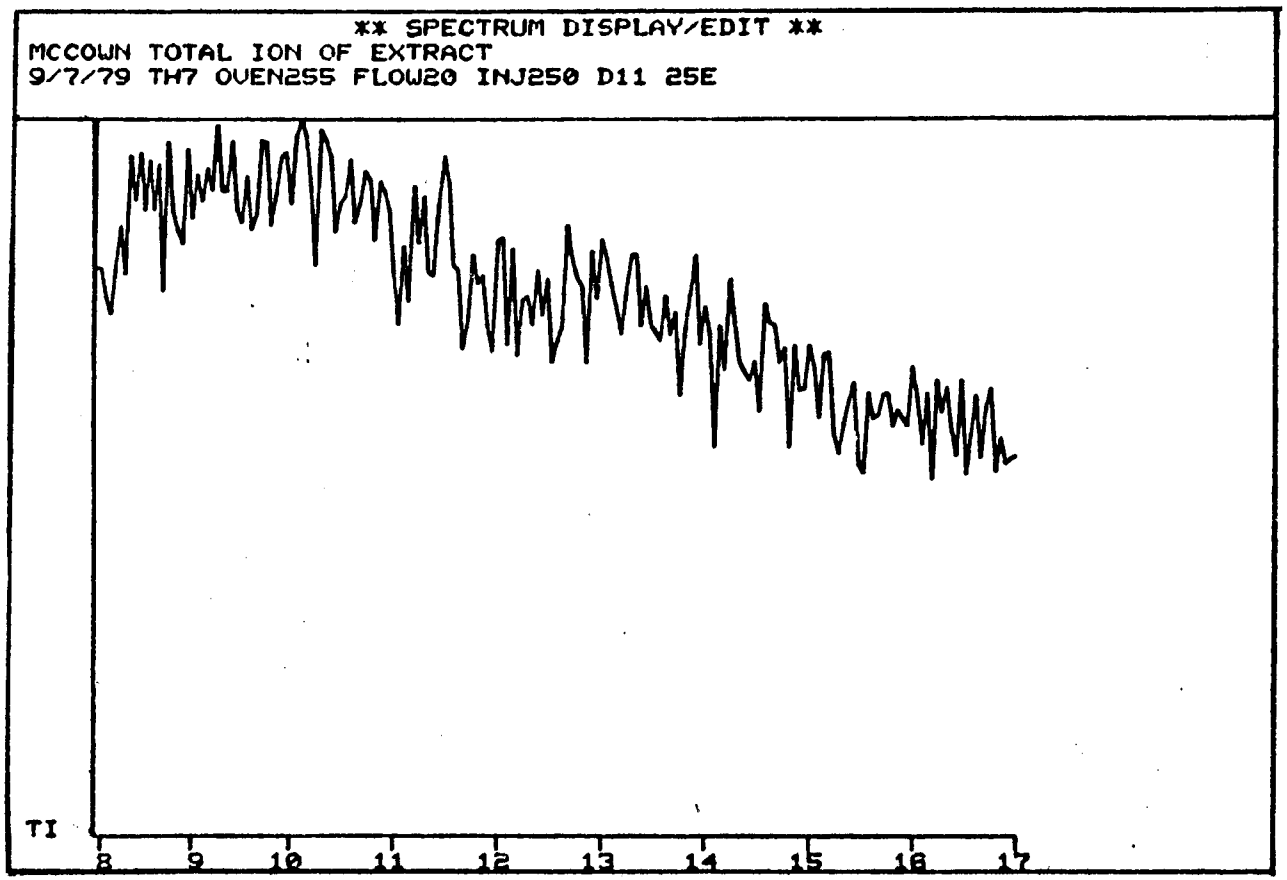


Figure 4. Total ion chromatogram of processed leaf extract obtained by scanning 0 to 400 amu.

nothing substantial rose above the noise. When the same size sample was injected and the mass spectrometer was set to scan the region of 100 to 200 amu, the chromatogram showed definite improvement, as shown in Figure 5 (p 31). Again the same size sample was injected, but this time the mass spectrometer was operated by the SIM program. Only one mass was chosen to be monitored, 183 amu. This mass was chosen because it was the most abundant mass, the base peak, found in the mass spectrum of permethrin. It can be seen in Figure 6 (p 32) that a mass spectrometer operated in selected ion monitoring mode creates a more selective detector and provides a much greater signal to noise ratio than does a mass spectrometer operated in a scanning mode.

C. Ionization Voltage

After choosing the SIM mode of operation, a voltage for the electron ionization must also be chosen. The ionization voltage must be selected to maximize the signal to be measured. In this study the base peak, 183 amu, was the only mass measured to obtain a chromatogram, so the ionization voltage was chosen to maximize this signal. The practical operating ionization voltage range in the Hewlett Packard 5980A source is from 25 to 70 electron volts. Generally speaking, the higher voltage settings produce greater fragmentation of a chemical molecular structure. The fragmentation of the permethrin molecule follows this general pattern. Intuitively one could say that higher voltages would produce more numerous, but smaller, fragments than lower voltages, and that the base peak of permethrin would

