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Title: Diamondback Moth (*Plutella xylostella* L.):

Toxicological Database, Resistance Monitoring

Techniques, and Intraplant Distribution.

Abstract approved: ___*Redacted for Privacy*___

René Feyereisen

The diamondback moth (*Plutella xylostella* L.) is a major pest of crucifer crops worldwide. It is a particularly important problem in Southeast Asia where climatic conditions favor a short generation time and rapid proliferation. In countries such as Thailand, the diamondback moth has apparently become resistant to all classes of insecticides, even the newest ones.

The first part of this thesis is the development of a computer database of all the available information on diamondback moth toxicology. About 1,550 records were extracted from more than 235 published or unpublished documents. These records include information on the geographical location of experiments, the insecticide evaluated, the testing method and the results observed. Most records in the database are from the last decade, and

are predominantly from Taiwan, Thailand, the USA and Japan. Trends in the use of various classes of insecticides can easily be followed. Thirty three records have been found of insecticide resistance of more than a thousand-fold. All the known mechanisms of resistance are found, and the diamondback moth has become resistant to all the classes of insecticides, including *Bacillus thuringiensis* but excluding abamectin. Case studies made possible by the use of the database showed a rapid succession in the use of 29 insecticides in the last 26 years in a single country (Thailand). Although this rapid succession is usually ascribed to the development of resistance, a case study on the regional differences in resistance to five insecticides showed marked variations in the level of resistance over distances of 20 miles or more. The possibility of managing insecticide resistance at the local level, already suggested by studies in Hawaii, is thus supported by this analysis.

In the second part of this thesis, the toxicity of *Bacillus thuringiensis* var. aizawai and of the bacterial toxin abamectin was tested by two bioassay techniques on populations of the diamondback moth from two Chinese kale fields at locations in Thailand at which the highest levels of resistance to most insecticides have been reported. The leaf-dip bioassay was found to be superior to a residue-vial assay or to the FAO-recommended assay. More than 70-fold resistance to *Bacillus thuringiensis* var.

aizawai was observed in the populations of DBM collected from these two fields, suporting the theory that resistance resulted in the control failures reported by growers. Resistance did not explain control failures with abamectin as no resistance to this compound could be detected.

The third part of this thesis analyzes the intraplant distribution of the diamondback moth larvae in an effort to explain the control failures with abamectin on Chinese kale. Extensive sampling of the various larval instars of the diamondback moth revealed that a majority of second instar larvae were sheltered on the young folded leaves of Chinese kale. The majority of fourth instar larvae and pupae were found on mature (lower) leaves. Inefficient spraying practices observed in the field with high volume, low pressure hydraulic pump sprayers may allow the young larvae to escape pesticide exposure possibly explaining control failures in the absence of physiological resistance.

This study shows that resistance in the diamondback moth is a worldwide problem of major proportions. The rapid development of resistance to *Bacillus thuringiensis* in the field is of practical concern. Resistance management programs in developing countries should include mechanisms to optimize insecticide delivery to the protected habitats of diamondback moth on Chinese kale.

Diamondback Moth (*Plutella xylostella* L.);

Toxicological Database, Resistance Monitoring

Techniques, and Intraplant Distribution

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INTRODUCTION

The diamondback moth (*Plutella xylostella* L. Family name: Plutellidae, Order: Lepidoptera, hereafter called DBM) is a cosmopolitan insect pest of crucifer crops including Chinese kale, *Brassica oleracea* L. var. acephala (Harcourt 1957, Rushtaprakornchai and Vattanatungum 1989 and 1990). Control failures in the field and resistance to numerous insecticides have been reported in many documents, for instance: Ankersmit (1953), Feng et al. (1978), Miyata et al. (1982), Rushtaprakornchai and Vattanatungum (1989), Tabashnik et al. (1990), and elsewhere (see details in the chapter "DBM Toxicological Database" ,page 30). The first reference and abstract compendium for the DBM was published in 1985 by The Asian Vegetable Research and Development Center, Shauhua, Taiwan and titled "The Annotated Bibliography of Diamondback Moth". This document contains approximately 1,200 abstracts in the areas of biology, physiology, ecology, chemical control, microbial control, biological control, toxicology, insecticide resistance, and communication by pheromones. However, the toxicological

data in this publication are not easily accessible, nor have they been analyzed for trends and patterns. Such an analysis can be useful in understanding past failures of insecticide control of this pest. It also can be useful in developing resistance management programs by documenting resistance development and occurrence.

Modern personal computer hardware and database management software makes possible the development of searchable databases from large information sets (Theiling 1987). In this thesis, the DBM toxicological database has been developed. It includes data from published and unpublished documents over the past 50 years. This database provided means to count, index, sort, search, and link data in each field of the subdatabase files. These fields are: code number, reference source, availability of hard copy, country of experiment, year of experiment, insecticide or chemical name, insecticide or chemical class, method of experiment, type of response, amount of response, response unit, resistance ratio, resistance mechanism, cross or multiple resistance insecticide, and comments.

The principal objectives of making a toxicological database for DBM were: (1.) to collect and group scattered toxicological data into a comprehensive information base accessible for analysis (2.) to characterize the data into their respective fields of content (above) (3.) to facilitate

the acquisition of data concerning specific questions or case studies.

One of the case studies (in the chapter DBM Toxicological Database, page 55) that addressed the question "What is the extent of insecticide resistance in DBM to phenthoate, cartap, cypermethrin, teflubenzuron, and *Bacillus thuringiensis*, var. kurstaki in different parts of Thailand?" identified two provinces named Bang-Kae and Bang Bua-Thong in central Thailand where DBM was highly resistant to these insecticides. Therefore, the field aspects of this study were located in commercial fields of these provinces.

Resistance monitoring techniques employed in this study were evaluated on DBM larvae collected from these two provinces. Although FAO (Food and Agriculture Organization) has recommended the topical bioassay method for detection and measurement of resistance in DBM since 1979 (FAO Plant Protection Bulletin 1979), it does not allow for the evaluation of stomach poison insecticides such as *Bacillus thuringiensis* (Tabashnik & Cushing 1987). Therefore, two resistance monitoring techniques, a residue-vial bioassay (Magaro & Edelson 1990) and a leaf-dip bioassay (Tabashnik & Cushing 1987) were modified for use in this study. Their methodologies are located in "Resistance Monitoring Techniques", page 12.

Insect control failures in commercial agriculture can be the result of factors other than resistance to insecticides. One reason for failure may be behavioral characteristic(s) such as the location of feeding and resting areas of the target insect on a host plant (Vegkrit 1985, Rushtaprakornchai and Vatanatungum 1989). Therefore, field observations on the intraplant distribution of immature stages of the DBM were made in Bang-Kae and Bang Bua-Thong provinces, where commercial growers of Chinese kale reported failures of both *Bacillus thuringiensis* and abamectin in controlling DBM. The distribution pattern of DBM larval and pupal stages on Chinese kales may relate to control failures in both fields after applications of insecticides at the recommended field rate.

MATERIALS AND METHODS

DBM Toxicological Database

Hardware and software

The personal computer IBM PS/2 55SX (IBM Corporation) run at 16 Mhz, 60 Mbytes hard disk, and 2 Mbytes RAM was used for input, storage, retrieval, and analysis of data. The database management software was dBASE IV (Ashton-Tate Corporation).

Searching documents

Published and unpublished toxicological documents concerning DBM were searched from Cambridge Life Science (series of compact disks database), Agricultural Science (series of compact disks database), The Oregon State University library, The University of Arizona library, The Kasetsart University library (Thailand), Diamondback moth management, The Asian Vegetable Research and Development Center (Taiwan), Annotated Bibliography of Diamondback moth, and also provided by René Feyereisen (present address: Department of Entomology, University of Arizona, Tucson, AZ.).

Structure of the DBM Toxicological Database

The toxicological database was constructed to contain two subdatabase files located in catalog (dBASE IV function) called DBM.CAT. The first subdatabase file is the reference source file called PX-SOURC.DBF which contains all the bibliographic information concerning <u>documents</u> listed in the database. I define in this thesis a <u>document</u> as a publication in the scientific literature (book, journal article, published conference proceedings, or a printed government report, or an unofficial letter). In PX-SOURC.DBF each document is characterized by 4 fields covering code number (CODE), reference source (SOURCE), (i.e. the full bibliographic information concerning document), availability of hard copy (HARDCOPY), and comments (COMMENTS). Because the database is an evolving working tool, updates of some fields in the reference source file is possible. For instance, a particular unpublished manuscript can become a published journal article. In that case the reference source can be updated, but the document retains the same code number. The second subdatabase file is the data file called PX-DATA.DBF which contains all the information about a particular <u>record</u> listed in the database. I define in this thesis a <u>record</u> as the smallest independent information set contained in a document. For instance, one document may report the toxicity of 2 insecticides to 5 different populations of DBM.

There would thus be 10 different records in that document. The data subdatabase file (PX-DATA.DBF) includes 13 fields referencing: code number (CODE), country of experiment (COUNTRY), year of experiment (YEAREXP), chemical or insecticide name (INSTICIDE), class of chemical or insecticide (INSTCLASS), method of experiment (METHOD), type of response (RESPTYPE), amount of response (RESPDOSE), response unit (RESPUNIT), resistance ratio (RESISRATIO), resistance mechanism (RESISMECHM), information on insecticides with cross or multiple resistance (CROSSRESIS), and comments (COMMENTS). The fields in both subdatabase files are described in Table 1 and 2.

Recording data

Recording of the toxicological documents was done in the catalog named DBM.CAT. Reference sources were first recorded in the reference source subdatabase file (PX-SOURC.DBF) and given code numbers (labeled on the first page of hard copies to allow quick retrieval). Then the data extracted from the toxicological documents were separated into records and stored in the data subdatabase file (PX-DATA.DBF). After all data were completely entered, a double checking process was performed by manual verification to insure the consistency of data input pattern and to allow for the correction of errors.

Table 1. Structure of the reference source subdatabase file (PX-SOURC.DBF).

No.	Field Name	Field Type	Width	Index	Field Description
1	CODE	Character	6	Y	Code number for linking the subdatabase files to each other and to the collection of hard copies
2	SOURCE	Character	254	N	Reference source
3	HARDCOPY	Character	1	Y	Availability of hard copies
4	COMMENTS	Character	50	N	Comments

Table 2. Structure of the data subdatabase file (PX-DATA.DBF).

No.	Field Name	Field Type	Width	Index	Field Description
1	CODE	Character	6	Y	Code number for linking the subdatabase files to each other and to the collection of hard copies
2	COUNTRY	Character	30	Y	Country of experiment
3	YEAREXP	Character	7	Y	Year of experiment
4	INSTICIDE	Character	80	Y	Insecticide or chemical name(s)
5	INSTCLASS	Character	40	Y	Insecticide or chemical class(s)
6	METHOD	Character	50	N	Method of experiment
7	RESPTYPE	Character	66	Y	Type of response
8	RESPDOSE	Character	12	Y	Amount of response
9	RESPUNIT	Character	30	N	Response unit
10	RESISRATIO	Character	10	Y	Resistance ratio
11	RESISMECHM	Character	100	N	Resistance mechanism
12	CROSSRESIS	Character	150	N	Insecticides showing cross resistance or multiple resistance
13	COMMENTS	Character	50	N	Comments

Database management

The database management was performed with the dBASE IV control center which contains the data application function (the inputting device) and queries application function (the searching device). The two subdatabase files (PX-SOURC.DBF and PX-DATA.DBF) are located in data application. This application allows to record data into the record fields. The two subdatabase files were linked together by code number in the code number field (CODE) and placed into a file named PX-LINK.QBE, located in the queries application. This configuration allows the user to quantify, search, and sort data in each record field.

Characterization and analysis of the database were performed with the PX-LINK.QBE file. Data in both subdatabase files (reference source and data files) were characterized by the attribution of documents, number of records, country of experiment, insecticide name, insecticide class, method of experiment, type of response, and resistance mechanism. The resistance data were attributed an arbitrary resistance index which is based on the resistance ratio (Table 3). Because the level of resistance can be extremely variable from one DBM population to another, and because resistance is a biological response, I chose to express the resistance ratio on a logarithmic scale. The arbitrary resistance index

Table 3. An arbitrary resistance index for the degree of resistance based on the resistance ratio.

RR[1]	Degree of resistance	Resistance index
RR > 1,000	Extremely high	4
1,000 > RR > 100	High	3
100 > RR > 10	Moderate	2
RR < 10	Low	1

[1] RR: Resistance ratio (LD_{50} or LC_{50} resistant strain divided by LD_{50} or LC_{50} susceptible strain)

permits a rapid overview of resistance levels in DBM populations.

Case studies

Two case studies are presented in this thesis. The first case study, pattern of insecticide use against DBM in Thailand from 1965 to 1990 (as evidenced by published and unpublished records) was performed with the queries application function and saved in a file named PX-CASE1.QBE. The data in the PX-DATA.DBF file were scanned both in the country of experiment record field by the command: Thailand, and in the year of experiment record field, by the command: >1964 and < 1991.

The second case study, what is the degree of resistance of DBM to phenthoate, cartap, cypermethrin, teflubenzuron, and *Bacillus thuringiensis*, var. kurstaki in different parts of Thailand ? was performed with the queries application function and stored in a file named PX-CASE2.QBE. The data in PX-DATA.DBF file were scanned both in the country of experiment record field by the command: Thailand, and in the insecticide name record field by the command: phenthoate, cartap, cypermethrin, teflubenzuron, and *Bacillus thuringiensis*, var. kurstaki.

Resistance Monitoring Techniques

Insects

Two hundred DBM larvae (mostly third and fourth instar) were collected from each of two commercial Chinese kale fields in Bang-Kae and Bang Bua-Thong provinces, respectively (field characteristics are described in the chapter "Intraplant Distribution"). Collected larvae were brought to the laboratory in Styrofoam boxes and were reared following the technique described on next page. Only fourth instar larvae from the first generation of the laboratory population were used in the resistance monitoring bioassays.

Chemicals

The following chemicals were used : Abamectin (Agrimec™ 1.8% EC., Agvet., Div. of Hertz Co.Ltd., Thailand. Imported from MSD Agvet., Div. of Merck & Co.,Inc., Netherlands) which contains Avermectin B_1: a mixture of Avermectins containing more than 80% of Avermectin B_{1a} and less than 20% of Avermectin B_{1b} (Agri-Mek 1987); *Bacillus thuringiensis*, var. aizawai, Serotype 7 (Florbac™ FC, Thep Watana Co.Ltd.,Thailand. Imported from Duphar B.V., Crop Protection Div., Netherlands) which contains 7,500 International Units per milligram of spore and crystalline delta-endotoxin; Wetting agent (Emulphor™ ,

GAF Chemical Corp.), a polyoxyethylated vegetable oil (non-ionic surfactant).

Mass rearing technique

The mass rearing technique used in this study was modified from Koshihara and Yamada (1976), Yamada and Koshihara (1978), Liu and Sun (1984). The minor modifications involve the use of Chinese kale seeds instead of rapeseed, and the use of metal instead of plastic containers for adult rearing. Chinese kale was the only food source used in order to standardize nutritional factors that may affect DBM toxicology (Gordon 1961).

Chinese kale seeds were soaked in distilled water over night, then transferred to 2" x 24" x 24" soil trays. Fifteen days after germination, the young plants were individually placed in 4" x 4" x4" plastic boxes with soil. Plants were watered once a day during the growing period.

DBM larvae were reared in larval rearing container (hard plastic boxes sized 4" x 8" x 12" with 100 mesh screen covers). The larvae were held under 12:12 (L:D), 65 ± 10 RH, and 27 ± 5 °C with air circulation in a greenhouse. Pupae were collected daily and kept at 8 °C (to delay/synchronize adult emergence) for 2-3 days. Fifty pupae were placed into the adult cage or oviposition cage (10" x 10" x 16" metal case) containing 1% sucrose solutions in distilled water and thirty-day-old live Chinese

kale in 4" x 4" x4" plastic boxes with soil. Watering was not necessary. Three days later, the adults emerged and laid eggs on the Chinese kale plants. The Chinese kale plants with DBM eggs were transferred into the larvae rearing container. Two to three days later, the eggs hatched, and the first instar larvae mined and fed inside the kale leaves. The kale leaves were placed in larval rearing container daily after the larvae molted into second instar.

Test for suitable concentrations of wetting agent for the residue-vial bioassay and for the leaf-dip bioassay

A 1% crystal violet solution with a series of wetting agent concentrations (1.0, 2.5, 5.0, 7.5, 10.0 ppt.) in acetone solvent (for residue-vial bioassay) and a similar series in distilled water (for leaf-dip bioassay) were prepared to determine the suitable concentration of wetting agent for each bioassay technique. For the residue-vial bioassay, 20 ml. of prepared solutions were transferred into a 30 ml. glass vial, capped and rolled for 10 sec. Vials were then emptied and air dried for 2 hours. For the leaf-dip bioassay, 6.5 cm. diameter Chinese kale leaf disks were dipped into prepared solutions for 5 sec. and the vials or leaf disks were left to dry for 2 hours, after which time the results were recorded. The suitable concentrations of wetting agent for the residue-vial bioassay and for the leaf-dip bioassay were 5.0 and 2.5 ppt., respectively. Lower

concentrations of the wetting agent caused inadequate coating as judged by the crystal violet (results not shown).

