AN ABSTRACT OF THE THESIS OF:

Hugo E. van de Baan for the degree of **Doctor of Philosophy** in **Entomology** presented on **September 29, 1988**.

Title: **Factors Influencing Pesticide Resistance in Psylla pyricola Foerster and Susceptibility in its Mirid Predator, Deraeocoris brevis Knight**.

Abstract approved: _____

Factors influencing pesticide susceptibility and resistance were studied in Psylla pyricola Foerster, and its mirid predator, Deraeocoris brevis Knight in the Rogue River Valley, Oregon. Factors studied were at the biochemical, life history, and population ecology levels.

Studies on detoxification enzymes showed that glutathione S-transferase and cytochrome P-450 monooxygenase activities were ca. 1.6-fold higher in susceptible D. brevis than in susceptible pear psylla, however, esterase activity was ca. 5-fold lower. Esterase activity was ca. 18-fold higher in resistant pear psylla than in susceptible D. brevis, however,

glutathione S-transferase and cytochrome P-450 monooxygenase activities were similar. Esterases seem to be a major factor conferring insecticide resistance in P. pyricola.

Although the detoxification capacities of P. pyricola and D. brevis were quite similar, pear psylla has developed resistance to many insecticides in the Rogue River Valley, whereas D. brevis has remained susceptible. Biochemical factors may be important in determining the potential of resistance development, however, they are less important in determining the rate at which resistance develops. Computer simulation studies showed that life history and ecological factors are probably of greater importance in determining the rate at which resistance develops in P. pyricola and D. brevis. High fecundity and low immigration of susceptible individuals into selected populations appear to be major factors contributing to rapid resistance development in pear psylla compared with D. brevis.

Implications of this study for pesticide resistance management of pear psylla are discussed.

Factors Influencing Pesticide Resistance in *Psylla pyricola* Foerster and Susceptibility in its Mirid Predator, *Deraeocoris brevis* Knight

by

Hugo E. van de Baan

A THESIS

submitted to

Oregon State University

In partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

Completed September 29, 1988

Commencement June 11, 1989

APPROVED:

Redacted for Privacy

Professor of Entomology in charge of major

Redacted for Privacy

Head of the Department of Entomology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented September 29, 1988

Acknowledgements

First of all I would like to thank my parents for all their support throughout my education. Thanks to my friends in Corvallis and everywhere else for 'being there'. I would like to thank Maurice Sabelis, Marjorie Hoy, and Dr. F.J. Oppenoorth for getting me interested in the basic and applied aspects of pesticide resistance. Thanks to Peter Westigard for his help, support, and sharing his knowledge about the world of pear pest management. Thanks to Rene Feyereisen, Dan Farnsworth, and Grahame Pratt for their excellence in trying to teach me some 'real science'. Thanks to Kevin Currans and Karen Theiling for their help with computer work. Thanks to Robert Allen, Toni Hoyman, and Keesjan van der Maas for their help with some of the practical aspects of this research. Thanks to Mike Burgett and the personnel of the Honey Bee lab for their personal support. And last but not least, thanks to Brian Croft who is able to keep his students humble until the last moment, for all his support and ideas during this study, and for the road trips.

This research was partially possible through grants from the USDA-WRCC-161 and the Winter Pear Control

Committee. I would like to thank the Netherlands America Commission for Educational Exchange for a Fulbright Fellowship.

"Wisdom is better than weapons of war"

Ecclesiastes 9:18a

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Factors Influencing Pesticide Resistance in Psylla pyricola Foerster and Susceptibility in its Mirid Predator, Deraeocoris brevis Knight

1. INTRODUCTION

Pear psylla, Psylla pyricola Foerster, is a major insect pest of pears in the Pacific Northwestern U.S.A. (Westigard and Zwick 1972; Westigard et al. 1979). This homopteran pest was introduced from the Eastern U.S.A. into Washington in 1939, and subsequently spread through Oregon (1949-1950) and northern California (1955-1958) (Westigard and Zwick 1972).

Pear psylla has two distinct forms, a summer-form and a larger, darker winter-form. In Oregon, summer-form psylla has three generations per year and the winter-form psylla has one generation per year (Westigard and Zwick 1972; Westigard et al. 1979). First summer adult psylla appear in late April and early May and overwintering adults are produced in October (Westigard and Zwick 1972; Westigard et al. 1979). Many

winter-form adults migrate out of pear orchards into surrounding vegetation by the end of October (Fye 1983). By the end of January these psyllids migrate back into orchards and oviposition occurs after mating.

Pear psylla feeds strictly on pear and there are even some Pyrus species which will not support psylla development (Williams et al. 1963; Kaloostian 1970; Westigard et al. 1979). Young succulent pear foliage appears to be important in determining the rate of pear psylla reproduction. Alhough pear is required for completion of the life cycle, overwintering psylla can be found on many other plant hosts (Wilde 1962, 1966, 1970; Kaloostian 1970; Ullman and McLean 1988).

Damage to pear trees by psylla includes the transmission of pear decline disease, caused by a mycoplasma-like organism which can kill trees, injection of a phytotoxin by nymphs which results in tree shock and injury, and secretion of honeydew by nymphs which causes fruit marking (Westigard and Zwick 1972; Westigard et al. 1979). In contrast to pear decline and the effects of psylla toxin, psylla honeydew causes direct fruit damage and is of utmost concern to growers. Of appoximately $700.00 annually spent per hectare on pest control in commercial orchards, ca. $400.00 is spent on psylla control to avoid direct fruit damage

(Westigard et al. 1986). Pear psylla is therefore the most expensive pest to control in commercial pear.

Natural enemies are important in controlling pear psylla in unsprayed orchards (Madsen 1961; Madsen et al. 1963; Madsen and Wong 1964; Westigard et al. 1968; Westigard 1973; Westigard et al. 1979). In the Rogue River Valley, Oregon, several coccinellids, the lacewing Chrysoperla carnea Steph. and the mirid Deraeocoris brevis Knight feed on pear psylla (Westigard et al. 1979). D. brevis is a generalist predator and appears to be the most effective natural enemy of pear psylla in southern Oregon pear orchards (Liss et al. 1986). Although all these predator species are potentially important biological control agents, they may be ineffective because of limited immigration and late build up in the early summer due to cool temperatures (Westigard et al. 1979). As a result, growers rely upon insecticides to achieve control of pear psylla.

The intensive use of broad-spectrum insecticides for the control of pear psylla and other pear pests has two rather dramatic effects on pest control. Natural enemies of psylla such as D. brevis seem to remain susceptible to most insecticides, and therefore populations are eliminated when these chemicals are used, and no biological control of psylla is possible (Westigard 1973; Westigard et al. 1979; Westigard et al.

1986). On the other hand pear psylla can quickly develop resistance to insecticides (Westigard and Zwick 1972; Westigard et al. 1979; Follett et al. 1985; Burts et al. in press). In the Pacific Northwest most compounds used for psylla control are only effective for a relatively short period of time, due to the development of resistance (Fig. 1.1).

The only insecticides presently registered and yet effective for psylla control are the pyrethroid fenvalerate, used as a dormant application for overwintering psylla, and the formamidine amitraz used as a nymphicide for summer psylla control (Westigard et al. 1986; Burts et al. in press). In view of these resistance problems, traditional chemical control practices of pear psylla must be replaced by pesticide resistance management programs in which one tries to retard or counteract resistance in pests and promote it in beneficials (Georghiou 1972, 1983; Croft 1982; Dover and Croft 1984).

The development of resistance to insecticides in pear psylla and the lack thereof in the predator D. brevis is a phenomenon observed in most arthropod pests and their natural enemies (Croft and Morse 1979; Theiling and Croft in press). While in 1986 more than 400 pest arthropods of agricultural and medical/veterinary importance had developed resistance

to one or more pesticides, only 12 species of natural enemies were known to be resistant (Georghiou 1986; Tabashnik and Johnson in press). Several hypotheses may explain the differences in resistance frequencies between pest and beneficial arthropods. Differences in previous evolutionary exposure to plant toxins may have resulted in different detoxification capabilities and therefore, different capabilities for resistance development (Croft and Brown 1975; Croft and Morse 1979; Croft and Strickler 1983). Life history attributes such as reproductive rate may differ for pest arthropods and natural enemies and may affect their potential to develop resistance (Georghiou and Taylor 1986). At the ecological level, differences in migration of susceptible individuals into a selected population, may slow down the rate of resistance development (Comins 1977; Taylor and Georghiou 1979; Tabashnik and Croft 1982). Another ecological factor may be food limitation (Tabashnik and Croft 1985; Tabashnik 1986a). Following selection with a pesticide a resistant pest usually has an unlimited food source, upon which a selected organism can reproduce and increase at a maximum rate. However, a natural enemy may encounter a limited food supply, the prey, and must either starve or emigrate to interbreed with susceptible individuals. The natural enemy's rate of increase is therefore less than that of its prey,

following selection. Interaction of these biochemical, life history, and ecological factors probably contribute to the rapid evolution of pesticide resistance in pests and the lack thereof in their predators and parasitoids.

This research is a comparative study of the factors influencing insecticide susceptibility and resistance in P. pyricola and its mirid predator, D. brevis, in the Rogue River Valley of Oregon. The factors studied ranged from the biochemical, to the life history, and population ecology levels (Fig. 1.2). Results of this study may contribute to better selective insecticide use, pesticide resistance management, and integrated control of pear psylla.

Figure 1.1. Chemical use patterns in pear psylla, P. pyricola, in the Pacific Northwest. Resistance as observed by field failure is indicated by R (after Westigard and Zwick 1972 and Follett et al. 1985).

Figure 1.1.

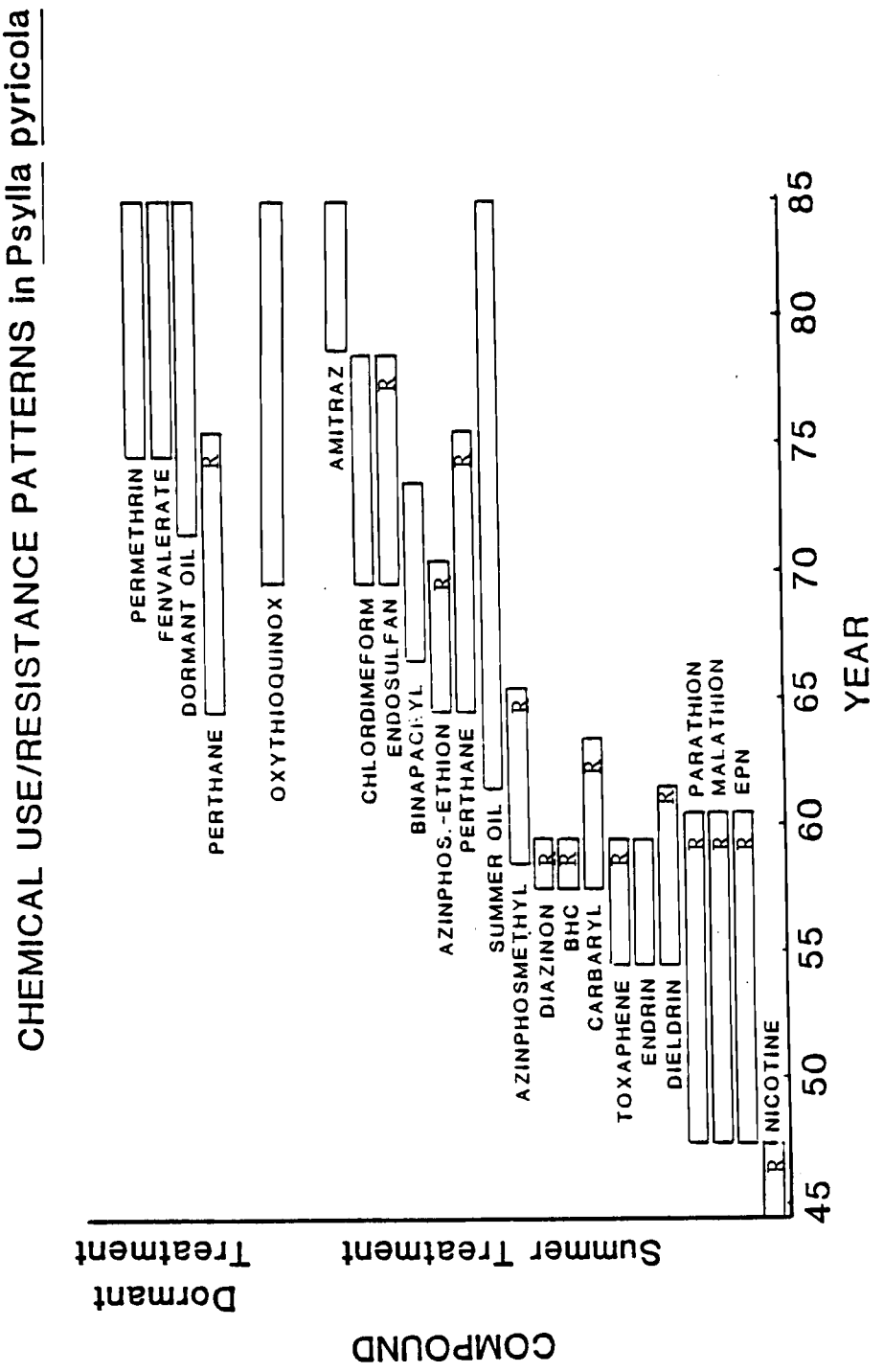
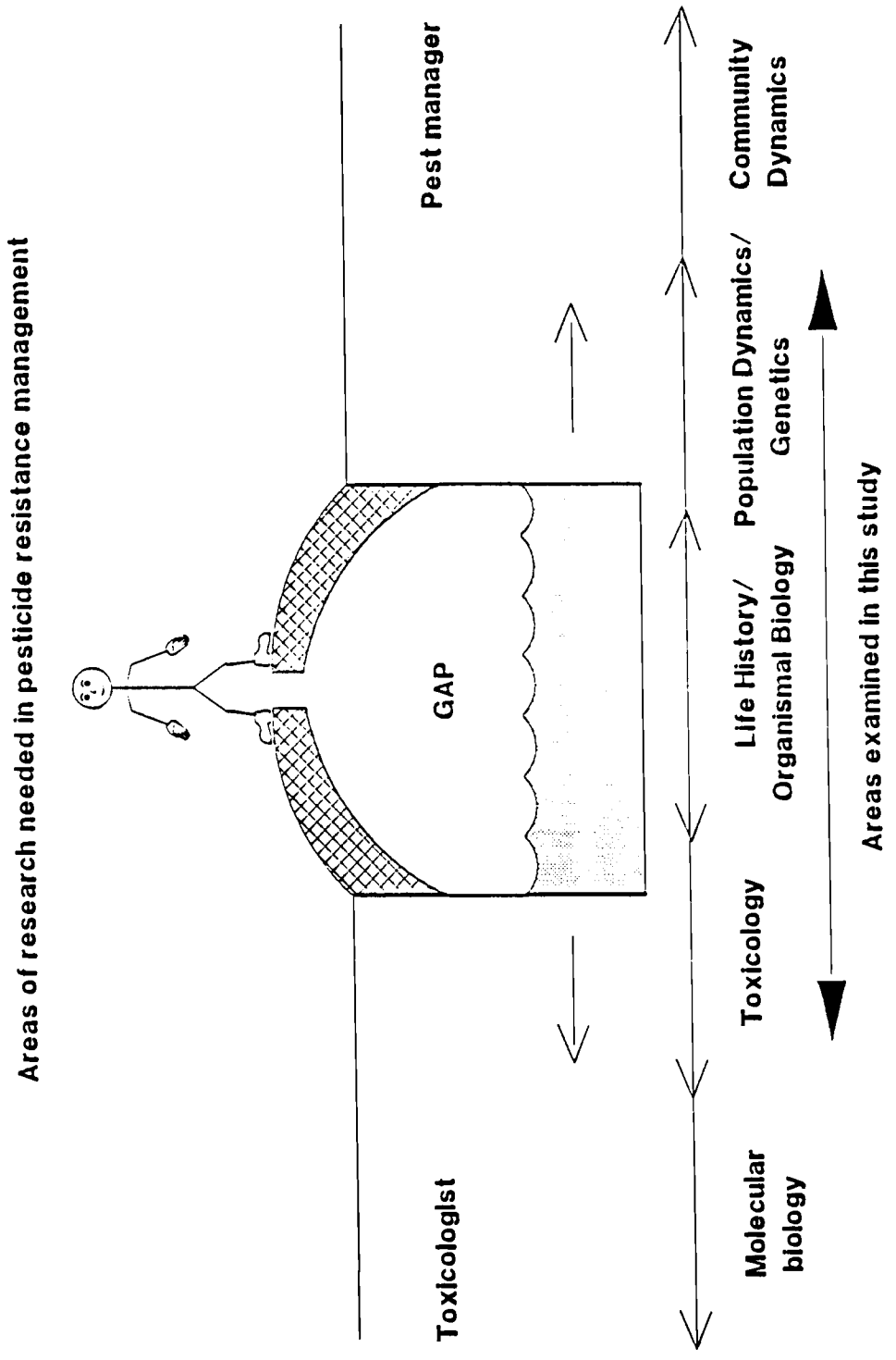

CHEMICAL USE/RESISTANCE PATTERNS in Psylla pyricola
PERMETHRIN
FENVALERATE
DORMANT OIL
PERTHANE
R
OXYTHIOQUINOX
AMITRAZ
CHLORDIMEFORM
ENDOSULFAN
BINAPACRYL
AZINPHOS.-ETHION
PERTHANE
SUMMER OIL
AZINPHOSMETHYL
DIAZINON
BHC
CARBARYL
TOXAPHENE
ENDRIN
DIELDRIN
PARATHION
MALATHION
EPN
NICOTINE
45
50
55
60
65
70
75
80
85
YEAR
COMPOUND
Summer Treatment
Dormant Treatment

Figure 1.2. Areas of research needed in pesticide resistance management. Areas considered in this study ranged from the biochemical, to the life history, and population ecology levels.

Figure 1.2.


Areas of research needed in pesticide resistance management
Pest manager
GAP
Toxicologist
Community Dynamics
Population Dynamics/ Genetics
Life History/ Organismal Biology
Toxicology
Molecular biology
Areas examined in this study

2. TOXICITY OF INSECTICIDES TO PEAR PSYLLA AND *DERAEOCORIS BREVIS*

2.1 Introduction

Before the mid 1980's, the status of insecticide resistance in populations of pear psylla was documented only when control failures occurred in the field. This is partly because pear psylla has been difficult to rear and assay for resistance in the laboratory (Fye 1981; Riedl et al. 1981). Follett et al. (1985) eventually developed a slide-dip bioassay test for studying regional resistance patterns in the Hood River, Willamette and Rogue River Valleys of Oregon. Early summer-form pear psylla from the Rogue River Valley, near Medford, were almost uniformly resistant to azinphosmethyl (20 to 41-fold), endosulfan (4 to 5-fold), Perthane (4-fold) and fenvalerate (2 to 4-fold). Follett (1984) noticed some difference, however, in the susceptibility of early and late summer-form pear psylla.

Although pear psylla in the Rogue River Valley has developed resistance to a variety of insecticides, its main predator *D. brevis* has remained susceptible to most

of these compounds (Westigard and Zwick 1972; Westigard 1973, 1979; Westigard et al. 1979). Many organophosphorous compounds (OPs) used for the control of codling moth, Cydia pomonella (L.), are highly toxic to D. brevis when applied either directly to nymphs or adults in laboratory bioassays (Westigard 1973) or to field populations (Westigard 1979; Westigard et al. 1979). Some nonorganophosphate insecticides only show moderate toxicity to D. brevis, however, these compounds do not fit into the total summer control program for pear pest control (Westigard 1973).

The studies of Follett (1984) and Follett et al. (1985) established the need to examine the seasonal and regional patterns of insecticide susceptibility and resistance in pear psylla in more detail. These toxicity relationships were tested in resistant pear psylla from the Rogue River Valley, Oregon, near Medford and a susceptible psylla population collected at the Oregon State University (O.S.U.) Entomology Farm, Corvallis, Oregon. A more detailed comparison of insecticide toxicity in P. pyricola and D. brevis was needed to provide basic information on susceptibility and resistance in prey and predator and to evaluate selectivity of some insecticides.

