Title: INTEGRATED PEST MANAGEMENT AND POLLINATOR PROTECTION ON ALFALFA PRODUCED FOR SEED 2018-2019

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Abstract: In early August 2018, six populations of two-spotted spider mites were collected from fields of alfalfa grown for seed in Walla Walla County, WA and the toxicity of 6 acaricides was tested on these populations. The tested doses for each acaricide ranged from 0 (control) to the maximum labelled field rate for TSSM on alfalfa or other crops if the acaricide was not registered on alfalfa grown for seed. The acaricides tested were abamectin, acequinocyl, bifenthrin, bifenazate, cyflumetofen and propargite. The responses of these populations were compared to the response of our acaricide naive susceptible lab colony of TSSM. Resistance ratios were calculated for each population by dividing the doses of each acaricide required to kill 10, 50, and 90% of each population by the calculated doses that killed 10, 50, and 90% of our acaricide naïve susceptible lab colony. Based on prior work we have concluded that populations with resistance ratios below 10 are considered susceptible. Populations with resistance ratios between 10 and 100 are low to moderately resistant and populations with resistance ratios that exceed 100 are resistant. We can conclude that most of the tested populations were either susceptible or very weakly tolerant to all tested acaricides. However in one field the mites were moderately resistant to bifenthrin. In that same field there was some resistance developing to abamectin in a portion of the population. The resistance ratios (RR_{10} and RR_{50}) implied that this population was susceptible to abamectin. However, the RR_{90} was 88.1 and this means that a small proportion of this population is developing resistance to abamectin. Our results for propargite were interesting. Propargite has been a commonly used acaricide on alfalfa seed in the Walla Walla Valley and this is reflected in the data. Five of 6 populations were in the moderately resistant range and one population could be considered resistant. Presently we have developed molecular makers associated with resistance to all 5 of 6 of these acaricides and we will complete this genetics work in 2019.

In another field study we attempted to increase the abundance of ground nesting halictid bees. This study basically failed since we calculated no differences in the number of emergence holes based on irrigation quantity or in adult bees captured in bee bowls.

Introduction: The two- spotted spidermite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a generalist herbivore pest with that feeds on over 1100 host plant species including alfalfa. Through its feeding activity coupled with rapid development and reproduction, TSSM can cause severe economic damage to alfalfa grown for seed especially when populations outbreak during the critical time period in high summer when seeds are being set. Broad-spectrum insecticides including organophosphates and pyrethroids are disruptive to populations of beneficial arthropod populations that in many cases would bioregulate TSSM populations. At present organophosphate and/or pyrethroid insecticides are mainstay insecticides in Lygus bug management. Pyrethroid and organophosphate insecticides are often applied pre-bloom prior to bee emergence or release in what is commonly called a clean-up spray. Pyrethroids in particular are renowned for flaring mite populations. We have completed substantial studies on TSSM in other south-central Washington State crops including hops, peppermint, and silage corn. When mite populations are exposed to pyrethroids they just tend to become more resistant to other acaricides applied later in the season for their control. We have seen that there is what we call an up-regulation of specific genes that associated with "housekeeping". These housekeeping genes code for a cascade of enzymes that in-turn detoxify poisons that TSSM are exposed to.

Presently synthetic acaricides are the cornerstone of TSSM management programs in most cropping systems including alfalfa produced for seed. Unfortunately, TSSM has an unrivalled ability to develop resistance to diverse chemistries of acaricides. This necessitates the need to identify the mechanisms of resistance to acaricides TSSM employs and how this contributes to the resistance status of endemic TSSM populations. Knowledge of the mechanisms enabling resistance and quantifying its prevalence in specific TSSM populations are essential to developing effective integrated TSSM management strategies. This has been the major motivation for this project.

An additional objective was completed in which we installed a drip irrigation system that delivered varying amounts of water in an attempt to create a "Goldilocks" zone that would attract larger ground-nesting bees, most notably *Agapostemon* spp. *Agapostemon* are commonly called metallic sweat bees. In prior surveys of bee populations in alfalfa seed fields and other crops grown in south-central Washington State crops *Agapostemon* have been the most abundant macro-sized bees. *Agaposemton* are about the same size as leafcutting bees and we have presumed that they would likely be contributing to pollination service in alfalfa seed fields. In wine grapes and hops we had observed an increase in nesting in soils near drip irrigation emitters. In both 2017 and 2018 we established a study site but never gained any substantive colonization rates of *Agapostemon* based on our irrigation treatments.