Compatibility tests of insecticides, wetting agent, and solvent

Mixtures of insecticide, wetting agent, and solvent (in Table 4) were prepared to test for physical compatibility. The results were recorded after 24 hours and showed that abamectin was compatible with acetone, distilled water, and wetting agent while *Bacillus thuringiensis* was incompatible with acetone but compatible with distilled water and wetting agent. A possible reason for the incompatibility of *Bacillus thuringiensis* with acetone may be that *Bacillus thuringiensis* contains spores and crystalline proteins which can be denatured by acetone.

Leaf-dip bioassay

6.5 cm. diameter leaf tissue disks were cut from unsprayed thirty-day-old Chinese kale leaves. Individual leaf disks were randomly selected and dipped into insecticide solutions for 5 sec. and air dried for 2 hours at room temperature. Each leaf disk from the respective treatments was placed on 9 cm. diameter filter paper in an 11 cm. diameter plastic Petri dish. Ten fourth instar larvae were placed in each Petri dish at 12:12 (L:D), 65 ± 10 RH, and 27 ± 5 °C with air circulation in a greenhouse. Mortality

Table 4. Compatibility tests of insecticide, wetting agent and solvents.

Mixtures		Results
Abamectin 1.8% EC	10 ml.	Miscible emulsions
Wetting agent[1]	10 ml.	
Distilled water to	1000 ml.	
Bt.[2] 7,500 IU./mg FC	100 ml.	Suspensions after shaking
Wetting agent	10 ml.	
Distilled water to	1000 ml.	
Abamectin 1.8% EC	1 ml.	Miscible emulsions
Wetting agent	1 ml.	
Acetone AR to	50 ml.	
Bt. 7,500 IU./mg FC	2 ml.	Caking
Wetting agent	1 ml.	
Acetone AR to	50 ml.	

[1] Polyoxyethylated vegetable oil

[2] Bt.: *Bacillus thuringiensis* var. aizawai

was recorded after 96 hours (see below). Larvae were considered to be dead if they did not move when probed with a hair tip.

Residue-vial bioassay

30 ml. glass vials were cleaned with detergent, rinsed 3 times with distilled water, finally rinsed with acetone and air dried overnight before being used in tests.

20 ml. of insecticide solutions was transferred into 30 ml. vials. The vials were capped, rolled for 10 sec., emptied, and air dried for 2 hours (acetone solvent) or 24 hours (for distilled water solvent). Ten fourth instar larvae were placed in each vial and kept at 12:12 (L:D), 65 ± 10 RH, and 27 ± 5 °C with air circulation in a greenhouse. Mortality was recorded after 24 hours (see below). Larvae were considered to be dead if they did not move when probed with a hair tip.

Recording mortality: Optimum time

Bacillus thuringiensis and abamectin were prepared at the concentrations of 5 mg AI per ml (1 mg AI = 7,500 International Units) and 0.0125 ppm. in water, respectively for leaf-dip bioassay (see details in the section "Leaf-dip Bioassay"). *Bacillus thuringiensis* was prepared at a concentration of 200 mg. per ml in water and abamectin was prepared at a concentration of 0.5 ppm. in acetone for

residue-vial bioassay (see details in the section "Residue-vial Bioassay"). Thirty and ten fourth instar larvae from Bang-Kae population were used for treatment and control, respectively. The cumulative mortalities were recorded at 12 hour intervals through 108 hours. Maximum mortality of fourth instar larvae for *Bacillus thuringiensis* and abamectin in the leaf-dip bioassay was observed 96 hours after treatment (Figure 1) and 24 hours after treatment in the residue-vial bioassay (Figure 2). From this experiment, 96 and 24 hours were hypothesized to be the optimum time to record mortality in the leaf-dip bioassay and the residue-vial bioassay, respectively. High mortality in controls in the residue-vial bioassay after 36 hours is explained by starvation and desiccation.

Preliminary tests

Bacillus thuringiensis and abamectin were evaluated for larval control on two DBM populations from Bang-Kae and Bang Bua-Thong provinces with the two different bioassay techniques (leaf-dip and residue-vial bioassays). Each treatment was replicated three times. Data were analyzed by Probit or Logit analysis computer software (Russell et al. 1977). The objectives of these preliminary tests were to estimate approximately LC_{10}, LC_{80}, LC_{85},

Figure 1. Cumulative percent mortality of fourth instar DBM larvae over time for Bacillus thuringiensis var. aizawai and abamectin in the leaf-dip bioassay.
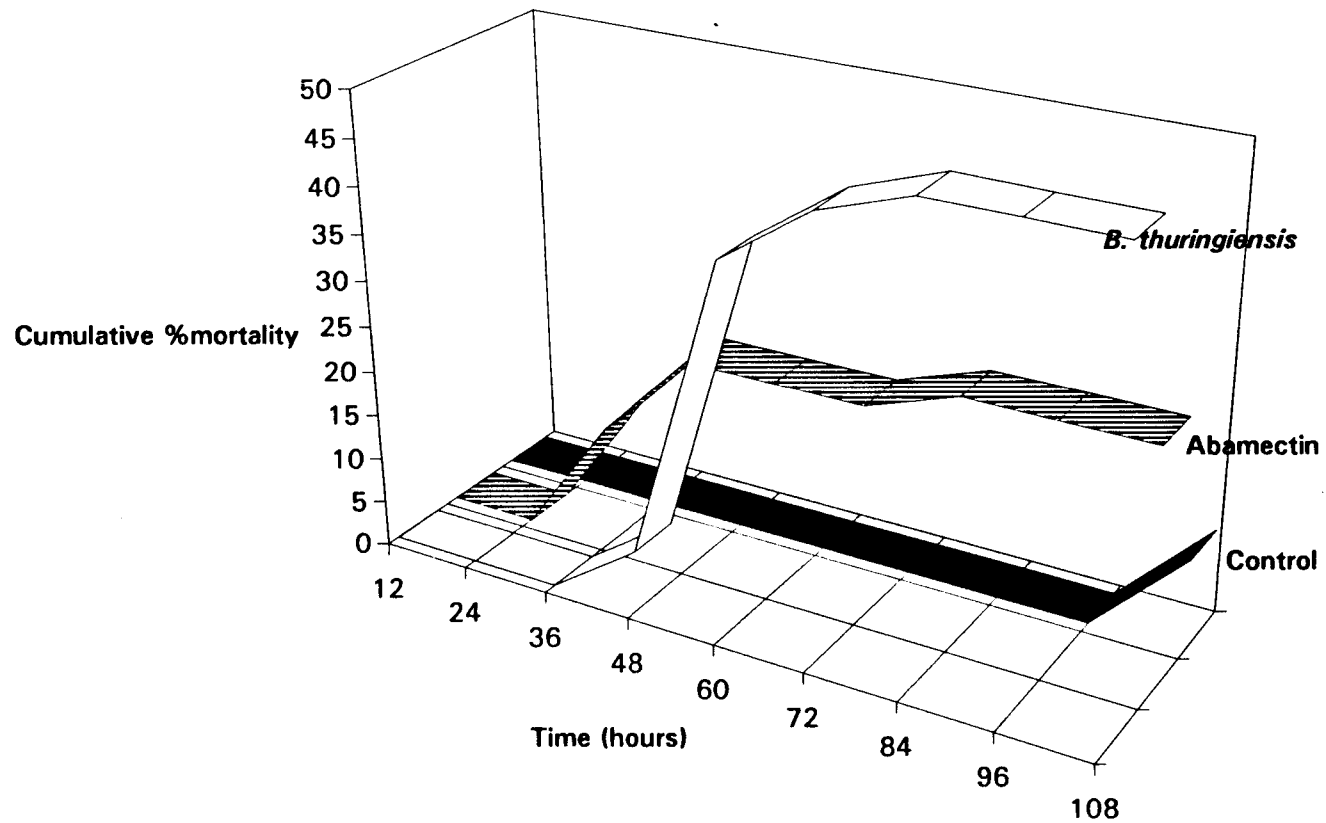
Figure 2. Cumulative percent mortality of fourth instar DBM larvae over time for abamectin in the residue-vial bioassay.
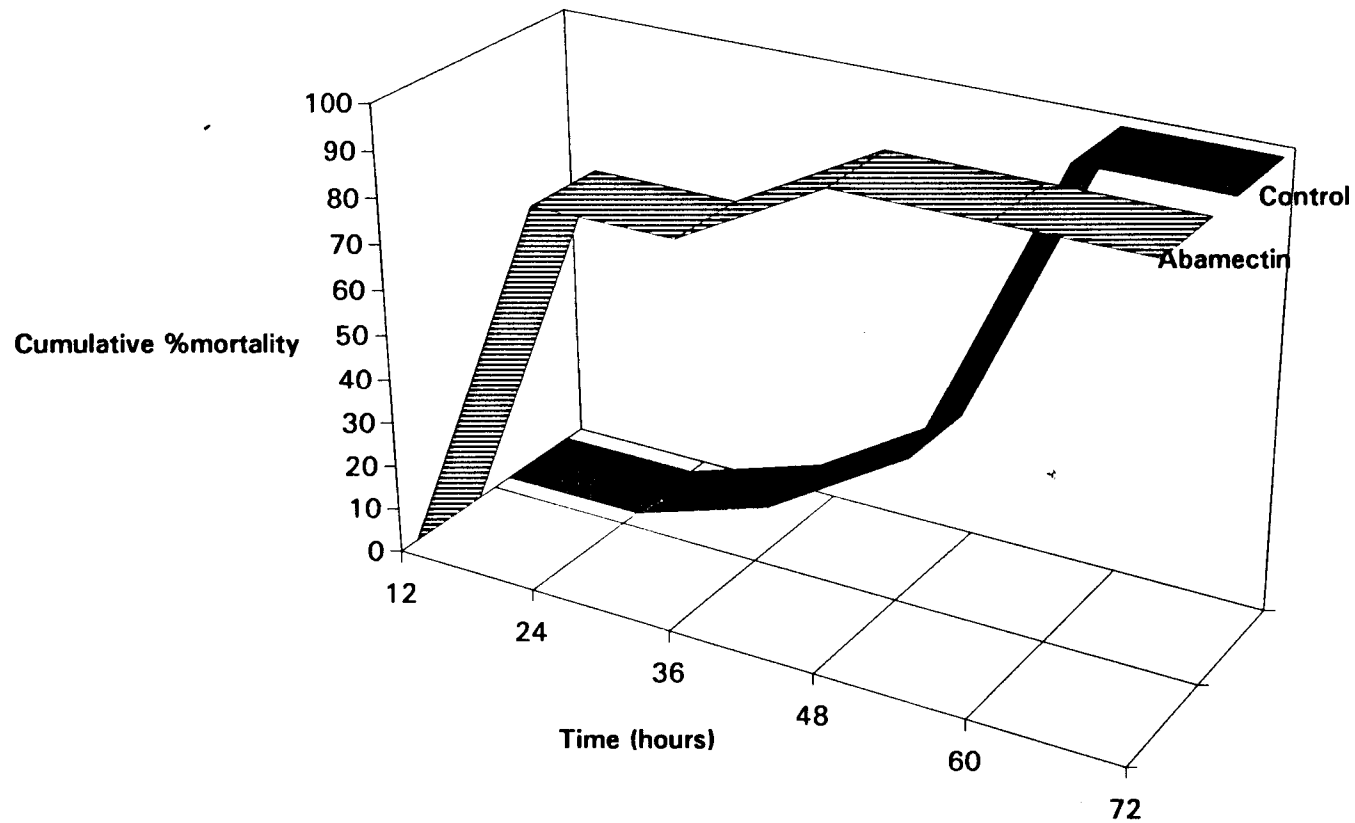
LC_{90}, and LC_{95} to be used in the final test (Robertson et al. 1984, Roush and Miller 1986, Robertson and Worner 1990).

Final tests

Bacillus thuringiensis and abamectin were prepared at concentrations of approximately LC_{10}, LC_{80}, LC_{85}, LC_{90}, and LC_{95} , respectively (LC values from the preliminary tests) and tested on two DBM larval populations (Bang-kae and Bang Bua-Thong) with two different bioassay techniques (leaf-dip and residue-vial bioassays). Each treatment was replicated five times. Data were analyzed by Probit or Logit analysis computer software (Russell et al. 1977).

Intraplant Distribution

Field characteristics

In October 1990 two commercial growers of Chinese kale in Bang-Kae and Bang Bua-Thong provinces located 20 and 40 kilometers northeast of Bangkok, respectively, reported control failures after applications of commercial formulations of *Bacillus thuringiensis*, var. aizawai and abamectin at recommend field rates (Growers, personal communications). Therefore a field in each province was rented from December 1990 to February 1991 to observe the intraplant distribution of immature stages of DBM. No pesticides were used in either field for the duration of the study. The Bang-Kae field, 8 kale growing beds, was divided into 104 plots (13 plots per bed), each plot sized 5 meters wide and 6 meters long (see Table 5 for details). Fifty six plots were selected to observe the intraplant distribution of the immature stages DBM (see Figure 3 for details). The Bang Bua-Thong field, 10 kale growing beds, was divided into 130 plots (13 plots per bed), each plot sized 5 wide and 6 meters long (see Table 5 for details). Seventy plots were selected to observe the intraplant distribution of the immature stages DBM (see Figure 4 for details).

Table 5. Field characteristics of the two Chinese kale study sites.

Characteristics	Bang-Kae	Bang Bua-Thong
Field size (ha.)	1	1.5
Number of beds	8	10
Bed size (m.)	5 by 78	5 by 80
Furrow	1 meter depth water	
Kale density	120 plants per 30 m^2	
Kale rows/bed	10 (0.5 m. wide and 78 m. long)	
Wind direction	typically southwest to northeast	
Wind speed (Km. per hour)	0-4	0-7
Temp. (°C)	18-34	18-36
Growing period	50 ± 3 days	
Planting pattern	Kales were transplanted when 30-day-old	
Irrigation	Once daily by hydraulic pump on small boat pulling water from furrow and spraying by two large nozzles (low pressure and high volume sprayers)	
Traditional Insecticide application method	Insecticides were mixed with water on the boat and sprayed with the same equipment used for irrigation	

Figure 3. Design and construction of the sampling plots in Bang-Kae field.
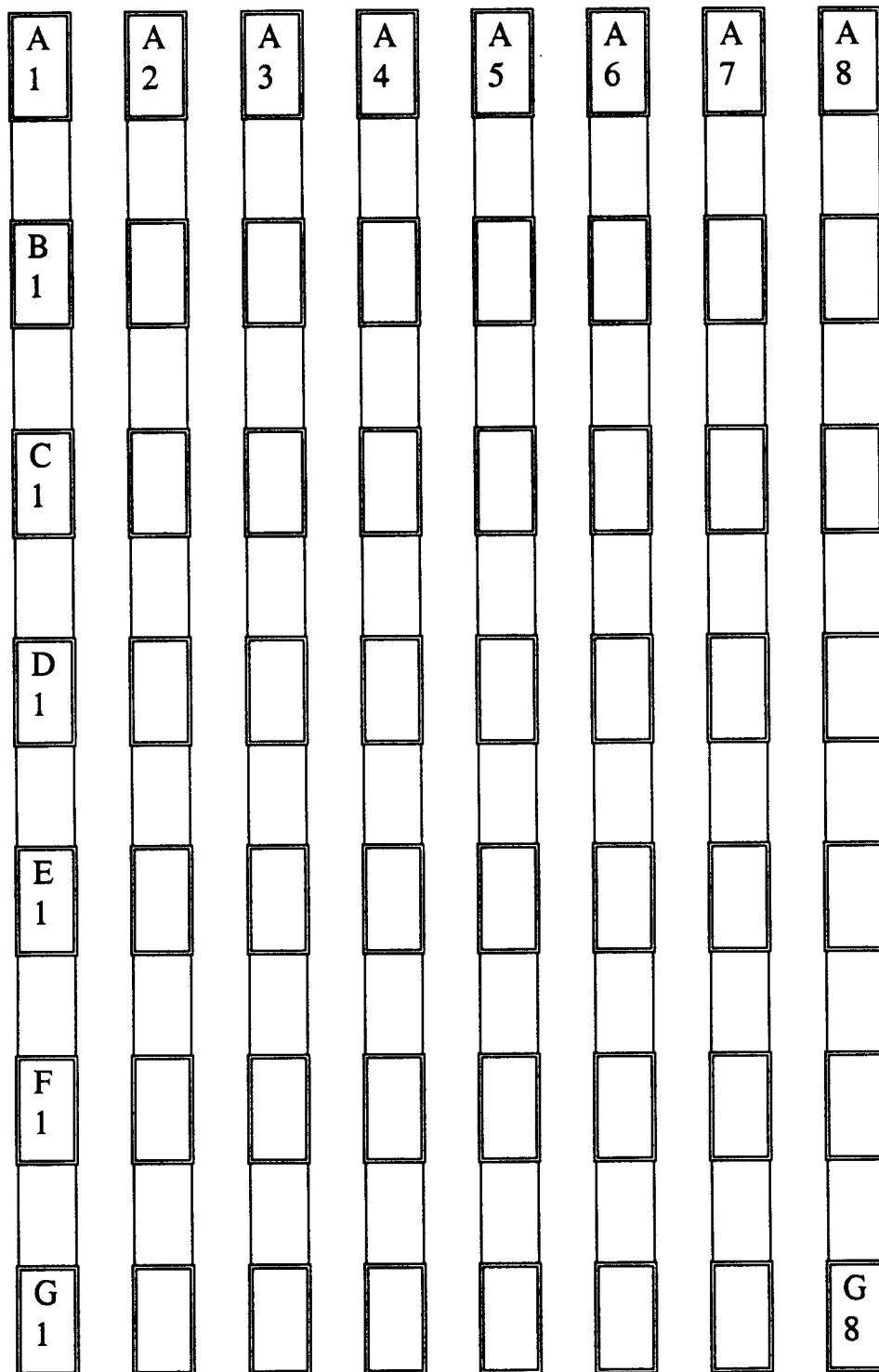
Note : Double line boxes indicate sampling plots