2.2 Materials and methods

Adult summer- and winter-form pear psylla from Medford, Oregon, were field collected throughout the winter and summer of 1985 by jarring trees using the limb-tap method (Retan and Burts 1984). They were then aspirated into a padded vial, cooled and transported to the laboratory for testing. As noted, pear psylla from Medford had previously been shown to be resistant to insecticides (R strain) (Follett et al. 1985). Insecticide susceptible (S) pear psylla from the O.S.U. Entomology Farm Corvallis (Follett 1984; Follett et al. 1985) were reared on small pear trees under long day photoperiod (16:8 [L:D]) and at 21 $\pm$ 5 ^{o}C to produce only summer forms. Summer forms of the susceptible laboratory colony were used for bioassays throughout 1986 and 1987. Winter forms of S psylla were field collected as described above at the O.S.U. Entomology Farm, Corvallis, in February 1987 and 1988.

Nymphs (3rd-5th instars) of D. brevis were collected from orchards near Medford in a similar way as pear psylla, in September 1985. Because 3rd to 5th instar nymphs are the most important stages which feed on pear psylla, these stages were subjected to toxicity tests. As noted, D. brevis tested were considered susceptible to insecticides, based on previous

laboratory tests and field observations (Westigard and Zwick 1972; Westigard 1973, 1979; Westigard et al. 1979).

Toxicity studies were performed using standardized laboratory bioassays, including a slide-dip method of Follett et al. (1985) as well as a bioassay test based on topical application. The slide-dip bioassay is relatively simple as it uses discriminating doses and formulated materials. This method is adequate for monitoring tolerance or resistance in field populations of pear psylla (Follett et al. 1985; Croft et al. in press). The topical bioassay was used for basic studies where technical compounds are used and greater precision in application is necessary.

Prior to bioassays, pear psylla were anesthetized with CO_2 and placed on strapping tape (Scotch 3M brand, St. Paul, Minnesota) on microscope slides using a # 1 camel-hair brush. They were secured to the strapping tape by pressing their wings to the tape surface. Double coated tape (Scotch 3M brand, St. Paul, Minnesota) was used to attach the strapping tape to the microscope slide. In assays with D. brevis, nymphs were placed on a small droplet of non-toxic adhesive (Elmer's glue, Borden Inc., Columbus, Ohio).

The following insecticides were tested in topical application assays for pear psylla: azinphosmethyl (O,O-

dimethyl S[(4-oxo-1,2,3,-benzotriazin-3(4H)-yl)methyl] phosphorodithioate), endosulfan ((3a,5aα,6β,9β,9aα)-6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexadro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide), fenvalerate (cyano(3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzene-acetate), methiocarb (3,5-dimethyl-4-(methylthio)phenyl-methylcarbamate), Perthane® (1,1'-(2,2-dichloroethylidene) bis[4-ethylbenzene]), amitraz (N'-(2,4-dimethylphenyl)-N-[[dimethylphenyl)imino] methyl]-N-methylmethanimidamide) and avermectin ([6S-[2aE,4E,6R*.7R*, 8dE,11S*,13R*[5'R*,6'S* (R*)] 15R*,17aS*,20S*,20aS*,20bR*]]-7-[[2,6-dideoxy-4-O-(2,6-dideoxy-3-O-methyl-α-L-arabino-hexopyranosyl)-3-O-methyl-α-L-arabino-hexopyranoxyl]oxy]-5',6,6',7,10,11,14,15,17a, 20,20a,20b-dodecahydro-20b-hydroxy-20-methoxy-5,6,8,19-tetramethyl-6'-(1-methylpropyl)spiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-17-one).

The insecticides tested in experiments included compounds previously used for psylla control (azinphosmethyl, endosulfan, methiocarb and Perthane), those currently in use (fenvalerate and amitraz), and a new compound being considered for registration for control of this pest (avermectin) (see Fig. 1.1). Azinphosmethyl and fenvalerate were tested on D. brevis.

Synergists tested on pear psylla were with piperonyl butoxide (PbO, (5-(2-(2 butoxyethoxy)ethoxymethyl)-6-propyl-1,3,benzodioxole)) and S,S,S-tributylphosphorotrithioate (DEF).

For all topical application bioassays, technical compounds of more than 91% purity were used, with analytical grade acetone as solvent. A 100 µl calibrated syringe (Hamilton Co., Reno, Nev.) was used to place a 0.21 µl droplet of insecticide to the ventral abdomen of each insect using a repeating dispenser. For each compound, a serial dilution of five doses or a diagnostic dose were tested with three replicates of 25 psylla or two replicates of 10 D. brevis. Controls in this test consisted of applying acetone to two replicates of slides containing 25 psylla or 10 D. brevis. In synergist tests for pear psylla, mixtures of insecticides and a constant non-lethal dose of 195.6 ng/mg insect DEF or 97.6 ng/mg insect PbO were applied. Controls consisted of applying synergists to two replicates of slides containing 25 individuals.

With the slide-dip assay for pear psylla, a diagnostic dosage (i.e. a dosage to kill an intermediate proportion of a population) of the following formulated compounds was evaluated : 2.4 g a.i.(active ingredient)/l azinphosmethyl (Guthion 50WP), 0.6 g a.i./l endosulfan (Thiodan 50WP), 0.3 g a.i./l

methiocarb (Mesurol 50WP), 0.06 g a.i./l fenvalerate (Pydrin 2.4EC) and 0.007 g a.i./l avermectin (Avermectin B1 0.15EC). Slides with psylla were dipped for 5 sec in a single diagnostic dosage of formulated material with water as diluent. Four replicates of 25 individuals were tested per diagnostic dosage for each compound tested. Two replicates of slides containing 25 individuals were dipped in water as controls.

Treated insects from both bioassays were kept for 48 h in holding chambers at 21 $\pm$ 2 ^{o}C and high relative humidity (95-100%). Holding chambers consisted of trays layered with wet paper towel and covered by thin plexiglas. Mortality was determined using a dissecting microscope. Absence of appendage movement and other mortality criteria (Follett et al. 1985) were used to assess mortality.

Data from serial dilution tests were subjected to probit analysis after adjusting for check mortality with Abbott's formula (Abbott 1925). For tests based on single diagnostic doses, LD_{50} values were estimated based on average slopes for probit analysis tests. Percent mortality values for slide-dip tests were corrected for control mortality and standard errors were calculated.

2.3 Results and discussion

2.3.1 Toxicity of insecticides to pear psylla

Results of topical application bioassays for endosulfan, methiocarb, Perthane, fenvalerate, and azinphosmethyl for summer-form S and R pear psylla (May/June) are shown in Table 2.1. Based on resistance ratios (LD_{50} resistant/LD_{50} susceptible strain), levels of resistance in pear psylla from Medford were relatively low for endosulfan and methiocarb (2.4 and 2.5-fold, respectively), intermediate for Perthane and azinphosmethyl (5.8 and 7.7-fold, respectively), and high for fenvalerate (40.1-fold).

Avermectin was highly active against R pear psylla with an LD_{50} value of 1.1 ng/mg insect. Because of the low tolerance to avermectin in R psylla, this compound was not tested in the susceptible strain.

After 48 h of exposure to amitraz, little mortality of adult psylla occurred regardless of the dose tested. Because amitraz mostly acts as an ovicide and nymphicide without affecting adults (Westigard et al. 1979; Burts 1983), bioassays using adult pear psylla were not appropriate for this compound. Therefore, no additional comparisons for amitraz were made with the other chemicals.

Results of these toxicity tests reflected intrinsic susceptibility or current levels of developed insecticide resistance in R pear psylla from Medford. Avermectin was highly toxic to R psylla, thus indicating that psylla were probably still susceptible to this compound. Avermectin has not been used for psylla control in the field and apparently no cross-resistance to avermectin from other insecticides used in the past is present.

Methiocarb and endosulfan have been used less frequently and for shorter periods of time in the Rogue River Valley, compared with Perthane and azinphosmethyl (Follett et al. 1985). This probably accounts for the relatively lower levels of resistance to these compounds and the low LD_{50} values observed in these studies.

The Medford strain showed intermediate levels of resistance to Perthane and azinphosmethyl. Perthane was used for many years for pear psylla control, previous to the introduction of fenvalerate and amitraz (period of use in the Rogue River Valley from 1965 to 1978; Follett et al. 1985). Azinphosmethyl has been used since the mid 60's for control of codling moth (Riedl et al. 1981; Follett et al. 1985). Intensive use of Perthane and azinphosmethyl in the Rogue River Valley possibly resulted in the intermediate levels of resistance to these compounds observed in pear psylla.

Fenvalerate has been used for dormant control of psylla in the Rogue River Valley since 1978 (Follett 1984). This material still provides effective control of overwintering populations of psylla, although topical bioassays showed relatively high levels of fenvalerate resistance. The levels of fenvalerate resistance observed in psylla from Medford by laboratory bioassays apparently are not indicative of field failure of this compound. However, low levels of susceptibility to fenvalerate in pear psylla from Medford compared with S psylla from the O.S.U. Entomology Farm may indicate that resistance to this compound is developing.

2.3.2 Seasonal variation of insecticide toxicity in pear psylla

Test results of topical application, expressed as LC_{50} values (g a.i./l), for azinphosmethyl, methiocarb, fenvalerate, Perthane and endosulfan, for both winter- (October, January, and March) and summer-form (June and August) pear psylla from Medford, are presented in Figure 2.1. Psylla were most tolerant to azinphosmethyl, methiocarb and fenvalerate in October just prior to entering overwintering. Susceptibility increased during the winter period, as mid-winter forms

(January) were more susceptible after migrating back into the orchard and late winter forms (March) were even more susceptible after beginning to feed, mate, and oviposit. For Perthane and endosulfan, tolerance levels remained about the same throughout the winter. Early summer forms in June were less susceptible to azinphosmethyl, methiocarb and fenvalerate than late winter forms (March). Early summer forms were more susceptible to endosulfan compared with late winter forms. Susceptibility, however, increased during the summer for all compounds as indicated by lower LC_{50} values for late summer forms in August. Based on the average LC_{50} values, summer forms were more susceptible to insecticides than winter forms.

LC_{50} values for avermectin varied from 0.003 to 0.006 g a.i./l throughout the year. However, differences in susceptibility were not related to the different seasonal periods as observed for the other compounds tested.

For the entire seasonal period of testing, LC_{50} values were lowest for avermectin followed by fenvalerate< methiocarb< endosulfan< azinphosmethyl< Perthane (Fig. 2.1). These results reflected levels of intrinsic susceptibility (avermectin) or current levels of developed insecticide resistance (fenvalerate,

methiocarb, endosulfan, azinphosmethyl, and Perthane) as discussed earlier (section 2.3.1).

Table 2.2 presents a comparison of the seasonal variation in insecticide mortality between R psylla from Medford and S psylla from Corvallis, collected in May/June (summer forms) and January/February (winter forms). Results show similar magnitudes of change in susceptibility in both strains. Changes in insecticide toxicity are therefore related to differences between summer- and winter-form psylla and are not related to the levels of resistance developed.

Overwintering pear psylla weigh ca. 1.2-fold more than summer-form adults (see Table 2.3). They may be more tolerant to insecticides than summer forms because they contain more detoxification enzymes or more fat body for sequestration of toxins. When LD_{50} values for both forms were corrected for body weight, the ratio of the LD_{50} of winter forms to the LD_{50} of summer forms was decreased, but not proportionally to that expected for this factor alone (Table 2.2). Probably, the relation between body weight and surface area of exposure accounts for some of the differences observed in toxicity between summer and winter-form pear psylla. However, differences in feeding behavior and condition of their host plant may also contribute to the tolerance and resistance differences observed.

Results of slide-dip tests with formulated azinphosmethyl, methiocarb and endosulfan showed similar trends in seasonal mortality of pear psylla to the microapplication test for technical formulations of these compounds (Fig. 2.2). Summer forms were more susceptible than winter forms. Slide-dip tests showed that avermectin was also more toxic to summer forms than to winter forms. However, slide-dip tests with fenvalerate, showed a different trend than did topical application tests. Summer forms were more tolerant to fenvalerate than winter forms (Fig. 2.2).

Table 2.4 shows results of more detailed monitoring studies using slide-dip tests for formulated fenvalerate during 1983-1985. Susceptibility to fenvalerate was high for winter and early summer forms but decreased for late summer forms in July and August. Differences in results between bioassays may have been due to use of formulated versus technical fenvalerate. Similar seasonal differences in achieving control between late winter/early summer and late summer generations of pear psylla with formulated fenvalerate have also been observed in field trials in Southern Oregon orchards (Westigard unpublished data).

2.3.3 Synergistic effects of PbO and DEF in pear psylla

Synergist ratios of mixtures of insecticides and PbO or DEF, respectively for summer and winter forms of R psylla from Medford are presented in Tables 2.5 and 2.6. Synergist ratios (SR) are expressed as LD_{50} insecticide/LD_{50} insecticide + synergist. Synergism was reported to occur if the SR $\geq$ 2, at which level LD_{50} values of insecticides and insecticides + synergists were significantly different as indicated by standard errors.

PbO was a synergist of azinphosmethyl, methiocarb, fenvalerate and endosulfan in early winter pear psylla from Medford in October. PbO also synergized fenvalerate and endosulfan in mid-winter forms in January and summer forms in June and August. DEF was effective as a synergist only in winter forms of psylla. DEF was a synergist of azinphosmethyl and methiocarb in early winter forms in October and of azinphosmethyl and fenvalerate in mid-winter forms in January.

Table 2.7 shows comparative data on the synergistic effects of PbO and DEF in summer and winter-form susceptible pear psylla. No synergism was observed for fenvalerate and endosulfan by PbO in summer forms of susceptible psylla. In winter pear psylla no synergism was observed for DEF and fenvalerate or azinphosmethyl,

or for PbO and endosulfan. Only low synergism was observed in susceptible winter pear psylla for PbO and fenvalerate.

The synergistic effect of PbO has been related to its ability to form metabolic complexes with the heme iron of cytochrome P-450 monooxygenases (Agosin 1985) and DEF acts as an inhibitor of esterase detoxification enzymes in insects (Oppenoorth 1976; Oppenoorth and Welling 1976). Synergism by PbO and DEF for various insecticides in R pear psylla and the lack thereof in S psylla, indicated that resistance in psylla from Medford is partly based on cytochrome P-450 monooxygenase and esterase detoxification enzymes. Differences of synergism by PbO and DEF for different compounds between summer and winter forms of psylla may be related to changes in levels of detoxification enzymes between these forms (see Chapter 3).

Based on SRs for DEF, esterases seemed to be most important in conferring resistance to azinphosmethyl (SR = 6.2/5.1) and less important in conferring resistance to methiocarb (SR = 2.6) and fenvalerate (SR = 2.3) in winter-form psylla. PbO showed relatively high SRs for fenvalerate in winter- (SR = 18.3/17.7) and summer-form (SR = 8.7/8.5) psylla and moderate SRs for endosulfan for winter (SR = 3.1) and summer forms (SR = 2.5/3.9). Lower SRs for PbO were observed in early winter-form

psylla for azinphosmethyl (SR = 2.3) and methiocarb (SR = 2.7). These results indicated that cytochrome P-450 monooxygenases were more important for detoxification of fenvalerate and endosulfan than for azinphosmethyl and methiocarb.

Synergist results with pear psylla agreed with the general trends that have been observed for esterases and cytochrome P-450 monooxygenases resistance mechanisms in other insects (Oppenoorth 1985). OP resistance is mostly due to esterases and these enzymes are less important in resistance to other esters such as carbamates and pyrethroids. Cytochrome P-450 monooxygenases detoxify a variety of insecticides and can be important in conferring resistance to organochlorines, pyrethroids, OPs and carbamates.

Caution is necessary in interpreting results of synergist tests. This is because of the relative specificity of synergists and other toxicodynamic interactions, which may interfere with their mode of action. Synergist tests are only indicative of the general importance of certain detoxification enzymes. Results of synergist tests need to be supplemented by biochemical studies of resistance mechanisms for more definitive assessment (see Chapter 3).

2.3.4 Toxicity of insecticides to D. brevis

Table 2.8 shows the toxicity of azinphosmethyl and fenvalerate to susceptible D. brevis compared with similar data for S and R pear psylla. LD_{50} values are expressed both as the amount of insecticide per insect and as the amount of insecticide per mg insect. LD_{50} values of azinphosmethyl and fenvalerate were similar in D. brevis.

Selectivity ratios (LD_{50} pear psylla/LD_{50} D. brevis) on a per mg weight basis showed that the level of intrinsic tolerance to azinphosmethyl was ca. 5-fold higher in S adult psylla than in susceptible predator nymphs (Table 2.8). Resistant psylla from Medford were ca. 37-fold more tolerant to azinphosmethyl than susceptible D. brevis. These data agreed with field observations of the effect of azinphosmethyl on both pest and predator populations. Azinphosmethyl is not effective in controlling pear psylla, but D. brevis are eliminated by this compound (Westigard 1973).

Tests with fenvalerate on a per mg weight basis, showed that the level of intrinsic tolerance was ca. 0.2-fold lower in S psylla than in susceptible D. brevis (Table 2.8). Predators were ca. 7-fold less tolerant to fenvalerate than resistant psylla. Although the intrinsic tolerance to fenvalerate was relatively low in

pear psylla compared with D. brevis, psylla had developed a 40-fold level of resistance to this compound under selection pressure (Table 2.1). This resulted in a 7-fold lower level of susceptibility to fenvalerate in susceptible D. brevis than in R psylla. Based on these results it was assumed that pear psylla is more able to develop resistance to insecticides than D. brevis.

2.4 Conclusions

Similar toxicity results obtained by slide-dip and topical application bioassays indicated that susceptibility and resistance to insecticides in pear psylla can be monitored using either method. For monitoring susceptibility or resistance in field populations of pear psylla the slide-dip bioassay seemed adequate. The slide-dip method is simpler, uses discriminating doses and formulated materials. Because formulated materials are used, the slide-dip bioassay may provide a better relationship between insecticide toxicity observed in the laboratory and in the field, as indicated by the results for fenvalerate. The topical application method seemed more appropriate for basic studies (e.g. studies on resistance mechanisms), because technical compounds are used, overcoming the variable

effects of formulation differences and greater precision in application is achieved.

Results of toxicity studies in pear psylla suggest several tactics which may limit further resistance development in this pest. Data confirmed the advisability of currently spraying pyrethroids for pear psylla control during late winter when their susceptibility is high, thereby reducing pest build up later in summer. Results indicated that summer sprays of fenvalerate should be avoided because of the greater tolerance of pear psylla in mid-summer. Fenvalerate should be used only for dormant season control. Amitraz should be used for summer control, thereby reducing selection pressure and resistance to fenvalerate.

Limiting fenvalerate use to dormant and amitraz to summer control, has thus far proven effective in limiting resistance in pear psylla in most areas of the Pacific Northwest as compared with areas where psylla has been exposed to intensive summer applications of pyrethroids (see Chapter 5; Riedl et al. 1981; Burts 1983; Croft et al. 1988). One example where resistance to pyrethroids developed rapidly due to extensive summer use is in Europe. This compound is now ineffective against the closely related Psylla pyri L. on a regional basis (L. Balzarotti personal communication).

An alternative for future control of psylla may be the use of avermectin. This compound showed low LD_{50} values in R pear psylla in bioassays and appeared to be effective in the field (Burts 1985). Rotation of avermectin with pyrethroids and amitraz may slow development of resistance to the latter two compounds.

Mixtures of synergists and insecticides may be used to counteract resistance development in pear psylla. This study indicated that both esterases and cytochrome P-450 monooxygenases are involved as detoxification enzymes in R psylla. DEF increased the effectiveness of azinphosmethyl, methiocarb, and fenvalerate in R winter-form psylla. PbO increased the effectiveness of fenvalerate and endosulfan in both R winter- and R summer-form psylla and synergized azinphosmethyl and methiocarb in R winter psylla. DEF was most effective as a synergist of azinphosmethyl and mixtures of DEF and OPs may enhance control of resistant early winter populations of psylla before they migrate out of orchards. PbO was highly active in increasing fenvalerate toxicity and in the case of pyrethroid resistance this synergist may be used as mixtures with pyrethroids. The effect of PbO as a synergist of pyrethroids and the potential of PbO as a tool for pyrethroid resistance management will be discussed further in Chapter 5.

Toxicity tests showed that pear psylla from Medford have developed resistance to a variety of insecticides. Susceptible psylla were more tolerant to azinphosmethyl than susceptible D. brevis but less tolerant to fenvalerate. Resistant psylla were more tolerant to azinphosmethyl and fenvalerate, than susceptible D. brevis. These data indicated that psylla is better adapted to toxins, as shown by both high intrinsic tolerance levels (e.g. azinphosmethyl in S psylla) and its potential to develop resistance (e.g. fenvalerate in R psylla).

