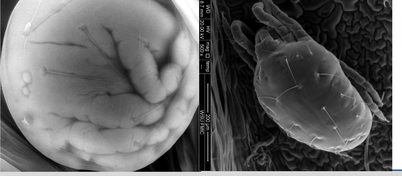

Multiple Acaricide resistance of the two-spotted spider mite in hop fields

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Abstract

Background: The two-spotted spider mite (TSSM) is a key pest of hop, especially in the pacific northwestern USA. Management of TSSM on hop is mainly by the application/rotation of different chemistries of acaricides. However, TSSM have developed resistance to these acaricides, leading to control failures and crop loss. Here, we evaluated resistance status of PNW hop TSSM populations to three acaricides: etoxazole, fenpyroximate, and spirodiclofen with dose-mortality bioassays. The mechanisms underlying resistance to six acaricides were also investigated in 36 field-collected TSSM populations.

Results: Resistance to abamectin, fenpyroximate, and spirodiclofen by enhanced metabolic detoxification was detected in 100%, 50%, and 20% of populations tested. Resistance-associated mutations were detected across target genes; I1017F in chitin synthase 1 gene, G126S in cytochrome b, and M918L, F1534S and F1538I in the voltage-gated sodium channel gene, with these mutations occurring in 97, 85.7, 25, 28 and 83 % of the tested population respectively.

Conclusion: Our study provides new information in understanding the complexity of TSSM adaptation to multiple acaricides, which will help in designing sustainable pest control strategies for TSSM on hops and other economic crops.

Introduction

- ✓ *Humulus lupulus* (hops) is a specialty crop that is cultivated mainly for its oils used in flavoring of craft beer. The US hop industry is worth >$500 million with key production areas in three Pacific Northwestern (PNW) states (Washington, Oregon and Idaho), which make up over 99% of the US and 30% of the world's hop acreage in 2015 [(1)].
- ✓ Successful cultivation of hops in PNW can be hindered by TSSM infestation[(2)].
- ✓ **Etoxazole(Mite Growth Inhibitor), fenpyroximate(Mitochondrion Electron Transport Inhibitor) and spirodiclofen(Lipid Biosynthesis Inhibitor)** are major TSSM management tools on hops based on farmers spray records.
- ✓ The use of these narrow-spectrum acaricides to reduce the ecological side-effects of earlier generations of broad-spectrum acaricides has resulted in increased rates of resistance development.
- ✓ Hence, it is important to know if populations of TSSM on hops harbor multiple resistant phenotypes and genotypes.

Research Goal and Objective

In order to design the most effective and sustainable TSSM management strategy, this study is aimed at:

(1) Knowing the resistant status of TSSM populations on PNW hops to 3 widely used acaricides with different mode of actions i.e. etoxazole, fenpyroximate and spirodiclofen.

(2) Understanding the underlying mechanisms of resistance; target-site mutations and metabolic resistance of hop TSSM to these acaricides.

Experimental Set-up (Methodology)

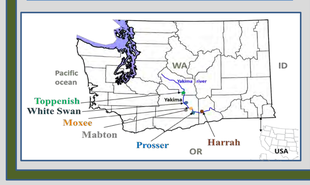
TSSM populations (36 in total) were collected from hop farms in **6** main districts of Yakima, Washington State (**Harrah, Mabton, Moxee, Prosser, Toppenish and White Swan**) and subjected to dose-mortality test with acaricides (etoxazole, fenpyroximate and spirodiclofen). The corresponding LC_{50} value of each population was compared to that of a susceptible TSSM strain to compute **a resistant ratio (RR)**. Genomic DNA and RNA were extracted from TSSM populations to characterize their resistant genotypes by diagnostic PCR and qRT-PCR respectively.