Figure 4.   Design and construction of the sampling plots in Bang
Bua-Thong field.
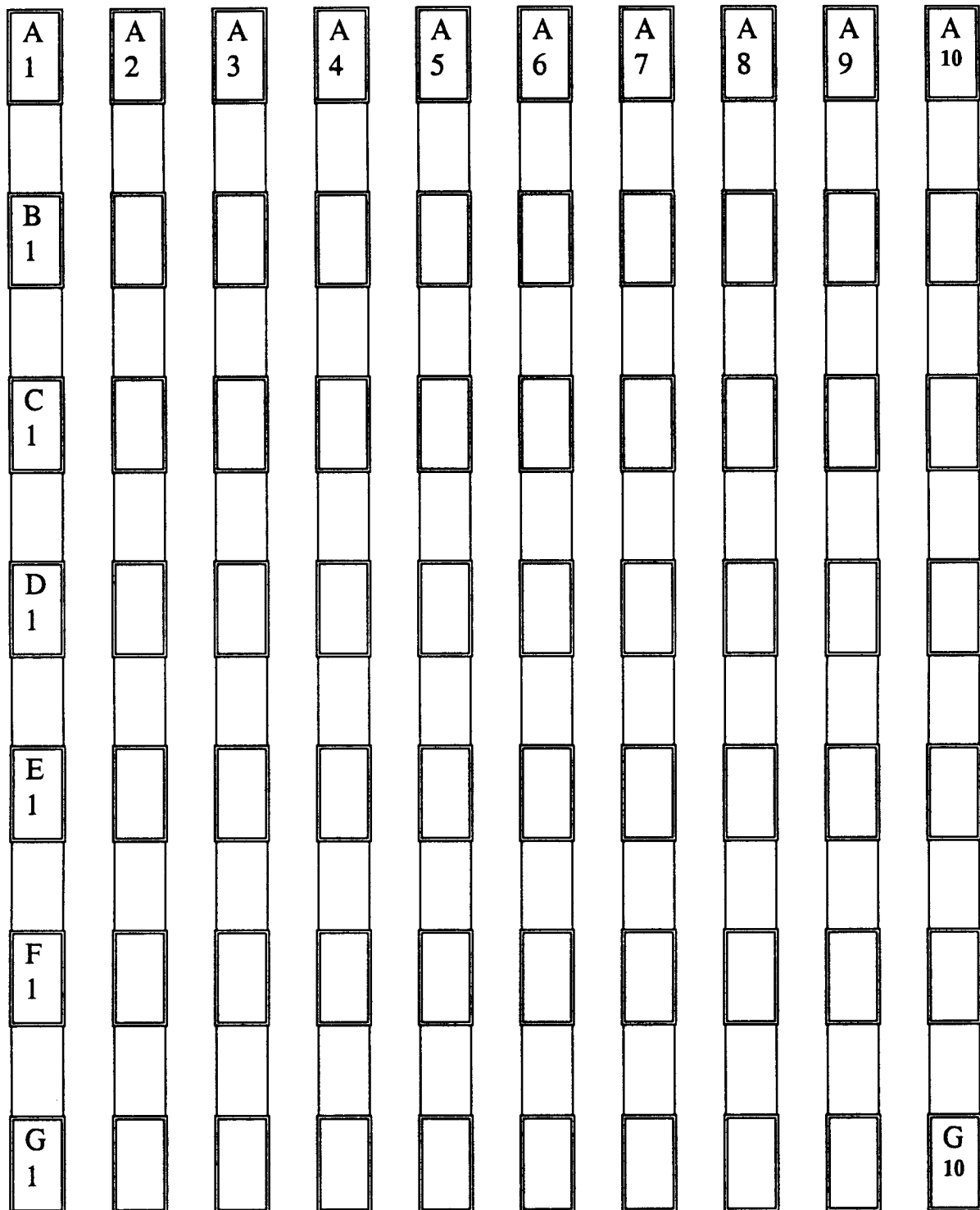
Note : Double line boxes indicate sampling plots

Pre-sampling experiment

The objective of this experiment was to determine an appropriate sample size. Literature reports the distribution of the immature stages of DBM as negative binomial or clumped, that is to say that individuals aggregate in certain areas of the field (Harcourt 1960, Chen & Su 1980, Kirby & Slosser 1981, and Sivaprakasam et al. 1986). The quantitative estimates of the immature stages of DBM populations are evidently improved by partitioning the sampling universe (one kale growing bed) into strata (sampling plots). Therefore the sampling universe chosen consisted of seven sampling plots, each sized 5 meters wide and 6 meters long (Barrett 1975). The total number of Chinese kale plants in the sampling universe (N) and the total number of Chinese kale plants in each strata or sampling plot (N_i) were measured. Each Chinese kale in each sampling plot was examined top to bottom, leaf by leaf to record the number of second, third, fourth instar and pupae of DBM (sampling organism). First instar larvae and eggs are too small and could not be counted in such a large scale experiment. Then the standard deviations of sampling organism in each sampling plot (s_i) were calculated. The total sample size required (n) was estimated by the formula in Appendix 1 which gave n equal to 42 Chinese kale plant per 7 sampling plots or 6 Chinese kale plant per plot (an appropriate sample size), (Cochran 1963, Barrett 1975).

Sampling experiment

Trips to the two fields were made at four ages of plant growth (Table 6). Each trip 336 and 420 Chinese kale plants were randomly selected from 56 sampling plots in Bang-Kae field and 70 sampling plots in Bang Bua-Thong field, respectively. Each selected kale plant was examined leaf by leaf to record the total number of second, third, fourth instar larvae and pupae of DBM (sampling organism) and to document their respective distributions on either young folded leaves or mature leaves (open leaves). These numbers were then transformed to distribution ratios [the number of individuals on young folded leaves divided by total number of individuals (on young folded and on mature leaves)] for analysis of their distribution. For instance, the distribution ratio of second instar larvae is the number of second instar larvae on young folded leaves divided by total number of second instar larvae (on young folded leaves and on mature leaves).

Table 6. Calendar dates and age of Chinese kale at different sampling times.

Trip	Plant growth stage	Bang-Kae (date)	Bang Bua-Thong (date)
1	35-day-old	01-16-91	01-13-91
2	40-day-old	01-21-91	01-18-91
3	45-day-old	01-26-91	01-23-91
4	50-day-old	01-31-91	01-28-91

RESULTS

<u>DBM toxicological Database</u>

DBM toxicological database management was performed to characterize each record field (Tables 1. and 2.) and to analyze data between record fields. These included the current 235 recorded documents in the reference source subdatabase file (PX-SOURC.DBF) and 1,556 records in data subdatabase file (PX-DATA.DBF). The record fields: reference source, country of experiment, insecticide, insecticide class, method of experiment, and response type were characterized by time trend curves to show how different aspects of insecticide use had progressed. The analysis of data between record fields follows the characterization of each record field in the results section of this thesis. Finally, two case studies were prepared to illustrate uses of this database.

Database management

Characterization of the number of records in reference source and data subdatabase files

The distribution of the number of recorded documents over time was estimated over five year intervals (Figure 5). The numbers of recorded documents increased after 1970, with a sharp upturn in slope after 1980. The rate of

Figure 5. Number of documents in the DBM toxicological database, chronological distribution during 5 year intervals.
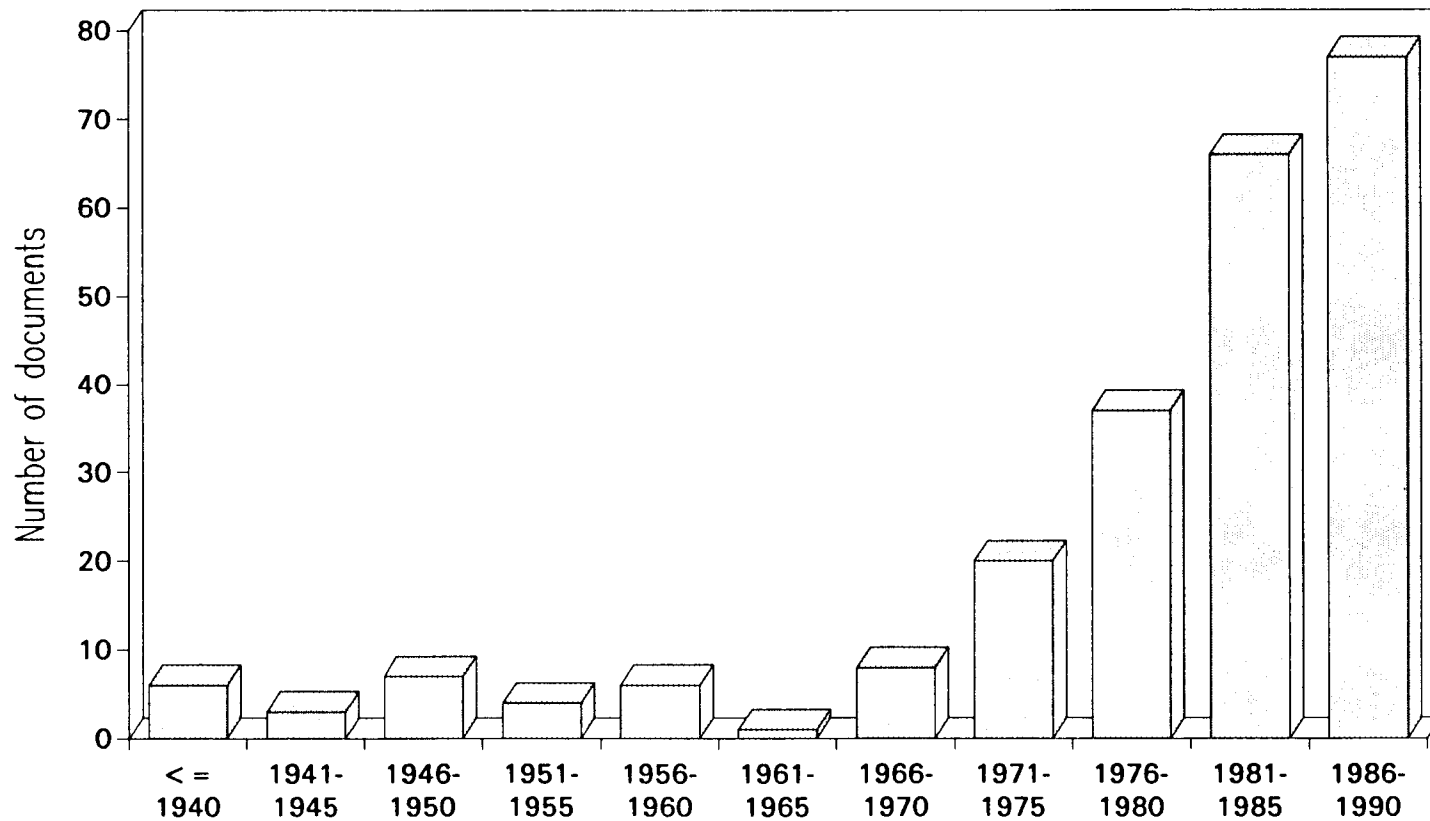
increase of recorded documents was negligible from 1940 to 1970, then rose from 1971 to 1990 (end of the current record).

The number of recorded data over five year intervals (Figure 6) shows that the number of records began to rise after 1965, with a sharp upturn in slope after 1980. The distribution pattern of the number of recorded documents and recorded data over time are similar, except for the increasing rate of record accumulation. Therefore the increasing rate of recorded data is more than that of recorded documents. Either the informational content of newer documents is greater or they contain data that fit the database structure better than the older documents.

Characterization of the number of records by country

The most numerous records in the DBM toxicological database came from Taiwan (572 records), Thailand (311 records), The United States of America (307 records), and Japan (219 records). The distribution of the number of records for each country in the DBM toxicological database is shown in Figure 7.

Characterization of the number of records by insecticide and insecticide class

The most commonly recorded insecticides in the insecticide record field were extracted from the database

Figure 6. Number of records in the DBM toxicological database, chronological distribution during 5 year intervals.
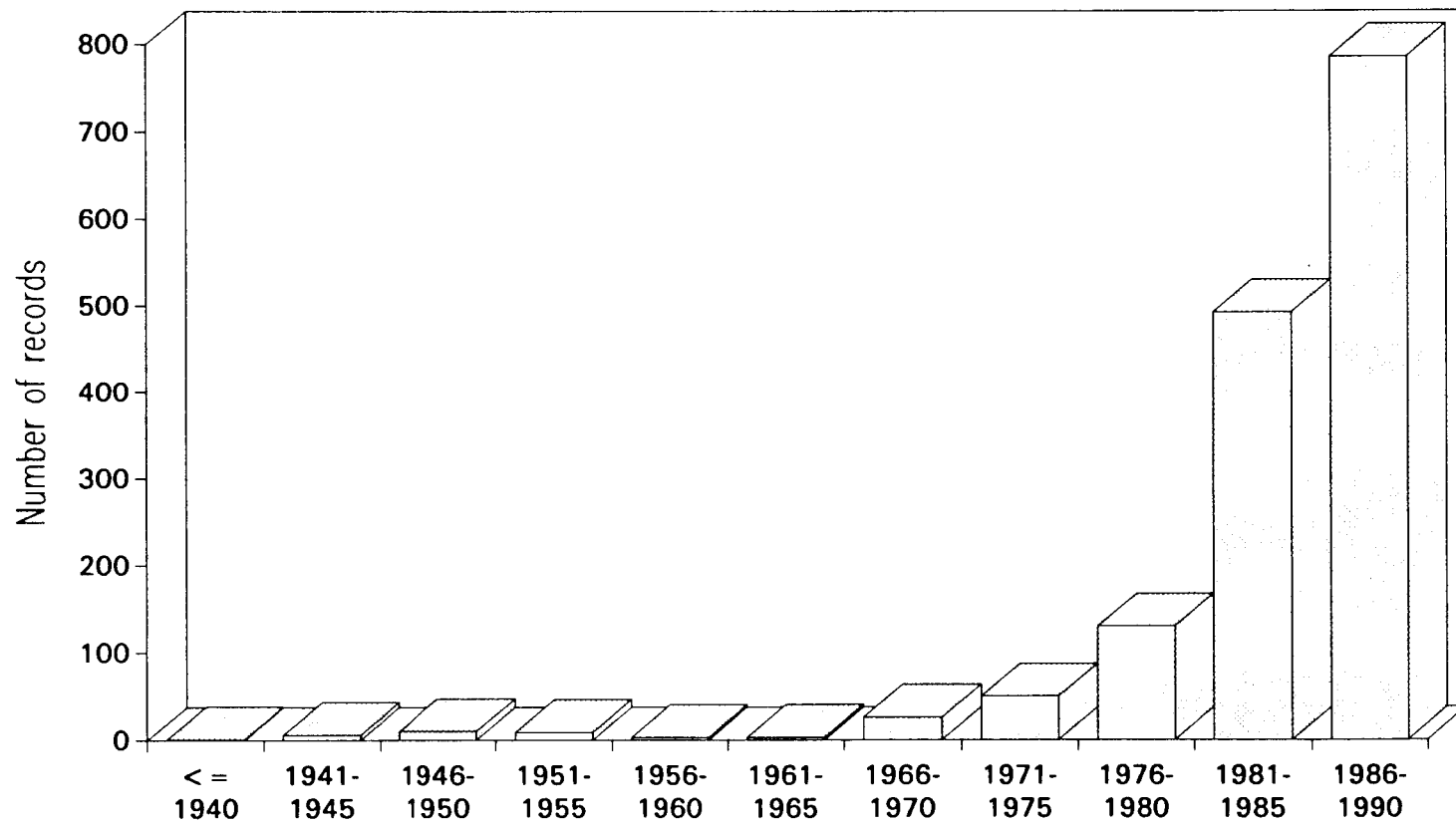
Figure 7. Distribution of the number of country records in the DBM toxicological database.
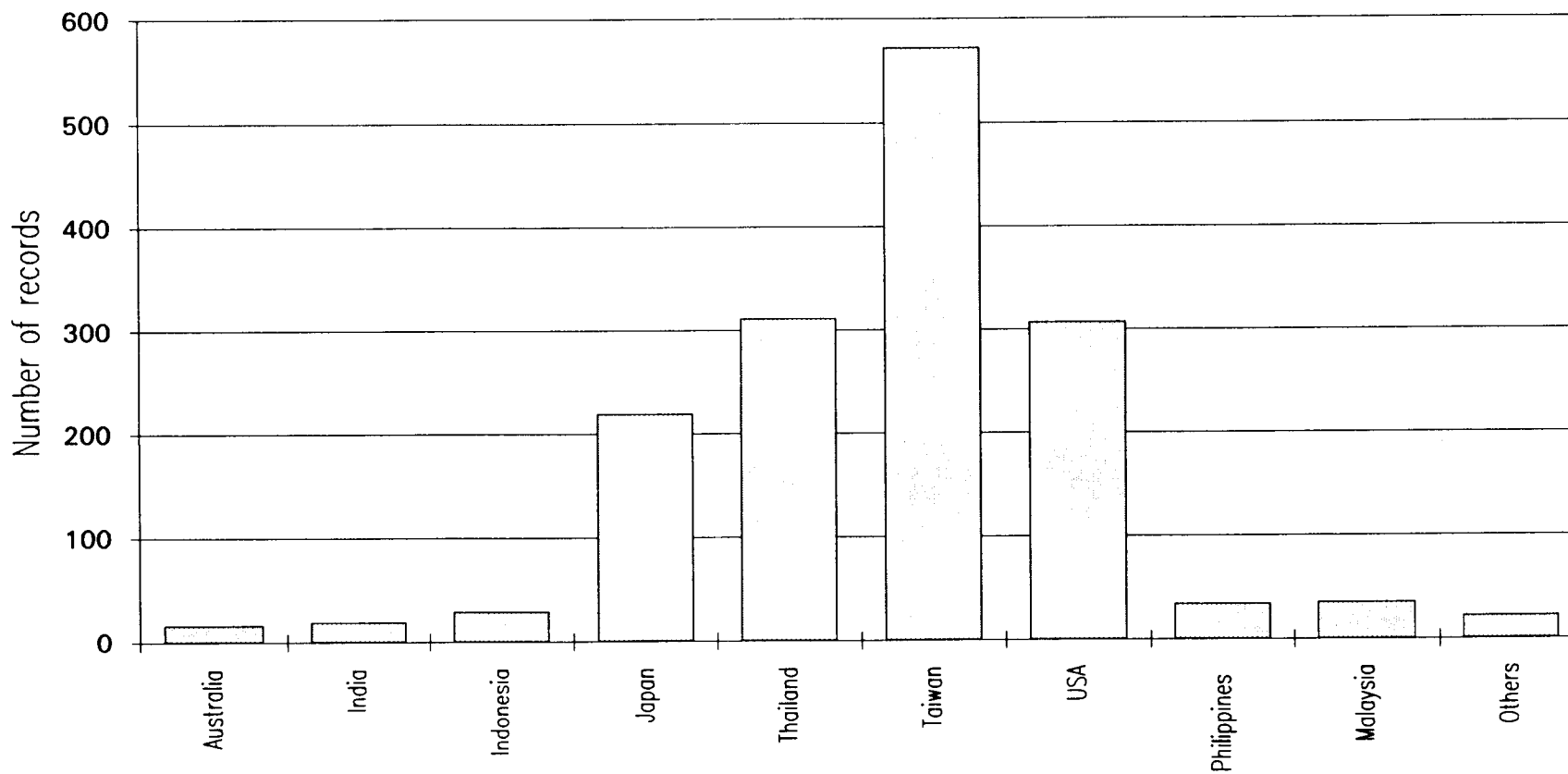
based on two year intervals (from 1981-1990) and are listed in Table 7. The pattern of insecticide use changed from (organophosphorus / carbamate / organochlorine) in the early 80's to (pyrethroid / *Bacillus thuringiensis*) to (*Bacillus thuringiensis* / insect growth regulator) then currently to (*Bacillus thuringiensis* / insect growth regulator / abamectin). The distribution of records for insecticides which account for more than 3.5% of the total number of records is shown in Figure 8. *Bacillus thuringiensis* accounts for the greatest number of individual records (10.02% of the total records). Organophosphorus and pyrethroid insecticides are the most common synthetic insecticide classes recorded in the DBM toxicological database (Figure 9). The records by insecticide class have evolved from organophosphorus, organochlorine, and carbamate (since 1966), to pyrethroid and bacterial insecticides (since 1971), and finally to insect growth regulators (since 1986), (Figure 10).

