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The Ohio State University

Ph.D. 1985

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DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Richard Walter Wadleigh, B.S., M.S.

* * * * *

The Ohio State University

1985

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LIST OF ABBREVIATIONS

ANOVA .............................................. Analysis of variance
CPM .................................................. Counts per minute
DEF .................................................. S,S,S- Tributyl phosphorotrithioate
DPM .................................................. Disintegrations per minute
GLC .................................................. Gas-liquid chromatography
HCL .................................................. Hydrochloric acid
MFO .................................................. Mixed function oxidase
NA .................................................... 1-Naphthyl acetate
NADPH .............................................. Nicotinamide adenine dinucleotide phosphate (reduced form)
PBO .................................................. Piperonyl butoxide
POPOP .............................................. p-bis [2-(5-Phenylloxazolyl)]-benzene
PPO .................................................. 2,5 Diphenyloxazole
SDS .................................................. Sodium dodecyl sulfate
SQRT ............................................... Square root
uCi .................................................... Micro curie
Chapter I
Introduction

A. Life Cycle.

The green peach aphid, *Myzus persicae* (Sulzer), is distributed throughout the temperate, subtropical, and tropical areas of the world. In temperate areas such as Ohio, *M. persicae* lives a host-alternating or holocyclic life cycle. Holocyclic strains overwinter as diapausing eggs on a woody primary or winter host (Hille Ris Lambers 1946) and migrate to a herbaceous secondary or summer host in late spring (Tamaki 1981). The eggs are produced in the fall by a single sexual generation consisting of apterous females (oviparae) and alate males, all other generations consisting of viviparous parthenogenic females (van Emden et al 1969). The overwintering eggs are deposited on the buds of peach trees, *Prunus persica*, and other members of the genus *Prunus* (Hille Ris Lambers 1946) which serve as primary hosts. In the spring, apterous females (fundatrices) emerge from the overwintering egg and mature while feeding on the developing buds of the winter host (van Emden et al 1969). The fundatrices are followed by several generations of apterous fundatrigenia, which feed
and mature on the leaves of the winter host. In Ohio, beginning in May, each generation of fundatrigeniae produces an increasing number of alate emigrants which colonize the summer hosts. The summer hosts include many species of cruciferous and solenacious plants and broad leaf weeds (van Emden et al 1969, Metcalf et al 1962, and Tamaki 1981). The offspring of the emigrants are either alate or apterous and are called alienicolae or virginopara (Hille Ris Lambers 1966). The alienicolae continue to feed on and colonize new hosts throughout the summer. In the autumn, in response to decreasing day length and temperature (Lees 1964), the alienicolae gradually begin to produce an increasing number of the alate gynoparae which fly to the winter host and produce the sexual generation.

In subtropical areas or temperate regions with mild winters, *M. persicae* may live either a holocyclic or anholocyclic life cycle (van Emden et al 1969). In the latter life cycle it is permanently parthenogenetic and overwinters as active stages on either the primary or secondary host (Hille Ris Lambers 1946). *M. persicae* living in the tropics or in greenhouses are anholocyclic.

B. Importance to Agriculture.

*M. persicae* has nearly 900 plant hosts (Tamaki 1981). While it can cause direct damage to crop plants when populations build up to sufficient numbers (Kennedy and
Stroyan 1959), it is most dangerous to agriculture as a transmitter of pathogenic plant viruses and is considered the most important arthropod vector of plant diseases (Ossiannlsson 1966). It transmits nearly 100 (van Emden et al 1969) of the 159 viral plant diseases spread by all aphid species (Ossiannlsson 1966). In fact, its efficiency in spreading plant virus diseases, combined with its extensive host range and wide geographic distribution, make *M. persicae* one of the world's most important agricultural insect pests. Some common viral diseases vectored by *M. persicae* are the potato leaf roll virus, *Corium soliani*, the bean mosaic virus, *Marmor phaseoli*, the crucifer mosaic virus, *M. cruciferarum*, the cucumber mosaic virus, *M. cucumeris*, and the yellow dwarf virus, *M. cepae* (Metcalf et al 1962).

The primary method of aphid-borne disease control is to control the aphid itself (van Emden et al 1969) because only one infected aphid is needed to spread a viral disease to crop plants (Tamaki 1981). Insecticidal control of *M. persicae*, complicated by the development of widespread resistance and cross resistance to organophosphate, carbamate, and pyrethroid insecticides (Georghiou 1963, Sudderuddin 1973a, Needham and Devonshire 1975, Sawicki et al 1978), has not been completely successful in preventing the spread of viral diseases (van Emden et al 1969). Thus,
chemical control methods have been supplemented by integrated pest management strategies which aim to reduce or eliminate the spread of pathogenic plant viruses by reducing the number of potential vectors (van Emden et al 1969, Tamaki 1981). Such strategies are aimed at critical weak points in the aphid's life cycle and result in either reduced habitat or interference with the growth and development of the nymphal stages (Tamaki 1981).

C. Insecticide Resistance.

Characteristics of resistance.

Strains of *M. persicae* have developed resistance and cross resistance to organophosphate, carbamate, and pyrethroid insecticides and resistance to some organochlorine compounds (Sudderuddin 1973a, Sawicki and Rice 1978). Resistant:susceptible toxicity ratios for organochlorine and carbamate insecticides are generally lower than those for organophosphate and pyrethroid compounds. Some typical resistance ratios for the organochlorines aldrin and DDT are 1.5 and 1.8 (Sudderuddin 1973a) and 4.1 for lindane (Georghiou 1963). Resistance values for the carbamate pirimicarb range from 2 to 8 (Sudderuddin 1973a, Sawicki and Rice 1978) and 3 for the carbamate ethiofencarb (Sawicki and Rice 1978). Five hundred-fold resistance has been recorded for the
organophosphate dimethoate in a laboratory strain of *M. persicae* (Devonshire 1977) while field collected strains are generally 10 to 130-fold resistant (Sudderruddin 1973a, Needham and Devonshire 1975, Sawicki et al 1978). Resistance ratios for other organophosphates are malathion, 2 to 103 (Georghiou 1963, Takada 1979); diazinon, 3; parathion, 182; phorate, 11; and demeton, 11 (Sudderruddin 1973a). Eighty seven-fold resistance and 65-fold resistance to the pyrethroids permethrin and cypermethrin have also been found (Sawicki and Rice 1978). And as we will see later in the section on the mechanisms of resistance, a strain resistant to either organophosphate, carbamate, or pyrethroid insecticides is also cross resistant to insecticides from all 3 classes because of the broad substrate specificity of the resistance-associated enzyme (Devonshire and Moores 1982).

There is, at this time, no standardized method for measuring the toxicity of insecticides among susceptible or resistant strains of *M. persicae*. While some workers have used the discriminating dose method to determine levels of toxicity (Needham and Devonshire 1975, Sawicki et al 1978), most prefer a multidose exposure experiment designed to obtain an LD$_{50}$, or a biochemical test developed by Devonshire (1975) and used routinely to determine levels of resistance among field-collected aphids (Devonshire and

Although much sophisticated work has been done to decipher the mechanism of insecticide resistance in *M. persicae*, the presentation of supporting toxicological data is, more often than not, incomplete. Two frequent flaws are the presentation of resistance ratios without the accompanying median lethal dose values (Needham and Sawicki 1971, Needham and Devonshire 1975) and the omission of the 95% fiducial limits of the median lethal dose (Georghiou 1963, Sawicki and Rice 1978, and Sawicki et al 1978), an error that seriously diminishes the reliability of toxicity data (Finney 1971). An additional error committed by researchers who conducted a 9 year study on the development of resistance to 29 insecticides in field populations of *M. persicae* in Ontario, Canada was the failure to make determinations of resistance based on comparisons of the LD$_{50}$ of the resistant strain to that of a susceptible strain (McClanahan and Founk 1983).
Mechanism of insecticide resistance.

Resistance and cross resistance in *M. persicae* to organophosphate, carbamate, and some pyrethroid insecticides is caused by the presence in resistant strains of high concentrations of an esterase that hydrolyzes 1-naphthyl acetate (NA) (Devonshire 1975, Devonshire and Moores 1982). The esterase, called E-4, has identical kinetic characteristics in susceptible and resistant strains (Devonshire 1977). Its $K_m$ for NA hydrolysis is 0.131 mM and 75 pM for paraoxon hydrolysis; E-4 hydrolyzes NA 2 x $10^6$ times faster than paraoxon (Devonshire 1977). The first order rate constant for both paraoxon hydrolysis and reactivation of E-4 inhibited by paraoxon is 0.01 minute$^{-1}$ (Devonshire 1977). The rate of ester hydrolysis is fastest with short side chain naphthyl esters such as 1-naphthyl-acetate (C$_2$), -propionate (C$_3$), and -butyrate (C$_4$), and decreases as the side chain length increases with naphthyl-valerate (C$_5$) and -caproate (C$_6$) and stops altogether with naphthyl esters having side chains longer than 6 carbon atoms (Beranek 1974). E-4 confers resistance by increasing the binding and hydrolysis of certain insecticide esters (Devonshire 1975, Devonshire and Moores 1982). Because insecticide resistance and the amount of E-4 are linearly correlated (Devonshire 1977, Sawicki et al 1980), it is possible to detect resistance in individual
aphids by either direct electrophoretic measurement of the enzyme or by measuring NA hydrolysis in whole body homogenates. These techniques are routinely used for monitoring resistance in field populations of *M. persicae* (Devonshire and Needham 1975, Needham and Devonshire 1975, Sawicki et al 1978, and Buchi and Hani 1984).

There are two studies which suggest the existence of alternate (non-esterase) resistance mechanisms in *M. persicae*. In these studies, resistance to organophosphate insecticides such as dimethoate and malathion is not always associated with high levels of E-4 (Blackman et al 1977, Takada 1979). Blackman et al (1977) found 4-fold differences in resistance levels in two strains of *M. persicae* with identical levels of total esterase activity and amounts of E-4 carboxylesterase. Takada (1979) demonstrated that malathion resistance ratios can vary from 2 to 16-fold among strains with similar amounts of E-4. Less convincing however, is a study by Buchi (1981) in which he putatively induced deltamethrin resistance with ethylmethane sulphonate in two organophosphate susceptible strains with low levels of E-4. Since deltamethrin is a repellent of *M. persicae* (Rice et al 1983) the observed 'resistance' may have been caused by reduced exposure to the compound during toxicity tests with sprayed chinese cabbage and not by an alternate resistance mechanism.
Some researchers have suggested that qualitative changes in oxidative metabolism may serve as a resistance mechanism in *M. persicae*. Needham and Sawicki (1971) suggested enhanced oxidative metabolism as a possible mechanism of resistance when they used the MFO inhibitor Sesamex to synergize dimethoate and antagonize disulphoton in an organophosphate resistant strain of *M. persicae*. Devonshire (1973) detected less omethoate, the toxic oxidative metabolite of dimethoate, in an organophosphate resistant strain of *M. persicae* than in a susceptible strain. Devonshire drew no conclusions from the data although he speculated that either reduced oxidative activation of dimethoate or enhanced metabolism of omethoate may function as a resistance mechanism.

**Genetic basis of resistance.**

The genetic basis of organophosphate, carbamate, and pyrethroid resistance in *M. persicae* is the subject of dispute between two schools of thought. One school believes that resistance is always associated with a structural rearrangement or heterozygosity in the chromosomes in strains with the normal diploid chromosome number (2n=12) and with strains having an extra chromosome (2n=13) (Blackman and Takada 1975, Blackman et al 1978). The structural heterozygosity in the 2n=12 variants, found throughout the world (Blackmen et al 1978), arises from a
translocation between nonhomologous autosomes 1 and 3 (Blackman and Takada 1975). Blackman and Takada (1975) stated that the translocation may be reciprocal, i.e. chromosome fragments are mutually exchanged by autosomes 1 and 3, or nonreciprocal, where a fragment is transferred from autosome 3 to autosome 1. The 2n=13 variant arises from a dissociation or break in autosome 3 at the same place where it occurs in autosome 3 during translocation in the 1,3 translocation heterozygote (Blackman and Takada 1975). The Danish researcher Lauritzen (1982) found a 1,2 autosomal translocation (2n=12) and a unique autosomal 3 dissociation (2n=13) that is structurally different from the autosomal 3 dissociation described by Blackman and Takada (1975), in two organophosphate resistant strains of *M. persicae*.

The translocation and dissociation heterozygositites are thought to cause insecticide resistance by increasing E-4 synthesis via a change in gene regulation (Blackman et al 1978). The 1,3 translocation brings into the same linkage group genes that were on autosomes 1 and 3. It is thought that the repositioning of the genes may increase E-4 synthesis by inactivating genes that suppress the production of E-4 (Blackman et al 1978). Such inactivation is called a variegated or V type position effect (Baker 1968). The dissociation is thought to increase E-4
synthesis by separating the E-4 genes from the suppressor genes (Blackman et al 1978).

The other school disputes the claim that insecticide resistance is always associated with the 1,3 translocation and 3 dissociation heterozygosities. Devonshire and Sawicki (1979) point out that the susceptible and the three least resistant of the 7 strains of *M. persicae* which they have studied lack the chromosomal rearrangements described by Blackmen and Takada (1975), while the 3 most resistant strains have only the 1,3 translocation heterozygosity. They believe that the 1,3 translocation results in the formation of multiple copies of the resistance-associated gene through a series of tandem duplications. Multiple copies of the resistance gene causes increased synthesis of the E-4 carboxylesterase and subsequent resistance. Furthermore, they suggest that resistance in the less resistant strains without the 1,3 translocation is due to gene duplication resulting from an unequal crossing over between homologous chromosomes. In both cases, modification of the gene regulation mechanism, as proposed by Blackman et al (1978), plays no role in resistance (Devonshire and Sawicki 1979).
Devonshire and Sawicki (1979) present as evidence of gene duplication the fact that both resistance and the amount of E-4 present in the 7 strain increase in a geometric series by a factor of 2, starting with the susceptible strain and ending with the most resistant strain (Devonshire and Sawicki 1979). Bunting and van Emden (1980) independently confirmed this observation and referred to the phenomena as gene amplification. The advocates of gene amplification or gene duplication reason that such a geometric doubling of resistance and E-4 content could only result from tandem duplications of the gene that transcribes E-4. The advocates of gene regulation as of yet have not presented experimental evidence in support of their hypothesis.

Inheritance of resistance.

Studies of the inheritance of insecticide resistance in *M. persicae* have progressed slowly because of the aphid's annual life cycle and the difficulty with rearing and mating the sexual generation in the laboratory (Blackman 1975). Despite these problems, Blackman et al (1977) conclusively demonstrated that increased E-4 carboxylesterase activity and organophosphate resistance are co-inherited in *M. persicae*. In further studies Blackman and Devonshire (1978) observed a basic difference in the nature of the inheritance between strains with low
and high levels of resistance. In strains with low levels of resistance the inheritance is monofactorial, i.e. resistance is due to inheritance of a single dominant or near dominant gene. However, in highly resistant strains the inheritance of a second resistance gene from a different linkage group is believed to occur because the segregation ratios resulting from breeding experiments did not fit the results expected for monofactorial inheritance.

