

# Bacterial Spot Management in Stone Fruit

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College of Agricultural Sciences

# Overview

- **Bacterial Spot**
  - Symptoms
  - Disease Cycle
  - Management
- **Antibiotic Resistance**



Severe bacterial spot lesions on apricot



# Bacterial Spot of Peach and Nectarine



'Snow King'



'Sweet Dream'



'Easternglo'

- Most Important Bacterial Disease of Peach and Nectarine
- *Xanthomonas arboricola* pv. *pruni* (Xap)
- Yield Limiting Symptoms
- 100% Fruit Loss Observed
- Few Effective Controls

# Bacterial Spot Symptoms - Fruit

## Early Season Lesions



- Irregularly shaped
- Extend deep into fruit

## Late Season Lesions



- Shallow
- Skin Cracking



# Bacterial Spot



**Bacterial Spot**

- Bacteria
- Angular lesions
- No lesion pattern
- Surface pitting
- Foliar symptoms
- Lesions are not fuzzy

# Peach Scab



**Peach Scab**

UGA1236149

- Fungus
- Circular lesions
- Lesions form pattern
- No fruit surface pitting
- No foliar symptoms
- Dark olive-brown, fuzzy lesions



# Foliar Bacterial Spot Symptoms



**Water-soaked Lesions**



**Angular Lesions with  
Chlorotic Halo**



**Leaf  
Yellowing**



**Copper Injury**



**Nitrogen  
Deficiency**

# Bacterial Spot Symptoms - Twigs

- Cankers
- Bark cracking
- Lack of vegetative growth
- Overwintering site for bacteria



Black Tip



# Bacterial Spot Disease Cycle

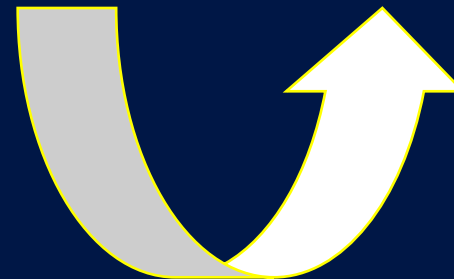
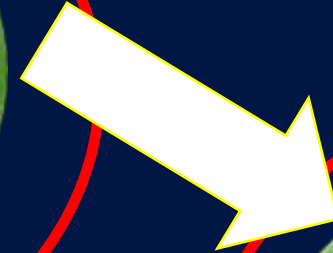
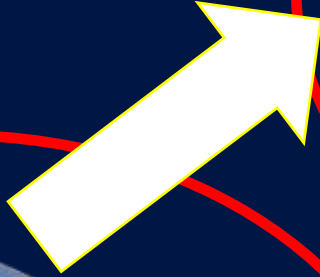
Spring



Summer



Fall/Winter





# Bacterial Spot Management

## Three Main Strategies:

- Less Susceptible Cultivars
- Cultural Management
- Chemical Bactericides



Bacterial Spot Symptoms  
on 'Sweet Dream'

# Cultivar Selection

- No cultivar completely resistance to bacterial spot
- Highly susceptible cultivars a source of inoculum
- Hide more susceptible cultivars inside orchard block
- Long-term strategy





# Cultural Management

- **Site Selection**
  - Well draining soil
  - Avoid low spots
- **Reduce Tree Stress**
  - Nutrition
  - Weed Management
- **Prune**
  - Increase Airflow
  - Remove Cankers



Severe flooding in peach orchard (clemson.edu)

# Bactericide Applications

- **Copper**
  - Dormant & cover sprays
  - Phytotoxic
- **Oxytetracycline**
  - Poor persistence on leaves
  - Label limitations