Characterization of the number of records by response type

Insecticide studies on DBM began with the determination of insecticide field rates in 1934. Insecticide toxicity tests began appearing in the literature in 1951. Enzyme activity tests appear in 1976 and neurophysiological experimentation is first noted in 1981. *In vivo* insecticide toxicity tests are the most common

Table 7. Ranking of most common insecticide records in the DBM toxicological database from 1981-1990.

Year	Insecticide
1981-1982	1. Dichlorvos
	2. Malathion
	3. Diazinon
	4. Methomyl
	5. DDT
1983-1984	1. Cypermethrin
	2. Fenvalerate
	3. Permethrin
	4. Deltamethrin
	5. *B. thuringiensis*, kurstaki
1985-1986	1. Fenvalerate
	2. Permethrin
	3. *B. thuringiensis*, kurstaki
	4. Dichlorvos
	5. Phenthoate

Table 7. (Continued)

Year	Insecticide
1987-1988	1. *B. thuringiensis*, kurstaki 2. Fenvalerate 3. Permethrin 4. Teflubenzuron 5. Malathion
1989-1990	1. Teflubenzuron 2. Chlorfluazuron 3. *B. thuringiensis*, kurstaki 4. Permethrin 5. Abamectin

Figure 8. Distribution of the number of insecticide records in the DBM toxicological database.

Legend:
- Abamectin
- B. thuringiensis
- Chlorfluazuron
- 106 insecticides, each contributing less than 3.5%
- Teflubenzuron
- Fenvalerate
- Phenthoate
- Permethrin
- Methomyl
- DDT

Pie chart values: 3.81%, 3.81%, 10.02%, 4.38%, 5.08%, 8.33%, 4.09%, 44.46%, 6.99%, 9.03%
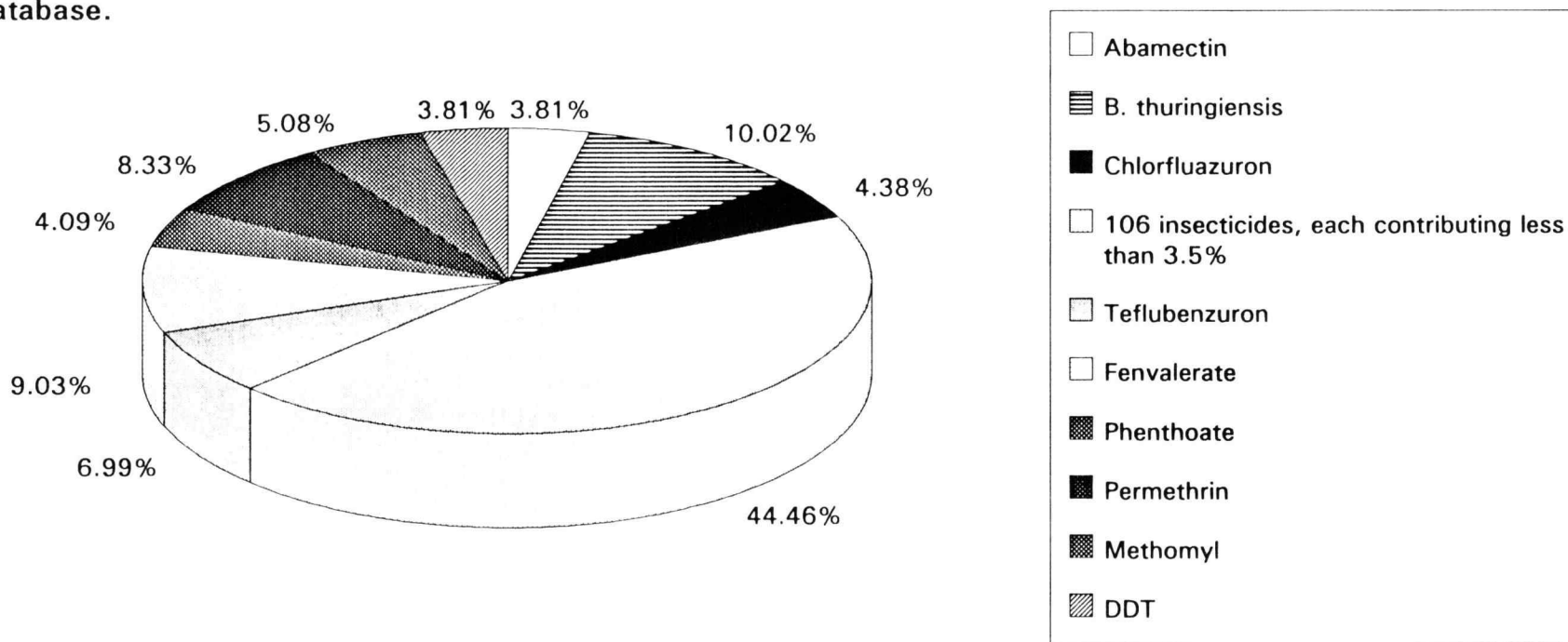
Figure 9. Distribution of the number of insecticide class records in the DBM toxicological database.
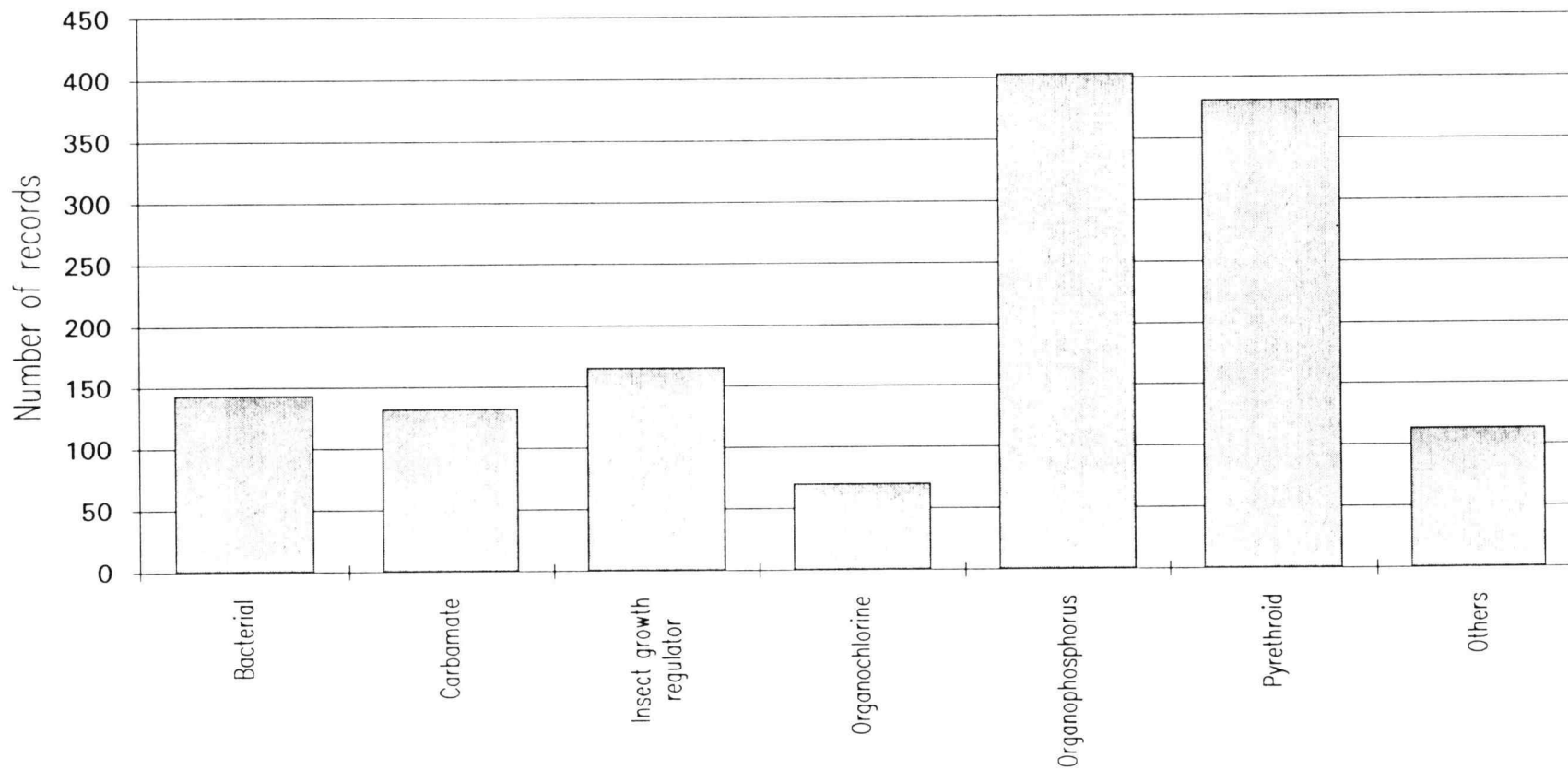
Figure 10. Cumulative number of insecticide class records in the DBM toxicological database.
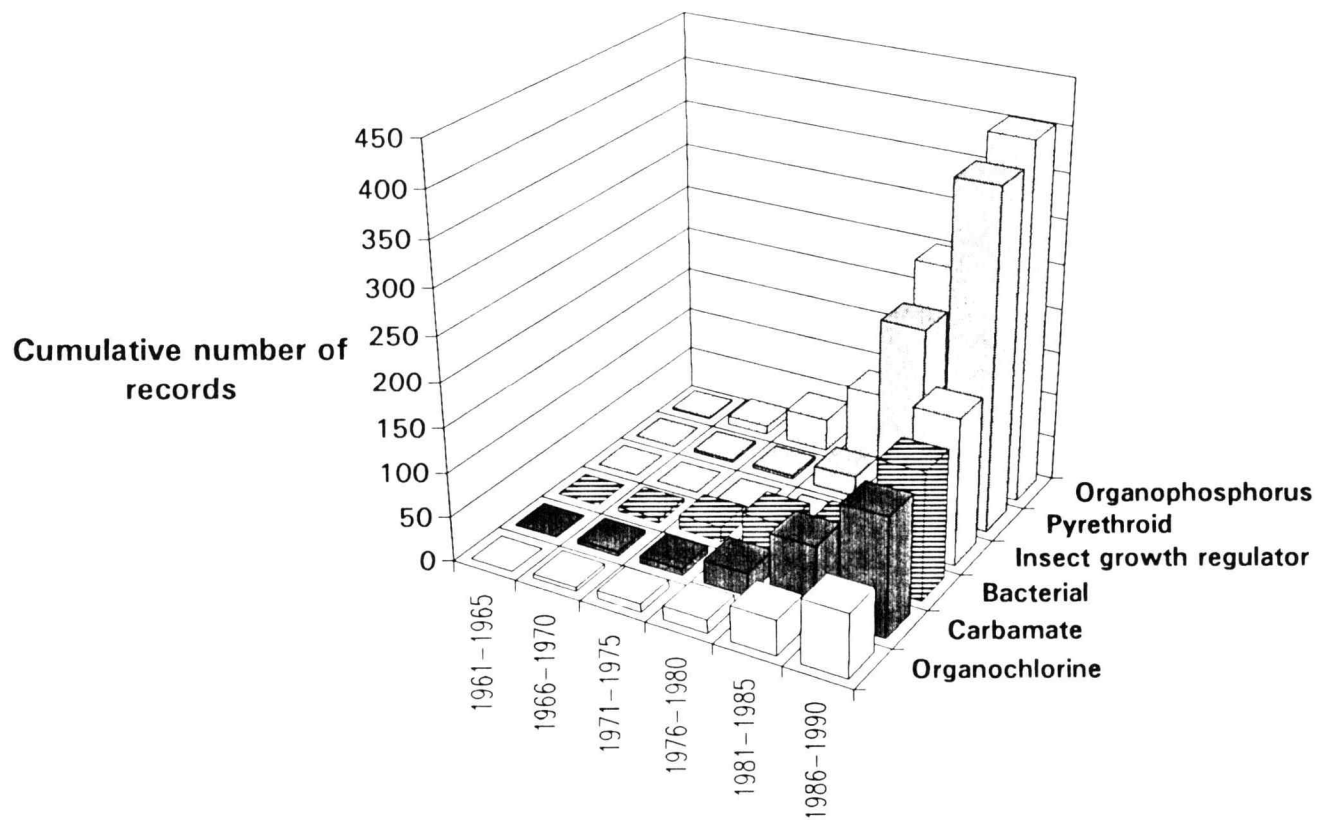
records in the record field "Type of Response". Data are shown in Figure 11.

Characterization of the number of records by method of experiment

The insecticide toxicity test methods were extracted from the database and the two most common methods were found to be: (1.) the leaf-dip bioassay (Tabashnik & Cushing 1987), (515 records) and (2.) the topical application (FAO Plant Protection Bulletin 1979), (298 records), as shown in Figure 12. The evolution of insecticide toxicity test methods is shown in Figure 13. The evolution of toxicity tests starts with direct spray (before 1951) to the topical application (since 1966 with sharp upturn in slope after 1980) to the leaf-dip bioassay (since 1971 with sharp upturn in slope after 1985) and to the residue-vial or Petri dish bioassay (since 1976).

