FINAL PROJECT REPORT

Project Title: Miticide resistance in spider mite pests of pears (PR-13-106)

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Total Project Funding: Year 1: 23,969 Year 2: 24,614

Budget History:

Item	2013	2014
Salaries	12,000	12,480
Benefits	4,666	4.853
Wages	5,720	5,949
Benefits	555	577
Equipment	0	0
Supplies	500	500
Travel	255	255
Plot Fees	0	0
Miscellaneous	0	0
Total	$23,696	$24,614

Objectives

1. Survey resistance status of spider mite populations on pear to key miticides.
2. Examine population genetics of resistance in spider mites.
3. Develop recommendations for effective control of spider mites and a resistance management plan.

Significant Findings

- The adulticides (Agri-Mek, Acramite, and FujiMite) were affected by resistance in most populations tested, in decreasing order of strength of effect.
- Agri-Mek is expected to provide little control in the field; Acramite may still be moderately effective outside the Wenatchee River Valley.
- FujiMite shows only incipient resistance, but field performance may still be retained.
- The ovicides (Onager, Zeal and Envidor) were less affected by resistance than the adulticides.
- There is evidence for cross-resistance between Onager (MOA 10A) and Zeal (MOA 10B). Where resistance to these materials occurred (lower Wenatchee River Valley), it was absolute.
- No evidence of resistance to Envidor was found in any population.

Results and Discussion: Obj. 1 - Survey

A total of 88 probit bioassays were performed on 9 twospotted spider mite populations, 8 collected from eastern Washington pear orchards, and 1 susceptible reference colony obtained from Cornell's Geneva Laboratory in New York. The latter has been reared in the laboratory for >15 years without exposure to pesticides. The bioassays were performed using commercial formulations of six acaricides (Table 1), including three adulticides and three ovicides. The acaricides chosen represent six different modes of action (MOAs); however, Onager and Zeal (10A and 10B, respectively) are considered closely related MOAs.

The eight commercial orchard populations were collected over two growing seasons (four per season), representing pear orchards in the Chelan, Douglas, Okanogan and Yakima Counties. Initiating a colony from the field was made by transferring individual mites with a fine-tipped paintbrush, taking care to avoid transferring other arthropods. The populations were reared on bean plants, *Phaseolus vulgaris* L., at a constant temperature of ca. 24 °C (75 °F), and 16:8 light:dark photoperiod. Colonies were kept isolated in different rooms, and supplied with fresh bean plants every 2 weeks.

Table 1. Acaricides tested against populations of twospotted spider mites from pear

Trade name	Common name	Group	MOA	bioassay type
Agri-Mek	Abamectin	avermectins	6	adulticide
Acramite	bifenazate	N/A	unknown	adulticide
FujiMite	fenpyroximate	METI	21A	adulticide
Envidor	spirodiclofen	tetronic/tetramic acid derivatives	23	ovicide
Onager	hexythiazox	mite growth inhibitors	10A	ovicide
Zeal	etoxazole	mite growth inhibitors	10B	ovicide

Each bioassay consisted of four to six concentrations of the acaricide and a distilled water check. All bioassays were conducted on bean leaf disks (3 cm/1.18 inch dia.) with the lower surface facing up in a 3.25 oz plastic cup with cotton and water. Acaricide concentrations were mixed by serial dilution of a 1 liter stock solution, and sprayed in a Potter Spray Tower (Burkard Mfg, Rickmansworth, England) with 2 ml (0.06766 fl oz) of mixture at 6.5 psi.

Adulticide bioassays used 20 adult female mites/disk and were evaluated after 24, 48, and 72 h (the 72 h data are shown throughout this report). For ovicidal bioassays, 10 adult females were transferred

to the disks and allowed to lay eggs for 24 h. Eggs were counted, and their positions marked with a felt-tip pen, and the females removed. The initial number of eggs was standardized to 20/disk by removing excess eggs. Eggs were treated and then held at 25 °C (77 °F) in a growth room for 10 days, when they were evaluated for treatment mortality (unhatched eggs). These methods are essentially the same as have been used historically in collecting information on mites from Washington tree fruits, allowing for comparisons across time.

The dose-response curves were calculated with POLO-Plus (LeOra software), which provided LC_{50}s (the concentration needed to kill 50% of mites) and associated 95% confidence intervals.