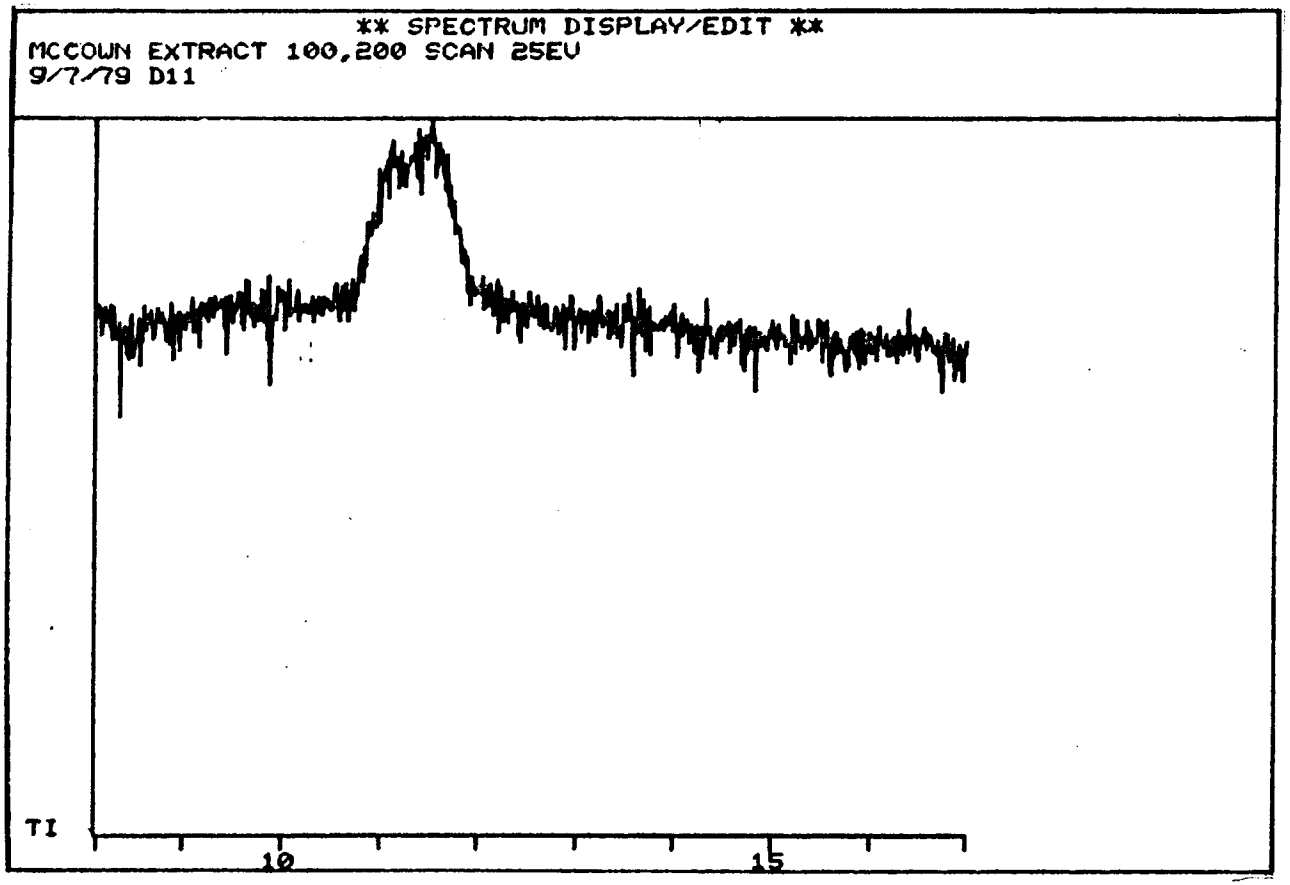


Figure 5. Total ion chromatogram of processed leaf extract obtained by scanning 100 to 200 amu.

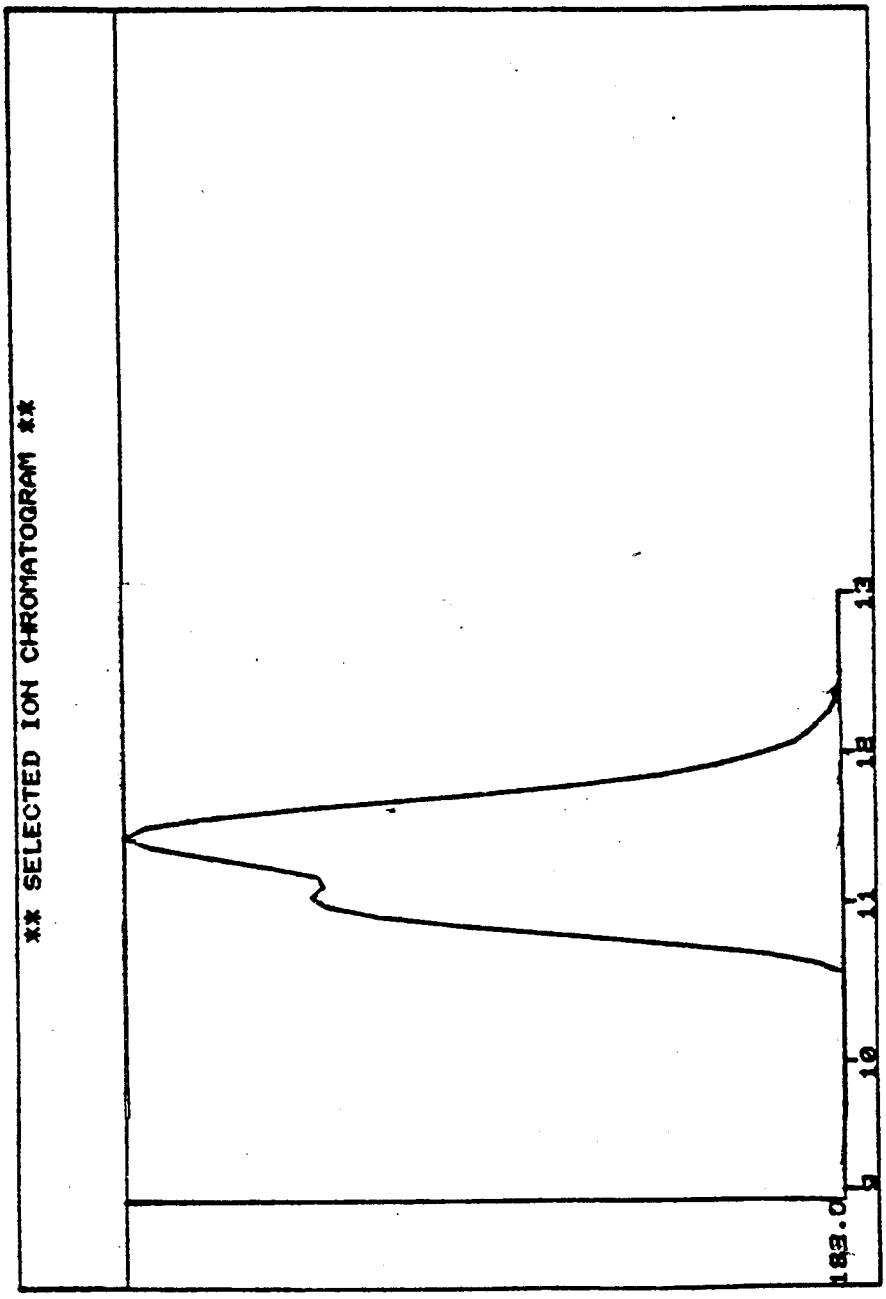


Figure 6. Single ion chromatogram of processed leaf extract obtained by monitoring 183 amu.