D. Host Plant Induction of Detoxification Enzymes.

The induction of detoxification enzymes such as mixed function oxidase (MFO) and glutathione S-transferase in herbivorous insects by host plants has been well documented. Induction refers to the increased synthesis of detoxification enzymes which occurs when herbivorous insects feed on certain plants. Induction of MFO has been demonstrated in the southern armyworm, *Spodoptera eridonia* (Cramer) (Brattsten et al 1977), variegated cutworm, *Peridroma saucia* (Hubner) (Berry et al 1980), fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Yu 1982a), cabbage looper, *Trichoplusia ni* (Hubner), and alfalfa looper, *Autographa californica* (Speyer) (Farnesworth et al 1981), Japanese beetle, *Popillia japonica* Newman (Ahmed 1983), and twospotted spider mite, *Tetranychus urticae* Koch (Mulins and Croft 1983). Similarly, host plant induction
of glutathione S-transferase has been seen in the fall armyworm (Yu 1982a,b).

The relative amount of induction in a species is specific to the host plant involved, with some host plants causing much more induction than others. For example, parsley and parsnip were the most potent inducers of glutathione S-transferase among 11 host plants fed to the fall armyworm (Yu 1984), while in the same species corn was the best MFO inducer of 9 host plants (Yu 1982a). Peppermint induced the most MFO of 5 host plants fed to the variegated cutworm (Berry et al 1980).

Brattsten et al (1977) demonstrated that detoxification enzymes are actually induced by the allelochemicals or secondary plant substances contained within a host plant. They argue that host plant induction of insect detoxification enzymes protects herbivores against "chemical stress" or poisoning by allelochemicals. Indeed, it has been shown that detoxification enzymes are induced by synthetic allelochemicals. For example, MFO was induced in the southern armyworm in a dose-dependant manner by the inclusion in the diet of the synthetic allelochemicals sinigrin, (+)-alpha-pinene, or trans-2-hexenal (Brattsten et al 1977). Yu (1984) induced both MFO and glutathione S-transferase in the fall armyworm with dietary synthetic xanthotoxin. He then isolated naturally
occuring xanthotoxin from the leaves of parsnip, the most potent host plant inducer of MFO and glutathione S-transferase in the fall armyworm (Yu 1984). Finding that xanthotoxin was present in the leaves at biologically active concentrations, Yu concluded that xanthotoxin was the active allelochemical in parsnip responsible for the induction of MFO and glutathione S-transferase in the fall armyworm.

The protection from allelochemical poisoning afforded by host plant induction of detoxification enzymes is also extended to poisoning by insecticides. Several researchers have demonstrated that insects are less susceptible to insecticides after induction of detoxification enzymes by host plants. Brattsten et al (1977) decreased the toxicity of dietary nicotine to the southern armyworm nearly 2-fold with dietary (+)-alpha-pinene. Berry et al (1980) found that variegated cutworm larvae were more tolerant of dietary acephate, methomyl, and malathion after feeding on peppermint leaves. Finally, Yu (1982a) found that corn-fed fall armyworm larvae were more tolerant of several organophosphate, carbamate, and pyrethroid insecticides than larvae fed soybeans, which is a less potent inducer of MFO than corn.
E. Function of MFO.

Mixed function oxidases are any enzymes catalyzing a reaction in which 1 atom from molecular oxygen is inserted into a substrate and the other is reduced to water (Lehninger 1975). The most common MFO is cytochrome P-450. The general function of MFO is the oxidation of lipophilic xenobiotics to excretable water soluble metabolites and the hydroxylation of steroids in synthetic pathways (Stryer 1981). P-450 is mostly found in the microsomal pellet of centrifuged tissues of plants, animals, and microbes. Insect sources of P-450 include the midgut, fat body, and malpighian tubules. The specific organelles from which it is isolated are the endoplasmic reticulum (=microsomes), lysosomes, golgi apparatus, and nuclear membranes (Hodgson and Dauterman 1978).

Cytochrome P-450 is so named because it is a hemoprotein with an absorption maximum of 450 nm when bound with carbon monoxide. Such data is obtained by placing a microsomal suspension in both chambers of a split beam spectrophotometer, reducing the suspensions in both chambers with dithionite and recording the difference spectra after CO is added to one of the suspensions (Hodgson and Dauterman 1978).

When catalyzing substrate oxidation, MFO systems require a second substrate, or cosubstrate, to donate.
electrons for the reduction of the second oxygen atom to water. The second substrate is NADPH. After the substrate receiving the first oxygen atom binds to cytochrome P-450, the enzyme flavoprotein NADPH reductase removes 2 electrons from NADPH. The flavoprotein transfers 1 electron to the oxidized cytochrome P-450-substrate complex which reduces the complex to the Fe$^{2+}$ form. Molecular oxygen then binds to the reduced P-450-substrate complex and the complex is further reduced by another electron from the reductase. At this point 1 oxygen atom is inserted into the substrate and the other is reduced to water after taking 2 protons from the matrix.

E. Purpose.

The current knowledge about insecticide resistance in *M. persicae*, although detailed and the result of high quality research, is limited in scope because of the exclusive attention paid to the contribution of esterases. The purpose of my research is to broaden the understanding of resistance mechanisms in this species by studying the role of oxidative metabolism in susceptible and resistant strains of *M. persicae* collected in Ohio. To accomplish this purpose I first measured the toxicity of 4 insecticides to the aphids and, in selected cases, use MFO and esterase inhibitors to diagnose potential resistance mechanisms. Then I used biochemical techniques to determine
the contribution, if any, of carboxylesterase to resistance, and finally investigate the role of MFO in resistance by studying the in vivo oxidative metabolism of aldrin and parathion among the strains of aphids. In addition, I studied the role of host plant induction of detoxification enzymes in the metabolism of aldrin and parathion by switching the host plant on which 1 of the aphid strains was reared and repeating some of the metabolism experiments.
Chapter II
Methods and Materials

A. Chemicals.

The sources and purity of the chemicals used in this study are listed in the following table.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Purity</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>Concentrated</td>
<td>Baker</td>
</tr>
<tr>
<td>Aldrin</td>
<td>99.5%</td>
<td>Chem Service</td>
</tr>
<tr>
<td>$^{14}$C Aldrin</td>
<td>83%</td>
<td>Amersham</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>96-99%</td>
<td>Sigma</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>99.5%</td>
<td>Chem Service</td>
</tr>
<tr>
<td>$S,S,S$- Tributyl phosphorothioate</td>
<td>95.4%</td>
<td>Mobay</td>
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<tr>
<td>Ethoxylated octylphenol</td>
<td>Scintillation grade</td>
<td>Fisher</td>
</tr>
<tr>
<td>Fast blue B salt</td>
<td>Reagent grade</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>Reagent grade</td>
<td>Mallinckrodt</td>
</tr>
<tr>
<td>Florisil (60/100)</td>
<td>Pesticide grade</td>
<td>Applied Science</td>
</tr>
<tr>
<td>Hydroxylamine hydrochloride</td>
<td>Reagent grade</td>
<td>Mallinckrodt</td>
</tr>
<tr>
<td>Methyl butyrate</td>
<td>Technical grade</td>
<td>Eastman</td>
</tr>
<tr>
<td>l-Naphthol</td>
<td>99%</td>
<td>Sigma</td>
</tr>
<tr>
<td>l-Naphthyl acetate</td>
<td>Technical grade</td>
<td>Sigma</td>
</tr>
<tr>
<td>Parathion</td>
<td>98.6%</td>
<td>City Chemical</td>
</tr>
</tbody>
</table>
14C Parathion 98% California Bionuclear
Piperonyl butoxide Technical grade Fairfield
p-bis[2-(5-Phenyl-oxazole)]-benzene Scintillation grade Research Products
2,5 diphenyloxazole Scintillation grade Packard
Resmethrin 94% Sumitomo
Sodium dodecyl sulfate 99% Sigma
Tissue solubilizer Scintillation grade Research Products
Trizma base 99.9% Sigma
Trizma HCL Reagent grade Sigma

All organic solvents were purchased as reagent grade and glass distilled before use.

B. Gas-Liquid Chromatography.

All gas-liquid chromatography (GLC) analyses were performed with a Varian 1400 gas chromatograph equipped with a 63Ni electron capture detector and a 1.83 m x 2 mm (id) glass column packed with 3% SE-30 (w/w) on 80/100 mesh Gas Chrome Q. The operating conditions were: N2 flow, 35 ml/minute; detector, column oven, and injector temperatures, 250°, 200°, and 235°C, respectively. The chromatogram was recorded by a Beckmen 10 inch strip chart recorder set at 0.25 inches per minute. Insecticide concentrations were determined by electronically
interpolating peak height against the regression line of a standard curve with a TI-55 calculator (Texas Instruments). The standard curve consisted of four or more points that deviated no more than 5% from the expected regression value. The concentration of each sample was based on the average of three injections. Additional injections were made if the coefficient of variation of the three injections was greater than 5%. A new standard curve was made each time samples were analyzed.

C. Insects.

Origin of the colonies.

The three colonies of aphids used in the experiments described in this thesis were reared as alienicolae in room 1 of the Botany and Zoology greenhouses. The aphids, collected in Ohio, were named after the municipality in which they were found and all three colonies were confirmed as *M. persicae* by Dr. Clyde Smith, Department of Entomology, North Carolina State University. The Wooster strain, already established as a laboratory culture at the Ohio Agricultural Research and Development Center in Wooster, was reared on tobacco. These aphids were large and dark green in color. The average weight of a newly matured aliencola was 1.09 mg. The Celeryville strain, collected in a commercial radish field, was reared on turnips. These
aphids were smaller than the Wooster strain alienicolae, light yellow in color, and weighed an average of 0.72 mg. The Columbus strain, reared on turnips, was collected in a research greenhouse at The Ohio State University from an infestation on wild radish plants that could not be controlled with acephate. The alienicolae were yellow-green in color and weighed an average of 0.62 mg.

The Wooster aphids used in experiments performed herein were usually reared on tobacco plants as described above. However, subcultures of the Wooster strain were occasionally established on turnip and used in experiments to test the effect of host plant on pesticide metabolism. The average weight of these aphids was 0.75 mg. Reciprocal rearing of the Celeryville and Columbus strains on tobacco could not be done since these strains did not survive on tobacco.

Aphid rearing and handling.

The Celeryville and Columbus colonies were reared in separate rectangular wooden frame cages (1.0 x 0.75 x 0.5 m). Each cage had a plywood bottom, plexiglass on the front, back and top, and fine mesh nylon screening on the sides. The Wooster colony was kept in a separate isolation room adjacent to the room were the Celeryville and Columbus strains were caged. The isolation room had metal screens
covering the air vents and doors. These arrangements proved sufficient to keep the cultures free of parasites, predators, and alate aphids during the course of the study. Cross contamination between the 3 colonies was avoided through a series of hygiene measures: All new host plants were inspected for aphids before use; host plants, reared in a separate room, were never returned to the plant propagation room once removed; people could not enter an isolation room after handling aphids or infested plants; hands and arms were washed with soapy water before and after aphids were handled; the forceps and aspirators used to move aphids were washed beforehand; and no aphid or plant, once removed from a colony, was returned to it. In addition, no alternate hosts were grown in the culture rooms on which aphids of any species could survive.

Aphids were reared in synchronized generations or cohorts at ambient greenhouse temperature (21-38°C) and a 15:9 hour photoperiod. A cohort was started by transferring 1 to 3 adult alienicola to each of several uninfested host plants with number 5 forceps. The plants were generally 2 to 4 weeks old with at least 2 sets of leaves above the cotyledon leaves. The aphids deposited nymphs, usually about 20 per female, for 48 hours and then were removed. The first instar nymphs, numbering about 20 to 60 per plant, reached adulthood in 7 to 9 days and were either
used for experimentation or discarded within 2 days after the first individuals became adults. Aphids were aspirated into 1 oz. glass jars covered with snap on lids so they could be carried to the laboratory for experimentation.

Rearing host plants.

The host plants were reared in an isolation room under the same temperature and photoperiod conditions as where the aphid colonies. The turnip plants (Burpee Tokyo Cross Hybrid) were grown from seed in 3 inch pots, 1 or 2 plants per pot. Tobacco transplants (variety unknown), grown from seed collected from mature plants in the greenhouse, were raised in trays and transplanted into 5 inch pots when the seedlings were 1 to 2 inches tall. The potting soil used for both host plants consisted of 40% peat moss, 25% loam, 25% perlite, and 10% sand. The plants were keep free of aphid contamination by employing the same hygienic methods described for the aphid colonies. On two occasions aphids from the Celeryville colony became established in the rearing room. The infestation was eliminated by throwing out badly infested plants and aspirating aphids from the other plants. For the most part, however, infestation of plants in the plant rearing room was not a problem during this study.
Insecticide selection.

Since the Columbus colony showed signs of insecticide resistance it was selected every 3 or 4 generations with dichlorovos (Vapona) vapors to prevent the reversion to susceptibility that occurred in other colonies of M. persicae (Needham and Sawicki 1971, Sawicki et al 1980). Forty to 60 adult aphids on a turnip plant were placed inside a 6 gallon trash bag with a Shell "No Pest Strip" (Vapona). The aphids were removed after 10 minutes. About 40% of the aphids survived this treatment and these survivors were used to start the next generation of aphids in the greenhouse. The Celeryville and Wooster strains were not selected.

D. Toxicity and Joint Action Tests.

Wooster aphids reared on tobacco and Celeryville and Columbus aphids reared on turnips were used in all experiments described in this section.

Toxicity tests to obtain the LD$_{50}$ of parathion, resmethrin, aldrin, and dieldrin were conducted using the method of Needham and Devonshire (1973) with the following variations: a 30 gauge needle was used instead of a 33 gauge needle and insecticides were applied in a 0.096 ul volume of 2-butanol. In certain cases as indicated in the results, joint toxicity tests were conducted with PBO, an MFO inhibitor (Casida 1970), or DEF, a carboxylesterase
inhibitor (Jao and Casida 1974). Dissolved in 2-butaneone with the insecticides, the inhibitors were applied to the Wooster and Columbus strains at a dose near the LD$_{0.01}$ of their respective dose-toxicity curves. The Celeryville strain received the dose of PBO applied to the Wooster strain and was not exposed to DEF. Both types of toxicity test were conducted using 4 doses of insecticide (+ inhibitor) estimated to give 16 to 84% mortality. Twenty aphids per dose were used and each experiment, except for dieldrin in the Wooster strain, was replicated 3 or more times. The treated aphids were kept at 25°C on excised host plant leaves using clip-on confinement cages (Ledieu 1979). Dead and moribund aphids were counted under a dissecting microscope after exposure for 24 hours (parathion and resmethrin) or 48 hours (aldrin and dieldrin). An aphid was considered moribund when it could no longer upright itself and walk after being placed on its dorsum. Control mortality was generally not a problem but some experiments were discarded when they did not give a dose-mortality curve. The LD$_{50}$ and 95% confidence limits, and the slope and standard error of the slope were calculated by the probit method (Finney 1971, SAS Institute 1982). The interaction ratios (synergism and antagonism) and the relative percent synergism (R%S) and and relative percent antagonism (R%A) (Brindley and Selim 1984) were calculated
for both inhibitors.

E. *In vitro* Carboxylesterase Assays in Three Strains of *M. persicae*.

Carboxylesterase assays were done with two substrates, NA and MEB. The aphids used in all experiments in this section were Wooster aphids reared on tobacco and Celeryville and Columbus aphids reared on turnips.

Protein determinations.

The amount of protein per aphid was determined spectrophotometrically according to the method of Bramhall et al (1969). The absorbance of protein in a 50 ul aliquot of homogenate was measured with a Spectronic 20 spectrophotometer at 605 nm. The concentration of protein was determined by interpolating against the regression line of a standard curve of bovine serum albumin with a TI-55 calculator.