An additional calculation was made using the probit regression parameters (slope, intercept, natural response). Using the maximum label field rate, the predicted percentage mortality of the various populations was estimated. It should be noted that these are relative indicators of activity because of the differences between laboratory studies and field conditions. However, they provide an index of predicted activity in the context of actual use rates, which is difficult to ascertain from the degree of change in the LC_{50}.

Rate ranges for the bioassays were chosen based initially on historical data, and adjusted if mortality was too high or too low to produce an LC_{50} using probit analysis. Because of the variable (and much higher than anticipated) levels of resistance, many of the bioassays failed probit analysis, and were re-run. Only those bioassays with six concentrations, an acceptable level of check mortality (<20%) and valid estimates of the LD_{10}, LD_{50}, LD_{90} and LD_{99} with 95% confidence intervals were retained (Table 2, Figs. 1a, b). Resistance ratios were (LC_{50}/baseline) calculated from the LC_{50} of the New York susceptible colony as the baseline; historical data are shown for reference. Resistance ratios (RRs) are useful metrics in assessing the degree of resistance and likelihood for it to spread in the field. Resistance ratio values <3 indicate no resistance, values 3-10 represent low levels of resistance that may spread in the field, values between 10-100 represent statistically significant resistance that may or may not cause field failure, and values >100 indicate high levels of resistance that are likely to lead to field failure of the acaricide.

Table 2. LC_{50}s and resistance ratios (LC_{50} of tested field-derived colony divided by LC_{50} of susceptible laboratory colony) of six acaricides tested against populations of twospotted spider mites collected from commercial pear orchards in eastern Washington.

				95% CI		
Acaricide	TSM population	New York TSM baseline	Calc LC_{50}	lower	upper	RR (New York)
Agri-Mek	C1-2013	0.004	271.20	142.38	409.74	**67,801**
Agri-Mek	C2-2013	0.004	503.04	413.28	604.14	**125,760**
Agri-Mek	C3-2013	0.004	389.33	277.54	508.77	**97,332**
Agri-Mek	Y1-2013	0.004	37.56	24.13	51.14	**9,391**
Agri-Mek	C1-2014	0.004	329.82	212.821	698.98	**82,455**
Agri-Mek	C2-2014	0.004	116.05	64.10	170.53	**29,012**
Agri-Mek	D1-2014	0.004	165.67	117.072	230.602	**41,417**
Agri-Mek	O1-2014	0.004	11.31	6.235	18.371	**2,827**
Acramite	C1-2013	2.29	1213.51	982.09	1476.08	**531**
Acramite	C2-2013	2.29	2165.29	1730.02	2626.31	**947**
Acramite	C3-2013	2.29	687.14	599.95	789.71	**300**
Acramite	Y1-2013	2.29	10.59	0.00	53.65	**5**
Acramite	C1-2014	2.29	739.75	520.39	994.44	**323**
Acramite	C2-2014	2.29	2845.92	2299.65	3533.12	**1,244**
Acramite	D1-2014	2.29	125.23	86.34	188.59	**55**
Acramite	O1-2014	2.29	3.47	2.64	4.35	**2**