decrease in size at the higher voltages due to the increased fragmentation of itself. To test this reasoning, several injections of permethrin standard were made at three different ionization voltages, 70 eV, 50 eV, and 25 eV. The mass spectrometer was operated by the SIM program, and the mass 183 amu was monitored after each injection. The chromatograms obtained showed increases in the permethrin peak area as the ionization voltage was lowered. The chromatograms obtained at 50 eV showed double the peak area of the ones obtained at the 70 eV setting, and the chromatograms obtained at 25 eV showed four times the peak area of the chromatograms obtained at 70 eV. The obvious choice of ionization voltage for this study was 25 eV

D. The Gas Chromatograph-Mass Spectrometer Sensitivity

To indicate the sensitivity of the gc-ms system when controlled by the SIM program, several dilutions of a standard permethrin solution were injected into the instrument. Only the mass 183 amu was monitored, and the ionization voltage was set at 25 eV. The gc-ms system was able to detect a sample as small as 150 picograms, with a sample to noise ratio of approximately 10 to 1. The sensitivity of this system is greatly dependent on the age and condition of the source filament. An old or dirty filament or an improperly tuned instrument will destroy the high sensitivity of this system.

E. Instrument Settings

GC column packing	3% OV-1 on Chromosorb W-HP 80-100 mesh
GC column type	6 ft. x 4 mm id glass column
Carrier gas	Helium
Carrier gas flow rate, ml per minute	20
Injection port temperature	250° C
Column temperature	255° C
Interface temperature	250° C
Mass monitored	183 amu
Thresholds	7
Dwell time	9000 milliseconds
Detector gain	11 (maximum setting)
Ionization voltage	25 eV

CHAPTER VI

EXPERIMENTAL RESULTS

A. Calibration Curve

To determine the concentrations of the 5-ml processed crop samples, a calibration curve had to be obtained from standard permethrin samples. Serial dilutions from a 3764-ppm permethrin stock solution were made until a series of dilutions from 7.53 ppm to 150 ppm were obtained. The combined areas of the partially resolved cis and trans isomer peaks were plotted versus the concentration of each sample, as shown in Figure 7 (p 36). Each point is the average of three 1-µl injections in which the peak areas varied less than 10%. Calibration curves like the one shown were made whenever the gc-ms system exhibited significant changes in response to known standards. The instrument maintained response to the standards so that a total of only three calibration curves were needed during the analysis of the crop samples.

The sensitivity of the gc-ms was as good as the 150 picogram figure mentioned in the sensitivity test section. Just after the sensitivity test and before any crop samples had been analyzed, the direct induction of a corrosive sample from another research project damaged the source filament and decreased the instrument's sensitivity. This damage was the main cause of the reduced sensitivity exhibited by the calibration plots.