Analysis of the extremely high resistance ratio and the extent of resistance

The analysis of resistance ratio was performed by extraction in the record field "Insecticide" from the DBM toxicological database. The insecticides which showed resistance ratios higher than 1,000 fold (extremely high resistance, resistance index = 4, Table 3.) were listed in Table 8. Thirty three records of extremely high resistance

Figure 11. Evolution of the types of insecticide studies on DBM during the last 40 years as evidenced by the number of records in the DBM toxicological database.
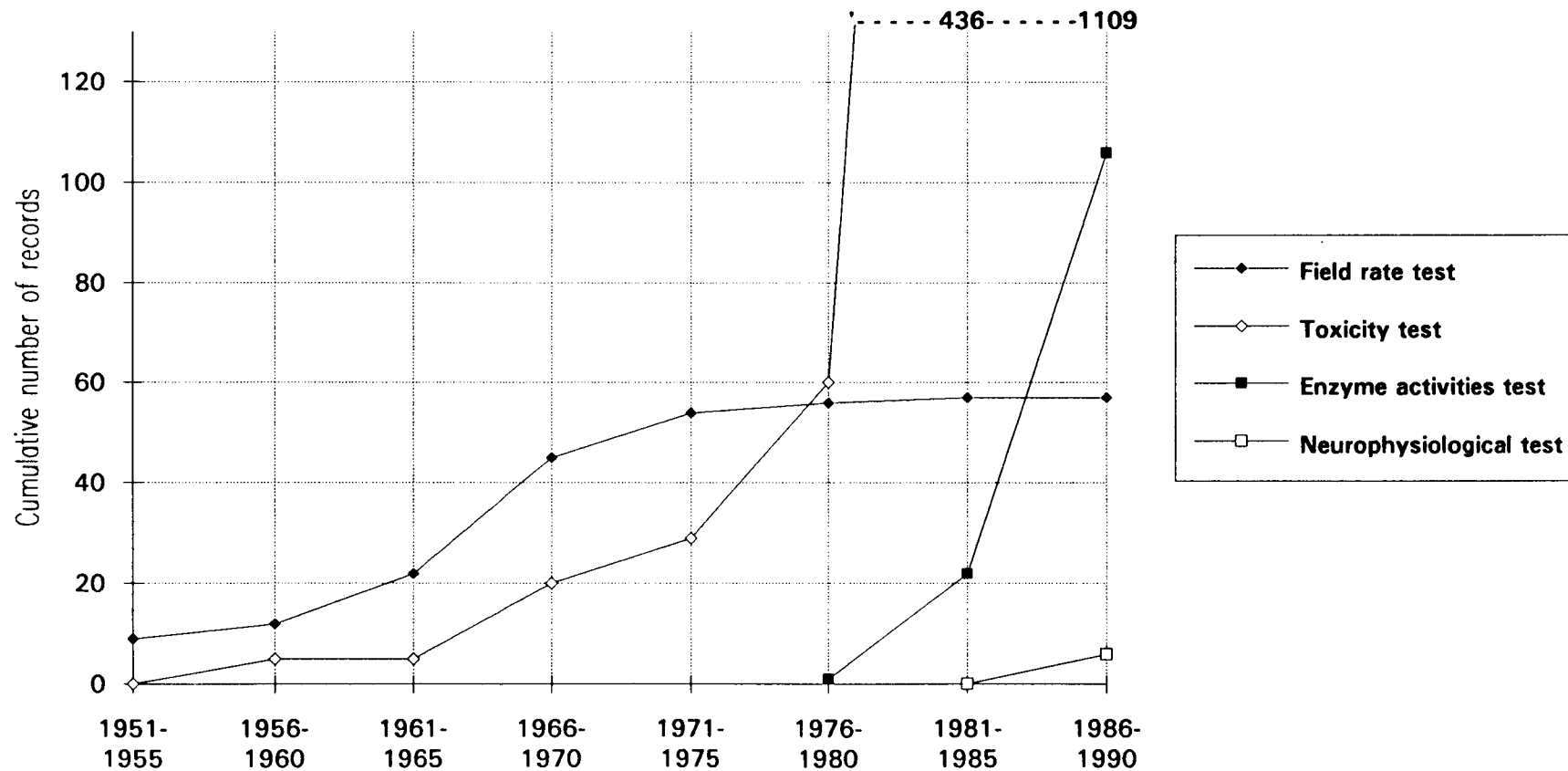
Figure 12.   Insecticide toxicity test methods in the DBM toxicological database.
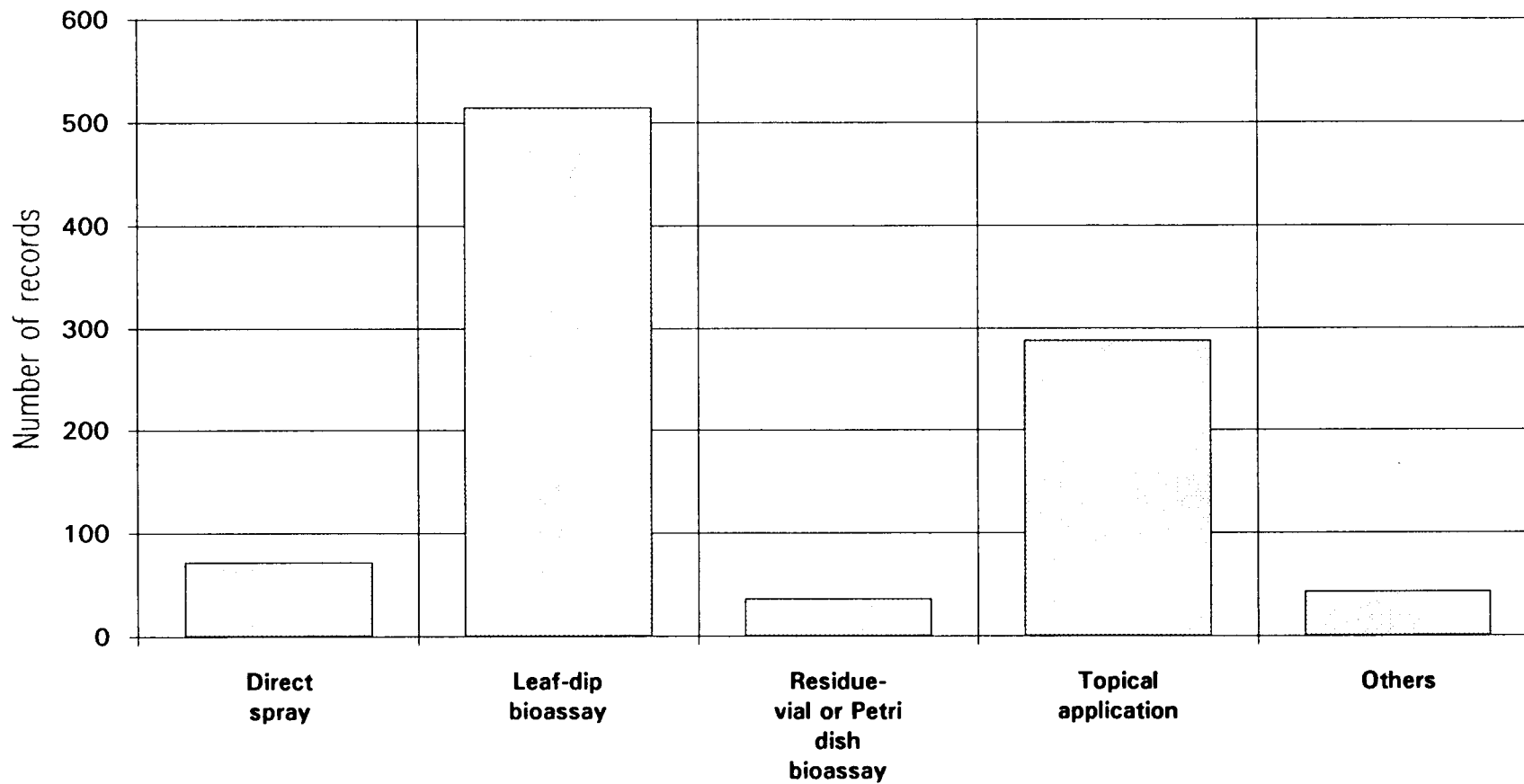
Figure 13. Evolution of the methods of insecticide toxicity testing on DBM during the
last 40 years as evidenced by the number of records in the DBM toxicological database.
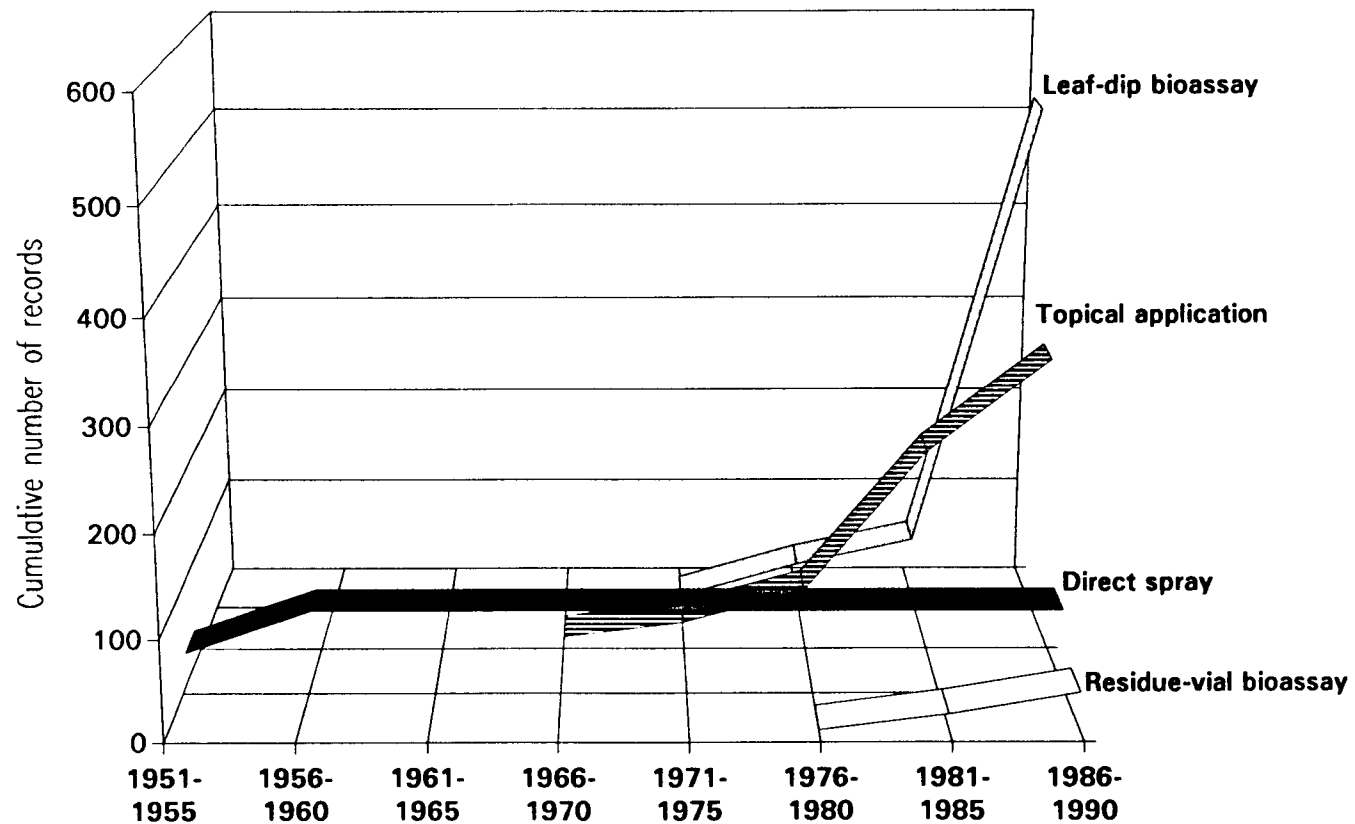
Table 8. Records of extremely high insecticide resistance ratios (> 1,000 fold) in DBM populations.

RR[1]	Insecticide	Class	Location	Year
54,706	Flucythrinate	PYR[2]	Taiwan	1985a
33,821	Tralomethrin	PYR	Taiwan	1985a
13,639	Chlorfluazuron	IGR[3]	Thailand	1990
11,028	Flucythrinate	PYR	Taiwan	1985b
10,508	Methyl parathion	OP[4]	Japan	1985
8,496	Teflubenzuron	IGR	Thailand	1990a
6,768	Tralomethrin	PYR	Taiwan	1985b
6,714	Profenophos	OP	Taiwan	1985
5,855	Methomyl	CAR[5]	Belize	1989
5,854	Prothiophos	OP	Taiwan	1985
3,231	DDT	OC[6]	Taiwan	1985
2,941	Malaoxon	OP	Taiwan	1985
2,880	Fenvalerate	PYR	Taiwan	1981
2,657	Methyl parathion	OP	Taiwan	1989
2,641	Phenothrin	PYR	Taiwan	1985
2,400	Fenvalerate	PYR	Japan	1984
2,327	Fluvalinate	PYR	Taiwan	1985
2,235	Methyl parathion	OP	Taiwan	1981
2,235	Decamethrin	PYR	Taiwan	1981
2,174	Deltamethrin	PYR	Taiwan	1984
2,100	Malathion	OP	Thailand	1988
2,067	Fenvalerate	PYR	Taiwan	1984

Table 8. (Continued).

RR	Insecticide	Class	Location	Year
1,965	Fenvalerate	PYR	Taiwan	1985
1,850	Deltamethrin	PYR	Taiwan	1985
1,840	Teflubenzuron	IGR	Thailand	1990b
1,753	Methyl parathion	OP	Philippines	1975
1,466	Fenpropathrin	PYR	Taiwan	1985
1,229	Parathion	OP	Taiwan	1989
1,200	Fenvalerate	PYR	Japan	1985
1,049	Methomyl	CAR	Taiwan	1985
1,049	Methomyl	CAR	Japan	1985
1,046	Parathion	OP	Taiwan	1990
1,039	Methyl parathion	OP	Taiwan	1990

[1] RR: Resistance ratio

[2] PYR: Synthetic Pyrethoids

[3] IGR: Insect growth regulators

[4] OP: Organophosphorus insecticides

[5] CAR: Carbamates

[6] OC: Organochlorines

were found for insecticides including organophosphorus insecticides, organochlorines, carbamates, synthetic pyrethroids, and insect growth regulators but not for bacterial insecticides. The analysis of the extent of resistance was performed as follows: Records were extracted for insecticides which were represented by more than 20 records. They were first sorted by resistance ratio from minimum to maximum, and then sorted by year of experiment from minimum to maximum. Insecticides, range of resistance ratio (minimum to maximum), year of experiment are shown in Table 9.

The analysis of resistance mechanisms

Most of the resistance mechanisms in the DBM toxicological database are biochemical and physiological resistance mechanisms (Table 10). The resistance mechanisms include increased metabolism (Esterase, Dehydrochlorinase, Glutathione S-transferase, Cytochrome P-450), target site insensitivity (Decreased nerve sensitivity, Altered acetylcholinesterase), reduced uptake (Decreased cuticular penetration), and behavior (Leg autotomy, Altered feeding behavior).

Table 9. Analysis of the extent of insecticide resistance in DBM.

Insecticide	Number of records	RR[1] (Min-Max)	Period
Abamectin	54	0.4-26	1987-1990
Bt., kurstaki[2]	142	0.8-820	1967-1991
Carbaryl	22	1.0-230	1967-1988
Methomyl	72	1.0-5,585	1972-1989
Cartap	33	0.9-199	1971-1990
DDT	54	0.5-3231	1949-1988
Chlorfluazuron	62	0.6-13,639	1983-1990
Teflubenzuron	99	1.0-8,496	1983-1990
Cypermethrin	50	1.0-30,000	1978-1990
Deltamethrin	29	0.6-6,667	1978-1985
Fenvalerate	128	0.3-12,000	1968-1990
Permethrin	118	0.3-700	1976-1990
Diazinon	48	0.3-413	1967-1988
Dichlorvos	30	1.0-300	1975-1988
Malathion	53	1.0-3,650	1965-1989
Methamidophos	38	1.0-145	1972-1989
Methyl parathion	20	1.0-21,000	1975-1990
Mevinphos	28	1.0-366	1965-1988
Phenthoate	62	1.3-236	1983-1990
Prothiophos	29	1.3-5,854	1975-1988

[1] RR: Resistance ratio

[2] Bt.,kurstaki: *Bacillus thuringiensis*, var.kurstaki

Table 10. Insecticide resistance mechanisms recorded in the DBM toxicological database.

Biochemical and physiological mechanisms	Insecticide	Class
Increased metabolism		
Esterases	Methomyl	CAR
	Carbofuran	CAR
	Dichlorvos	OP
	Malathion	OP
	Mevinphos	OP
	Profenophos	OP
	Phenthoate	OP
	Permethrin	PYR
	Deltamethrin	PYR
	Cypermethrin	PYR
Glutathione-S-transferase	Mevinphos	OP
	Dichlorvos	OP
	Profenophos	OP
Dehydrochlorinase	DDT	OC
Cytochrome P-450	Methomyl	CAR
	Carbofuran	CAR
	Diazinon	OP
	Dichlorvos	OP

Table 10. (Continued).