Copper Injury



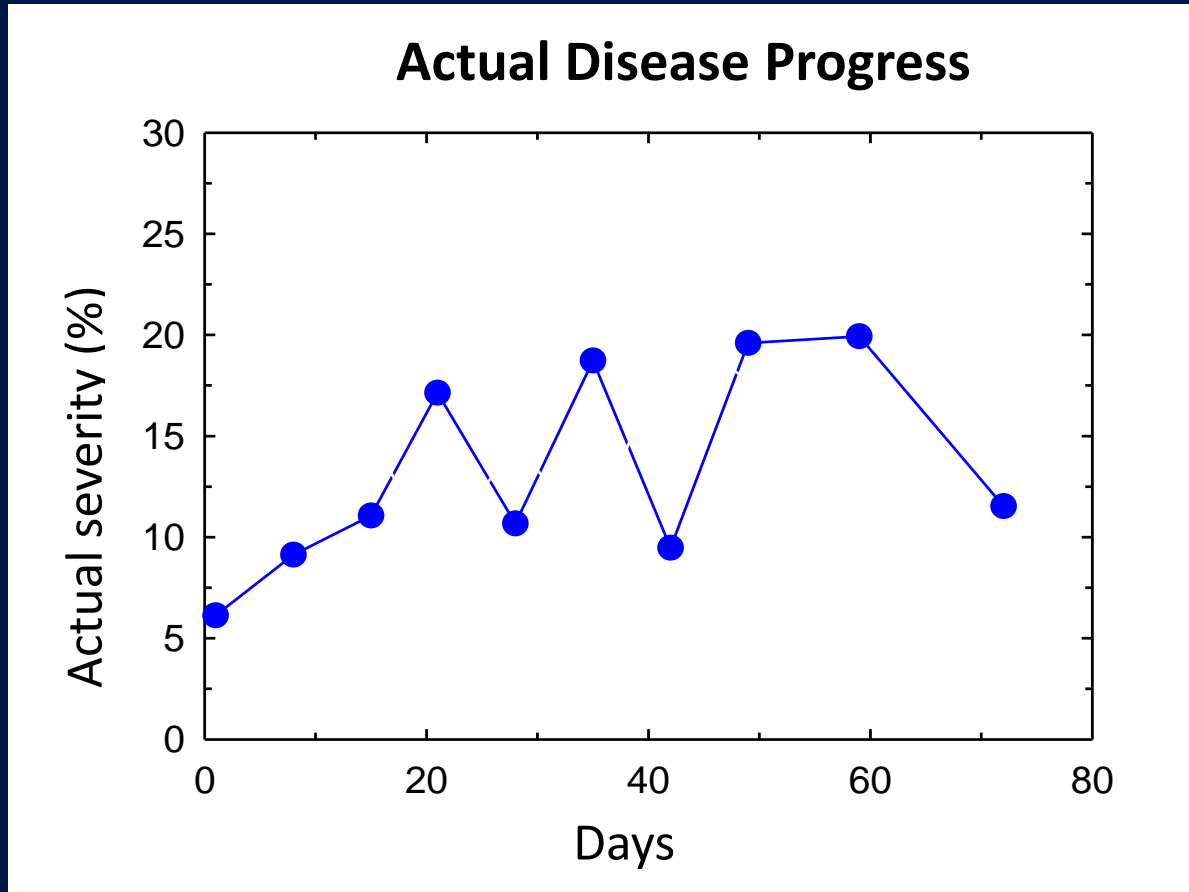
# Refining Management

- **Disease progress**
  - Target weak points
- **Defoliation**
  - Significant factors
- **Alternative Bactericide Programs**
  - Reduce the use of a single product



**Bacterial Spot and Brown Rot**

# Disease Progress



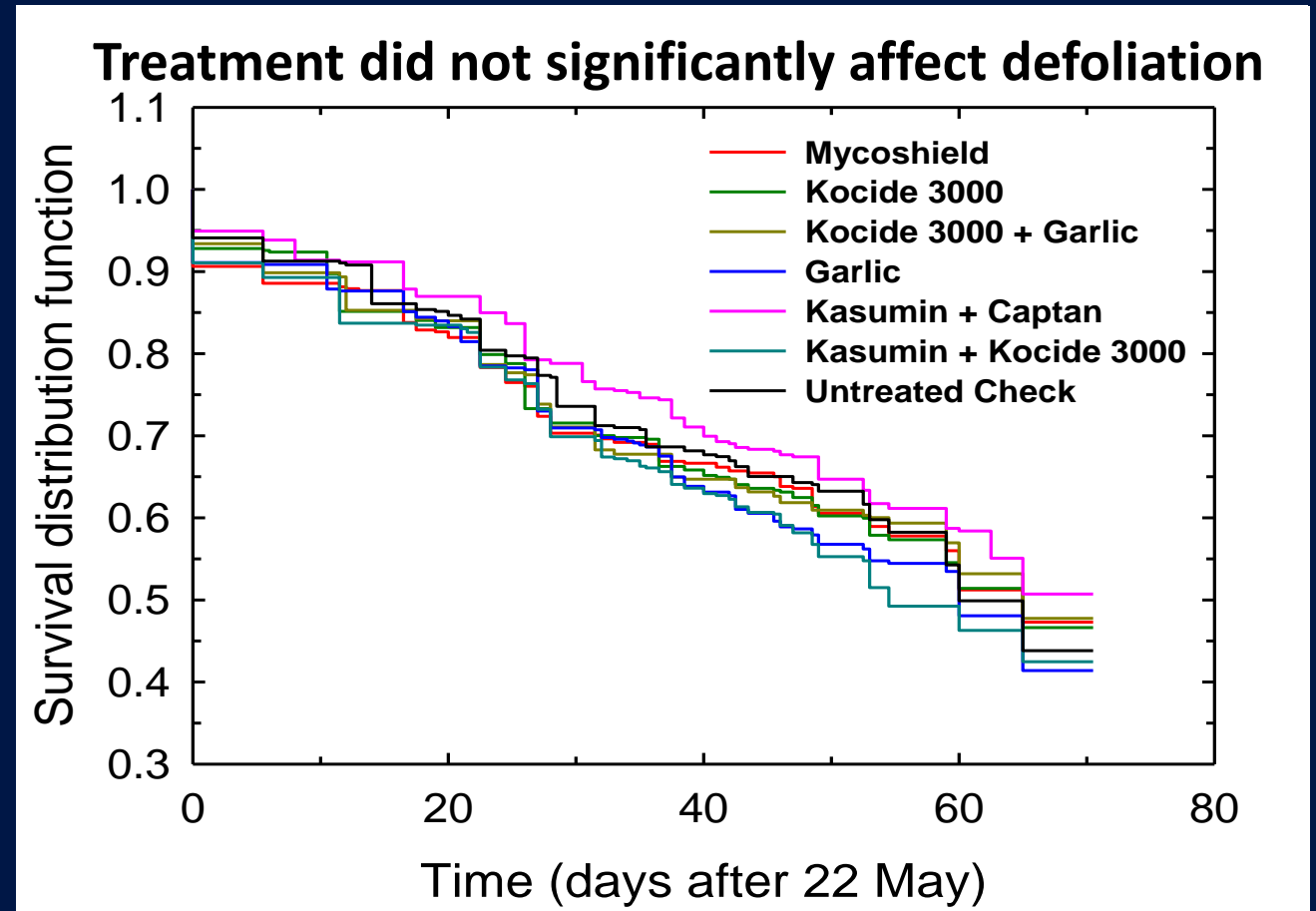
- **Highly Variable**
- **Severe Defoliation**
- **Significant Influences:**
  - **Bactericide treatment**
  - **Cultivar**