Project Objectives:

1. Test selected field populations of spider mites from a representative sample of alfalfa seed fields and compare their dose response curves to acaricide naïve populations and mite populations from other crops including hops and peppermint.
2. Expand robust molecular diagnostics to predict (multiple) acaricide resistance in the field.
3. Test a novel method for enhancing populations of soil-nesting bees by identifying the species and quantifying the change in bee emergence rate achieved by irrigating soil at field margins.

Materials and Methods:

Objective 1. Test selected field populations of spider mites from a representative sample of alfalfa seed fields and compare their dose response curves to acaricide naïve populations and mite populations from other crops including hops and peppermint.

Mites

The susceptible TSSM population was initially collected from feral grape vines in Montana in 1995 and has never been exposed to any pesticides since. Six TSSM populations were collected from alfalfa seed fields grown in the Walla Walla Valley of Washington State. Alfalfa leaves from each alfalfa seed field were randomly sampled covering all the areas of the farm in an X-shaped pattern. Leaves were collected from the upper, middle, and lower parts of alfalfa plant to ensure that each sample was representative of the actual mite population occurring in the alfalfa seed field. The leaves were kept in Ziploc® plastic bags in ice coolers and transported immediately to the laboratory, where the TSSM from each of the 6 fields sampled were transferred with a small camel hair brush onto lima bean seedlings (*Phaseolus lunatus* L.) and reared for one generation under laboratory conditions (28 ± 2 °C, 70 ± 5 RH). This permitted the buildup of enough individual adult female mites for us to complete experiments detailed below.

Bioassays

TSSM were subjected to leaf disk bioassays with 7 acaricides. These acaricides included abamectin, acequinocyl, bifenthrin, bifenazate, cyflumetofen and propargite. Abamectin, bifenthrin, bifenazate, and propargite are registered for use in Washington State on alfalfa or alfalfa grown for seed. Acequinocyl and cyflumetofen and not registered on alfalfa or alfalfa grown for seed. Growers in states other than Washington should check for the regulatory status of all these products prior to their use and should follow all label restrictions detailed on the labels.

Adult females were used for the bioassay of the 7 acaricides. Briefly, ten gravid adult female TSSM were placed on fresh lima bean leaf disks (2 cm in diameter). These mites were then subjected to exposure to these 7 acaricides using our Potter precision spray system. The tested doses for each acaricide ranged from 0 (control) to the labelled field rate for TSSM on alfalfa if the acaricide is registered for use on alfalfa or alfalfa grown for seed. Otherwise the upper limit for the dose used was the maximum labelled dose.on other crops. After 48 h, the mortality was scored for the sprayed adult females. Mites were counted as dead if they did not respond to a gentle tap with a fine camel-hair brush. The dose–mortality response was adjusted to the control treatment using Abbot’s formula. Probit analysis was used to estimate LC_{10}, LC_{50}, and LC_{90} values (lethal concentration required to kill 10, 50, ad 90% of the population, respectively), slopes and 95% confidence intervals (POLO Probit 2014). We develop resistance ratios (RR) by dividing the lethal concentration of the acaricide being tested that is required to kill to kill 10, 50 or 90% of the candidate population (LC_{50}) divided by the LC_{50} of our acaricide naïve laboratory population. Our susceptible lab colony was field collected from volunteer grape vines in Montana over 10 years ago. It has been maintained in colony and exposed to no poisons since. Hence we call this population our “acaricide naïve population”. We conclude that if RRs are less than 10 (10<RR) the population is susceptible. If the RR is between 10 and 100 (10<RR<100) that the mites are moderately tolerant to resistant. If the RR for a population exceeds 100 (RR>100) we conclude that the mite population is resistant and resistance increases as the RR value exceeds 100.