Table 2.1. Toxicity of several insecticides to adult summer forms (May/June) of susceptible (S) and resistant (R) pear psylla, P. pyricola, using topical application bioassay.

compound	R pear psylla				S pear psylla	fold-R
	LD_{50}[1] (ng/mg insect)	95% CI (ng/mg insect)	slope	r^2	LD_{50}[2] (ng/mg insect)	
endosulfan	135.8	135.6-135.9	3.63	0.80	57.4	2.4
methiocarb	83.2	82.6-83.8	2.92	0.99	33.3	2.5
perthane	1166.5[2]	-	-	-	199.8	5.8
fenvalerate	41.7	30.3-57.5	2.54	0.93	1.04	40.1
azinphosmethyl	208.7	202.9-214.7	1.63	0.97	27.0	7.7
avermectin	1.11	1.07-1.15	2.09	0.93	-	-

[1] LD_{50} values calculated based on probit analysis. n = 375.

[2] LD_{50} values estimated based on a single diagnostic dose and an average probit analysis slope of 2.56. n = 75.

Table 2.2. Seasonal toxicity of several insecticides to adult summer (May/June) and winter (January/February) forms of susceptible (S) and resistant (R) pear psylla, P. pyricola, using topical application bioassay.

compound	strain	LD_{50} (ng/insect)		LD_{50} winter/ LD_{50} summer (ng/insect)	LD_{50} (ng/mg insect)		LD_{50} winter/ LD_{50} summer (ng/mg insect)
		summer[1]	winter[2]		summer[3]	winter[2]	
azinphosmethyl	R	118.5	180.6	1.52	208.7	266.6	1.28
	S	15.3	18.1	1.18	27.0	26.7	0.99
fenvalerate	R	23.7	23.1	0.98	41.7	34.1	0.82
	S	0.59	1.51	2.56	1.04	2.23	2.14
endosulfan	R	77.1	138.6	1.80	135.8	204.6	1.51
	S	32.6	81.9	3.47	57.4	120.9	2.11
methiocarb	R	47.2	77.7	1.65	83.2	114.7	1.38
	S	18.9	31.5	1.67	33.3	46.5	1.40
perthane	R	661.5	2520.0	3.81	1165.5	3720.0	3.19
	S	113.4	100.8	0.98	199.8	148.8	0.75

[1] LD_{50} values calculated from data presented in Table 2.1.

[2] LD_{50} values for R psylla calculated from data presented in Fig. 2.1. LD_{50} values for S psylla estimated based on a single diagnostic dose and an average probit analysis slope of 2.56. $n = 75$.

[3] LD_{50} values from Table 2.1.

Table 2.3. Weight (mg) of adult winter and summer form pear psylla, P. pyricola, and of nymphs (3rd-5th instars) of D. brevis.

species	weight (mg)[1]
P. pyricola:	
male summer	0.46
female summer	0.67
average male and female summer	0.57
male winter	0.54
female winter	0.81
average male and female winter	0.68
D. brevis	2.09

[1] Weight derived by weighing 100 adult male or female P. pyricola of each form and 50 nymphs (3rd-5th instars) of D. brevis, no variance estimates were obtained.

Table 2.4. Seasonal variability in mortality to fenvalerate (0.06 g a.i./l) in adult summer and winter forms of resistant pear psylla, P. pyricola, using slide-dip bioassay (data from van de Baan et al. in press).

date	psylla form	% survival ± SE
1983		
February 16	winter	0
May 29	summer	49.1 ± 7.5
July 5	summer	97.0 ± 3.0
August 22	summer	97.9 ± 3.6
1984		
January	winter	15.0 ± 3.4
December 11	winter	8.3 ± 3.5
1985		
January 29	winter	6.0 ± 1.2
March 4	winter	0
July 25	summer	64.1 ± 12.0
October 14	winter	2.0 ± 1.2

Table 2.5. Synergist ratios for mixtures of insecticides and piperonyl butoxide (PbO) in adult resistant pear psylla, P. pyricola, using topical application bioassay.

compound	synergist ratio (LD_{50} insecticide[1]/LD_{50} insecticide+PbO[2])			
	January	June	August	October
azinphosmethyl	0.9	1.5	-	2.3
methiocarb	1.0	0.7	-	2.7
avermectin	1.1	1.7	-	1.5
fenvalerate	17.7	8.7	8.5	18.3
endosulfan	3.1	2.5	4.0	3.2
perthane	1.0	1.7	0.9	1.0

[1] LD_{50} values calculated from data presented in Table 2.1 and Fig. 2.1.

[2] LD_{50} values estimated based on a single diagnostic dose and an average probit analysis slope of 2.35. n = 75.

Table 2.6. Synergist ratios for mixtures of insecticides and DEF in adult resistant pear psylla, P. pyricola, using topical application bioassay.

compound	synergist ratio (LD_{50} insecticide[1]/LD_{50} insecticide+DEF[2])		
	January	June	October
azinphosmethyl	5.1	1.1	6.2
methiocarb	1.2	0.6	2.6
avermectin	0.8	-	0.9
fenvalerate	2.3	0.7	1.4
endosulfan	1.5	0.7	1.0
perthane	1.3	-	1.0

[1] LD_{50} values calculated from data presented in Table 2.1 and Fig. 2.1.

[2] LD_{50} values estimated based on a single diagnostic dose and an average probit analysis slope of 2.35. n = 75.

Table 2.7. Synergist ratios for mixtures of insecticides and piperonyl butoxide (PbO) or DEF in adult susceptible pear psylla, P. pyricola, using topical application bioassay.

mixture	synergist ratio (LD_{50} insecticide[1]/LD_{50} insecticide+syn.[2])	
	June	January
fenvalerate + PbO	0.8	2.4
endosulfan + PbO	1.6	1.5
fenvalerate + DEF	-	1.6
azinphosmethyl + DEF	-	1.5

[1] LD_{50} values from Tables 2.1 and 2.2.

[2] LD_{50} values estimated based on a single diagnostic dose and an average probit analysis slope of 2.35. n = 75.

Table 2.8. Toxicity of azinphosmethyl and fenvalerate to nymphs (3rd-5th instars) of susceptible D. brevis, and adult summer forms of susceptible (S) and resistant (R) pear psylla, P. pyricola, using topical application bioassay. Selectivity ratio (Sel-R) expressed as LD_{50} pear psylla/LD_{50} D. brevis.

compound	D. brevis				P. pyricola		Sel-R	
	LD_{50}[1] (ng/insect)	95% CI (ng/insect)	slope	r^2	LD_{50}[2] (ng/insect) S	R	S	R
azinphosmethyl	11.94	11.80-12.08	3.26	0.93	15.33	118.46	1.28	9.92
fenvalerate	12.05	12.03-12.07	4.53	0.89	0.59	23.69	0.05	1.97
	LD_{50}[1] (ng/mg insect)	95% CI (ng/mg insect)	slope	r^2	LD_{50}[2] (ng/mg insect)			
azinphosmethyl	5.69	5.62-5.76	3.26	0.93	27.0	208.7	4.75	36.68
fenvalerate	5.74	5.73-5.75	4.53	0.89	1.04	41.7	0.18	7.26

[1] LD_{50} values calculated based on probit analysis. n = 100.

[2] LD_{50} values from Table 2.2.

Figure 2.1. Seasonal variability in LC_{50} values to five insecticides for adult summer and winter forms of resistant pear psylla, P. pyricola, using topical application bioassay. LC_{50} values (plus 95% confidence intervals [CI]) for March, June, and October were calculated using probit analysis based on five different dosages. n = 375 per test date. LC_{50} values for August and January were estimated based on a single diagnostic dosage and an average slope of 2.81 obtained from probit analysis. n = 75 per test date.

Figure 2.1.

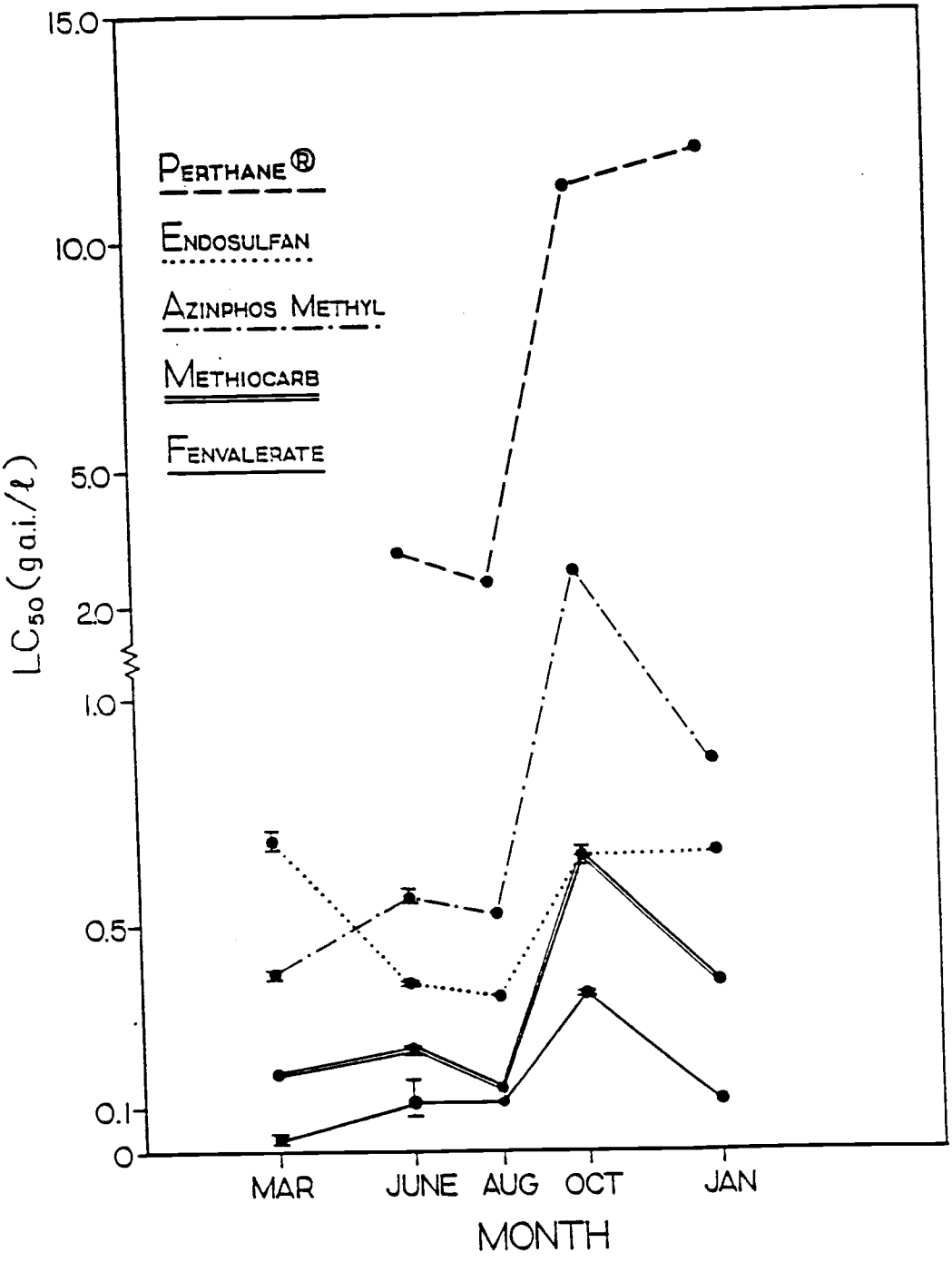

15.0
10.0
5.0
2.0
1.0
0.5
0.1
0
LC50 (g a.i./ℓ)
PERTHANE®
ENDOSULFAN
AZINPHOS METHYL
METHIOCARB
FENVALERATE
MAR
JUNE
AUG
OCT
JAN
MONTH

Figure 2.2. Seasonal variability in percent survival (± standard error [SE]) to five insecticides in adult summer and winter forms of resistant pear psylla, P. pyricola, using slide-dip bioassay. n = 100 per test date.

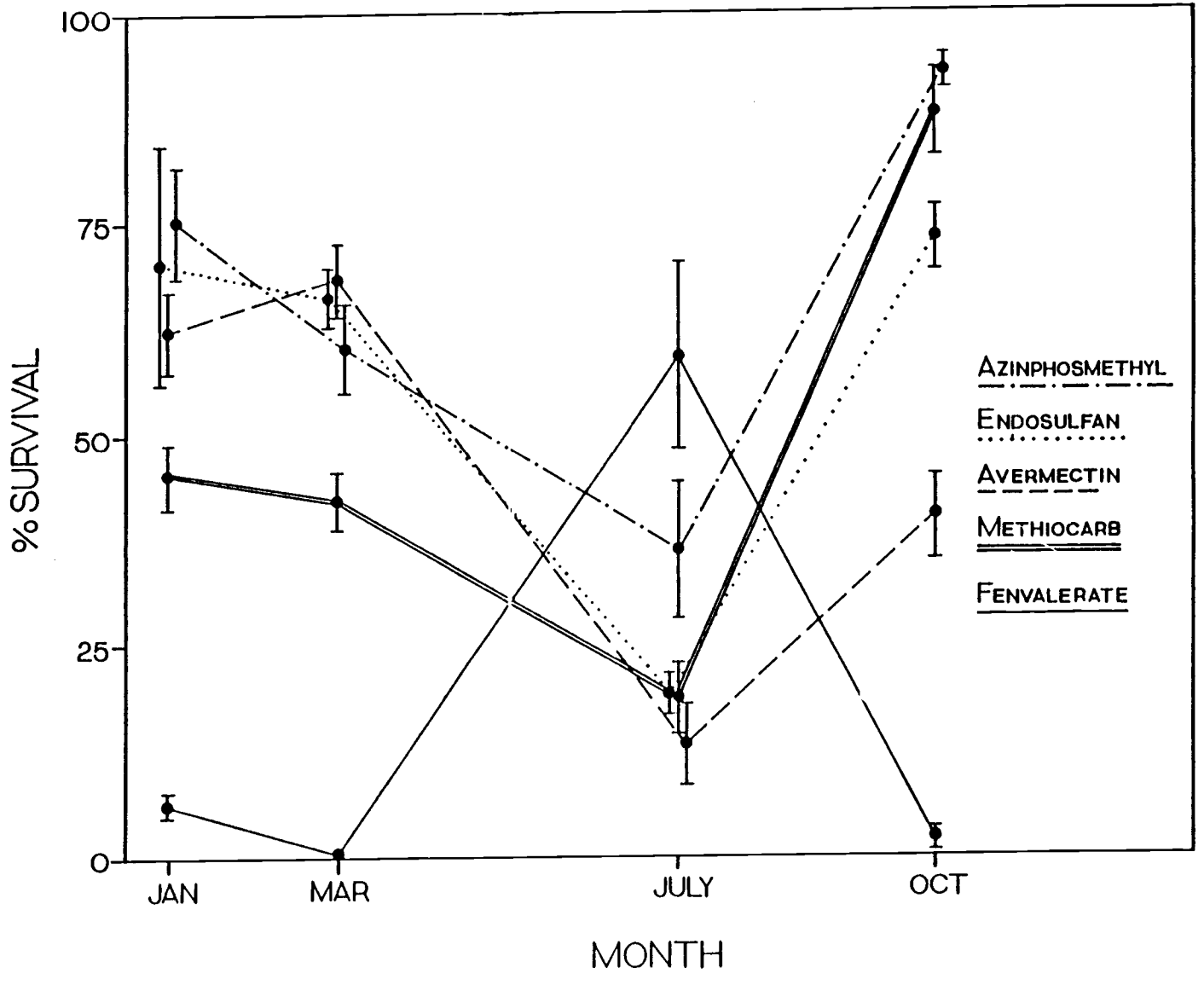

100
75
50
25
0
% SURVIVAL
JAN
MAR
JULY
OCT
MONTH
AZINPHOSMETHYL
ENDOSULFAN
AVERMECTIN
METHIOCARB
FENVALERATE

Figure 2.2.

3. BIOCHEMICAL RESISTANCE MECHANISMS IN PEAR PSYLLA AND COMPARATIVE TOXICOLOGY OF DERAEOCORIS BREVIS

3.1 Introduction

Biochemical mechanisms of resistance to insecticides in arthropods include altered site of action, increased detoxification, and reduced penetration (Oppenoorth and Welling 1976; Plapp 1976; Oppenoorth 1985). These mechanisms have evolved in arthropods as adaptations to environmental toxins (Gordon 1961; Brattsten and Metcalf 1970; Krieger et al. 1971; Brattsten et al. 1977; Dowd et al. 1983; Mullin 1986). Studies on detoxification enzyme systems have suggested that polyphagous or generalist herbivores have a higher detoxification capability, because they are exposed to a larger variety of plant toxins than monophagous or specialist herbivores (Krieger et al. 1971; Brattsten et al. 1977). Also differences in feeding modes among herbivores such as leaf chewing or phloem sucking result in different exposure to plant allelochemicals and therefore to differences in detoxification capabilities (Mullin 1986).

Similarly, differences in detoxification capabilities could be expected between phytophagous and entomophagous arthropods. A preadaptation hypothesis has been put forth to explain the intrinsic differences in susceptibility or tolerance between herbivores and their natural enemies (Croft and Brown 1975; Croft and Morse 1979). This hypothesis states that herbivores are more adapted to counteract toxic effects and therefore develop resistance to insecticides than predators and parasitoids (Croft and Morse 1979; Croft and Strickler 1983). The preadaptation hypothesis may therefore be a factor explaining resistance differences in these groups (Croft and Strickler 1983).

Recently, the importance of the relationship between detoxification mechanisms and herbivore polyphagy has been questioned (Gould 1984; Rose 1985), and other factors such as predation has been proposed as major factors influencing the evolution of herbivore diets (Bernays and Graham 1988). However, plant chemistry seems still of major importance in mediating plant-herbivore coevolution (Schultz 1988; Ehrlich and Murphy 1988; Mullin 1986), and may therefore encounter for differences in detoxification capabilities between phytophagous pests and their entomophagous natural enemies.

A better knowledge of the similarities and dissimilarities of detoxification enzyme systems between phytophagous and entomophagous arthropods may help explain the observed differences in evolution of resistance between these trophic levels. This knowledge may also explain differences in selectivity of insecticides and be useful for designing selective insecticides. Selective insecticides that are effective against pest species but relatively safe for beneficial arthropods will reduce problems of pest resurgence and development of pest resistance associated with broad-spectrum insecticides.

Biochemistry of resistance was studied in an insecticide resistant and susceptible strain of pear psylla from Medford and Corvallis, Oregon, respectively. Resistance mechanisms studied were increased detoxification, target site insensitivity, and reduced penetration. Because psylla showed seasonal variation in insecticide mortality (see Chapter 2), these resistance mechanisms were studied in both summer and winter pear psylla.

Detoxification enzyme systems in susceptible P. pyricola and D. brevis were compared, to evaluate intrinsic detoxification capabilities of prey and predator. The importance of detoxification in conferring insecticide resistance in pear psylla and

susceptibility in D. brevis was evaluated in resistant prey and susceptible predators.

3.2 Materials and methods

Insects

Adult summer and winter forms of insecticide resistant (R) pear psylla were field collected from pear orchards near Medford, Oregon, throughout the winter and summer of 1986 and 1987 as described in Chapter 2. Winter forms of insecticide susceptible (S) psylla were field collected at the O.S.U. Entomology Farm, Corvallis in February of 1986 and 1987. Summer forms of S psylla were collected from a laboratory colony that originated from the O.S.U. Entomology Farm throughout 1986 and 1987 (see Chapter 2). Susceptible nymphs (3rd-5th instars) of D. brevis were collected from pear orchards near Medford in a similar way as psylla in September 1986 and 1987 (see Chapter 2).

Insects collected for enzyme preparations were stored at -80 ^{o}C.

Enzyme preparations

Abdomens of 10 psylla or 5 D. brevis were homogenized in 500 µl 0.1 M sodium phosphate buffer pH 6.0 or 7.2 in a 2 ml potter tube with a motor-driven Teflon pestle. The homogenate was centrifuged at 10,000 x g for 10 min in a 1.5 ml Eppendorf tube in a Beckman Microfuge 11 instrument. The resulting supernatant was used for enzyme assays.

