AN ABSTRACT OF THE THESIS OF

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Title: **Genetics and Gene Flow of Organophosphate Resistance in Three Predatory Mites, *Amblyseius andersoni* Chant, *Typhlodromus pyri* Scheuten, and *Metaseiulus occidentalis* Nesbitt (Acarina: Phytoseiidae), in Oregon**

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Abstract approved: _____

Genetics, gene flow, and distribution of pesticide resistance traits were examined for organophosphate (OP) resistance in three beneficial phytoseiid mites. Levels and genetics of OP resistance in *Amblyseius andersoni* were examined first. Laboratory strains from Italy and Oregon, USA, were compared in susceptibility to insecticides used in western Oregon fruit crops. The Italian strain was 80-100 times more resistant to the OPs azinphosmethyl, diazinon, malathion, and phosalone, as well as carbaryl, a carbamate. Significant differences were not found between strains for endosulfan or fenvalerate. Using backcross analysis, response of F_1 hybrids to azinphosmethyl indicated OP resistance was semidominant. Through novel statistical analysis, backcross of F_1 to parent strains revealed resistance was polygenic, with at least two loci. Reciprocal crosses

demonstrated the presence of maternal effects, with increased variation associated with progeny of Oregon females.

In the next set of experiments, electrophoresis of allozymes was used to estimate gene flow for *Typhlodromus pyri*. Ten populations from two apple growing valleys of Oregon were compared. Subpopulations were collected from in and around commercial apple orchards. Four loci unaffected by pesticide use were examined. F_{ST} was calculated at 0.115, and Nm as 2.08. No allelic patterns could be discerned for populations among or within valleys; however, more variation was present for mite populations within valleys than between them. Some inbreeding was found within populations. While from dispersal studies one would conclude *T. pyri* is non-dispersive, allozymic analysis indicates there is moderate gene flow.

Factors affecting OP resistance distribution in *T. pyri* and *Metaseiulus occidentalis* were examined. A diagnostic concentration of azinphosmethyl was used to determine OP resistance frequencies for populations of each species, collected in and near commercial apple orchards in two valleys. OP resistance in *T. pyri* populations was localized: mites from 10 m or more outside orchards were OP susceptible, while those within orchards were resistant. This indicated limited gene flow. All *M. occidentalis* populations were resistant, indicating a regional resistance pattern and high gene flow. Factors which were not significant in the distribution of OP resistance were: valley, degree of orchard isolation, host plant, and seasonality.

Genetics and Gene Flow of Organophosphate Resistance in Three Predatory Mites, *Amblyseius andersoni* Chant, *Typhlodromus pyri* Scheuten and *Metaseiulus occidentalis* Nesbitt (Acarina: Phytoseiidae), in Oregon

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Anybody can make the simple complicated.
Creativity is making the complicated simple.

Charlie Mingus, jazz musician

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INTRODUCTION

Resistance to pesticides used to control arthropod pests is a persistent problem of increasing magnitude in agriculture (Roush and Tabashnik 1990). The number of available pesticides is declining because of decreasing effectiveness or loss of registration, while replacement by new pesticides is low (Croft 1990). Biological control of pests by predators, while always desirable, is becoming more important in integrated pest management (IPM) programs. As problems associated with pesticide resistance increase, more emphasis must be placed on preserving natural enemies through use of selective pesticides. Selective pesticides are those which are appreciably more toxic to pests than beneficials when applied to crops (Croft 1990). Pesticide resistance in beneficial arthropods is one manner in which previously toxic pesticides can be rendered selective. High levels of pesticide resistance in natural enemies may allow

biological control to be maintained within pesticide-structured agroecosystems. This principle has become a tenet of pesticide resistance management: reduce pesticide resistance in pests while encouraging it in beneficials (Croft 1990).

The importance of tetranychid mites as agricultural pests is well-documented (Helle and Sabelis 1985), as is their propensity to develop resistance to acaricides (Helle and Sabelis 1985; Croft and van de Baan 1987; Croft 1990; Roush and Tabashnik 1990). Because few acaricides remain effective very long, predatory phytoseiid mites are of increasing importance for maintaining biological control of spider mites (Croft et al. 1987; Dunley and Croft 1992). An important feature of phytoseiids in IPM is their tendency to develop resistance to insecticides used in tree fruit production (Croft and van de Baan 1987). Pesticide resistant phytoseiids can often survive pesticide applications. In particular, resistance to organophosphate (OP) pesticides is important in preventing mite outbreaks in apple orchards. OPs such as azinphosmethyl are regularly used in commercial apple orchards for control of key pests, such as the codling moth, *Laspyresia pomenella* L.

Three phytoseiid species, *Amblyseius andersoni* Chant, *Typhlodromus pyri* Scheuten, and *Metaseiulus occidentalis* (Nesbitt), are responsible for successful

IPM programs in deciduous tree fruits in many areas of the world (Helle and Sabelis 1985). Much of their successful use is specifically attributed to development of OP resistance in individual strains of these species (McMurtry 1982; Babcock and Tanigoshi 1988; Croft et al. 1990, Dunley et al. 1991).

OP resistant strains of *A. andersoni* have been widely used in controlling spider mites in deciduous tree fruit orchards as well as grape vineyards throughout southwestern Europe (Duso 1985, Genini and Baillod 1987, Ivancich-Gambaro 1975, 1986). In western Oregon this species occurs in a wide variety of agricultural crops and native vegetation (Hadam et al. 1986), but these populations are susceptible to OPs (Dunley et al. 1991).

Typhlodromus pyri and *M. occidentalis* are the two dominant phytoseiid species found in commercial apple orchards of the Pacific Northwest, where they are largely responsible for effective integrated spider mite management (Hoyt 1969, Croft et al. 1990). Within the Pacific Northwest, the geographic distribution of *T. pyri* is primarily in the western valleys of Oregon and Washington, while *M. occidentalis* is present in the more arid regions east of the Cascade mountains. Both species commonly occur together in the Hood River (HR) Valley of Oregon which lies in a gradient in decreasing rainfall toward the east (Croft et al. 1990). The greatest

concentration of apple orchards in the state of Oregon occurs in HR, with the remainder in the Willamette Valley (WV) of western Oregon. HR has many large, contiguous orchards, while WV orchards are smaller and scattered throughout the region. More land is managed agriculturally relative to unsprayed vegetation in HR than WV, and OPs are applied more widely and regularly in HR than WV.

Dispersal and gene flow of pesticide resistance traits are major influences in the development and maintenance of resistance. Dispersal, as emigration or immigration, is the movement of individuals between populations (Taylor 1978), while gene flow is the movement of genes between populations (Falconer 1989). Dispersal and gene flow impact resistance in several ways. First, gene flow provides possible introduction of novel genes for resistance into a population. Gene flow also reduces or increases the rate of resistance development in a population, depending on the amount of immigration of susceptible or resistant phenotypes. In addition, dispersal influences residency time, directly affecting the amount of pesticide exposure and the proportion of a population selected by sprays. Together, these elements determine the distribution of resistant phenotypes through movement of individuals between populations on both local and regional spatial scales.

For instance, if an organism is highly vagile with high levels of gene flow, pesticide resistance can show a regional distribution over a large area (*e.g.*, *Psylla pyri* Foerster: Follett *et al.* 1985, Croft *et al.* 1989; *Heliothis armigera* (Hubner): Daly 1989). Alternatively, low levels of dispersal limit distribution of resistance genes and may result in more localized, heterogenous patterns of resistance.

Ecological factors also influence the distribution of pesticide resistance in phytoseiid mites (Croft and van de Baan 1988). In addition to dispersal characteristics, reproduction and genetics can effect the rate of pesticide resistance development. As such, examination of the ecological genetics of pesticide resistance are often necessary, however few studies have been made of these relationships (Croft and van de Baan 1988, Roush and Tabashnik 1990, Dunley and Croft 1992).

Three experiments were conducted to examine the genetics and gene flow of pesticide resistance traits in populations of phytoseiid mites. These studies are arranged in this thesis by increasing levels of population genetic structure. Chapter 1 considers the genetics of resistance in a single species, Chapter 2 examines the genetic variation and gene flow within and between populations of a species, and Chapter 3 considers

the area-wide distribution and ecological factors which influence resistance occurrence in two species.

At the single species level, genetic crossing studies of OP resistant and susceptible *A. andersoni* were conducted to estimate the number of genes associated with resistance and to determine the mode of inheritance. Gene flow, using measures of genetic variation for *T. pyri* populations in two apple growing regions of Oregon, was determined at the population genetic level to ascertain the effects of dispersal on pesticide resistance. Finally, at the regional or area-wide levels, the ecological factors which influence the occurrence and spatial distribution of resistance were examined. In this study, the distribution of organophosphate resistance in *T. pyri* and *M. occidentalis* was related to density and size of apple production areas, presence of alternate host plants, and relative areas under selection pressure from pesticide sprays.

CHAPTER I

Levels and Genetics of Organophosphate Resistance in Italian and Oregon Biotypes of *Amblyseius andersoni* Chant (Acarina: Phytoseiidae)

Introduction

Disruption of arthropod biological control agents by chemical pesticides and pesticide resistance evolution in pests are key factors which favor adoption of integrated pest management (IPM). As part of IPM, pesticide resistance management seeks to lessen resistance in pests while maximizing resistance in natural enemies, thus allowing biological control to occur in pesticide-structured agroecosystems (Croft 1990). Use of resistant natural enemies is an integral part of spider mite management on several crops including peaches (Field 1978), almonds (Hoy 1985), and glasshouse vegetables (Hussey & Scopes 1985).

Resistance to pesticides has been found in only 31 biological control agents, 35% of which are phytoseiid mites (Croft 1990). These acarines have rapid reproductive rates, functional arrhenotoky, and exhibit unique immigration and colonization attributes which favor resistance evolution on a microgeographic scale (Croft & van de Baan 1988). Practical uses have been made of both naturally occurring (i.e., serendipitous, field-developed) and induced (i.e., deliberate, laboratory-selected) resistance in several phytoseiid species (Hoy 1985, Croft 1990). Introduction of field-developed resistant phytoseiids to new geographic regions

often results in effective biological control (Croft & Barnes 1972, Field 1978, Penman et al. 1979), and is analogous to classical biological control using unique biotypes of natural enemies.

The genetics of organophosphate resistance at the species level have already been characterized for *Typhlodromus pyri* (Overmeer and van de Baan 1984) and *Metaseiulus occidentalis* (Hoy 1985). Further study of their resistance genetics would be less novel, although new insight might be gained using the more advanced techniques reported herein. Instead, the inheritance and genetics of organophosphate resistance was examined in the less studied phytoseiid, *Amblyseius andersoni* Chant.

Resistance of *Amblyseius andersoni* Chant to azinphosmethyl was first observed in peach orchards in the upper Po River Valley, near Verona, Italy (Ivancich-Gambaro 1975). Later, Ivancich-Gambaro (1986), Duso (1985), Caccia *et al.* (1985), and Genini & Baillod (1987) documented resistance levels and cross-resistance to compounds used on peaches, apples, and grapes in Italy and Switzerland. In contrast, Oregon *A. andersoni* populations are highly susceptible to most organophosphates (OPs) and carbaryl (Croft & AliNiazee 1983). Also, the distribution of *A. andersoni* in commercial crops and native vegetation in the Willamette Valley indicates that standard sprays exclude it from

many crops (Hadam *et al.* 1986). Although efficacy as a biological control agent for spider mites has been amply demonstrated for *A. andersoni*, susceptibility to pesticides prevents it from becoming important in Oregon crops.

In the following experiments, *A. andersoni* from Italy was evaluated for levels of resistance and potential use where normal insecticide treatments for other insect pests exclude native predators (Hadam *et al.* 1986). After tests revealed no reproductive isolation between these two races (Messing & Croft, 1990), hybridizations and bioassays of F_1 and backcross generations were made to analyze resistance genetics, with the intent to introduce and manage resistance in the field.

Materials and Methods

Resistance Tests

A colony of *A. andersoni* from Italy was started with approximately 100 individuals obtained from a laboratory colony in Switzerland (from M. Baillod, Station Federale de Recherches Agronomiques de Changins). The strain was originally collected from commercial peach near Verona. The Oregon strain was collected from several native plant species in the Willamette Valley and has been maintained in the laboratory since 1986.

Strains were reared in separate rooms on four to six trays (10 by 10 cm) containing a rearing substrate and a water moat (McMurtry & Scriven 1964). Each tray was lined with adhesive (Tanglefoot^{R}, Tanglefoot Co., Grand Rapids, Mich.) and maintained at constant photoperiod of 16:8 (L:D) and temperature ($20^{o}+2^{o}$ C). Predators were fed mixed stages of *Tetranychus urticae* Koch and corn pollen three times weekly.

The following commercial formulations were tested: azinphosmethyl (Guthion 50 Wettable Powder [WP], Mobay Corp., Kansas City, Mo.), carbaryl (Sevin 1.9 Emulsifiable Concentrate [EC], Lilly Co., Portland, Ore.), diazinon (4 EC, Ciba-Geigy, Greensboro, NC), endosulfan (Thiodan 3 EC, FMC, Phila., Penn.), fenvalerate (Pydrin 2.4 EC, DuPont, Wilmington, Del.),

malathion (5 EC, FMC, Phila., Penn.), and phosalone (Zolone 3.0 EC, Rhone-Poulenc, Monmouth Junction, NJ). At least 80 mites were tested at each of three concentrations per insecticide. Controls were treated with water.

Bioassays were conducted by modified slide-dip technique (Croft *et al.* 1976). Fifteen to 30 gravid adult females per replicate were mounted dorsally on double-sided sticky tape affixed to a glass microscope slide. The slide was dipped in each pesticide concentration for 5 s then air dried for 20 minutes. Slides were placed in a closed plastic box (40 by 25 by 15 cm) which contained moistened paper towels to maintain humidity (*approximately* 80%). Mortality was measured under a dissecting microscope at 48 h: mites that failed to respond with leg movements after a gentle touch with a fine camel hair brush were scored as dead. Two to eight replicates were used per concentration per strain or cross, with replication occurring across days. Probit regressions lines were estimated using the probit option of POLO (Russell *et al.* 1977). Likelihood ratio tests were used to test the hypotheses of equality and parallelism of response lines.