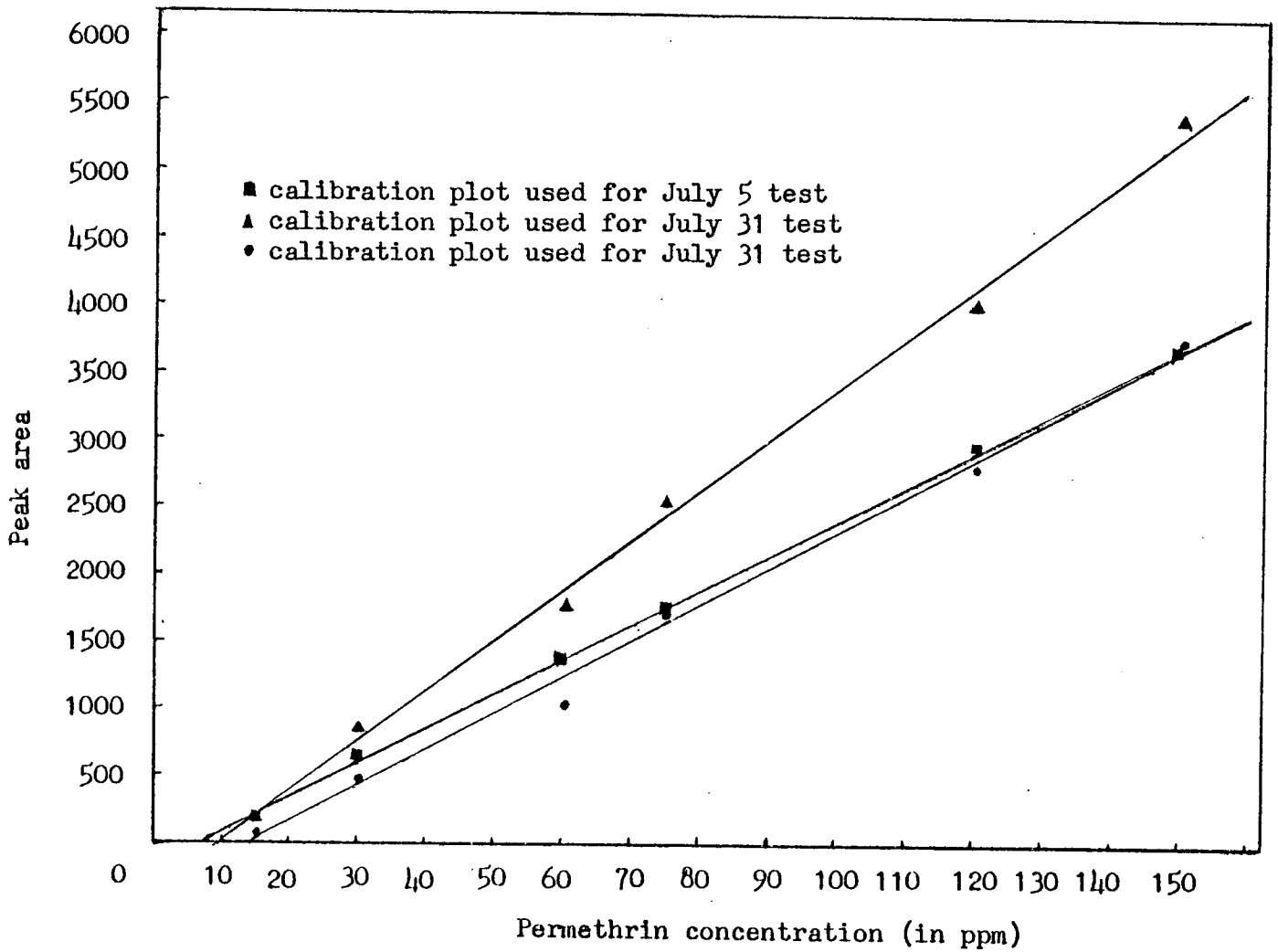


Figure 7. Calibration plots.

B. Crop Sample Analysis

The 5-ml processed crop samples were analyzed in the same manner and under the same instrumental conditions as the standard permethrin samples used for the calibration plots. At the beginning of each day's analysis, 3 µl of a standard permethrin solution containing at least 150 ppm was injected into the gc-ms. The mass spectrometer was set to scan the mass range of 100 to 200 amu. The resulting mass spectrum was used to establish, to the nearest 0.1 amu, the value of the base peak. Since 183 amu was the base peak in the mass spectrum, it would be used as the only mass monitored to produce the chromatograms during the SIM runs. Establishing the proper value for the base peak was important because the SIM program used a very narrow signal window. Assuming the same value each day for the base peak could produce poor and inconsistent results. The mass spectrometer usually gave reproducible values from day to day, with only slight variations occasionally. The base peak values, however, never fell below 182.8 amu nor rose above 183.3 amu. Values varying as little as 0.2 amu from the actual value produced greatly reduced output. An alternate procedure consisting of calibrating the instrument each day with perfluorotributylamine proved slower, and at best no more accurate. After the base peak value had been obtained, it was used during the entire day's analysis.

To analyze the crop samples, 1.0-µl injections of the processed extracts were made. In the resulting chromatograms, the total area of the partially resolved cis and trans isomer peaks was measured

with the aid of the CRT display and the gc-ms data system. Three peak areas which varied less than 10% were used to obtain an average peak area value for each crop sample. The average peak area value for each sample was compared to the appropriate calibration plot to obtain its particular concentration. The actual concentrations were obtained by using a Texas Instrument SR-51-II calculator program which gave X values corresponding to any Y area value on the calibration plot. Visual checks of the calibration plots were also made in order to insure the reality of any value. Since all leaf samples received the same processing, were diluted to the same amounts, and analyzed in the same manner, their concentrations can be viewed directly in TABLES II, III, IV, and V (p 39-42). The tabular data is plotted in Figures 8, 9, 10, and 11 (p 43-46). The averages from each day's samples plotted for each spraying rate are shown in Figure 12 (p 47).

In the July 5 test period, samples were taken only three days. Rain, which measured 0.65 inches in a simple rain gauge at the test site and 0.80 inches at the airport a few miles away, occurred on the second day after application. Sample collection was discontinued after the third sample was taken because of continual rain. It was also decided that the 100.0-gram sample size was too large and depleted the tomato plants.

For the second test spraying which began on July 31, clear, warm weather prevailed with rain occurring on August 11, one day after the "Day 10" samples were taken. Data collected for the 0.1 lb

TABLE II

DATA OF PERMETHRIN RESIDUE CURVES[a]
JULY 5 APPLICATION AT 0.1 LB/ACRE

Day	Block I	Block II	Block III	Block IV	Temperature, °F High	Low
0	79.4	84.0	100.	52.6	78	63
1	43.2	59.0	39.3	35.7	74	63
2*	--	--	--	--	72	65
3	22.8	22.1	21.8	19.7	73	67

* indicates rain on that day
-- indicates no samples taken that day
a. all concentrations in ppm with no corrections made for processing losses

TABLE III

DATA OF PERMETHRIN RESIDUE CURVES[a]
JULY 5 APPLICATION AT 0.2 LB/ACRE

Day	Block I	Block II	Block III	Block IV	Temperature, °F High	Low
0	150.	164.	158.	179.	78	63
1	89.4	108.	96.1	81.8	74	63
2*	--	--	--	--	72	65
3	22.8	31.2	38.6	26.7	73	67

* indicates rain on that day
-- indicates no samples taken that day
a. all concentrations in ppm with no corrections made for processing losses

TABLE IV

DATA OF PERMETHRIN RESIDUE CURVES[a]
JULY 31 APPLICATION AT 0.1 LB/ACRE

Day	Block I	Block II	Block III	Block IV	Temperature, °F High	Temperature, °F Low
0	40.0	45.7	46.8	44.8	88	73
1	--	--	--	--	89	73
2	27.2	24.8	22.7	24.4	86	71
3	23.0	19.5	22.6	21.0	85	68
4	--	--	--	--	87	66
5	22.7	17.9	22.2	19.6	88	67
6	--	--	--	--	89	69
7	15.8	16.0	16.1	14.8	91	71
8	--	--	--	--	94	73
9	--	--	--	--	93	73
10	--	--	--	13.7	92	75

-- indicates no samples taken that day
a. all concentrations in ppm with no corrections made for processing losses

TABLE V

DATA OF PERMETHRIN RESIDUE CURVES[a]
JULY 31 APPLICATION AT 0.2 LB/ACRE

Day	Block I	Block II	Block III	Block IV	Temperature, °F High	Low
0	141.	160.	127.	83.2	88	73
1	--	--	--	--	89	73
2	78.7	80.0	61.6	49.9	86	71
3	36.5	42.6	35.5	30.4	85	68
4	--	--	--	--	87	66
5	30.7	33.5	29.5	25.3	88	67
6	--	--	--	--	89	69
7	26.5	24.2	23.3	22.1	91	71
8	--	--	--	--	94	73
9	--	--	--	--	93	73
10	23.0	19.5	20.2	--	92	75
11*	--	--	--	--	84	68
12	--	--	--	--	76	65
13	--	--	--	--	82	58
14	14.1	11.7	12.0	--	83	67