				95% CI		
Acaricide	TSM population	New York TSM baseline	Calc LC_{50}	lower	upper	RR (New York)
FujiMite	C1-2013	1.29	8.94	7.95	10.02	**6.93**
FujiMite	C2-2013	1.29	11.68	8.87	14.39	**9.05**
FujiMite	C3-2013	1.29	20.82	13.77	26.29	**16.14**
FujiMite	Y1-2013	1.29	1.35	0.13	3.37	**1.04**
FujiMite	C1-2014	1.29	8.90	6.793	11.198	**6.90**
FujiMite	C2-2014	1.29	15.19	13.09	17.43	**11.77**
FujiMite	D1-2014	1.29	4.43	3.49	5.46	**3.43**
FujiMite	O1-2014	1.29	3.84	2.57	5.24	**2.98**
Onager	C1-2013	0.014	0.51	0.37	0.74	**36**
Onager	C2-2013	0.014	0.39	0.34	0.45	**28**
Onager	C3-2013	0.014	1785.18	1573.97	1995.69	**127,513**
Onager	Y1-2013	0.014	0.42	0.29	0.51	**30**
Onager	C1-2014	0.014	2052.76	1806.379	2293.881	**146,626**
Onager	C2-2014	0.014	1182.94	1019.41	1367.30	**84,496**
Onager	D1-2014	0.014	0.15	0.11	0.18	**10**
Onager	O1-2014	0.014	0.24	0.18	0.28	**17**
Zeal	C1-2013	0.062	5.02	2.81	7.25	**81**
Zeal	C2-2013	0.062	5.77	5.00	6.47	**93**
Zeal	C3-2013	0.062	[x]			--
Zeal	Y1-2013	0.062	1.57	1.29	1.83	**25**
Zeal	C1-2014	0.062	[x]			--
Zeal	C2-2014	0.062	[x]			--
Zeal	D1-2014	0.062	0.313	0.443	0.639	**5**
Zeal	O1-2014	0.062	1.418	1.012	1.879	**23**
Envidor	C1-2013	5.96	9.76	5.57	13.32	**1.64**
Envidor	C2-2013	5.96	11.41	9.20	14.15	**1.91**
Envidor	C3-2013	5.96	8.22	6.24	10.09	**1.38**
Envidor	Y1-2013	5.96	9.70	6.08	12.94	**1.63**
Envidor	C1-2014	5.96	13.62	11.144	15.586	**2.28**
Envidor	C2-2014	5.96	6.43	5.66	7.21	**1.08**
Envidor	D1-2014	5.96	9.277	8.038	10.539	**1.56**
Envidor	O1-2014	5.96	7.559	6.311	8.809	**1.27**

[x]Unable to obtain significant mortality at 200,000 ppm AI (near limits of solubility).