Mechanisms	Insecticide	Class
Cytochrome P-450	Mevinphos	OP
	Phenthoate	OP
	Profenophos	OP
	Cypermethrin	PYR
	Deltamethrin	PYR
	Fenvalerate	PYR
	Teflubenzuron	IGR
Target site insensitivity Decreased nerve sensitivity	Fenvalerate	PYR
Altered acetylcholine-	Malathion	OP
esterase	Phenthoate	OP
Reduced uptake Decreased cuticular penetration	Fenvalerate	PYR
Behavior Leg autotomy	Fenvalerate	PYR
Altered feeding behavior	Permethrin	PYR

Analysis of the resistance index of Organophosphorus insecticides

Organophosphorus insecticides records from Thailand and Taiwan were extracted from the record fields "Resistance Ratio" and "Country of Experiment". Resistance ratios were converted to resistance index (as shown in Table 3). Resistance indexes of organophosphorus insecticides were averaged each year (1985-1990). The average resistance indexes from both countries are shown in Table 11. The pattern of resistance index in both countries is similar, except that the rate of increase of the resistance index in Taiwan is faster than in Thailand. This illustrates the positive feedback between increased resistance and increased selection pressure (Georghiou & Saito 1983).

Case studies

Case 1. Pattern of insecticide use against DBM in Thailand from 1965 to 1990

Various classes of insecticides such as organophosphorus insecticides, organochlorines, carbamates, synthetic pyrethroids, insect growth regulators, bacterial insecticides were used to control DBM in Thailand (Figure 14). During 1965 to 1970 only organophosphorus insecticides were recorded in the DBM toxicological

Table 11. Average resistance index of DBM populations from Thailand and Taiwan to various organophosphorus insecticides.

Country	Average resistance index[1]					
	1985	1986	1987	1988	1989	1990
Thailand	1.15	1.11	1.28	1.67	2.11	2.78
Taiwan	1.31	NA.[2]	1.96	2.36	2.88	3.50

[1] See table 3 for the definition of the resistance index

[2] NA: Non available

Figure 14. DBM toxicological database records of insecticides use in DBM studies from Thailand (1965-1990).
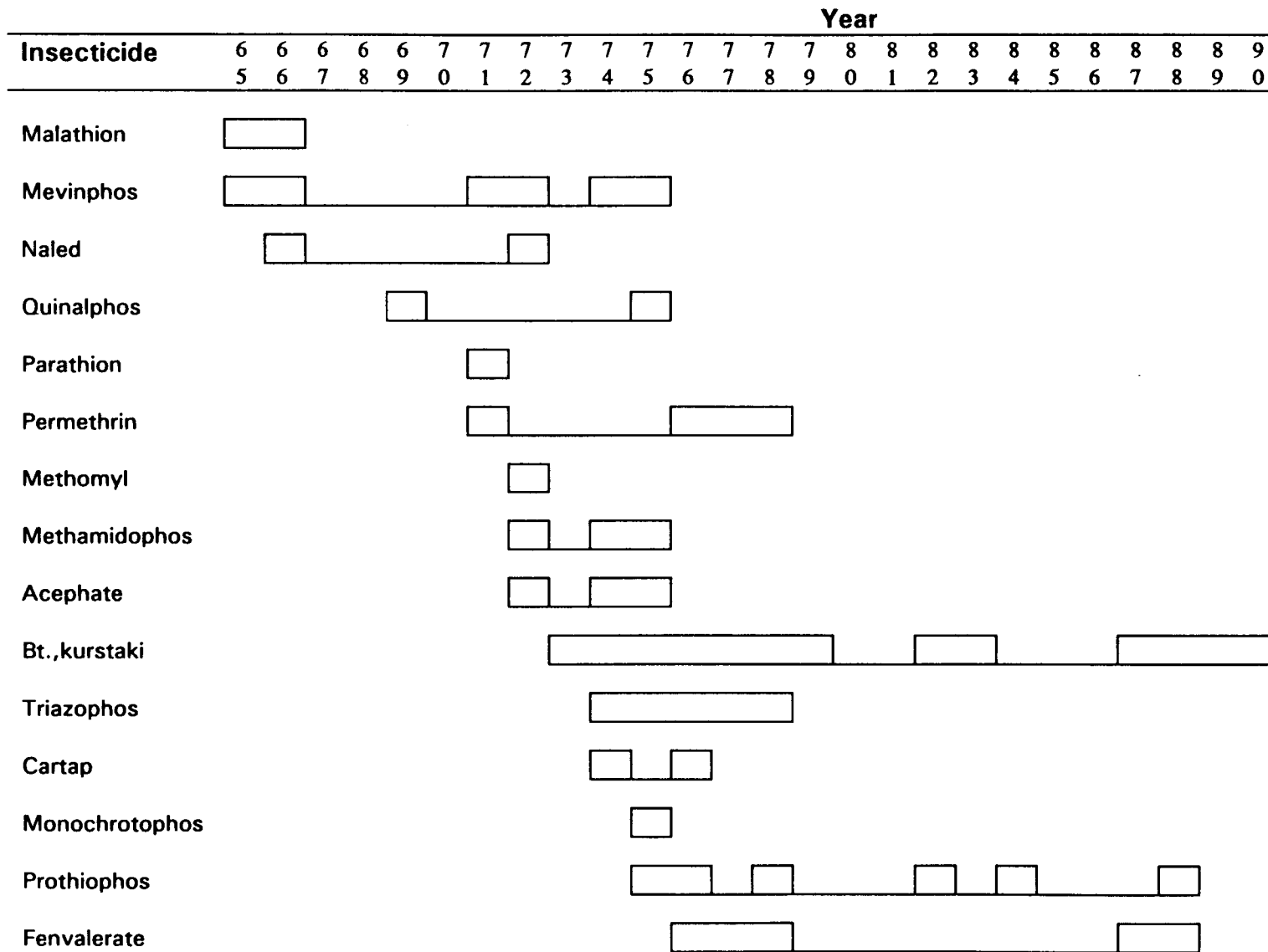
Year

Insecticide	6 5	6 6	6 7	6 8	6 9	7 0	7 1	7 2	7 3	7 4	7 5	7 6	7 7	7 8	7 9	8 0	8 1	8 2	8 3	8 4	8 5	8 6	8 7	8 8	8 9	9 0
Malathion																										
Mevinphos																										
Naled																										
Quinalphos																										
Parathion																										
Permethrin																										
Methomyl																										
Methamidophos																										
Acephate																										
Bt.,kurstaki																										
Triazophos																										
Cartap																										
Monochrotophos																										
Prothiophos																										
Fenvalerate																										

Note: Boxes indicate that records for each insecticide were found in the DBM toxicological database for that year.

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Figure 14. (Continued).

Insecticide	Year																									
	6 5	6 6	6 7	6 8	6 9	7 0	7 1	7 2	7 3	7 4	7 5	7 6	7 7	7 8	7 9	8 0	8 1	8 2	8 3	8 4	8 5	8 6	8 7	8 8	8 9	9 0

Cyanophenphos

Profenophos

Deltamethrin

Cypermethrin

Trifumuron

Diflubenzuron

Teflubenzuron

Chlorfluazuron

Ethofenprox

Cycloprothrin

Abamectin

Phenthoate

Cyhalothrin

Bt.,aizawai
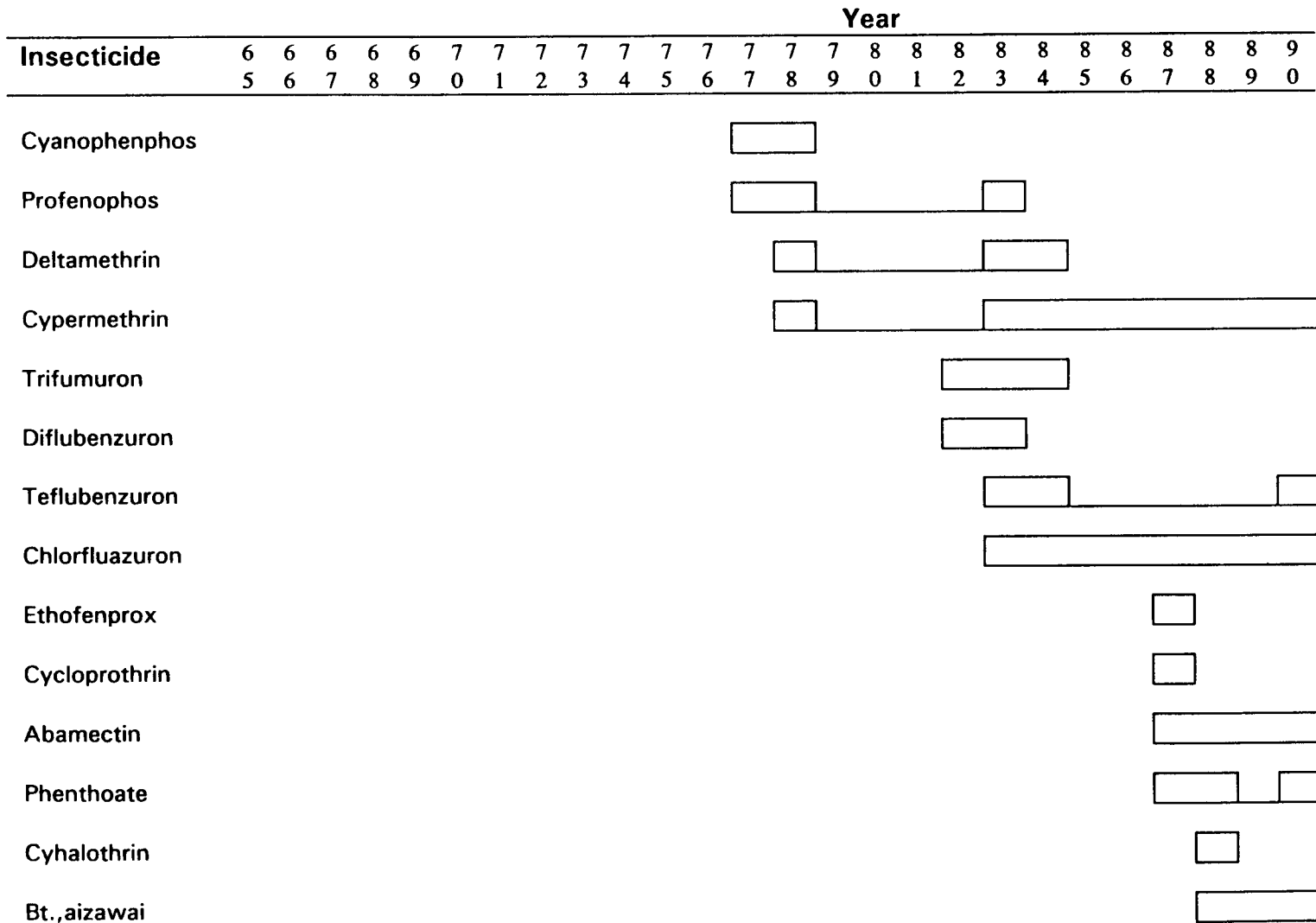
Note: Boxes indicate that records for each insecticide were found in the DBM toxicological database for that year.

database, then during 1971 to 1972 insecticidal control of DBM entered the new era of synthetic pyrethroid and bacterial insecticides when permethrin and *Bacillus thuringiensis* var. kurstaki were first introduced and continuously used until 1990 (end of the current records). Fenvalerate, permethrin, deltamethrin, cycloprothrin, cyhalothrin were among other synthetic pyrethroid that were introduced later. Cycloprothrin and cyhalothrin were used extensively for just 1 year in 1988 and 1989, respectively. The first insect growth regulator, diflubenzuron was introduced in 1983 and continuously used until 1990. Between 1970 and 1990 some new organophosphorus insecticides were used and abandoned in a short period of time (1 to 5 years). The evolution of insecticide use for DBM control in chronological order by insecticide class is: organophosphorus insecticides / carbamates / synthetic pyrethroids / *Bacillus thuringiensis* var. kurstaki, / insect growth regulators / *Bacillus thuringiensis* var. aizawai.

Case 2. "What is the extent of insecticide resistance in DBM to phenthoate, cartap, cypermethrin, teflubenzuron, and *Bacillus thuringiensis*, var. kurstaki in different parts of Thailand?"

The locations of DBM populations are shown in Figure 15. The resistance ratios of phenthoate, cartap,

Figure 15. Locations of DBM populations tested for insecticide toxicity in Thailand.
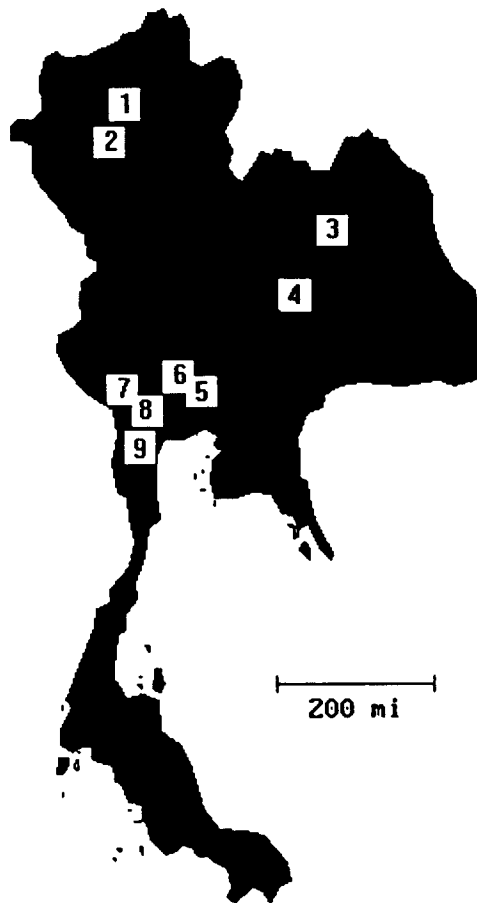
[1] Chiangmai
[2] Lumpoon
[3] Khorn-Kaen
[4] Korat
[5] Bang-Kae
[6] Bang Bua-Thong
[7] Karnchanaburi
[8] Tub Lung
[9] Petchaburi

cypermethrin, teflubenzuron, *Bacillus thuringiensis* var. kurstaki are shown in Table 12. The DBM showed low resistance (RR < 10, Table 3) in two locations in the northern part of Thailand, Chiangmai (1) and Lumpoon (2) to all five insecticides, although the Lumpoon population showed a moderate resistance (10 < RR < 100) to teflubenzuron (Insect growth regulator: Chitin synthesis inhibitor). DBM populations from two northeastern locations, Korn-Kaen (3) and Korat (4), showed low resistance to all 5 insecticides. DBM populations from Bang-Kae (5) and Bang Bua-Thong (6) in the central part of Thailand showed extremely high resistance (RR > 1,000) to teflubenzuron and showed moderate resistance (10 < RR < 100) to high resistance (100 < RR < 1,000) to phenthoate, cypermethrin, and *Bacillus thuringiensis* var. kurstaki. In those populations only low resistance (RR < 10) to cartap was observed. This is interesting because nearly all available insecticides have been used and eventually failed in these two locations (Rushtaprakornchai and Vattanatungum 1990). DBM populations from Karnchanaburi (7) and Tub Lung (8), the western part of Thailand, showed high resistance to Teflubenzuron and moderate resistance to phenthoate, cartap, and cypermethrin. DBM populations from Petchaburi (9) in the southwestern part of Thailand, showed high resistance to teflubenzuron and phenthoate

Table 12. Degree of insecticide resistance to five conventional insecticides in 1989 and 1990 in different populations of DBM in Thailand.

Location	Resistance ratio				
	Phenthoate (OP)	Cartap	Cypermethrin (PYR)	Teflubenzuron (IGR)	*Bacillus thuringiensis,* kurstaki (BAC)
(1)Chiangmai	4.9	7.2	7.2	0.1	4.9
(2)Lumpoon	3.2	4.3	3.4	69.5	4.8
(3)Khorn-kaen	3.0	1.7	4.7	1.9	12.3
(4)Korat	3.9	1.4	1.9	1.4	6.2
(5)Bang-Kae	236.5	3.1	59.4	8496.4	77.3
(6)Bang Bua-Thong	55.2	4.4	58.3	1840.0	36.2
(7)Kanchanaburi	20.6	10.0	20.6	384.4	13.6
(8)Tub Lung	14.4	28.5	33.8	284.6	5.9
(9)Petchaburi	112.6	0.9	21.0	227.3	21.1

and moderate resistance to cypermethrin, and *Bacillus thuringiensis* var. kurstaki but no resistance to cartap.

These two case studies illustrate uses of the DBM toxicological database. Case study 1. "Pattern of insecticide use against DBM in Thailand from 1965 to 1990" documents the chronological pattern of chemical control of this pest. Case study 2. "What is the extent of insecticide resistance in DBM to phenthoate, cartap, cypermethrin, teflubenzuron, *Bacillus thuringiensis* var. kurstaki in different parts of Thailand ?" demonstrates the pattern of insecticide resistance development in DBM: Different degrees of resistance to the same insecticide were observed in different locations separated by approximately 50 miles.