*In vitro* hydrolysis of 1-naphthyl acetate.

The hydrolysis of NA was studied using the method of Devonshire (1977). Ten to 15 aphids were counted and weighed to the nearest 0.01 mg before homogenization in 1.0 ml of 20 mM phosphate buffer (pH 7.0) kept at room temperature. Aliquots of homogenate containing the equivalent of 0.5 insect (Wooster and Celeryville) or 0.2 insect (Columbus) were incubated at 25°C in a 6.0 ml
reaction mixture of 0.25 mM NA for 30 minutes. The reaction was stopped with the addition of 0.5 ml of a solution containing two parts fast blue B dye (1% w/v) and 5 parts sodium dodecyl sulfate (5% w/v). The absorbance of the 1-naphthol-dye complex was measured at 605 nm 15 minutes later in a Varian DMS-100 split beam spectrophotometer. The uMoles of NA hydrolyzed was determined with a TI-55 calculator for each replicate by interpolating from the regression line of a standard curve of uMoles of 1-naphthol vs. absorbance at 605 nm.

Effect of time on the hydrolysis of methyl butyrate.

In this assay, modeled after Robbins et al (1958) and modified by Bigley and Plapp (1960), methyl butyrate (MEB) was used as a substrate and Tris buffer (pH 7.5, 0.05 mM) was substituted for acetate and phosphate buffers. All other reagents were the same as those used by Robbins et al (1958). About 80 to 100 aphids from the Wooster or Columbus strains were counted and weighed to the nearest 0.01 mg before homogenization in 3 ml of cold Tris buffer. The concentration of the homogenate was adjusted to 5 mg aphid/ml. Two duplicate sets of 7 test tubes, containing 1.0 ml of 5 mM MEB per tube, were placed in a water bath shaker (New Brunswick Scientific Co., Inc.) under gentle agitation (100 RPM) at 25°C. The hydrolysis reaction was
initiated in one set of tubes with the addition of 1.0 ml of homogenate per tube. The other set of tubes received 1.0 ml of buffer. The latter tubes served as controls for nonenzymatic hydrolysis and contained, at the end of the reaction, the amount of MEB available for enzymatic hydrolysis in the experimental tubes. The reaction in one homogenate and one control tube was stopped every 5 minutes, starting at time 0, by adding 4.0 ml alkaline hydroxylamine. Two minutes later 2.0 ml of HCL solution was added followed by 2.0 ml ferric chloride solution. A blank correction for nonspecific color was prepared in the same manner as the controls except that HCL was added 2 minutes before alkaline hydroxylamine. The solutions were filtered through filter paper (Reeve Angel, grade 202, 11.0 cm) into cuvettes and the absorbance at 540 nm was read on a Spectronic 20 spectrophotometer. After correcting for nonspecific color in both the homogenate and control tubes, the uMoles of MEB per tube was mathematically determined with a TI-55 calculator by interpolating from a standard curve, umoles of MEB against absorbance. The uMoles of MEB hydrolyzed then was determined by subtracting uMoles of MEB in the homogenate tubes from the uMoles available for hydrolysis in the control tubes.
Effect of substrate concentration on MEB hydrolysis.

One ml of homogenate (5 mg aphid/ml) was incubated with 1.0 ml of 5, 7, 8.5, or 10 mM MEB for 25 minutes. The experimental conditions and procedures were identical to those used above except that Celeryville aphids were used as well as Columbus and Wooster aphids. Controls for nonenzymatic color were prepared for each substrate concentration. Lineweaver-Burk plots (Lineweaver and Burk 1934), using the reciprocals of reaction velocity (umoles MEB/ug protein/minute) and substrate concentration, were constructed to determine the Michaelis constant (Km) and maximum velocity of the reaction (Vmax). The plots were corrected for substrate depletion according to the method of Glick et al (1968).

F. Aldrin Epoxidation Experiments.

The Celeryville and Columbus aphids used in experiments described in this section were reared on turnips. Wooster aphids were reared on either tobacco or turnips, as indicated in the text.

Analysis and purification of 14C aldrin.

The 14C aldrin (1,2,3,4,10-14C, 208 uCi/mg) used in the aldrin epoxidation experiments was analyzed for chemical purity using gas chromatography. A 9.9 ul aliquot of the manufacturer's stock solution was diluted with 2.5
ml of glass distilled benzene. A 0.5 ml aliquot of the
diluted solution was than diluted 1:3 for analysis of
dieldrin and an additional 1.0 ml aliquot was diluted 1:300
for analysis of aldrin. The results showed the
manufacturer's stock solution to contain 83.3% aldrin, 6.8%
dieldrin, and by inference, 9.9% other compounds.

Column chromatography was used to purify separate 5.0
ul aliquot of the manufacturer's stock solution. The
chromatographic columns consisted of a 9 inch Pasteur
pipette (9.0 mm O. D.) with a glass wool plug and 0.6 g of
oven-activated pesticide grade florisil (60/80 mesh). Each
5.0 ul aliquot of aldrin was eluted with 25 ml of glass
distilled petroleum ether and collected in a 25 ml Kuderna-
Danish concentrator tube equipped with a modified Snyder
column. The eluates were concentrated to 0.5 ml in a 41-
65°C water bath and combined. GLC analysis showed the
combined concentrated eluate to be a 0.017 mg/ml solution
of uncontaminated aldrin.

The purified eluate was reduced to near dryness and
reconstituted with 2-butanol to 0.25 ml or 1/2 the volume
used for GLC analysis. To it was added an equal volume of
nonradiolabeled aldrin in 2-butanol, 0.47 mg/ml, to give a
final concentration of 0.295 mg/ml or 29.5 ng/0.1 ul of $^{14}$C
aldrin. Six-10 ul aliquots of this solution were pipetted
into separate 6.0 ml polyethylene scintillation vials
containing 4.0 ml of toluene scintillation cocktail (Toluene (1000 ml)/PPO (5 g)/POPOP (0.1 g)/Ethoxylated octylphenol (330 ml)). The activity was measured on a Beckman 6800 scintillation counter with automatic quench control. There were 487 DPM/0.1 ul or 16.5 DPM/ng of $^{14}$C aldrin.

Penetration and epoxidation of $^{14}$C aldrin.

The penetration and epoxidation of a sublethal dose of $^{14}$C aldrin was studied by topically applying 24 ng per female of $^{14}$C aldrin to groups of 20 aphids from the Wooster strain reared on tobacco or the Celeryville and Columbus aphids reared on turnips. After a 2 hour exposure, the aphids were rinsed 4 times with 0.5 ml of glass distilled petroleum ether to remove the unpenetrated aldrin. The rinse was pipetted into 6.0 ml polyethylene scintillation vials containing 4.0 ml of toluene scintillation cocktail and saved for liquid scintillation counting. The aphids were homogenized in 2.5 ml of petroleum ether to extract the penetrated aldrin and its toxic oxidative metabolite, dieldrin. The extract was pipetted into a 10 ml Kuderna-Danish concentrator tube and concentrated to <0.5 ml in a 41-65°C water bath. The concentrate was pipetted into a chromatographic column (see above) and eluted with 25 ml of petroleum ether to remove the aldrin and 25 ml of 1% methanol in hexane to elute the
dieldrin. The eluates were collected into 25 ml Kuderna-Danish concentrator tubes and reduced to 0.5 ml volumes in a water bath. A few ul of the aldrin and dieldrin fractions were analyzed by GLC to insure that each fraction was pure aldrin or dieldrin. The concentrates and 3-0.5 ml petroleum ether rinses of the concentrator tubes were pipetted into 6.0 ml polyethylene scintillation vials containing 4.0 ml of toluene scintillation cocktail and, along with the rinse, counted by a Beckman 6800 scintillation counter with automatic quench control.

Aldrin penetration and epoxidation over time.

A second set of experiments was performed to investigate the penetration and epoxidation of aldrin over an 8 hour exposure period. The cumbersome and time consuming column separation of aldrin and dieldrin used above was replaced with GLC and unlabeled aldrin was used instead of $^{14}$C aldrin. Thirty ng/female of aldrin was applied to groups of 100 or more aphids from the Celeryville and Columbus strains and the Wooster aphids reared on tobacco. After an exposure period of 0.05, 1, 2, 4, or 8 hours a group of aphids was rinsed 3 times in 0.5 ml of glass distilled petroleum ether to remove the unpeneetrated aldrin from the cuticle. The aphids were homogenized in petroleum ether and centrifuged for 5
minutes at 2020 g's in a tabletop centrifuge. The extract and rinse were analyzed with GLC for the presence of aldrin and its toxic oxidative metabolite, dieldrin.

Effects of host plant on aldrin metabolism.

Wooster aphids reared on turnips for 4 generations were treated with aldrin in order to determine if host plant affected aldrin metabolism. The procedure was identical to that used to investigate the epoxidation of aldrin over time in Wooster aphids reared on tobacco except that in this case exposure was limited to a single 4 hour period. The results of this experiment were compared with the 4 hour aldrin epoxidation data, gathered previously in the experiment testing aldrin exposure over time, for Wooster aphids reared on tobacco and Celeryville and Columbus aphids reared on turnips.

G. Oxidative Metabolism of \(^{14}\text{C}\) Parathion.

The Celeryville and Columbus aphids used in the experiments described in this section were reared on turnips. The Wooster aphids were reared on either tobacco or turnips.

Purity and specific activity of \(^{14}\text{C}\) parathion.

Fifty uCi of \(^{14}\text{C}\) parathion (O,O-diethyl-1-\(^{14}\text{C}\) O-p-nitrophenyl phosphorothioate, 1.54 mCi/mMole) containing 9.448 mg of parathion was dissolved in 1.0 ml of glass
distilled benzene. A 0.50 ml aliquot of this solution was diluted in 1.0 ml of 2-butanone to give a concentration of 0.448 mg/ml.

The purity of the $^{14}$C parathion was determined with thin layer chromatography. A 50 ul aliquot of the 0.448 mg/ml solution containing 26 ug of $^{14}$C parathion and a 50 ul aliquot of a solution containing 50 ug each of nonradiolabeled parathion and paraoxon were applied to a single spot on the preabsorbent layer of a thin layer chromatography plate (Anatech Preabsorbent Silica Gel GF, 250 um, 20 x 5 cm). The plate was developed in hexane:ethyl acetate:acetic acid (250:125:0.5) for 15 cm. The location of the uv-visible standards was marked and each standard was scraped into separate 20 ml glass scintillation vials containing 5.0 ml of toluene scintillation cocktail. The rest of the plate was divided into zones 1.0 cm in width and scraped by zone into scintillation vials. The gel was held in the dark for 98 hours before counting in a Beckman 6800 scintillation counter with automatic quench control. Counted also was 5.0 cm$^2$ of silica gel scraped from above the 15 cm development area. This gel was used as a control to correct for residual phosphorescence when the DPM per zone were estimated. These estimates were calculated according to the method of Wang et al (1975). First, the phosphorescence control and sample vials were corrected for
background radiation and counting efficiency to obtain DPM per vial. Then the control DPM were corrected for the amount of silica gel in the sample vial (this correction was bypassed if the sample vial contained the same amount of silica gel as the control, 5.0 cm²). Next, 95% confidence limits were calculated for both vials using the formula

$$X + \sqrt{X} (1.96),$$

where X is the DPM per vial. The activity in the sample vial was judged to be significantly above the control DPM if the lower confidence limit of the sample did not overlap with the upper confidence limit of the control. The ¹⁴C parathion was 98% pure, matching the percent purity claimed by the manufacturer.

The specific activity of the ¹⁴C parathion was determined by GLC and liquid scintillation counting. Another 0.05 ml aliquot of the 9.448 mg/ml solution was diluted 1:10,000 with benzene and used for GLC. Four 50 ul aliquots of the parathion solution used for GLC analysis were pipetted into separate 20 ml glass scintillation vials containing 5.0 ml of toluene scintillation cocktail. The activity was counted on a Beckman 6800 scintillation counter with automatic quench control. The specific activity was calculated at 1.54 uCi/uMole or 14.097 DPM/ug, matching that claimed by the manufacturer.
Preparation of \textsuperscript{14}C parathion for metabolism study.

A 0.05 ml aliquot of labeled parathion (9.448 mg/ml) was dissolved in 1.2 ml 2-butanone to give a final concentration of 0.404 mg/ml or 40.4 ng/0.1 ul. Since the volume of application was 0.0965 ul, the amount applied per female was 39 ng.

Oxidative metabolism of \textsuperscript{14}C parathion.

The oxidative metabolism of parathion was studied by applying 39 ng/female of \textsuperscript{14}C parathion to groups of 100 or more aphids. The aphids used were those from the Wooster strain reared on tobacco and the Celeryville and Columbus strain reared on turnips. After 1 hour exposure the aphids were rinsed 3 times with 0.5 ml petroleum ether, homogenized in 1.5 ml of methanol/acetone (1:1) containing 50 ul of HCL, and centrifuged for 5 minutes on a tabletop centrifuge at 2020 g's. The pellet was dissolved in tissue solubilizer and saved for liquid scintillation counting. The supernatant solution was evaporated to just dryness in a 35 °C water bath under nitrogen, reconstituted with 0.3 ml of ethyl acetate and spotted in a broad band across the preabsorbent layer of a thin layer chromatography plate (Whatman LK5F 250 um, 20 x 10 cm). The plate was developed in hexane:ethyl acetate:acetic acid (400:100:1). After the solvent front migrated for 15 cm, the location of the UV-
visible zones were noted and each 0.5 cm zone of silca gel was scraped into a scintillation vial. The gel was stored in darkness for 98 hours before counting in a Beckman 6800 liquid scintillation counter equipped with automatic quench control. Under the same experimental conditions, controls for nonenzymatic hydrolysis of parathion were obtained by adding $^{14}$C parathion to an acidified reaction mixture before homogenization.

Effects of host plant on $^{14}$C parathion metabolism.

Wooster aphids reared on turnips for 4 generations were subjected to the procedure described above for the oxidative metabolism of $^{14}$C parathion. The results were compared with those for the Wooster strain reared on tobacco that were subjected to the same experimental procedure.

H. Analysis of Data

Analysis of variance methods (SAS Institute 1982) were used for the statistical treatment of all but the toxicity data. Tukey's honestly significant difference test (SAS Institute 1982) was used to tests differences between significant means generated by ANOVA.
Chapter III

Results

A. Toxicity Tests.

The toxicity and joint action data described in this section were obtained in experiments with turnip-reared Celeryville and Columbus aphids and tobacco-reared Wooster aphids.