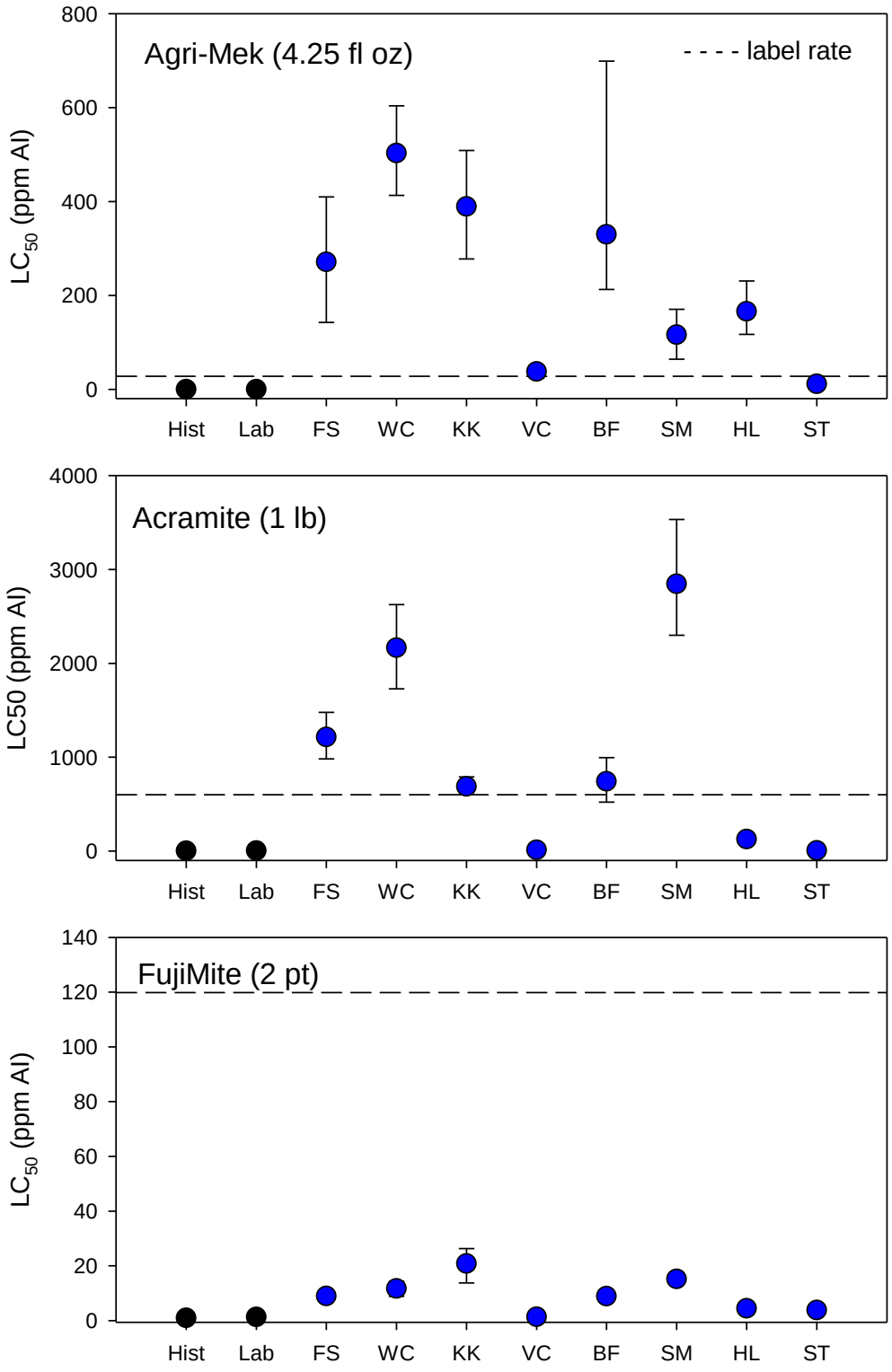

Fig. 1a. LC_{50}s of adulticidal acaricides for populations of twospotted spider mite from pear.