**Bacterial spot progress curves do not follow standard disease progress curves due to abscission of heavily infected leaves**



# Bacterial Spot and Defoliation

- 1,460/ 3,052 leaves abscised
- Factors
  - Leaf age
  - Cultivar
  - Bactericide Treatment
  - Initial Disease Onset



For every small increase ↑ of initial disease severity, the time the leaves remain on the tree is greatly reduced ↓

# Alternative Bactericide Programs



## Evaluation of Alternative Chemicals for Bacterial Spot Management and Mitigation of the Risk of Antibiotic Resistance Development in Pennsylvania Stone Fruit Orchards

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### Bacterial Spot and Antibiotic Use

Bacterial spot, caused by *Xanthomonas arboricola* pv. *pruni* (Xap), is the most important bacterial disease of stone fruit in the eastern US, where severe epidemics can result in 100% yield loss on susceptible cultivars. Leaf infections (A) lead to fruit infections (B) and early season fruit infections can lead to deep lesions that extend to the pit (C). Bacterial spot is managed with repeated applications of the antibiotic oxytetracycline as well as copper. The intensive use of antibiotics applies a strong selective pressure on bacterial populations favoring antibiotic resistant bacteria. The overall goal of this project was to evaluate alternative chemicals to oxytetracycline to manage bacterial spot and to monitor the effects of these alternatives on the prevalence of tetracycline resistance genes, conferring resistance to oxytetracycline, in epiphytic bacteria. This insight will help shape more effective approaches to bacterial spot management as well as to mitigate the risk of antibiotic resistance.



### Alternatives to Oxytetracycline

#### Copper

Copper has been used to reduce bacterial inoculum when trees are dormant but has also been used, with success, to manage bacterial spot during the season. Copper is often phytotoxic (D), however, sometimes causing severe injury to leaves and premature defoliation of entire trees. Kocide and Cueva were evaluated alone and mixed with a biofungicide.

#### Biofungicides

Biofungicides are microorganisms and naturally occurring compounds that control disease. They often have multiple modes of action, thereby reducing selection pressure favoring resistance against the active ingredient. Serenade, Optimum, and Double Nickel were investigated mixed with copper.

#### Hydrated Lime

Hydrated lime, or calcium hydroxide, is a chemical compound used to raise soil pH. It was investigated as a safener, meant to reduce the phytotoxicity associated with copper.



### Acknowledgements

State Horticultural Association of PA (SHAP)



### Evaluation of Bacterial Spot Incidence and Severity

Table 1:		Leaves - defoliation <sup>1</sup>		Fruit	
Treatment & Dates	Timing	# Missing	% late	% incidence <sup>2</sup>	% area affected <sup>2</sup>
1 Untreated		14.3 d	13.5 c	31.7 ab	3.7 b
2 Petalure 1, 3, 5	PF/SS - TC	9.7 a	15.5 c	32.3 ab	3.8 a
3 Cueva 2 q + Double Nickel 1 q	PF/SS - TC	21.2 bc	41.0 a	68.0 bc	4.1 c
4 Cueva 2 q	PF/SS - TC	26.4 a	38.5 b	55.2 ab	3.5 a
5 Kocide 0.5 lb	PF/SS - TC	21.4 bc	27.7 c	32.4 b	3.8 c
6 Kocide 0.2 lb + Serenade Optimum 14 oz	TC - TC	16.7 c	17.3 c	45.3 cd	3.6 c
7 Cueva 2 q + Serenade Optimum 14 oz	PF/SS - TC	29.2 a	33.6 ab	68.0 bc	3.4 c
8 Kocide 0.2 lb + Double Nickel 1 q	TC - TC	22.5 b	18.4 c	47.7 cd	4.0 c
9 Kocide 0.2 lb	TC, AC, SC, TC	15.2 d	12.7 c	38.9 a	4.0 c
Hydrated lime 2.0 lb					

Table 2:		Leaves - defoliation <sup>1</sup>		Fruit	
Treatment & Dates	Timing	# Missing	% late	% incidence <sup>2</sup>	% area affected <sup>2</sup>
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2 Petalure 1, 3, 5	PF/SS - TC	9.7 a	15.5 c	32.3 ab	3.8 a
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### Tetracycline Resistance Genes in Bacterial Epiphytes