Objective 2. Expand robust molecular diagnostics to predict (multiple) acaricide resistance in the field. We have sequenced the genomes from TSSM from hops, peppermint, and silage corn from the Yakima Valley, from alfalfa produced as a seed crop in the Walla Walla Valley and from berry fields near both Oxnard and Watsonville, CA. The presence of markers associated acaricide resistance is low in alfalfa produced for seed. This is directly reflected by the history of acaricide use in each crop. TSSM from California berries were by far the most resistant to acaricides and this was reflected in their genetics. Specifically, we have found specific point mutations in TSSM populations from California berry fields that are associated with abamectin resistance. We have never found these mutations in any TSSM population ever tested in the Pacific Northwest. Among the crops tested in Washington State populations with the greatest incidence of point mutations associated acaricide resistance was hops. Hops were followed by silage corn, alfalfa seed, and peppermint. Among alfalfa seed, silage corn and peppermint this was greatest for pyrethroid resistance. Pyrethroids are routinely applied to alfalfa grown for seed and silage corn. There are no pyrethroids presently registered on peppermint. At present the data to finalize this objective are being processed. We anticipate that this work will be completed in early 2019. We did have one field we monitored (see results and discussion and table 6) in which we observed the greatest RR for propargite among all the crops we have sampled. We are interested in how the genetics of this population will play out.

Objective 3. Test a novel method for enhancing populations of soil-nesting bees by identifying the species and quantifying the change in bee emergence rate achieved by irrigating soil at field margins. We established a pilot study site on the Roza unit on a 1 acre block of forage alfalfa that was grown in summer 2017 like it was being grown for seed production. The field was 384' long and 121' wide. On the parallel lengthwise edges a series of drip lines was placed with irrigation drip pipe & emitters that delivered 0.5, 1.0 or 2.0 gallons of water per hour when water was being applied. Control plots that had no water delivery were established as well. A grid of white bee bowls was established in transects across the field from the field edges and then at 24' intervals. Bees were sampled for a 24 hr period by pouring a 4 oz solution of ethylene glycol into the bee bowls and returning 24 hrs later to recover what was captured in the bee bowls. Bee bowls were sampled twice in June and twice in July, 2018. We never had any differences in the emergence of bees from any of the irrigation treatments and the data from our bee bowl collections in June and July were meaningless. We discontinued sampling and scraped this study at the end of July 2018 to focus on the spider mite project.

Results and Discussion:

Objective 1: Test selected field populations of spider mites from a representative sample of alfalfa seed fields and compare their dose response curves to acaricide naïve populations and mite populations from other crops including hops and peppermint.

The toxicity of abamectin varied significantly among the alfalfa TSSM populations based on the resistance ratios (RR) in table 1(low to moderate resistance). Mortality at field dose of abamectin ranged between 56.8-100 % and LC50 of all the populations were below the field dose of abamectin.

The toxicity of acequinocyl (Kanemite) is summarized in table 2. At the labeled field rate, mortality ranged between 60-100% and was above 50% for all the 6 populations. Compared to the laboratory susceptible strain, the resistance ratio of acequinocyl ranged between 0.4- 1.6, 7.5-10.3, and 75

Toxicity of bifenthrin is presented in table 3 and the mortality at the field dose ranged between 33.6-85.6%. The LC_{50} of the field populations were all below the labelled field dose except for field 2. The level of resistance to bifenthrin based on the RR_{10}, RR_{50} and RR_{90} ranged 0.9-4.5, 1.5-53.3, and 4-64 respectively.

Bifenazate was relatively effective on TSSM populations from the sampled alfalfa fields except for Field 2 where the mortality at field dose was 42%, others ranged between 69-100% (table 4). Furthermore, the LC50 of the field populations except for field 2 were below the labeled dose for bifenazate (acramite).

Cyflumetofen was also very effective on the TSSM populations, causing 67-100 mortality of the sampled populations at labeled field dose (Table 5). Relative to the susceptible strain, the level of resistance at RR_{10}, RR_{50}, and RR_{90} ranged between 1.9-7.2, 5-15.8, and 4-35.2 respectively. Furthermore, the LC_{50} of the 6 field populations were all below the labelled field dose of cyflumetofen suggesting that resistance that will lead to control failure in the field is very unlikely as at now.

