Biology and Management of Spider Mites on hops

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Pest Management Strategic Plan for U.S. Hops

Summary of a workshop held on
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Pest Management Strategic Plan for Hops in Oregon, Washington, and Idaho
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Pest Management Strategic Plans are stored on the Southern Region IPM Center's website.

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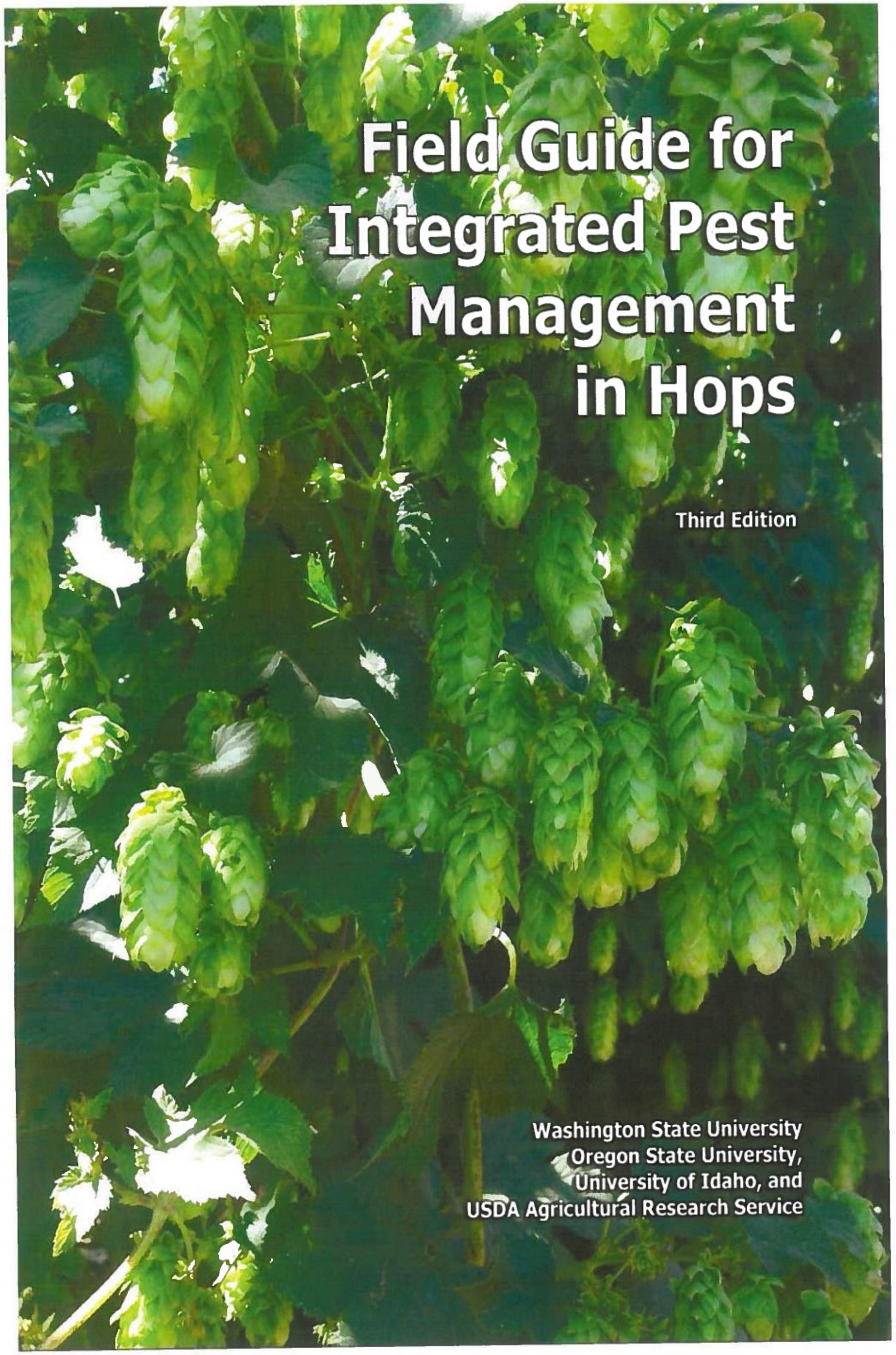

Field Guide for Integrated Pest Management in Hops

Third Edition • 2015

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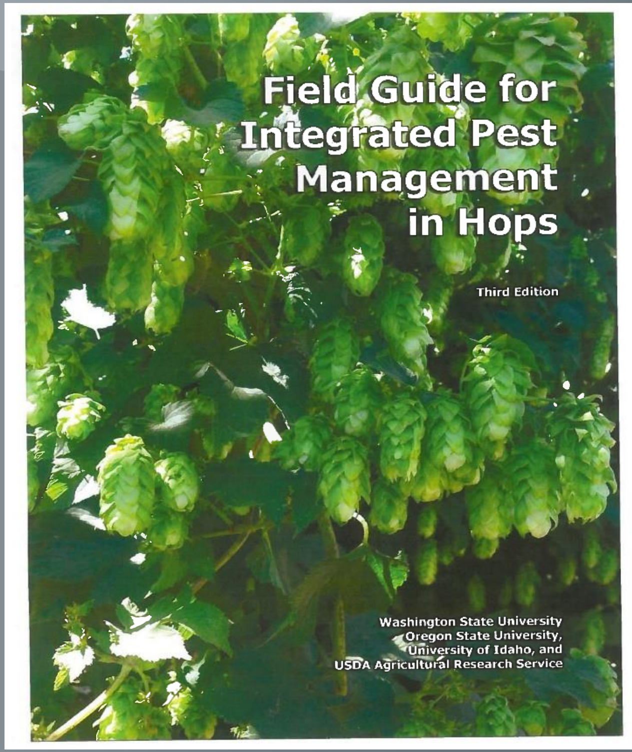
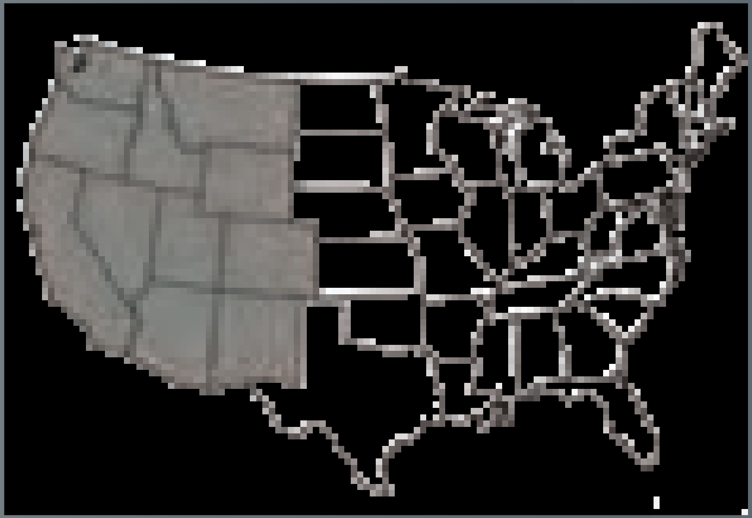
Arthropod Pests of Hops

- Aphids
- Beetles
  - California Prionus
  - Hop Flea Beetle
  - Japanese Beetle
  - Root Weevils
  - Rose Chafer
- Garden Symphylan
- Leafhoppers
- Caterpillars
- Spider mites