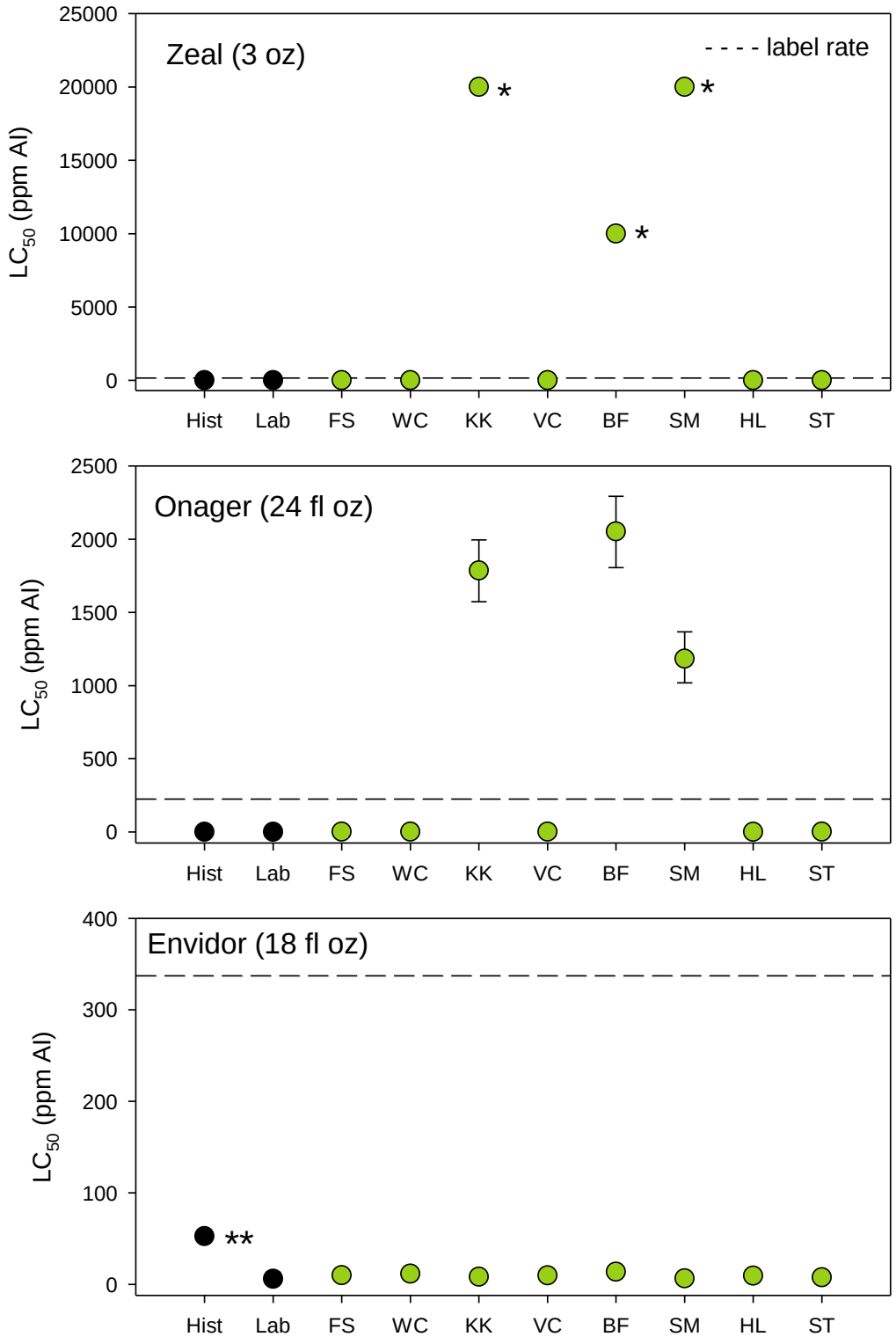

Fig. 1b. LC_{50}s of ovicidal acaricides for populations of twospotted spider mite from pear. LC_{50}s with single asterisks indicate the highest rate used when the probit bioassay failed due to resistance. The double asterisk indicates data from European red mite rather than twospotted spider mite.

Agri-Mek. The RRs for this material were extremely high for all populations tested (Table 2), ranging from ca. 2,827 to 125,760- fold increase in the LC_{50}. Of the mite populations examined, the lowest RR was from Okanogan county population; all those from Chelan County (in this case, the Wenatchee River Valley [WRV]), were uniformly high. This high level of resistance is the probable cause for field failure as a miticide for spider mites. However, it may still be useful for rust mites and pear psylla. The elevated resistance levels reflect its continued and frequent use since the late 1980s in Washington's pear industry. The predicted percentage mortality at the maximum label rate of Agri-Mek varied from 1 to 72% (Fig. 2a).

Acramite. The RRs for Acramite were considerably lower than those for Agri-Mek (4.63-947). This material has been used for a much shorter period of time. However, with the exception of the Y1-2013 colony from Yakima and the O1-2014 colony for Okanogan county, RRs were still very high, indicating a major shift in the LC_{50}s. The predicted percentage mortality at the maximum label rate of Acramite varied from 13 to 100% (Fig. 2b).

FujiMite. The RRs were lower for FujiMite than the other two adulticides (1.04-16.14); the Yakima colony showed no increase in resistance, and the five of the colonies only a moderate increase. The predicted percentage mortality at the maximum label rate of FujiMite was 99-100% for all populations (Fig. 2c).

Onager. The RRs were quite variable for this material. Three of the populations (all from the WRV east of Dryden), were very high (84,496-146,626). Two other populations slightly to the west but still in the WRV growing region were much lower. All populations outside the WRV had low RRs (10-36), indicating some change in susceptibility. However, the predicted percentage mortality at the maximum label rate of Onager was 100% for all populations except the three resistant ones, where the predicted mortality was zero (Fig. 2d).

Zeal. The RRs for Zeal all indicated that a low to moderate level of resistance has occurred in five of the eight populations. Three of the WRV were highly resistant, such that no significant mortality was measured at 200,000 ppm AI, making the RR >3.2 million. The populations are the same ones with high (but measurable) levels of resistance to Onager, the other IRAC group 10 material. The predicted percentage mortality with Onager at the maximum label rate (Fig. 2e) is 100%, with the exception of the three highly resistant populations (0% predicted mortality).

Envidor. None of the populations tested showed any measureable resistance to Envidor; all RRs were <2.5. Envidor is one of the more recent materials to be used on pear. It is classed as IRAC MOA group 23, the same MOA as Ultor, which is routinely used on pears for psylla. All populations tested had a predicted mortality of 100% based on probit regression (Fig. 2f).

Results and Discussion: Obj. 2. Dominance of Resistance

Making crosses. Crosses were made on whole bean plants by adding at least 80 female *T. urticae* deutonymphs in teleochrysalises from the resistant mite colony and 40 males from the susceptible colony to the plants. Mites were taken from the same colonies used in Objective 1. For adulticide tests, crosses were observed for 1-2 wk until F_1 larvae began hatching. At this point, all adults were removed from the cross. This was done by removing a leaf from the plant, removing all adults from the leaf, then attaching that leaf to a new plant using a paper clip. The juveniles from the leaf moved to the new plant as the old leaf desiccated. This was done until the entire original plant was harvested. These juveniles were observed for ~1 week until all had matured and adult females were available for use in bioassays.