Propargite (table 6) displayed some very interesting results among the 6 populations tested. Five of 6 populations exhibited moderate resistance and we would categorize 1 population as being resistant to highly resistant. Mortality at the field rate ranged from 48.5-80.3%. Relative to the susceptible strain, the level of resistance at RR_{10}, RR_{50}, and RR_{90} ranged between 4.9-35.2, 12.7-155.7, and 9.1-1,507 respectively. Field 3 had our most interesting results. The genetics of this population should prove to be interesting. The population contains susceptible adults with an RR_{10} of 8.8. However, the RR_{50} was 115.7 and the RR_{90} was 1,507. This means that 10% of the population in this field will survive a dose of propargite that exceed 384 times the registered field rate of propargite on alfalfa produced for seed in Washington State.

Based on the toxicology of these tested acaricides on TSSM populations collected from alfalfa seed fields we can conclude for the most part that TSSM have not developed substantive resistance to most acaricides. Propargite is somewhat of an outlier. It had been the predominant acaricide applied to alfalfa grown for seed in the past and this is reflected in the data we have collected. Fields 2 and 3 are of some concern and I will make it a point to schedule a meeting with these specific growers to directly discuss their insect and mite management programs. All is not lost, compared to berries from California resistance except for propargite in field 3 the TSSM from alfalfa seed fields are not resistant to acaricides. Compared to other specialty crops in the Pacific Northwest The TSSM populations from alfalfa seed fields are comparable TSSM populations in peppermint. Acaricide use in peppermint is similar to acaricide use in alfalfa grown for seed. The TSSM population in hop yards and in fields of silage corn are more resistant than TSSM from alfalfa seed fields. This is reflected in the substantially greater amount of acaricides that are typically applied to hops and the routine treatment of silage corn with pyrethroid insecticides.

Objective 2. Expand robust molecular diagnostics to predict (multiple) acaricide resistance in the field. Based on previous study done on TSSM populations from hops, silage corn and peppermint, we have identified markers to diagnose resistance to multiple acaricides via diagnostic PCR for resistant-associated mutation and qRT_PCR for metabolic resistance. This objective is currently on-going with the mite populations we have collected from fields of alfalfa produced for seed and will be completed in the first quarter of 2019.

Objective 3. Test a novel method for enhancing populations of soil-nesting bees by identifying the species and quantifying the change in bee emergence rate achieved by irrigating soil at field margins. We put 2 years into this objective and basically have nothing to show for it. We discontinued this study in July 2018 to focus on the TSSM objectives detailed above.

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Keywords: Acaricide resistance, toxicology, two-spotted spider mite.

Table 1. Toxicity of **Abamectin** to TSSM populations collected from alfalfa seed fields

Population	% Mortality[a]	N	Slope±SE	LC_{10} (95%CL)	LC_{50} (95%CL)	LC_{90} (95%CL)	χ^2 (df)	RR10	RR50	RR90
Susceptible	100	241	1.4±0.2	0.02(0.007-0.1)	0.8(0.5-1.3)	6.7(4.2-13.4)	6.2(16)	1	1	1
Field 1	78.2	224	1.4±0.3	0.5(0.1-1.1)	4.7(2.7-2.7)	37.2(16.7-189.3)	4.4(13)	2.5	5.9	5.6
Field 2	56.8	306	0.6±0.1	0.05(0.008-0.16)	5.8(3.5-12.3)	590.5(111.5-1720)	3.8(13)	25	7.3	88.1
Field 3	71.3	234	1.1±0.2	0.13(0.04-0.26)	2.4(1.5-3.4)	43.7(19.7-138)	9.1(13)	6.5	3	6.5
Field 4	78	249	0.9±0.1	0.08(0.02-0.19)	2.4(1.6-3.4)	63.7(21.8-266)	9.2(13)	4	3	9.5
Field 5	59	278	0.7±0.2	0.07(0.008-0.23)	3.8(2.3-6.9)	207.2(60.8-2601))	4.3(13)	3.5	4.8	30.9
Field 6	100	236	1.1±0.2	0.1(0.03-0.22)	1.5(0.8-2.6)	21.1(10.6-68.4)	18.9(13)	5	1.9	3.1

a % Mortality stands for the % mortality at field dose of abamectin, which is 11 ppm a.i