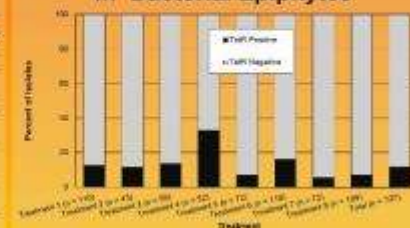


Fig.1: A total of 727 epiphytic bacteria (e.g.: bacteria living on the surface of the leaves) (E, F, G) were collected in 2013 and 2014 at the PSU FREC from peach trees treated with the following treatments: 1 = copper, 2 = copper rotated with Rampart, 3 = copper rotated with Serenade, 4 = copper and vegetable oil, 5 = lime sulfur rotated with copper, 6 = oxytetracycline, 7 = Regalia rotated with Serenade, and 8 = untreated. These bacteria were screened for tetracycline resistance genes (*tetA*, *tetB*, and *tetC*). Out of those isolates, 3.16% were positive for *tetA*, 5.78% carried *tetB*, and an additional 3.16% of isolates were positive for *tetC*. Combined data indicated that the incidence of bacteria positive for tetracycline resistance genes significantly differed among bacteria collected from different treatments ( $X^2 = 32.98$ ,  $P < 0.0001$ ).

### Conclusions

On leaves, Cueva treatments were associated with greater levels of defoliation compared to Kocide, oxytetracycline, and untreated treatments. The addition of Double Nickel or Serenade Optimum did not further reduce infection on fruit, nor did it reduce the phytotoxic effect of the copper on leaves. Tetracycline resistance genes were found in 12.1% of the 727 epiphytic bacteria collected from trees treated with different bactericides. Although the incidence of resistance genes was significantly associated with bactericide treatment, resistance genes were not associated with oxytetracycline use alone. In fact, all of the treatments, even the untreated control supported bacteria carrying tetracycline resistance genes. Therefore, oxytetracycline use does not increase the prevalence of resistance genes in the orchard environment.



# Antibiotic Resistance in *Xap*

**Objective: To determine the sensitivity of *Xap* isolates to oxytetracycline**

- **Persistent yield loss**
- **Potential resistance development**



Peach leaves with bacterial spot

# Antibiotic Resistance in *Xap*



- **Sampled:**
  - 12 Orchards
  - 830 *Xap* Isolates
- **Management Survey:**
  - Oxytetracycline and Copper Use, Spray Method, Tree Age



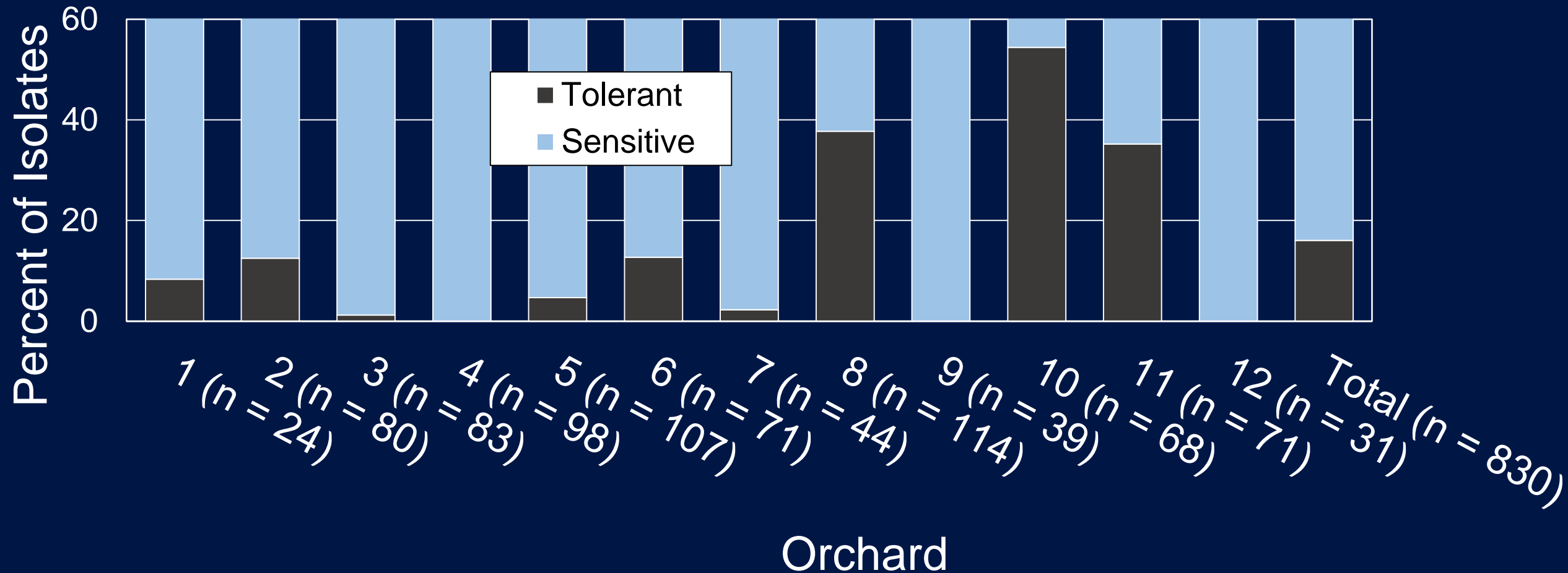
# Antibiotic Resistance in *Xap*

- No *Xap* Resistant to 150 ppm oxytetracycline
- No *Xap* Positive for Antibiotic Resistance Genes
- Variable Sensitivity