Inheritance of OP Resistance

Inheritance of resistance to azinphosmethyl was tested in crosses and backcrosses of parent (P) strains (Italy and Oregon) and progeny (F_1, F_2), each with eight pesticide concentrations. Hybridizations between the Italy (resistant, hereafter referred to as R) and Oregon (susceptible, hereafter S) strains occurred on rearing trays by introducing deutonymph females and adult males for each reciprocal cross: Italy female X Oregon male (R X S), and Oregon female X Italy male (S X R). Trays were checked daily for F_1 eggs, which were collected and transferred to separate trays with a fine brush, then reared to adults for bioassay. For backcrosses, F_1 deutonymph females from parental crosses were put on separate hybridization arenas with males from the same strain as their mother. Backcrosses were made in both directions (to the R and to the S) to obtain the F_2: (Italy female X Oregon male) female X Italy male (RS X R), and (Oregon female X Italy male) female X Oregon male (SR X S). Bioassays were replicated over time, and the reciprocal crosses were performed simultaneously.

Degree of dominance for the resistance trait was estimated in reciprocal F_1 females using the formula:

$$D = \frac{2X_2 - X_1 - X_3}{X_1 - X_3}$$

where X_1 is the logarithm (base 10) of the LC_{50} for the Italy (R) population, X_2 is the log LC_{50} of heterozygous hybrids (RS), and X_3 is the log LC_{50} of the Oregon (S) population (Stone 1968).

Concentration-response lines were compared with expected lines for a single gene model of inheritance. Expected response at each concentration was calculated as:

$$X_y = 0.5\ W_{F1} + 0.5\ W_P$$

where X_y is the expected response in the backcross generation at concentration y, W_{F1} is the observed response at concentration y of the hybrid (RS or SR) parent (Italy female X Oregon male or Oregon female X Italy male), and W_P is the observed at concentration y of the resistant (R) or susceptible (S) homozygous parent (Italy or Oregon) (Georghiou 1969). Backcross results at each concentration were compared to expected results by both χ^2 goodness-of-fit test and 2x2 contingency table.

The 2x2 contingency test is a conservative test, and χ^2 is the standard analysis statistic.

Results from each backcross were also compared with a two-locus model, assuming equal and additive contributions to resistance, with independent segregation and no epistasis. Expected mortality of the backcross between parent and F_1 was calculated as:

$$X_y = 0.25\ W_{F1} + 0.25\ W_P + 0.5\ W_H$$

where X_y is the expected percent response in the backcross generation at concentration y, and W_H is the calculated percentage response of the progeny which is heterozygous at only one locus (RSRR, SRRR, SSSR, or SSRS). W_H is the area under a truncated mortality normal distribution curve, and was calculated as:

$$W_H = \frac{1}{\sqrt{2\Pi}} \int_{-\varpi}^{Y_H-5} e^{-1/2u^2} du$$

and,

$$Y_H = b' (y - m') + 5$$

where Y_H is the expected probit mortality at concentration y of the genotype heterozygous at only one

locus, b' is the mean of the probit line slopes of the homozygous parent (SSSS or RRRR) and F_1 cross (RSRS), and m' is the mean of the logarithms of LC_{50}'s of the homozygous parent and F_1.

Results

Resistance Analysis

Amblyseius andersoni from Italy was more resistant than the Oregon biotype to all OPs tested (Table I.1). The average recommended field rate of each OP (Fisher et al. 1990) caused 100% mortality to the susceptible population but essentially no mortality to the resistant population. The Italy strain was also highly resistant to carbaryl, with the field rate causing 100% mortality to the Oregon strain and about 15% mortality to the Italy biotype.

Endosulfan, a chlorinated hydrocarbon, was only moderately toxic to both biotypes of *A. andersoni*; fenvalerate, a pyrethroid, was highly toxic to each biotype (Table I.1). Thus, there was no evidence of cross-resistance from OPs and carbamates to the insecticides with different modes of action (e.g., GABA receptor, cellular ion transfer).

Inheritance of OP Resistance

Response lines to azinphosmethyl for each parent strain and reciprocal crosses and backcrosses are shown in Figure I.1. The statistical characteristics of these lines are in Table I.2. Maternal effects from Oregon

Table I.1. Percentage mortality of Italy and Oregon strains of *Amblyseius andersoni* to commercial pesticides at field rates, and their factors, when assessed using slide-dip bioassay

Compound	Strain	Rate tested[a] 0.01X	0.1X	1X	5X	10X
malathion	Italy	-	-	0.8	46.6	98.3
	Oregon	24.4	40.2	100	-	-
azinphosmethyl	Italy	-	-	0	7.5	31.0
	Oregon	2.5	58.0	100	-	-
phosalone	Italy	-	-	1.3	18.2	70.7
	Oregon	23.9	95.3	98.3	-	-
diazinon	Italy	-	-	10.1	64.7	82.6
	Oregon	5.6	71.1	99.1	-	-
carbaryl	Italy	-	-	14.6	45.2	100
	Oregon	0	77.3	100	-	-
endosulfan	Italy	-	-	9.8	15.1	-
	Oregon	-	-	12.3	16.5	45.0
fenvalerate	Italy	5.4	45.9	90.4	-	-
	Oregon	15.4	54.6	84.2	-	-

[a] - 1X = field rate, estimated as 0.5% active ingredient for malathion and carbaryl, 0.01% for fenvalerate, and 0.1% for all other compounds.

Figure I.1 Concentration-mortality lines for parental strains, crosses, and backcrosses of *Amblyseius andersoni* in response to azinphosmethyl.

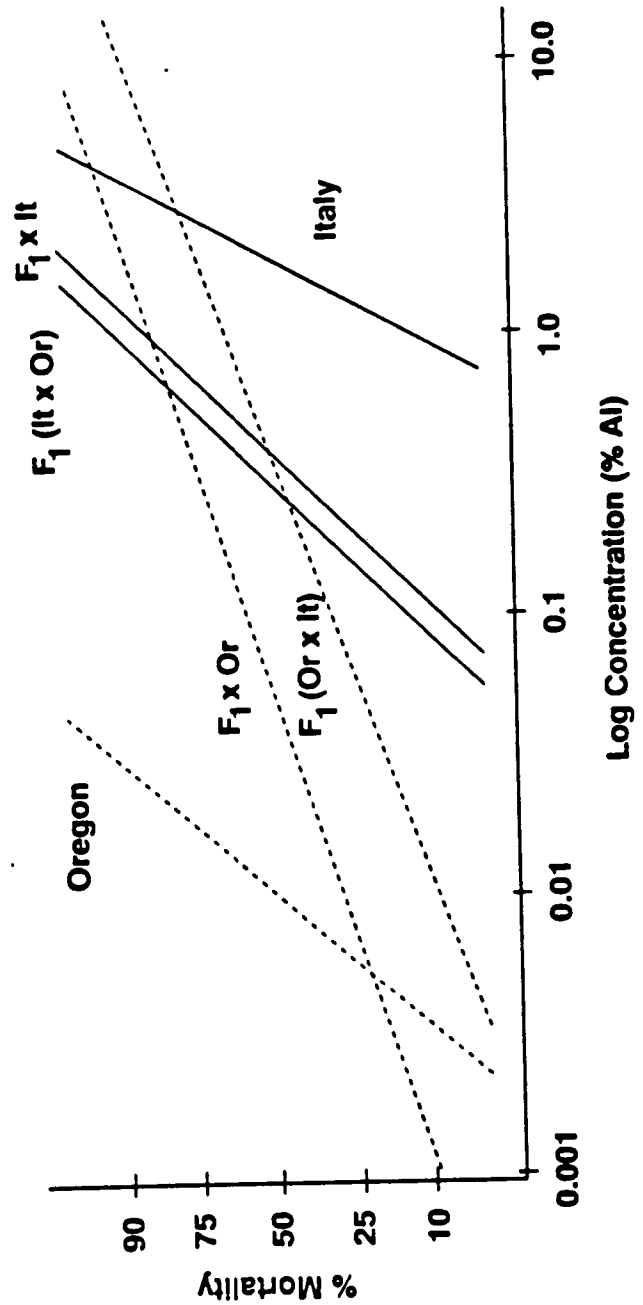
% Mortality
90
75
50
25
10
0.001
0.01
0.1
1.0
10.0
Log Concentration (% Al)
Oregon
F1 x Or
F1 (Or x It)
F1 (It x Or)
F1 x It
Italy

Figure I.1

Table I.2. Toxicity of azinphosmethyl to parental strains and crosses of Italy and Oregon populations of *Amblyseius andersoni*.

Colony	*n*	Slope ± SE	LC_{50} [a]	95% FL
Oregon	774	2.612 ± 0.241	0.012	0.009-0.015
Italy	634	4.786 ± 0.750	1.572	1.282-2.029
OR X IT	988	0.918 ± 0.093	0.436	0.249-0.764
IT X OR	536	2.051 ± 0.235	0.294	0.219-0.375
(OR X IT) X OR	684	0.772 ± 0.099	0.063	0.026-0.127
(IT X OR) X IT	281	1.786 ± 0.212	0.336	0.235-0.454

[a]- determined by probit analysis using slide-dip bioassay

females were evident in reciprocal F_1 crosses (Fig. I.1). Variation in response to treatment concentration, which is inversely proportional to the slope of the regression line, was higher in Oregon female X Italy male F_1 than in the Italy female X Oregon male cross. Increased variation was also seen in backcross progeny of Oregon males and F_1 from Oregon female X Oregon male cross. Because of these maternal effects, results from crosses of Oregon females and Italian females were analyzed separately.

In crosses involving Oregon females, χ^2 analysis of observed versus expected for a single gene model was significantly different at four of eight concentrations (Table I.3). However, more conservative 2x2 contingency table tests showed no differences at any concentration. The overall probit line was not significantly different from the expected backcross line ($\chi^2 = 2.54$; df = 2; $p = 0.28$). Analysis for degree of dominance, assuming a single gene, described resistance as incompletely dominant (D = 0.47). When compared with a two-locus model, observed mortality was significantly different from expected at three of eight concentrations, but the expected probit line was not significantly different ($\chi^2 = 4.21$; df = 2; $p = 0.12$).

Table I.3. Backcross analysis for azinphosmethyl resistance mode of inheritance in *Amblyseius andersoni*.

		1-locus Model			2-locus Model	
Conc. (%AI)	Obs. % Mort.	Exp. % Mort.[a]	X^{2b}	2x2 Cont. Table[b]	Exp. % Mort.[c]	2x2 Cont Table[b]
Backcross: (Oregon X Italy) X Oregon						
0.001	10.0	7.0	0.82	0.18	3.5	3.36
0.005	22.0	12.9	6.32 *	1.67	10.5	4.82 *
0.01	32.6	33.4	0.04	0.01	26.5	0.91
0.05	50.7	59.6	4.85 *	1.60	62.2	2.70
0.10	58.4	66.2	4.13 *	1.20	63.0	0.44
0.50	63.9	76.4	5.28 *	3.12	84.8	11.42 *
1.0	72.6	76.1	0.50	0.25	87.0	6.44 *
2.0	93.0	95.4	0.97	0.12	94.9	0.35
Backcross: (Italy X Oregon) X Italy						
0.001	3.6	10.3	1.26	0.88	5.1	0.28
0.005	3.6	10.3	1.26	0.88	5.1	0.28
0.01	3.9	9.9	1.06	0.73	4.9	0.14
0.05	6.8	12.1	1.16	1.05	6.1	0.05
0.10	24.4	17.2	1.65	1.05	8.7	8.96 *
0.50	60.7	41.3	4.34 *	7.52 *	37.0	11.30 *
1.0	77.8	55.9	10.45 *	9.54 *	66.4	3.22
2.0	90.0	82.3	2.44	1.18	88.3	0.14

[a] - expected mortality based on the one-locus model

[b] - X^2 test of independence, df = 1, $P < 0.05$

[c] - expected mortality based on the two-locus model

For crosses with Italy females, mortality at two of eight concentrations was significantly different from expected for a one-locus model (Table I.3). Significant differences were also apparent in the 2x2 contingency table analysis. However, the probit line deviated significantly from expected (χ^2 = 45.68; df = 2; p < 0.001). Resistance in Italy female crosses was also incompletely dominant (D = 0.31). For the two gene model, observed mortality deviated significantly at two of eight concentrations, and the overall line was not significantly different (χ^2 = 1.54; df = 2; P = 0.46).

Discussion

Increased variation (reduced slope of regression lines) that was observed with Oregon females makes clear interpretation of our backcross experiments difficult. However, the large number of mites tested, repeatability of tests, and small variation in responses of the parental strains suggest that experimental error is not a likely cause for increased variation. Instead, the mixed results from reciprocal crosses probably result from maternal effects. Results from each cross can be discussed separately.

The increased variation present in crosses with Oregon females masks any discernible relationship between observed and expected mortalities. However, when standard methods to evaluate backcross results (i.e., χ^2) are used, the single gene model would be rejected; this conflicts with the results from 2x2 contingency table analysis and overall probit line. Alternatively, if the model line for a two gene model was accepted, then three of eight data points were significantly different from expected. The most conclusive evidence that maternal effects obscured interpretation of data from Oregon female crosses was the distribution of differences between observed and expected for a single gene model. In Figure I.2a, the differences between observed and

Figure I.2. Patterns of deviation between observed percentage mortality in the backcross of *Amblyseius andersoni* and expected percentage mortality from a one-locus model. a. backcross: (OR X IT) X OR ; b. backcross: (IT X OR) X IT . Dashed line represents expected difference between one- and two-locus models.