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Spider mites

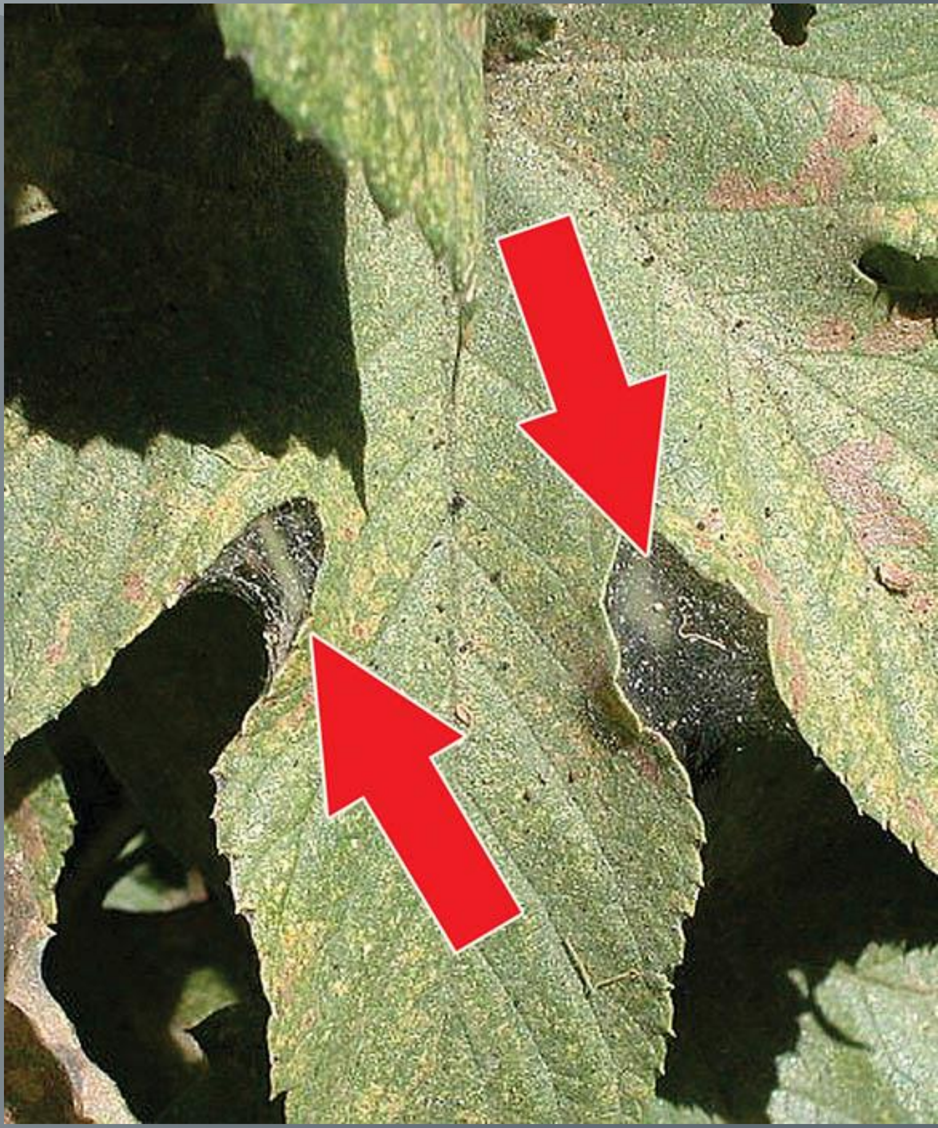
Twospotted Spider Mite, *Tetranychus urticae*

Twospotted spider mites are present throughout the U.S.

Pest Description and Crop Damage

- Adult female twospotted spider mites are small, oval, yellow to yellow-green arthropods, approximately 1/50 inch long, with a large black feeding spot on each side of the abdomen.
- Newly hatched spider mites (larvae) have three pairs of legs, whereas all other life stages (protonymphs, deutonymphs, and adults) have four.

- Spider mites at all life stages produce webs from silk glands located near their mouthparts.
- Webbing may protect the mite from wind, rain, natural enemies, and exposure to chemicals.



- Spider mites damage hops while feeding damaging parenchyma cells, and removing chlorophyll and other cell contents.
- The loss of chlorophyll results in a visibly patchy discoloration of leaf tissue as well as a reducing photosynthetic.
- At extreme populations complete defoliation can occur.

- The most economic damage is caused by spider mites feeding on cones, which results in dry, brittle, discolored (red) cones that tend to shatter, reducing both quality and quantity of yield.
- Late-season mite feeding on both leaves and cones has been documented to reduce the alpha-acids content in hop cones at harvest. Spider mites in hop cones are also considered contaminants that lower cone quality.
- When infestations are severe, brewer rejection or total crop loss can occur.

- The life cycle of *T. urticae* progresses through four stages (egg, larva, protonymph, deutonymph) before molting into its fifth and final stage as an adult male or female.
- Newly laid eggs appear as translucent pearl-like spheres, 0.005 inch in diameter, and are deposited singly.
- Eggs become opaque as they mature, until hatching into a larv.
- At optimal temperatures of 86 to 90° F, twospotted spider mites can develop from egg to adult in as few as seven or eight days.
- Outbreaks usually occur during the hottest summer months of July and August when their populations can increase rapidly.

- Monitoring and Thresholds
- Spider mite (and predatory mite) abundance can be monitored during the dormant season using a simple but effective method.
- In the hop yard, collect a small trowel of soil litter from the top inch around at least 25 dormant or semi-dormant hop crowns

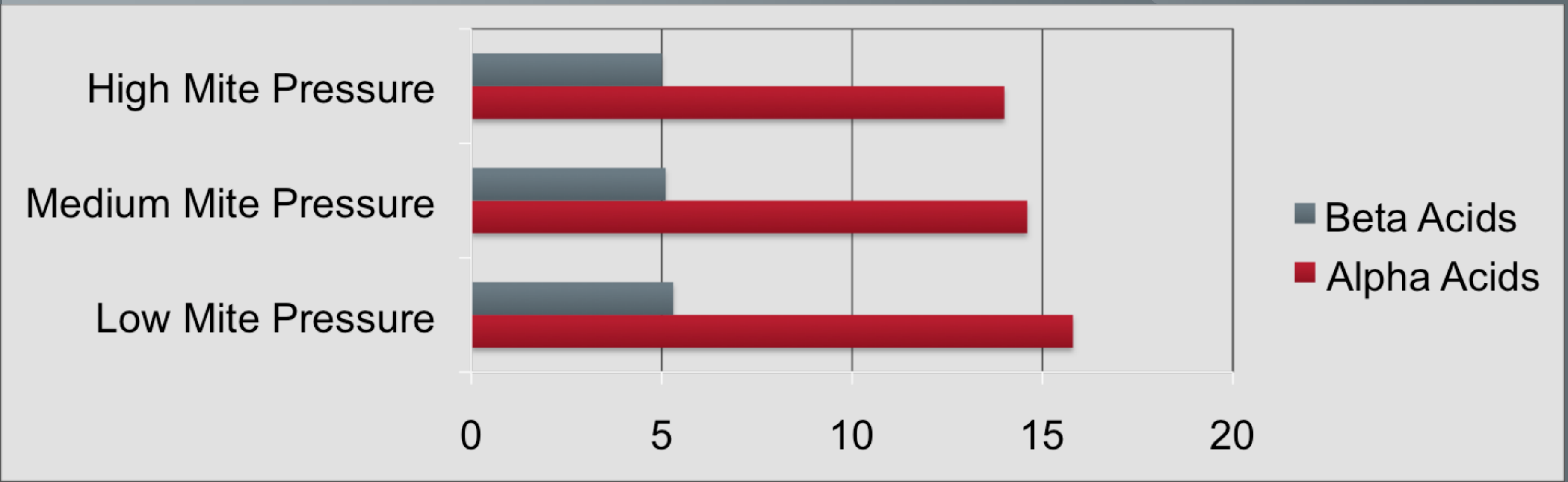
- Place these samples all together, mixing them lightly, in the gallon bag.
- Indoors, fill 25 five-oz disposable cups approximately halfway with material.
- Place each cup upright on a 3- by 5-inch yellow insect sampling sticky card on a table or countertop at heated room temperatures of roughly 70° F for a week

- At the end of this week, remove the cups and use a hand lens to count the pest and beneficial mites present on the sticky cards.
- Be aware that the adult female spider mites will be in their winter orange/red-colored morph and should not be confused with several species of predatory mites.
- This sampling technique is recommended in hop yards that had severe infestation the prior growing season.

In Season Foliar Samples

- Samples should be taken weekly beginning in mid- to late May by removing leaves and examining the undersides for the presence of spider mites, mite eggs, and webbing, as well as stippling and yellowing of leaves associated with spider mite feeding.
- After approximately mid-June, as the vines approach the trellis, samples should be taken from leaves higher in the canopy.
- Several leaves from each of 10 to 30 plants should be sampled depending on field size and the amount of time available.
- A 10X to 20X hand lens and a pole pruner are useful mite-sampling tools

Thresholds: Mite Feeding Acid Results: Laboratory Study



- High >50, Medium 15 to 50, Low <15 mites per leaf
- Tomahawk hop variety
- Acids standardized to 8% moisture
- SE range ±0.1 to ±0.3
- Significantly lower alpha acids on medium and high mite pressure samples at *p*<0.05 than samples collected from low mite infestation plots

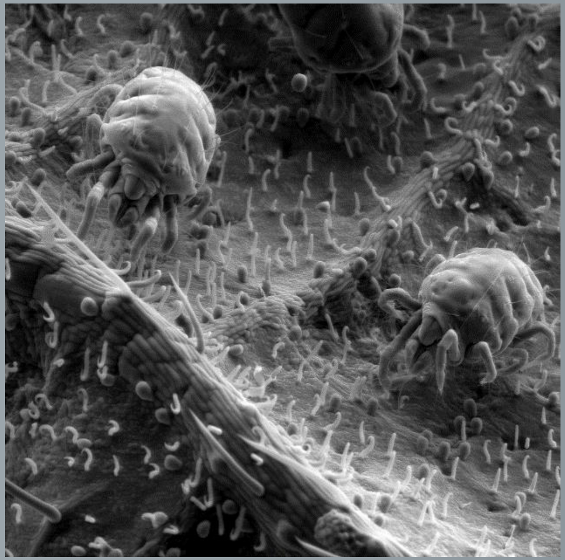
Primary method for control is still applying miticides

- Abamectin*
- Spirodiclofen
- Hexythiozox
- Etoxazole
- Fenpyroximate
- Spirotetramat
- Bifenazate*

*/ Well documented instances of field failures (e.g. Resistance) in Washington

Developing and validating robust diagnostics including a quantitative sequencing protocol and PCR to follow acaracide-based resistance frequencies in the field.