Late Season Bacterial Spot Lesions on Nectarine

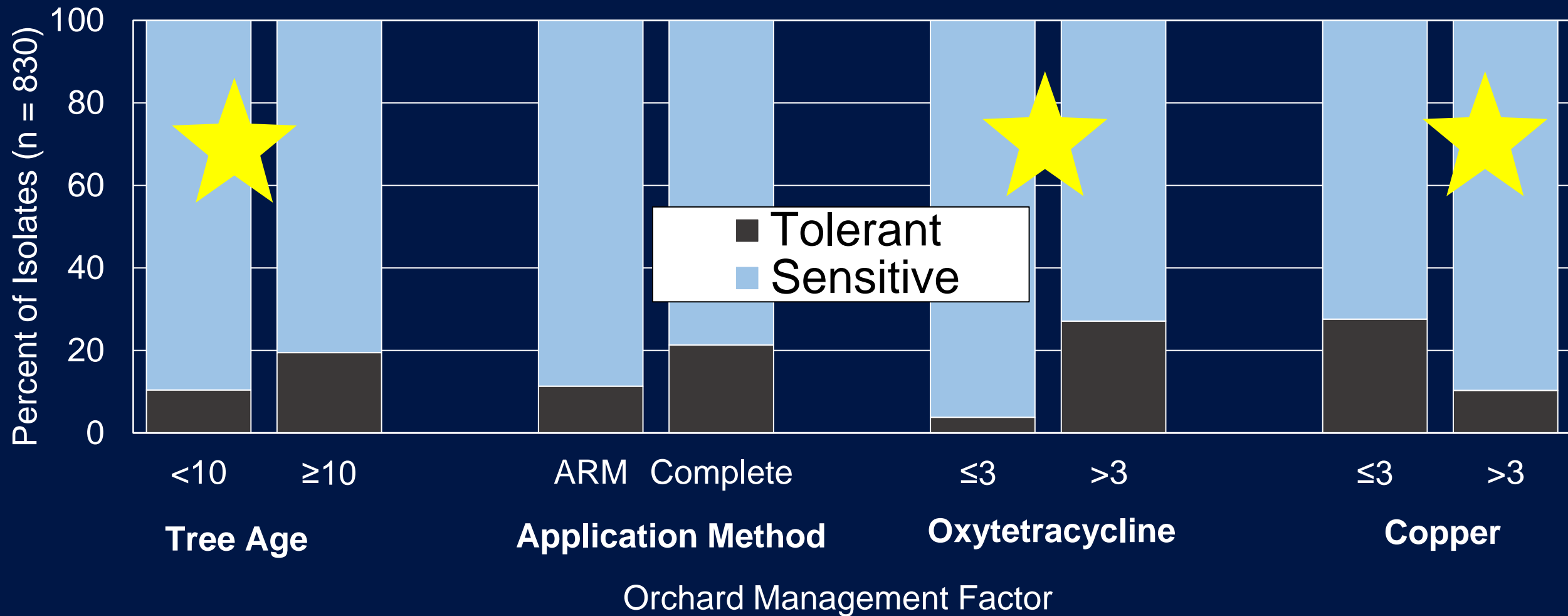
# *Xap* Sensitivity



***Xap* sensitivity to oxytetracycline significantly varied among isolates collected from different orchards**



# *Xap* Sensitivity



**Tree age, oxytetracycline, and copper were significantly associated with the sensitivity of *Xap* isolates to oxytetracycline**

# Antibiotic Resistance and Oxytetracycline

- No resistance in *Xap*
- Gradual shift in antibiotic sensitivity in *Xap*
- Oxytetracycline does not persist on leaves for long – 2 days max!



# Summary

- Bacterial spot disease progress is variable
- Reduce initial inoculum in order to reduce defoliation
  - Dormant copper
  - Prune out cankers
- Reduce tree stressors
- *Xap* remains sensitive to oxytetracycline in PA

# Acknowledgments

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- **Penn State University**
- **PSU Fruit Research & Extension Center**

## Funding

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- **PA Dept. of Ag. – Peach & Nectarine Marketing Board**
- **PSU College of Agricultural Sciences Graduate Student Competitive Grant Program**



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# Questions





# Alternative Bactericides

Leaves			
Treatment	Incidence	Severity	Phytotoxicity
1 Untreated	85.2a	4.1a	0.2g
2 FireLine (1.5lb)	89.3a	3.4b	0.2g
3 MasterCop (1pt)	58.4bc	1.9cd	6.3b
4 MasterCop (1.5pt)	55.6c	1.8cd	8.1a
5 Kocide3000 (0.5lb)	49.1d	1.2e	4.2d
6 MasterCop/ (1.0pt) Rampart (1.0qt)	62.7bc	2.1cd	3.7e
7 MasterCop (1.0pt)/ Serenade Optimum (14oz) ★	56.8c	1.8cd	3.1f
8 MasterCop (1.0pt) + hydrated lime (2.0lb) + vegetable oil (3.0 qt) ★	56.5c	1.5de	2.7f
9 MasterCopt (1.0pt) + vegetable oil (3qt)	58.0c	1.6d	5.6c