* indicates rain on that day
-- indicates no samples taken that day
a. all concentrations in ppm with no corrections made for processing losses

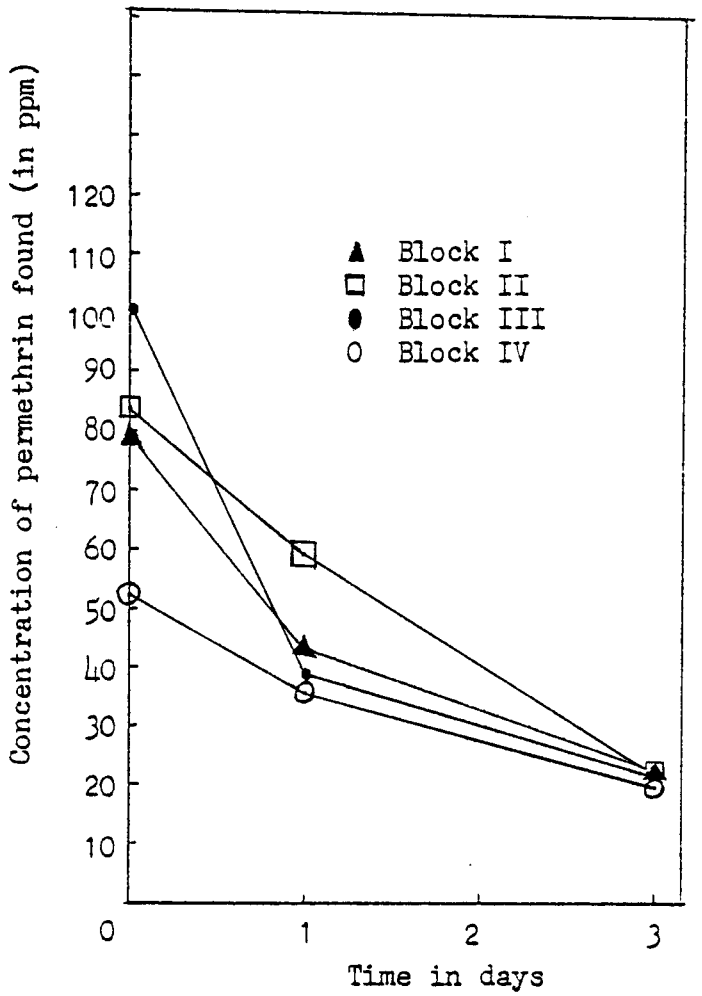


Figure 8. Permethrin residue curve showing July 5 application of 0.1 lb per acre.

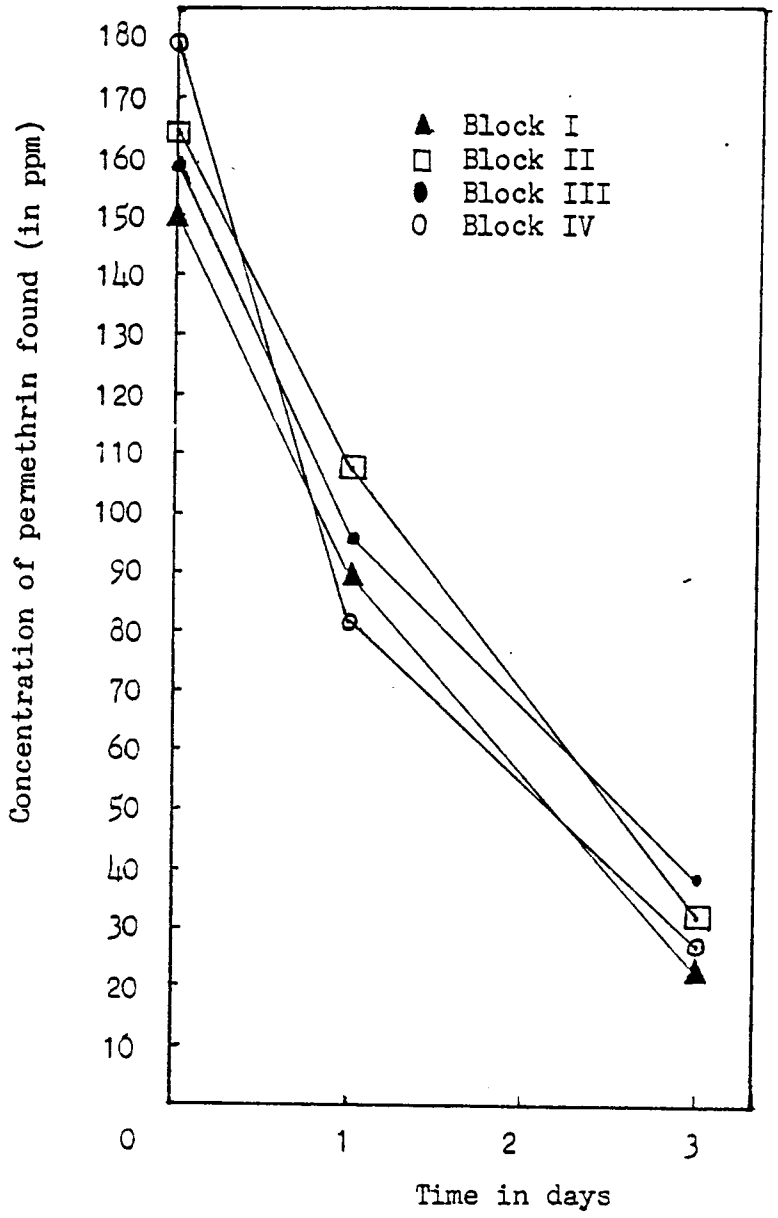


Figure 9. Permethrin residue curve showing July 5 application of 0.2 lb per acre.