Microsomes were prepared by a method modified after Feyereisen et al. (1985) and Baldridge and Feyereisen (1986). One hundred abdomens of psylla or 50 abdomens of D. brevis were homogenized in 350 µl 50 mM morpholinopropanesulfonic acid (MOPS) buffer pH 7.2 containing 1 mM EDTA, 10% sucrose, and 0.4 mM phenylmethylsulforylfluoride (PMSF, freshly made) as described above. The homogenate was centrifuged at 1,000 x g for 10 min and the supernatant was loaded on a 5 ml linear 45 to 15% sucrose gradient in a polyallomer Quick-seal tube (Beckman) and centrifuged at 65,000 rpm in a Beckman VTi 80 rotor for 20 min ($\omega^2 t = 3.7 \times 10^{10}$ $rad^2.sec^{-1}$). For psylla, the bottom 1.2 ml of the gradient was discarded and the middle 1.4 ml (which contained the majority of aldrin epoxidase activity) were used as microsomal enzyme source, whereas for D. brevis, the bottom 1.6 ml of the gradient was discarded

and the middle 1.6 ml were used as microsomal enzyme source.

Supernatant of psylla heads were obtained by homogenizing 100 heads in 1 ml 64 mM potassium phosphate buffer pH 7.5 and centrifuging this homogenate at 1,000 x g for 1 min as described above.

Protein levels were determined according to Bradford (1976) using γ-globulins as standard.

Enzyme assays

Esterase: α-naphthyl esterase activity was determined spectrophotometrically by using a method modified after Gomori (1953). Incubations of 5 µl supernatant of psylla or D. brevis abdomen homogenate in 0.1 M sodium phosphate buffer pH 7.2 and 495 µl buffer were initiated with 500 µl α-naphthylacetate in buffer containing 1% (v/v) acetone and 5% (v/v) ethanol. Incubations took place for various periods of time and were terminated with 50 µl of an aqueous solution of 5% (w/v) sodiumdodecylsulfate and 0.5% (w/v) fast blue RR salt. Absorbance was read at 600 nm in a Beckman DU-40 spectrophotometer with α-naphthol standards. Blanks without α-naphthylacetate were run for comparison.

Glutathione S-transferase (GSH S-transferase): GSH S-transferase activity was measured according to Habig

et al. (1974) with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The rate of product formation was measured at 340 nm during a 5 min incubation period at 25 ^{o}C in an Aminco DW2a spectrophotometer. The reaction mixture contained 480 µl 0.1 M sodium phosphate buffer pH 6.0 containing 1 mM EDTA, 500 µl 10 mM reduced glutathione (GSH) in buffer, 20 µl 50 mM CDNB in acetone and 10 µl supernatant of abdomen homogenate of pear psylla or D. brevis in buffer. This reaction mixture was run against blanks without GSH for comparison.

Cytochrome P-450 monooxygenase: Cytochrome P-450 monooxygenase activity was determined by measuring the epoxidation of aldrin into dieldrin according to a modified method after Baldridge and Feyereisen (1986). One hundred eighty µl of sucrose gradient containing microsomal fraction was mixed with 16 µl NADPH regenerating system containing 10 units glucose-6-phophate dehydrogenase, 106 mM glucose-6-phosphate and 3.8 mM $NADP^{+}$ in 50 mM MOPS buffer pH 7.2 containing 1 mM EDTA. The reaction was started by addition of 4 µl 20 uM aldrin in methylcellosolve and incubated for 30 min at 30 ^{o}C. Blanks lacked the NADPH regenerating system. The reaction was stopped by addition of 16 µl 1 M HCl. Dieldrin was extracted with 250 µl hexane after addition of 20 µl 9% (w/v) NaCl to suppress emulsion formation. Two µl aliquots of the hexane phase were assayed for

dieldrin by gas chromatography with a Varian series 1200 instrument equipped with a tritium electron capture detector.

Acetylcholinesterase (AChE): AChE activity and inhibition were measured according to Ellman et al. (1961). One hundred µl supernatant of psylla head homogenate in 64 mM potassium phosphate buffer pH 7.5 was incubated with 10 µl of various concentrations of paraoxon (1-(4-hydroxyphenyl)-1-propanone), propoxur (2-(1-methylethoxy)phenylmethyl carbamate), diisopropyl fluorophosphate (DFP), or eserine ((3aS-cis)-1,2,3,3a,8,8a hexahydro-1,3a,8-trimethylpyrrolo [2,3-b]indol-5-ol methyl carbamate(ester)) in 20% acetone for 10 min at 25 ^{o}C. Inhibitors were of more than 99% purity. Uninhibited activity was measured by similar incubation with 10 µl 20% acetone. After this incubation the mixture was diluted by adding 850 µl buffer and 25 µl 20 mM acetylthiocholine iodide (ASChI) in water and 25 µl 10 mM dithionitrobenzene (DTNB) in acetone to stop the inhibition. Enzyme activity was measured at 421 nm in an Aminco DW2a spectrophotometer thermostated at 25 ^{o}C. Blanks without enzyme or substrate were used to correct for non-enzyme activity.

Penetration and metabolism of [14C] azinphosmethyl

Pear psylla were treated with [^{14}C] azinphosmethyl (specific activity 27.8 mCi/mmol) using topical application as described in Chapter 2. Ten psylla were placed on a microscope slide with 3 replicates for each time period that penetration and metabolism measurements were taken. Per insect, 15.3 ng a.i. [^{14}C] azinphosmethyl (= 2975 dpm) was applied which is equivalent to the LD_{50} value of this compound for S summer psylla (Table 2.2). After 0, 1, 3, 6, 12 and 24 h, psylla which survived the treatment were removed from the microscope slides and placed in 1 ml glass tubes. Psylla were washed with 50 µl acetone per insect. Radioactivity was counted for 100 µl aliquots of acetone wash in Formula-963 aqueous counting cocktail (Du Pont) on a Searle Isocap/300 liquid scintillation counter.

After washing with acetone, psylla were homogenized in 50 ul acetone per insect using a glass tissue grinder. Large body parts were discarded and the remaining homogenate was centrifuged for 10 min at 10,000 x g in a 1.5 ml Eppendorf tube in a Beckman Microfuge 11 instrument and supernatant was saved. One hundred ul aliquots of supernatant were spotted on precoated silica gel 60 F-254 TLC plates (0.20 mm thickness; Merck). The TLC plate was run in a solvent

system of n-hexane-ethylacetate-benzene (2:4:1 v/v). The R_f value of azinphosmethyl in this solvent system is 0.75 and various possible water-soluble metabolites, i.e. desmethyl azinphosmethyl, desmethyl azinphosmethyl oxygen analog, dimethyl phosphorodithioic acid, dimethyl phosphoric acid, and monomethyl phosphoric acid remain at the origin (Motoyama et al. 1971). Areas that corresponded with these R_f values were cut out and radioactivity was determined by liquid scintillation spectrometry.

3.3 Results and discussion

3.3.1 Increased detoxification

Esterase and GSH S-transferase activities for S and R psylla and susceptible D. brevis are shown in Tables 3.1 and 3.2. Esterase and GSH S-transferase activity per ug protein was 3.8 and 1.8-fold higher, respectively in R summer psylla than in S summer psylla. Higher esterase and GSH S-transferase activities in summer R psylla versus summer S psylla may be indicative of resistance mechanisms, which contribute to greater detoxification of insecticides.

Esterase and GSH S-transferase activities per ug protein were lower in winter-form pear psylla than in summer-form pear psylla for both R and S strain. In R winter psylla enzyme activities decreased during the season. However, winter forms weighed ca. 1.2-fold more than summer forms (Table 2.3) and contained ca. 4.5-fold more protein. Therefore, enzyme activity expressed per insect showed that R winter forms in October had 6.4-fold higher esterase and 4.9-fold higher GSH S-transferase activity than S summer forms. These levels decreased to 1.9 and 0.9-fold for R psylla in March compared with S psylla in March.

Winter-form psylla were more resistant to insecticides than summer-form psylla (Fig. 2.1 and 2.2) and higher levels of protein in winter-form psylla may contribute to a quantitative increase in detoxification enzymes conferring higher levels of insecticide resistance. Synergism of azinphosmethyl, methiocarb and fenvalerate by DEF in R winter psylla also indicated the importance of esterases as a resistance mechanism (Table 2.6). However, increased amounts of proteins can also play a role in sequestration of insecticides in winter psylla.

Susceptible D. brevis showed 4.7-fold lower esterase activity and 1.5-fold higher GSH S-transferase activity than S summer psylla per ug protein. GSH S-transferase

activity was only 1.2-fold higher but esterase activity was 17.8-fold higher in R summer psylla than in susceptible predator. High susceptibility of D. brevis to azinphosmethyl and fenvalerate (Table 2.8) compared with R pear psylla, may be due to lack of detoxification by esterases, whereas GSH S-transferases may not be important for detoxification of these compounds.

Profiles of sucrose gradients for pear psylla and D. brevis abdomen homogenates are shown in Figures 3.1 and 3.2. Microsomal oxidase activity, measured by aldrin epoxidase activity was focused at fraction 4-7 in pear psylla (Fig. 3.1) and fraction 5-8 in D. brevis (Fig. 3.2). These fractions were collected and pooled as microsomal enzyme source for assays.

Cytochrome P-450 monooxygenase activity for pear psylla and D. brevis is shown in Table 3.3. Cytochrome P-450 monooxygenase activity in R summer psylla was 1.6-fold higher than in S summer psylla. Resistant winter psylla in October showed a 4.1-fold higher enzyme activity than S summer forms and enzyme activity decreased in winter forms with R psylla in March showing a 2.2-fold higher activity than S summer psylla. Higher cytochrome P-450 monooxygenase activity in R psylla than in S psylla may be important in the detoxification of insecticides and may provide a mechanism for insecticide resistance. The effect of PbO as a synergist for

azinphosmethyl, methiocarb, endosulfan, and fenvalerate in R winter psylla and for endosulfan and fenvalerate in R summer psylla, further verified the role of cytochrome P-450 monooxygenases in insecticide detoxification (Table 2.5).

Susceptible D. brevis had a 1.6-fold higher cytochrome P-450 monooxygenase activity than S summer psylla. Enzyme activity was similar in the susceptible predator and in R summer psylla. Similar levels of enzyme activity in susceptible predator and resistant prey apparently do not explain the predator's lower tolerance to azinphosmethyl and fenvalerate as compared with R pear psylla (Table 2.8).

Table 3.4 summarizes the relative activity per amount protein of all detoxification enzyme studies for summer and winter forms of susceptible and resistant P. pyricola and for susceptible D. brevis.

3.3.2 Altered site of action

Low AChE activities of 0.78 and 0.63 nmole/µg protein/h measured in S and R summer psylla were not significantly different at the $P \leq 0.05$ level. In order to determine the inhibition constant K_i for R and S psylla, experiments were performed with paraoxon. No

inhibition of AChE was observed in either strain of pear psylla for concentrations of paraoxon as high as 1 mM. Similar experiments with head homogenate of a susceptible strain of the housefly, Musca domestica L., showed that the paraoxon used, was an effective inhibitor (unpublished data). Lack of inhibition in both strains of psylla was also observed for propoxur, DFP and eserine.

Lack of AChE inhibition in both S and R psylla indicated that other enzymes interferred with AChE activity, that AChE levels were too low or that the enzyme was too unstable to perform inhibition experiments. In order to stabilize the enzyme and increase its activity, the effects of various buffers were studied containing 1% (v/v) Triton X-100, or 1 mM EDTA, 1 mM DTT, and 20% glycerol, or 0.5 to 1.5% BSA, or 0.1 M NaF. Using these buffers, no effect of inhibitors was observed in either strain of psylla. Centrifugation of homogenates at various speeds and periods of time (ranging from 1 min at 1,000 x g to 15 min at 10,000 x g) or increasing the number of psylla heads per homogenate did not affect inhibition or enzyme activity either. Due to these problems, inhibition of AChE in psylla was not further investigated.

3.3.3 Penetration and metabolism of [^{14}C] azinphosmethyl

The amount of external [^{14}C] azinphosmethyl present on S summer and R summer and R winter psylla, is shown in Figure 3.3. After 24 h, the amount of external azinphosmethyl was ca. 2-fold higher in R summer psylla than in S summer psylla, and ca. 4-fold higher in R winter psylla than in S summer psylla. Assuming that the amount of external azinphosmethyl is inversely correlated to the amount of insecticide penetrated, slower penetration through the cuticle in R psylla may contribute to azinphosmethyl resistance.

In vivo degradation of ^{14}C azinphosmethyl by S and R strains of psylla is presented in Figure 3.4. No significant difference in the percent azinphosmethyl degraded was observed in summer forms of both strains. These results were contrary to earlier results from detoxification enzyme assays (Tables 3.1, 3.2, and 3.3). Resistant summer-form psylla showed higher activities of esterases, GSH S-transferases and cytochrome P-450 monooxygenases than S summer-form psylla and therefore a higher percent degradation of azinphosmethyl would be expected. However, resistance to azinphosmethyl may be based on other resistance mechanisms such as altered site of action or a combination of reduced penetration and detoxification.

Although winter-form psylla were more resistant to azinphosmethyl than summer forms and higher activity of detoxification enzymes per insect were observed in winter forms than in summer forms, the percent degradation of azinphosmethyl was lower in R winter forms than in R and S summer forms. This may indicate that sequestration is important as a resistance mechanism for azinphosmethyl. Esterase and GSH S-transferase activity per amount protein was lower in resistant winter-form psylla than in resistant summer-form psylla (Tables 3.1 and 3.2). Thus, a higher amount of protein in winter than in summer-form psylla does not imply that the total amount of these detoxification enzymes was also higher in winter-form psylla. Higher amounts of protein in winter-form psylla, instead may provide a mechanism for sequestration.

It may still be possible that degradation of azinphosmethyl was higher in R than in S psylla, but this difference was not detected using the TLC method as described by Motoyama et al. (1971). Azinphosmethyl degradation in P. pyricola may differ from that found in the predatory mite Amblyseius fallacis Garman. This will result in the production of other metabolites in psylla than in the predatory mite, which may not be separated by the same solvent system used in this study.

3.4 Conclusions

Higher esterase, GSH S-transferase and cytochrome P-450 monooxygenase activities in R versus S summer psylla indicated that increased detoxification probably contributes to the general insecticide resistance observed in this insect. Together, these detoxification enzymes probably cause psylla to be resistant to many insecticides. Synergism of fenvalerate, azinphosmethyl, endosulfan, and methiocarb by PbO and DEF (Tables 2.5 and 2.6) supported this conclusion and further confirmed the importance of cytochrome P-450 monooxygenases and esterases in resistance. Based on relative enzyme activities, esterases were a more important detoxification mechanism of resistance in P. pyricola than GSH S-transferases and cytochrome P-450 monooxygenases (Table 3.4).

Studies on OP and carbamate resistance in other homopterans such as the green rice leafhopper, Nephotettix cincticpes Uhler, the brown planthopper, Nilaparvata lugens (Stal), the smaller brown planthopper, Laodelphax striatellus Fallen, and the green peach aphid, Myzus persicae (Sulz.), demonstrated the importance of esterases as a detoxification mechanism in homopterans (Miyata and Saito 1976; Chang

and Whalon 1987; Ozaki 1969; Devonshire and Sawicki 1977). Mullin (1986) studied detoxification in aphids and suggested that differences in enzyme activity between phloem-sucking homopterans and leaf-chewing herbivores, such as lepidopterans was due to adaptation to different secondary plant compounds. Compared with chewing herbivores, aphids showed low levels of cytochrome P-450 monooxygenases, whereas general carboxylesterase levels were similar. These enzymes could be used by aphids to hydrolyze potentially toxic carboxylesters. Higher esterase activity in S pear psylla compared with susceptible D. brevis may indicate a greater adaptation to feeding on phloem. Esterases are apparently important detoxification enzymes in conferring insecticide resistance in psylla, as indicated by higher esterase activity in R than S summer psylla.

Esterase and GSH S-transferase activities per ug protein were lower, whereas cytochrome P-450 activity per mg protein was higher in R winter psylla than in R summer psylla. Winter psylla, however, were more resistant to insecticides than summer forms (Fig. 2.1 and 2.2). Due to higher levels of proteins in winter forms than summer forms, total activity of esterases and GSH S-transferases per insect was higher in winter forms than in summer forms. As discussed by Oppenoorth (1984,

1985), resistance mechanisms could theoretically consist of quantitative changes of an enzyme (more site of action or more detoxification enzyme) or qualitative changes (less sensitive site of action or more efficient detoxification enzyme). This study indicated that higher levels of resistance in winter-form psylla may be based on a quantitative increase of esterases and GSH S-transferases and a qualitative increase in cytochrome P-450 monooxygenases. However, due to higher protein levels in winter psylla than in summer psylla, sequestration may also contribute to resistance.

Slower penetration of [^{14}C] azinphosmethyl through the cuticle of R psylla than S psylla, suggested that reduced penetration of insecticides may contribute to resistance. Reduced penetration was more important in winter than in summer-form psylla, apparently due to differences in cuticle composition between these two distinct morphological forms. Reduced penetration can act as an 'opportunity' factor (Oppenoorth 1985), increasing the effect of detoxification enzymes.

GSH S-transferase and cytochrome P-450 monooxygenase activities were about 1.5-fold higher in susceptible D. brevis than in S summer psylla and similar to the enzyme activity of R summer psylla. Esterase activity was 4.8-fold higher in S summer psylla than in susceptible D. brevis, and R summer psylla

showed a 17.8-fold higher esterase activity than the susceptible predator. Low esterase activity may contribute to the high susceptibility of D. brevis to insecticides observed in laboratory bioassays (Table 2.8; Westigard 1973) and field trials (Westigard 1973, 1979).

Comparative toxicological studies on susceptible strains of the predatory mite Amblyseius fallacis Garman demonstrated lower levels of aldrin epoxidase and trans-epoxide hydrolase activities than the spider mite Tetranychus urticae Koch, but higher levels of GSH S-transferase and cis-epoxide hydrolase activities (Mullin et al. 1982). Esterase activity was similar for predator and prey. Larvae of the common lacewing, Chrysoperla carnea Steph., showed high esterase activity compared with cotton pests, which accounted for the predator's high levels of resistance (Ishaaya and Casida 1981). The hemipteran predator, Podisus maculiventris (Say), which is more closely related to D. brevis, had lower cytochrome P-450 monooxygenase, GSH S-transferase, and hydrolase activities than four species of lepidopterous prey (Yu 1987). However, P. maculiventris had higher levels of cytochrome P-450 microsomal desulfurase and GSH S-transferase (CDNB) activities. In addition, the predator contained relatively more high-spin form of cytochrome P-450. P. maculiventris was

generally more susceptible to organophosphorous and carbamate insecticides but more tolerant to pyrethroids compared with its prey (Yu 1988). These studies indicate that differences in activity of detoxification enzymes between predators and their prey apparently contribute to differences in toxicity of pesticides and therefore to differences in selectivity of these chemicals.

Detoxification capacity was similar in P. pyricola and D. brevis based on GSH S-transferase and cytochrome P-450 monooxygenase activities. Based on the preadaptation hypothesis (see section 3.1), no significant differences in detoxification capacity were expected between a monophagous phloem-sucking phytophage such as P. pyricola and a polyphagous entomophage such as D. brevis. Esterase activity, however, was higher in the prey than in the predator. Esterases may be a major biochemical resistance mechanism conferring resistance to insecticides in pear psylla.

Although their detoxification capacity was quite similar, P. pyricola is resistant to a variety of insecticides, whereas D. brevis is susceptible (see Chapter 2; Westigard 1973, 1979; Westigard et al. 1979). The potential to develop resistance seems to be determined by biochemical factors (e.g. detoxification enzymes), which enable an insect to detoxify pesticides.

However, non-biochemical factors are probably more important in determining the rate of resistance development (Tabashnik and Croft 1985). The importance of such factors (e.g. life history and ecological factors) influencing insecticide resistance in pear psylla and D. brevis will be discussed in Chapter 4.