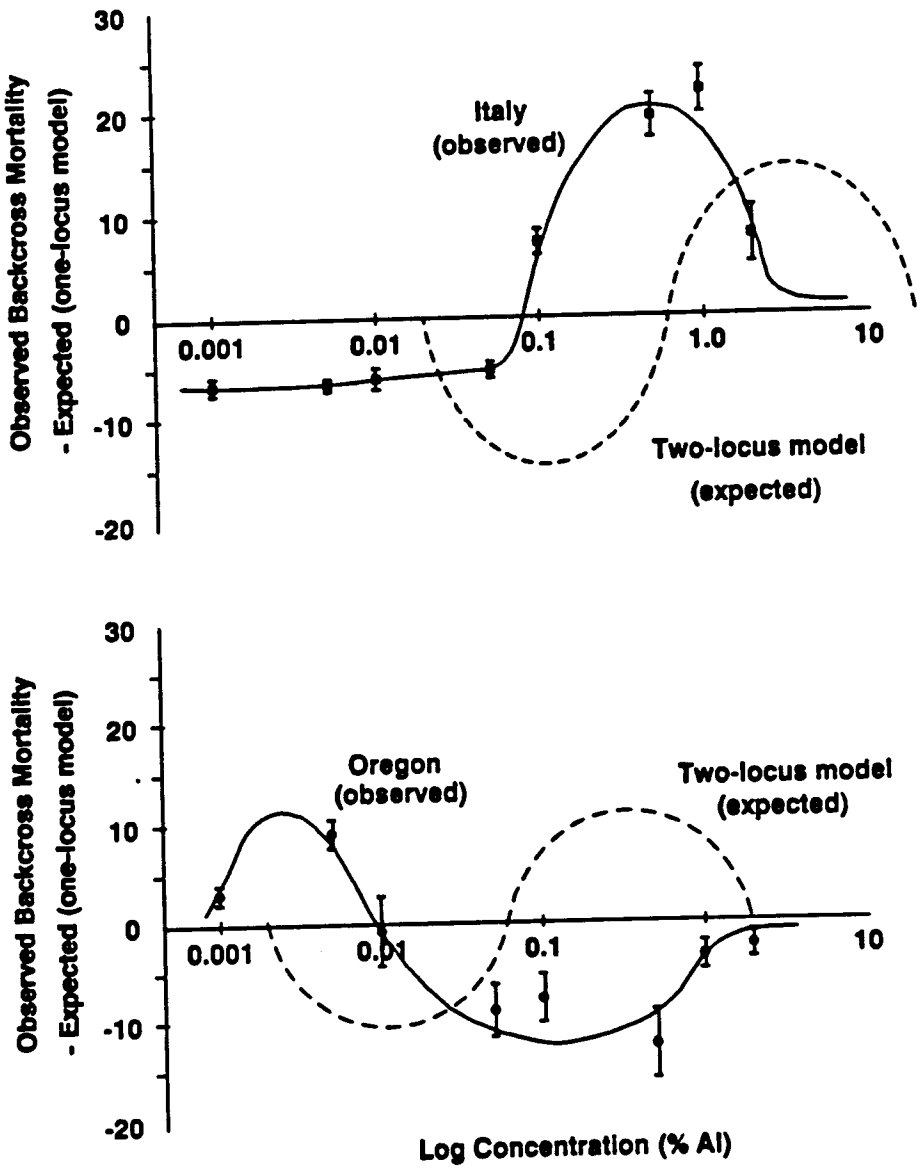
Observed Backcross Mortality
- Expected (one-locus model)
30
20
10
0
-10
-20
0.001
0.01
0.1
1.0
10
Italy
(observed)
Two-locus model
(expected)
Oregon
(observed)
Two-locus model
(expected)
Log Concentration (% AI)

Figure I.2

expected (single gene model) percentage mortalities are shown, along with the pattern of differences which would occur if resistance were polygenic. This figure shows that the deviation is opposite from that expected for a polygenic trait.

In crosses using Italy females, statistical analysis of the probit line indicates that the polygenic model fits the data more closely. This conclusion is further supported by a plot of differences between observed and expected mortalities (Fig. I.2b). The plot shows that the direction of deviations in the backcross more closely fits a polygenic model; however, deviations are shifted somewhat downward from the LC_{50}. These deviations may indicate epistasis, an interaction of genes, which reduces resistance in some heterozygotes. As opposed to an expected bimodal curve, the unimodal curve is particularly indicative of epistasis (Tabashnik, 1991). Because influences of individual resistance genes in this polygenic system are unknown (and may be unequal), the expected response values for the heterozygotes at a single locus (SSRS, RSSS, RRRS, RSRR) cannot be calculated.

In summary, the results from Italy crosses are most clear. They indicate that OP resistance in *A. andersoni* is polygenic and semidominant. This conclusion agrees with that of Anbar & Oppenoorth (1989) who, using an

Italian race of *A. andersoni* from similar sources as ours, isolated two genetic mechanisms for parathion resistance: a hydrolyzing esterase and an altered acetylcholinesterase.

In most IPM programs which have used pesticide resistant phytoseiids successfully, resistance has been conferred by a single dominant or semi-dominant allele at one locus (evidence reviewed in Croft & van de Baan 1988). Although polygenic resistance in phytoseiids has been found occasionally, it most often has been seen in small populations which were artificially selected in the laboratory, with each factor contributing a low resistance level (e.g., mechanism studies of pyrethroid resistance in *Amblyseius fallacis* Garman [Scott *et al.* 1983]). In only one case has polygenic resistance been reported in field-selected populations (dimethoate resistance in *Metaseiulus occidentalis* (Nesbitt) [Roush & Hoy 1981]). Most polygenic resistances have been recessive (Croft & van de Baan 1988).

There are disadvantages in using polygenic rather than monogenic resistance in genetically improved beneficials (Croft 1990). Polygenic resistance is more likely to be diluted, and eventually lost, by hybridization with susceptible individuals. Because of this dilution effect, resistance will likely spread from field to field much more slowly (Roush & McKenzie 1987).

Conventional wisdom puts a high value on monogenic resistance as being more manageable (i.e., resistance maintenance) under field conditions (Hoy *et al.* 1985). However, most discussions of polygenic resistance assume that field-level resistance is based on additive effects of several genetically distinct physiological mechanisms, and that each mechanism alone is insufficient to provide resistant phenotypes.

In *A. andersoni*, there is no evidence that additive effects are necessary, and in fact some evidence that suggests each of two mechanisms is sufficient in itself to provide a resistant phenotype. Anber & Oppenoorth (1989) reported resistance ratios of 10,900 and 5,560 for azinphosmethyl in two sub-strains of *A. andersoni*, each of which contained a separate (i.e., non-allelic) gene for resistance. Although their method of testing differed from ours and although neither method is directly applicable to field situations, high resistance ratios indicate tolerance in each substrain to field rates of azinphosmethyl. If each mechanism does confer field level resistance, then although OP resistance in the imported *A. andersoni* is polygenic, resistance may be managed as though it were monogenic. Resistance dilution in the field by hybridization with native susceptible individuals will be unlikely, assuming no reduced fitness in resistant phenotypes and continued selection pressure.

Stable resistance in the field is not only dependent upon genetics of resistance, but also upon ecological attributes of the species (Croft & van de Baan 1988). Factors such as background resistance in native interbreeding populations, ratio of absolute population density of introduced and native strains, immigration and emigration rates, and relative fitness of each population (including behavioral and physiological reproductive measures) can be important (Croft 1990). To predict and evaluate mass-releases of the Italy biotype of *A. andersoni* in western Oregon, ecological attributes of both the Italy and Oregon biotypes, as well as the resistance genetics, should be determined.

This study indicates a need to recognize the ecological genetics and interactions that influence polygenic resistance. Presently, at least five predatory mite species are known to have two or more resistance mechanisms present in the same strain (Croft 1990); any of these mechanisms may confer resistance to field rates of pesticides. Until now, factors such as maternal effects and epistasis have been largely ignored in research to genetically improve pesticide-resistant natural enemies, but these genetic phenomena must be better understood if sustained progress is to be made using these biocontrol agents.

CHAPTER II

Estimation of gene flow in *Typhlodromus pyri* Scheuten using allozymic analysis

Introduction

Gene flow, the movement of genes between populations, is an important factor in the adaptation of insects and mites to agriculturally managed ecosystems. Artificial selection by pesticides used in agriculture both induces and alters the rate of evolution of pesticide resistance in pest arthropods and their natural enemies (Crow 1957). Because selection for resistance occurs only in pesticide treated areas, knowledge of dispersal and gene flow between populations in treated and untreated areas is important in predicting the rate of resistance evolution (Tabashnik and Croft 1982).

Understanding the potential for and rate of evolution of pesticide resistance is necessary for development of pesticide resistance management programs. Resistance management seeks to decrease the rate of evolution of resistance in pest insects and mites, while increasing the likelihood of resistance in their natural enemies (Croft 1990). One group of pestiferous mites, tetranychid or spider mites, has a high propensity to develop resistance to acaricides (Croft and van de Baan 1988). This propensity has led to a need for resistance management programs for spider mites, particularly in deciduous tree fruit cultivation. However, predatory phytoseiid mites, important natural enemies of spider

mites, have also developed resistance to many chemicals used in fruit production. The occurrence of resistance in predatory mites enables them to survive pesticide sprays, increasing the likelihood of biological control of spider mites. Thus, knowledge of dispersal and gene flow of pesticide resistance in predatory mites is important in integrated management of spider mites.

There are two approaches to studying gene flow of pesticide resistance. First, gene flow between populations in the absence of selection pressure from pesticide sprays can be estimated through examination of allele frequencies, presumably for enzymes unaffected by pesticides. Alternatively, gene flow can be examined though distribution of a resistance trait. This entails examination of the distribution of the pesticide resistance and its movement through populations.

Studies of gene flow use measurements of variation in allele frequencies from different populations to estimate how much genetic fragmentation has occurred. The more genetic isolation, i.e., the more disparate the allele frequencies between populations, the less likely that dispersal occurs between them. Eventually populations become genetically distinct as genetic drift alters allele frequencies at random. Several methods can be used to estimate genetic differences, the foremost

being Wright's coefficient of inbreeding (Wright 1951), defined as

$$F_{ST} = \frac{\sigma_p^2}{p(1-p)}$$

with σ^2_p as the variance in frequency of an allele among subpopulations, while p is the mean allele frequency among subpopulations. Several modifications have been made to remove limitations to the original estimate of F_{ST}. One modification, called G_{ST}, was developed to correct for more than two alleles at a locus (Nei 1973), and to correct for multiple loci (Nei and Chakravarti 1977). Another estimate, θ_w, corrects for differences in sample sizes among populations (Weir and Cockerham 1984). These three methods of estimating genetic variation within and between populations all are expressed as a value from 0 to 1. A value of 0 indicates no genetic differentiation between populations. This usually results from a high level of gene flow which makes the populations genetically identical. A value of 1 indicates total differentiation, i.e., no gene flow is occurring between populations. Intermediate values depend on the amount of variation which occurs between populations, however most estimates of gene flow in natural populations are close to 0 (Weir 1990). Finally, the value may be negative, indicating a surplus of heterozygotes.

To determine the actual amount of migration occurring between populations, Wright (1951) suggested that F_{ST} could be used to estimate the average number of migrants between populations per generation. The proportion of a population which migrates per generation is defined as

$$N_em = 1/4\ (1/F_{ST} - 1)$$

where N_e is the effective population size for a population and m is the average migration rate. Estimates of N_em which are greater than 1 indicate "high" gene flow (Loxdale 1991), although somewhat larger estimates have been considered "moderate" (Roush and Daly 1990).

While there are no examples of studies of gene flow in predatory mites or spider mites, there are several for pesticide resistant insects. Populations of resistant *Spodoptera frugiperda* were estimated to have high gene flow, with an F_{ST} of 0.034 (Pashley et al. 1985). *Spodoptera exempta* populations were also characterized by high gene flow (Den Boer 1978). Two species of *Yponomeuta*, *Y. cagnagella* and *Y. padellus*, had high levels of gene flow, with F_{ST} values of 0.027 and 0.03 respectively (Menken et al. 1980, Menken 1981). Diamondback moth, *Plutella xylostella*, was found to have

high levels of gene flow and an F_{ST} of 0.011 (Caprio and Tabashnik 1993). Finally, Daly (1989) estimated levels of gene flow in resistant *Heliothis armigera* to be 0.037.

Several studies have attempted to determine levels of gene flow in insects and relate them to ecological observations. For *Cacopsylla pyricola*, Unruh (1990) related an initial founder event in the northwestern U.S., and subsequent southward dispersal, to levels of genetic variation in psylla populations. Counter to expectations of high dispersal levels, gene flow estimates were only moderate, with $F_{ST} = 0.08$.

A second example, discussed by Slatkin (1987), demonstrated that direct observations of dispersal may disagree with estimates of gene flow. In this case, McKenchie et al. (1975) found low levels of genetic variation between populations of *Euphdryas editha*, indicating high levels of gene flow. However, when they used mark-release-recapture studies to directly measure dispersal of *E. editha*, they found very little movement of individuals between populations. Selection was given as a possible explanation for the lack of variation between populations, although unidirectional simultaneous selection at several loci was unlikely (Slatkin 1987). Instead, Slatkin proposed that the seeming discrepancy in

results could occur through bursts of gene flow which happened relatively recently. Small amounts of gene flow, even only over limited time periods, could account for a lack of differentiation between populations.

While dispersal has been examined in several predatory mite species (Bernstein 1983, Hoy et al. 1984, Dunley and Croft 1990), amounts of gene flow have not been determined. Dispersal in one species, *Typhlodromus pyri*, has been described both experimentally (Dunley and Croft 1990) and observationally (Boller et al 1988, Genini and Baillod 1987, Duso 1990). All studies concluded that dispersal in *T. pyri* was very low, with little emigration over distances as small as 10 m (Dunley and Croft 1990). This limited dispersal suggests that there should be relatively little gene flow occurring between populations of *T. pyri*.

In this chapter, estimates of gene flow are made through electrophoretic analysis of the predatory phytoseiid, *T. pyri*. Allozymes were examined from loci which were presumably not affected by pesticides, or correlated with pesticide use. Possible explanations for the findings are discussed in relation to the biology of *T. pyri*. In Chapter III, the results from this chapter are then used to make predictions regarding the

distribution of organophosphate resistance in *T. pyri*. Finally, the application of this study to development of pesticide resistance management programs using *T. pyri* is discussed in the final conclusion section.

Materials and Methods

Samples of *T. pyri* were collected from commercial apple orchards and nearby blackberry bushes at ten sites in the Willamette and Hood River Valleys, Oregon. Four populations were collected in the Willamette Valley, in and around two commercial apple orchards. At Hood River Valley, six populations were collected from in and around two orchards. Four populations were collected from one of the two Hood River orchards, with a pair collected from opposite ends of the orchard (greater than 1 km distant). Samples were arranged in a simple hierarchical manner: two populations from each orchard (one orchard population, one population 100 m outside of the orchard), two orchards from each valley, with two valleys.