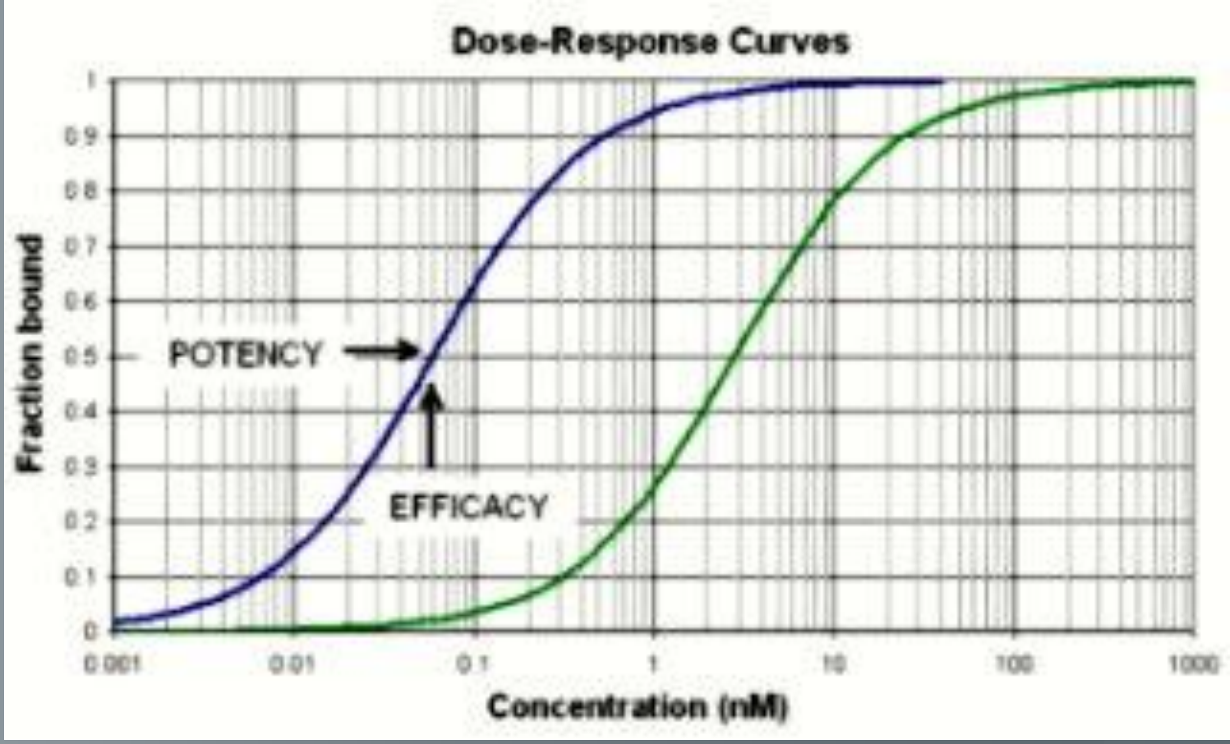
Acaricide Resistance



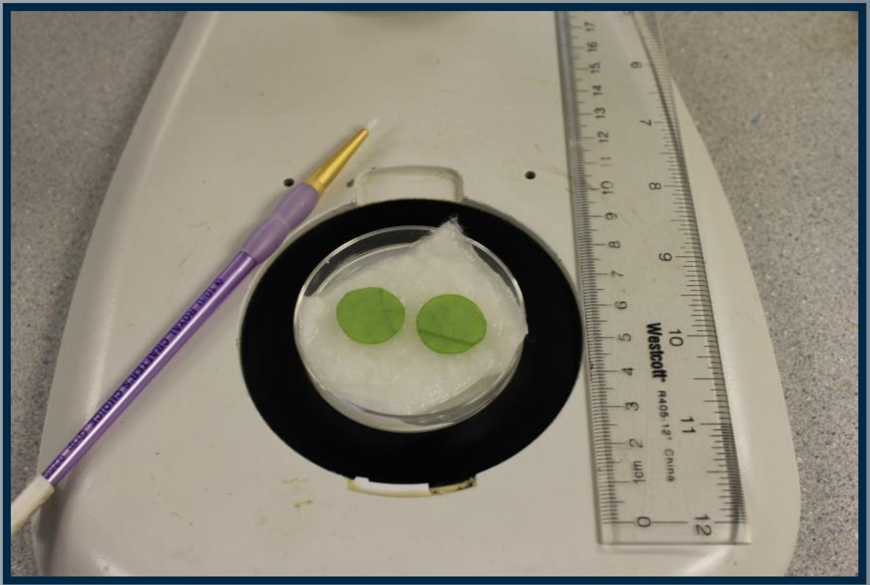
Spider Mites

- The two-spotted spider mite is the arthropod pest with the greatest documented number of resistance events worldwide
- Genomics/transcriptomic resources are available
- Genomic- All DNA (genes) present
- Transcriptomic- what genes are actully being upregulated (used)

We have Developed baseline dose response curves of spider mite populations susceptible to abamectin, bifenazate, bifenthrin, hexythiozox, etoxazole, and clofentazine.



Leaf Disc Bioassay for Adulticidal acaricides

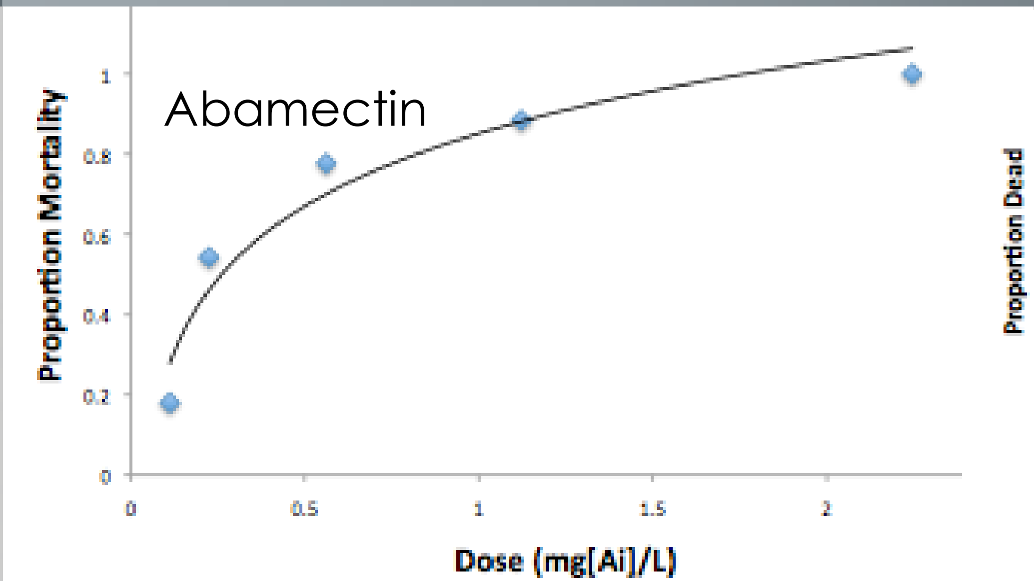


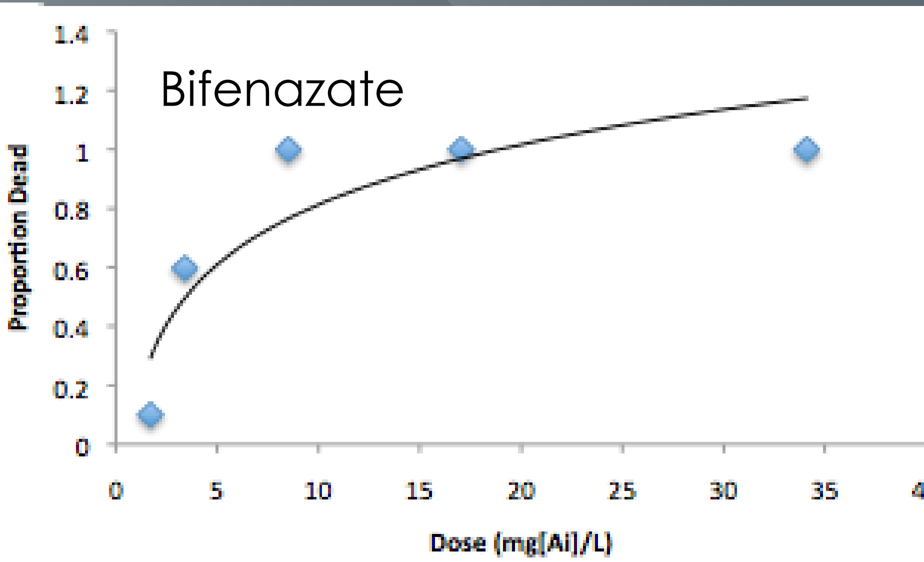
Transferred ten adult female mites to leaf discs placed on top of soaked cotton

Exposed to 2 mL of varying concentration of candidate acaricides.