Phenotype Characterization

Precision spray of acaricides

bioassay

Levels of resistant phenotypes
RR < 2 susceptible
2<RR< 10 =Low Resistance
10 < RR < =100 Moderate
RR > 100 =High Resistance



Genotype Characterization

Diagnostic PCR for resistance-associated mutations

Extract gDNA

Design primers around mutation region on gDNA

Amplify mutation region

Sanger sequencing of amplicon

Examine presence of mutation

qRT-PCR to measure mRNA expression

Extract total RNA

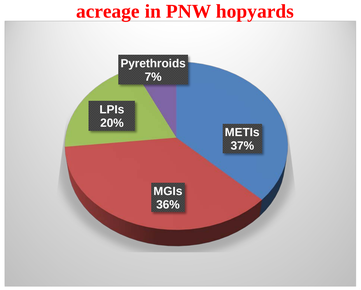
Synthesize cDNA

Perform PCR to measure mRNA expression of resistant-associated metabolic genes i.e. cytochrome P450, glutathione S transferase and carboxylesterases

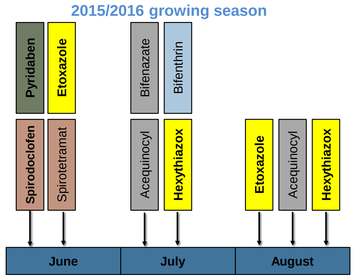
Results

Acaricide Spray Model at Hopyards: Several acaricides with different modes of actions were applied for the control of TSSM in PNW hops based on the spray records obtained from hop growers in the PNW (Figures below).

Acaricide usage based on acreage in PNW hopyards



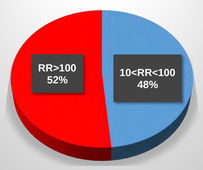
TSSM management regime in PNW Hops
2015/2016 growing season



*** Acaricides with same color box have similar mode of action**

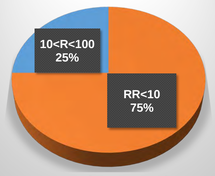
Acaricide Resistance Levels in TSSM Field Populations on hops: the toxicities of 3 registered acaricides i.e. etoxazole(MGI), fenpyroximate (METI) and spirodoclofen (LPI) for TSSM control, were assessed on 31, 28, and 21 hop TSSM populations respectively.

Etoxazole(MGI)
- ✓ LC_{50s} ranged from 16 to 217 ppm a.i. which is below its field dose(300ppm a.i.).
- ✓ RRs ranged from 24.7 to 216.
- ✓ Mortality at the field dose ranged from 63.2 to 86.7%.



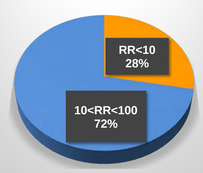
Etoxazole resistant ratio In hop TSSM

Fenpyroximate(METI)
- ✓ LC_{50s} ranged from 25 to 228.5 ppm a.i. (field dose=180 ppm a.i.).
- ✓ RRs of the populations ranged from 2.6 to 24.2.
- ✓ Mortality at the field dose ranged from 52 to 100 %.



Fenpyroximate resistant ratio in hop TSSM

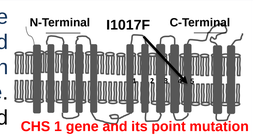
Spirodiclofen(LPI)
- ✓ LC_{50s} ranged from 6.0 to 83.5 ppm a.i. which is below its field dose(300ppm a.i.).
- ✓ RRs varied from 3.2 to 44.1.
- ✓ Mortality at field dose ranged from 71-100%



Spirodiclofen resistant ratio in hop TSSM

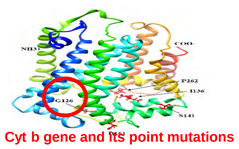
Evaluation of Resistance-Associated Target Site Mutations: the presence of mutations across 7 acaricide target genes in hop TSSM were evaluated.

Mutation in Chitin synthase 1 gene (I1017F): 5.4% of the TSSM populations had susceptible allele(I), while 89.2% had heterozygous allele (I/F). Interestingly, 5.4% of the population had resistant allele (F) fixed i.e. homozygous resistant allele. Indicating target insensitivity is responsible for the observed phenotypic resistance to MGIs like etoxazole.



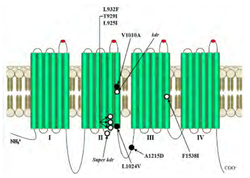
CHS 1 gene and its point mutation

Bifenazate (METI class II): 5 mutations that are associated with bifenazate resistance in the mitochondrial cytochrome b (**Cyt b**) gene were assessed, but only the **G126S** mutation was detected in 90% of the population which all had the heterozygous allele(G/S).