Table 3.1. Esterase activities for adult summer and winter forms of susceptible (S) and resistant (R) pear psylla, P. pyricola, and for 3rd-5th nymphal instars of susceptible D. brevis.

species	esterase activity[1]	
	nmole/min/ µg protein	nmole/min/ insect
S psylla summer June	1.84 ± 0.10	17.06 ± 0.93
R psylla summer June	6.93 ± 0.45	57.40 ± 3.73
R psylla winter October	3.07 ± 0.14	109.78 ± 5.01
R psylla winter January	1.25 ± 0.04	39.90 ± 1.28
R psylla winter March	0.50 ± 0.02	23.75 ± 0.71
S psylla winter March	0.26 ± 0.04	12.64 ± 2.06
D. brevis August	0.39 ± 0.02	7.80 ± 0.40

[1] Enzyme activities significantly different at $P<0.05$ by Student's t test. Mean ± SE for 3 enzyme samples with 15 determinations per sample.

Table 3.2. Glutathione S-transferase activities for adult summer and winter forms of susceptible (S) and resistant (R) pear psylla, P. pyricola, and for 3rd-5th nymphal instars of susceptible D. brevis.

species	GSH S-transferase activity[1]	
	nmole/min/ µg protein	nmole/min/ insect
S psylla summer June	0.34 ± 0.07a	1.42 ± 0.29f
R psylla summer June	0.60 ± 0.04b	3.78 ± 0.25g
R psylla winter October	0.27 ± 0.02a	6.93 ± 0.51h
R psylla winter January	0.17 ± 0.01c	4.73 ± 0.28i
R psylla winter March	0.07 ± 0.01d	2.55 ± 0.19j
S psylla winter March	0.13 ± 0.01e	2.95 ± 0.12j
D. brevis August	0.51 ± 0.03b	10.20 ± 0.60k

1 Enzyme activities with the same letter are not significantly different at $P<0.05$ by Student's t test. Mean ± SE for 3 enzyme samples with 5 determinations per sample.

Table 3.3. Cytochrome P-450 monooxygenase activities for adult summer and winter forms of susceptible (S) and resistant (R) pear psylla, P. pyricola, and for 3rd-5th nymphal instars of susceptible D. brevis.

species	P-450 monooxygenase activity[1] pmole/min/mg protein
S psylla summer June	1.32 ± 0.05a
R psylla summer June	2.09 ± 0.18b
R psylla winter October	5.39 ± 0.16c
R psylla winter January	3.60 ± 0.13d
R psylla winter March	2.88 ± 0.11e
D. brevis August	2.07 ± 0.11b

[1] Enzyme activities with the same letter are not significantly different at $P<0.05$ by Student's t test. Mean ± SE for 2 enzyme samples with 5 determinations per sample.

Table 3.4. Relative activities of detoxification enzymes in adult summer and winter forms of susceptible (S) and resistant (R) pear psylla, P. pyricola, and 3rd-5th nymphal instars of susceptible D. brevis.

species	relative enzyme activity[1]		
	esterase	GSH S-transferase	P-450 mono-oxygenase
S psylla summer June	1	1	1
R psylla summer June	3.76	1.77	1.58
R psylla winter October	1.76	0.79	4.08
R psylla winter January	0.70	0.50	2.73
R psylla winter March	0.27	0.21	2.18
S psylla winter March	0.14	0.38	-
D. brevis August	0.21	1.50	1.57

[1] Enzyme activities are relative to enzyme activities of S summer psylla per amount protein. Ratios calculated from data presented in Tables 3.1, 3.2, and 3.3.

Figure 3.1. Characterization of a 65,000 rpm ($\omega^2 t = 3.7 \times 10^{10}$ $rad^2 sec^{-1}$) sucrose gradient for R winter (January) pear psylla, P. pyricola. Gradient was formed with 45, 35, 25, and 15% sucrose/MOPS solutions and loaded with 350 ul 1,000 x g supernatant of pear psylla abdomen tissue homogenate. Each point is the mean of duplicate samples.

Figure 3.1.

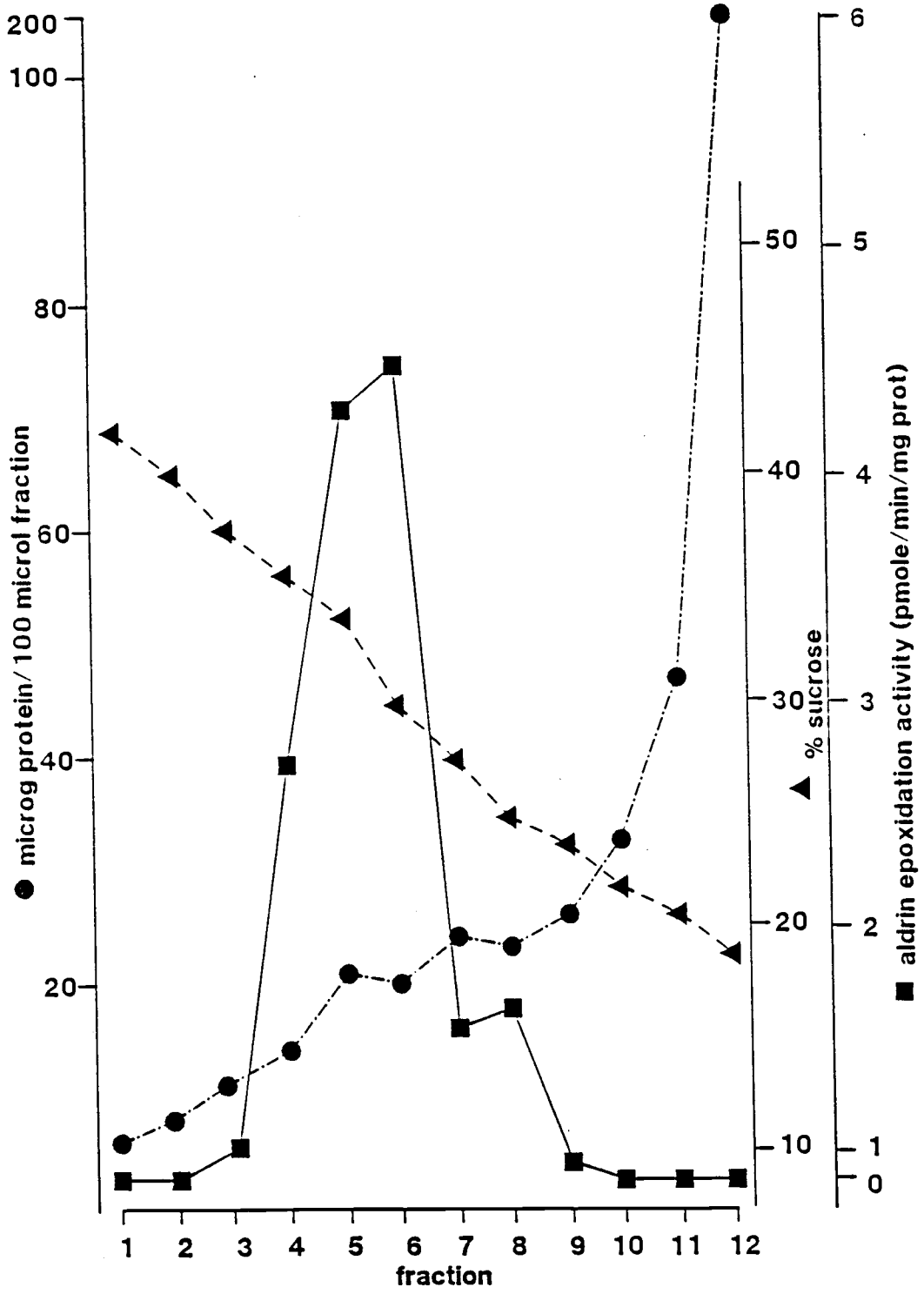

200
100
80
60
40
20
● microg protein/100 microl fraction
50
40
30
20
10
▲ % sucrose
6
5
4
3
2
1
0
■ aldrin epoxidation activity (pmole/min/mg prot)
1 2 3 4 5 6 7 8 9 10 11 12
fraction

Figure 3.2. Characterization of a 65,000 rpm ($\omega^2 t = 3.7 \times 10^{10}$ $rad^2 sec^{-1}$) sucrose gradient for D. brevis. Gradient was formed with 45, 35, 25, and 15% sucrose/MOPS solutions and loaded with 350 ul 1,000 x g supernatant of D. brevis abdomen tissue homogenate. Each point is the mean of duplicate samples.

Figure 3.2.

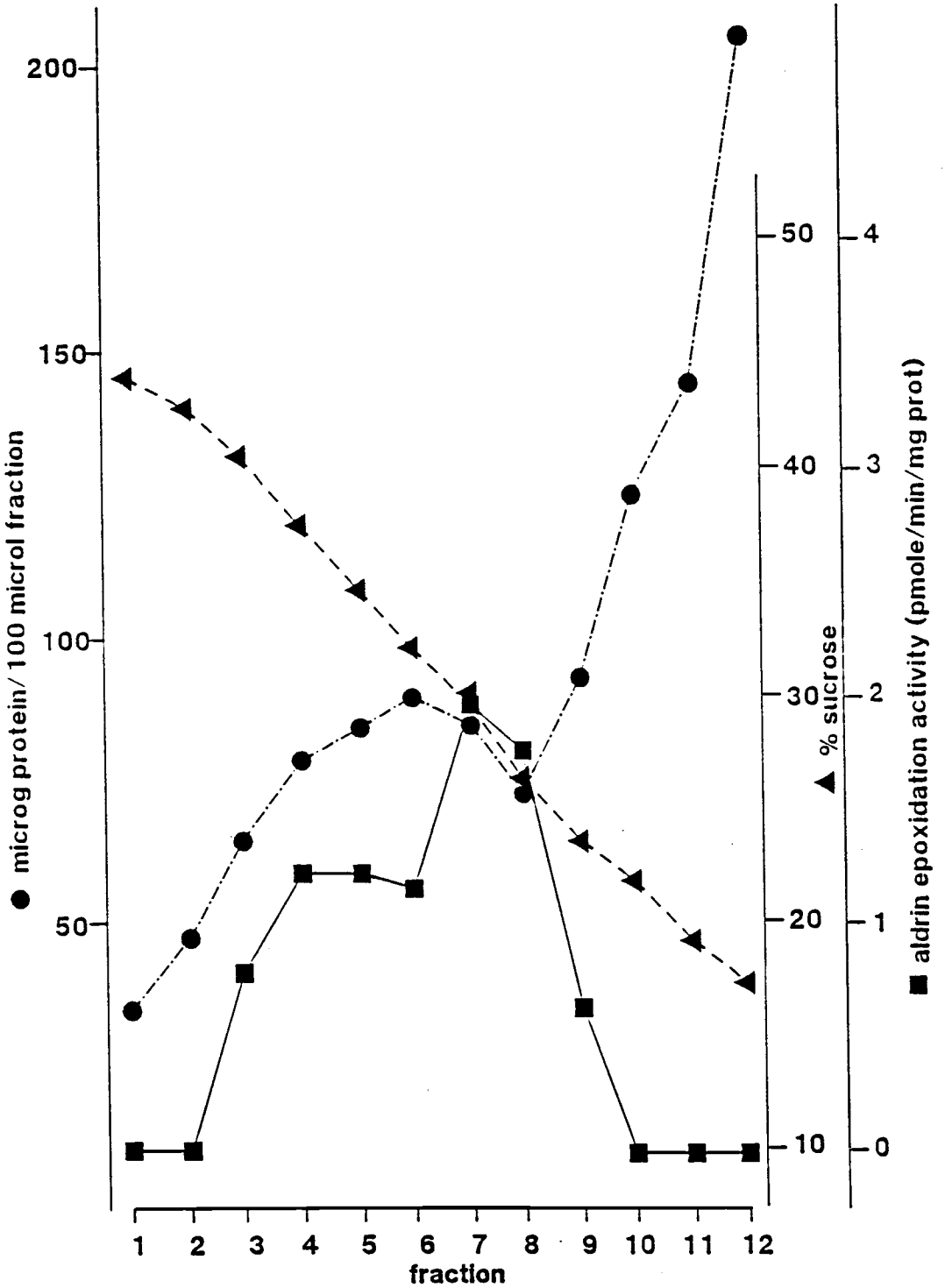

200
150
100
50
● microg protein/ 100 microl fraction
50
40
30
20
10
▲ % sucrose
4
3
2
1
0
■ aldrin epoxidation activity (pmole/min/mg prot)
1 2 3 4 5 6 7 8 9 10 11 12
fraction

Figure 3.3. Radioactivity recovered from the cuticle by a surface wash with acetone at various times after treatment for susceptible (S) summer and resistant (R) summer and winter pear psylla, P. pyricola. Each point represents the mean ± SE of 3 replicates of 10 insects.

Figure 3.3

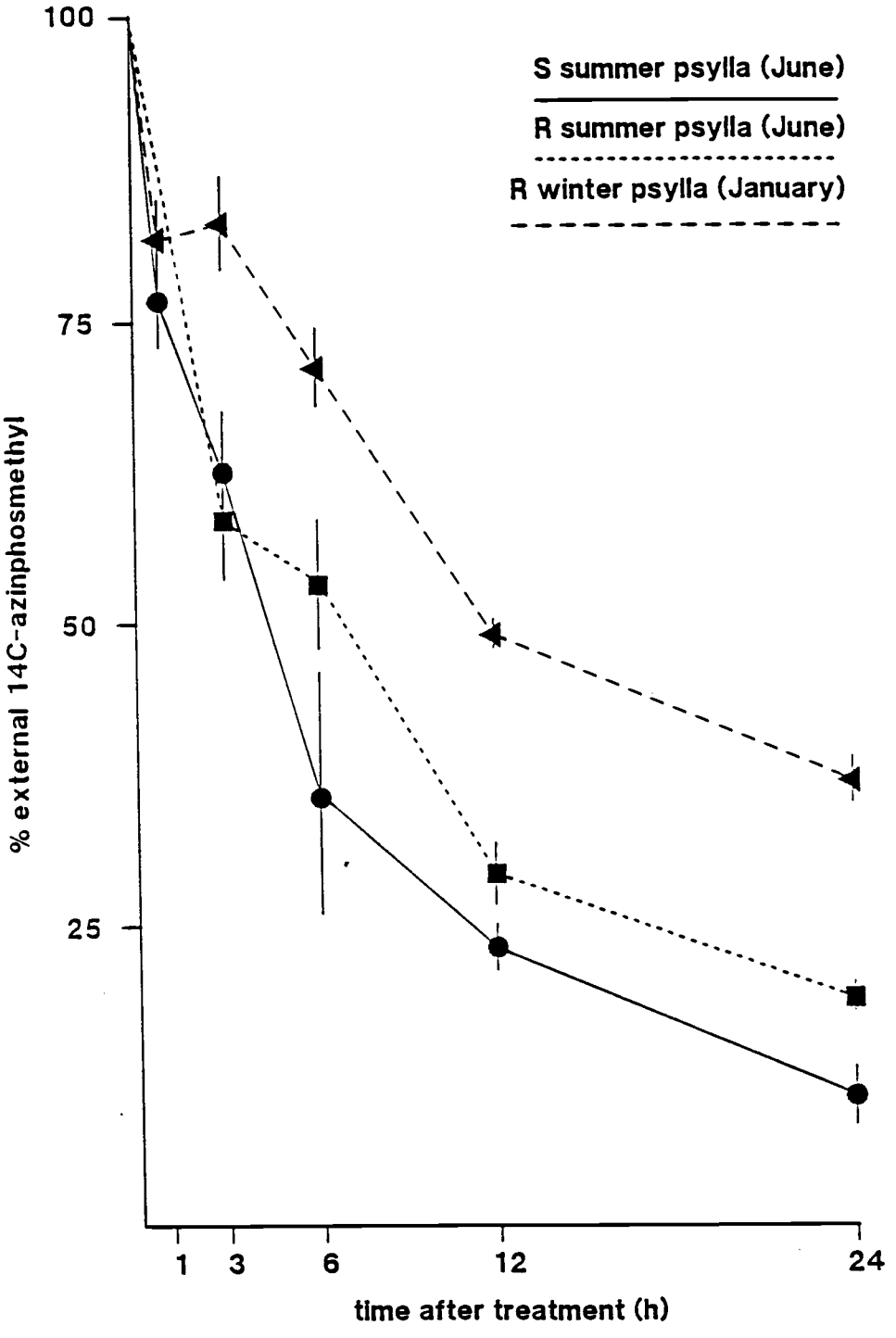
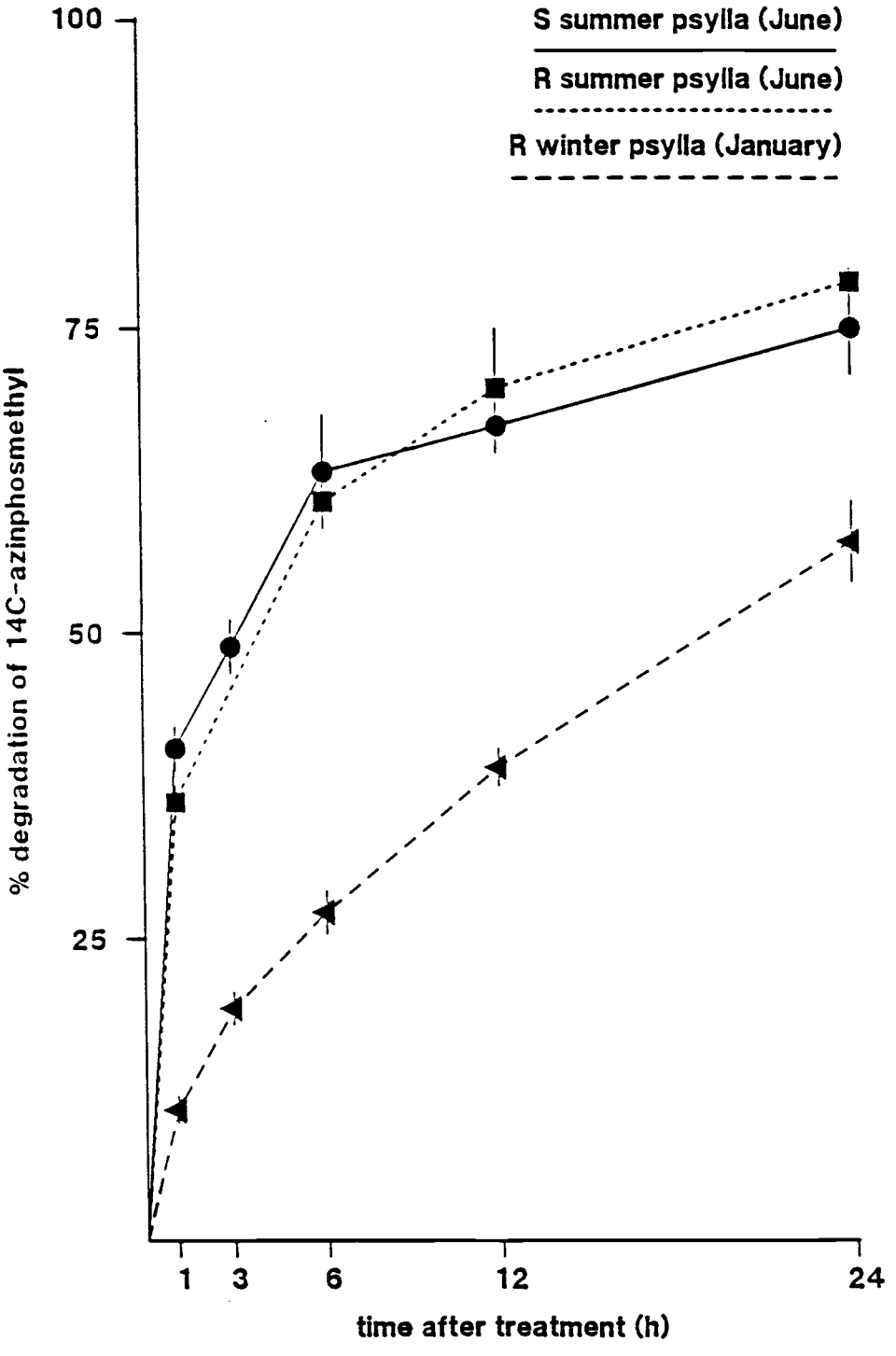

100
75
50
25
% external 14C-azinphosmethyl
S summer psylla (June)
R summer psylla (June)
R winter psylla (January)
1
3
6
12
24
time after treatment (h)

Figure 3.4. *In vivo* degradation of azinphosmethyl by susceptible (S) summer and resistant (R) summer and winter pear psylla, *P. pyricola*, at various times after treatment. Each point represents the mean ± SE of 3 replicates of 10 insects.

Figure 3.4.