Mites are held at 24C for 24 hrs, and evaluated for mortality

Results:
Dose response curves of susceptible colony





Miticide	Slope ±SEM	LC_{50} (mg[Ai]/L)	LC_{90} (mg[Ai]/L)
Abamectin	2.1398±0.2707	**0.216** (0.4826-0.8937)	1.18 (0.8937-0.9194)
Bifenture	1.217±0.097	**91.2** (0.4735-0.542)	218.7 (0.35-0.867)
Bifenazate	4.723±0.240	**3.05** (0.478-0.539)	5.54 (0.863-0.918)

Bioassay methods to evaluate the efficacy of ovicidal miticides.



- <u>We have validated 3 methods for screening ovicides.</u>

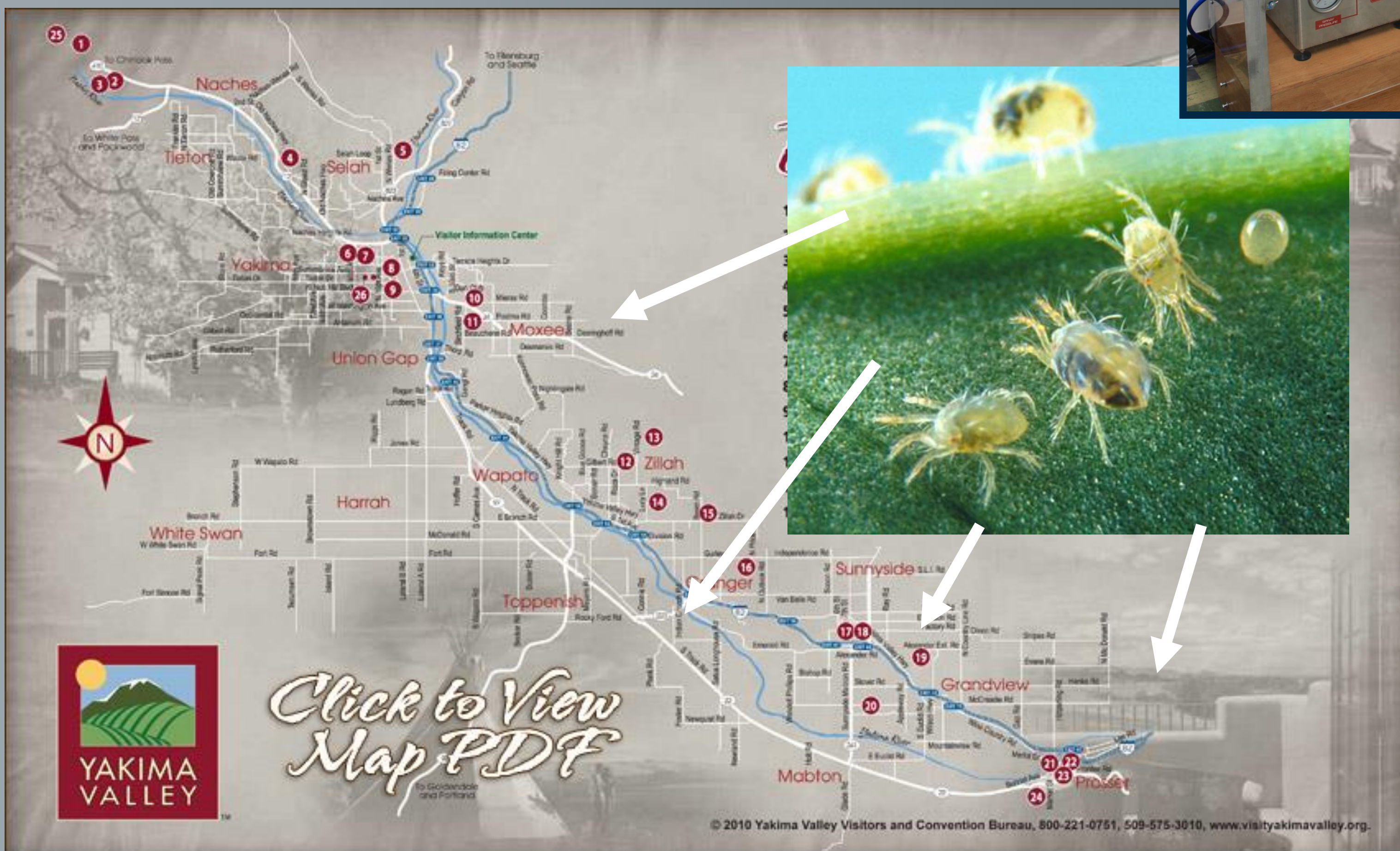
1. Direct exposure of mite eggs to ovicidal miticides
2. Exposure of gravid females and then monitor the eggs she lays
3. Spray the leaf disk and then place the female on the disk and permit her to lay eggs.

Baseline dose response curves of spider mite populations susceptible to hexythiozox, etoxazole and clofentazine

Dose response of ~24 hr old *T. urticae* eggs from susceptible colony

Acaricide	LC10 (ppm)	LC50 (ppm)	LC90 (ppm)	Slope ± Std error
Zeal (etoxazole)	0.215	0.646	1.937	2.68±0.21
Savey (hexythiozox)	0.011	0.715	48.115	0.7±0.067
Apollo (clofentazine)	0.073	2.173	64.67	0.87±0.09

In 2013 we tested 25 field populations of spider mites from hop yards and compared their dose response curves to abamectin, bifenazate, and bifenthrin to susceptible acaracide naïve mites.

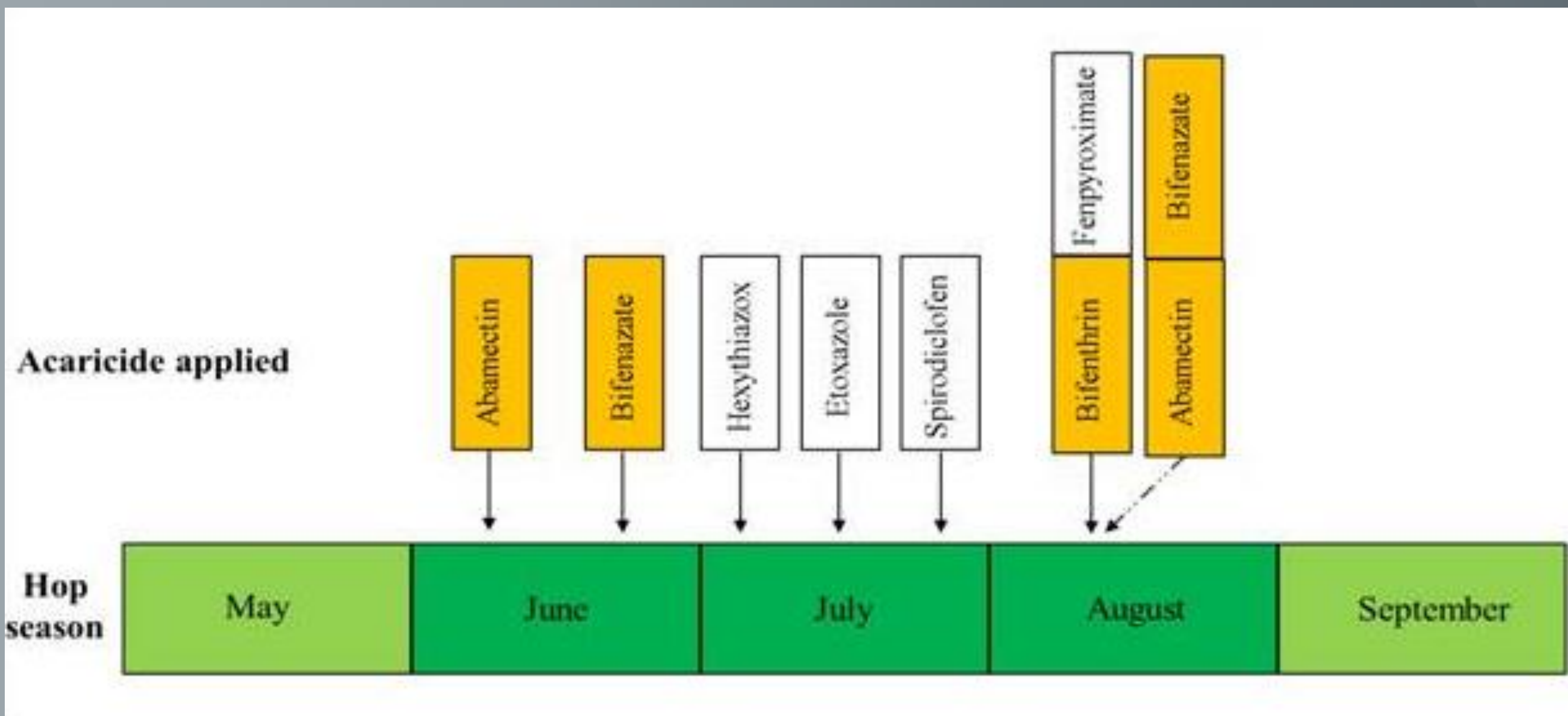


FIGHT THE MITE

- Mechanisms of miticide resistance can be <u>categorized broadly into two categories.</u>
- 1. Target site insensitivity conferred by conserved point mutations in specific target genes such as sodium gated ion channels
- 2. Metabolic resistance mediated by detoxification enzymes such as carboxylases, cytochrome P450s, and the multi-drug resistant ATP-binding cassette transporters.