# Alternative Bactericides

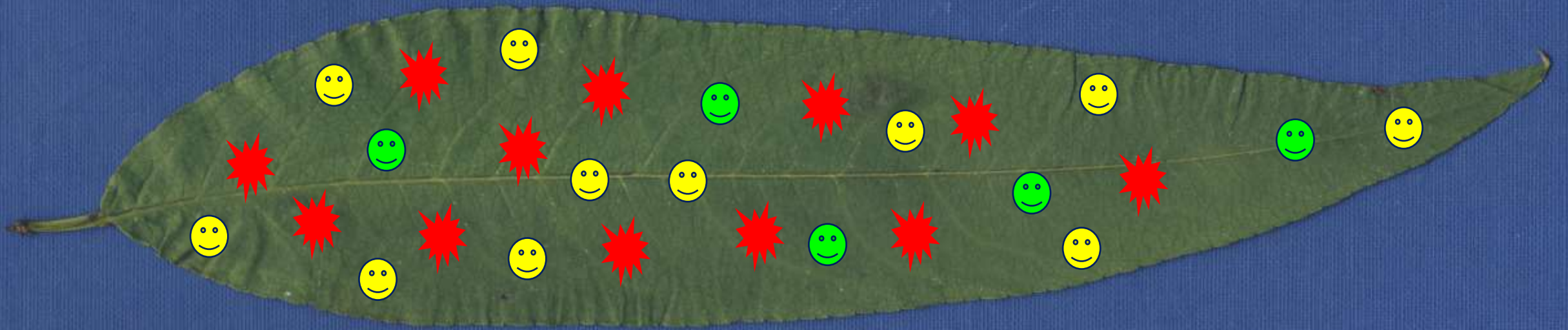
Fruit		
Treatment	Incidence	Severity
1 Untreated	89.6a	10.6a
2 FireLine (1.5lb)	84.8ab	7.8b
3 MasterCop (1.0pt)	75.5de	6cd
4 MasterCop (1.5pt)	73e	4.8de
5 Kocide3000 (0.5lb)	76.3de	3.2f
6 MasterCop (1.0pt)/ Rampart (1.0qt)	86.4ab	6.7bc
7 MasterCop (1.0pt)/ Serenade Optimum (14 oz) ★	74.7de	4.2ef
8 MasterCop (1.0pt) + hydrated lime (2.0lb) + vegetable oil (3.0qt) ★	80.0cd	5.5c-e
9 MasterCopt (1.0pt) + vegetable oil (3.0qt)	82.1 bc	6.4c

# Antibiotic Resistance in PA

- **Persistent Yield Loss**
- **Potential Development of Antibiotic Resistance**
- **Research Goal: To determine the consequences associated with repeated oxytetracycline applications**