Leaf samples were collected and adult females were removed under a 30X dissection microscope. Adult female mites without visible internal eggs were used. Sample size varied from 14 to 32 mites per population, with a total of 221 females collected.

Individual mites were placed in microcentrifuge tubes in 500 µl Tris glycine buffer (pH 6.0), and macerated using a glass pestle. 50 µl aliquots were placed in a sample well of a Super-Z sample loader (Helena Labs, Beaumont, Tex.). The sample loader was used to place 0.25 µl of mite extract per column on

cellulose acetate plates (Helena Labs, Beaumont, Tex.). Eight columns were used, with one individual per column.

Electrophoresis was conducted in Tris glycine buffer, using Whatcom #3 filter paper as wicks. Cellulose acetate plates were placed such that the wicks touched only the last mm of each side. Electrical charge was run for 20 minutes, 2 ma, 210 mv. Enzyme stains and reaction mixtures followed Easteal and Boussy (1987). At the end of each run, 2 ml of liquid agar containing the appropriate stain were poured on the surface of the cellulose acetate and allowed to cool. This procedure held the stain on the substrate long enough to produce dark staining bands.

Seven enzymes were initially tested for polymorphisms: arginine phosphokinase (APK), phospho-glucose isomerase (PGI), phospho-glucose mutase (PGM), mannose phosphate isomerase (MPI), alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), and aldehyde dehydrogenase (AO). Only APK, PGI, PGM and MPI were polymorphic in early samples, so ADH, MDH and AO were not run for all mites and are not included in the analyses. Genotype for each enzyme was determined for each mite.

Allozyme frequencies, F_{ST} and G_{ST} were analyzed using BIOSYS-1 (Swofford & Selander 1981). Additionally, θ_w and contribution of each subpopulation to overall χ^2 were calculated using GENESTAT (Weir 1990). F_{ST}, G_{ST}, and θ_w

were estimated for each locus, and over all four loci. Overall F_{ST} was used in calculating Nm. Additionally, F_{IS} (Workman and Niswander 1970) was included to determine whether significant deviations from random mating were occurring within subpopulations. Finally, genetic distances were calculated between populations to further estimate genetic relationships between populations (Nei 1972, 1978).

Results

Allele frequencies for the 10 *T. pyri* populations tested are given in Table II.1. Of these, there was significant deviation ($p < 0.05$) from expected frequencies using chi-square analysis for 7 of 40 locus x population combinations. This suggested that significant deviation from random mating occurred between subpopulations and indicated increased inbreeding or reduced gene flow.

Table II.2 lists the relative contribution to overall chi-square by each subpopulation for each locus. These data can be used to determine whether there are any patterns associated with locus or population. While for each locus there was significant variation among subpopulations ($p=0.001$ for each locus), no pattern was seen; MC1 was the only population which contributed >.10 to more than two loci. Moreover, the population most closely associated with MC1 did not deviate in the same manner, particularly for PGI.

Mean F-statistics over all populations are given in Table II.3. Values for F_{ST} for each locus indicated moderate genetic differentiation, suggesting moderate gene flow. Overall θ_w, averaged over the four loci, was 0.107 (std. dev. = 0.014). This gave an estimate of Nm as 2.08. F_{IS}, the fixation index, was significant for

Table II.1 Allozyme frequencies for 10 populations of *T. pyri* at four polymorphic loci. Populations were collected from commercial apple orchards and blackberry in surrounding vegetation, in Hood River and Willamette Valleys, Oregon.

	Population[a]									
Locus	MCN1	MCN2	MC1	MC2	BR1	BR2	ZL1	ZL2	AW1	AW2
PGI										
(N)	32	32	24	28	20	14	15	22	18	16
A	.500	.844	.792	.786	.775	.857	.733	.727	.944	.688
B	.500	.156	.208	.214	.225	.143	.267	.273	.056	.313
PGM										
(N)	32	32	24	28	20	14	15	22	18	16
A	1.000	1.000	.833	.982	.850	1.000	.700	.818	.667	.906
B	.000	.000	.167	.018	.150	.000	.300	.182	.333	.094
APK										
(N)	32	32	24	28	20	14	15	22	18	16
A	1.000	.891	.979	1.000	.675	.929	.700	.932	.889	.969
B	.000	.109	.021	.000	.325	.071	.300	.068	.111	.031
MPI										
(N)	32	32	24	28	20	14	15	22	18	16
A	.672	.672	1.000	.893	.850	.857	1.000	1.000	1.000	1.000
B	.328	.328	.000	.107	.150	.143	.000	.000	.000	.000

[a] Key to populations

Population name	Valley	Orchard	Vegetation
MCN1	HR	MC	Blackberry
MCN2	HR	MC	Apple
MC1	HR	MC	Blackberry
MC2	HR	MC	Apple
BR1	HR	BR	Blackberry
BR2	HR	BR	Apple
ZL1	WV	ZL	Blackberry
ZL2	WV	ZL	Apple
AW1	WV	AW	Blackberry
AW2	WV	AW	Apple

Table II.2 Contribution of *T. pyri* subpopulations to genetic structuring of the total population.

Allele		Chi Square	*p.*	Relative Contributions to Overall Chi-Square									
				MCN1	MCN2	MC1	MC2	BR1	BR2	ZL1	ZL2	AW1	AW2
PGI	A	35.393	.0001	.625	.079	.011	.009	.003	.046	.002	.005	.200	.021
	B	35.393	.0001	.625	.079	.011	.009	.003	.046	.002	.005	.200	.021
PGM	A	59.869	.0001	.127	.127	.031	.077	.013	.056	.198	.044	.326	.001
	B	59.869	.0001	.127	.127	.031	.077	.013	.056	.198	.044	.326	.001
APK	A	59.711	.0001	.107	.005	.047	.093	.448	.002	.268	.004	.003	.023
	B	59.711	.0001	.107	.005	.047	.093	.448	.002	.268	.004	.003	.023
MPI	A	72.671	.0001	.300	.300	.100	.004	.002	.000	.062	.091	.075	.067
	B	72.671	.0001	.300	.300	.100	.004	.002	.000	.062	.091	.075	.067

Table II.3. Mean F-statistics for 10 populations of *T. pyri* at four polymorphic loci. Populations were collected from commercial apple orchards and blackberry in surrounding vegetation, in Hood River and Willamette Valleys, Oregon.

Locus	F_{IS}[a]	F_{ST}[a]	G_{ST}[b]	θ_W[c]
PGI	0.292	0.070	0.080	0.062
PGM	0.272	0.126	0.135	0.123
APK	0.103	0.133	0.135	0.126
MPI	0.557	0.167	0.164	0.152
Mean	0.301	0.115	0.121	0.107
σ^2	--	--	--	0.014

[a] Wright (1978)

[b] Nei (1977)

[c] Weir and Cockerham (1984)

all four loci, indicating that there was deviation from random mating. This supports the chi-square analysis of allele frequencies.

Hierarchical F-statistics comparing variation between each of the subpopulations are given in Table II.4. These F-values were all low to moderate, indicating little differentiation between orchards (II.4.A) and little differentiation between valleys (II.4.B). (The negative value for the F-statistic comparing orchard and valley variation is due to an excess in heterozygotes). Thus, differentiation appeared to be at the population level, with no discernible patterns of genetic relationships.

Results from Nei's genetic distance analysis are given in matrix form in Table II.5. Two calculations are given: Nei's original genetic distance (1972), given above the diagonal, and below the diagonal is Nei's unbiased estimate of genetic distance (1978), after correction for differences in sample size between populations. Once again, no genetic relationships could be discerned.

Table II.4. Hierarchical F-statistics for 10 populations of *T. pyri* across four polymorphic loci. Populations were collected from commercial apple orchards and blackberry in surrounding vegetation, in Hood River and Willamette Valleys, Oregon.

Comparison		
X	Y	F_{XY}
POPULATION-	ORCHARD	.093
POPULATION-	VALLEY	.078
POPULATION-	TOTAL	.093
ORCHARD -	VALLEY	-.016
ORCHARD -	TOTAL	.000
VALLEY -	TOTAL	.016

Table II.5 Matrix of genetic distance coefficients for 10 populations of *T. pyri* across four polymorphic loci. Populations were collected from commercial apple orchards and blackberry in surrounding vegetation, in Hood River and Willamette Valleys, Oregon. Above the diagonal is Nei's genetic distance (1972), while below the diagonal is Nei's unbiased minimum distance (1978).

Population	MCN1	MCN2	MC1	MC2	BR1	BR2	ZL1	ZL2	AW1	AW2
MCN1	*****	.033	.055	.033	.059	.042	.086	.049	.107	.038
MCN2	.029	*****	.036	.016	.026	.009	.062	.039	.057	.037
MC1	.052	.033	*****	.009	.029	.014	.025	.002	.015	.004
MC2	.030	.013	.006	*****	.031	.003	.046	.012	.037	.007
BR1	.053	.020	.023	.026	*****	.023	.012	.023	.033	.030
BR2	.037	.004	.009	.000	.016	*****	.044	.018	.035	.015
ZL1	.078	.054	.018	.039	.002	.036	*****	.017	.020	.029
ZL2	.045	.035	.000	.008	.016	.012	.009	*****	.018	.003
AW1	.103	.053	.010	.033	.026	.030	.012	.013	*****	.032
AW2	.034	.032	.000	.003	.023	.009	.021	.000	.027	*****

Discussion

Results from allozymic analysis of *T. pyri* suggest that moderate amounts of genetic differentiation occur between populations in the Willamette and Hood River Valleys of Oregon. In these cases, gene flow estimates are only moderate relative to values for more vagile winged insects. While gene flow was less than expected between populations, based on the limited dispersal described for *T. pyri*, there was a lack of pattern in allelic variation that would exist if some gene flow were occurring. However, the evidence of significant inbreeding indicates that gene flow is still quite restricted within populations.

Hierarchical structuring of the samples does provide some information on the patterns of allelic variation. For allele frequencies, there was more variation between orchards and between valleys than within orchards or within valleys, respectively. Overall, the moderate inconsistency in the results may have arisen from several factors.

One possible explanation for the mixed results was the relatively small sample size. Reduced sample size, such as those smaller than 32 individuals, may incorporate too much sample variation to determine

patterns in allele frequency (Weir 1990). Also, the ability to detect alleles at low frequencies is reduced.

Another possible explanation for variation in results is the temporal limitation to estimating gene flow. Electrophoretic data samples one instant, while gene flow can occur over a long period of time. If there are periodic or rare events which stimulate dispersal, such as change in prey availability or decrease in host plant suitability, subsequent bursts of gene flow can bias future estimates. Depending on how much migration occurs at any one time, estimates of gene flow can be much higher than the actual amount of dispersal that occurs in each generation.

For *T. pyri*, like the previously discussed example of *Euphdryas editha* (McKenchie et al. 1975), it is likely that repeated colonization events occur. Several factors can impact *T. pyri* populations to cause localized extinction, including chemical sprays and prey depletion. Pesticides may directly impact the predators, or may cause prey mortality leading to predator starvation. *Typhlodromus pyri* avoids starvation more than some other phytoseiid species by using alternate food resources (Dicke 1988). However, sporadic outbreaks of spider mites can reach high densities and allow *T. pyri* to increase to relatively large numbers. Subsequent suppression of spider mites can then expose *T. pyri* to

extreme food stress. It is unknown whether density dependent dispersal associated with a reduction in prey levels occurs in *T. pyri,* and whether it affects measurements of gene flow. However, subsequent extinction and recolonization would give estimates of gene flow higher than expected from a fairly sessile arthropod.

In summary, estimates of gene flow in *T. pyri* were higher than expected for a phytoseiid species which has dispersal limited to < 10 m per year (Dunley and Croft 1990). These estimates may have been influenced by the population dynamics of *T. pyri,* or by limited sample size, or both. The differing appraisals of dispersal lead to opposing hypotheses regarding the evolution of OP resistance in *T. pyri.* If gene flow is low, resistance would evolve relatively rapidly in habitats with OP use. Immigration of susceptible mites into treated area would be low, and the frequency of resistant alleles would not be reduced following selection by interbreeding with susceptible mites. In this case, OP resistance would most likely be distributed primarily in treated habitats. If dispersal is higher, as indicated by the gene flow estimates, OP resistance could rapidly evolve because of higher rate of spread. In this model, resistance would develop in one area, then would be introduced through dispersal into other areas. Selection from OP use would then increase the occurrence of resistance in the new

areas, followed by further spread. This scenario predicts that resistance would be present outside of OP treated habitats, but in decreasing in frequency with distance. Chapter III of this dissertation will address the distribution of OP resistance in *T. pyri*, and provide more information on possible mechanisms for resistance evolution.

CHAPTER III

Factors Affecting The Distribution of Organophosphate Resistance in *Typhlodromus pyri* and *Metaseiulus occidentalis* in the Hood River and Willamette Valleys of Oregon

Introduction

While genetic and population genetic studies of pesticide resistance are used to examine how pesticide resistance may evolve, studies of ecological factors which influence these processes are relatively uncommon (Roush and McKenzie 1987). The components of ecological genetics may be divided into two classes: intrinsic and extrinsic (Futuyma 1986). Intrinsic ecological factors are those which are inherent to the genetics of the organism, such as reproductive fitness, reproductive strategy, resource utilization, and propensity for dispersal. Extrinsic ecological factors are those which are not directly related to the biology of the organism, but instead act upon the organism. Intensity of selection and habitat heterogeneity are examples of extrinsic factors. Knowledge of intrinsic and extrinsic factors together are important not only in understanding and predicting resistance evolution, but also in practicing resistance management (Roush and Tabashnik 1991).

Many ecological genetic factors have been studied in phytoseiid mites. This has been due to the importance of phytoseiids in agriculture and their relative propensity to develop resistance compared to other biological control agents. Factors demonstrated to influence the

rate of resistance evolution in phytoseiids are intrinsic biological traits such as rapid reproduction (Tabashnik and Croft 1982), pseodo-arrhenotoky (Hoy 1985), prey density dependence (Tabashnik 1986), and dispersal (Dunley and Croft 1992). In addition, extrinsic factors such as availability of refugia (Tabashnik and Croft 1982), intensity of pesticide use (Tabashnik and Croft 1982, Tabashnik 1986), and habitat heterogeneity affect the occurrence and distribution of resistance (Taylor and Georghiou 1979).