Pesticide Resistance through Decreased Target Site Sensitivity

- A representative spray program for hopyards during the 2013 hop season in the Yakima Valley. Several acaricides with different modes of action were often applied to control *T. urticae*. Among them, abamectin, bifenazate, and bifenthrin were the most common used miticides in hopyards



Target Site Insensitivity

- A total of 16 mutations in four target genes were observed and evaluated.

- We successfully identified one mutation on cytochrome b (G126R) in 36% of the field populations tested including 24% double alleles (G/R) and 12% resistant alleles (R).
  This is linked to bifenazate resistance.

- The other mutation on sodium channel (F1538I) was identified in 66.7% field populations tested including 50% double alleles (F/I) and 16.7% resistant allele (I).
  This is linked to bifenthrin resistance

Code	Location	Date Sampled	TuGluCl1 (G323D)	TuGluCl3 (G326E)	Cytb (	KDR II	KDR II-III (A1215D)	KDR III (F1538I)
Susceptible	Prosser	06/10/13	G	G	No	No	A	F
Grandview [a)]	McCoomb	09/23/13	G	G	-	No	A	I
Granger 1[a)]	Carpenter-F 1	07/16/13	G	G	No	No	A	F/I
Granger 1	Carpenter-F 1	08/20/13	G	G	No	No	A	F
Granger 2[b)]	Carpenter-F 2	07/16/13	G	G	No	No	A	F
Granger 3	Carpenter-F 3	07/25/13	G	G	No	No	A	F
Granger 3	Carpenter-F 3	08/20/13	G	G	**G126G/R**	No	A	F/I
Granger 4	Carpenter-F 4	07/25/13	G	G	**G126G/R**	No	A	F/I
Granger 5	Carpenter-F 5	07/25/13	G	G	No	No	A	F/I
Mabton 1	Sauve-F 1	07/15/13	G	G	No	No	A	F/I
Mabton 1[a)]	Sauve-F 1	07/16/13[c)]	G	G	No	No	A	F/I
Mabton 2	Sauve-F 2	07/15/13[c)]	G	-	No	No	-	-
Mabton 3	Puterbaugh	07/02/13	G	G	No	No	A	F/I
Moxee 1[a)]	Roy Farms-F1	07/18/13	G	G	**G126R**	No	A	F
Moxee 2	Roy Farms-F2	08/29/13	G	G	**G126R**	No	A	F/I
Prosser 1	Olsen-Hanks	07/14/13[c)]	G	G	**G126G/R**	No	A	F/I
Prosser 2	Pleasant	07/14/13	G	G	No	No	A	F/I
Prosser 2	Pleasant	07/28/13	G	G		No	A	F
Prosser 2[a)]	Pleasant	08/19/13	G	G	No	No	A	F
Prosser 3	Roza/Pleasant	07/14/13	G	G	No	No	A	F
Prosser 3	Roza/Pleasant	08/19/13	G	G	No	No	A	F
Prosser 4	Roza/Hogue	07/17/12	G	G	No	No	-	I
Prosser 4	Roza/Hogue	09/03/13	G	G	**G126G/R**	No	A	F/I
Prosser 4	Roza/Hogue	09/08/13	G	G	**G126G/R**	No	A	I
Prosser 5	Goldengate	07/24/13	G	G	**G126G/R**	No	A	F/I
Prosser 5	Goldengate	08/21/13	G	G	**G126R**	No	A	I

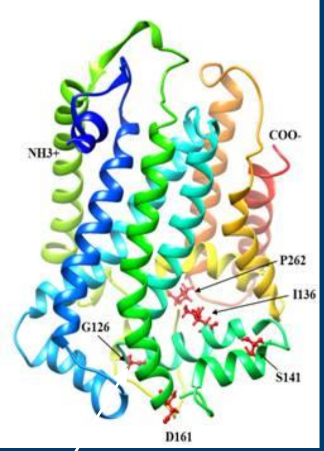
Code	Location	Date Sampled	TuGluCl1 (G323D)	TuGluCl3 (G326E)
Susceptible	Prosser	06/10/13	G	G
Grandview [a)]	McCoomb	09/23/13	G	G
Granger 1[a)]	Carpenter-F 1	07/16/13	G	G
Granger 1	Carpenter-F 1	08/20/13	G	G
Granger 2[b)]	Carpenter-F 2	07/16/13	G	G
Granger 3	Carpenter-F 3	07/25/13	G	G
Granger 3	Carpenter-F 3	08/20/13	G	G
Granger 4	Carpenter-F 4	07/25/13	G	G
Granger 5	Carpenter-F 5	07/25/13	G	G
Mabton 1	Sauve-F 1	07/15/13	G	G
Mabton 1[a)]	Sauve-F 1	07/16/13[c)]	G	G
Mabton 2	Sauve-F 2	07/15/13[c)]	G	-
Mabton 3	Puterbaugh	07/02/13	G	G
Moxee 1[a)]	Roy Farms-F1	07/18/13	G	G
Moxee 2	Roy Farms-F2	08/29/13	G	G
Prosser 1	Olsen-Hanks	07/14/13[c)]	G	G
Prosser 2	Pleasant	07/14/13	G	G
Prosser 2	Pleasant	07/28/13	G	G
Prosser 2[a)]	Pleasant	08/19/13	G	G
Prosser 3	Roza/Pleasant	07/14/13	G	G
Prosser 3	Roza/Pleasant	08/19/13	G	G
Prosser 4	Roza/Hogue	07/17/12	G	G
Prosser 4	Roza/Hogue	09/03/13	G	G
Prosser 4	Roza/Hogue	09/08/13	G	G
Prosser 5	Goldengate	07/24/13	G	G
Prosser 5	Goldengate	08/21/13	G	G

These are mutations that are linked to abamectin resistance in *T. urticae* Korea

This implies that target site insensitivity mediated resistance is not the mechanism of abamectin resistance in our hopyards

Code	Location	Date Sampled	Cytb
Susceptible	Prosser	06/10/13	No
Grandview [a]	McCoomb	09/23/13	-
Granger 1[a]	Carpenter-F 1	07/16/13	No
Granger 1	Carpenter-F 1	08/20/13	No
Granger 2[b]	Carpenter-F 2	07/16/13	No
Granger 3	Carpenter-F 3	07/25/13	No
Granger 3	Carpenter-F 3	08/20/13	**G126G/R**
Granger 4	Carpenter-F 4	07/25/13	**G126G/R**
Granger 5	Carpenter-F 5	07/25/13	No
Mabton 1	Sauve-F 1	07/15/13	No
Mabton 1[a]	Sauve-F 1	07/16/13[c]	No
Mabton 2	Sauve-F 2	07/15/13[c]	No
Mabton 3	Puterbaugh	07/02/13	No
Moxee 1[a]	Roy Farms-F1	07/18/13	**G126R**
Moxee 2	Roy Farms-F2	08/29/13	**G126R**
Prosser 1	Olsen-Hanks	07/14/13[c]	**G126G/R**
Prosser 2	Pleasant	07/14/13	No
Prosser 2	Pleasant	07/28/13	No
Prosser 2[a]	Pleasant	08/19/13	No
Prosser 3	Roza/Pleasant	07/14/13	No
Prosser 3	Roza/Pleasant	08/19/13	No
Prosser 4	Roza/Hogue	07/17/12	No
Prosser 4	Roza/Hogue	09/03/13	**G126G/R**
Prosser 4	Roza/Hogue	09/08/13	**G126G/R**
Prosser 5	Goldengate	07/24/13	**G126G/R**
Prosser 5	Goldengate	08/21/13	**G126R**

We have investigated for 5 mutations in *T.urticae* cytochrome b that are linked to bifenazate resistance in Korea and Israel.