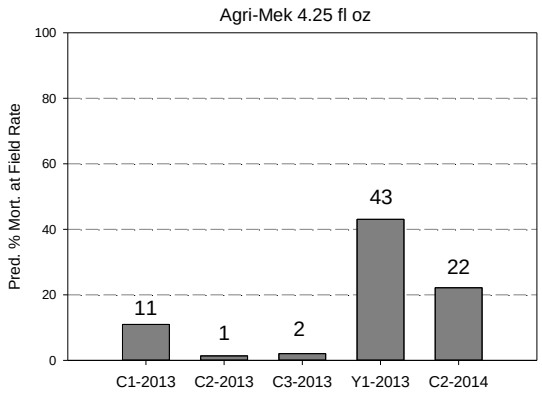

Fig. 2a. Predicted percentage mortality at the field rate of Agri-Mek (4.25 fl oz/acre).

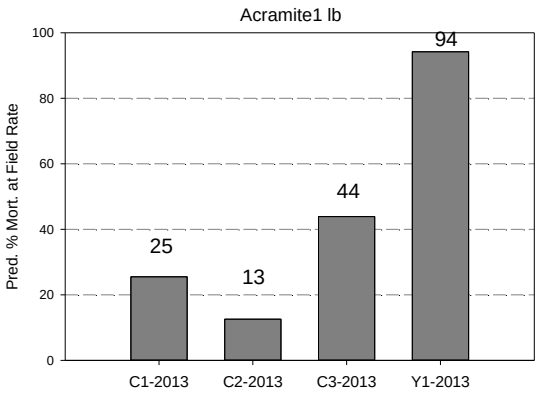

Fig. 2b. Predicted percentage mortality at the field rate of Acramite (1 lb/acre).

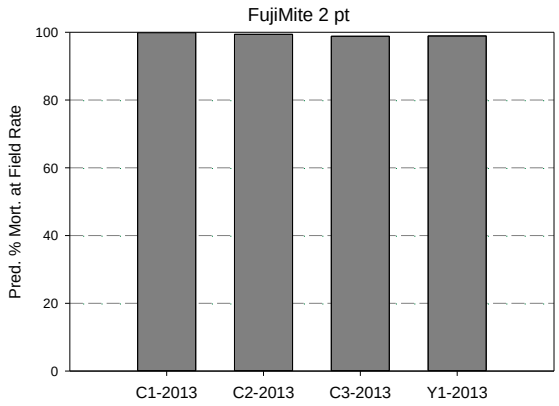

Fig. 2c. Predicted percentage mortality at the field rate of FujiMite (2 pt/acre).

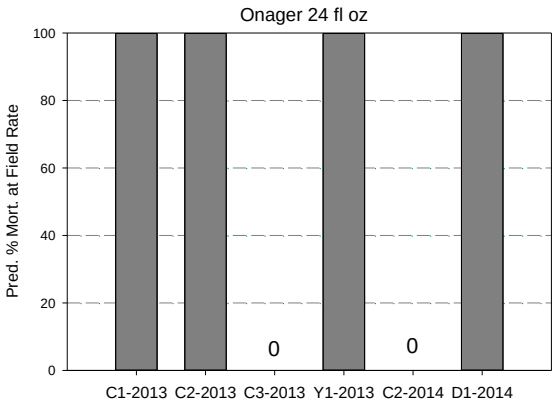

Fig. 2d. Predicted percentage mortality at the field rate of Onager (24 fl oz/acre).

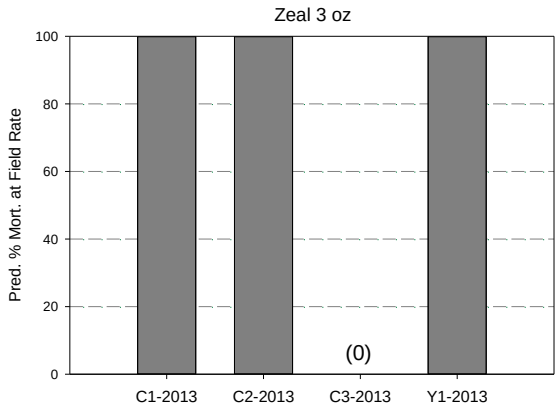

Fig. 2e. Predicted percentage mortality at the field rate of Zeal (3 oz/acre).