# Antibiotic Resistance Development



*Xap*



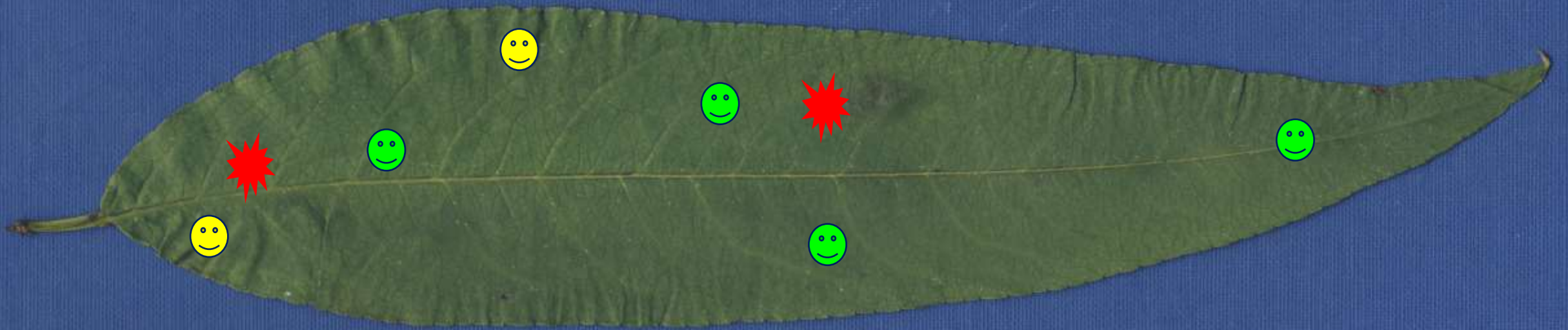
*Tet<sup>R</sup>* Epiphytic Bacteria



Sensitive Epiphytic Bacteria



# Antibiotic Resistance Development

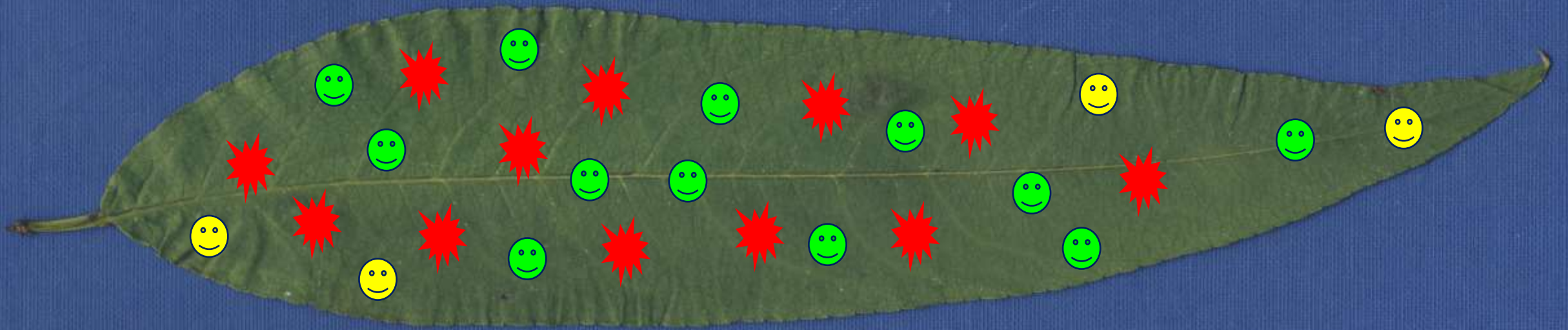


*Xap*

 *Tet<sup>R</sup>* Epiphytic Bacteria

 Sensitive Epiphytic Bacteria

# Antibiotic Resistance Development



*Xap*



*Tet<sup>R</sup>* Epiphytic Bacteria



Sensitive Epiphytic Bacteria

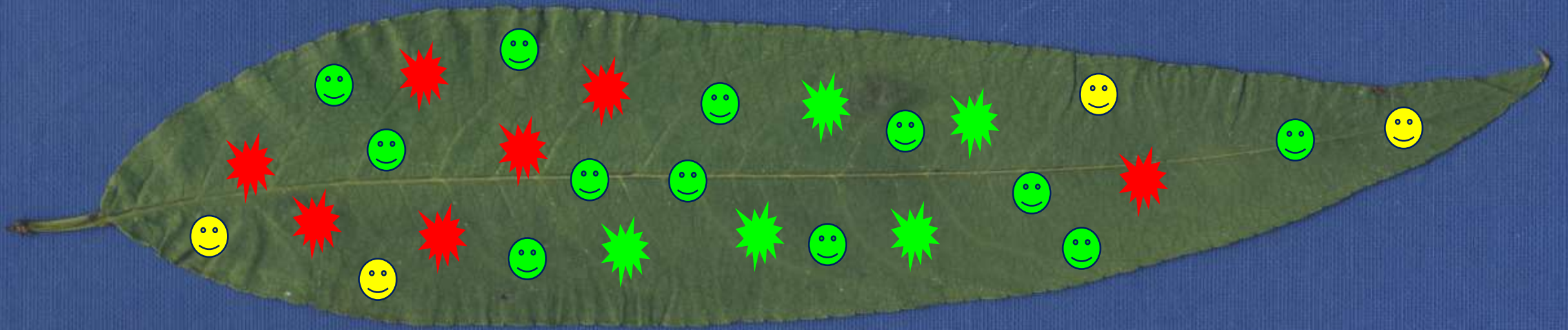
# Antibiotic Resistance Development



-  *Xap*
-  *Tet<sup>R</sup> Xap*
-  *Tet<sup>R</sup> Epiphytic Bacteria*
-  Sensitive Epiphytic Bacteria



# Antibiotic Resistance Development



-  *Xap*
-  *Tet<sup>R</sup> Xap*
-  *Tet<sup>R</sup> Epiphytic Bacteria*
-  Sensitive Epiphytic Bacteria



Peach Leaves

# Epiphytic Bacteria and Antibiotic Resistance

- Foliar bacteria
- Already antibiotic resistant
- Potential source of genetic material
- Objective: Monitor antibiotic resistance in epiphytic bacteria from commercial stone fruit orchards