Only one mutation G126R was observed in 36% field samples tested including

24% double alleles (G/R) (partial resistance)

12% resistant allele (R) (fully resistant)

Code	Location	Date Sampled	KDR II	KDR II-III (A1215D)	KDR III (F1538I)
Susceptible	Prosser	06/10/13	No	A	F
Grandview [a)]	McCoomb	09/23/13	No	A	I
Granger 1[a)]	Carpenter-F 1	07/16/13	No	A	F/I
Granger 1	Carpenter-F 1	08/20/13	No	A	F
Granger 2[b)]	Carpenter-F 2	07/16/13	No	A	F
Granger 3	Carpenter-F 3	07/25/13	No	A	F
Granger 3	Carpenter-F 3	08/20/13	No	A	F/I
Granger 4	Carpenter-F 4	07/25/13	No	A	F/I
Granger 5	Carpenter-F 5	07/25/13	No	A	F/I
Mabton 1	Sauve-F 1	07/15/13	No	A	F/I
Mabton 1[a)]	Sauve-F 1	07/16/13[c)]	No	A	F/I
Mabton 2	Sauve-F 2	07/15/13[c)]	No	-	-
Mabton 3	Puterbaugh	07/02/13	No	A	F/I
Moxee 1[a)]	Roy Farms-F1	07/18/13	No	A	F
Moxee 2	Roy Farms-F2	08/29/13	No	A	F/I
Prosser 1	Olsen-Hanks	07/14/13[c)]	No	A	F/I
Prosser 2	Pleasant	07/14/13	No	A	F/I
Prosser 2	Pleasant	07/28/13	No	A	F
Prosser 2[a)]	Pleasant	08/19/13	No	A	F
Prosser 3	Roza/Pleasant	07/14/13	No	A	F
Prosser 3	Roza/Pleasant	08/19/13	No	A	F
Prosser 4	Roza/Hogue	07/17/12	No	-	I
Prosser 4	Roza/Hogue	09/03/13	No	A	F/I
Prosser 4	Roza/Hogue	09/08/13	No	A	I
Prosser 5	Goldengate	07/24/13	No	A	F/I
Prosser 5	Goldengate	08/21/13	No	A	I

We have examined nine mutations on the voltage-gated sodium channel that are known to link with pyrethroid resistance in *T. urticae* or other insect pests

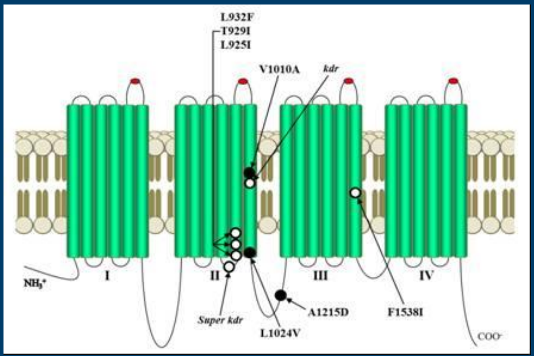
For bifenthrin resistance

Only one mutation (F1538I) was observed in T. urticae from hopyards.

66.7% of the populations in hopyards had this mutation

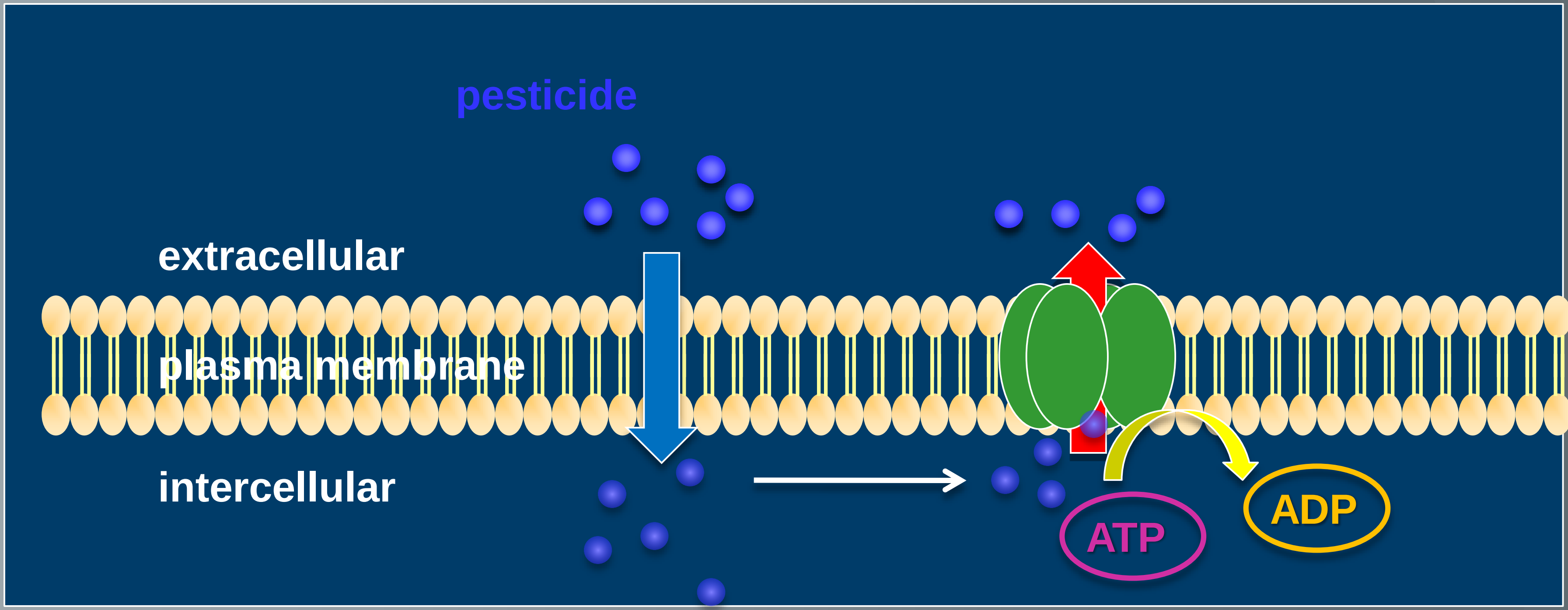
50% double alleles (F/I)) (partial resistance)

16.7% resistant allele (I) (fully resistant)

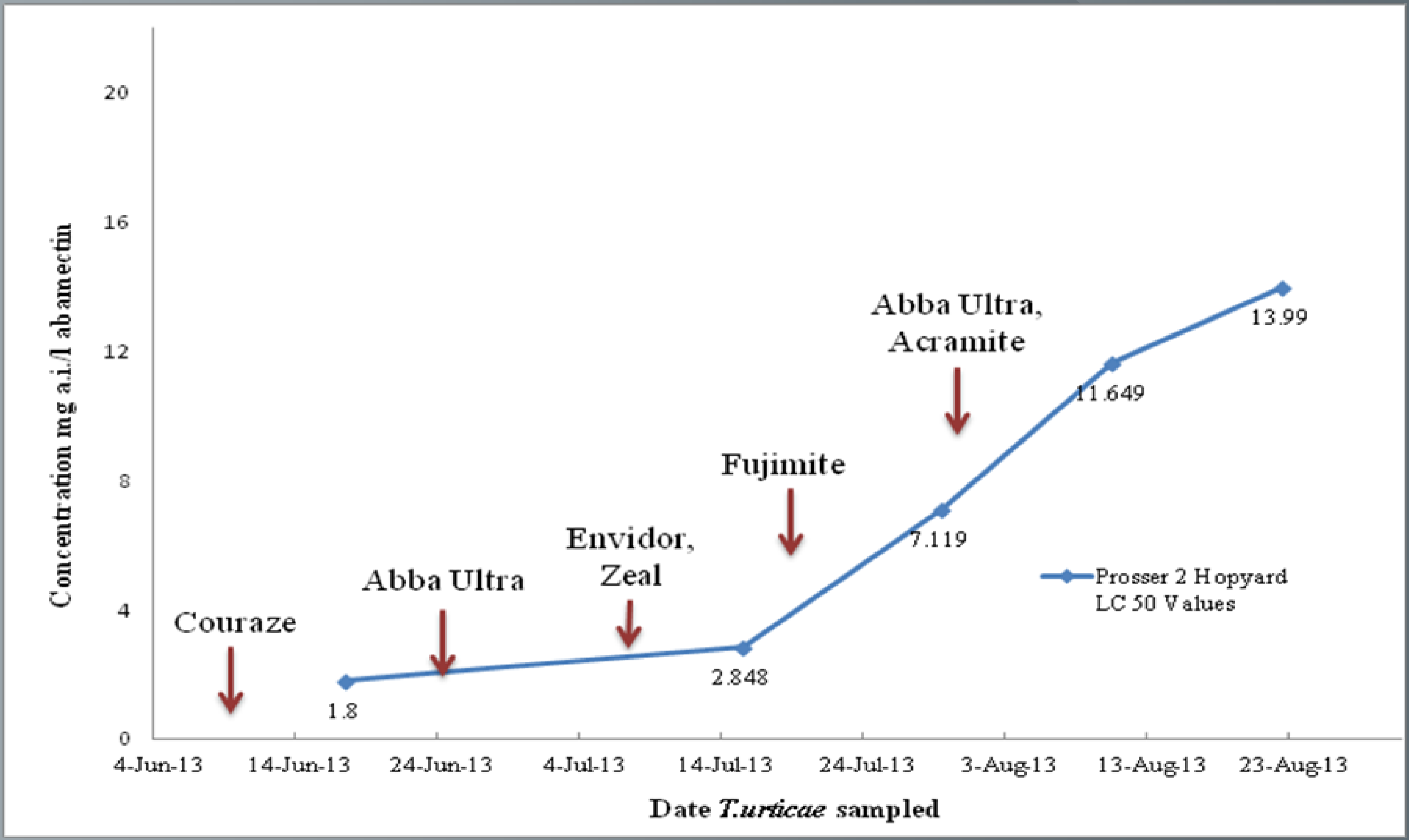


Where are we with Abamectin Resistance?