Cyt b gene and its point mutations

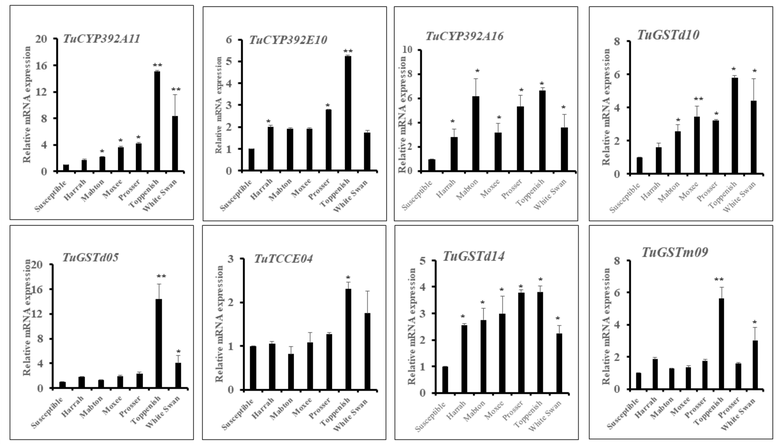
Mutations in the voltage-gated sodium channel gene(VGSC): As showed by the acaricide spray record on hops, pyrethroids (bifenthrin) are also used for TSSM management. Hence, we amplified fragments of the VGSC gene from field TSSM to check for mutations. In domain II, super kdr resistance M918L was detected in 25% of the population. In the domain III, F1534S (heterozygous=29%) and F1538I (heterozygous 43.3%, homozygous =53%) mutations were also observed.



No mutation was observed at the acetyl-coenzyme A carboxylase (K645E) and NADH dehydrogenase 1 of mitochondrial inner memeberane(H92R) and the Glutamate chloride channels (G323D &G326E) ; indicating other point mutations or/and mechanisms of resistance to METI class I (fenpyroximate), LPIs (Spirodiclofen), and abamectin.

Expression Pattern of Resistance-Associated Metabolic Genes

Molecular analysis of spirodiclofen resistance in TSSM indicate a P450, ***CYP392E10*** and a carboxyl/choline esterase-4 ***CCE04*** gene are strongly associated with high levels of resistance to spirodiclofen (Figure below). These two genes can be used as molecular markers to screen for spirodiclofen resistance. Gene expression of 2 P450s i.e. ***CYP392A11***, and ***CYP392A16*** that have been functionally studied[(4)] to metabolize fenpyroximate and abamectin respectively were significantly overexpressed (>2 fold) in TSSM populations from PNW hops. ***TuGSTd10***, ***TuGSTd14*** and ***TuGSTm09*** that are involved in resistance to abamectin was also highly expressed. Thus suggesting polygenic resistance in hop TSSM. ***TuGSTd05*** which confer resistance to METI class II acaricides was over expressed in some of the hop TSSM populations.



Relative expression of resistance-associated metabolic genes in hop TSSM populations.** *P< 0.01,* P<0.05* (Resistance/Susceptible>=2 fold)

Outcome and Significance of Study

- ✓ Narrow-spectrum and reduced-risk acaricides are cardinal tools for sustainable TSSM management in most cropping systems. To remain IPM-compatible as long as possible, it is necessary to develop a comprehensive strategy to frequently examine the phenotypic resistance status and underlying mechanisms of adaptation to acaricides in field populations.
- ✓ This study shows that populations of TSSM on hops in the PNW harbor multiple acaricide-resistant phenotypes and genotypes. This pattern and level of resistance reflects the frequency of use of acaricides and their mode of action.
- ✓ There was significant variation in the level of phenotypic and genotypic resistance across the hop TSSM populations. This might be a result of differences in factors such as individual grower practices.
- ✓ Our data revealed a unique acaricide adaptation pattern for six commonly used acaricide in PNW hopyards. Resistance to abamectin, fenpyroximate, and spirodiclofen was by enhanced metabolic detoxification. While, target site insensitivity conferred resistance to bifenthrin, bifenazate and etoxazole was observed in a wide range of hop TSSM populations
- ✓ The resistant alleles present in TSSM populations on hops should be taken into consideration in the adoption of new chemistries of acaricides and in the design of future IPM programs.
- ✓ The approach and findings in this study are also extendible to other cropping systems, especially in pesticide management programs.

Acknowledgment and References

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1. USDA National Agriculture Statistics Service (NASS), National Hop Report (2013).
2. Piraneo et al., 2015 Scientific Report.
3. Van Leeuwen et al., 2012. PNAS
4. Van Leeuwen et al., 2015 Pesticide Physiology and Biochemistry.