The dose-mortality curves for toxicity tests with parathion, resmethrin, aldrin, and dieldrin for the three strains of aphids are illustrated in Figures 1-4, respectively. The LD$_{50}$'s with 95% confidence limits, slopes and standard errors, and resistance ratios are seen in Table 1. The data show that the Columbus strain, with resistance ratios of 24 and greater, is resistant to all of the insecticides. Its resistance to parathion, 2930-fold, is higher than any reported contact or topical resistance ratio found in the literature (Needham and Sawicki 1971, Sudderuddin 1973a, and Devonshire and Needham 1974). Its resistance to aldrin and dieldrin is so high that a dose-mortality curve was unobtainable as the maximum dose applied (5000 ng/female) of either compound caused only 5-15% mortality. The Columbus strain's resistance to resmethrin, 24-fold, is moderate when compared to its
Figure 1. Dose-mortality curve for parathion in the Wooster (●----●), Celeryville (○----○), and Columbus (△----△) strains.
Figure 2. Dose-mortality curves for resmethrin in the Wooster (●----●), Celeryville (○----○), and Columbus (△----△) strains.
Figure 3. Dose mortality curves for aldrin in the Wooster (●---●) and Celeryville (〇----〇) strains.
Figure 3

Mortality (Probit Scale) vs. Dose, ng/female (log scale)
Figure 4. Dose mortality curves for dieldrin in the Wooster (●——●) and Celeryville (○——○) strains.
Table 1. LD$_{50}$ values, 95% confidence limits, and resistance ratios from toxicity tests among three strains of *M. persicae*.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Strain</th>
<th>LD$_{50}$ (95% C.L.)</th>
<th>Ratio</th>
<th>Slope (±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WOO</td>
<td>0.37 (0.28-0.47)</td>
<td></td>
<td>1.9 (+1.3)</td>
</tr>
<tr>
<td></td>
<td>CEL</td>
<td>0.78 (0.62-1.1)</td>
<td>2</td>
<td>5.2 (+0.6)</td>
</tr>
<tr>
<td></td>
<td>COL</td>
<td>1082 (985-1189)</td>
<td>2930</td>
<td>3.3 (+0.7)</td>
</tr>
<tr>
<td></td>
<td>CEL</td>
<td>4.4 (3.9-5.0)</td>
<td></td>
<td>3.3 (+1.0)</td>
</tr>
<tr>
<td></td>
<td>WOO</td>
<td>13 (9-23)</td>
<td>3</td>
<td>1.4 (+0.9)</td>
</tr>
<tr>
<td></td>
<td>COL</td>
<td>106 (86-149)</td>
<td>24</td>
<td>2.6 (+1.1)</td>
</tr>
<tr>
<td></td>
<td>WOO</td>
<td>58 (40-79)</td>
<td></td>
<td>1.8 (+1.8)</td>
</tr>
<tr>
<td></td>
<td>CEL</td>
<td>280 (214-364)</td>
<td>5</td>
<td>1.7 (+1.8)</td>
</tr>
<tr>
<td></td>
<td>COL</td>
<td>&gt;5000 (-)</td>
<td>&gt;86</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>WOO</td>
<td>14 (-)</td>
<td></td>
<td>0.8 (+12.1)</td>
</tr>
<tr>
<td></td>
<td>CEL</td>
<td>257 (199-323)</td>
<td>19</td>
<td>2.2 (+1.4)</td>
</tr>
<tr>
<td></td>
<td>COL</td>
<td>&gt;5000 (-)</td>
<td>&gt;370</td>
<td>-</td>
</tr>
</tbody>
</table>

1Wooster (WOO) strain reared on tobacco, Celeryville (CEL) and Columbus (COL) strains reared on turnips.

2Resistance ratio = LD$_{50}$ resistant strain/LD$_{50}$ susceptible strain.

324 hour LD$_{50}$.

448 hour LD$_{50}$.
resistance to the other three compounds.

The Celeryville strain is tolerant (less than 8-fold resistant) to parathion and aldrin and is resistant (greater than 8-fold resistant) to dieldrin. The Wooster strain is the most susceptible of the three strains to all compounds except resmethrin, to which it is 3-fold more tolerant than the Celeryville strain.

The dose-mortality curves for joint action experiments using parathion with PBO or DEF and resmethrin with PBO are plotted in Figures 5-7, respectively. The LD₅₀'s with 95% confidence limits, joint action ratios, and slopes and standard errors are in Table 2. The data in Table 2 show that PBO antagonizes the toxicity of parathion in the Wooster and Celeryville strains while apparently synergizing it to a small degree in the Columbus strain. The overlapping 95% confidence limits for the Wooster and Celeryville strains show that PBO eliminates the differential toxicity of parathion to the two strains. PBO synergizes the toxicity of resmethrin in all three strains and causes the most interactive effect overall of any insecticide-inhibitor combination used, with the greatest effect in the Wooster strain (45-fold) and the least in the Celeryville strain (3.3-fold). The Columbus strain's interaction ratio for resmethrin and PBO is approximately one fourth that of the Wooster strain. DEF synergizes
Figure 5. Dose-mortality curves for parathion and PBO in the Wooster (●----●), Celeryville (○----○), and Columbus (△----△) strains.
Figure 5

% MORTALITY (PROBIT SCALE)

DOSE, NG/FEMALE (LOG SCALE)
Figure 6. Dose-mortality curves for parathion and DEF in the Wooster (●----●) and Columbus (△----△) strains.
Figure 6

% MORTALITY (PROBIT SCALE)

DOSE, NG/FEMALE (LOG SCALE)
Figure 7. Dose-mortality curves for resmethrin and PBO in the Wooster (●---●), Celeryville (○---○), and Columbus (△---△) strains.
Table 2. LD_{50} values, 95% confidence limits, and interaction ratios from joint action toxicity tests among three strains of *M. persicae*.\(^1\)\(^2\)

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>+Inhibitor</th>
<th>Strain</th>
<th>LD_{50} (95% C.L.)</th>
<th>Interaction(^3)</th>
<th>Ratio</th>
<th>Slope (±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathion</td>
<td>WOO</td>
<td>1.6 (1.4-1.9)</td>
<td>0.23</td>
<td>4.0 (±0.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+PBO</td>
<td>CEL</td>
<td>1.4 (1.3-1.5)</td>
<td>0.55 (±0.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL</td>
<td>864 (741-976)</td>
<td>1.3 (±0.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resmethrin</td>
<td>CEL</td>
<td>1.3 (0.98-1.8)</td>
<td>3.3</td>
<td>6.0 (±1.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+PBO</td>
<td>WOO</td>
<td>0.29 (0.22-0.36)</td>
<td>45</td>
<td>2.0 (±3.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL</td>
<td>11.0 (7.0-21.0)</td>
<td>9.6</td>
<td>1.1 (±1.2)</td>
<td></td>
</tr>
<tr>
<td>Parathion</td>
<td>WOO</td>
<td>0.32 (0.25-0.49)</td>
<td>1.2</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+DEF</td>
<td>COL</td>
<td>267 (160-375)</td>
<td>4.1</td>
<td>2.2 (±1.4)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)See Table 1 for LD_{50} values without inhibitor.

\(^2\)Wooster (WOO) strain reared on tobacco, Celeryville (CEL) and Columbus (COL) strains reared on turnips.

\(^3\)Interaction ratio = LD_{50} insecticide/LD_{50} insecticide + inhibitor.
parathion in the Columbus strain but the Wooster strain's overlapping 95% confidence limits for the LD$_{50}$ of parathion alone and its LD$_{50}$ with DEF indicates no significant synergism of toxicity.

The R&S and R&A computations (Table 3) provide another approach to analyzing joint action data (Brindley and Selim 1984). The R&S and R&A compare the amount of synergism or antagonism between a susceptible and resistant strain by comparing the percent change, on a logarithmic scale, of LD$_{50}$ relative to the total change of LD$_{50}$ values due to synergism or antagonism. These calculations show that PBO causes the largest change in the toxicity of resmethrin and parathion in the Wooster strain. The Wooster strain's R&S value for resmethrin with PBO is 3-fold greater than the Celeryville strain's R&S value (166% vs. 54%) and is 2-fold greater than the comparable R&S value for the Columbus strain (64% vs. 39%). The R&A value for parathion with PBO is nearly 5-fold greater in the Wooster strain than it is in the Celeryville strain (116% vs. 25%). Finally, DEF synergizes the toxicity of parathion 17% in the Columbus strain and an insignificant 2% in the Wooster strain.

B. In vitro Carboxylesterase Assays.

All carboxyesterase assays were conducted using Wooster strain aphids reared on tobacco and Celeryville and Columbus aphids reared on turnips.
Table 3. Relative percent synergism (R%S) and relative percent antagonism (R%A) calculations from toxicity tests (Table 1) and joint action experiments (Table 2) with susceptible (S) and resistant (R) strains of *M. persicae*.

<table>
<thead>
<tr>
<th>Insecticide/ Synergist</th>
<th>R%S</th>
<th>R%S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Resmethrin PBO</td>
<td>54</td>
<td>166</td>
</tr>
<tr>
<td>Celeryville (S)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resmethrin PBO</td>
<td>64</td>
<td>39</td>
</tr>
<tr>
<td>Wooster (S)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parathion DEF</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Wooster (S)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Celeryville (R) Columbus (R)

<table>
<thead>
<tr>
<th>Insecticide/ Antagonist</th>
<th>R%A</th>
<th>R%A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Parathion PBO</td>
<td>116</td>
<td>25</td>
</tr>
<tr>
<td>Wooster (S)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values in the column "R" represent the R%S or R%A for the resistant strain used for comparison with the comparable value of the susceptible strain in the "S" column.
Hydrolysis of 1-naphthyl acetate.

The rate of NA hydrolysis (Table 4) differs significantly among all three strains (P<0.001, ANOVA). Naphthyl acetate hydrolysis rates rank Columbus > Celeryville > Wooster with a maximum 1.3-fold difference between the parathion resistant Columbus strain and the parathion susceptible Wooster strain.

Hydrolysis of a single concentration of MEB (10mM) from the MEB kinetics experiment (see below) was examined in order to compare MEB hydrolysis in the three strains. Although the rate of MEB hydrolysis (Table 4) does not differ significantly in the Wooster and Celeryville strains, both of these strains hydrolyze significantly less MEB than the Columbus strain (P<0.0001, ANOVA). The rate of MEB hydrolysis in the Columbus strain is 1.6-fold greater than the rate averaged from the means of the other two strains.

Hydrolysis of MEB over time.

A study of the linearity of MEB hydrolysis over time was undertaken to determine the reaction conditions for the enzyme kinetics experiment. One ml of homogenate (5 mg/ml), prepared from the Columbus and Wooster strains, was incubated at 25°C with 1 ml of MEB (5 mM) for periods of time up to 30 minutes. A plot of the uMoles of MEB hydrolyzed vs. time (Figure 8) shows the reaction in both
Table 4. Means and standard errors of NA and MEB hydrolyzed by whole body homogenates of three strains of *M. persicae*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>NA Hydrolyzed</th>
<th>MEB Hydrolyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n uMoles mg⁻¹ hr⁻¹</td>
<td>n uMoles mg⁻¹ hr⁻¹</td>
</tr>
<tr>
<td>Wooster</td>
<td>3 0.35±0.006 a</td>
<td>8 1.25±0.05 a</td>
</tr>
<tr>
<td>Celeryville</td>
<td>3 0.43±0.002 b</td>
<td>12 1.08±0.04 a</td>
</tr>
<tr>
<td>Columbus</td>
<td>3 0.46±0.005 c</td>
<td>8 1.83±0.04 b</td>
</tr>
</tbody>
</table>

¹Means in the same column followed by identical letters are not significant at P<0.05, Tukey's honestly significant difference test.
strains to be linear up to 25 minutes. Based on these results, the conditions for the enzyme kinetics experiment were: reaction time and temperature, 25 minutes and 25°C, respectively; homogenate concentration, 5 mg aphid/ml; and minimum substrate concentration, 5 mM or 5 uMoles/tube.

Effect of substrate concentration on MEB hydrolysis.

The kinetics of MEB hydrolysis were investigated using the reaction conditions established above. Double reciprocal plots (Figure 9) of uMoles MEB hydrolyzed per ug protein per minute vs. the corrected substrate concentration (Glick et al 1968) and uMoles hydrolyzed per mg body weight per minute vs. the corrected substrate concentration (Figure 10) were used to determine the maximum reaction velocity (Vmax) and Michaelis constant (Km) (Table 5). The Columbus strain's Vmax is 1.5-fold and 1.6-fold greater than the respective values for the Wooster and Celeryville strains when calculated on the basis of activity per mg body weight per minute. When calculated on the basis of activity per ug protein per minute, the Columbus strain's Vmax is 1.8-fold greater than the Wooster strain's and 2.1-fold greater than the Celeryville strain's. The Km is 12.5 mM for all strains regardless of whether the Km is calculated as activity per mg body weight per minute or ug protein per minute.
Figure 8. Effect of time on the hydrolysis of 5 mM MEB at 25 °C by whole body homogenates (5mg/ml, pH 7.5) of the Wooster (●—●) and Columbus strains (△—△).
Figure 8

MOLES MEB HYDROLYZED

TIME (MINUTES)
Figure 9. Effect of substrate concentration on MEB hydrolysis: Double reciprocal (Lineweaver-Burk) plots of substrate concentration (1/S) vs. reaction velocity (1/uMoles/ ug protein/ minute) (Wooster ○----○, Celeryville ○----○, and Columbus △----△ strains).
Figure 9
Figure 10. Effect of substrate concentration on MEB hydrolysis: Double reciprocal (Lineweaver-Burk) plot of substrate concentration (1/S) vs. reaction velocity (1/μMoles/ mg body weight/ minute) (Wooster ●—● , Celeryville ○—○ , and Columbus △—△ strains).
Figure 10

\[ \frac{1}{V_0} \text{ vs. } \frac{1}{[S]} \]

Graph showing the double reciprocal plot.
Table 5. Michaelis constants (Km) and maximum reaction velocities (Vmax) of *in vivo* MEB hydrolysis in three strains of *M. persicae*<sup>1</sup>.

<table>
<thead>
<tr>
<th></th>
<th>Km (mM)</th>
<th>Vmax (umoles mg body wt&lt;sup&gt;-1&lt;/sup&gt; minute&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Vmax (umoles ug protein&lt;sup&gt;-1&lt;/sup&gt; min&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celeryville</td>
<td>12.5</td>
<td>0.045</td>
<td>5.1 x 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wooster</td>
<td>12.5</td>
<td>0.054</td>
<td>5.5 x 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Columbus</td>
<td>12.5</td>
<td>0.095</td>
<td>8.2 x 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Wooster strain reared on tobacco, Celeryville and Columbus strains reared on turnips.
C. Epoxidation of Aldrin.

The epoxidation of aldrin was studied in Wooster aphids reared on tobacco and Celeryville and Columbus aphids reared on turnips.

Metabolism of $^{14}$C aldrin.

The amount of internal aldrin, internal dieldrin, and aldrin rinsed off the cuticle were determined 2 hours after the topical application of 24 ng per female of $^{14}$C aldrin. The amount of aldrin which penetrated during each exposure period was determined by adding the extracted aldrin and dieldrin, while the total amount of insecticide recovered at each exposure period was calculated as the penetrated insecticide plus rinsed aldrin. The data were analyzed with a one way ANOVA (Table 6).

The analysis of the epoxidation of $^{14}$C aldrin show that there are significant differences among the strains in the amount of dieldrin formed from aldrin ($P<0.02$, ANOVA). The parathion-susceptible Wooster strain produces significantly more dieldrin than the parathion-tolerant Celeryville strain and parathion-resistant Columbus strain. The latter two strains are statistically indistinguishable in this regard. However, there are no significant differences among the strains in the amount of internal aldrin, aldrin rinsed off the cuticle, penetrated aldrin, and total aldrin recovered from the aphids (Table 6).
Table 6. Means and standard errors of in vivo epoxidation study with $^{14}$C aldrin in three strains of M. persicae (n=3)¹,²,³.