RR represents resistance ratio = LC50 of field population/LC50 of susceptible population

Table 2. Toxicity of Acequinocyl to TSSM populations collected from alfalfa seed fields

Population	% Mortality[a]	N	Slope±SEM	LC_{10} (95%CL)	LC_{50} (95%CL)	LC_{90} (95%CL)	χ^2 (df)	RR_{10}	RR_{50}	RR_{90}
Susceptible	100	228	1.4±0.2	4.5(1.6-7.4)	20.7(13.2-27)	95.7(66.6-167)	5.3(16)	1	1	1
Field 1	90.7	245	1.3±0.2	7.6(2.4-14.1)	67.6(49.1-94))	605(346-1512)	14.7(13)	1.7	3.27	6.3
Field 2	85.2	240	1.2±0.2	3.6(1.4-7.2)	46.4(32.9-68.4)	603(348-1408)	4.5(13)	0.8	2.24	6.3
Field 3	90.5	240	1.1±0.2	3.4(0.8-7.4)	45.7(28-74)	610(254-2441)	21.6(13)	0.8	2.21	6.4
Field 4	93.9	228	1±0.2	1.9(0.6-4.2)	33.7(21-53)	587(284-1568)	10.8(13)	0.4	1.6	6.1
Field 5	60.4	250	2±0.4	44.1(22-63)	189.5(148-271)	813(444-2414)	2(13)	9.8	9.2	8.5
Field 6	100	219	1.5±0.2	7.3(2-14.1)	49.5(31-78.7)	337(180-1106)	31.5(13)	1.6	2.4	3.5

a % Mortality stands for the % mortality at field dose of Kanemite, which is 245 ppm a.i

b RR represents resistance ratio = LC50 of field population/LC50 of susceptible population

Table 3. Toxicity of bifenthrin to TSSM populations collected from alfalfa seed fields

Population	% Mortalitya	N	Slope±SEM	LC_{10} (95%CL)	LC_{50} (95%CL)	LC_{90} (95%CL)	χ^2 (df)	RR_{10}	RR_{50}	RR_{90}
Susceptible	100	230	1.73 ± 0.08	2.4(0.8-4.2)	17.97(8.4-44.6)	85.2(45-112.2)	73.6(13)	1	1	1
Field 1	61	230	1.0±0.2	4.2(1-8)	73.7(47-145)	1321.3(463-12232)	5.4(13)	1.8	4.1	15.5
Field 2	33.6	222	0.7±0.2	10.8(1-25)	958.2(234-3564)	5464(4340-94323)	7.3(13)	4.5	53.3	64
Field 3	71	239	1.3±0.2	6.3(3-11)	60.6(43-96)	580.9(281-2222)	7.4(13)	2.6	3.4	6.9
Field 4	69.8	230	1.3±0.2	6.4(2-11)	61.9(43-99)	602.9(286-2501)	11.6(13)	2.7	3.4	7.1
Field 5	81.5	283	1.2±0.2	2.3(0-6)	29.0(16-50)	357.4(165-1625)	32.3(16)	1	1.6	4.2
Field 6	85.6	237	1.2±0.2	2.1(1-4)	26.8(18-40)	344.2(173-1148)	5.9(13)	0.9	1.5	4

a % Mortality stands for the % mortality at field dose of bifenthrin, which is120 ppm a.i

b RR represents resistance ratio = LC50 of field population/LC50 of susceptible population

Table 4. Toxicity of bifenazate to TSSM populations collected from alfalfa seed fields