In this chapter, the effects of various intrinsic and extrinsic ecological factors on the respective distributions of organophosphate resistance in two phytoseiid mites were examined. Comparison were made of the distributions of OP resistance in populations of *T. pyri* and *M. occidentalis* from orchards versus surrounding vegetation in HR and WV. Additionally, the effect of the relative amount of surrounding managed and unmanaged vegetation within valley each valley was examined. This study compared centrally located orchards surrounded primarily by other orchards with orchards located on the margins of the growing areas, surrounded by unsprayed vegetation. Finally, the impact of host plant substrate and effect of indirect exposure to pesticides were determined. These factors are discussed within the context of OP resistance management in phytoseiid mites.

Materials and Methods

Laboratory Bioassay

Diagnostic Concentration

Laboratory colonies of *T. pyri* and of *M. occidentalis* were established in 1988 from 200-500 field collected adult female mites. OP-resistant (OP-R) *T. pyri* were collected from commercial apple orchards in HR and WV known to have a history of successful integrated mite management (i.e., OP sprays did not disrupt spider mite control). An OP-R *M. occidentalis* strain was collected from non-disrupted orchards in HR. OP-susceptible (OP-S) *T. pyri* were collected from wild blackberry in Lebanon and Corvallis, Oregon. These two populations were far removed (>10 k) from any commercial agriculture. We were unable to locate OP-S *M. occidentalis*, so tolerance lines were derived from Babbcock and Tanigoshi (1988).

Each strain was reared on four trays (10 by 10 cm), containing a paper substrate and a water moat (McMurtry & Scriven 1964). Each tray was lined with adhesive (Tanglefoot^{R}, Tanglefoot Co., Grand Rapids, Mich.) and maintained at constant photoperiod of 16:8 (L:D) and temperature (20^{o}+2^{o} C). Predators were fed mixed stages of *Tetranychus urticae* Koch and corn pollen three times weekly. Mites were tested at each of five to seven

concentrations of azinphosmethyl (Guthion 50 Wettable Powder [WP], Mobay Corp., Kansas City, Mo.) per strain. Controls were treated with distilled water.

Bioassays were conducted by modified slide-dip technique (Croft *et al.* 1976). Twenty to 30 gravid adult females per replicate were mounted dorsally on adhesive tape affixed to a glass microscope slide using double-sided sticky tape. The slide was dipped in each pesticide concentration for 5 s then air dried for 20 minutes. Slides were placed in a closed plastic box (40 by 25 by 15 cm) which contained moistened paper towels to maintain humidity (approximately 80%). Mortality was measured under a dissecting microscope at 48 h: mites that failed to respond with leg movements after a gentle touch with a fine camel hair brush were scored as dead. Four replicates were tested per concentration per strain. Probit regressions lines were estimated using the probit option of POLO (Russell *et al.* 1977). Likelihood ratio tests were used to test the hypotheses of equality and parallelism of response lines. The diagnostic concentration for determining frequency of OP resistance in field populations was determined to be 0.10 % a.i. for both species. This concentration caused maximum mortality in the OP-S strains while having least toxicity to the OP-R strains.

Diagnostic Bioassay on Field Populations

In the laboratory, phytoseiid mites from field samples were identified and counted under a 30X binocular dissecting microscope. The number, stage, and species of all mites on 40 randomly chosen leaves was recorded. Adult females were separated by species and removed from leaves with a fine camel hair brush. Representative subsamples of mites were placed on microscope slides in Hoyer's solution for clearing and identification using a phase-contrast microscope. All other adult females were subjected to bioassay for determination of resistance frequency using 0.10 % a.i. azinphosmethyl. At least three replicates of more than 20 adult females each was tested for most populations. In a few cases, the low phytoseiid density present did not allow this level of replication.

Statistical analysis of resistance frequency was by analysis of variance using orthogonal contrasts of percent mortality at the diagnostic concentration. Normality of data was achieved through arcsine transformation.

Field Studies

Experiments were conducted in the Hood River (HR) Valley of north central Oregon, and the Willamette Valley

(WV) of western Oregon. Eight orchards were used, four in HR that contained *T. pyri* and *M. occidentalis* and four in WV with *T. pyri*. Sites were chosen based on three criteria: presence of predatory mite species in the orchards; suitable habitat for phytoseiid mites in surrounding vegetation; correlation to predetermined test categories (Tables III.1 and III.2). Different sampling schemes were used in 1989 and 1990.

1989

Linear transects were established at each of the orchards beginning 100-200 m outside of the orchard in the surrounding vegetation. Transects were 800-1000 m in length at two sites in each valley (HR: MC,BR; WV: ZS,AW), while the remaining transects were 300 m (HR: BW,WM; WV: RJ, NU). Transects traversed the unsprayed habitat, went into the orchard, and continued into the surrounding vegetation on the opposite side. Leaf samples (2-4 *l*) were taken from blackberry (*Rubus* spp.) in the native vegetation, and from commercial apple trees, apple root suckers and blackberry within orchards. Samples were located along the transects at 200 m, if logistically possible, and at 100 and 10 m into the native vegetation. Within the orchards, samples were taken 10 m inside the orchard margin (hereafter edge

Table III.1. Hierarchical organization of factors influencing spatial distribution of organophosphate resistance in *T. pyri* and *M. occidentalis*

Factor	Level		
Valley	WV	HR	
Orchard Type	Isolated	Non-Isolated	
Location	Orchard	Groundcover	Surrounding Vegetation
Distance	middle, edge		10 m, 100 m, 200 m
Host Plant	Apple	Blackberry	
Season	Early	Mid	Late

Table III.2. Description of orchard sites with regard to valley and location in the valley (center = non-isolated = surrounded primarily by other commercial orchards; margin = isolated = surrounded by native vegetation), length of linear sampling transect, and phytoseiid fauna.

Orchard	Valley	Location in Valley	Transect Length	Phytoseiid Species
MC	HR	Center	1000 m	*T. pyri, M. occidentalis*
WM	HR	Center	300 m	*T. pyri, M. occidentalis*
BW	HR	Margin	300 m	*T. pyri, M. occidentalis*
AB	HR	Margin	800 m	*T. pyri, M. occidentalis*
ZS	WV	Center	1000 m	*T. pyri*
RJ	WV	Center	300 m	*T. pyri*
NU	WV	Margin	300 m	*T. pyri, M. occidentalis*
AW	WV	Margin	800 m	*T. pyri, M. occidentalis*

samples), and from the transect midpoint as it passed through the orchard (hereafter middle samples). The sampling scheme was mirrored on the reciprocal end of the transect. Samples were collected three times: in early season (May 10-17), midseason (June 23-29), and late season (August 4-11).

For samples from the surrounding vegetation, mature leaves were removed from the blackberry bushes using hand pruners. Apple leaves were collected from six apple trees (approx. 500 ml each) over two rows for edge and middle samples. Additional samples were made of orchard groundcover vegetation, taking apple root suckers and blackberry. All leaves were placed in brown paper sacks, then collected in plastic garbage sacks and kept cool (4 $C^{o)}$ until processing.

1990

Experiments in 1990 were based on a five by five grid sampling scheme, using only one area in each valley. The HR grid encompassed the MC orchard, while the WV grid contained the ZS orchard, along with the associated surrounding habitat. Leaf samples were collected at equally spaced points within the grid, separated by 200 m. Samples were taken over the same time of the season as 1989, and were treated in the same manner.

An additional site was used in 1990 and 1991 to test the effects of OP spray cessation on resistance persistence. Following the 1989 growing season, the grower at the AB orchard in HR followed organic growing practices and stopped using OP sprays . The transect sampling method established in 1989 was followed for 1990. Samples were collected during the same time of the season as 1989. One final sample was taken from AB in Oct. 1991, two full growing seasons after cessation of OP sprays.

Results

Diagnostic Concentration

Results from probit analysis for the development of a diagnostic concentration of azinphosmethyl are shown in Table III.3 and Figure III.1. A discriminating dose, one that kills all susceptible mites and no resistant mites, was not found because the regression lines for both *T. pyri* and *M. occidentalis* overlapped. Instead, a diagnostic dose of 0.10% a.i. was found to cause approximately 10-20% mortality in the resistant lab populations of both species, and 80% mortality in the susceptible populations.

Field Populations - 1989

The mean frequency of azinphosmethyl resistance for each sample and location in HR and WV for 1989 are given in Tables III.4 for *T. pyri* and III.5 for *M. occidentalis*. There were no significant differences among orchards in levels of azinphosmethyl resistance at each of the sample locations. While there was a general decrease in levels of resistance over the season for *T. pyri*, changes were not significant within sample locations (Table III.6). The pattern seen for *M. occidentalis* was quite different from that of *T. pyri*.

Table III.3. Toxicity of azinphosmethyl to susceptible (OP-S) and resistant (OP-R) laboratory strains of *T. pyri* and *M. occidentalis*.

	Strain	*n*	Slope ± SE	LC_{50}	95% FL
T. pyri	OP-S	541	2.59 ± 0.31	0.013	0.007 - 0.022
	OP-R	603	2.94 ± 0.55	0.230	0.152 - 3.56
M. occidentalis	OP-S[a]	--[b]	.86	0.0073	-- [b]
	OP-R	622	2.32 ± 0.42	1.74	1.20 - 2.44

[a] information for OP-S *M. occidentalis* derived from Babcock and Tanagoshi 1988

[b] information unavailable

Figure III.1 Concentration-mortality lines for organophosphate susceptible and resistant laboratory populations of *T. pyri* and *M. occidentalis*. The line for the susceptible *M. occidentalis* population was derived from Babcock and Tanigoshi (1988).

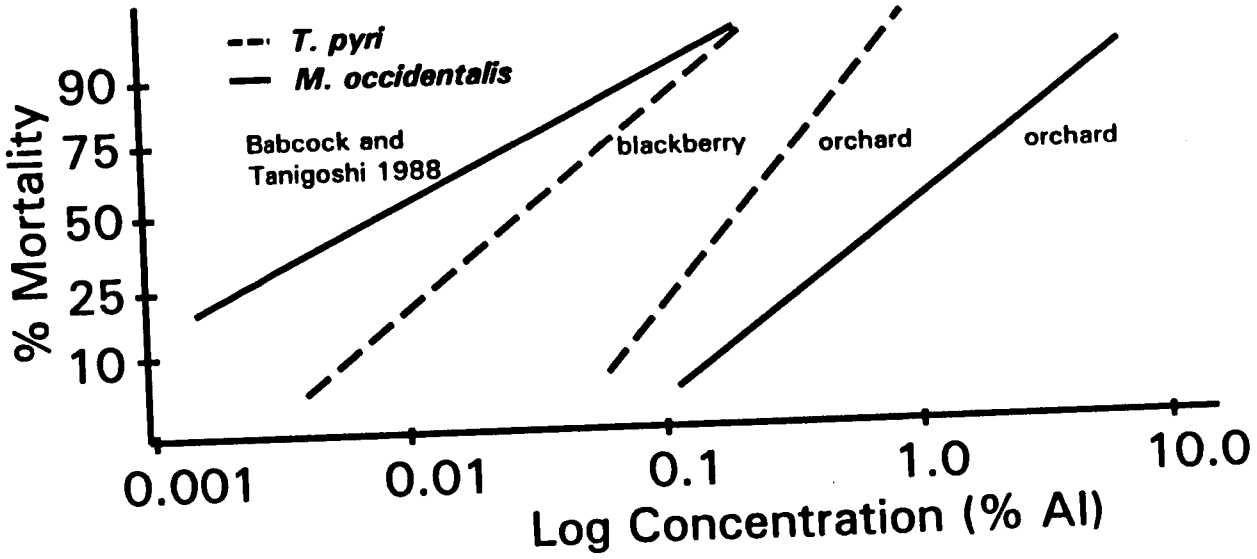
T. pyri
M. occidentalis
Babcock and
Tanigoshi 1988
blackberry
orchard
orchard
% Mortality
90
75
50
25
10
0.001
0.01
0.1
1.0
10.0
Log Concentration (% AI)

Figure III.1

Table III.4. Frequency of azinphosmethyl resistance as percent survival for *T. pyri* populations in the Hood River and Willamette Valleys of Oregon in 1989. Numbers in parentheses are sample sizes. Populations were sampled along transects. Resistance frequency was calculated using slide-dip bioassay, and was corrected for control mortality.

	Early Season Sample Location					Mid Season Sample Location					Late Season Sample Location				
	Orchard		Surr.Veg.			Orchard		Surr.Veg.			Orchard		Surr.Veg.		
Orch.	mid	edge	10 m	100 m	200 m	mid	edge	10 m	100 m	200 m	mid	edge	10 m	100 m	200 m
MC	94.2	87.4	2.7	7.1	9.0	79.0	90.0	14.0	8.5	8.1	94.3	81.1	10.8	14.7	8.4
	(180)	(172)	(191)	(178)	(193)	(182)	(148)	(172)	(140)	(67)	(108)	(84)	(101)	(84)	(91)
AB	84.8	90.0	7.2	20.6	4.5	73.9	89.6	7.1	7.6	9.9	75.8	81.3	8.1	12.5	6.9
	(72)	(124)	(88)	(35)	(74)	(87)	(76)	(59)	(68)	(57)	(77)	(127)	(42)	(34)	(93)
WM	68.8	67.4	4.9	0.0	-----	78.9	90.0	15.7	12.0	-----	69.3	65.4	18.8	12.8	-----
	(31)	(54)	(40)	(40)		(97)	(71)	(98)	(18)		(52)	(93)	(60)	(85)	
BW	74.0	77.1	18.0	17.2	-----	75.0	91.7	12.9	25.0	-----	80.0	90.0	15.3	10.0	-----
	(19)	(29)	(60)	(40)		(24)	(48)	(22)	(16)		(20)	(16)	(31)	(15)	
ZL	77.9	69.3	18.0	6.5	6.0	58.1	79.9	21.0	18.8	10.0	57.8	54.9	19.1	14.3	11.1
	(96)	(78)	(39)	(78)	(80)	(69)	(76)	(72)	(61)	(21)	(48)	(64)	(101)	(14)	(18)
RJ	76.8	72.0	10.0	16.1	-----	72.0	81.1	17.7	15.5	-----	80.0	86.9	12.1	14.6	-----
	(55)	(50)	(49)	(79)		(240)	(148)	(72)	(104)		(208)	(68)	(54)	(184)	
NU	86.9	92.5	14.3	17.1	-----	69.6	65.7	14.7	19.6	-----	74.8	77.4	12.9	21.0	-----
	(137)	(112)	(92)	(180)		(106)	(117)	(127)	(39)		(93)	(89)	(76)	(91)	
AW	85.5	65.9	16.1	8.0	17.6	67.8	71.1	19.1	11.3	16.0	67.4	84.6	20.6	14.8	22.0
	(67)	(75)	(80)	(81)	(93)	(82)	(84)	(91)	(51)	(43)	(111)	(62)	(67)	(57)	(96)

Table III.5. Frequency of azinphosmethyl resistance as percent survival for *M. occidentalis* populations in the Hood River and Willamette Valleys of Oregon in 1989. Numbers in parentheses are sample sizes. Populations were sampled along transects. Resistance frequency was calculated using slide-dip bioassay, and was corrected for control mortality.