S summer psylla (June)
R summer psylla (June)
R winter psylla (January)
100
75
50
25
% degradation of 14C-azinphosmethyl
1
3
6
12
24
time after treatment (h)

4. ECOLOGICAL AND LIFE HISTORY FACTORS INFLUENCING THE EVOLUTION OF INSECTICIDE RESISTANCE IN PEAR PSYLLA AND *DERAEOCORIS BREVIS*

4.1 Introduction

The evolution of pesticide resistance in arthropods is primarily determined by pesticide selection pressure. Genetic, life history, and ecological attributes of an organism as well as operational factors associated with pesticide use influence the rate at which resistance evolves (Georghiou 1972, 1983; Georghiou and Taylor 1976, 1977a, 1986). Simulation studies have shown that life history and ecological factors such as number of generations per year, fecundity, and immigration of susceptible individuals into a selected population are of major importance for determining the rate at which resistance develops (Taylor and Georghiou 1979; Tabashnik and Croft 1982; Tabashnik 1986b). However, validation of these models have been limited (Tabashnik and Croft 1985).

A better understanding of these ecological and life history factors and their influence on resistance may explain some of the differences in resistance

development between pest arthropods and their natural enemies. This knowledge will also facilitate manipulation and exploitation of certain operational factors for managing pesticide resistance and utilizing pesticide selectivity (Leeper et al. 1986).

The evolution of resistance to azinphosmethyl in P. pyricola and the lack thereof in D. brevis was evaluated using a computer simulation model developed by Tabashnik and Croft (1982, 1985). Azinphosmethyl has been used for more than 25 years in commercial pear orchards in the Rogue River Valley for the control of codling moth, C. pomonella (Westigard et al. 1979; Follett et al. 1985). In the Rogue River Valley, pear psylla developed resistance to azinphosmethyl within 4 years, whereas D. brevis has remained susceptible to this compound (Westigard and Zwick 1972; Westigard 1973, 1979; Westigard et al. 1979; Follett et al. 1985). Ecological and life history attributes of prey and predator were evaluated by modeling and sensitivity analysis, in order to determine their relative importance on the evolution of resistance or the lack thereof in these two species.

4.2 Life history and ecology of pear psylla and D. brevis

Features of the ecology and life history of pear psylla and D. brevis are summarized in Table 4.1 (Burts and Fischer 1967; Westigard and Zwick 1972; Westigard 1973; Brunner 1975; Westigard et al. 1979). Differences in these ecological and life history attributes between pear psylla and D. brevis may account for differences in the rate of resistance development between prey and predator. For example, the reproductive rate is higher in pear psylla than in D. brevis due to higher fecundity (psylla 200 eggs/female, D. brevis 25), shorter developmental time (psylla 26 days, D. brevis 40), and more generations per year (psylla 4 generations/year, D. brevis 2).

Also the migration characteristics and occurrence in refugia attributes, are different for predator and prey (Table 4.1). Adult winter pear psylla migrate out of orchards by the end of October (Fye 1983). Mixing of resistant and susceptible populations may occur during this period in their overwintering sites. However, little mixing of psylla populations occurs once psylla migrate back into orchards by the end of January (Westigard and Zwick 1972). Summer pear psylla feed

strictly on pear (Williams et al. 1963; Kaloostian 1970; Westigard et al. 1979) and therefore, no individuals will migrate into orchards from surrounding unsprayed vegetation. Thus the overall immigration/residency ratio (i.e. number of susceptible immigrants moving into the treated habitat per generation relative to the number of residents present in the treated population) will be extremely low for pear psylla during its life cycle. Because dilution of resistant individuals in orchards by susceptible individuals from surrounding vegetation is almost negligible, resistance development is favored in psylla populations residing in the orchards which are exposed to high selection pressure. On the other hand D. brevis, as a generalist predator, feeds on different prey species in orchards as well as in surrounding vegetation (Liss et al. 1986). Therefore, only part of the predator population will be exposed to insecticides while residing in orchards. Dilution of resistant individuals often occurs by immigration into orchards by susceptible individuals from the surrounding habitats.

Another ecological factor that may contribute to differences in rate of resistance development between P. pyricola and D. brevis is the food limitation factor due to differences in availability of food sources (Tabashnik and Croft 1985; Tabashnik 1986a). After

insecticide applications psylla normally will find an unlimited food source, its host the pear, whereas, D. brevis will encounter reduced populations of prey, pear psylla, which may cause the predator to migrate out of orchards in order to feed on prey in surrounding vegetation. However, D. brevis is a generalist predator and feeds on a variety of prey species and therefore, food limitation will probably be less of a factor, than for a more specialized predator which may only feed on one or a few prey species.

4.3 Methods

4.3.1 The simulation model

As noted, the simulation model used in this study was that of Tabashnik and Croft (1982, 1985). The insect's life cycle for both P. pyricola and D. brevis was divided into 20 substages, with transition probabilities between substages determined by natural and pesticide mortality. Winter survival rates per substage determined the transition of the overwintering generations to the next year. For each substage, the fraction not exposed to the pesticide and therefore not subjected to pesticide mortality, was defined (i.e. the

fraction in stage long refuge). Temporary refuge simulated the escape from pesticides by adults temporarily leaving the treated habitat.

It was assumed that resistance was determined by a single semi-dominant gene, which is often true in the case of resistance developed under field conditions (Oppenoorth 1985; Roush and Croft 1986; Roush and Mckenzie 1987). LC_{50} values of azinphosmethyl for susceptible (SS) and resistant (RR) pear psylla were based on probit analysis of toxicity tests (Table 2.1). These data were expressed in field rates for a better comparison with actual field application of azinphosmethyl and were 0.27 and 2.08 kg/ha for SS and RR genotypes, respectively. It was assumed that the LC_{50} value for RS psylla was 1.68 kg/ha. The LC_{50} value of azinphosmethyl for SS D. brevis was based on probit analysis of toxicity tests (Table 2.8) and was 0.21 kg/ha. It was assumed that LC_{50} values of azinphosmethyl for RR and RS individuals were similar to those for pear psylla. The initial frequency of the resistance gene was set at 0.0001. It was assumed that the insect had developed resistance if the resistance (R) gene frequency exceeded 0.50.

Selection pressure of azinphosmethyl was simulated based on the historical use pattern of azinphosmethyl in the Rogue River Valley with 3 foliar sprays being

applied each year at a recommended field rate of 2.24 kg/ha. Based on field data, pesticide concentration decayed exponentially, with a half-life of seven days (Tabashnik and Croft 1985). Pesticide mortality was determined by the current pesticide concentration, the fraction of the population in refuges and the distribution of individuals among genotypes with their dose-mortality lines.

Reproductive disadvantage of RR and RS females relative to SS females was assumed to be 0.75 and 0.90 %, respectively. RR and RS males had no reproductive disadvantage.

4.3.2 Parameter values and sensitivity analysis

Physiological, biochemical and genetic parameters were held constant for both species while the following ecological and life history parameters were estimated (Table 4.2): generations/year, immigration rate of susceptible individuals, initial overwintering size, fecundity, natural (non-pesticide) mortality, development rate, sex ratio, pesticide exposure in orchards, and percent of time spent in orchards by adults. Parameter values for both species were mostly based on data presented by Burts and Fischer 1967;

Westigard and Zwick 1972; Westigard 1973; Brunner 1975; Westigard et al. 1979, and Tabashnik and Croft 1985.

Results of simulations were compared with historical data of resistance development to azinphosmethyl in pear psylla and the lack thereof in D. brevis (see Chapter 2; Westigard and Zwick 1972; Westigard 1973, 1979; Westigard et al. 1979; Follett et al. 1985; van de Baan et al. in press). The model was used to evaluate the importance of certain parameters for resistance development, in the case of agreement between observed and predicted time of resistance development. Sensitivity analyses were run in which ecological and life history factors were changed in order to determine the importance of these factors on the evolution of resistance.

4.4 Results and dicussion

Table 4.3 shows the observed and predicted time of resistance development to azinphosmethyl in pear psylla and D. brevis. Predicted time for pear psylla was 2 years and the observed time of field failure to occur in the Rogue River Valley was 3 to 4 years (Westigard and Zwick 1972; Follett et al. 1985). Simulations showed that after 20 years no resistance to azinphosmethyl

developed in D. brevis. These results agreed with field observations of lack of resistance development in the predator (Westigard 1973, 1979; Westigard et al. 1979). Agreement between observed and predicted time of resistance development in both predator and prey showed that the input parameters of the model were in the right order of magnitude in the case of uncertain parameter estimates. The model could therefore be further tested to evaluate the importance of ecological and life history factors for resistance development.

Under the initial set of conditions the R gene frequency after 2 years of selection with azinphosmethyl was 0.79 for pear psylla and 0.00074 for D. brevis (Table 4.4). Under the assumption that resistance had developed when the R gene frequency exceeded 0.50, pear psylla was resistant whereas D. brevis remained susceptible (see also Table 4.3). Main differences in ecology and life history between pear psylla and D. brevis are a higher fecundity and a lower immigration/residency ratio in pear psylla as compared with D. brevis (Table 4.2). Changing the fecundity either to a value similar to that of pear psylla in D. brevis or a value similar to that of D. brevis in pear psylla, resulted in a dramatic change of the R gene frequency in both species (Table 4.4). In these situations the R gene frequency was 0.013 for pear

psylla and 0.33 for D. brevis, indicating that the prey was still susceptible after 2 years of selection with azinphosmethyl, whereas the predator had almost developed resistance at the 0.50 frequency level.

Similar results were obtained when the rate of immigration of susceptible individuals was changed (Table 4.4). In the case of the predator having an immigration rate proportional to that of pear psylla, D. brevis developed resistance to azinphosmethyl after 2 years (R gene frequency = 0.69). The prey, however, was still susceptible after 2 years, if its immigration rate was made proportional to that of the predator (R gene frequency = 0.024).

Other ecological and life history parameters than fecundity and immigration of susceptible individuals are quite similar in P. pyricola and D. brevis (Table 4.2). Sensitivity analyses showed that these other factors do not affect the development of resistance as do fecundity and immigration. Apparently fecundity and immigration of susceptible individuals are both more important than other ecological and life history factors in determining the rate at which resistance develops in P. pyricola and D. brevis.

4.5 Conclusions

Computer simulations showed that fecundity and immigration of susceptible individuals into a selected population, are important factors that determine the rate of development of resistance to azinphosmethyl in P. pyricola and D. brevis. Two important differences in life history and ecology between pear psylla and D. brevis that contribute to different propensities for resistance development are reproductive rate and migration/residency ratio. A high reproductive rate apparently contributes to rapid resistance development in pear psylla. Lack of dilution of resistance by immigration of susceptible individuals into orchards during a period of selection pressure may be an even more important factor influencing resistance evolution in P. pyricola. The opposite is true for D. brevis. A low reproductive rate and a high migration/residency ratio cause D. brevis to remain susceptible to azinphosmethyl.

The importance of food limitation for determining the rate at which resistance develops could not be evaluated, because the model used was a single species model and did not couple predator and prey dynamics. Therefore, food supply was not incorporated in the model. However, food limitation may be a factor slowing

down resistance development in D. brevis. In a coupled predator-prey model Tabashnik (1986a) showed that in certain cases food limitation indeed can be an important factor that slows down resistance development in natural enemies. However, D. brevis as a generalist predator feeds on a variety of prey (Liss et al. 1986). Therefore, reduction in pear psylla density by spraying with insecticides will probably not have a large impact on the availability of food because other prey species may still be available.

Comparative toxicological studies between P. pyricola and D. brevis showed that the detoxification capacity of prey and predator are quite similar. Therefore, their potential to develop resistance to insecticides based on this factor should be similar also (see Chapter 3). However, pear psylla has developed resistance to many insecticides in the Rogue River Valley, whereas D. brevis remained susceptible (see Chapter 2). Biochemical factors may be important in determining a species' potential to develop resistance to a particular pesticide (Tabashnik and Croft 1985). However, they are less important in determining the rate at which resistance develops. Apparently ecological and life history factors are more important in determining the rate at which resistance to pesticides develops in arthropods, as supported by these simulation studies.

Table 4.1. Ecological and life history differences between pear psylla, P. pyricola, and D. brevis[1].

parameter	P. pyricola	D. brevis
# generation/year	4	2
fecundity (progeny/female)	200	25
developmental time (days)	26	40
migration/residency	low	high
food limitation	no	yes

[1] Data from Burts and Fischer 1967; Westigard and Zwick 1972; Westigard 1973; Brunner 1975; Westigard et al. 1979.

Table 4.2. Ecology and life history parameter values for pear psylla, P. pyricola, and D. brevis, used in the simulation model of Tabashnik and Croft (1985)[1].

parameter	P. pyricola	D. brevis
generations/year	4	2
SS immigration/tree/year	1	20
initial overwintering pop. size	500	5
fecundity (progeny/female)	200	25
survivorship	0.30	0.60
overwintering survivorship	0.55	0.75
development time (days)	26	40
sex ratio (females/males)	1.0	2.0
fraction of time in orchard (adults)	1.0	0.5
fraction in stage long refuge (in orchard):		
eggs	0.03	0.98
larvae	0.03	0.05
pupae	0.03	0.25
adults	0.05	0.05

[1] Data from Burts and Fischer 1967; Westigard and Zwick 1972; Westigard 1973; Brunner 1975; Westigard et al. 1979; Tabashnik and Croft 1985.

Table 4.3. Time (years) of resistance development to azinphosmethyl in pear psylla, P. pyricola, and D. brevis, based on simulation studies using the simulation model of Tabashnik and Croft (1985).

	rate of R development (years)	
	P. pyricola	D. brevis
observed[1]	3-4	none
predicted	2	> 20

[1] Data from Westigard and Zwick 1972; Westigard 1973, 1979; Westigard et al. 1979; Follett et al. 1985; van de Baan et al. in press.

Table 4.4. Predicted R gene frequencies after 2 years of selection with azinphosmethyl (3 applications/year) for pear psylla, P. pyricola, and D. brevis, using the simulation model of Tabashnik and Croft (1985). Initial R gene frequency = 0.0001.

parameter	R gene frequency	
	P. pyricola	D. brevis
initial conditions	0.79	0.00074
fecundity:		
10 fold increase		0.33
10 fold decrease	0.013	
immigration:		
proportional to P. pyricola		0.69
proportional to D. brevis	0.024	

5. FENVALERATE RESISTANCE AND RESISTANCE MANAGEMENT IN PEAR PSYLLA

5.1 Introduction

For the past 10 years, pear psylla has been controlled in the pear growing areas of Washington and Oregon by dormant sprays of pyrethroids. These sprays are directed at post-diapause winter-form adults when they begin to lay eggs. Full cover sprays of amitraz are used to control nymphs of summer-form pear psylla (Riedl et al. 1981; Westigard et al. 1979; Westigard et al. 1986). In the spring of 1987 preliminary field evidence of control failures with fenvalerate and permethrin near Wenatchee, Washington, was reported (Burts personal communication). Investigations showed that surviving populations of winter-form adults exceeded the retreatment threshold of Burts and Brunner (1981), even after two applications of these materials (Burts et al. in press).

Because of the importance of pyrethroids for control of pear psylla, fenvalerate resistance was studied in more detail in psylla populations from the main pear growing areas in the Pacific Northwestern

U.S.A.. Laboratory bioassays were used to determine the level of fenvalerate resistance in pear psylla from the Wenatchee area. Psylla populations from Yakima, Washington, and the Hood River, Willamette and Rogue River Valleys, Oregon, where fenvalerate resistance has not been reported yet, were also monitored to detect fenvalerate resistance development at an early stage. The genetics of fenvalerate resistance was studied through classical crossing experiments to determine the nature and stability of this resistance factor in the field.

For effective pesticide resistance management, tools are needed to counteract resistance and resistance development. Previous laboratory studies have shown that the synergist piperonyl butoxide (PbO) was effective in enhancing the activity of some insecticides in the case of resistance in pear psylla (see Chapter 2). Therefore, the effects of PbO as a synergist of fenvalerate were studied in greater detail.

5.2 Materials and methods

Adult summer-form pear psylla used in laboratory bioassays were collected as described in Chapter 2 in the spring of 1987 from commercial orchards near

Wenatchee and Yakima, Washington, and near Hood River, Corvallis and Medford, Oregon.

For genetic studies, resistant psylla from Wenatchee (R) were collected to start a laboratory colony. Individuals from this laboratory colony were crossed with psylla from a susceptible laboratory colony (S) that was originated from the O.S.U. Entomology Farm, Corvallis (see Chapter 2). The F_1 offspring of the R x S crosses were backcrossed with the S strain. Psylla of the S, R, and F_1 strain used for crosses, were held as immature nymphs on isolated caged trees before adults emerged and mating occurred. Within 12 h after emergence, adults of the S, R or F_1 strain were placed on caged trees for appropriate crosses. No mating occurred among individuals of the same strain, because psylla only start mating 24 h after emergence (Burts personal communication). All strains of psylla were reared under long day photoperiod (16:8 [L:D]) and at 21 $\pm$ 5 ^{o}C.

Both slide-dip and topical application methods were used for toxicity tests (Chapter 2). For topical application tests, technical fenvalerate (cyano (3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzene-acetate) and piperonyl butoxide (PbO, (5-2-(2 butoxyethoxy)ethoxymethyl)-6-propyl-1,3 benzodioxole) with purity of 93.5 and 97.6 percent respectively, were

diluted with analytical grade acetone. Toxicity tests with fenvalerate consisted of five doses with three replicates of 25 individuals or a high (185 ng/mg insect) and low (37 ng/mg insect) diagnostic dose with four replicates of 25 individuals. Controls were treated with acetone only. Synergist tests consisted of applying a high (185 ng/mg insect) or low (37 ng/mg insect) dose of fenvalerate mixed with a non-lethal dose of PbO (97.6 ng/mg insect) to four replicates of 25 psylla. Controls were treated with PbO only.

Compounds used in slide-dip tests were formulated as emulsifiable concentrates. Using slide-dip tests, the effect of the rate of PbO (Butacide 8EC) on synergism of fenvalerate (Pydrin 2.4EC) was studied for four serial rates of PbO combined with each of two rates of fenvalerate (90 and 180 mg a.i./l) representing low and high field rates. Two replicates of 25 psylla were treated with each combination. Controls were treated with fenvalerate only.

Psylla were held post-treatment for 48 h before mortality was determined (see Chapter 2). When mortality occurred in controls, data were adjusted accordingly using Abbott's formula (Abbott 1925). Mortality data from dilution series tests were subjected to probit analysis. For data based on a single diagnostic dose, LD_{50} values were estimated based on a

probit analysis slope of 2.56, which is an average value for dose-probit mortality lines for moderate resistant strains, using topical application (see Chapter 2).

LC_{50} and LD_{50} values for psylla populations from various pear production areas were compared with results of previous bioassays to show changes in susceptibility, since fenvalerate resistance monitoring was initially conducted in 1983 (Chapter 2; Follett et al. 1985; Burts et al. in press; Burts unpublished data; Westigard unpublished data).

5.3 Results and discussion

5.3.1 Monitoring for fenvalerate resistance

All populations of pear psylla collected from commercial pear growing areas were less susceptible to fenvalerate than those from the O.S.U. Entomology Farm, Corvallis (Table 5.1). Psylla populations from the Willamette Valley showed low levels of fenvalerate resistance (ca. 10-fold), psylla from Medford, Hood River and Yakima showed intermediate levels of resistance (ca. 26-fold) and psylla from Wenatchee showed high levels of resistance (ca. 242-fold). Comparison with results of previous bioassays showed

that no increase in fenvalerate resistance had occurred over the past five years in psylla populations from Hood River, Willamette Valley and Medford (Table 5.2). Psylla populations from the Wenatchee area, however, seem to be in a phase of rapidly increasing resistance (Table 5.2).

Levels of fenvalerate resistance in the different psylla populations tested, appear to be unrelated to use patterns of pyrethroids for control of this pest. Fenvalerate use in the Wenatchee area has not been substantially different from that in other pear growing areas in the Pacific Northwestern U.S.A. (Burts et al. in press; Croft et al. in press). However, insecticides used in the past have been different for these areas and this may have resulted in the development of fenvalerate resistance to a greater extent in pear psylla from Wenatchee, than in pear psylla from other pear growing areas (Croft et al. in press). Another contributing factor may be the overall size and intensity of production in the Wenatchee area, which influences the size and composition of species pools of resistant and susceptible pear psylla (Croft et al. in press).