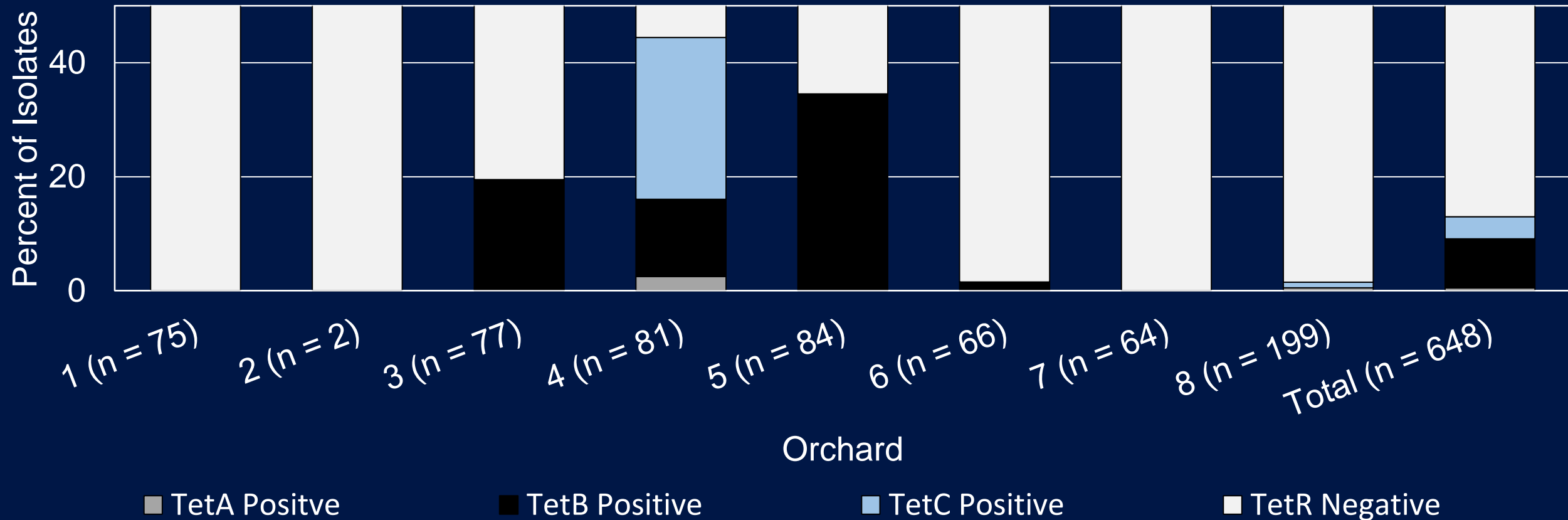
Epiphytic Bacteria

# Epiphytic Bacteria and Antibiotic Resistance

- **Sampled:**
  - 6 Conventional Orchards
  - 2 Organic Orchards
- **Management Survey:**
  - Oxytetracycline Use, Spray Method, Tree Age

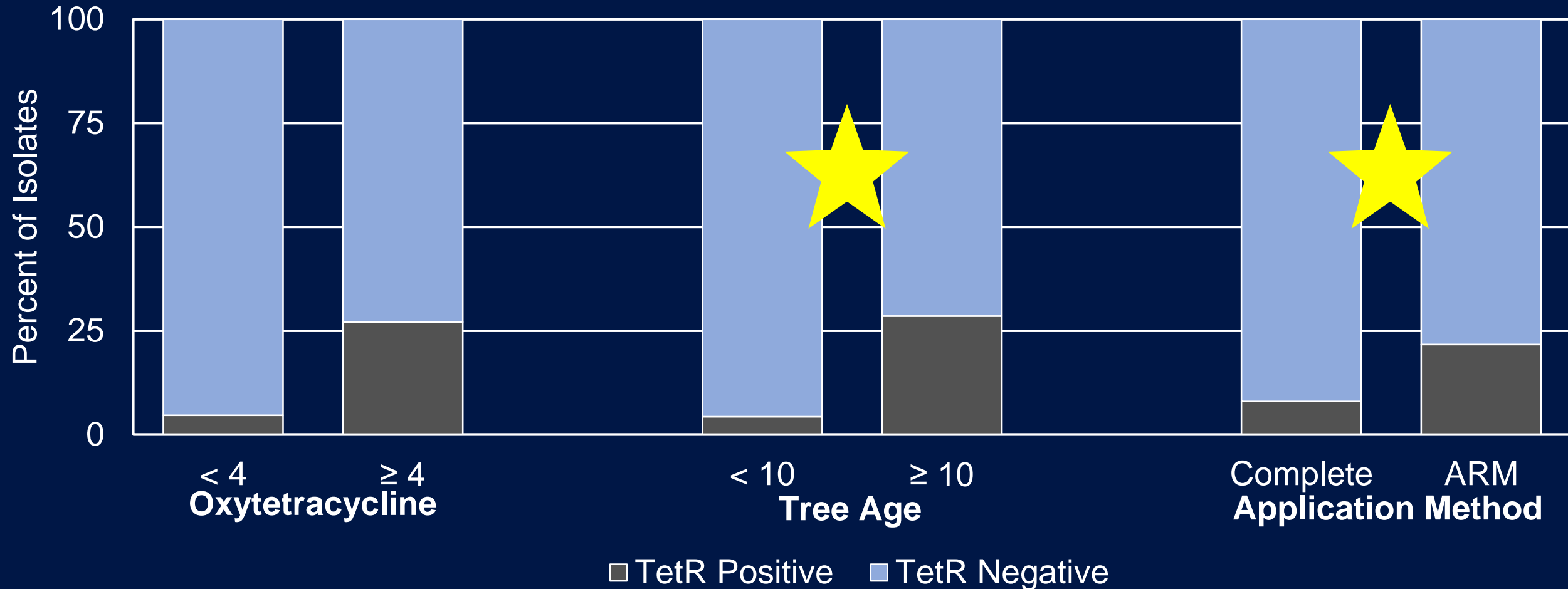


# *Tet*<sup>R</sup> Genes in Epiphytic Bacteria



***Tet*<sup>R</sup> genes were found in 12.96% of epiphytic bacteria.**  
***Tet*<sup>R</sup> were not limited to orchards that used oxytetracycline.**

# Disease Management and *Tet<sup>R</sup>* Genes



**Tree age and application method were significantly associated with the incidence of *tet<sup>R</sup>* genes but oxytetracycline use was not**