- ABC transporters are transmembrane proteins that utilize the energy of adenosine triphosphate (ATP) hydrolysis to translocate toxins across membranes

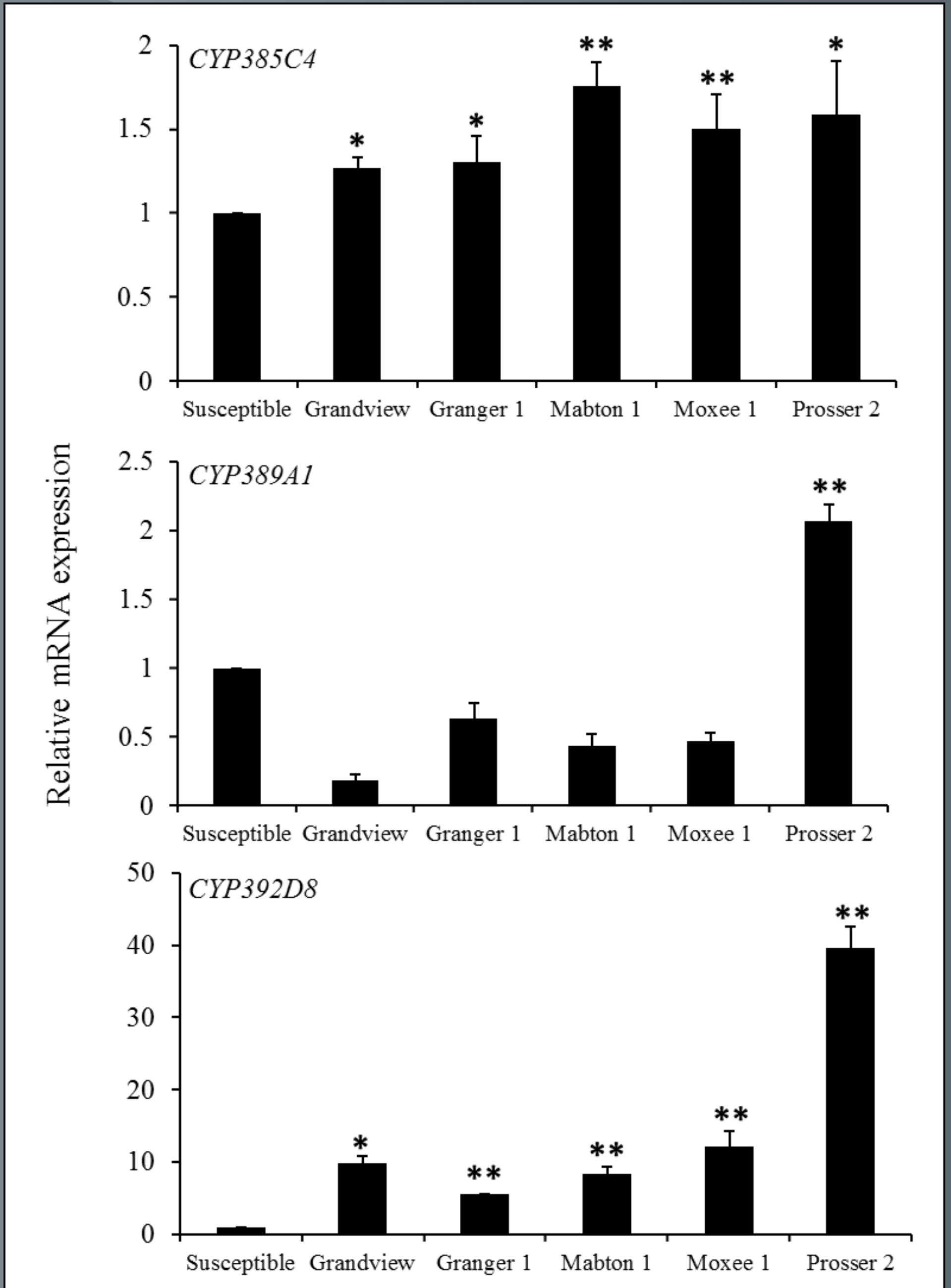


Increases in the titer of ABC transporters in the mite population over the course of the season is making them more tolerant to many toxins including abamectin.



Acaricide Resistance through Increased Acaricide Detoxification

- Relative expression of *CYP385C4*,
- *CYP389A1* and *CYP392D8* in the susceptible and field *T. urticae* populations.
- The mRNA levels were quantified by qRT-PCR and normalized with reference genes *Actin* and *RP49*.
- The data shown are mean + SEM (n = 3).
- Statistical significance of the gene expression between two samples was calculated using Student's *t* test.
- * *p*-value < 0.05, ***p*-value < 0.01.



Conclusions (so far w/ abamectin)

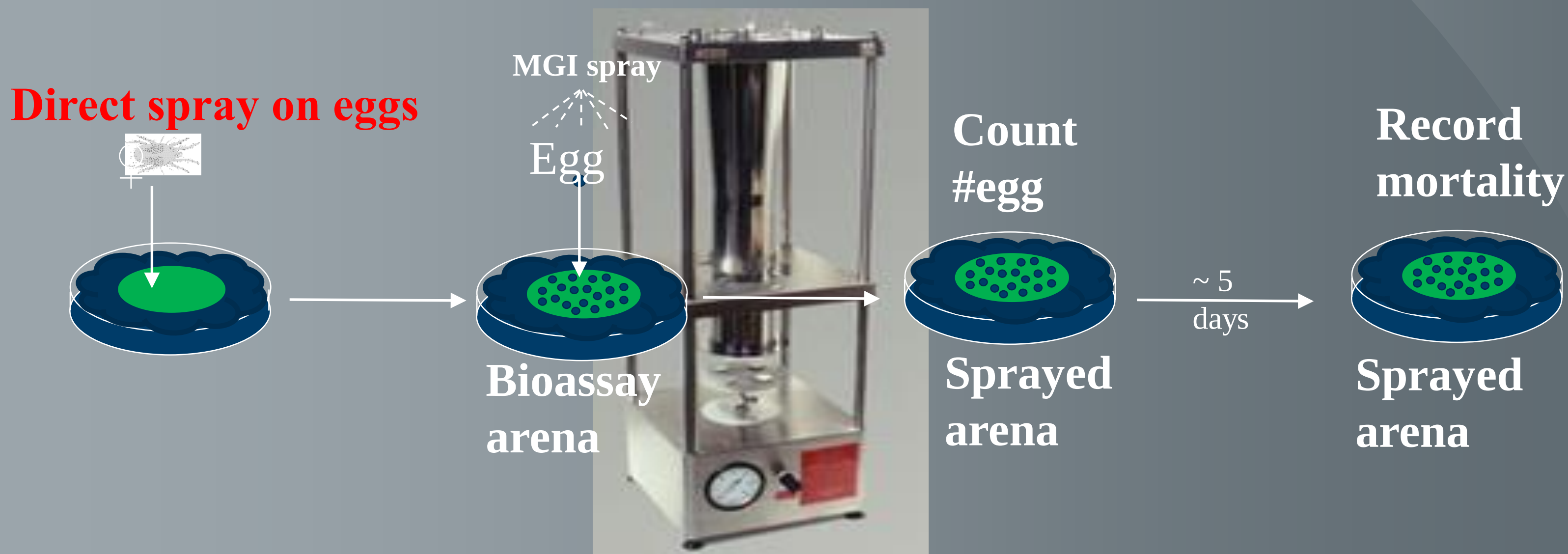
- Target site insensitivity is not the mechanism involved in *T. urticae* resistance to abamectin since there is no mutation on Glutamate-gated chloride channel subunits detected.
- Other mechanisms such as enhanced metabolic detoxification may play a role in the resistance to abamectin, such as that which led to *T. urticae* control failure with abamectin in hopyards during 2013.

Test selected field populations of spider mites from a representative sample of hopyards and compare their dose response curves to etoxazloe, hexythiozox and clofentazinemite populations as detailed above.

35 *T. urticae* populations were sampled from hopyards in 2016

Methods

Characterizing phenotypic resistance to ovicidal acaricides (Zeal®) in field-collected spidermite population from hopyards



Parameters

eggs laid mortality LC_{50} RR

Surveying the prevalence of MGI resistance phenotypes and genotypes in hopyards populations of *T. urticae*.