<table>
<thead>
<tr>
<th></th>
<th>Wooster</th>
<th>Celeryville</th>
<th>Columbus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>0.41 ± 0.0 a</td>
<td>0.87 ± 0.2 a</td>
<td>0.81 ± 0.2 a</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>1.99 ± 0.2 a</td>
<td>0.91 ± 0.2 b</td>
<td>0.98 ± 0.1 b</td>
</tr>
<tr>
<td>Rinse</td>
<td>5.00 ± 0.8 a</td>
<td>3.83 ± 0.2 a</td>
<td>4.63 ± 0.3 a</td>
</tr>
<tr>
<td>Penetration⁴</td>
<td>2.4 ± 0.1 a</td>
<td>1.78 ± 0.2 a</td>
<td>1.78 ± 0.2 a</td>
</tr>
<tr>
<td>Total⁵</td>
<td>7.4 ± 1.0 a</td>
<td>5.61 ± 0.2 a</td>
<td>6.46 ± 0.2 a</td>
</tr>
</tbody>
</table>

¹Means in the same row followed by identical letters are not significant at $P<0.05$, Tukey's honestly significant difference test.

²Wooster strain reared on tobacco, Celeryville and Columbus strains reared on turnips.

³24 ng $^{14}$C aldrin per female aphid applied.

⁴Penetrated = ng aldrin + ng dieldrin.

⁵Total = ng penetrated + ng rinsed.
Effect of exposure time on the epoxidation of aldrin.

The epoxidation of aldrin over time was studied by applying aldrin to groups of aphids from each of the 3 strains. The amounts of internal aldrin and dieldrin extracted from the aphids, penetrated aldrin (ng internal aldrin + ng internal dieldrin), unpenetrated aldrin rinsed off the cuticle, and total amount of insecticide recovered (ng penetration + ng rinse), both as ng per female aphid and ng per mg aphid, were quantified at 0.05, 1, 2, 4, and 8 hours after application. A two way ANOVA was used to analyzed the data (internal aldrin and dieldrin, penetrated aldrin, rinsed aldrin, and total aldrin recovered) by time after application, i.e. exposure time, and strain of aphid. The analysis by exposure time determined if the average interstrain amount of aldrin, dieldrin, etc., changes significantly during the course of the experiment, while the analysis by strain tested for significant differences in the amount of aldrin, dieldrin, etc. among the three aphid strains. The two way ANOVA was also used to determine if changes among the strains in any single variable, such as the amount of internal aldrin, etc. were proportional (nonsignificant) or nonproportional (significant) over time by testing for statistical interaction between exposure time and time after application. Tukey's honestly significant difference test was used to find differences
among means found significantly different by ANOVA.

Results of the two-way ANOVA.

The two-way ANOVA shows significant differences among the strains of aphids and exposure times in the amount of total insecticide recovered, aldrin rinsed off the cuticle, and internal aldrin and dieldrin, whether the data are analyzed as ng per female aphid (Table 7) or ng per mg aphid (Table 8). There are also significant differences among the strains of aphids in the amount of penetrated insecticides but no difference in penetration among exposure times (Tables 7 and 8). Tukey's analysis of the significant means is as follows:

Total insecticide recovered.

The amount of total insecticide recovered, when taken as the average of the 3 strains, decreases for the first hour after exposure and remains constant for the duration of the experiment, regardless if the data are calculated on a ng per female basis (Figure 11, Table 9) or a ng per mg aphid basis (Figure 12, Table 10). However, there are differences among the strains in the total amount of insecticide recovered. The amount recovered from the Columbus strain on a ng per female basis is significantly lower than it is in the Celeryville and Wooster strains, which do not differ in this respect (Figure 11, Table 11).
And total recovery on a ng per mg aphid basis is lower in the Wooster strain than it is in the Celeryville and Columbus strains, in which the total amount recovered is equal (Figure 12, Table 12).

Aldrin rinsed from the cuticle.

The average amount of aldrin rinsed off the cuticle in the 3 strains of aphids, on both a ng per female (Figure 13, Table 9) and ng per mg aphid basis (Figure 14, Table 10), decreases for the first two hours after exposure and remains unchanged thereafter. However, analysis of the differences among the strains show significantly more aldrin is rinsed off the Wooster strain than the Celeryville and Columbus strains when the data are calculated as ng per female aphid (Figure 13, Table 11). There are no difference among the latter 2 strains in this respect. When expressed as ng per mg aphid, the amount of aldrin rinsed from the Columbus strain is significantly higher than it is in the Wooster and Celeryville strains, which show no difference in this regard (Figure 14, Table 12).

Internal aldrin and dieldrin.

The average internal aldrin content of the 3 strains decreases (Figures 17 and 18) as the average internal dieldrin content increases (Figures 19 and 20), regardless
of whether the data are calculated as ng per female aphid or ng per mg aphid. The average amount of aldrin decreased for the first 2 hours after exposure and remained unchanged thereafter, while the average amount of dieldrin increased for the first 4 hours after exposure and remained the same for the duration of the experiment (Tables 9 and 10).

Analysis of the differences among the strains show that the Wooster strain has significantly less aldrin and more dieldrin than the Columbus and Celeryville strains, regardless of whether the data are considered on a ng per female aphid basis (Figures 17 and 19, Table 11) or a ng per mg aphid basis (Figures 18 and 20, Table 12). The Columbus strain itself has less aldrin than the Celeryville strain but equal amounts of dieldrin on a ng per female basis (Table 11). However, the converse is true when the data are considered on a ng per mg aphid basis: the Columbus aphids have an equal amount of aldrin but more dieldrin than the Celeryville strain (Table 12).

Penetrated insecticide.

The average amount of penetrated insecticide does not differ over time whether the data are analyzed as ng per female aphid (Figure 15, Table 11) or ng per mg aphid (Figure 16, Table 12). However, there are differences in the amount of aldrin penetrating the individual strains. On a ng per female aphid basis the Columbus strain has
significantly lower amounts of penetrated aldrin than the Wooster and Celeryville strains, which do not differ in this respect (Figures 15, Table 11). On a ng per mg aphid basis, penetration is significantly lower in the Wooster strain than in the Celeryville and Columbus strains (Figure 16, Table 12). Equal amounts of aldrin penetrated the later 2 strains.

Interaction of exposure time and strain of aphid.

Finally, the interaction of exposure time and strain of aphid is significant for the variables dieldrin and total insecticide recovered when the data are tabulated as ng per female aphid (Table 7), and for aldrin rinsed off the cuticle and total insecticide recovered when the data are considered as ng per mg aphid (Table 8). These interactions indicate that changes in the variables over time are not proportional among the strains. For example, the analysis by time after application of the total amount of insecticide recovered (ng per female aphid) shows no change in the interstrain average after the first hour after application (Figure 11, Table 7). However, the amount of insecticide recovered in each individual strain is not constant during this period, and in fact the amount recovered in the Wooster strain increases relative to the Celeryville and Columbus strains at about one hour after
Table 7. In vivo aldrin epoxidation over time (data of Figures 11, 13, 15, 17, and 19). Mean squares of analysis of variance for ng per female of aldrin, dieldrin, penetrated insecticide, rinsed insecticide, and total insecticide recovered for three strains of M. persicae.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Aldrin</th>
<th>Dieldrin</th>
<th>Penetration</th>
<th>Rinse</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (T)</td>
<td>4</td>
<td>76*</td>
<td>72*</td>
<td>1.45</td>
<td>203*</td>
<td>218*</td>
</tr>
<tr>
<td>Strain (S)</td>
<td>2</td>
<td>87*</td>
<td>87*</td>
<td>30***</td>
<td>6.9*</td>
<td>41*</td>
</tr>
<tr>
<td>(T) x (S)</td>
<td>8</td>
<td>1.4</td>
<td>6.6**</td>
<td>4.71</td>
<td>0.7</td>
<td>6.7****</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>1.5</td>
<td>1.2</td>
<td>3.1</td>
<td>0.3</td>
<td>2.7</td>
</tr>
</tbody>
</table>

1Wooster strain reared on tobacco, Celeryville and Columbus strains reared on turnips.

*P<0.0001.

**P<0.0003.

***P<0.0006.

****P<0.0321.
Table 8. *In vivo* aldrin epoxidation over time (data of Figures 12, 14, 16, 18, and 20). Mean squares of analysis of variance for ng per mg aphid of aldrin, dieldrin, rinsed aldrin, penetrated aldrin, and total insecticide recovered for three strains of *M. persicae*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Aldrin</th>
<th>Dieldrin</th>
<th>Penetration</th>
<th>Rinse</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (T)</td>
<td>4</td>
<td>122*</td>
<td>104*</td>
<td>2.6</td>
<td>356*</td>
<td>398*</td>
</tr>
<tr>
<td>Strain (S)</td>
<td>2</td>
<td>291*</td>
<td>18*</td>
<td>164*</td>
<td>3.4**</td>
<td>184*</td>
</tr>
<tr>
<td>(T) X (S)</td>
<td>8</td>
<td>2.4</td>
<td>2.6</td>
<td>7.8</td>
<td>12.8*</td>
<td>33*</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>3.1</td>
<td>1.5</td>
<td>5.1</td>
<td>0.5</td>
<td>4.3</td>
</tr>
</tbody>
</table>

1Wooster strain reared on tobacco, Celeryville and Columbus strains reared on turnips.

*P<0.001.

**P<0.0136.
Table 9. *In vivo* aldrin epoxidation over time. Summary of Tukey's honestly significant difference test of exposure time means (ng per female).\(^1\)

<table>
<thead>
<tr>
<th>Hours after application</th>
<th>0.05</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aldrin</strong></td>
<td>10.96 a</td>
<td>6.50 b</td>
<td>5.05 bc</td>
<td>3.71 bc</td>
<td>3.50 c</td>
</tr>
<tr>
<td><strong>Dieldrin</strong></td>
<td>0.00 a</td>
<td>4.14 b</td>
<td>5.05 bc</td>
<td>5.62 cd</td>
<td>9.98 d</td>
</tr>
<tr>
<td><strong>Penetration</strong></td>
<td>10.96 a</td>
<td>10.64 a</td>
<td>10.10 a</td>
<td>9.33 a</td>
<td>13.48 a</td>
</tr>
<tr>
<td><strong>Rinse</strong></td>
<td>11.34 a</td>
<td>1.41 b</td>
<td>0.77 bc</td>
<td>0.42 c</td>
<td>0.45 c</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>22.30 a</td>
<td>12.05 b</td>
<td>10.87 b</td>
<td>9.75 b</td>
<td>13.93 b</td>
</tr>
</tbody>
</table>

\(^1\)Means within rows followed by different letters are significant at \(P<0.05\), Tukey's honestly significant difference test.
Table 10. *In vivo* aldrin epoxidation over time. Summary of Tukey's honestly significant difference test of exposure time means (ng per mg aphid).

<table>
<thead>
<tr>
<th>Hours after application</th>
<th>0.05</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>13.40 a</td>
<td>6.41 b</td>
<td>7.06 bc</td>
<td>6.17 bc</td>
<td>4.69 c</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>0.00 a</td>
<td>5.44 b</td>
<td>6.05 bc</td>
<td>7.53 cd</td>
<td>8.86 d</td>
</tr>
<tr>
<td>Penetration</td>
<td>13.40 a</td>
<td>11.85 a</td>
<td>13.11 a</td>
<td>13.70 a</td>
<td>13.55 a</td>
</tr>
<tr>
<td>Rinse</td>
<td>14.78 a</td>
<td>1.41 b</td>
<td>0.81 bc</td>
<td>0.45 c</td>
<td>0.47 c</td>
</tr>
<tr>
<td>Total</td>
<td>28.18 a</td>
<td>13.26 b</td>
<td>13.92 b</td>
<td>14.15 b</td>
<td>14.02 b</td>
</tr>
</tbody>
</table>

1 Means within rows followed by different letters are significant at P<0.05, Tukey's honestly significant difference test.
Table 11. In vivo aldrin epoxidation over time. Summary of Tukey's honestly significant difference test of between strain means (ng per female aphid)\(^1\).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Wooster</th>
<th>Celeryville</th>
<th>Columbus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>3.64 a</td>
<td>8.59 b</td>
<td>6.03 c</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>7.43 a</td>
<td>3.44 b</td>
<td>3.15 b</td>
</tr>
<tr>
<td>Penetration</td>
<td>11.07 a</td>
<td>12.03 a</td>
<td>9.18 b</td>
</tr>
<tr>
<td>Rinse</td>
<td>3.66 a</td>
<td>2.41 b</td>
<td>2.39 b</td>
</tr>
<tr>
<td>Total</td>
<td>14.73 a</td>
<td>14.44 a</td>
<td>11.57 b</td>
</tr>
</tbody>
</table>

\(^1\)Means within rows followed by different letters are significant at P<0.05, Tukey's honestly significant difference test.
Table 12. *In vivo* aldrin epoxidation over time. Summary of Tukey's honestly significant difference test of between strain means (ng per mg aphid).\(^1\)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Wooster</th>
<th>Celeryville</th>
<th>Columbus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>3.34 a</td>
<td>11.93 b</td>
<td>9.71 b</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>6.82 a</td>
<td>4.78 b</td>
<td>5.07 c</td>
</tr>
<tr>
<td>Penetration</td>
<td>10.16 a</td>
<td>16.71 b</td>
<td>14.78 b</td>
</tr>
<tr>
<td>Rinse</td>
<td>3.36 a</td>
<td>3.35 a</td>
<td>3.85 b</td>
</tr>
<tr>
<td>Total</td>
<td>13.51 a</td>
<td>20.06 b</td>
<td>18.63 b</td>
</tr>
</tbody>
</table>

\(^1\)Means within rows followed by different letters are significant at \(P<0.05\), Tukey's honestly significant difference test.
Figure 11. Ng per female of total insecticide recovered (aldrin + dieldrin + rinse) after application of 24 ng aldrin per female to three strains of *M. persicae*. Each data point is the average for three replicates.
Figure 11

NG PER FEMALE

HOURS AFTER APPLICATION

- Wooster
- Celeryville
- Columbus
Figure 12. Ng per mg body weight of total insecticide recovered (aldrin + dieldrin + rinse) after application of 24 ng aldrin per female to three strains of *M. persicae*. Each data point is the average for three replicates.
Figure 12

NG PER MG BODY WEIGHT

HOURS AFTER APPLICATION

Celeryville
Columbus
Wooster
Figure 13. Ng per female of external (rinsed) aldrin after application of 24 ng aldrin per female to three strains of *M. persicae*. Each data point is the average for three replicates.
Figure 14. Ng per mg body weight of external (rinsed) aldrin after application of 24 ng aldrin per female to three strains of *M. persicae*. Each data point is the average for three replicates.
Figure 14

- Celeryville
- Columbus
- Wooster

NG PER MG BODY WEIGHT

HOURS AFTER APPLICATION
Figure 15. Ng penetrated insecticide (internal aldrin + dieldrin) per female after application of 24 ng aldrin per female to three strains of *M. persicae*. Each data point is the average for three replicates.
Figure 16. Ng penetrated insecticide (internal aldrin + dieldrin) per mg body weight after application of 24 ng aldrin per female to three strains of *M. persicae*. Each data point is the average for three replicates.
Figure 16

NG PER MG BODY WEIGHT

HOURS AFTER APPLICATION

- Columbus
- Celeryville
- Wooster
Figure 17. Ng internal aldrin per female after application of 24 ng aldrin per female to three strains of *M. persicae*. Each data point is the average for three replicates.
Figure 17

NG per Female vs. Hours after Application

- Celeryville
- Columbus
- Wooster

HOURS AFTER APPLICATION

NG PER FEMALE
Figure 18. Ng internal aldrin per mg body weight after application of 24 ng aldrin per female to three strains of *M. persicae*. Each data point is the average for three replicates.
NG PER MG BODY WEIGHT

HOURS AFTER APPLICATION

Figure 18

Celeryville
Columbus
Wooster
Figure 19. Ng internal dieldrin per female after application of 24 ng of aldrin per female to three strains of *M. persicae*. Each data point is the average for three replicates.
Figure 19

NG PER FEMALE vs. HOURS AFTER APPLICATION

Wooster

Columbus

Celeryville
Figure 20. Ng internal dieldrin per mg body weight after application of 24 ng aldrin per female to three strains of *M. persicae*. Each data point is the average for three replicates.
application, while the recovery in the Columbus strain increases between 2 and 4 hours after application (Figure 11). A similar disproportionate increase in the total amount recovered occurs in the Celeryville strain at about 1/2 and 6 hours after application when the data are considered as ng per mg aphid (Figure 12). The interaction for dieldrin (ng per female aphid) is significant (Table 7) because the accumulation of internal dieldrin in the Wooster strain increases after 4 hours while the average interstrain value for dieldrin accumulation levels off after 4 hours (Figure 19, Table 9). The significant interaction for external or rinsed aldrin (ng per mg aphid, Figure 14) is due to the relatively slower decline in the amount of aldrin rinsed off the Wooster strain between 0.05 and 4 hours after application (Figure 13). The average interstrain values show no decrease after 2 hours (Table 10).

