

Implicating early fruit fly life stages in sour rot and spray timing applications

Show Me Grape and Wine Symposium

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04 March, 2020

Overview



❖ What we know about sour rot

Disease Description

Causal Organisms

❖ Experiments

1. Reducing number of applications after sour rot develops
2. Effects of fruit fly life stage on disease progression





From beautiful to rotten...



Vignoles: most planted white grape in Missouri (15% total acres)

Chardoneel: 4%

Traminette: 7%

Vidal Blanc: 8%

Chambourcin: 10%

*tight cluster and/or thin skin varieties

Sour Rot Characteristics



Browning of berries



Liquification of berry pulp



Smell of acetic acid



Fruit flies present





Berry Damage Caused By:

- Birds
- Insects
- Berry splitting (thin skin & tight clusters)



Separation from pedicle



Yellowjackets

*Fruit flies need wound site to establish



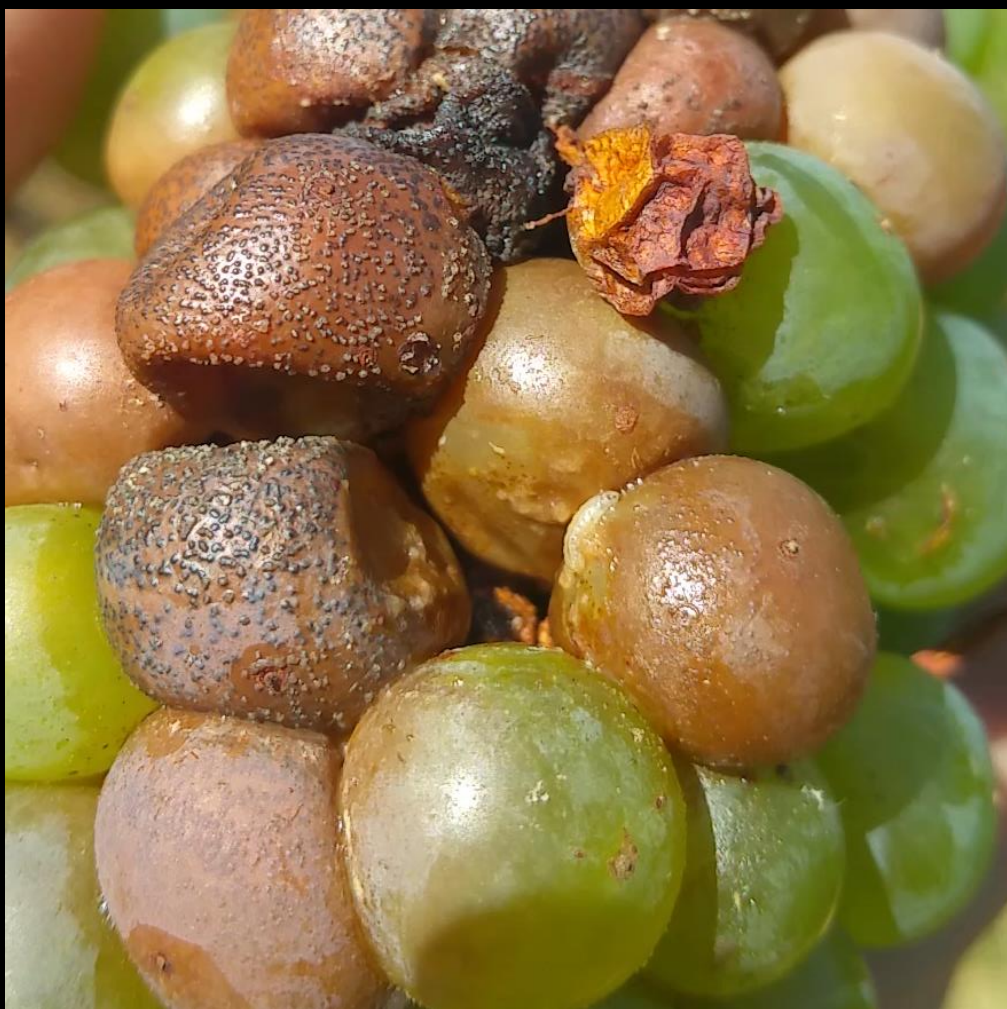
Why Wounds Are Important



- Wounds expose sugary carbon source for yeast and bacteria which increase in immediate area (Barata et al., 2012)
- Wounds make an aerobic environment, converting ethanol to acetic acid (Hall et al., 2018)
- *D. melanogaster* are attracted to volatiles in overripe grapes that contain fermenting yeast and acetic acid (Becher et al., 2010)

Fruit Flies Everywhere!







Sour Rot: what we know



This Riesling cluster affected with sour rot is shown shortly before harvest. Note the association of multiple *Drosophila* fruit flies (arrows) with diseased berries and the lack of fungal mold growth.

Research Focus 2017-3: Cornell Viticulture and Enology, Megan Hall, Gregory Loeb, Wayne Wilcox

Causal Organisms:

- ❖ Yeast
- ❖ Acetic Acid Bacteria
- ❖ *Drosophila* fruit flies are necessary

Wounds & humidity are very important!