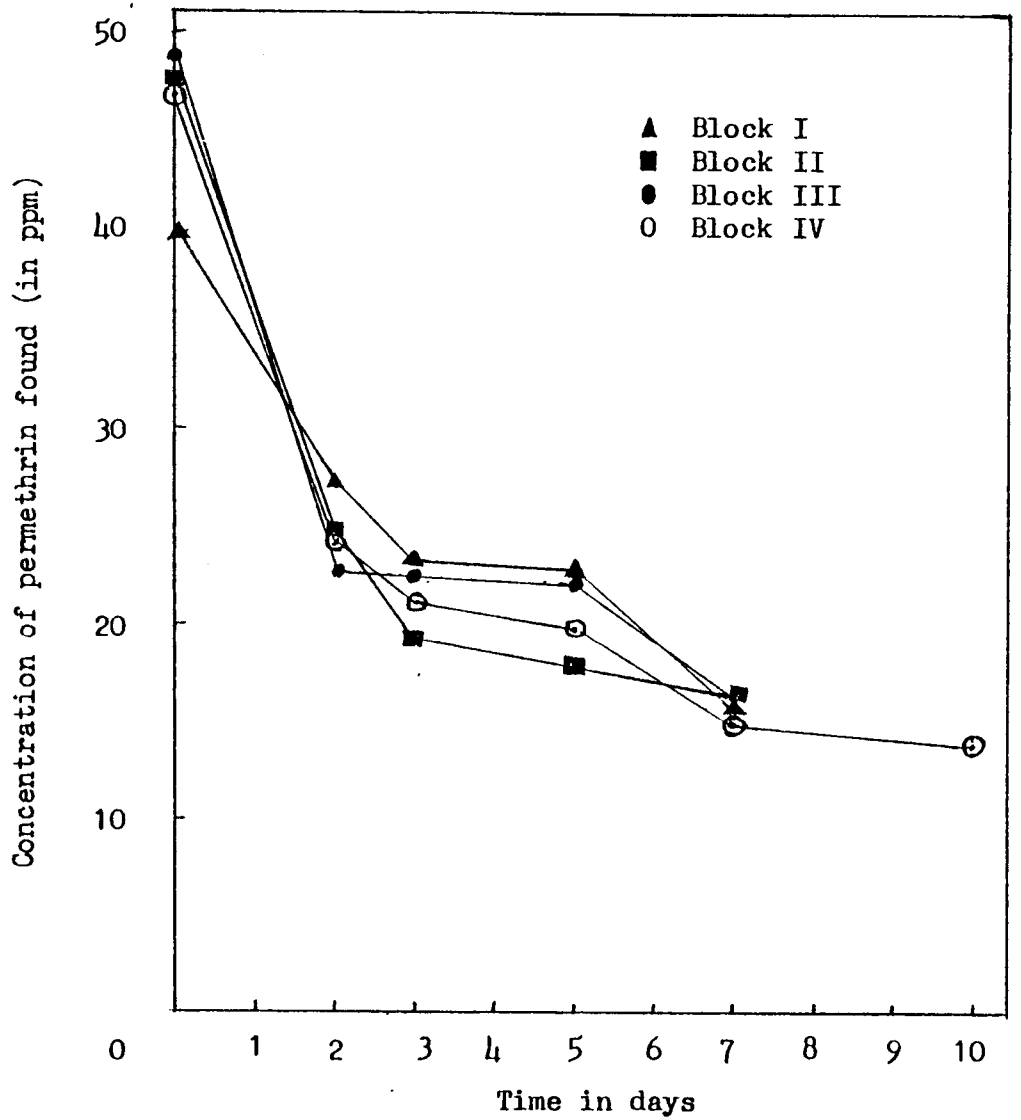


Figure 10. Permethrin residue curve showing July 31 application of 0.1 lb per acre.