Host plant effect on the epoxidation of aldrin.

A culture of parathion-susceptible Wooster aphids reared on turnips for 4 generations was treated with 24 ng per female of aldrin to determine if the interstrain differences (Tables 11 and 12) in aldrin epoxidation described in the last section can be attributed to the selection of tobacco, rather than turnip, as the host plant for the Wooster strain. The amount of aldrin, dieldrin, and
penetrated insecticide present in the turnip-reared Wooster aphids was determined after a 4 hour exposure and compared with the 4 hour exposure data reported above for the tobacco-reared Wooster strain aphids and Celeryville and Columbus strain aphids (Figures 15, 17, and 18).

The results of the host plant experiment shows that there is no difference in the level of aldrin epoxidation or the amount of penetrated aldrin between subcultures of Wooster aphids reared on tobacco or turnips (Table 13). However, the Wooster aphids reared on turnips differ significantly from the Celeryville and Columbus aphids in the amount of internal aldrin \((P<0.002)\) and internal dieldrin \((P<0.005)\) (Table 14). The Wooster aphids have significantly lower levels of aldrin and higher levels of dieldrin than do the Celeryville and Columbus aphids. The level of aldrin epoxidation and the amount of penetrated insecticide is the same in the latter 2 strains.

D. Metabolism of \(^{14}\text{C}\) Parathion.

The metabolism of \(^{14}\text{C}\) parathion was studied in Wooster aphids reared on tobacco and Celeryville and Columbus aphids reared on turnips. After a 1 hour exposure to 39 ng per female of \(^{14}\text{C}\) parathion, parathion and its metabolites were extracted from the aphids and then separated by thin layer chromatography. Parathion \((\text{Rf 0.46})\) and 3 metabolites
Table 13. Means and standard errors of in vivo aldrin epoxidation experiment with Wooster aphids reared on tobacco or turnips (n= 3)\textsuperscript{1,2}.

<table>
<thead>
<tr>
<th></th>
<th>Aldrin</th>
<th>Dieldrin</th>
<th>Penetration\textsuperscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wooster (Tobacco)</td>
<td>1.7±0.3 a</td>
<td>9.1±0.77 a</td>
<td>10.8±0.77 a</td>
</tr>
<tr>
<td>Wooster (Turnip)</td>
<td>1.2±0.07 a</td>
<td>7.9±0.33 a</td>
<td>9.0±0.35 a</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Means in the same column followed by identical letters are not significant at P<0.05, ANOVA.

\textsuperscript{2}24 ng aldrin per female aphid applied.

\textsuperscript{3}Penetration = ng aldrin + ng dieldrin.
Table 14. Means and standard errors of \textit{in vivo} aldrin epoxidation experiment with Wooster, Celeryville, and Columbus aphids reared on turnip (n=3)\textsuperscript{1,2}.

<table>
<thead>
<tr>
<th></th>
<th>Aldrin</th>
<th>Dieldrin</th>
<th>Penetration\textsuperscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wooster</td>
<td>1.2±0.07 a</td>
<td>7.9±0.33 a</td>
<td>9.0±0.35 a</td>
</tr>
<tr>
<td>Celeryville</td>
<td>6.6±0.63 b</td>
<td>4.3±0.47 b</td>
<td>10.9±0.48 a</td>
</tr>
<tr>
<td>Columbus</td>
<td>4.9±0.58 b</td>
<td>5.1±0.4 b</td>
<td>9.9±0.86 a</td>
</tr>
</tbody>
</table>

\textsuperscript{1}M eans in the same column followed by identical letters are not significant at P<0.05, Tukey's honestly significant difference test.

\textsuperscript{2}24 ng per female aphid applied.

\textsuperscript{3}Penetration = ng aldrin + ng dieldrin.
were detected (Table 15). One metabolite was paraoxon (Rf 0.13) and the other two were unidentified compounds found in the preabsorbent zone and at Rf 0.013.

The amount of internal parathion and paraoxon, other metabolites, unextractable parathion in the pellet, unpenetrated parathion rinsed from the cuticle, penetrated parathion (internal parathion + paraoxon + other metabolites + pellet), and total insecticide recovered (penetrated parathion + rinse) were converted to ng parathion equivalent per female aphid and analyzed with a one way ANOVA (Table 16). This analysis shows that there are no significant differences among the strains in the amount of penetrated and rinsed parathion, parathion in the pellet, and total amount of insecticide recovered. There are, however, differences among the strains in the fate of the penetrated insecticide (Table 16). Significantly more paraoxon, more other metabolites, and significantly less parathion are found in the parathion susceptible Wooster strain than in the parathion-tolerant Celeryville strain and parathion-resistant Columbus strain (Table 17). There is no difference in this respect between the Celeryville and Columbus strain (Table 17).

There are no differences among the strains in the amount of other metabolites and parathion in the pellet when the data are considered on a ng parathion equivalent
Table 15. In vivo metabolism of $^{14}$C parathion in tobacco-reared Wooster aphids and Wooster, Celeryville, and Columbus aphids reared on turnips: Rf values of parathion and metabolites separated by thin layer chromatography.

<table>
<thead>
<tr>
<th>Rf</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00$^2$</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.0013</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.08</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.13$^3$</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.46$^4$</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

1"+" indicates the presence of a metabolite in the indicated Rf zone, "-" signals the absence of the metabolite.

$^2$Preabsorbent origin.

$^3$Paraoxon.

$^4$Parathion.
Table 16. Mean square of analysis of variance for *in vivo* metabolism of \(^{14}\)C parathion in three strains of *M. persicae* for data tabulated as ng parathion equivalent per female aphid. See Table 17 for data\(^1\).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Parathion</th>
<th>Paraoxon</th>
<th>Other Metabolites</th>
<th>Pellet</th>
<th>Penetration</th>
<th>External Rinse</th>
<th>Total Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>2</td>
<td>2.50***</td>
<td>0.20*</td>
<td>0.04**</td>
<td>0.0002</td>
<td>1.05</td>
<td>5.99</td>
<td>1.96</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>0.25</td>
<td>0.001</td>
<td>0.004</td>
<td>0.0004</td>
<td>0.25</td>
<td>2.14</td>
<td>1.61</td>
</tr>
</tbody>
</table>

\(^1\)Wooster aphids reared on tobacco, Celeryville and Columbus aphids reared on turnip.

*P<0.0001

**P<0.001.

***P<0.0119.
Table 17. Means and standard errors of in vivo tracer studies with $^{14}$C parathion among three strains of *M. persicae* (n= 3)\textsuperscript{1,2,3}.

<table>
<thead>
<tr>
<th>ng$^{14}$C parathion equivalents female$^{-1}$ (±S.E.)</th>
<th>Wooster</th>
<th>Celeryville</th>
<th>Columbus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathion</td>
<td>0.91±0.05 a</td>
<td>2.6±0.35 b</td>
<td>2.3±0.17 b</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>0.45±0.03 a</td>
<td>0.08±0.06 b</td>
<td>0.0±0.0 b</td>
</tr>
<tr>
<td>Other Metabolites</td>
<td>0.37±0.02 a</td>
<td>0.2±0.006 b</td>
<td>0.14±0.02 b</td>
</tr>
<tr>
<td>Pellet</td>
<td>0.08±0.02 a</td>
<td>0.07±0.003 a</td>
<td>0.08±0.006 a</td>
</tr>
<tr>
<td>Penetration</td>
<td>1.8±0.03 a</td>
<td>3.0±0.35 a</td>
<td>2.5±0.17 a</td>
</tr>
<tr>
<td>External Rinse</td>
<td>20.5±0.81 a</td>
<td>17.8±0.58 a</td>
<td>18.7±0.4 a</td>
</tr>
<tr>
<td>Total Recovered</td>
<td>22.4±0.81 a</td>
<td>20.8±0.29 a</td>
<td>21.3±0.5 a</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Wooster aphids reared on tobacco, Celeryville and Columbus strains reared on turnips.

\textsuperscript{2}Means in the rows followed by identical letters are not significant at the P<0.05 level, Tukey's honestly significant difference tests.

\textsuperscript{3}Treatment of 39 ng per female aphid.
per mg aphid basis (Table 18). There are, however, differences among the strains in the amount of extracted parathion and paraoxon, penetrated insecticide, external rinse, and total insecticide recovered (Table 18). Analysis of significant means with Tukey's test shows that the Wooster strain has significantly more paraoxon and less parathion than the Celeryville and Columbus strains (Table 19). The Celeryville strain has significantly more paraoxon and the same amount of parathion than the Columbus strain (Table 19). The amount of parathion rinsed off the cuticle and total insecticide recovered are significantly higher in the Columbus strain than in the Celeryville strain, which in turn has higher values for rinsed insecticide and total recovery than the Wooster strain (Table 19).

Effect of host plant on the metabolism of parathion.

A subculture of Wooster strain aphids was reared on turnips for 4 generations to determine if the interstrain differences in parathion metabolism reported above can be attributed to the use of tobacco as the host plant for the Wooster strain. The turnip-reared Wooster aphids were treated with 39 ng per female of $^{14}$C parathion for 1 hour. The amounts of internal parathion and paraoxon, other metabolites, parathion in the pellet, unpenetrated
parathion, and total insecticide recovered were determined exactly as they were for the metabolism of $^{14}$C parathion in the Wooster strain reared on tobacco and the Celeryville and Columbus strains reared on turnips (Tables 16-19). These data are compared to the comparable data for the tobacco-reared Wooster strain and analyzed with a one way ANOVA.

Rearing the Wooster aphids on turnip as opposed to tobacco has both qualitative and quantitative effects on their ability to metabolize parathion. Turnip-reared Wooster aphids produced an additional metabolite of unknown identity with an Rf value of 0.08 (Table 15). The amounts of parathion, paraoxon, and penetrated insecticide are higher in turnip-reared Wooster aphids whether the data were analyzed on a ng per female aphid (Table 20) or ng per mg aphid (Table 21) basis. Analysis of the data on a body weight basis also showed significantly higher amounts of other metabolites and total insecticide recovered in the turnip-reared Wooster aphids. The amount of parathion rinsed off the cuticle and parathion left in the pellet after extraction does not differ with host plant.
Table 18. Mean squares of analysis of variance for *in vivo* metabolism of $^{14}$C parathion in three strains of *M. persicae* for data tabulated as ng parathion equivalent per mg aphid. See Table 19 for data.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Parathion</th>
<th>Paraoxon</th>
<th>Other Metabolites</th>
<th>Pellet</th>
<th>Penetration</th>
<th>External</th>
<th>Total</th>
<th>Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>2</td>
<td>2.33*</td>
<td>0.65*</td>
<td>0.25</td>
<td>0.001</td>
<td>7.80**</td>
<td>31.46***</td>
<td>264</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>0.56</td>
<td>0.0009</td>
<td>0.05</td>
<td>0.0006</td>
<td>0.63</td>
<td>6.69</td>
<td>6.75</td>
<td></td>
</tr>
</tbody>
</table>

1Wooster aphids reared on tobacco, Celeryville and Columbus aphids reared on turnips.

*P<0.0001.

**P<0.0076.

***P<0.0266.
Table 19. Means and standard errors of *in vivo* tracer studies with $^{14}$C parathion among three strains of *M. persicae* (n=3)\(^1\).

<table>
<thead>
<tr>
<th></th>
<th>Wooster(^2)</th>
<th>Celeryville(^3)</th>
<th>Columbus(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathion</td>
<td>0.84±0.04 a</td>
<td>3.7±0.52 b</td>
<td>3.7±0.26 b</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>0.42±0.03 a</td>
<td>0.11±0.01 b</td>
<td>0.00±0.00 c</td>
</tr>
<tr>
<td>Other metabolites</td>
<td>0.34±0.02 a</td>
<td>0.3±0.01 a</td>
<td>0.22±0.03 a</td>
</tr>
<tr>
<td>Pellet</td>
<td>0.08±0.01 a</td>
<td>0.09±0.003 a</td>
<td>0.12±0.01 e</td>
</tr>
<tr>
<td>Penetration(^5)</td>
<td>1.7±0.03 a</td>
<td>4.0±0.51 b</td>
<td>4.0±0.29 b</td>
</tr>
<tr>
<td>External rinse</td>
<td>17.6±0.74 a</td>
<td>24.7±0.77 b</td>
<td>30.2±1.1 c</td>
</tr>
<tr>
<td>Total recovery</td>
<td>20.5±0.76 a</td>
<td>28.8±0.40 b</td>
<td>34.2±0.8 c</td>
</tr>
</tbody>
</table>

\(^1\)Means in the same row followed by identical letters are not significant at P<0.05, Tukey's honestly significant difference test.

\(^2\)Reared on tobacco; treated with 35.8 ng per mg aphid.

\(^3\)Reared on turnips; treated with 54.2 ng per mg aphid.

\(^4\)Reared on turnips; treated with 62.8 ng per mg aphid.

\(^5\)Penetration = ng parathion + ng paraoxon + ng other metabolites + ng in pellet.

\(^6\)Total recovery = ng penetration + ng rinse.
Table 20. Means and standard errors of *in vivo* tracer studies comparing the effects of host plant on $^{14}$C parathion metabolism by Wooster strain aphids. Data are tabulated as ng parathion equivalent per female$^{1,2}$.

<table>
<thead>
<tr>
<th></th>
<th>Wooster (tobacco)</th>
<th>Wooster (turnip)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathion</td>
<td>$0.91\pm0.05$ a</td>
<td>$3.91\pm0.16$ b</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>$0.45\pm0.03$ a</td>
<td>$0.65\pm0.02$ b</td>
</tr>
<tr>
<td>Other metabolites</td>
<td>$0.37\pm0.02$ a</td>
<td>$0.58\pm0.06$ a</td>
</tr>
<tr>
<td>Pellet</td>
<td>$0.08\pm0.02$ a</td>
<td>$0.090\pm0.22$ a</td>
</tr>
<tr>
<td>Penetration$^3$</td>
<td>$1.8\pm0.03$ a</td>
<td>$5.22\pm0.22$ b</td>
</tr>
<tr>
<td>External rinse</td>
<td>$20.5\pm0.81$ a</td>
<td>$17.1\pm0.58$ a</td>
</tr>
<tr>
<td>Total$^4$ recovery</td>
<td>$22.4\pm0.81$ a</td>
<td>$22.3\pm3.67$ a</td>
</tr>
</tbody>
</table>

$^1$Means in rows followed by identical letters are not significantly different at $P<0.05$, ANOVA.