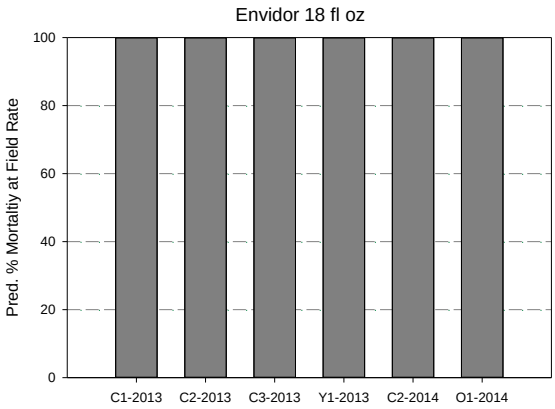

Fig. 2f. Predicted percentage mortality at the field rate of Envidor (18 fl oz/acre).

Bioassays of crosses. Disks (3.5 cm diam) were cut from clean beans and placed with the lower surface facing up in a plastic cup (30 ml) filled with cotton and water Twenty F_1 *T. urticae* females were placed on each disk. There were five replications per concentration tested, with a total of 5-7 concentrations (including the check). The number of concentrations was dependent on the number of F_1 individuals available. The treatments were applied by contact to females on the disks. The concentrations range used was set so that it included values that approximated the LC_{95} of the resistant colony, LC_{25} of the resistant colony, LC_{75} of the susceptible colony, LC_5 of the susceptible colony, and some intermediate values. The LC values were determined in Objective 1. The solutions were made by mixing the appropriate amount of the formulated pesticide in 1 liter of water. Pesticides were applied with a Potter Spray Tower set at 44.8 kPa using the intermediate nozzle.

Adulticide bioassays were held in a growth room at 22 °C (72 °F) and evaluated every 24 h for 3 days after treatment (DAT). Mites were counted as live, dead, runoff, or moribund. All juveniles from hatched eggs were moved onto fresh arenas and observed until mature so the number of males and females for each replication can be recorded.

Calculating *h*. Dominance of resistance (h) is defined as: $h = (W_h - W_s)/(W_r - W_s)$, where W_s, W_r, and W_h are the survival of susceptible, resistant, and hybrid (the cross) females, respectively. When $W_s \leq W_h \leq W_r$, h=0 indicates completely recessive resistance and h=1 indicates completely dominant resistance, with calculated values falling in between these two extremes. Resistance is expected to evolve slower when h is close to 0; resistance evolves more quickly as h approaches 1.

This value (h) was calculated for all doses of each pesticide assayed against a specific cross at 3 DAT. Values of W_h for these doses were obtained directly from the assays. Because the doses used in the cross bioassays were different from those used to assay resistant and susceptible colonies (Objective 1), survival at the doses used in the cross bioassay for the resistant and susceptible colonies was estimated using the probit curve for each colony.

Crosses were performed with two of the resistant pear populations (FS and KK) with the lab (susceptible) colony, and the progeny were assayed with FujiMite. Summary results of the crosses are reported in Tables 3 and 4. Calculations of h are reported in Tables 5 and 6. Except for the two doses on the extreme ends of the range, all values of h for the KK cross were <0.5, indicating recessive inheritance. At the extreme doses, survival of the crosses was lower than that of the susceptible individuals, resulting in negative values. In these cases, resistance is assumed to be completely recessive and results are due to variation in survival.

Twospotted spider mites are a haplodiploid species, which means males are produced from unfertilized (i.e., haploid) eggs and females are produced from fertilized (i.e., diploid) eggs. This differs from most generalist predators that feed on twospotted spider mites and are diploid, where both males and females are produced from fertilized eggs. The implications of haplodiploidy for resistance evolution are well known. If you assume resistance is controlled by two alleles, where R is recessive and S is susceptible, then diploid species have three genotypes: RR, RS, and SS. This is important for resistance evolution because most of the resistance alleles in diploids are carried by heterozygotes (i.e., RS). If these individuals are killed by the pesticide because dominance is recessive then resistance will evolve slowly. However, in haplodiploid species more resistance alleles are carried by homozygotes (i.e., male R or female RR), and this speeds up resistance evolution.