	Early Season Sample Location					Mid Season Sample Location					Late Season Sample Location				
	Orchard		Surr.Veg.			Orchard		Surr.Veg.			Orchard		Surr.Veg.		
Orch.	mid	edge	10 m	100 m	200 m	mid	edge	10 m	100 m	200 m	mid	edge	10 m	100 m	200 m
MC	88.6	81.0	78.5	89.7	92.0	82.1	91.1	78.5	77.0	84.0	100	82.0	75.5	70.0	75.0
	(121)	(56)	(32)	(53)	(25)	(155)	(164)	(80)	(67)	(27)	(54)	(64)	(52)	(33)	(21)
AB	83.5	87.0	84.6	69.8	90.3	79.3	83.2	76.6	77.6	90.0	73.0	74.0	79.5	84.5	72.0
	(56)	(87)	(65)	(62)	(48)	(85)	(79)	(49)	(68)	(40)	(45)	(50)	(60)	(24)	(18)
WM	85.9	94.5	88.8	75.4	-----	79.8	87.4	79.4	92.0	-----	71.0	76.4	84.0	76.8	-----
	(78)	(57)	(42)	(67)		(90)	(83)	(39)	(29)		(24)	(47)	(35)	(38)	
BW	97.0	84.5	78.9	79.2	-----	85.0	78.8	76.5	75.0	-----	78.9	82.3	82.2	76.8	-----
	(87)	(59)	(51)	(59)		(90)	(76)	(41)	(36)		(54)	(47)	(39)	(31)	
ZL	80.0	76.6	83.5	100	-----	88.9	82.5	80.0	76.0	-----	80.0	70.0	80.0	85.0	-----
	(26)	(65)	(22)	(20)		(16)	(44)	(24)	(25)		(21)	(20)	(20)	(20)	
RJ	82.2	84.7	60.0	85.6	-----	90.0	86.3	69.8	80.0	-----	82.0	75.0	76.6	75.0	-----
	(54)	(50)	(20)	(43)		(48)	(71)	(29)	(25)		(36)	(30)	(28)	(40)	
NU	85.0	90.0	86.8	75.0	-----	74.6	68.8	72.4	82.6	-----	72.5	71.4	82.5	79.0	-----
	(121)	(84)	(54)	(25)		(58)	(66)	(37)	(42)		(45)	(60)	(40)	(36)	
AW	90.0	88.1	82.7	91.0	60.0	80.0	66.7	75.4	77.3	52.2	100	80.2	75.0	81.3	51.0
	(31)	(38)	(42)	(16)	(20)	(45)	(15)	(25)	(23)	(35)	(24)	(28)	(16)	(29)	(45)

Table III.6. Seasonality of levels of OP resistance in *T. pyri* and *M. occidentalis* populations in commercial apple orchards and the surrounding vegetation in Hood River and Willamette Valley, Oregon, 1989. Values in columns followed by the same letter are not significantly different.

	T. pyri		*M. occi.*	
	Orchard	Surr. Veg.	Orchard	Surr. Veg.
Early	76.9 a	18.5 a	88.0 a	82.3 a
Mid	74.3 a	17.4 a	84.5 a	79.6 a
Late	73.9 a	18.1 a	81.4 a	80.1 a

Mean resistance frequencies over all sample locations within valleys are given in Figure III.2. There were no significant differences between valleys for resistance frequencies at each of the sample distances. Populations in the Hood River Valley were consistently higher in resistance levels, however these differences were not significant. Furthermore, resistance frequencies among valleys between locations (sample distances) were not significantly different for *M. occidentalis*, whereas levels of OP resistance were significantly different for *T. pyri* between distances. *Typhlodromus pyri* populations within orchards remained significantly more resistant than populations in surrounding vegetation, even when pooled over orchards and valleys.

No effect of orchard type (location in the valley) was found (Table III.7). Isolated orchards located on the margin of the growing regions did not differ significantly in the levels of resistance within the orchards or in the surrounding vegetation.

Host plant and population location also did not affect levels of OP resistance in either *T. pyri* or *M. occidentalis* (Table III.8). Populations from the same location had the same levels of resistance whether the plant substrate was apple or blackberry. Furthermore, populations from orchard trees which received direct

Figure III.2 Mean frequencies of organophosphate resistance in adult female phytoseiid mites sampled in 1989-1990 from apple orchards and surrounding vegetation in the Hood River and Willamette Valleys, Oregon. Bars represent 2 s.e.m.

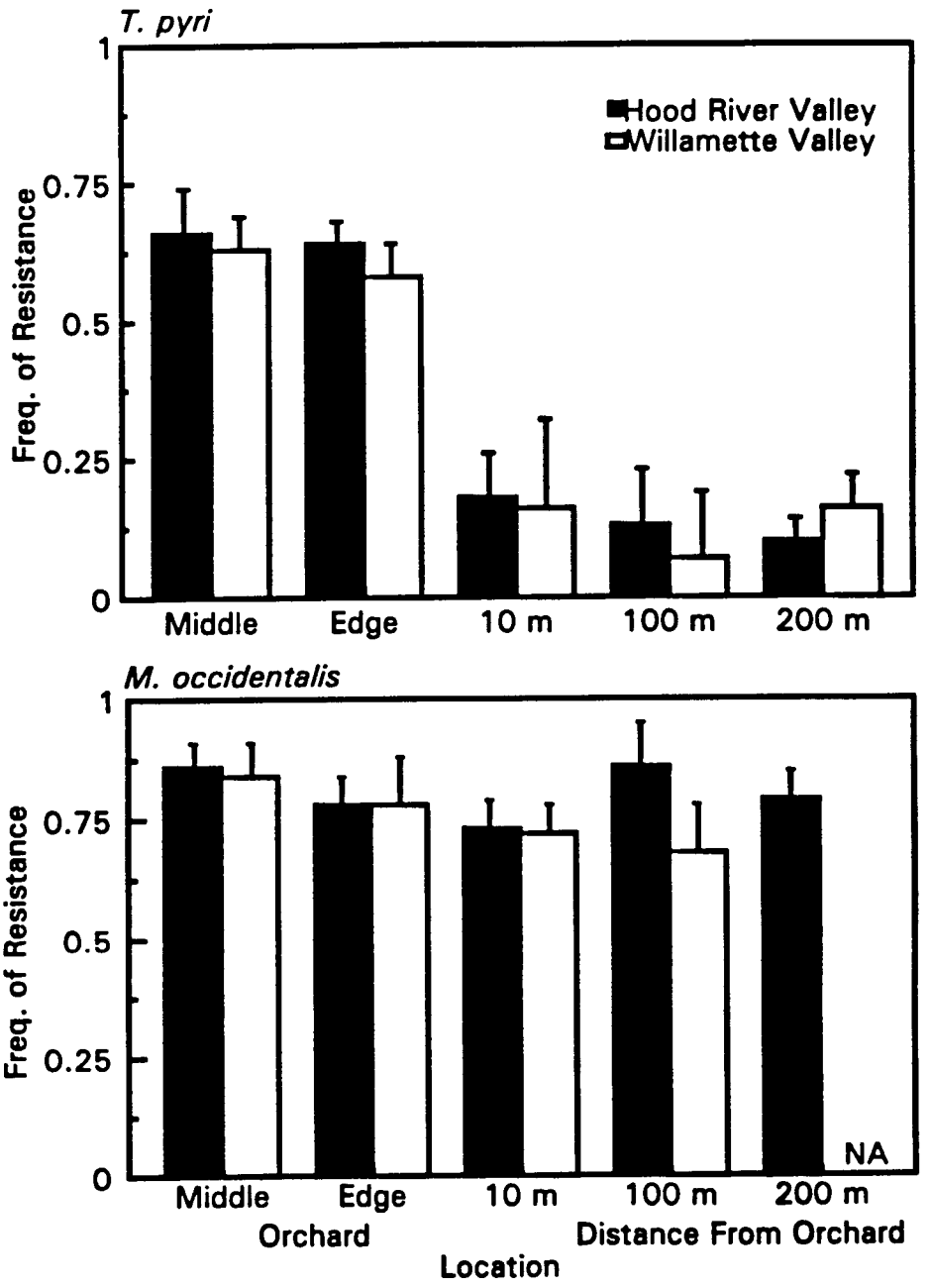
T. pyri
Hood River Valley
Willamette Valley
Freq. of Resistance
1
0.75
0.5
0.25
0
Middle
Edge
10 m
100 m
200 m
M. occidentalis
Freq. of Resistance
1
0.75
0.5
0.25
0
NA
Middle
Edge
10 m
100 m
200 m
Orchard
Distance From Orchard
Location

Figure III.2

Table III.7. Effect of orchard location on levels of organophosphate resistance in *T. pyri* and *M. occidentalis* within the HR and WV apple growing regions of Oregon. Isolated orchards are on the edge of the growing area and are surrounded primarily by native vegetation, while non-isolated orchards are surrounded primarily by other orchards. Values in columns followed by the same letter are not significantly different.

T. pyri				
Isolated	Orchard		Surr. Veg.	
HR	77.0	a	17.8	a
WV	74.3	a	18.4	a
Non-isolated				
HR	74.4	a	18.1	a
WV	74.0	a	16.8	a
M. occi.				
Isolated				
HR	85.6	a	82.6	a
WV	83.4	a	76.0	a
Non-isolated				
HR	84.6	a	80.0	
WV	86.3	a	78.8	

Table III.8. Effect of host plant and sample location on levels of organophosphate resistance in *T. pyri* and *M. occidentalis*.

Habitat Plant Host	Orchard Apple	Groundcover Apple	Blackberry	Surr. Veg. Apple	Blackberry
T. pyri	75.2	74.8	75.3	17.5	18.2
M. occi.	85.6	84.5	84.7	56.0	80.8

pesticide applications did not differ in levels of resistance from population in the groundcover that received more indirect selection pressure.

Examples of the patterns of OP resistance levels found at specific orchards along transects for *T. pyri* and *M. occidentalis* are given in Figures III.3 and III.4. In both HR and WV, the frequency of OP resistance in *T. pyri* was significantly lower in samples taken outside of orchards, including samples immediately adjacent (10 m). For *M. occidentalis*, however, levels of OP resistance were much more consistent along the entire transect.

Hood River Grid: 1990

Results from the HR grid in 1990 are shown in Figures III.5 for *T. pyri* and III.6 for *M. occidentalis*. No significant changes in population resistance levels were found over the 1990 season. For *T. pyri*, resistance frequencies were significantly different between orchard populations and the populations in unsprayed vegetation. Populations within the commercial orchard were not different, and there were no differences between populations sampled from the surrounding unsprayed vegetation. No significant differences were found between any populations of *M. occidentalis*.

Figure III.3 Frequencies of organophosphate resistance in *T. pyri* and *M. occidentalis* at the MC orchard in Hood River, Oregon. Samples were taken along transects beginning in surrounding vegetation, travelled through the apple orchard, crossed unsprayed native vegetation, then continued across another apple orchard. Mites were collected and bioassayed for resistance in May, July, and September 1989.