$^2$Treated with 39 ng per female aphid.

$^3$Penetration = ng parathion + ng paraoxon + ng other metabolism + ng pellet.

$^4$Total recovered = ng penetration + ng rinse.
Table 21. Means and standard errors of *in vivo* tracer studies comparing the effects of host plant on metabolism of $^{14}$C parathion by Wooster strain aphids. Data calculated as ng parathion equivalents per mg aphid$^{-1}$.

<table>
<thead>
<tr>
<th></th>
<th>Wooster$^2$ (tobacco)</th>
<th>Wooster$^3$ (turnips)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathion</td>
<td>0.84±0.04 a</td>
<td>5.15±0.21 b</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>0.42±0.03 a</td>
<td>0.86±0.02 b</td>
</tr>
<tr>
<td>Other metabolites</td>
<td>0.34±0.02 a</td>
<td>0.75±0.07 b</td>
</tr>
<tr>
<td>Pellet</td>
<td>0.08±0.01 a</td>
<td>0.13±0.02 a</td>
</tr>
<tr>
<td>Penetration$^4$</td>
<td>1.66±0.03 a</td>
<td>6.88±0.29 b</td>
</tr>
<tr>
<td>External rinse</td>
<td>18.9±0.74 a</td>
<td>22.5±1.64 a</td>
</tr>
<tr>
<td>Total recovery$^5$</td>
<td>20.5±0.76 a</td>
<td>29.4±1.90 b</td>
</tr>
</tbody>
</table>

$^1$Means followed by identical letters are not significant at P<0.05, ANOVA.

$^2$Treated with 35.8 ng per mg aphid.

$^3$treated with 51.4 ng per mg aphid.

$^4$Penetration = ng parathion + ng paraoxon + ng other metabolites + ng pellet.

$^5$Total recovery = ng penetration + ng rinse.
Parathion metabolism data as a percent of the total dose applied.

The parathion metabolism data are displayed in Table 22 as a percent of the total dose applied in order to present a clear picture of the influence of host plant and strain of aphid on the metabolism of parathion by *M. persicae*. Included in this table are the data from $^{14}$C parathion metabolism experiments with Wooster aphids reared on tobacco and Wooster, Celeryville, and Columbus aphids reared on turnips.

Considering the data as a percent of the dose shows that the strains do not differ in the total amount of the dose recovered or in the amount of unextractable parathion found in the pellet. Total recovery of the dose was highest in the turnip-reared Wooster aphids, 57.4 %, and lowest in the Celeryville aphids, 53.3 %. There are small differences among strains in the amount of unextracted parathion in the pellet. The percent parathion in the pellet ranged from a low of 0.2 % in the Celeryville aphids to a high of 0.3 % in the turnip-reared Wooster aphids. However, there are larger interstrain differences in the amount of parathion rinsed off the cuticle and in the amount and fate of penetrated parathion. The rankings of the strains by the amount of parathion rinsed off the cuticle are Wooster (tobacco) (52.6 %) > Columbus (48.1 %) > Celeryville (45.6 %) > Wooster (turnip) (43.8 %). The order of ranking by
Table 22. Parathion metabolism data as a percent of the dose applied to Wooster strain aphid reared on tobacco and Wooster, Celeryville, and Columbus strain aphids reared on turnips. See Tables 17 and 20 for data used in calculations.

<table>
<thead>
<tr>
<th></th>
<th>Wooster</th>
<th></th>
<th>Celeryville</th>
<th></th>
<th>Columbus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tobacco</td>
<td>Turnip</td>
<td>Turnip</td>
<td>Turnip</td>
<td>Turnip</td>
<td></td>
</tr>
<tr>
<td>Parathion</td>
<td>2.3</td>
<td>10.0</td>
<td>6.7</td>
<td>5.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraoxon</td>
<td>1.2</td>
<td>1.7</td>
<td>0.2</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other metabolites</td>
<td>1.0</td>
<td>1.5</td>
<td>0.5</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pellet</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penetration</td>
<td>4.6</td>
<td>13.4</td>
<td>7.7</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External rinse</td>
<td>52.6</td>
<td>43.8</td>
<td>45.6</td>
<td>48.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total recovery</td>
<td>57.4</td>
<td>57.2</td>
<td>53.3</td>
<td>54.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
penetration of parathion is the opposite of that for the external rinse; the percent of penetrated parathion is highest in the turnip-reared Wooster aphids (13.4 %), and second and third highest, respectively, in the Celeryville (7.7 %) and Columbus (6.4 %) strains, and lowest in the tobacco-reared Wooster aphids (4.6 %). The turnip-reared Wooster aphids have the largest proportion of the dose present as internal parathion (10.0 %), paraoxon (1.7 %), and other metabolites (1.5 %). The tobacco-reared Wooster aphids are perhaps more efficient at metabolizing penetrated parathion since they have the smallest percent of the dose present as internal parathion (2.3 %) but have the second largest percent present as paraoxon (1.2 %) and other metabolites (1.0 %). The Celeryville strain has the second highest percent of the dose present as parathion (6.7 %) and ranks third in the percent paraoxon (0.2 %) and other metabolites (0.5 %). The Columbus strain ranks third in the percent internal parathion (5.9 %) and the last in the percent other metabolites (0.4 %). No paraoxon was detected in this strain.

In order to determine the relative efficiency of the strains in metabolizing penetrated parathion, the amounts of internal parathion, paraoxon, and other metabolite data are presented as a percent of penetrated parathion.
(Table 23). The tobacco-reared Wooster strain aphids clearly metabolize a greater percentage of the penetrated compound to paraoxon and other metabolites than the turnip-reared Wooster, Celeryville, and Columbus strains. Twenty-five % of the penetrated dose is metabolized to paraoxon in the tobacco-reared Wooster aphids compared to 12.5 % in the turnip-reared Wooster aphids, 2.8 % in the Celeryville aphids, and 0.0 % in the Columbus strain. The percent of other metabolites in the tobacco-reared aphids is nearly double that in the turnip-reared Wooster aphids (20.6 % vs. 10.9 %) and higher still than that present in the Celeryville (6.7 %) and Columbus (5.6 %) aphids. The total percent metabolism of penetrated insecticide is about 45.6 % in the Wooster aphids reared on tobacco, 23.4 % in the turnip-reared Wooster aphids, 9.5 % in the Celeryville strain, and 5.6 % in the Columbus strain.

These data show that the choice of tobacco as a host plant is not responsible for the higher level of parathion metabolism in the tobacco-reared Wooster aphids relative to the turnip-reared Celeryville and Columbus aphids. This is because rearing Wooster aphids on turnips increases, not decreases, the absolute amount of parathion metabolism by this strain.
Table 23. Parathion metabolism data as a percent of the penetrated parathion in the Wooster strain reared on tobacco and the Wooster, Celeryville, and Columbus strains reared on turnips. See Tables 17 and 20 for data used in calculations.

<table>
<thead>
<tr>
<th></th>
<th>Wooster</th>
<th>Celeryville</th>
<th>Columbus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tobacco</td>
<td>Turnip</td>
<td>Turnip</td>
</tr>
<tr>
<td>Parathion</td>
<td>50.6</td>
<td>74.9</td>
<td>86.7</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>25.0</td>
<td>12.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Other metabolites</td>
<td>20.6</td>
<td>10.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Total % metabolized</td>
<td>45.6</td>
<td>23.4</td>
<td>9.5</td>
</tr>
</tbody>
</table>
The aldrin epoxidation data reported in this section and the last indicate that the level of aldrin epoxidation is higher in the parathion-susceptible Wooster strain than it is in the parathion-tolerant Celeryville strain and parathion-resistant Columbus strain.
Chapter IV
Discussion

A. Levels of Insecticide Resistance.

The Columbus strain is unique among the strains of aphids studied here because of its high resistance to the 4 insecticides tested. The Columbus strain is particularly set apart from the Celeryville and Wooster strains and strains of *M. persicae* described in the literature by its nearly 3000-fold resistance to parathion (Table 1). Its high resistance ratio for parathion is not due to abnormal susceptibility in the Wooster strain because the susceptible Wooster strain has nearly the same LD$_{50}$ value and 95% confidence limits as the susceptible strain of *M. persicae* investigated by Sudderuddin (1973a). Sudderuddin's (1973a) resistant strain was only 182-fold resistant to parathion and 1.8-fold resistant to aldrin, compared to the Columbus strain's 58-fold resistance to aldrin.

Unfortunately, further comparisons of LD$_{50}$ values from the literature are not possible since I was unable to find other examples of resistance evaluations where LD$_{50}$ values for parathion and aldrin (or dieldrin and resmethrin) are reported. Resistance in *M. persicae* is typically measured with the organophosphate dimethoate (Needham and Devonshire 122
1975) and the carbamate pirimicarb (Sawicki et al 1978) and the results frequently reported as resistance ratios only. Resistance ratios for field-collected *M. persicae* range from 2 to 200 for dimethoate and 2 to 8 for pirimicarb (Needham and Devonshire 1975, Sawicki et al 1978). Laboratory reared strains of *M. persicae* are up to 500-fold resistant to dimethoate (Devonshire 1977) and 12-fold resistant to topically applied pirimicarb (Needham and Sawicki 1971). The latter laboratory strain, 12-fold resistant to pirimicarb, is also 171-fold resistant to topically applied parathion and 212-fold resistant to topically applied dimethoate (Needham and Sawicki 1971).

Although metabolic resistance is probably the major contributor to the Columbus strain's high LD$_{50}$ of 1082 ng/female for parathion (Table 1), one should consider the contributions of biophysical factors that can influence insecticide efficacy. These factors may include (i) rapid evaporation of parathion from the cuticle (Devonshire and Needham 1974) and (ii) the decreased percent cuticular penetration associated with high doses of insecticides (Busvine 1968). However, the affect of differential evaporation and penetration using unequal doses was not determined. The data from Table 17 show that evaporation and penetration of an equal dose of parathion is the same in the resistant and susceptible strains, indicating that
cuticular permeability and evaporation do not contribute to parathion resistance in this case. Devonshire and Needham (1974) have drawn similar conclusions from data showing no differences in cuticular evaporation and penetration of organophosphate insecticides among susceptible and resistant strains of *M. persicae*.

B. Joint Action Data.

It has long been recognized that methylenedioxyphenyl inhibitors of MFO, such as PBO, inhibit *in vivo* aldrin epoxidation and phosphorothionate activation (Sun and Johnson 1960). Moreover, the respective synergism and antagonism of the toxicity of pyrethroid (Farnham 1973) and organophosphate (Wilkinson 1971) insecticides by methylenedioxyphenyl MFO inhibitors can be used to determine if MFO contributes to resistance to these classes of insecticides. Brindley and Selim (1984) demonstrated how calculations of the R&S and R&A values from joint action data allow between-strain comparisons of the amount of synergism or antagonism induced by metabolic inhibitors such as PBO. Furthermore, the R&S and R&A values are more useful than simple interaction or joint action ratios in the diagnosis of resistance mechanisms with metabolic inhibitors because they provide a relative measurement of the contribution of the target enzyme system to the level
of insecticide resistance in the resistant strain relative to the susceptible strain (Brindley and Selim 1984, Rose and Brindley 1985). The joint action data of my study, as expressed by R%S and R%A values (Table 3), suggest that the substantial capacity for oxidative metabolism present in all 3 strains of aphids is highest in the organophosphate-susceptible, tobacco-reared Wooster strain. This conclusion is supported by the respective 2-fold and 3-fold differences in R%S values for resemthrin with and without PBO in the Wooster and Columbus strains and the Wooster and Celeryville strains and the nearly 5-fold difference in R%A values for parathion with and without PBO in the Wooster and Celeryville strains (Table 3). This striking diagnosis is at odds with the conventional explanation of the role of oxidative metabolism in insecticide resistance, namely that such resistance depends on increased, not decreased, oxidative metabolism (Wilkinson 1983). On the other hand, the R%S value for parathion with and without DEF in the Columbus strain, 5-fold greater than it is in the Wooster strain, suggests the possibility of enhanced esterases as a resistance mechanism in the Columbus strain, a phenomenon frequently found in resistant M. persicae (Devonshire 1977). The role of MFO and esterases in parathion resistance will be further discussed in the section on carboxylesterase hydrolysis and in vivo insecticide
metabolism.

The failure of PBO to antagonize the toxicity of parathion in the Columbus strain (Table 2) is perhaps an indication that the rate of parathion oxidation to paraoxon in this strain is too slow to be much affected by MFO inhibition. The combined dose of parathion and PBO chemical at the LD$_{50}$ is about 1000 ng/female. Hence, the contribution of PBO to the toxic effect could be biophysical in nature i.e. PBO increased the proportion of the parathion absorbed by decreasing the evaporation of parathion from the cuticle.

C. In vivo Carboxylesterase Assays.

Effect of substrate concentration on MEB hyrolysis.

The Michaelis constant (Km) of 12.5 mM (Table 5) for the 3 strains of aphids indicates no qualitative differences among the strains in the ability of the carboxylesterases in whole body homogenates to hydrolyze MEB. However, the differences among the strains in the maximum reaction velocity (Vmax) of MEB hydrolysis (Table 5) shows that the Columbus strain has more carboxylesterase activity per mg body weight or ug protein than the Celeryville and Wooster strains. It is difficult, however, to link increased MEB hydrolysis among resistant strains _M. persicae_ to insecticide resistance since it has not been shown, as with the substrate NA (Beranek and Oppenoorth
1977 and Devonshire 1977), that the resistance-associated enzyme, E-4, hydrolyzes both MEB and organophosphate insecticides. In fact, Sudderuddin (1973a, 1973b) found little difference in the kinetics or amount of ethyl butyrate hydrolysis among a susceptible and organophosphate resistant strain of *M. persicae* which were previously found different with respect to the amount of NA hydrolyzed.

Research with other species gives conflicting data about the relationship between resistance and the amount of MEB hydrolysis among resistant and susceptible strains of insects. Bigley and Plapp (1960) found that rate of MEB hydrolysis was actually lower in organophosphate resistant house flies than in susceptible ones. However, Salleh (1980), found that both the Km and Vmax of MEB hydrolysis were higher in 2 resistant strains of the cockroach, *Blatella germanica*, than in a susceptible strain.

Hydrolysis of 1-naphthyl acetate.