Bioassay

<u>Level of resistance or susceptibility</u>

RR < 2 susceptible or tolerance

2<RR< 10 Low Resistance

10 < RR < 100 Moderate

RR > 100 High Resistance

Toxicity of Etoxazole to *T. urticae* populations from WA hop yards, Summer, 2015

Population	LC_{50} (ppm a.i)	RR
Susceptible	**0.65**	1
Prosser_1	**15.7**	3.3
Prosser_2	**2.2**	5.6
Mabton	**17.5**	3.8

[redacted]

Susceptible

Low resistance

Lab selection

Toxicity of Etoxazole to *T. urticae* populations from WA hop yards, Summer, 2016

Prosser_2016

Name	% Mortality at field dose	LC_{50} (ppm a.i.)	RR
Susceptible	100	0.65	1.00
Prosser_1	63.2	217.4	336.6
Prosser_2	68.2	66.9	103.6
Prosser_3	84	60.5	93.6
Prosser_4	86.5	72.9	112.8
Prosser_6	76.4	30.1	46.6
Prosser_7	83.1	16	24.7
Prosser_8	74.2	21.7	33.6
Prosser_9	81.1	25.5	39.5

Toxicity of Etoxazole to *T. urticae* populations from WA hop yards, Summer, 2016

Harrah_2016

Name	% Mortality at field dose	LC_{50} (ppm a.i.)	RR
Susceptible	100	0.65	1
Harrah_1	66.7	78	120.7
Harrah_1_1	69.4	79	122.2
Harrah_2	69.3	140	216.7
Harrah_3	75.2	61.0	94.5
Harrah_4	74.1	85.1	131.7
Harrah_5	79.7	66.5	102.9
Harrah_6	77.9	19.7	30.5
Harrah_8	85.6	29.7	45.9

Toxicity of Etoxazole to *T. urticae* populations from WA hop yards, Summer, 2016

Name	**% Mortality at field dose**	**LC_{50} (ppm a.i.)**	**RR**
Susceptible	100	0.65	1
Toppenish_1	309	34.4	53.3
Toppenish_2	82.9	34.5	53.4
Toppenish_3	67.3	90.7	140.4
Toppensih_4	77	77.6	120.1
Toppensih_4_1	75.1	72.8	112.7
Toppenish_5	70.38	44.7	69.2
Toppenish_5_1	72.5	75.2	116.5

Toxicity of Etoxazole to *T. urticae* populations from WA hop yards, Summer, 2016

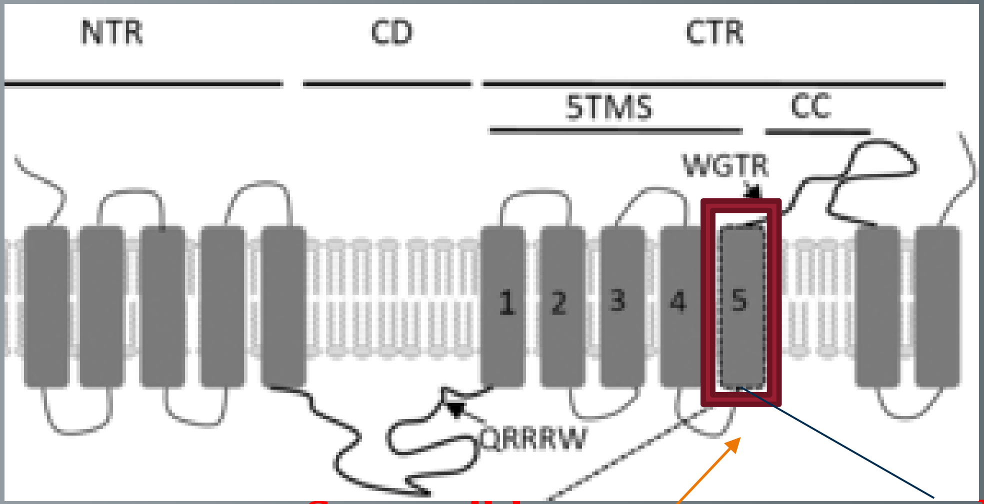
Name	% Mortality at field dose	LC_{50} (ppm a.i.)	RR
Susceptible	100	0.65	1.00
Moxee_1	82.5	45.4	70.3
Mabton_1	80.8	31.1	48.1
Mabton_2	65	126.3	195.4
White_swan_1	67.6	92.6	143.3
White_swan_2	66.1	89	137.9
White_swan_3	67.1	95	147
White_swan_4	72.6	86.2	133.5

In 2016 we screened *T. urticae* populations for the presence of resistance-associated mutation at the target site of ovicidal acaricides (mite growth inhibitors)

We are trying to find the underlying genotypes responsible for the resistance phenotypes observed in *T. urticae* from hopyards.

Screen *T. urticae* populations for the presence of resistance-associated mutation at the target site of MGIs.

Chitin synthase
(CHS 1)



Susceptible (isoleucine) (ATT)

Resistant (phenylalanine) (TTT)

Chitin forms the "structure" of mites.
MGIs inhibit the formation of chitin.
This point mutation confers resistance in mites to MGIs

Van Leeuwen et al., (2012)

Result

Target site mutations in the susceptible and field *T. urticae* populations

Sequence result for ATT(isoleucine) —TTT(phenylalanine) mutation at position 1107 in chitin synthase 1 gene (CHS1)

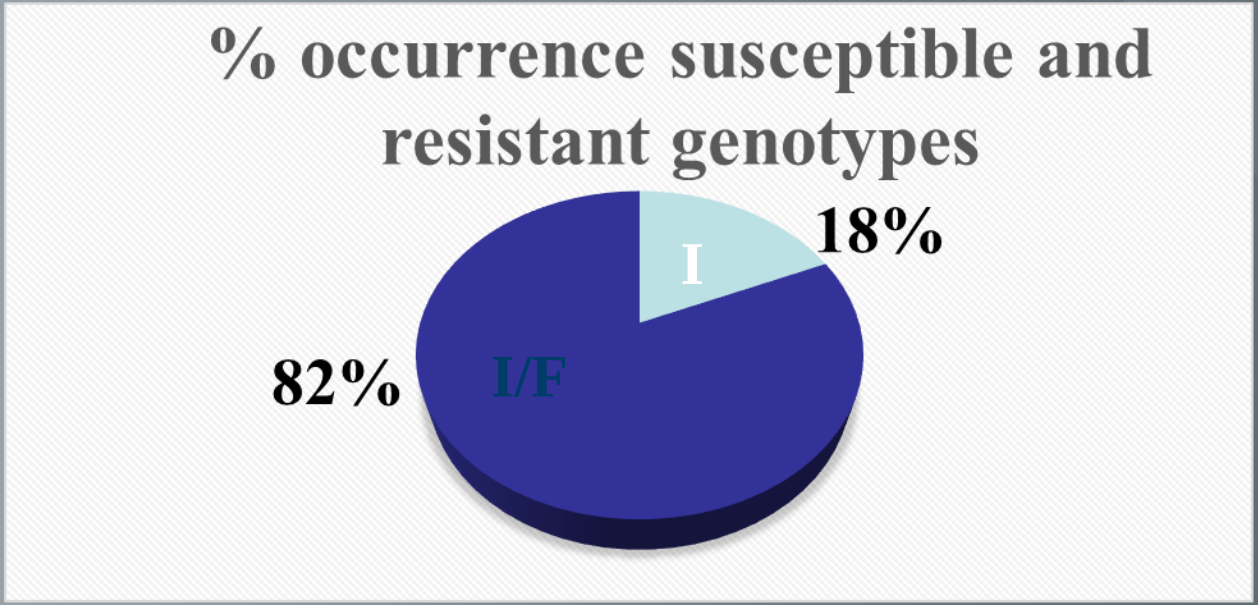
Summer, 2015

I1017F mutation in CHS 1 from the susceptible, field-collected and ovicidal acaricide-selected populations of TSSM from PNW, USA

TSSM Population	I107F mutation	Response to MGIs
Susceptible	ATT	Sensitive
Prosser 1/OL_MR	ATT/TTT	Low resistance
Prosser 1	ATT	Tolerance
Moxee	ATT	Tolerance
Mabton	ATT	Tolerance
CLOF_RS	ATT/TTT	High resistance
ETOX_RS	ATT/TTT	High resistance
HEXY_RS	ATT/TTT	High resistance

Presence of the
*I*1017*F* CHS 1 mutation in PNW *T. urticae*

Summer, 2016



- ✓ **Low to high resistance to ovicidal acaricide in *T. urticae* populations on hops is mediated by target site mutation.**

Although they restricted themselves to one drink at lunch time, Howard and Tom still found they were not at their most productive in the afternoons

I PREDICT BEER IN YOUR FUTURE

Best Psychic Ever