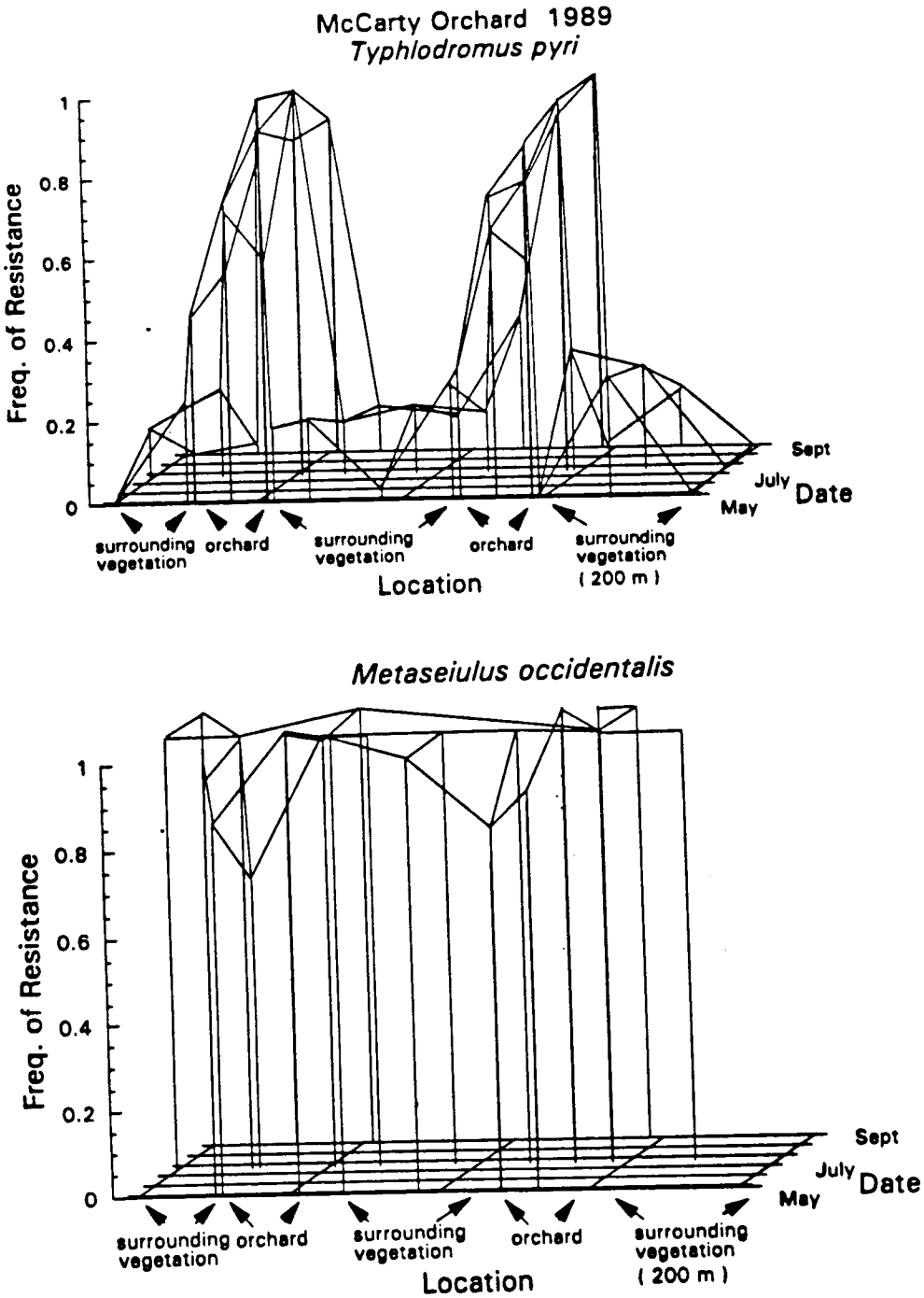
McCarty Orchard 1989
Typhlodromus pyri
Freq. of Resistance
1
0.8
0.6
0.4
0.2
0
Sept
July
May
Date
surrounding vegetation
orchard
surrounding vegetation
orchard
surrounding vegetation (200 m)
Location
Metaseiulus occidentalis
Freq. of Resistance
1
0.8
0.6
0.4
0.2
0
Sept
July
May
Date
surrounding vegetation
orchard
surrounding vegetation
orchard
surrounding vegetation (200 m)
Location

Figure III.3

Figure III.4 Frequencies of organophosphate resistance in *T. pyri* and *M. occidentalis* at the AB orchard in Hood River, Oregon. Samples were taken along transects in May, July, and September 1989. Transects began and ended in unsprayed surrounding vegetation, crossing through the apple orchard.

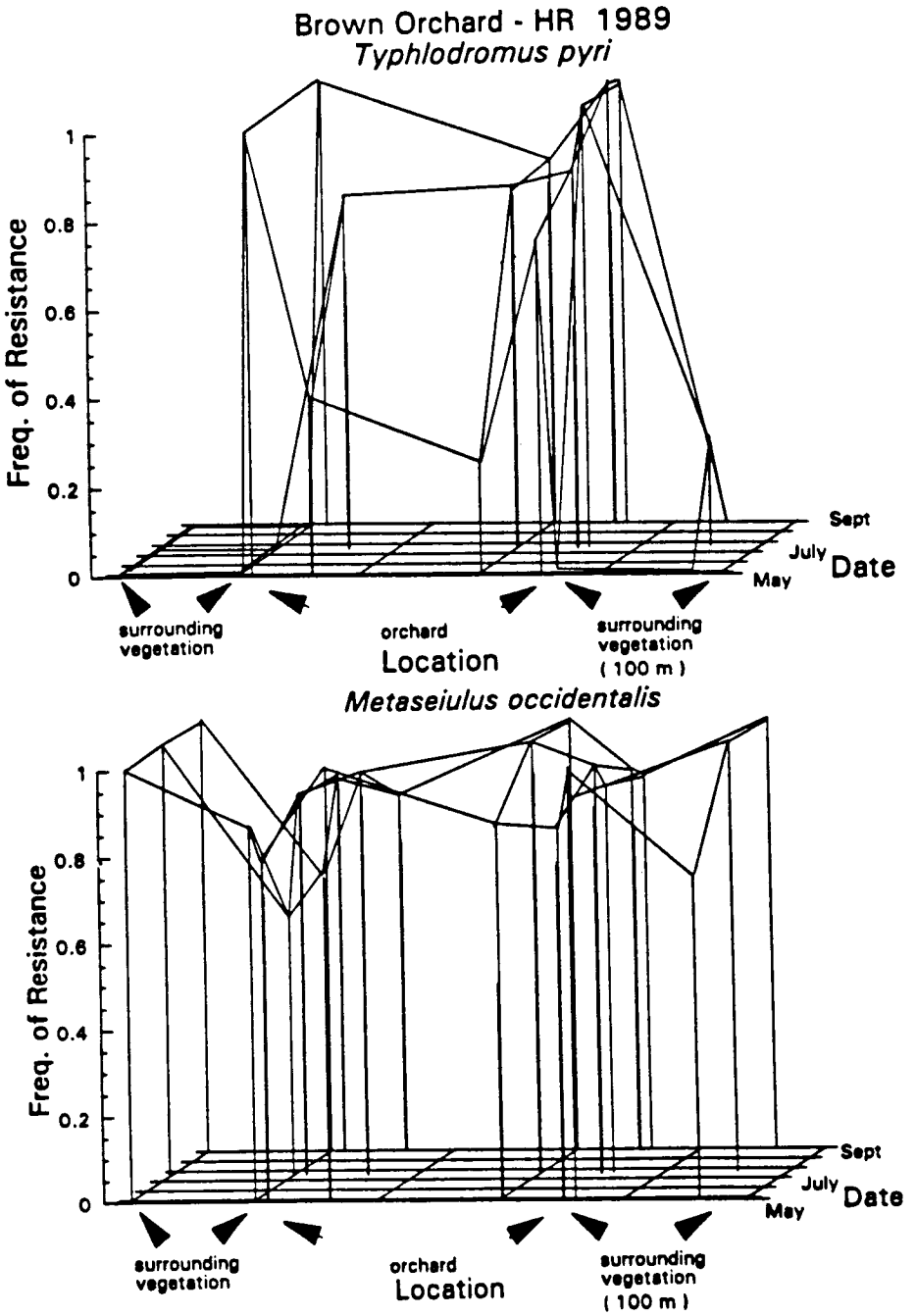
Brown Orchard - HR 1989
Typhlodromus pyri
Freq. of Resistance
1
0.8
0.6
0.4
0.2
0
Sept
July
May
Date
surrounding vegetation
orchard
Location
surrounding vegetation (100 m)
Metaseiulus occidentalis
Freq. of Resistance
1
0.8
0.6
0.4
0.2
0
Sept
July
May
Date
surrounding vegetation
orchard
Location
surrounding vegetation (100 m)

Figure III.4

Figure III.5 Frequencies of organophosphate resistance in *T. pyri* using a 5 x 5 grid in Hood River, Oregon, in 1990. Nearest neighbor analysis was used to interpolate between sample points. Shaded areas represent commercial apple orchard, unshaded areas are unsprayed surrounding vegetation.

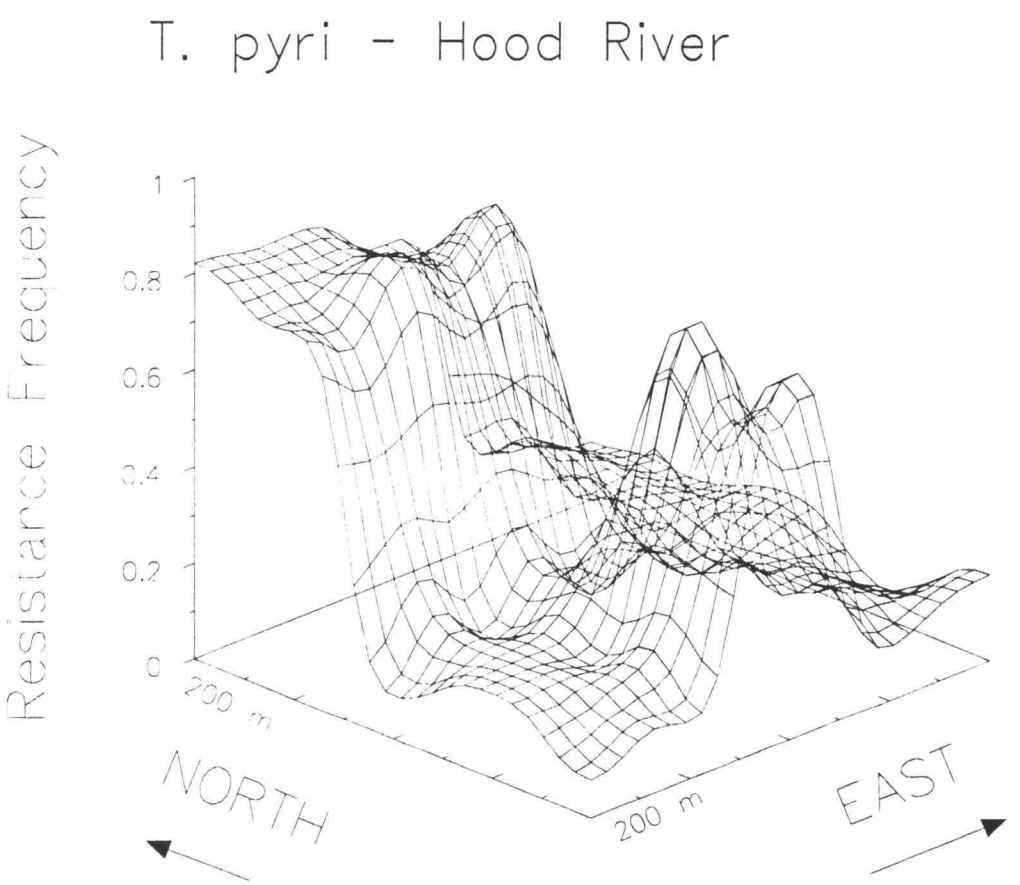
T. pyri - Hood River
Resistance Frequency
1
0.8
0.6
0.4
0.2
0
200 m
NORTH
200 m
EAST

Figure III.5

Figure III.6 Frequencies of organophosphate resistance in *M. occidentalis* using a 5 x 5 grid in Hood River, Oregon, in 1990. Nearest neighbor analysis was used to interpolate between sample points. Shaded areas represent commercial apple orchard, unshaded areas are unsprayed surrounding vegetation.

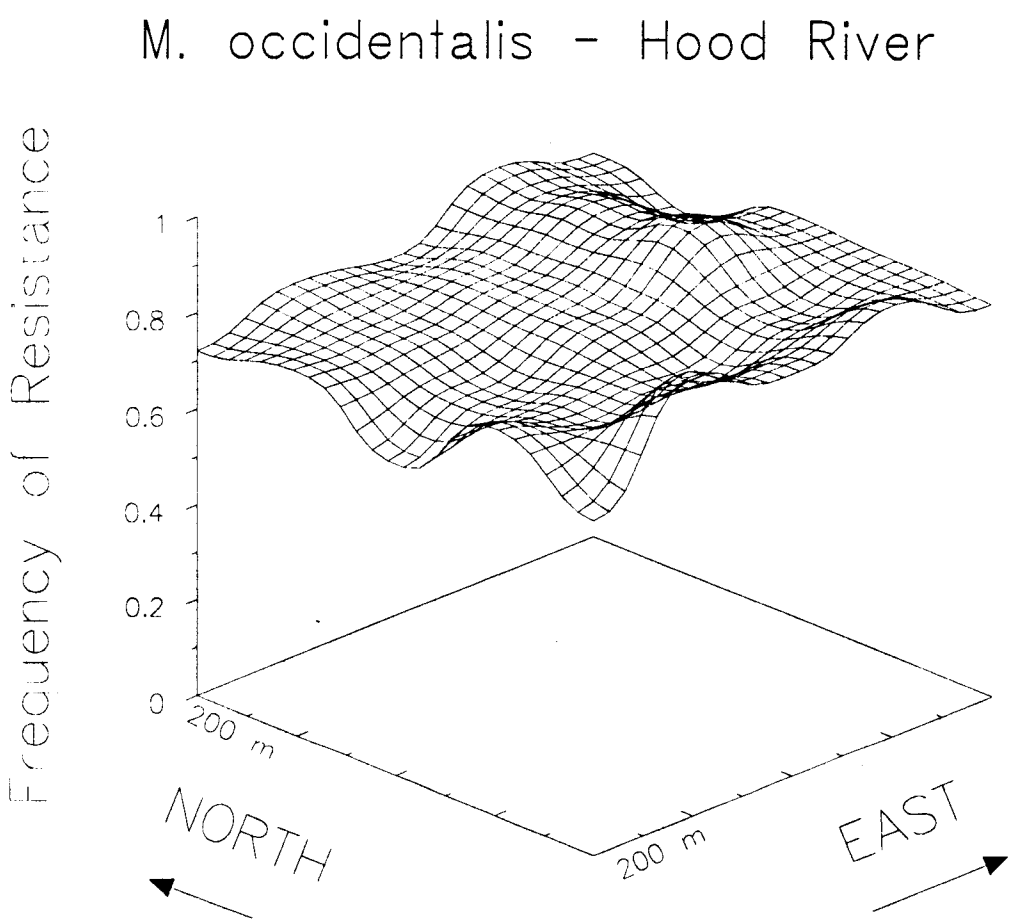
M. occidentalis - Hood River
Frequency of Resistance
1
0.8
0.6
0.4
0.2
0
200 m
NORTH
200 m
EAST

Figure III.6

Willamette Valley Grid: 1990

Frequencies of OP resistance in *T. pyri* from the WV grid in 1990 are shown in Figure III.7. Frequencies of OP resistance did not change significantly over the season at any given site. Significant differences in levels of resistance in *T. pyri* were found between populations in orchards and unsprayed vegetation. Samples within the commercial orchard were not significantly different, as was the case among populations sampled from surrounding vegetation.

Unsprayed Orchard: 1990-1991

Results from the AB orchard in 1990 and 1991, years when the orchard was not sprayed with synthetic pesticides, are shown in Table III.9. Levels of resistance did not change significantly from one year to the next, even following the cessation of sprays for two entire growing seasons. Additionally, resistance frequencies were not significantly different between the orchard and surrounding vegetation for *M. occidentalis*, however, significant differences remained for *T. pyri*.