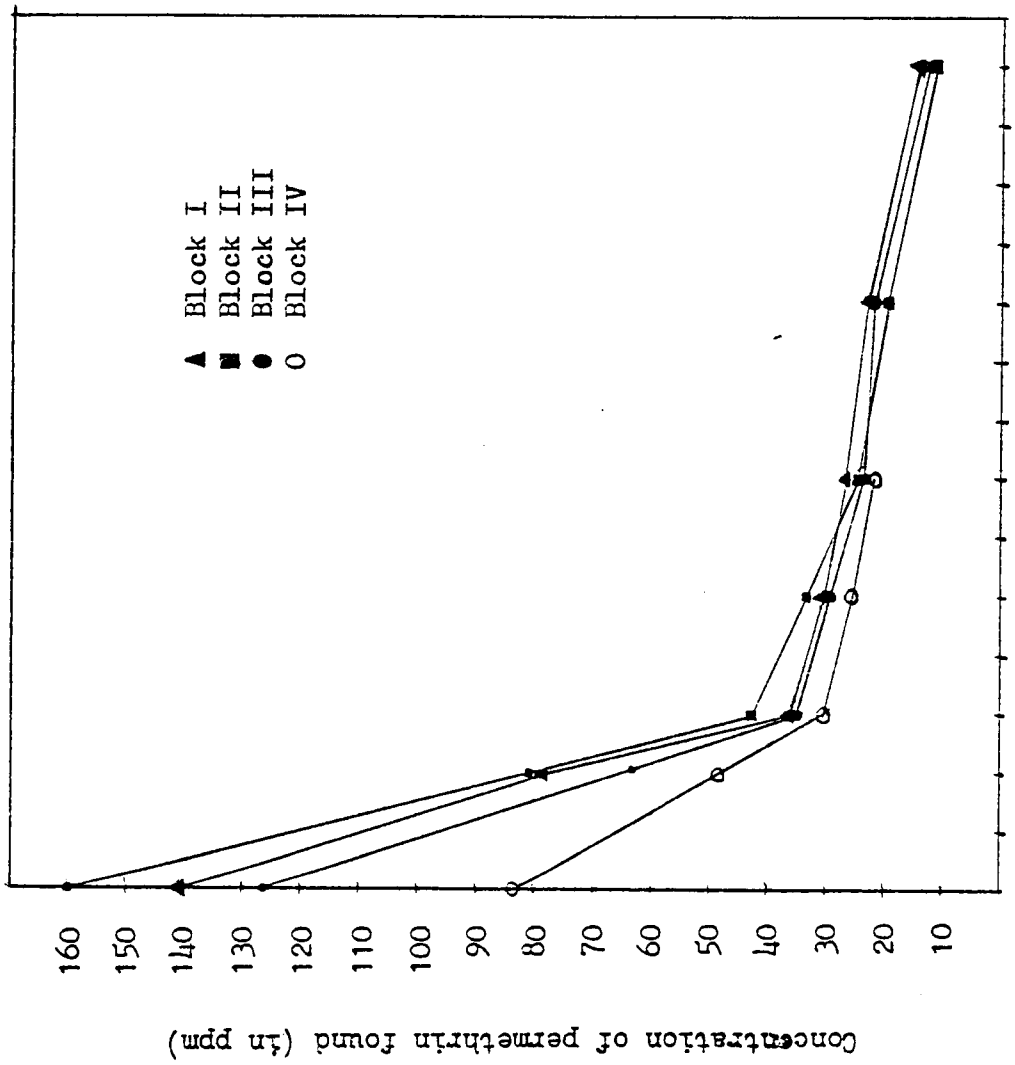


Figure 11. Permethrin residue curve showing July 31 application of 0.2 lb per acre.

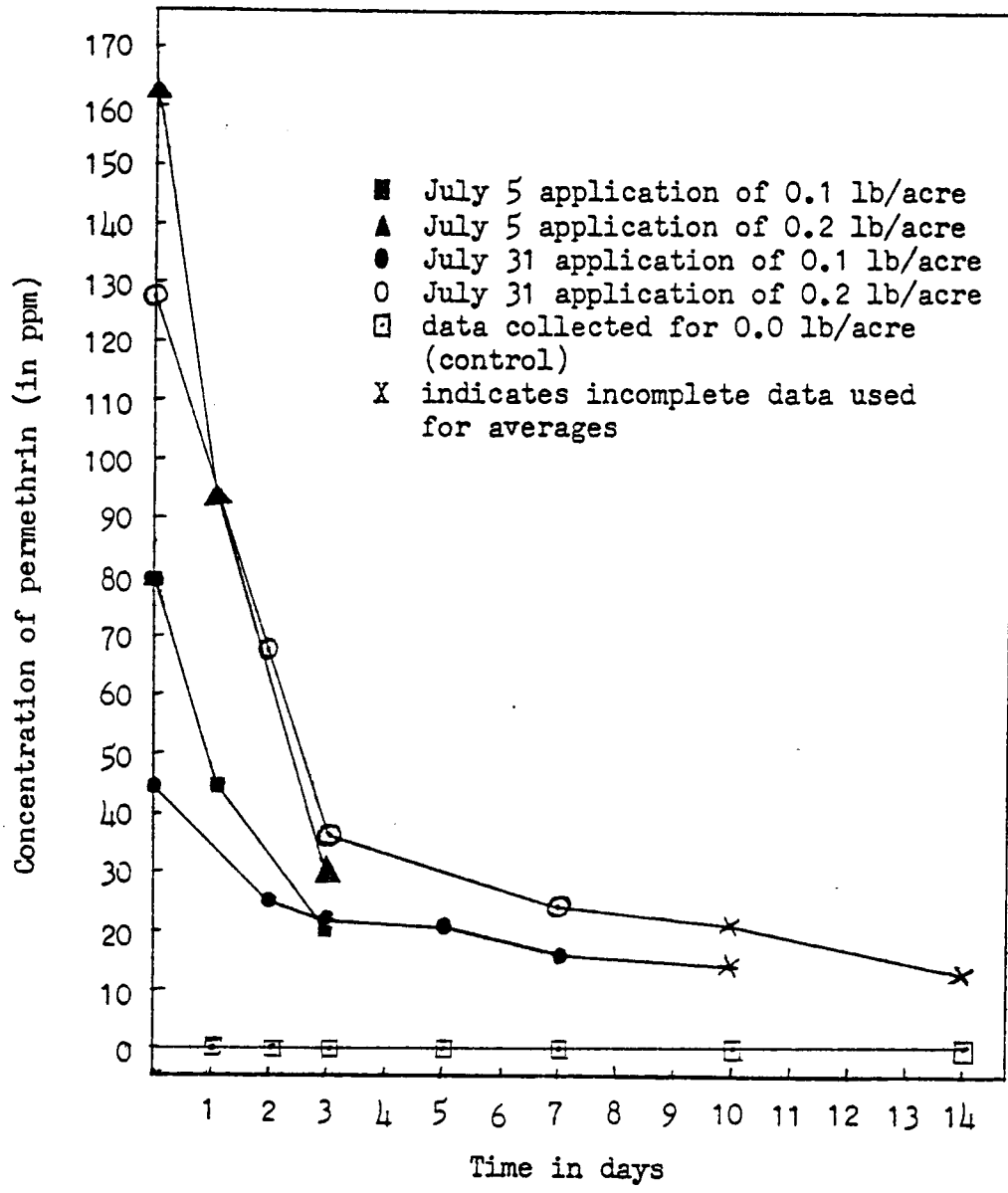


Figure 12. Plots of the average residues for the July 5 and July 31 applications.

per acre rate was discontinued because of the deteriorating condition of the tomato plants. The main cause of the plant deterioration was early blight. The 0.2 lb per acre rate was obtainable for three replications on the tenth day and fourteenth day. Samples were obtainable from the untreated check through the fourteenth day after spraying. However, no permethrin was detected in any of these samples.

C. Interpretation of Results

A sharp decline in residual permethrin during the first two to three days after application and an equally drastic change to a more gentle decline occurred after the third day. In Figure 13 (p 49) average values obtained from Figure 10 (p 45) and Figure 11 (p 46) plotted as log of concentration versus time in days are shown. The plots show a linear relationship which may indicate a first order loss rate after the first three days. The average values indicate that after the third day, the lower application rate showed nearly two-thirds as much residual permethrin as the greater application rate. This means that apparently the three times as great a concentration at "Day 0" did not guarantee three times as much residue at "Day 3" or later. This trend might be explained by possible uptake or binding of a small amount of permethrin by the leaf itself, thereby leaving the rest exposed to greater risk of degradation or volatilization. This explanation might also be applied to the cases in which considerable rainfall did not seem to cause drastic decreases in expected permethrin residue. Also the fact that permethrin is