Resistance Monitoring Techniques

Two resistance monitoring techniques were evaluated with insects collected from Bang-Kae and Bang Bua-Thong, Thailand. The first technique, leaf-dip bioassay, was modified from Tabashnik & Cushing (1987) and the second technique, residue-vial bioassay, was modified from Magaro & Edelson (1990) (see Materials and Methods).

Preliminary tests

Bacillus thuringiensis with leaf-dip bioassay technique

The LC_{10}, LC_{80}, LC_{85}, LC_{90}, and LC_{95} values from Bang-Kae larval population were 0.22, 6.43, 8.78, 12.9, and 23.2 mg. AI per ml (1 mg. AI equal to 7,500 International Units), respectively and from Bang Bua-Thong larval population were 0.73, 8.99, 11.3, 15.1, and 23.2 mg. AI per ml, respectively. The results are shown in Table 13.

Bacillus thuringiensis with residue-vial bioassay technique

The concentration mortality responses shown in Table 14 indicated that *Bacillus thuringiensis* cannot be assayed accurately by the residue-vial bioassay technique. The reasons are explained in the Discussion.

Table 13. Toxicity of *Bacillus thuringiensis* var. aizawai and abamectin to fourth instar DBM larvae in preliminary tests.

Strain	Insecticide	Bioassay technique	LC_{10}	LC_{80}	LC_{85}	LC_{90}	LC_{95}	Slope $\pm$ SE[5]	n[6]	Chi-square (df[7])
			$LCs^{[1]}$ in mg. AI / ml[2] or ppm.[3] (95% $FL^{[4]}$)			
BK[8]	Bt.[9]	Leaf-dip	0.22 (0.09- 0.38)	6.43 (3.91- 12.9)	8.78 (5.14- 19.2)	12.9 (7.20- 31.6)	23.2 (11.7- 67.2)	1.49 $\pm$ 0.20	270	0.18 (2)
BBT[10]	Bt.	Leaf-dip	0.73 (0.35- 1.17)	8.99 (5.92- 16.9)	11.3 (7.22- 23.1)	15.1 (9.2- 34.2)	23.2 (13.1- 61.8)	1.99 $\pm$ 0.31	270	0.12 (2)
BK	Abamectin	leaf-dip	0.0003 (0.0- 0.001)	0.02 (0.01- 0.04)	0.03 (0.02- 0.06)	0.04 (0.02- 0.11)	0.08 (0.04- 0.25)	1.25 $\pm$ 0.17	270	0.70 (2)

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Table 13. (Continued)

Strain	Insecticide	Bioassay technique	LC_{10}	LC_{80}	LC_{85}	LC_{90}	LC_{95}	Slope $\pm$ SE[5]	n[6]	Chi-square (df[7])
			span: LCs^1 in mg. AI / ml^2 or ppm.^{3} (95% FL^4)							
BBT[10]	Abamectin	Leaf-dip	0.001 (0.0-0.002)	0.09 (0.04-0.24)	0.13 (0.06-0.40)	0.22 (0.10-0.78)	0.47 (0.18-2.15)	1.11 ± 0.16	180	0.34 (1)
BK[8]	Abamectin	Residue-vial	0.004 (0.0-0.008)	0.66 (0.38-1.29)	1.06 (0.59-2.23)	1.92 (1.01-4.48)	4.64 (2.21-12.8)	0.96 ± 0.10	320	7.45 (3)
BBT	Abamectin	Residue-vial	0.02 (0.01-0.05)	3.37 (1.96-6.84)	5.31 (2.94-11.8)	9.42 (4.88-23.5)	22.0 (10.2-66.4)	1.01 ± 0.11	320	6.51 (3)

Table 13. (Continued)

Legend:

[1] LCs: Lethal concentrations

[2] mg. AI / ml: For *Bacillus thuringiensis*

(1 mg. AI equal to 7,500 International Units)

[3] ppm: For Abamectin

[4] FL: Fiducial limits

[5] SE: Standard error

[6] n: Fourth instar from the first generation reared in the laboratory without exposure to insecticide

[7] df: Degree of freedom

[8] BK: Bang-Kae field

[9] Bt: *Bacillus thuringiensis*

[10] BBT: Bang Bua-Thong field

Table 14. Concentration-mortality response of fourth instar DBM larvae to *Bacillus thuringiensis* var. aizawai by residue-vial bioassay.

Bt.[2] (mg. AI / ml)	Mean percent mortality (SE[1])	
	Bang-Kae strain	Bang Bua-Thong strain
0.0	0.0 (0.0)	0.0 (0.0)
0.1	2.5 (1.0)	0.0 (0.0)
0.5	0.0 (0.0)	20.0 (5.3)
2.5	65.5 (14.8)	2.5 (1.0)
12.5	35.0 (10.9)	0.0 (0.0)
62.5	0.0 (0.0)	0.0 (0.0)
312.5	2.5 (1.0)	5.0 (1.7)
Chi-square value	248.2	50.7

[1] SE: Standard error

[2] Bt: *Bacillus thuringiensis*(1 mg. AI equal to 7,500 International Units)

Abamectin with leaf-dip bioassay technique

The LC_{10}, LC_{80}, LC_{85}, LC_{90}, and LC_{95} values from Bang-Kae larval population were 0.0003, 0.02, 0.03, 0.04, and 0.08 ppm., respectively and from Bang Bua-Thong larval population were 0.001, 0.09, 0.13, 0.22, and 0.47 ppm., respectively. The results are shown in Table 13.

Abamectin with residue-vial bioassay technique

The LC_{10}, LC_{80}, LC_{85}, LC_{90}, and LC_{95} values from the Bang-Kae larval population were 0.004, 0.66, 1.06, 1.92, and 4.64 ppm., respectively and from Bang Bua-Thong larval population were 0.02, 3.37, 5.31, 9.42, and 22.0 ppm., respectively. The results are shown in Table 13.

Final tests

The final tests were designed to determine the precision of LC_{90} estimation in concentration mortality regression. Precision of LC_{90} estimation required at least one concentration at approximately the LC_{10} ,a majority of concentrations between the LC_{75} and LC_{95} , and a sample size of 240 or more (Robertson et al. 1984 and Robertson & Worner 1990).

The LC_{90} values of *Bacillus thuringiensis* to Bang-Kae and Bang Bua-Thong larval populations with leaf-dip bioassay technique were 11.1 and 11.9 mg. AI per ml (1

mg. AI equal to 7,500 International Units), respectively. The LC$_{90}$ values of abamectin to Bang-Kae and Bang Bua-Thong larval populations with leaf-dip bioassay technique were 0.05 and 0.33 ppm., respectively. And the LC$_{90}$ values of abamectin to Bang-Kae and Bang Bua-Thong larval populations with Residue-vial bioassay technique were 1.82 and 11.0 ppm., respectively. The results are shown in Table 15.

Table 15. Toxicity of *Bacillus thuringiensis* var. aizawai and abamectin to fourth instar DBM larvae in the final tests.

Strain	Insecticide	Bioassay technique	LC_{90} in mg. AI / ml[1] or ppm.[2] (95% FL[3])	Slope ± SE[4]	n[5]	Chi-square (df[6])
BK[7]	Bt.[8]	Leaf-dip	11.1 (8.26-15.8)	1.19 ± 0.23	300	7.28 (4)
BBT[9]	Bt.	Leaf-dip	11.9 (9.21-16.6)	2.00 ± 0.21	300	5.50(4)
BK	Abamectin	Leaf-dip	0.05 (0.03-0.09)	1.12 ± 0.13	300	3.09 (4)
BBT	Abamectin	Leaf-dip	0.33 (0.20-0.67)	0.99 ± 0.11	300	5.38 (4)
BK	Abamectin	Residue-vial	1.82 (1.11-3.38)	1.04 ± 0.11	300	5.22 (4)
BBT	Abamectin	Residue-vial	11.0 (6.75-20.6)	1.06 ± 0.12	300	5.13 (4)

[1] mg. AI / ml (1mg AI equal to 7,500 International Units): For *Bacillus thuringiensis* , [2] ppm: For Abamectin

[3] FL: Fiducial limits , [4] SE: Standard error

[5] n: Fourth instar from the first generation reared in the laboratory without exposure to insecticide

[6] df: Degree of freedom , [7] BK: Bang-Kae field

[8] Bt: *Bacillus thuringiensis* , [9] BBT: Bang Bua-Thong field

Intraplant Distribution

The experiment on intraplant distribution of the immature stages DBM was performed to understand the pattern of distribution of insects on young folded kale leaves and on mature kale leaves. The pattern of insects distribution on kale plants may relate to the control failures after applications of insecticide like abamectin at the recommended field rate.

The distribution patterns of each immature stage DBM (except eggs and first instar larvae) on young folded kale leaves and on mature kale leaves were analyzed quantitatively by a distribution ratio [the number of sampling organisms on young folded leaves divided by the number of total sampling organisms (on young folded leaves and on mature leaves)] to understand the pattern of insect distribution on plants in both fields (Table 16 and 17). The distribution ratios from each sampling trip and each stage of DBM show a similar pattern of distribution that the second instar was found mostly on young folded leaves rather than mature leaves except for the fourth sampling trip in Bang Bua-Thong field where the majority of the second instar was distributed on mature leaves. The majority of third instar larvae was found on young folded leaves when Chinese kale plants were young (35-day-old) then the distribution was shifted to mature leaves on older

Table 16. Distribution of the immature stages of DBM on Chinese kale in the Bang-Kae field.

Sampling trip (Plant growth stage)	Type of kale leaves	Total number of immatures per field				
		2nd instar	3rd instar	4th instar	pupae	total
First time (35-day-old)	Young folded	15	5	0	0	20
	Mature	0	0	3	1	4
	DR[1]	1.00	1.00	0.00	0.00	0.83
Second time (40-day-old)	Young folded	14	13	1	0	28
	Mature	1	2	2	1	6
	DR[1]	0.93	0.87	0.33	0.00	0.82
Third time (45-day-old)	Young folded	20	23	3	1	47
	Mature	3	7	15	5	30
	DR[1]	0.87	0.77	0.17	0.17	0.61
Fourth time (50-day-old)	Young folded	14	12	1	0	27
	Mature	4	26	81	16	127
	DR[1]	0.78	0.32	0.01	0.00	0.18

[1] DR: Distribution ratio, the number of insects on young folded leaves divided by the number of total insects (on young folded and on mature leaves).

Table 17. Distribution of the immature stages of DBM on Chinese kale in the Bang Bua-Thong field.

Sampling trip (Plant growth stage)	Type of kale leaves	Total number of immatures per field				
		2nd instar	3rd instar	4th instar	pupae	total
First time (35-day-old)	Young folded	59	8	0	0	67
	Mature	0	4	1	1	6
	DR[1]	1.00	0.67	0.00	0.00	0.92
Second time (40-day-old)	Young folded	44	21	3	2	70
	Mature	1	13	38	10	64
	DR[1]	0.94	0.67	0.07	0.17	0.52
Third time (45-day-old)	Young folded	30	11	1	4	46
	Mature	9	104	194	46	353
	DR[1]	0.77	0.10	0.01	0.08	0.12
Fourth time (50-day-old)	Young folded	6	1	0	1	8
	Mature	29	209	439	71	748
	DR[1]	0.17	0.00	0.00	0.01	0.01

[1] DR: Distribution ratio, the number of insects on young folded leaves divided by the number of total insects (on young folded and on mature leaves).

Chinese kale plants (45-day-old and 50-day-old), the majority of fourth instar larvae and pupae were found on mature leaves much more than young folded leaves during plant 35-day-old to 50-day-old.

The percent distribution of the immature stages DBM on Chinese kale from both fields (Table 18) indicates that 81 percent of second instar larvae was distributed on young folded leaves, approximately 50 percent of third instar larvae was distributed on young folded leaves, 93 percent of the fourth instar larvae was distributed on mature leaves, and 95 percent of pupae was distributed on mature leaves.

Table 18. Summary of the distribution of the immature stages of DBM on Chinese kale.

Type of leaves	Percent distribution of immature stages[1] (SE)[2]				
	2nd instar	3rd instar	4th instar	pupae	total
Young folded leaves	81(9)	55(12)	7(4)	5(3)	50(12)
Mature leaves	19(9)	45(12)	93(4)	95(3)	50(12)

[1] Calculated from results in Table 16 and 17.

[2] SE: Standard error

DISCUSSION

DBM Toxicological Database

The new technology of computer hardware and database management software makes it possible to combine and analyze the toxicological information scattered in the literature on DBM. The IBM PS/2 55SX and dBASE IV have been used in this thesis for the computer hardware and database management software, respectively. Analysis of literature data for use in decision making for pest control and environmental impact assessment has been achieved with the SELCTV database (Theiling 1987). That database covers pesticide side effects on arthropod natural enemies. The DBM toxicological database is, to my knowledge, the first database devoted to a single insect pest. This work attempts to demonstrate the usefulness of a database approach of the problem of insecticide resistance in a pest for which limited progress in resistance management has been achieved (Croft 1990), despite its being characterized as a critical case of resistance (Georghiou 1990).

The DBM toxicological database contains two subdatabase files called the reference source subdatabase file (PX-SOURC.DBF) and the data subdatabase file (PX-DATA.DBF). The reference sources and the extracted data were separately inputted into PX-SOURC.DBF and PX-

DATA.DBF, respectively to reduce the time for data entry and quick retrieval. Both subdatabase files were linked in the PX-LINK.QBE file for the practical use or management of the database.

The studies on DBM toxicology are expanding in quantity (the number of published and unpublished documents, Figure 5.), in diversity (eg. tests of various kinds of insecticide classes: organochlorine, organophosphorus, synthetic pyrethroid, bacterial, insect growth regulator in Figure 9 and 10.), and in specificity (eg. pattern of insecticide studies on DBM: Field rate test, Toxicity test, Enzyme activities test, and neurophysiological test in Figure 11.).

Database management

The greater number of documents published on the toxicology of DBM, as well as the larger number of records that were extracted from these documents in the last decade may be caused by four factors: (1.) it may reflect the overall growth in the scientific literature. (2.) it may reflect a greater concern for pests of crucifers in the economies of Asian countries. (3.) it may reflect the recent emergence of DBM as a major pest following the destruction of its natural enemies by the early waves of non-selective insecticides. (4.) it may reflect an increase in the production of crucifers by consolidation from family-

sized plots into larger, commercial operations, with the insect pest control problems associated with such a shift (qualitative and quantitative) in production.

The two most common insecticide toxicity test methods for the DBM larvae recorded are topical application and leaf-dip bioassay. FAO has recommended the topical application method since 1979 (FAO method No. 21, 1979), and this method is widely used in Taiwan (e.g. Sun et al. 1978, Chang & Sun 1979, Liu et al. 1984, Maa & Guh 1988, Cheng et al. 1990). The Leaf-dip bioassay is widely used in The United States of America, Japan, and Thailand (e.g. Miyata et al. 1988, Sinchaisri et al. 1989, Rushtaprakornchai & Vattanagum 1990, Jackson & Graham 1979, Shelton & Wyman 1989, Tabashnik et al. 1987, Adam et al. 1990, Magaro & Edelson 1990). The advantages and disadvantages of both methods are discussed in chapter "Resistance Monitoring Techniques".

The most common insecticides recorded in the DBM toxicological database during 1989-1990 are teflubenzuron (insect growth regulator) chlorfluazuron (insect growth regulator), *Bacillus thuringiensis* var. kurstaki, permethrin (synthetic pyrethroid), and abamectin (microbial toxin). Teflubenzuron and chlorfluazuron are insect growth regulator insecticides which inhibit chitin synthesis in insects. Results in Table 8 show that DBM has already developed extremely high resistance to these insecticides in

many locations including such as Thailand and Taiwan. This can be a lesson for other crucifer growing countries, to carefully use and manage these insecticides in the control of DBM. *Bacillus thuringiensis* is one of the most selective and safe insecticides. The mode of action of this insecticide is still not completely understood. However, the protoxin in the formulation must be hydrolyzed in insect midgut into an active toxin which binds to a receptor protein in the midgut epithelium. Disruption of ionic balance (K^+) then causes death. Recently, Rushtaprakornchai and Vattanatungum (1990) and Tabashnik et al. (1991) reported that DBM has developed resistance to *Bacillus thuringiensis* var. kurstaki or Dipel™ in Thailand and in Hawaii, respectively. This can be another lesson of the abuse of a useful insecticide like *Bacillus thuringiensis* in the control of DBM in crucifer crops. Permethrin, a synthetic pyrethroid insecticide, is another insecticide which is still used to control DBM but does not give good control in many locations such as Bang-Kae, Thailand and Ban-Chau, Taiwan (Rushtaprakornchai and Vattanatungum 1990 and Liu et al. 1984). This may be because synthetic pyrethroid insecticides were introduced to control DBM in the 1970's (Figure 10) and DBM has already developed resistance to this class of insecticides.