Figure III.7 Frequencies of organophosphate resistance in *T. pyri* using a 5 x 5 grid in the Willamette Valley, Oregon, in 1990. Nearest neighbor analysis was used to interpolate between sample points. Shaded areas represent commercial apple orchard, unshaded areas are unsprayed surrounding vegetation.

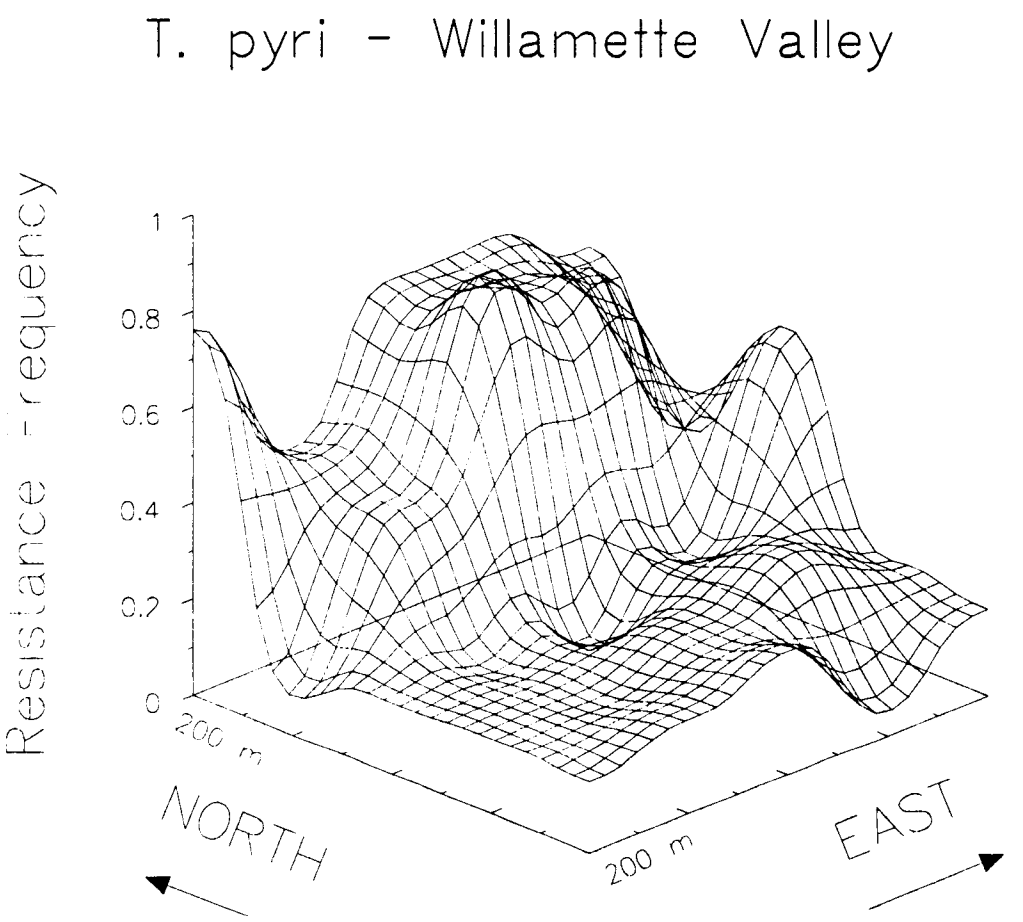
T. pyri - Willamette Valley
Resistance - requency
1
0.8
0.6
0.4
0.2
0
200 m
NORTH
200 m
EAST

Figure III.7

Table III.9. Levels of organophosphate resistance in *M. occidentalis* and *T. pyri* at AB orchard in HR for 1989-1991. Applications of organophosphates to the orchard ceased in 1989. Values for each phytoseiid species in columns followed by the same letter are not significantly different.

Species		Sample Location			
	Orchard		Surr.Veg.		
Year	mid	edge	10 m	100 m	200 m
T. pyri					
1989	78.2a	87.0a	7.5b	13.6b	7.1b
1990	84.0a	85.0a	10.0b	0.0b	7.5b
1991	76.7a	73.9a	5.0b	5.0b	8.4b
M. occi.					
1989	78.5a	83.2a	80.7a	76.0a	84.0a
1990	77.5a	80.0a	75.0a	82.5a	82.5a
1991	85.0a	82.5a	78.0a	84.0a	85.0a

Discussion

Typhlodromus pyri was the only species to have heterogeneous frequencies of OP resistance. All *T. pyri* populations within sprayed commercial orchards had high survivorship at the diagnostic concentration, while populations taken at 10, 100, and 200 m outside orchards consistently were much more susceptible to the diagnostic concentration. This pattern occurred at all orchards in HR and WV. From these data, it appeared that gene flow was limited from the commercial orchards into the surrounding habitat. Also, the observation that resistance levels increased only slightly after the early season samples (and application of OP sprays) demonstrates that susceptible mites were not immigrating in appreciable numbers into the margin of the orchards. The distribution of the OP resistance trait was limited in *T. pyri* to sprayed orchards, and was very low in populations immediately adjacent to the sprayed area (10m). *T. pyri* may not seem to disperse in adequate numbers from the treated area to build up resistance in the surrounding vegetation; those individuals that do disperse have little impact in the large susceptible reservoir in the surrounding vegetation. An alternative explanation is that there may be reduced fitness associated with OP resistance. However, this does not

appear to be the case, as the frequency of OP resistance remained constant both in the laboratory and in the AB orchard two years after selection pressure was removed.

Metaseiulus occidentalis had near homogeneous levels of OP resistance, both within sprayed and unsprayed habitat. No populations were even marginally susceptible in HR. In WV, however, one population in an abandoned apple tree at AW was moderately susceptible (R freq. = 0. 56). A possible explanation for the lower resistance at this site was the low population size; the single tree from which these mites were collected was not maintained and diseased and thus supported a relatively sparse acarine fauna.

Nevertheless, the general lack of susceptibility in *M. occidentalis* could be associated with its natural history. While *T. pyri* has many alternate foods, such as pollen and fungi, *M. occidentalis* feeds primarily on mites, with few alternate foods. *Typhlodromus pyri*'s ability to reproduce on pollen allows large populations to be maintained in undisturbed habitats, which are less favorable to large spider mite buildups. *M. occidentalis* favors *Tetranychus urticae* (Dicke 1988) as tetranychid prey; two-spotted spider mite is largely associated with pesticide disturbed habitats. Because the native vegetation is less favorable to *M. occidentalis* than the sprayed orchards, a very large portion of the population

is subjected to OP sprays. Subsequently, founders of new populations in native vegetation are more likely to have come from the orchards, and thus would be resistant. Considering the long-term and widespread use of OPs throughout HR, the susceptible phenotype may be very rare. With less OP use in WV, susceptible phenotypes may still occur (as at AW), but at a very low frequency.

Overall, results from the distribution of OP resistance in *T. pyri* and *M. occidentalis* can be summarized into two distinct patterns: *T. pyri* displays a localized resistance pattern, while *M. occidentalis* has regional resistance. The fact that *T. pyri* developed resistance in many orchards over a wide area suggests an ability to evolve resistance to OPs at a relatively fast rate, particularly in comparison to codling moth, which is the primary target pest of the OP sprays. The level of resistance in the *T. pyri* populations also is not significantly different between regions; however, the increased amount of overall variation in resistance levels in WV may demonstrate the impact of sprayed acreage on the resistance distribution patterns. Apple growing in WV is much less intense, and the orchards tend to be small and scattered. This is in opposition to HR, where orchards tend to be larger and are spread contiguously over a greater area. Thus, the overall area under selection pressure from OP sprays is greater in HR.

Also, growers are much more uniform in their timing of OP sprays in HR because of the greater codling moth pressure.

The location of the orchard, and the proximity of the orchard to surrounding sprayed areas, also had no effect on either the resistance status of orchard populations, nor populations in unsprayed native habitat. Sites in the center of the valleys (WV: AW, NU; HR: MC, BW) did not have more variable frequencies of resistance than those on the margin, surrounded by native vegetation.

OP resistance was consistent also between phytoseiid populations in orchard trees and groundcover. While groundcover would receive less direct sprays of OPs, it is likely that indirect application (run-off) to groundcover provided enough selection pressure to maintain resistance. As grower spray nozzles are often pointed downward, and trees are sprayed to drip with an excess of pesticide, it is likely that phytoseiid populations in the groundcover would contact OPs. Moreover, migration occurs between mites in the groundcover and trees; *T. pyri* and *M. occidentalis* readily dispersed to small trees placed within orchards (Dunley and Croft 1990). Thus, groundcover does not appear to harbor a susceptible reservoir within an orchard.

The host plant substrate, either apple or blackberry, also did not affect T. *pyri* and *M. occidentalis* resistance levels. This provides evidence that OP detoxification mechanisms in these phytoseiid species are neither induced by either plant through the prey, nor are they reduced by mites feeding on prey which develop on them.

In summary, the distribution of organophosphate resistance in *Typhlodromus pyri* is such that gene flow appears to be minimal in this species. The rapid change in frequency of resistance between the orchards and surrounding vegetation implies that little dispersal is occurring. This conclusion is supported by direct sampling studies of dispersal (Dunley and Croft 1990).

Metaseiulus occidentalis, on the other hand, appears to exhibit a regional pattern of resistance such that little variation in resistance levels occur over space. This is seen even in situations where selection pressure is small or non-existent; thus, the high levels of resistance must originate from sprayed orchards. A high proportion of *Metaseiulus occidentalis* within the growing areas must be selected for resistance, such that dispersal must bring a high proportion of the gene pool under selection pressure by pesticides, or populations outside of sprayed areas are much smaller relative to populations within orchards.

There is need to further understand the movement and dynamics of pesticide resistance traits among pest and beneficial arthropods. As crop management systems continue to move away from monocultures toward multi-crop mosaics and become more diversified, more effort will be made to manage resistance under an array of selection types and pressures (Croft 1992). Future resistance management programs will likely require a regional, multi-tactic, multi-crop perspective, with dispersal and gene flow being key input variables for decision making.

CONCLUSION

The presence of organophosphate resistance in populations of phytoseiid mites which provide biological control of phytophagous pest mites will continue to be important for pesticide-structured agroecosystems such as apple orchards, particularly until more selective alternatives to broad-spectrum pesticides become available. The nature of the information obtained in this thesis on the genetics and gene flow of OP resistance in three important phytoseiid species, *A. andersoni*, *M. occidentalis*, and *T. pyri*, is the type needed to implement better pesticide resistance for these beneficial species and their prey. From these results, more specific strategies for resistance management of each individual species can be made.

Biological control of spider mites utilizing *A. andersoni* where organophosphate use is common will largely be dependent on the presence of an OP resistant strain. Differences described between resistant and susceptible strains demonstrate that field rates of OPs are likely to eliminate susceptible *A. andersoni*. Also, because at least two genes are involved in conferring OP resistance, dilution of resistance by interbreeding with native susceptible populations may slow establishment of

resistant *A. andersoni* after release. However, the expression of resistance in the field is more likely because the resistant phenotype is semi-dominant, thus increasing the potential for biological control.

Much information has also been gained for practical use in the management of pesticide resistance in *T. pyri* and *M. occidentalis*. The difference in dispersal and gene flow between *T. pyri* and *M. occidentalis* provides different outlooks for managing pesticide resistance in populations relative to inoculation, stability, and the persistence of resistance. Inoculation means a resistance trait can be introduced or carried into an agroecosystem. This assumes that the trait has evolved elsewhere. Stability refers to the maintenance of the resistance trait at high frequencies with continued selection pressure, without reduction from interbreeding with susceptible phenotypes or from fitness differences. Persistence is the continuation of the trait at a relatively high frequency in a population once the selection pressure has been reduced or eliminated, for example when pesticide spray regimes are altered.

Management of pesticide resistance in *T. pyri* has been successful, as noted earlier. However, OP resistance in *T. pyri* has a patchy distribution and is associated only with sprayed orchards. Considering the low dispersal tendency of *T. pyri*, and the absence of OP

resistance in populations from native unsprayed habitat, populations in a new planting would be slow in developing resistance as the few immigrants that invaded would most likely be susceptible. Resistance would also spread quite slowly. Nevertheless, once a population became resistant, the low level of immigration by susceptible phenotypes would have little dilution effect on the frequency of resistance in the population. In this manner, resistance would remain stable, particularly when pesticide selection was maintained over a large area. When pesticide pressure was relaxed, however, resistance would persist for some time. This is again because of limited immigration of susceptible phenotypes from the surrounding habitat.

Pesticide resistance management in *M. occidentalis* has also been possible, but for different reasons than in *T. pyri*. As noted by Hoy *et al.* (1985), the high level of dispersal by this mite allows resistant phenotypes to spread rapidly, moving the resistance traits into new orchards. Stability of resistance within *M. occidentalis* populations may be high as well. For OP resistance, this predatory mite shows a regional distribution, thus any immigrants from surrounding habitat are likely to be resistant and the possibility of dilution by susceptible phenotypes is low. For a newly developed trait, however, it would be necessary to maintain selection pressure

until the trait had spread regionally. The stability of resistance for *M. occidentalis* would also be reflected in its persistence: for a regionally distributed resistance, the trait would remain stable simply because in these two valley areas there were no susceptible individuals to interbreed with. However, if selection pressure were eliminated for a resistance trait which was novel and only locally distributed, then a high degree of dispersal and immigration of susceptible phenotypes would rapidly reduce resistance within the locality. Again, widespread resistance traits which show regional features would be the most stable and persistent.

In conclusion, knowledge of genetics, dispersal, and gene flow of pesticide resistance traits are important in the development of stable integrated pest management programs through pesticide resistance management. For pests, this knowledge provides the framework for strategies and tactics which prevent or reduce evolution of pesticide resistance. In natural enemies, such as *A. andersoni, T. pyri, and M. occidentalis*, determination of the genetics, dispersal, and gene flow of resistance provide information necessary to implement pest management practices advantageous to beneficial arthropods. This will increase overall effectiveness of biological control programs. Used in concert, resistance management programs for both pest and beneficial

arthropods will reduce reliance on insecticides in agriculture and thereby benefit society and the environment.

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