Population	% Mortality[a]	N	Slope±SEM	LC_{10} (95%CL)	LC_{50} (95%CL)	LC_{90} (95%CL)	χ^2 (df)	RR_{10}	RR_{50}	RR_{90}
Susceptible	100	236	0.7±0.1	0.24(0.05-0.880	11.5(5.1-26.1)	148(118-236)	8.8(13)	1	1	1
Field 1	69.8	232	1.8±0.4	51.5(17-84)	248.8(184-360)	1202.5(678-4527)	5.7(13)	214.6	21.6	8.1
Field 2	42.1	239	1.6±0.4	92.9(40-138)	600.9(390-1611)	3885.0(1496-48166-)	6.2(13)	387	52.3	26
Field 3	95.1	290	5.1±1.1	133.7(75-170)	239.3(199-274)	428.2(355-652)	6.5(13)	557	20.8	3
Field 4	72.7	247	1.5±0.2	34.7(12-59)	234.2(156-437)	1580.3(716-9553)	19.1(13)	144.6	20.4	10.7
Field 5	100	244	1.5±0.2	11.2(2-25)	78.6(42-134)	549.6(280-2296)	29.5(13)	46.7	6.8	3.7
Field 6	85.5	237	1.2±0.2	7.8(2-15)	100.3(68-150)	1290.8(647-4307)	5.9(13)	32.5	8.7	8.7

a % Mortality stands for the % mortality at field dose of bifenazate, which is 450 ppm a.i

b RR represents resistance ratio = LC_{50} of field population/LC50 of susceptible population

Table 5. Toxicity of cyflumetofen to TSSM populations collected from alfalfa seed fields

Population	%Mortalitya	N	Slope±SEM	LC10 (95%CL)	LC50 (95%CL)	LC90 (95%CL)	χ^2 (df)	RR_{10}	RR_{50}	RR_{90}
Susceptible	100	291	4.6±0.2	1.3(0.6-2.1)	7.9(5.7-10.4)	47.9(33.5-78.9)	6.9(19)	1	1	1
Field 1	100	238	1.6±0.2	7.4(1-16)	46.0(23-95)	285.4(125-2885)	46.3(13)	5.7	5.9	6
Field 2	67.3	296	1.1±0.3	9.3(1-20)	124.8(83-240)	1684.7(606-24657)	4.5(13)	7.2	15.8	35.2
Field 4	95.1	280	4.7±0.9	53.9(33-69)	101.7(85-118)	191.9(159-275)	5.5(13)	41.5	12.9	4
Field 5	80.2	245	1.3±0.2	7.8(3-14)	82.1(57-129)	867.2(426-3012)	10.0(13)	6.1	10.4	18.1
Field 6	80.8	234	1.0±6.5	2.3(1-5)	39.7(25-61)	671.8(322-2426)	5.4(13)	1.9	5	14

a % Mortality stands for the % mortality at field dose of cyflumetofen, which is 210 ppm a.i

b RR represents resistance ratio = LC50 of field population/LC50 of susceptible population

Table 6. Toxicity of propargite to TSSM populations collected from alfalfa seed fields

Population	% Mortality[a]	N	Slope±SE	LC_{10} (95%CL)	LC_{50} (95%CL)	LC_{90} (95%CL)	χ^2 (df)	RR10	RR50	RR90
Susceptible	100	209	1.4±0.2	6.9(1.9-16.8)	56.(25.6-87.2)	459(283-792)	11.1(13)	1	1	1
Field 1	80.3	245	2±0.5	242.8(106.8-362.6)	1006(760-1332)	4169(2453-15713)	10.9(13)	35.2	17.9	9.1
Field 2	74.7	296	1.9±0.5	247.3(110-415)	1274.5(1026-1726)	5922(3464-30462)	6.1(13)	35.8	22.7	12.9
Field 3	48.5	228	0.6±0.2	60.9(8.6-140)	6490(2412-7309)	691780(64810-3884300)	11.9(13)	8.8	115.7	1507
Field 4	83.8	321	0.8±0.2	61(9.7-134)	712(461-962)	8301(3632-45448)	11.5(13)	8.8	12.7	18.1
Field 5	74.3	265	0.8±0.2	33.9(4.7-85)	1157(727-2208)	39522(12004-577340)	9(13)	4.9	20.6	86.1
Field 6	53.3	211	0.9±0.2	94.7(28-178)	2580(1308-11442)	70256(14376-432950)	7.9(13)	13.7	45.9	153

a % Mortality stands for the % mortality at field dose of propargite, which is1797 ppm a.i

RR represents resistance ratio = LC50 of field population/LC50 of susceptible population