5.3.2 Genetics of fenvalerate resistance

Dose-response relationships for fenvalerate for resistant psylla (R) from Wenatchee, susceptible psylla (S) from the O.S.U. Entomology Farm, Corvallis, and their F_1 offspring (R x S), and backcross (F_1 x S) are presented in Figure 5.1. Resistance to fenvalerate was 242-fold higher in R psylla than in S psylla. The F_1 offspring and the backcross showed a ca. 8-fold level of fenvalerate resistance compared with the susceptible parent population (Table 5.3).

A low level of fenvalerate resistance (8-fold) in the F_1 offspring indicated that this resistance is of a semi-recessive or intermediate nature. It was concluded that lack of segregation of susceptible and resistant dose response at the 50% mortality level in the backcross is an indication of the polygenic nature of fenvalerate resistance.

Pesticide resistance evolved under field selection is often based on a semi-dominant monogenic trait (Oppenoorth 1985; Roush and Croft 1986; Roush and McKenzie 1987; Croft and van de Baan 1988). Polygenic resistance from field selection with pesticides is usually less common than monogenic resistance (Via 1986; Roush and McKenzie 1987). However, situations that favor the development of polygenic resistance in the

field are those in which resistance alleles have been selected by previously used insecticides in intensely sprayed or relatively isolated populations or in situations in which the intensity of selection is lowered by reduction of exposure to pesticides (Via 1986; Roush and McKenzie 1987). Selection by previously used insecticides under intense spraying conditions in the Wenatchee area may have contributed to the polygenic nature of fenvalerate resistance in psylla from that area.

5.3.3 Synergism of fenvalerate by piperonyl butoxide (PbO)

Synergist tests using topical application bioassay for psylla populations with moderate levels of fenvalerate resistance, showed that mixtures of fenvalerate and PbO increased mortality over fenvalerate alone (Fig. 5.2). Synergistic effect of PbO, however, was higher in Wenatchee psylla with high levels of fenvalerate resistance than in psylla populations with moderate levels of fenvalerate resistance. Synergism of fenvalerate by PbO indicated that cytochrome P-450 monooxygenases were involved in detoxification of this compound. Detoxification was probably more important in

the case of high levels of resistance and therefore, a high synergistic effect was observed in pear psylla from Wenatchee.

Synergist tests using slide-dip bioassay in which the rate of PbO was varied with a constant rate of fenvalerate (90 or 180 mg a.i./l), showed an increase in synergism for concentrations of PbO up to 225 µl a.i./l (Fig. 5.3). A two-fold increase of this rate did not significantly increase mortality. These data indicated that there is a minimum rate of PbO (225 µl a.i./l) that provides an optimum synergistic effect. Synergist data also showed that a minimum rate of PbO necessary for optimum effect of fenvalerate-PbO mixtures may be independent of fenvalerate rate (Fig. 5.3) but proportional to the level of fenvalerate resistance in the population (Fig. 5.2).

Similar synergistic effects of PbO for fenvalerate were obtained for P. pyricola from the Wenatchee area by Burts et al. (in press) using slide-dip bioassays. They also studied the effect of PbO for the pyrethroids permethrin, fenpropanate, cyfluthrin, and flucythrinate. Using field rates of fenvalerate, flucythrinate, and permethrin mixed with PbO showed more than 90% mortality, whereas these compounds alone produced 36, 40 and 15% mortality, respectively. No synergistic effect of PbO was shown for fenpropanate and cyfluthrin. At

dosages tested, these pyrethroids alone provided high mortality of 95%. Lack of strong synergism by PbO and their high level of toxicity alone to pear psylla from the Wenatchee area indicated an absence of cross-resistance to fenpropanate and cyfluthrin.

5.4 Conclusions

Field failure of fenvalerate during dormant control of pear psylla in the Wenatchee area in 1987 (Burts et al. in press) suggested that high levels of resistance had developed to this compound. This conclusion was further supported by results of laboratory bioassays (Table 5.1). Bioassays indicated intermediate levels of resistance to fenvalerate in psylla populations from Yakima, Hood River and Medford. In these areas, fenvalerate is still effective at labeled rates for the control of psylla (Burts unpublished data; Westigard unpublished data). However, pear psylla is able to develop resistance to fenvalerate quickly as observed in Wenatchee. Careful monitoring for fenvalerate resistance in areas with intermediate resistance levels such as at Yakima, Hood River, and Medford is therefore necessary.

Amitraz is the only compound available that is effective as full cover spray for the control of summer-form psylla. Because of the importance of amitraz for summer control of psylla, resistance development to amitraz needs to be assessed. However, bioassays using adult pear psylla are not appropriate tests for evaluating the toxicity of amitraz, because this compound mainly acts as a nymphicide (Westigard et al. 1979; Burts 1983). Therefore, standardized laboratory bioassays for amitraz need to be developed in which the toxicity to pear psylla nymphs is tested.

Genetic tests for fenvalerate resistance in pear psylla from the Wenatchee area indicated the presence of a semi-recessive or intermediate polygenic trait (Table 5.3 and Fig. 5.1). Polygenic resistance may develop slowly in the field due to poor genotypice discrimination (Roush and McKenzie 1987). Polygenic resistance is also less likely to spread than monogenic resistance, because resistant alleles are more likely to be diluted by hybridization with non resistant alleles. However, this does not agree with the rapid resistance development in pear psylla to fenvalerate as has been observed in the Wenatchee area.

Persistence of resistance will be affected by immigration of susceptible individuals which will result in a dilution of resistant genes (see Chapter 4; Comins

1977; Taylor and Georghiou 1979; Tabashnik and Croft 1982, 1985). The immigration/residency factor will have more effect on the dilution or maintenance of less stable polygenic resistance than of monogenic resistance. However, because only a few susceptible psylla occur outside orchards after adult winter-form psylla migrate back into orchards, dilution of fenvalerate resistance will be negligible (see Chapter 4). Therefore, if fenvalerate resistance is polygenic, it appears to be stable in the field. If migration of susceptible individuals would occur, low levels of resistance would be maintained as shown by similar resistance levels of the F_1 and backcross (Fig. 5.1). Only multiple backcrosses with susceptible individuals may eventually reduce resistance to a susceptible baseline level. Semi-recessive or intermediate polygenic resistance to fenvalerate in pear psylla from the Wenatchee area may therefore be expected to be as stable as semi-dominant monogenic resistance.

The effectiveness of PbO synergism (Fig. 5.2 and 5.3) indicated that resistance to fenvalerate in pear psylla is, at least in part, due to increased activity of detoxification by cytochrome P-450 monooxygenases. The importance of this detoxification enzyme system in conferring pesticide resistance was shown in pear psylla from Medford (see Chapter 3). Cytochrome P-450

monooxygenase activity was higher in psylla from Medford with moderate levels of fenvalerate resistance than in susceptible psylla from the O.S.U. Entomology Farm, Corvallis (Table 3.3).

Results of toxicity tests and synergist studies suggest means to manage fenvalerate resistance in pear psylla. PbO was an effective synergist for fenvalerate (Fig. 5.1 and 5.2). In situations where labeled rates of fenvalerate are not effective anymore, mixtures with PbO may render them effective again for dormant season control of post-diapause winter-form adults. PbO was less effective as a synergist when resistance levels to fenvalerate were intermediate (Fig. 5.2). Use of PbO to control moderately resistant psylla is therefore not recommended and may not be economically effective depending on the relative costs of fenvalerate to PbO. However, in the situation of moderate levels of resistance, lower rates of fenvalerate could be effectively used as mixtures with PbO. Such mixtures may be less selective for increased resistance.

Other chemical tactics that may be useful in retarding resistance development to fenvalerate or that may provide alternative control in situations where this compound is not effective anymore, are the following (see Chapter 2; Burts 1985; van de Baan et al. in press; Burts et al. in press):

- Rotation of fenvalerate with pyrethroids such as cyfluthrin and fenpropanate without cross-resistance to fenvalerate for dormant control.
- Use of mixtures of PbO and previously used insecticides such as endosulfan for dormant or full cover control (see Chapter 2).
- Use of mixtures of previously used insecticides and DEF in the fall before psylla migrate out of orchards (DEF can only be used in the fall because it is a defoliant) (see Chapter 2).
- Rotation of fenvalerate with new effective compounds such as avermectin for dormant control (see Chapter 2).

These chemical counter measures, however, will probably only provide short term solutions for resistance problems in psylla because of the ability of this pest to quickly develop resistance to many insecticides (see Chapter 2 and 4). More long term solutions for resistance management are therefore necessary.

Long term solutions for resistance management are possible only if natural enemies of pear psylla are more integrated in pest management programs. Selective or soft control programs based on the use of selective compounds or on a more selective use of compounds, are effective to control pear psylla (Westigard 1974, 1979; Burts 1983, 1985; Westigard et al. 1986). Because these

selective pest management programs allow the integration of natural enemies such as D. brevis, these programs will provide more stable control of pear psylla.

In summary, monitoring for resistance using laboratory bioassays will remain an important aspect of resistance management of pear psylla (Croft et al. in press). Based on the biochemistry of resistance, development of more sensitive tests for identifying detoxification enzymes may provide means for detecting resistance development in an earlier stage of evolution. Resistance risk assessment of new compounds and potential alternative compounds for psylla control, using laboratory bioassays, may be helpful in avoiding resistance development in a short period of time after a new compound is introduced. Continuing research on chemical counter measures for resistance development is necessary for short term solutions of resistance problems in pear psylla. Research on alternative more selective insecticides is necessary to provide effective psylla control in the long term.

Table 5.1. Toxicity of fenvalerate to adult early summer pear psylla, P. pyricola, from several Western fruit growing areas compared with a susceptible (S) strain from Corvallis, using topical application bioassay.

population	LD_{50} (ng/mg insect)	fold-R
Corvallis (S)	1.04[1]	-
Willamette Valley	9.99[2]	9.6
Medford	26.64[2]	25.6
Hood River	27.38[2]	26.3
Yakima	26.64[2]	25.6
Wenatchee	251.45[1]	241.8

[1] LD_{50} values calculated based on probit analysis (see Table 5.3).

[2] LD_{50} values estimated based on a single diagnostic dose and an average probit analysis slope of 2.56. n = 100.

Table 5.2. Susceptibility of adult pear psylla, P. pyricola, from several Western fruit growing areas to fenvalerate for the period 1983-1987.

area	year	bioassay[a]	form[b]	LC_{50}[c]	fold-R
Wenatchee	1984[1]	SD	W	12 (4.3-3.7)	6
	1985[1]	SD	W	39 (29-49)	19
	1985	T	W	47 (45-48)	7
	1986[1]	SD	W	49 (32-65)	23
	1987[1]	SD	W	170 (119-297)	81
	1987[1]	SD	S	240 (180-405)	92
	1987	T	S	680 (639-723)	243
Yakima	1987	T	S	72[d]	26
Hood River	1982-83[2]	SD	S	59 (± 12)	23
	1987	T	S	74[d]	26
Medford	1982-83[2]	SD	S	70 (± 23)	27
	1984[3]	SD	W	11[e]	5
	1984[3]	SD	S	36[e]	14
	1985	T	W	110[d]	15
	1985	T	S	113 (82-155)	40
	1987	T	S	72[d]	26
Willamette Valley[f]	1982-83[2]	SD	S	70 (± 12)	27
	1987	T	S	27[d]	10

Table 5.2. continued.

Corvallis	1986	T	S	4.6[d]	2
	1987	SD	W	2.1 (2.0-2.2)	BL[g]
	1987	SD	S	2.6 (2.3-2.8)	BL[g]
	1987	T	W	7.2[d]	BL[h]
	1987	T	S	2.8 (2.7-2.9)	BL[h]

a T = topical application; SD = slide-dip application.

b W = winter form; S = summer form.

c Expressed as mg a.i./l and 95% CI or ± SE.

d Estimates based on a single diagnostic dose and an average probit analysis slope of 2.56.

e Estimates based on 2-3 data points.

f Excludes data from the O.S.U. Entomology Farm, where populations are considered most susceptible to pesticides (see Chapter 2; Follett et al. 1985).

g Baseline data used in calculating resistance ratios for slide-dip bioassays.

h Baseline data used in calculating resistance ratios for topical application bioassays.

1 Data from Burts et al. (in press).

2 Data from Follett et al. (1985).

3 Data from Follett (unpublished).

Table 5.3. Toxicity of fenvalerate to adult summer psylla, P. pyricola, of parent R (Wenatchee) and S (Corvallis) populations, and their F_1 (R x S) and backcross (F_1 x S) offspring, using topical application bioassay.

population	LD_{50}[1] (ng/mg insect)	95 % CI (ng/mg insect)	slope	r^2	fold-R
S	1.04	1.03-1.07	2.61	0.92	-
R	251.45	236.32-267.58	0.87	0.89	241.8
F_1 (R x S)	7.84	7.67-8.03	1.99	0.95	7.5
Backcross (F_1 x S)	8.07	7.66-8.52	1.69	0.92	7.8

[1] n = 375.

Figure 5.1. Dose-mortality responses for adult summer pear psylla, P. pyricola, of susceptible (S) and resistant (R) parent populations and their F_1 (R x S) and backcross (F_1 x S) offspring to fenvalerate. n = 75 per dose.

Figure 5.1.

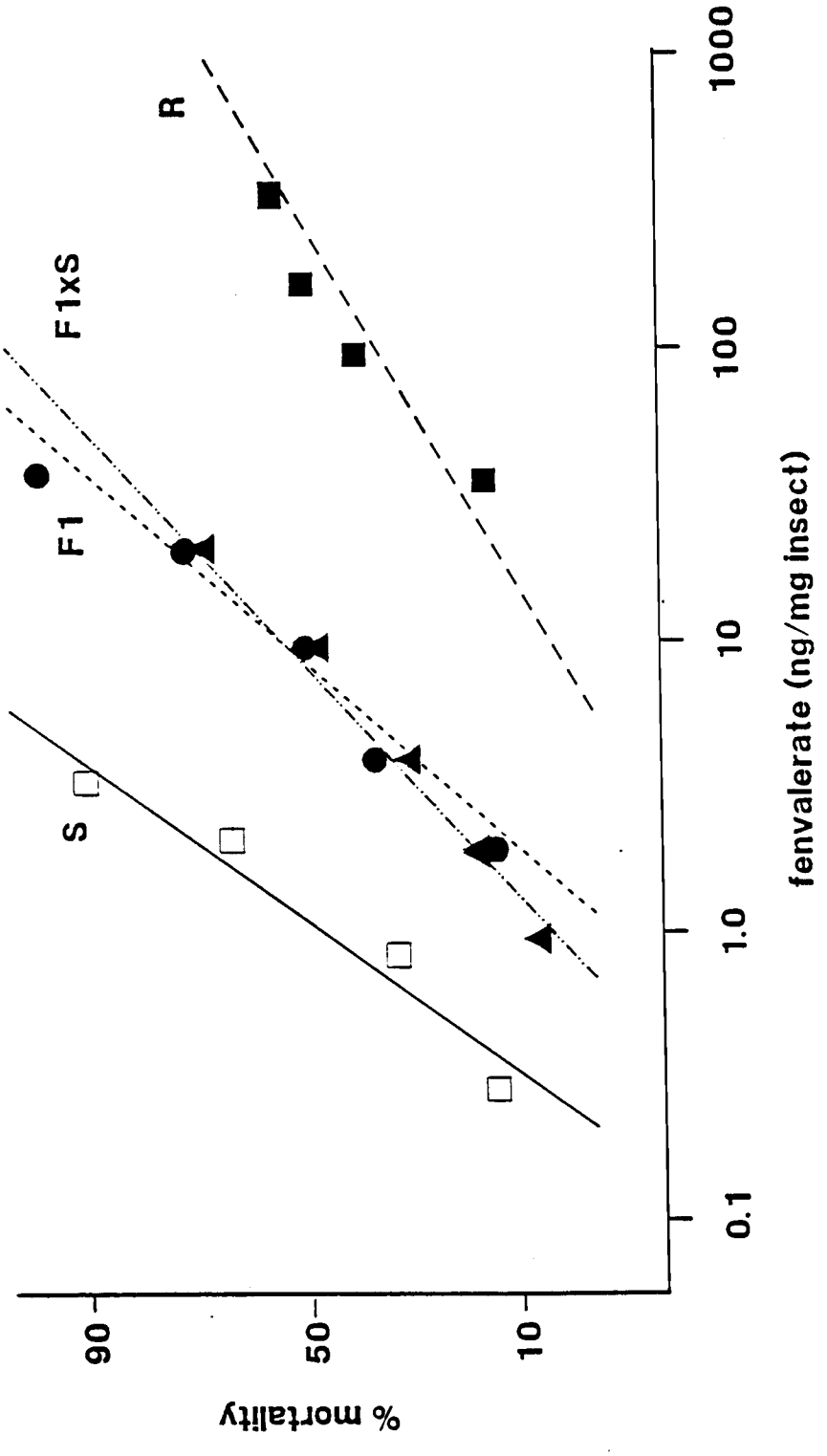

% mortality
90
50
10
0.1
1.0
10
100
1000
fenvalerate (ng/mg insect)
S
F1
F1xS
R

Figure 5.2. Effect of piperonyl butoxide (PbO) on toxicity of fenvalerate to adult summer pear psylla, P. pyricola, from several Western fruit growing areas, using topical application bioassay. Each bar represents the mean ± SE of 4 replicates of 25 insects.