The contribution of carboxylesterases to parathion resistance in the three strains of aphids was also determined by measuring the hydrolysis of NA. Esterase-4, the only resistance-related esterase in *M. persicae* (Beranek 1974, Baker 1977, Beranek and Oppenoorth 1977, Devonshire 1977, and Takada 1979) hydrolyzes both NA and organophosphate insecticide and is responsible for a large
share of NA hydrolysis in resistant strains of *M. persicae* (Beranak and Oppenoorth 1977, Devonshire 1977). Measurements of NA hydrolysis is a reliable assay for esterase-associated resistance in *M. persicae* because NA hydrolysis increases linearly with the amount of E-4 present in resistant strains of *M. persicae* while the level of organophosphate resistance increases geometrically with the amount of E-4 (Devonshire and Sawicki 1979). Comparison of the NA hydrolysis rates of the strains studied herein (Table 4) with values for other strains of *M. persicae* suggests that esterases do not contribute significantly to parathion resistance in my 3 strains. The Columbus strain, 3000-fold resistant to parathion, hydrolyzes only 1.3-fold more NA than the Wooster strain. Needham and Sawicki (1971) reported a strain 171-fold resistant to parathion and 215-fold resistant to dimethoate hydrolyzed 6.1-fold more NA than the susceptible strain. Another strain, 500-fold resistant to dimethoate (parathion was not used), hydrolyzed 34-fold more NA than the susceptible strain (7.13 vs. 0.21 uMoles/mg/hr) (Devonshire 1977). Overall, NA hydrolysis rates in the present study fall between the rate of NA hydrolysis of Devonshire's MSIG strain (0.42 uMoles/mg/hr), 8-fold resistant to dimethoate, and the susceptible USIG stain (0.21 uMoles/mg/hr) (Devonshire 1977). The low rates of NA hydrolysis in the strains
studied here are near the level of E-4 activity associated with organophosphate susceptibility (Devonshire 1975 and Devonshire and Sawicki 1979). I therefore conclude that enhanced esterase activity plays a minor role in resistance in the Celeryville and Columbus strains.

D. In vivo Metabolism of Aldrin and Parathion.

The R%S and R%A calculations of the joint action data (Table 3) suggest reduced MFO activity in the parathion tolerant and resistant Celeryville and Columbus strains. Studies of the in vivo metabolism of aldrin and parathion were used to further investigate the level of oxidative metabolism present in the 3 strains of aphids.

Epoxidation of $^{14}$C aldrin.

The greater production of dieldrin in the Wooster strain is the only significant result found in this single-exposure time experiment (Table 6). These data indicate that oxidative metabolism of aldrin is highest in the organophosphate-susceptible Wooster strain, a result predicted by the R%S and R%A values presented in Table 3, and equal in the tolerant and resistant Celeryville and Columbus strains. However, one anomaly of the aldrin epoxidation data is that there are no significant differences among the strains in the amount of aldrin found per aphid when there are significant differences in the
amount of dieldrin. The within treatment group variation or error, which is much higher for the aldrin data than for the dieldrin data, may have masked the substantial differences among the strains in the amount of internal aldrin (Table 6). The variation in the data due to error, calculated during the variation partitioning phase of ANOVA, is 50% for the aldrin data and 27% for the dieldrin data. The high within treatment variation found here is probably due to the error introduced by the use of column chromatography to separate aldrin and dieldrin. GLC was substituted for column chromatography in the study of aldrin epoxidation over time and the resulting variation due to error is usually quite low (<10%), as can be seen in the error mean square terms of Tables 7 and 8.

Epoxidation of aldrin over time.

The study of the epoxidation of aldrin over time confirmed the earlier conclusion drawn from the joint action data (Table 3) and supported by the result of the epoxidation of $^{14}$C aldrin (Table 6) that the level of oxidative metabolism, as measured by aldrin epoxidation, is highest in the parathion-susceptible, tobacco-reared Wooster strain and lowest in the turnip-reared, parathion tolerant and resistant Celeryville and Columbus strains (Tables 11 and 12). Furthermore, conversion of aldrin to dieldrin is not affected by the weight of the aphids
(Tables 11 and 12). Proof of this is that the epoxidation of aldrin is highest in the Wooster strain on both a per animal (Table 11, Figures 17 and 19) and per body weight basis (Table 12, Figures 18 and 20). Calculating results based on the body weight of the aphids affect the statistical relationships of the penetration, rinse, and total recovery data because the Wooster strain is nearly 40% heavier than the Columbus strain. Thus, on a ng/mg body weight basis, penetration is lowest in the Columbus strain and highest in the Wooster strain (Table 12, Figure 16), rinsed insecticide is lowest in the Wooster strain and highest in the Columbus strain (Table 12, Figure 14), and total insecticide recovered is lowest in the Wooster strain and higher in the Columbus strain (Table 12, Figure 12). Meanwhile, the Celeryville strain's relative ranking in these catagories remains unchanged from what it was on a ng/female basis.

Host plant effect on aldrin epoxidation.

Host plant induction of MFO provides herbivorous insects with substantial protection from poisonous plant substances and insecticides (Brattsen et al 1977, Yu 1982a). The basis of this protection is the induction of increased synthesis of detoxification enzymes, such as MFO, by specific allelochemicals produced by the plants.
(Brattsen et al. 1977 and Yu 1984). Since the amount of induction varies with the species of host plant (Yu 1982a, 1984 and Berry et al. 1980) it was necessary in my study to rear the Wooster strain on turnip and repeat a portion of the aldrin epoxidation experiment to determine if the Wooster strain's superior capacity to epoxidize aldrin is due to greater induction of MFO by tobacco over what occurs if the strain was reared on turnips. The results of this experiment (Table 13) clearly show that the epoxidation and penetration of aldrin is equal in both the turnip-reared and tobacco-reared Wooster aphids. Furthermore, aldrin epoxidation in the turnip-reared Wooster strain is significantly higher than it is in the turnip-reared Celeryville and Columbus strains (Table 14). In the present study, therefore, the Wooster strain's increased capacity for oxidative metabolism, as measured by aldrin epoxidation, is independent of host plant.

Metabolism of $^{14}$C parathion.

The study of $^{14}$C parathion metabolism supports the conclusion drawn earlier from the joint action and aldrin epoxidation data that oxidative metabolism is higher in the parathion-susceptible Wooster strain than it is in the parathion-tolerant and resistant Celeryville and Columbus strains. The oxidative metabolism of parathion to paraoxon is greatest in the Wooster strain (Tables 17 and 19). At
least on a ng/female basis, the production of other metabolites is greater in the Wooster strain as well (Table 17). Furthermore, the differences in oxidative metabolism among these strains cannot be attributed to host plant induction of MFO in the tobacco-reared Wooster strain since turnip-reared Wooster aphids actually produce more paraaxon than tobacco-reared Wooster aphids (Table 20 and 21). The contrast between the tobacco-reared Wooster strain and the turnip-reared Celeryville and Columbus strains is even more pronounced in light of the equal amounts of penetrated, rinsed, and total recovered insecticide found in these strains on a ng/female basis (Table 17). Indeed, parathion oxidation is still significantly greater in the Wooster strain on a ng/mg body weight basis even though the Wooster strain has significantly less penetrated, rinsed, and total recovered insecticide than the other 2 strains (Table 19). The significant differences in the ng/female penetration, rinse, and recovery variables occur because these values were divided by the weight of the aphids, which differ with each strain.

Rearing the Wooster aphids on turnip changes the amount of penetrated parathion so it is nearly 3-fold that of the tobacco-reared Wooster aphids (Table 20). The turnip-reared Wooster aphids are also smaller than their tobacco-reared counterparts so the differences in
penetration as well as total recovery are, on a ng/mg body weight basis, accentuated (Table 21). However, viewing the parathion data of all strains, both turnip and tobacco-reared Wooster aphids as well as the Celeryville and Columbus strains, shows that the percent of the penetrated compound metabolized to paraoxon and other metabolites is negatively correlated with increased resistance to parathion while the percent of parathion found in each strain is positively correlated with insecticide resistance (Table 23). These figures indicate that the parathion-susceptible Wooster strain aphids are more efficient at oxidatively converting parathion to its toxic metabolite, paraoxon, and at parathion metabolism in general.

E. Implication of Data Concerning Parathion Resistance.

I have produced 3 independent lines of evidence showing reduced oxidative metabolism of insecticides occurs in the parathion resistant or tolerant strains of *M. persicae* studied here: (1) interaction data demonstrating greater antagonism of parathion toxicity and greater synergism of resmethrin toxicity by PBO in the the parathion-susceptible Wooster strain than in the parathion-tolerant and resistant Celeryville and Columbus strains (Tables 2 and 3); (2) the *in vivo* epoxidation of aldrin is greater in the Wooster strain than in the other
two strains (Tables 6, 11, and 12, Figures 11-20); and (3) greater \textit{in vivo} parathion oxidation and metabolism in the Wooster strain (Table 19-23). In the latter 2 cases additional experiments demonstrated that the increased oxidative metabolism of aldrin and parathion in the Wooster strain cannot be attributed to allelochemical or host plant induction of MFO in the tobacco-reared Wooster strain (Tables 13, 14, 20, and 21). In fact rearing Wooster aphids on tobacco rather than turnip had no effect on aldrin epoxidation and actually reduced the oxidation of parathion to paraoxon. In terms of esterase activity, the 3 aphid strains have similar capacities to hydrolyze MEB and while the capacity among the strains to hydrolyze NA, though small, is nonetheless positively correlated with parathion resistance. The levels of NA hydrolysis seen in this study are indicative of organophosphate susceptibility or tolerance as outlined by Devonshire (1977).

These findings on the levels of oxidative metabolism and esterase activity suggest that parathion resistance in the Columbus strain is due to interaction between two mechanisms of resistance, namely low MFO activity which prevents formation of toxic metabolites such as paraoxon, and slightly elevated esterase activity which increases hydrolysis of paraoxon. Parathion tolerance in the Celeryville strain is due to reduced oxidative production
of paraoxon since esterase activity in this strain is lower than that in the Columbus strain. This mechanism of resistance implies insecticide selection of resistant aphid biotypes possessing either quantitatively or qualitatively different MFO needed to impart such resistance. I hypothesize that parathion resistance in this study is, in part, the result of such selection. A role for other resistance mechanisms cannot be ruled out since they were not studied.

The many mechanisms of insect resistance to organophosphate insecticides are discussed by the contributing authors of the book "Pest Resistance to Pesticides" (Georghiou and Saito 1983). The mechanisms listed are (1) decreased penetration, (2) insensitive target enzymes, (3) enhanced metabolism, (4) sequestering the toxicant, and (5) increased conjugation. To this list I will add reduced oxidative activation. Reduced oxidative activation by aphids is a unique resistance mechanism among insects and I know of no similiar example in the literature.

The existence of a resistance mechanisms in *M. persicae* other than Devonshire's enhanced esterase model would explain the anomalous data of Takada (1979) in which strains of *M. persicae* with high levels of malathion resistance did not have the correspondingly high levels of
esterase activity predicted by the Devonshire model. Malathion resistance in Takada's low esterase aphids may have been caused by interaction between 2 resistance mechanisms, i.e. enhanced E-4 and reduced oxidative activation. The data from my study also confirm Devonshire's suggestion (Devonshire 1973) that the organophosphate-resistant strain of *M. persicae*, which produce less omethoate, the toxic oxidative metabolite dimethoate, than the susceptible strain, used decreased oxidative metabolism as a resistance mechanism (Devonshire 1973)

Finally, my study and others (Blackman et al 1978 and Takada 1979) indicate that the NA hydrolyzing activity of E-4 or whole body homogenates may not be the only indicator of insecticide resistance in *M. persicae*. The biochemical assay developed by Devonshire (1977) and others (Needham and Sawicki 1971) should not be the only test used to diagnose organophosphate resistance in this species. Assays for oxidative metabolism and other potential resistance mechanisms should be developed and employed when monitoring resistance in field populations of *M. persicae*.
SUMMARY

In this dissertation I described my investigation of the contribution of carboxylesterase activity and oxidative metabolism to parathion resistance in two strains of the aphid, *M. persicae*, collected in Ohio. In the first part of my research I established three colonies of *M. persicae* in a greenhouse and tested them for resistance to parathion. One colony, reared on tobacco and called the Wooster strain, was susceptible to parathion. Another colony, reared on turnips and called the Celeryville strain, was 2-fold tolerant or slightly resistant to parathion. The last colony, also reared on turnip and called the Columbus strain, was 2930-fold resistant to parathion. I then studied the role of carboxylesterase and oxidative metabolism in parathion resistance by subjecting the aphids to three groups of experiments. The first group of experiments consisted of joint action toxicity tests, using parathion with PBO or DEF and resmethrin with PBO, designed to measure relative carboxylesterase and oxidative activity among the three aphid strains. The results of these experiments showed greater antagonism of parathion toxicity and greater synergism of resmethrin toxicity by PBO in the
Wooster strain while DEF synergized parathion toxicity more in the Columbus strain than in the Wooster strain. From this evidence I was able to predict decreased carboxylesterase activity and elevated oxidative metabolism in the susceptible Wooster strain relative to the resistant Celeryville and Columbus strains.

In the second group of experiments I determined the role of carboxylesterases in parathion resistance by assaying the level of NA and MEB hydrolysis in whole body homogenates. Other researchers have demonstrated that increased amounts of a single carboxylesterase causes resistance to organophosphate and carbamate insecticides in *M. persicae*. Furthermore, their research shows that measuring NA hydrolysis in whole body homogenates of this species serves as a reliable biochemical indicator of resistance. The assays I performed show that the levels of NA and MEB hydrolysis of the two resistant strains were significantly higher than the susceptible strain, but the carboxylesterase activity ratios for the susceptible and resistant strains, 1.6-fold for MEB and 1.3-fold for NA, were not great enough to account for the 2930-fold resistance ratio in the Columbus strain.

In the third set of experiments I investigated the contribution of oxidative metabolism to parathion resistance by measuring the *in vivo* oxidation of parathion
and aldrin to their toxic oxidative metabolites, paraoxon and dieldrin. In addition, I repeated portions of the experiments with parathion and aldrin using susceptible Wooster aphids reared on turnips, the same host plant on which the Celeryville and Columbus strains were reared. These latter experiments, where the host plant of the Wooster strain was changed, were done to determine if differences in oxidative metabolism were due to differential induction of detoxification enzymes by the two host plants, tobacco and turnips, a well documented phenomena among polyphagous herbivorous insects. The in vivo assays demonstrated that, relative to the susceptible Wooster strain, the two resistant strains were less able to oxidize parathion and aldrin to paraoxon and dieldrin, regardless of the host plant the Wooster aphids were reared on, and were able to metabolize less parathion overall.

Based on the findings outlined above I have concluded the following: (1) The Columbus strain is exceptionally resistant to parathion. Its LD$_{50}$ of 1080 ng/female and its resistance ratio of 2930 are the highest I know of for this species; (2) the joint action toxicity tests proved to be reliable indicators of the metabolic mechanisms and their relative activity among the aphid strains. The conclusions drawn from these data were confirmed by in vitro esterase assays and in vivo oxidative metabolism studies; (3) the
enhanced carboxylesterase model of organophosphate and carbamate insecticide resistance in *M. persicae*, elucidated by Alan Devonshire and others, does not explain para-thion resistance in the Celeryville and Columbus strains. The *in vitro* esterase assays demonstrated that carboxylesterase activity in all strains was at the level associated with insecticide susceptibility or tolerance in *M. persicae*; (4) para-thion resistance in the Columbus strain is due to a combination of low oxidative activity that prevents formation of toxic metabolites and enhanced hydrolysis of the toxic metabolite by slightly elevated esterase activity. However, low oxidative activity is the primary resistance mechanism since, as stated before, esterase activity in this strain is too low to account for the high level of resistance; (6) para-thion tolerance in the Celeryville strain is due only to reduced oxidative production of paraoxon since esterase activity in this strain is lower than that in the Columbus strain; and (7) since enhanced E-4 carboxylesterase activity, contrary to the conclusions of other researchers, is not the universal resistance mechanism in *M. persicae*, measurements of NA hydrolysis, used by some laboratories to routinely assay for organophosphate and carbamate insecticide resistance in *M. persicae*, should not be used as the sole biochemical indicator of resistance. Biochemical assays for oxidative
metabolism and other potential mechanism should be
developed and used in conjunction with the NA assay of
esterase activity.
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