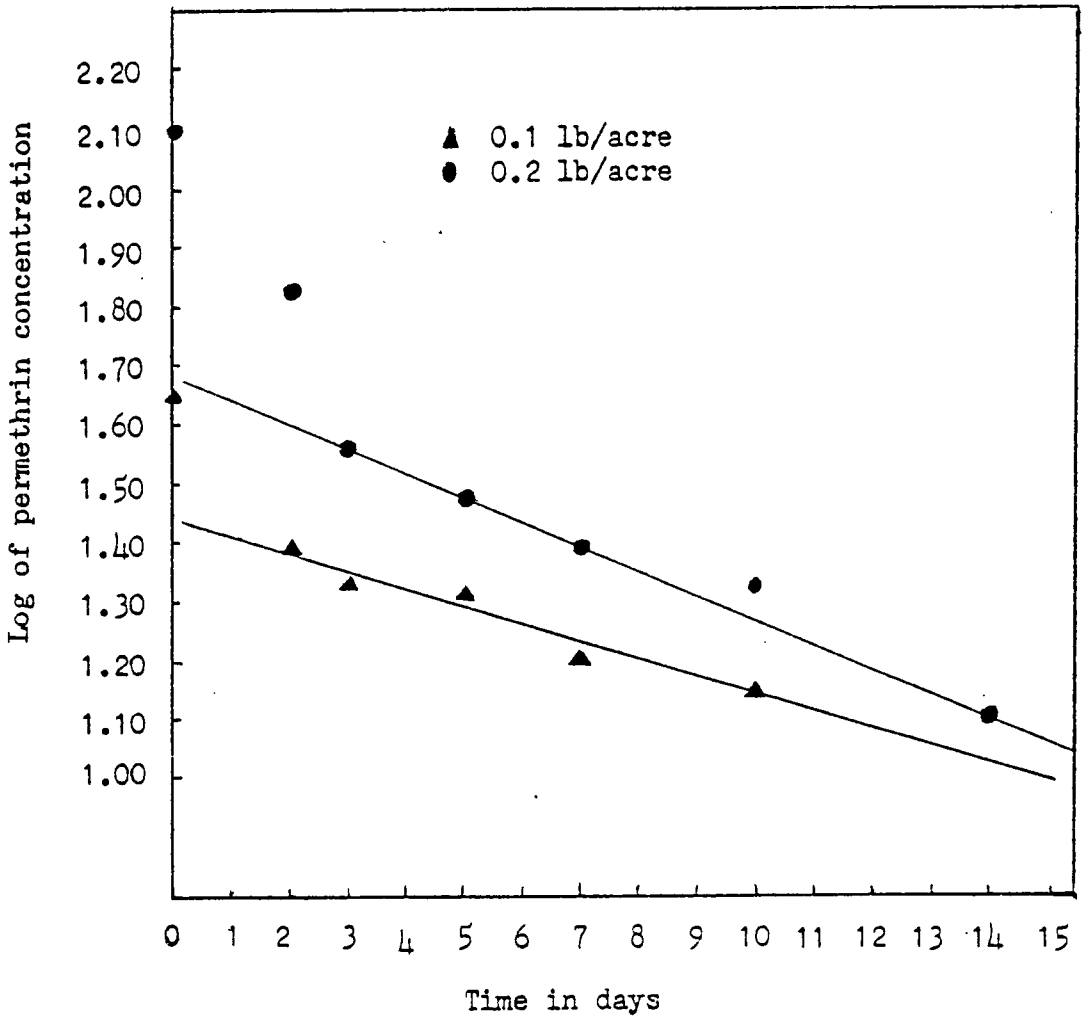


Figure 13. Log of permethrin concentration versus time in days.

sparingly soluble in water must be a contributing factor.

A Duncan's Multiple Range Test was applied to the July 5 and July 31 applications to determine whether there were significant differences between the three spraying rates tested. The test was applied to each day where complete data was available to determine significant differences for those days. The computer calculations showed that for all days, the two chemical treatments were significantly different from the untreated check at the 95% probability level.

CHAPTER VII

SUMMARY

This study indicates that permethrin residues follow two trends. During the first two to three days, permethrin residue underwent rapid loss in which the residue of the 0.1 and 0.2 lb per acre spraying rates declined to similar low levels. The second trend developed after about the third day and showed a much more gradual first order loss rate. These results resemble those of Henzell and Lauren.[43] In considering future application rates, the results of this study indicate that the practice of using greatly increased spray concentrations is not practical for increasing the field life of this insecticide.

Samples collected after rain did not show greatly reduced residue levels from that which might be expected without rain. This conclusion is similar to the one reached by Ruscoe[28] in his study on cabbage.

Due to permethrin's low dermal toxicity[27] and the rapid loss during the first few days after application, health hazards to field workers seem minimal, and reentry time may be as little as 24 hours.

This study indicates that, at least for permethrin, the gc-ms provides a useful analytical tool for the study of insecticide residue. By using the tc-ms one gains the advantage of the separating power of normal gas chromatography plus something a normal gas chromatograph does not have, an identifying detector. In normal

gas chromatography retention time is the only identifying characteristic. By using the gc-ms the operator has retention time and a sensitive, nearly substance specific detector. With the gc-ms one does not worry as much about rigorous cleanup procedures to remove all interferring peaks as one has to do when using electron capture detection.[47] Comparing the detection limits of the gc-ms to electron capture, one finds that electron capture is probably the most sensitive detector known in gas chromatography with detection limits extending into the low picogram range. However, with permethrin the detection limits are about the same.[1]

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VITA

Timothy Edward McCown was born on Otis Air Force Base in Buzzards Bay, Massachusetts on June 11, 1954. His elementary school years were spent in Pensacola, Florida and Birmingham, Alabama. In May 1972 he graduated from Shelby County High School, Columbiana, Alabama. The following fall he enrolled in Auburn University, Auburn, Alabama. After spending two years at Auburn, he transferred to Carson-Newman College, Jefferson City, Tennessee where he earned a B. S. degree in chemistry and history in May 1977. The author entered the Graduate School of the University of Tennessee in the fall of 1977.

Mr. McCown is married to the former Rebekah Hobbs, and they are eagerly awaiting the arrival of their first child in June 1980.