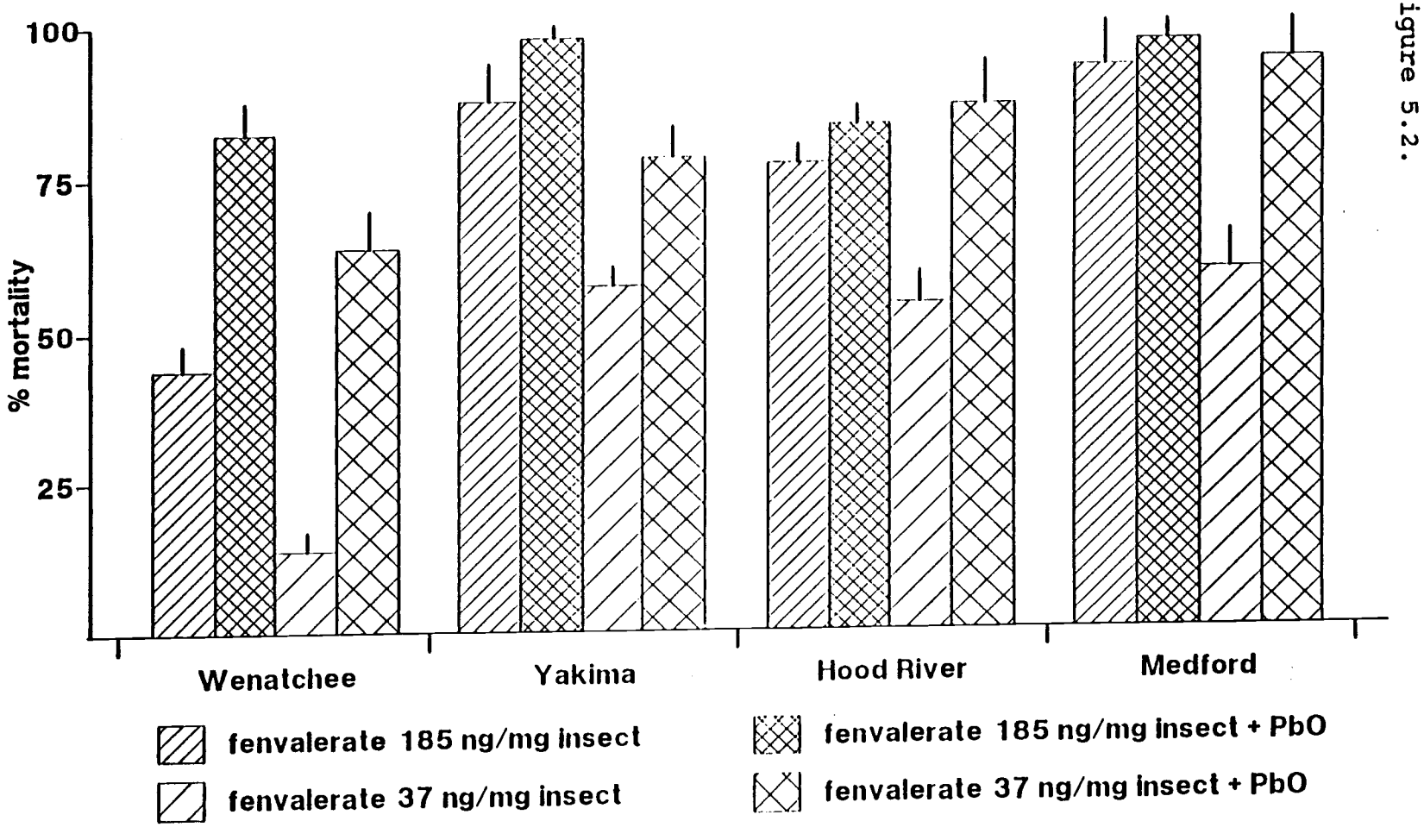
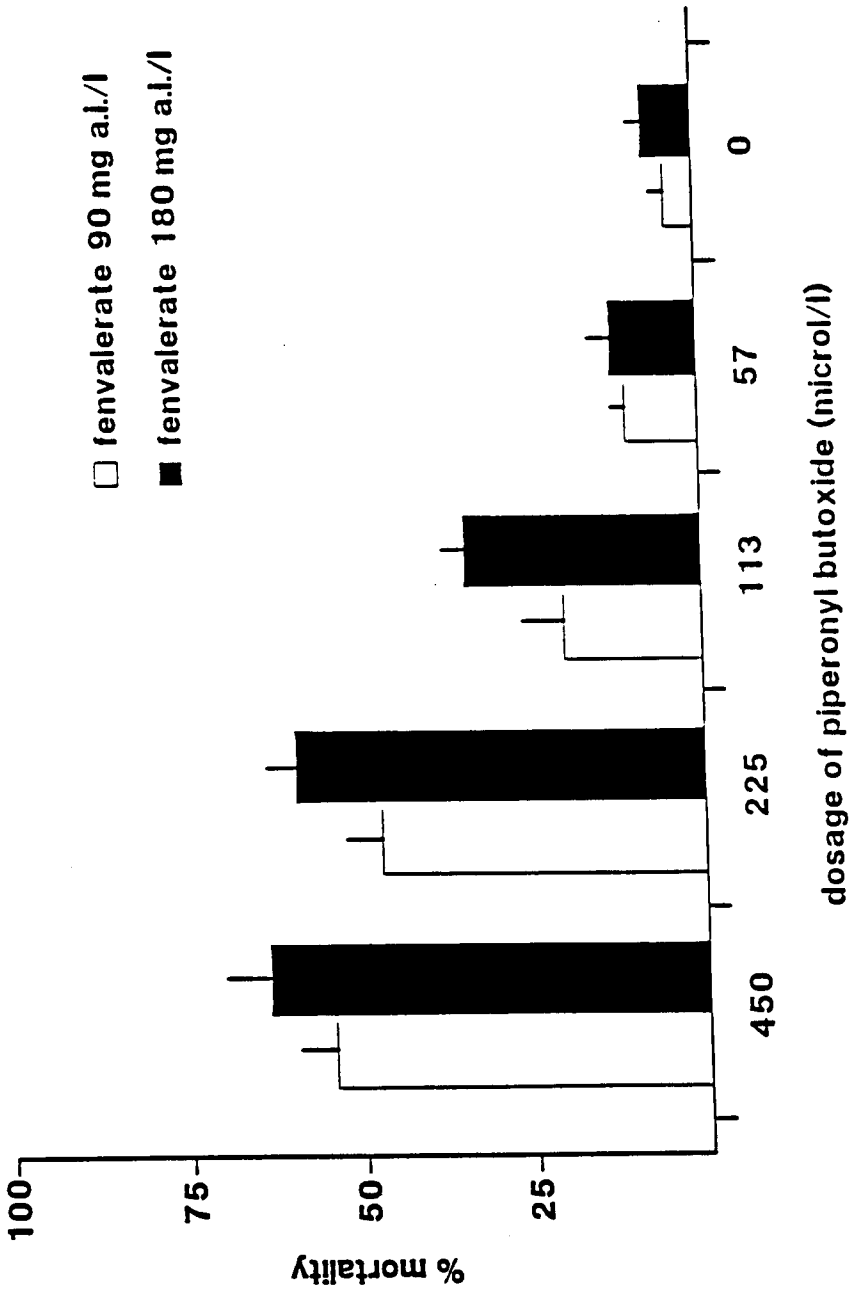

100
75
50
25
% mortality
Wenatchee
Yakima
Hood River
Medford
fenvalerate 185 ng/mg insect
fenvalerate 37 ng/mg insect
fenvalerate 185 ng/mg insect + PbO
fenvalerate 37 ng/mg insect + PbO

Figure 5.2.

Figure 5.3. Effect of the rate of piperonyl butoxide (PbO) on toxicity of fenvalerate to adult summer pear psylla, P. pyricola, from Wenatchee, using slide-dip bioassay. Each bar represents the mean ± SE of 4 replicates of 25 insects.

Figure 5.3.


fenvalerate 90 mg a.i./l
fenvalerate 180 mg a.i./l
% mortality
100
75
50
25
0
57
113
225
450
dosage of piperonyl butoxide (microl/l)

BIBLIOGRAPHY

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265-267.

Agosin, M. 1985. Role of microsomal oxidation in insecticide degradation. pp. 647-712. in: Comprehensive insect physiology, biochemistry and pharmacology. Vol. 12: Insect control. G.A. Kerkut and L.I. Gilbert, eds., Pergamon Press, Oxford.

Baldridge, G.D. and R. Feyereisen. 1986. Blood meal and cytochrome P-450 monooxygenases in the northern house mosquito, Culex pipiens. Pest. Biochem. Physiol. 25, 407-413.

Bernays, E. and M. Graham. 1988. On the evolution of host specifity in phytophagous arthropods. Ecology 69, 886-892.

Bradford, M.M. 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72, 248-254.

Brattsten, L.B. and R.L. Metcalf. 1970. The synergistic ratio of carbaryl with piperonyl butoxide as an indicator of the distribution of multifunction oxidases in the Insecta. J. Econ. Entomol. 63, 101-104.

Brattsten, L.B., C.F. Wilkinson and T. Eisner. 1977. Herbivore-plant interaction: Mixed-function oxidases and secondary plant substances. Science 196, 1349-1352

Brunner, J.F. 1975. Economic injury level of the pear psylla, Psylla pyricola Foerster, and a descrete time model of a pear psylla-predator interaction. Ph. D. Thesis, Washington State University, Pullman, 94pp.

Burts, E.C. 1983. Effectiveness of a soft-pesticide program on pear pests. J. Econ. Entomol. 76, 936-941.

Burts, E.C. 1985. SN 72129 and Avermectin B_1, two new pesticides for control of pear psylla, Psylla pyricola (Homoptera: Psyllidae). J. Econ. Entomol. 78, 1327-1330.

Burts, E.C. and W.R. Fischer. 1967. Mating behavior, egg production, and egg fertility in the pear psylla. J. Econ. Entomol. 60, 1297-1300.

Burts, E.C. and J.F. Brunner. 1981. Dispersion statistics and sequential sampling plan for adult psylla. J. Econ. Entomol. 74, 291-294.

Burts, E.C., H.E. van de Baan and B.A. Croft. in press. Pyrethroid resistance in pear psylla, (Homoptera: Psyllidae) and synergism of pyrethroids with piperonyl butoxide. Can. Entomol.

Chang, C.K. and M.E. Whalon 1987. Substrate specificities and multiple forms of esterases in the brown planthopper, Nilaparvata lugens (Stal). Pest. Biochem. Physiol. 27, 30-35.

Comins, H.N. 1977. The development of insecticide resistance in the presence of migration. J. Theor. Biol. 64, 177-197.

Croft, B.A. 1982. Arthropod resistance to insecticides: a key to pest control failures and successes in North American apple orchards. Entomol. Exp. & Appl. 31, 88-110.

Croft, B.A. and A.W.A. Brown. 1975. Responses of arthropod natural enemies to pesticides. Ann. Rev. Entomol. 20, 285-335.

Croft, B.A. and J.G. Morse. 1979. Recent advances in natural enemy-pesticide research. Entomophaga 24, 3-11.

Croft, B.A. and K. Strickler. 1983. Natural enemy resistance to pesticides: Documentation, characterization, theory and application. pp. 669-702. in: Pest resistance to pesticides. G.P. Georghiou and T. Saito, eds., Plenum Press, New York and London.

Croft, B.A. and H.E. van de Baan. 1988. Ecological and genetic factors influencing evolution of pesticide resistance in tetranychid and phytoseiid mites. Exp. & Appl. Acarol. 4, 277-300.

Croft, B.A., E.C. Burts, H.E. van de Baan, P.H. Westigard and H.W. Riedl. in press. Local and regional resistance to fenvalerate in Psylla pyricola (Homoptera: Psyllidae) in western North America. Can. Entomol.

Devonshire, A.L. and R.M. Sawicki. 1977. The properties of a carboxylesterase from the peach-potato aphid, Myzus persicae (Sulz.), and its role in conferring insecticide resistance. Biochem. J. 167, 675-683.

Dover, M.J. and B.A. Croft. 1984. Getting tough: Public policy and the management of pesticide resistance. Washington D.C.: World Resources Institute, 80 pp.

Dowd, P.F., C.M. Smith and T.C. Sparks. 1983. Detoxification of plant toxins by insects. Insect Biochem. 13, 453-468.

Ellman, G.L., K.D. Courtney, V. Anders and R.M. Featherstone. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-95.

Ehrlich, P.R. and D.N. Murphy. 1988. Plant chemistry and host range in insect herbivores. Ecology 69, 893-895.

Feyereisen, R., G.D. Baldridge and D.E. Farnsworth. 1985. A rapid method for preparing insect microsomes. Comp. Biochem. Physiol. 82b, 559-562.

Follett, P.A. 1984. Monitoring pesticide resistance in Psylla pyricola Foerster from western Oregon pear orchards. M.S. Thesis, Oreg. St. Univ., Corvallis, 65 pp.

Follett, P.A., B.A. Croft and P.H. Westigard. 1985. Regional resistance to insecticides in Psylla pyricola from pear orchards in Oregon. Can. Entomol. 117, 565-573.

Fye, R.E. 1981. Method for rearing pear psylla. J. Econ. Entomol. 74, 490-491.

Fye, R.E. 1983. Dispersal and winter survival of the pear psylla. J. Econ. Entomol. 76, 311-315.

Georghiou, G.P. 1972. The evolution of resistance to pesticides. Ann. Rev. Ecol. System. 17, 133-168.

Georghiou, G.P. 1983. Management of resistance in arthropods. pp. 769-792. in: Pest resistance to pesticides. G.P. Georghiou and T. Saito, eds., Plenum Press, New York.

Georghiou, G.P. 1986. The magnitude of the problem. pp. 14-43. in: Pesticide resistance: Strategies and tactics of management. Nat. Acad. Press, Washington, D.C.

Georghiou, G.P. and C.E. Taylor. 1977a. Genetic and biological influences in the evolution of insecticide resistance. J. Econ. Entomol. 70, 319-323.

Georghiou, G.P. and C.E. Taylor. 1977b. Operational influences in the evolution of insecticide resistance. J. Econ. Entomol. 70, 653-658.

Georghiou, G.P. and C.E. Taylor. 1986. Factors influencing the evolution of resistance. pp. 157-169. in: Pesticide resistance: Strategies and tactics of management. Nat. Acad. Press, Washington, D.C.

Gomori, G. 1953. Human esterases. J. Lab. Clin. Med. 42, 445-453.

Gould, F. 1984. Mixed function oxidases and herbivore polyphagy: the devil's advocate position. Ecol. Entomol. 9, 29-34.

Gordon, H.T. 1961. Nutritional factors in insect resistance to chemicals. Ann. Rev. Entomol. 6, 27-54.

Habig, W.H., M.J. Pabst and W.B. Jacoby. 1974. Glutathione S-transferases, the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

Ishaaya,I and J.E. Casida 1981. Pyrethroid esterase(s) may contribute to natural pyrethroid tolerance of larvae of the common green lacewing. Environ. Entomol. 10, 681-684.

Kaloostian, G.H. 1970. Transitory hosts of the pear psylla. J. Econ. Entomol. 63, 1039-1041.

Krieger, R.I., P.P. Feeny and C.F. Wilkinson. 1971. Detoxification enzymes in the guts of caterpillars: An evolutionary answer to plant defenses? Science 172, 579-581.

Leeper, J.R., R.T. Roush and H.T. Reynolds. 1986. Preventing or managing resistance in arthropods. pp. 335-346. in: Pesticide resistance: Strategies and tactics for management. Nat. Acad. Press, Washington, D.C.

Liss, W.J., L.J. Gut, P.H. Westigard and C.E. Warren. 1986. Perspectives on arthropod community structure, organization, and development in agricultural crops. Ann. Rev. Entomol. 31, 455-478.

Madsen, H.F. 1961. Notes on Anthocoris melanocerus Reuter (Hemiptera: Anthocoridae) as a predator of the pear psylla in British Columbia. Can. Entomol. 93, 660-662.

Madsen, H.F., P.H. Westigard and R.L. Sisson. 1963. Observations on the natural control of the pear psylla, Psylla pyricola Foerster, in California. Can. Entomol. 95, 837-844.

Madsen, H.F. and T.T.Y. Wong. 1964. Effects of predators on control of pear psylla. Calif. Agric. 18, 2-3.

Miyata, T. and T. Saito. 1976. Mechanism of malathion resistance in the green rice leafhopper, Nephotettix cincticeps Uhler (Hemiptera: Deltocephalidae). J. Pestic. Sci. 1, 23-29.

Motoyama, N., G.C. Rock and W.C. Dauterman. 1971. Studies on the mechanism of azinphosmethyl resistance in the predaceous mite, Neoseiulus (T) fallacis (family: Phytoseiidae). Pestic. Biochem. Physiol. 1, 205-215.

Mullin, C.A. 1986. Adaptive divergence of chewing and sucking arthropods to plant allelochemicals. pp. 175-209. in: Molecular aspects of insect-plant associations. L.B. Brattsten and S. Ahmad, eds., Plenum Publ. Corp.

Mullin, C.A., B.A. Croft, K. Strickler, F. Matsumura and J.R. Miller. 1982. Detoxification enzyme differences between a herbivorous and predatory mite. Science 217, 1270-1272.

Oppenoorth, F.J. 1976. Development of resistance to insecticides. pp. 41-59. in: The future for insecticides, needs and prospects. R.L. Metcalf and J.J. McKelvey, eds., John Wiley & Sons, New York.

Oppenoorth, F.J. 1984. Biochemistry of insecticide resistance. Pest. Biochem. Physiol. 22, 187-193.

Oppenoorth, F.J. 1985. Biochemistry and genetics of insecticide resistance. pp. 731-773. in: Comprehensive insect physiology, biochemistry and pharmacology. Vol. 12: Insect control. G.A. Kerkut and L.I. Gilbert, eds., Pergamon Press, Oxford.

Oppenoorth, F.J. and W. Welling. 1976. Biochemistry and physiology of resistance. pp. 507-551. in: Insecticide biochemistry and physiology. C.F. Wilkinson, ed., Plenum Press, New York and London.

Ozaki, K. 1969. The resistance to organophosphorus insecticides of the green rice leafhopper Nephotettix cincticeps Uhler and the smaller brown planthopper Laodelphax striatellus Fallen. Rev. Plant Protect. Res. 2, 1-15.

Plapp, F.W. 1976. Biochemical genetics of insecticide resistance. Ann. Rev. Entomol. 21, 179-197.

Retan, A.R. and E.C. Burts. 1984. Pear psylla detection and control. Wash. State Univ. Coop. Ext. Bull. 1230.

Riedl, H.W., P.H. Westigard, R.S. Bethell and J.E. DeTar. 1981. Problems with chemical control of pear psylla. Calif. Agric. 35, 7-9.

Rose, H.A. 1985. The relationship between feeding specialization and host plants to aldrin epoxidase activities of midgut homogenates in larval Lepidoptera. Ecol. Entomol. 10, 455-467.

Roush, R.T. and B.A. Croft 1986. Experimental population genetics and ecological studies of pesticide resistance in insects and mites. pp. 257-270. in: Pesticide resistance: Strategies and tactics of management. Nat. Acad. Press, Washington, D.C.

Roush, R.T. and J.A. McKenzie. 1987. Ecological genetics of insecticide and acaricide resistance. Ann. Rev. Entomol. 32, 361-380.

Schultz, J.C. 1988. Many factors influence the evolution of herbivore diets, but plant chemistry is central. Ecology 69, 896-897.

Tabashnik, B.E. 1986a. Evolution of pesticide resistance in predator/prey systems. Bull. Entomol. Soc. Amer. 32, 156-161.

Tabashnik, B.E. 1986b. Computer simulation as a tool for pesticide resistance management. pp. 194-206. in: Pesticide resistance: Strategies and tactics for management. Nat. Acad. Press, Washington, D.C.

Tabashnik, B.E. and B.A. Croft. 1982. Managing pesticide resistance in crop-arthropod complexes: Interactions between biological and operational factors. Environ. Entomol. 11, 1137-1144.

Tabashnik, B.E. and B.A. Croft. 1985. Evolution of pesticide resistance in apple pests and their natural enemies. Entomophaga 30, 37-49.

Tabashnik, B.E. and M.W. Johnson. in press. Evolution of pesticide resistance in natural enemies. in: Biological control treatise. Vol. II, T. Fisher, ed..

Taylor, C.E. and G.P. Georghiou. 1979. Suppression of insecticide resistance by alteration of gene dominance and migration. J. Econ. Entomol. 72, 105-109.

Theiling, K.M and B.A. Croft. in press. Pesticide side-effects on arthropod natural enemies: a database summary. Agric. Ecosystems Environ.

Ullman, D.E. and D.L. Mclean. 1988. Feeding behavior of the winter-form pear psylla, Psylla pyricola (Homoptera: Psyllidae), on reproductive host plants. Environ. Entomol. 17, 675-678.

van de Baan, H.E., P.H. Westigard, E.C. Burts and B.A. Croft. in press. Seasonal susceptibility to insecticides in insecticide resistant pear psylla, Psylla pyricola (Homoptera: Psyllidae). Crop. Prot.

Via, S. 1986. Quantitative genetic models and the evolution of pesticide resistance. pp. 222-235. in: Pesticide resistance: Strategies and tactics for management. Nat. Acad. Press, Washington, D.C.

Westigard, P.H. 1973. The biology of and effect of pesticides on Deraeocoris brevis piceatus (Heteroptera: Miridae). Can. Entomol. 105, 1105-1111.

Westigard, P.H. 1974. Control of the pear psylla with insect growth regulators and prelimary effects on some non-target species. Environ. Entomol. 3, 256-258.

Westigard, P.H. 1979. Codling moth: control on pears with diflubenzuron and effects on nontarget pest and beneficial species. J. Econ. Entomol. 72, 552-554.

Westigard, P.H., L.G. Gentner and D.W. Berry. 1968. Present status of biological control of the pear psylla in southern Oregon. J. Econ. Entomol. 61, 740-743.

Westigard. P.H. and R.W. Zwick. 1972. The pear psylla in Oregon. Agr. Exp. Sta. Tech. Bull. 122, 22 pp.

Westigard, P.H., P.B. Lombard and D.W. Berry. 1979. Integrated pest management of insects and mites attacking pears in southern Oregon. Agr. Exp. Stat. Bull. 634, 40 pp.

Westigard. P.H., L.J. Gut and W.J. Liss. 1986. A selective control program for the pear pest complex in southern Oregon. J. Econ. Entomol. 79, 250-257.

Wilde, W.H.A. 1962. Bionomics of the pear psylla, Psylla pyricola Foerster, in pear orchards of the Kootenay Valley in British Columbia. Can. Entomol. 94, 845-849.

Wilde, W.H.A. 1966. Climbing night-shade as a host of pear psylla. Can. Entomol. 98, 558-559

Wilde, W.H.A. 1970. Common plantain as a host of pear psylla (Homoptera: Psyllidae). Can. Entomol. 102, 384.

Williams, M.W., L.P. Batjer, E.S. Deyman and E.C. Burts. 1963. Susceptibility of some pear species to injury from pear psylla. Proc. Amer. Soc. Hort. Sci. 82, 109-113.

Yu, S.J. 1987. Biochemical defense capacity in the spined soldier bug (Podisus maculiventris) and its lepidopterous prey. Pest. Biochem. Physiol. 28, 216-223.

Yu, S.J. 1988. Selectivity of insecticides to the spined soldier bug (Heteroptera: Pentatomidae) and its lepidopterous prey. J. Econ. Entomol. 81, 119-122.