Case studies

Case study 1. "Pattern of insecticide use against DBM in Thailand from 1965 to 1990" showed that synthetic pyrethroids were extensively used to control DBM for a short period of time. Cycloprothrin and cyhalothrin were each extensively used for just 1 year 1987 and 1988, respectively (Figure 14). They were then removed from the market because of resistance. This rapid expression of resistance may be explained as follows: In Thailand before 1965, DDT was used to control DBM until DBM became resistant. The database shows a record of DDT resistance in Java as far back as 1953 (Ankersmit, 1953). One of the resistance mechanisms of insects to DDT is *kdr* (knockdown resistance) resistance mechanism, reduced sensitivity of the sodium channels proteins in the nerve membrane (Carino and Feyereisen 1990). Because pyrethroid and DDT both act on sodium channels, cross resistance to synthetic pyrethroids like cycloprothrin and cyhalothrin, may be explained.

B. thuringiensis var. kurstaki was introduced in 1973, and *B. thuringiensis* var. aizawai was first introduced in 1988 (Figure 14). In 1989 and 1990, Rushtaprakornchai et al. reported that DBM had shown resistance to *B. thuringiensis* var. kurstaki. However, *B. thuringiensis* var. aizawai can be used to control the populations of DBM resistant to *B. thuringiensis* var. kurstaki. This may be

because *B. thuringiensis* var. kurstaki and *B. thuringiensis* var. aizawai do not share the same complement of crystalline toxins (Hofte and Whiteley 1989, Shimizu et al. 1988, Jarrett 1985). Cross or multiple resistance to different crystalline toxins has not yet been observed. For instance, selected strains of the Indian meal moth (*Plodia interpunctella,* Hübner) have shown resistance to the cry IA toxin but not to the cry IC toxin at all (Van Rie et al. 1990, McGaughey and Johnson 1987, McGaughey and Beeman 1988). It is possible that resistance in the DBM is similar to the case of the Indian meal moth, i.e. resistance may be limited to some crystalline toxin(s) in *B. thuringiensis* var. kurstaki but not to those of *B. thuringiensis* var. aizawai.

Case study 2. "What is the extent of insecticide resistance in DBM to phenthoate, cartap, cypermethrin, teflubenzuron, *Bacillus thuringiensis* var. kurstaki in different parts of Thailand ?", revealed that DBM from different locations show different degree of resistance to the same insecticide (Table 12). This suggest a geographical limitation for DBM migration from one location to another. The shortest distance from one location to another is approximately 20 miles (Bang-Kae to Bang Bua-Thong). Tabashnik et al. (1987) showed great differences in DDT and diazinon resistance in DBM populations in Hawaii. Although long-range movements of DBM are possible, these authors were surprised by the local

differentiation of DBM populations. They concluded that DBM insecticide resistance can be managed locally; the data from Thailand support this conclusion.

Resistance Monitoring Techniques

The LC$_{90}$s (leaf-dip bioassay) of *Bacillus thuringiensis* and abamectin for DBM larval populations from the Bang-Kae and Bang Bua-Thong fields were compared to the LC$_{90}$s of *Bacillus thuringiensis* and abamectin to the susceptible laboratory populations (Osaka susceptible strain, insects were collected from Osaka, Japan and reared in the laboratory more than 30 generations without exposure to insecticide, Rushtaprakornchai and Vatanatungum 1989 and Rushtaprakornchai 1990). The results of the comparison are shown in Table 19.

The resistance ratios shown in Table 19 indicate that both field populations (Bang-Kae and Bang Bua-Thong) developed resistance to *Bacillus thuringiensis* var. aizawai almost to the same level (resistance ratios from Bang-Kae and Bang Bua-Thong larval populations were 73.8 and 79.8, respectively). I propose the following reasons to explain this similarity: (1.) Chinese kales in both fields were grown throughout the year and *Bacillus thuringiensis* var. aizawai was continuously used (every 3 days) during the latter part of the plant growing cycle (last 30 days of a 53 day cycle). There are thus seven crops per year and at least 70 insecticide applications. (2.) *Bacillus thuringiensis* var.aizawai (Florbac™) was introduced in both fields in 1988 (Growers, personal communications). *Bacillus*

Table 19. Insecticide resistance of fourth instar DBM larvae from two Chinese kale fields in central Thailand.

Strain	Insecticide	F^1	LC_{90}^2 mg. AI / ml^3 or ppm.^{4}	RR^5
Osaka susceptible	Bt.^{6}	>30	0.15	1.0
Bang-Kae	Bt.	1	11.07	73.8
Bang Bua-Thong	Bt.	1	11.98	79.8
Osaka susceptible	Abamectin	>30	0.09	1.0
Bang-Kae	Abamectin	1	0.05	0.6
Bang Bua-Thong	Abamectin	1	0.33	3.7

[1] F: Generation rearing in the laboratory without exposure to insecticide

[2] LC_{90} : With leaf-dip bioassay technique

[3] mg. AI / ml: For *Bacillus thuringiensis*(1 mg. AI equal to 7,500 International Units)

[4] ppm: For Abamectin

[5] RR: Resistance ratio (LC_{90} from field population divided by LC_{90} from susceptible population)

[6] Bt: *Bacillus thuringiensis* var. aizawai

thuringiensis resistance development of DBM larval populations from Bang-Kae and Bang Bua-Thong fields may be one of the reasons why control failures were observed after applications of *Bacillus thuringiensis* at the recommended field rate.

The LC_{90} of larval population from the first generation (reared in the laboratory without exposure to insecticide) is an accurate reflection of that of the field population. However, rearing DBM in the laboratory without exposure to insecticide more than 3 generations significantly reduced LC_{90} for populations derived from the field population for *Bacillus thuringiensis* (Tabashnik et al. 1991) as well as other insecticides (Rushtapakornchai & Vattanatungum 1990).

The mean percent mortality of DBM larvae to *Bacillus thuringiensis* with residue-vial bioassay technique shown in Table 14 indicates that *Bacillus thuringiensis* cannot be assayed by the residue-vial bioassay technique. Indeed, immediate evaluation of data and a large Chi-square (50.7-248.2) indicated a poor fit of the data by the probit analysis model (Finney 1971, Robertson et al. 1980). The most likely reason for this failure is that *Bacillus thuringiensis* is a stomach poison insecticide which must be ingested and hydrolyzed in the larval midgut in order to show toxicity. With this bioassay technique, larvae may accidentally ingest some coated *Bacillus thuringiensis* from

the vial, in contrast to the leaf-dip bioassay technique where larvae positively ingest coated *Bacillus thuringiensis* from the tissue leaf disk while they are feeding on leaf disk. The leaf-dip bioassay seems to be one of the most logical resistance monitoring technique for *Bacillus thuringiensis.* The advantages and disadvantages of both techniques are shown in Table 20.

The resistance ratios shown in Table 19 indicate that both field populations (Bang-Kae and Bang Bua-Thong) have not yet developed resistance to abamectin (resistance ratios from Bang-Kae and Bang Bua-Thong larval populations were 0.6 and 3.7, respectively). Abamectin is a new insecticide containing a mixture of two bacterial toxins (avermectin B_{1a} and avermectin B_{1b}) which are large (molecular weight 859) and complex molecules (Lasota & Dybas 1991). It had been applied on crucifer crops in Thailand including both study fields for only one year (1989 to 1990). These toxins had never been used to control commercial DBM larval infestations in Thailand prior to 1989. No obvious cross resistance pattern was seen. However, both growers of Chinese kale from fields used in this study reported control failure after application of abamectin at the recommended field rate. Reasons for this failure may not relate to DBM resistance to abamectin but may relate to other factors, for instance: the method of insecticide application which may not be efficient enough to deliver the insecticide to the

Table 20. Advantages and disadvantages of two resistance monitoring techniques.

Resistance monitoring techniques	Advantages	Disadvantages
Leaf-dip bioassay	1. Both stomach and contact poison insecticides can be tested 2. Equipment can be reused 3. Closely approximates natural conditions (insect on food)	1. Higher cost of assay* 2. Longer observation time*
Residue-vial bioassay	1. Lower cost of assay* 2. Shorter observation time* 3. Equipment can be reused	1. Only contact poison insecticide can be tested 2. Insect removed from food (may affect physiology)

* See Table 21 and 22.

target site (such as high volume and low pressure spraying technique) or environmental factors (such as sunlight, heat, water pH) which may affect abamectin activity.

The concentration-mortality responses of DBM larval populations from both fields to abamectin with residue-vial bioassay technique were not compared to those responses of a susceptible larval population as there was no susceptible strain available. No published document reports the concentration-mortality responses of the susceptible larval population to abamectin with this bioassay technique. However, the objective of this study was to evaluate the two resistance monitoring techniques.

Finally, an economical analysis of the two resistance monitoring techniques was performed and is shown in Table 21 for Thai Baht unit and in Table 22 for US dollar unit. Not surprisingly, the cost of the bioassays is much cheaper in Thailand than it would be in The United States of America ($118-154 in Thailand vs. $602-678 in USA). However, in each country the cost of the leaf-dip bioassay is only 10-30% higher than the cost of the residue-vial bioassay. The topical application method (FAO Plant Protection Bulletin 1979) would require additional specialized equipment and labor, making it even more expensive. The benefit of the leaf-dip bioassay outweigh the small increase in cost.

Table 21. Economical analysis of two resistance monitoring techniques for field populations of DBM in Thailand.

Type of cost	Unit cost (Baht)	Quantity[1]	Bioassay Leaf-dip	Bioassay Residue-vial
Material cost				
Vial	3.50	30	NR[2]	105
Plastic cap	1.50	30	NR	45
Petri dish	25	30	750	NR
Filter paper	1.50	30	45	NR
Acetone	319/gal	0.3	NR	95
Isopropanol	25/liter	0.5 , 1	12.50	25
Kale seeds	20/Kg	0.1	2	NR
Time cost				
Preparation time	15/hr	5 , 2	75	30
Observation time	105	4 , 1	420	105
Clean up time	15/hr	0.5 , 1	7.5	15
Insect rearing cost				
Larvae cage	95	10	950	950
Pupae cup	5	3	15	15
Adult cage	10	3	30	30
Labor cost	105/day	14	1,470	1,470
Insect food	14/Kg	5	70	70
Net cost (Thai Baht)			B 3,847	B 2,955
Net cost (US dollar) and	$ 1 = 25 Baht		($ 154)	($ 118)

[1] Amount based on 5 test replications   [2] NR: Not required

Table 22. Economical analysis of two resistance monitoring techniques for field populations of DBM in The United States of America.

Type of cost	Unit cost (Baht)	Quantity[1]	Bioassay	
			Leaf-dip	Residue-vial
Material cost				
Vial	0.50	30	NR[2]	15
Plastic cap	0.10	30	NR	3
Petri dish	0.50	30	15	NR
Filter paper	0.10	30	3	NR
Acetone	20/gal	0.3	NR	6
Isopropanol	4/liter	0.5 , 1	2	4
Kale seeds	5/lb	0.2	1	NR
Time cost				
Preparation time	4.50/hr	5 , 2	22.5	9
Observation time	24/day	4 , 1	96	24
Clean up time	4.50/hr	0.5 , 1	2.25	4.50
Insect rearing cost				
Larvae cage	6	10	60	60
Pupae cup	3	3	9	9
Adult cage	40	3	120	120
Labor cost	24/day	14	336	336
Insect food	1/lb	12	12	12
Net cost			$ 678.75	$ 602.50

[1] Amount based on 5 test replications   [2] NR: Not required

Intraplant Distribution

The distribution of the immature stages DBM in crucifer crop fields has been reported by Harcourt (1960), Chen & Su (1980), Kirby & Slosser (1981), and Sivaprakasam et al. (1986). These authors all showed a negative binomial distribution of DBM in the field, which means that the insects aggregate in certain areas of the field. But in this experiment, I studied the distribution of immature stages DBM not at the field level, but at the level of single Chinese kale plants. This was done to understand the pattern of intraplant distribution which may relate to control failures after applications of insecticides at the recommended field rate.

The sampling experiments were performed with kales aged between 35-day-old and 50-day-old because growers replanted kales thirty days after germination, to provide larger space for each plant. The growers harvested kales when they were 50-day-old. Intraplant distribution experiments were designed to provide a qualitative analysis of the immature stages DBM distribution on Chinese kale. The distribution ratios (the number of insects on young folded leaves divided by total number of insects) were used to explain the dispersion behavior of each immature stage DBM on Chinese kale. The control failures reported by growers after applications of insecticides at the

recommended field rate may not only result from DBM resistance to those insecticides (Vegkrit 1985, Rushtaprakornchai and Vatanatungum 1989) but may also relate to the dispersion behavior of insects and to the insecticide application equipment / technique (see details in Table 5). The high percentage of immature stages DBM on young folded leaves (for instance: 81% of second instar larvae distributed on young folded leaves, Table 18) may cause control failure after application of insecticide at the recommended field rate. Indeed the spray equipment / technique (use of wide nozzle, high volume plus low pressure spray, and combination of irrigation with pesticide application, Table 5) may not be efficient enough to deliver the small insecticide particles (droplets) to young folded leaves (Vegkrit 1982 and 1984).

The data in Table 16 and 17 showed that the majority of the second instar larvae was distributed on young folded leaves. However, the fourth sampling trip in Bang Bua-Thong field revealed a majority on mature leaves. This may be explained by the high DBM and other pests damage on young folded leaves which resulted in very few young folded leaves being left in the Bang Bua-Thong field. The high percentage of second instar larvae on young folded leaves may be related to the egg-laying habit of the female moth (Vegkrit 1985, Rushtaprakornchai and Vatanatungum 1989).

CONCLUSIONS & FUTURE PROSPECTS

The problem of DBM resistance to insecticides has been mentioned in the literature in an anecdotal fashion (Georghiou 1990, Croft 1990). This work documents the extent of resistance in DBM populations around the world. Perhaps the most encouraging information extracted from the toxicological database is the fact that resistance appears to be a regional phenomenon, even though DBM is reputed to migrate easily over long distances (Tabashnik et al. 1987). If resistance to a particular insecticide, insecticide class, or more precisely if a particular resistance mechanism is present in only some DBM populations, then the implementation of resistance management programs (Croft 1990) should be encouraged. Resistance management programs must rely on easy, low-cost monitoring techniques. The leaf-dip bioassay appears to be the best choice because it reproduced the field conditions. The cost analysis shows that it can be implemented at low cost in developing countries.

Resistance management alone will not work unless the technology of pesticide application is improved. The very large droplets of insecticide that result from the use of inadequate nozzles (low pressure, high volume, irrigation technology using fire-fighting equipment) lead to a patchy

distribution of the chemical on the plant. Some plant parts remain unexposed to the chemical, and the top part of the plant is less exposed than the bottom part, because of gravity. It has been suggested (Vegkrit 1985) that such a patchy distribution of the insecticide may affect the behavior of DBM. The insect may learn to avoid treated parts of the plant and feed or oviposit on untreated surfaces. Problems caused by poor pesticide application methods are difficult to solve. Although a suitable spraying technology exists, it involves a high cost to the grower and its acceptance is limited. Education efforts should emphasize the risks of environmental contamination (including high residues on food), and the high costs caused by the rapid succession of different chemicals.

Alternatives to the use of pesticides as the sole control strategy for DBM have been discussed in the literature, and need to be implemented on a large scale through an effective extension program. These alternatives range from the very simple to the very sophisticated: (1) An observation of the oviposition behavior revealed that adult females fly towards their preferred oviposition sites between 6:00 pm and 9:00 pm (Chavalitpongporn 1985). It has been suggested that irrigation of the host plant at that time of the day may disrupt oviposition behavior or wash off newly laid eggs (Tabashnik and Mau 1986, Nakahara et al. 1985). (2) Attraction of adult DBM of both sexes by

plastic sheets of various colors showed that polyethylene envelops (0.04 mm. thickness) painted yellow and coated with a sticky substance such as Kinryu spray™ (SDS Biotech, Tokyo) could serve as a very effective DBM trap (Saito et al. 1988). This "fatal attraction" strategy is easy to monitor and serves as an effective recycling method for polyethylene envelops. (3) The sex pheromone of DBM has also been studied as a tool for mass capture of adults and for mating disruption (Chavalitpongporn 1985). However, this technique is more expensive to implement because of the cost of the pheromone and its low environmental stability.

There are many aspects of DBM ecology and toxicology that deserve future research. The development of resistance monitoring techniques which would be able to detect resistance in single insects would be very helpful. Indeed such techniques would provide information needed in the validation of models of DBM population genetics (Denholm 1990). This toxicological database shows that the range of tests needed includes virtually all the known insecticide resistance mechanisms.

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APPENDIX

APPENDIX 1

<u>The equations required to estimate sample size in the intraplant distribution of the immature stages of DBM.</u>

$$n = \frac{\displaystyle\sum_{i=1}^{L} \frac{N_i^2 s_i^2}{W_i}}{N^2 D + \displaystyle\sum_{i=1}^{L} N_i s_i^2} = \frac{\dfrac{N_1^2 s_1^2}{W_1} + \dfrac{N_2^2 s_2^2}{W_2} + \ldots + \dfrac{N_L^2 s_L^2}{W_L}}{N^2 D + N_1 s_1^2 + N_2 s_2^2 + \ldots + N_L s_L^2}$$

$$D = \frac{E^2}{t^2 N^2}$$

Where:

n = Sample size required

N = Total number of sample units in the population

N_i = Total number of sample units in stratum i

s_i = Standard deviation in stratum i

E = Allowable error

t = t-value

W_i = N_i / n