As an example of the impacts of haplodiploidy, assume that a pesticide kills >90% of susceptible individuals and 0% of resistant individuals (such as Agri-Mek), and the initial frequency of resistance is low (1 allele out of 1,000). If the pesticide is sprayed on 90% of orchards then a diploid species will take more than 100 generations to evolve resistance under these conditions, while a haplodiploid species will evolve resistance in less than 10 generations. Moreover, if our hypothetical pesticide is used on only 50% of pear acreage then the diploid species would not be expected to evolve resistance in over 1,000 generations while the haplodiploid species would be expected to evolve resistance in

20-30 generations. These differences are staggering, and indicate that the genetic pre-disposition of haplodiploid species to evolve resistance to pesticides is one reason species such as mites and whiteflies are such major crop pests.

Table 3. Percentage mortality ± SE for the offspring of KK ♀ crossed with NY♂ treated with FujiMite.

	% Mortality		
Conc (mg AI/liter)	1 DAT	2 DAT	3 DAT
75	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
12	81.89 ± 6.60	83.00 ± 5.15	88.89 ± 5.09
9	74.00 ± 7.97	73.24 ± 6.07	85.74 ± 6.24
7	65.00 ± 2.24	67.68 ± 6.43	74.89 ± 7.84
4.55	52.00 ± 7.00	42.42 ± 6.80	45.00 ± 6.89
0.06	5.00 ± 1.58	9.16 ± 3.31	17.00 ± 4.06
0	0.00 ± 0.00	0.00 ± 0.00	2.22 ± 2.22

Table 4. Percentage mortality ± SE for FS ♀ crossed with NY♂treated with FujiMite.

	% Mortality		
Conc (mg AI/liter)	1 DAT	2 DAT	3 DAT
23	98.82 ± 1.18	100.00 ± 0.00	100.00 ± 0.00
6	96.95 ± 2.01	95.89 ± 1.96	98 ± 1.22
0.6	38.97 ± 3.87	37.04 ± 4.10	41.53 ± 3.69
0.06	8.33 ± 3.79	5.23 ± 2.74	11.56 ± 4.38
0	2.23 ± 1.37	4.11 ± 1.89	5.22 ± 2.30

Table 5. Survival (*W*) and dominance of inheritance (*h*) calculations for doses of FujiMite tested against the KK cross.

Conc (mg AI/liter)	W_r	W_s	W_h	*h*
75	0.05	0.01	0.00	0.00*
12	0.75	0.09	0.11	0.03
9	0.85	0.12	0.14	0.03
7	0.92	0.15	0.25	0.13
4.55	0.97	0.21	0.55	0.44

Table 6. Survival (*W*) and dominance of inheritance (*h*) calculations for doses of FujiMite tested against the FS cross.

Conc. (mg AI/liter)	W_r	W_s	W_h	*h*
7	0.76	0.17	0.02	0.00*
4.55	1.00	0.61	0.58	0.00*
0.06	1.00	0.94	0.88	0.00*

* Values rounded up to 0.0

Executive Summary

A survey of twospotted spider mite from pear orchards indicated that resistance to several acaricides is present in moderate to high levels. As a group the adulticides were more affected by resistance than the ovicides. Of the adulticides, Agri-Mek (a material used for both mites and pear psylla since the late 1980s) had the highest resistance ratios (RRs), and mite control at the field rate is predicted to be poor in most orchards. Acramite also had had high levels of resistance in all populations in Chelan County, while those from Yakima and Okanogan counties showed only a minor increase in resistance. FujiMite overall had the lowest RRs, and is predicted to give good control at the field rate.

Of the ovicides, only three populations from the Wenatchee River Valley showed a significant level of resistance to Onager and Zeal; all other populations appeared to be susceptible. However, the populations that were resistant to Zeal and Ongager were virtually immune to this product. The resistance to Zeal and Onager appear to be related, which is not surprisingly given that they are have closely related MOAs. All populations tested were susceptible to Envidor, the most recently introduced miticide. However, it is in the same MOA group as Ultor, which has also been widely adopted in pear production, and caution is advised in its use.

With the exception of Agri-Mek, all of the acaricides tested are limited by their labels to a single application per year, presumably for the purposes of resistance management. Despite this, the development of resistance in spider mite populations appears to be progressing rapidly in pear orchards.