Where are the yeast and bacteria?



- Yeast and bacteria are diverse and make up the natural microbiota of the grape (Fleet et al., 2003)
- Microbial populations increase as berries ripen (Barata et. al., 2012)
- Isolated not only from the surface of berries, but within berry pulp (Hall & Wilcox 2019)



How is Sour Rot Managed?

Current industry practice:

Weekly sprays of insecticide and antimicrobial mixture every week starting around 15 Brix until harvest (4-5 sprays)

- * Does not account for fruit fly populations or whether disease is present
- ❖ This is costly to the grower and impacts non-target organisms



Resistant Fruit Flies

Resistant *D. melanogaster* populations have been found in Finger Lakes, NY

*Few products (modes of action) available
Inexpensive and short pre-harvest interval

Insecticides

Venom- (Dinotefuran)

Delegate-
(Spinetoram)

Mustang Maxx-
(zeta cypermethrin)

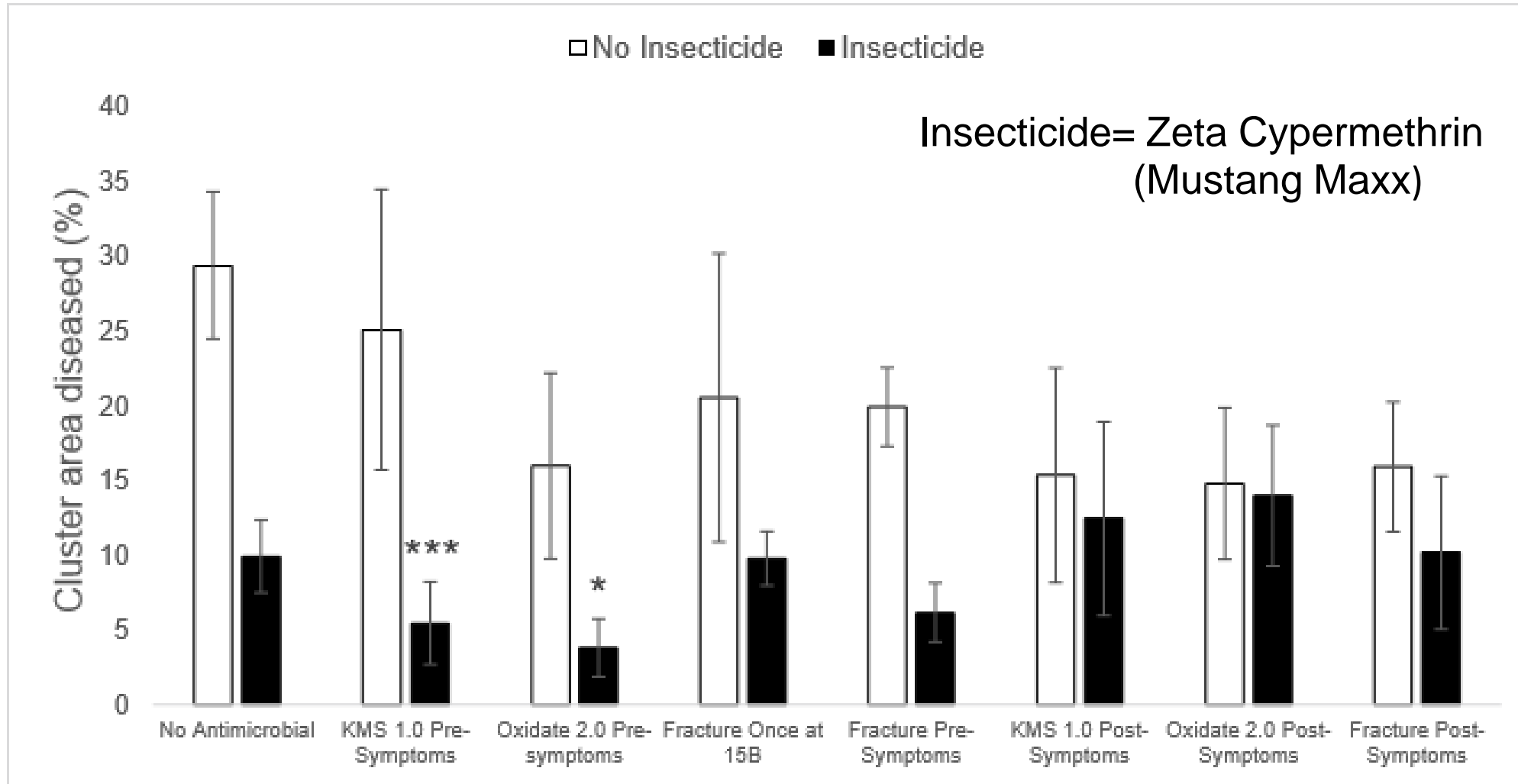
Malathion-
(organophosphate)

Acetamiprid-
(neonicotinoid)

**We want to save
these chemistries!



Antimicrobials alone do not reduce sour rot

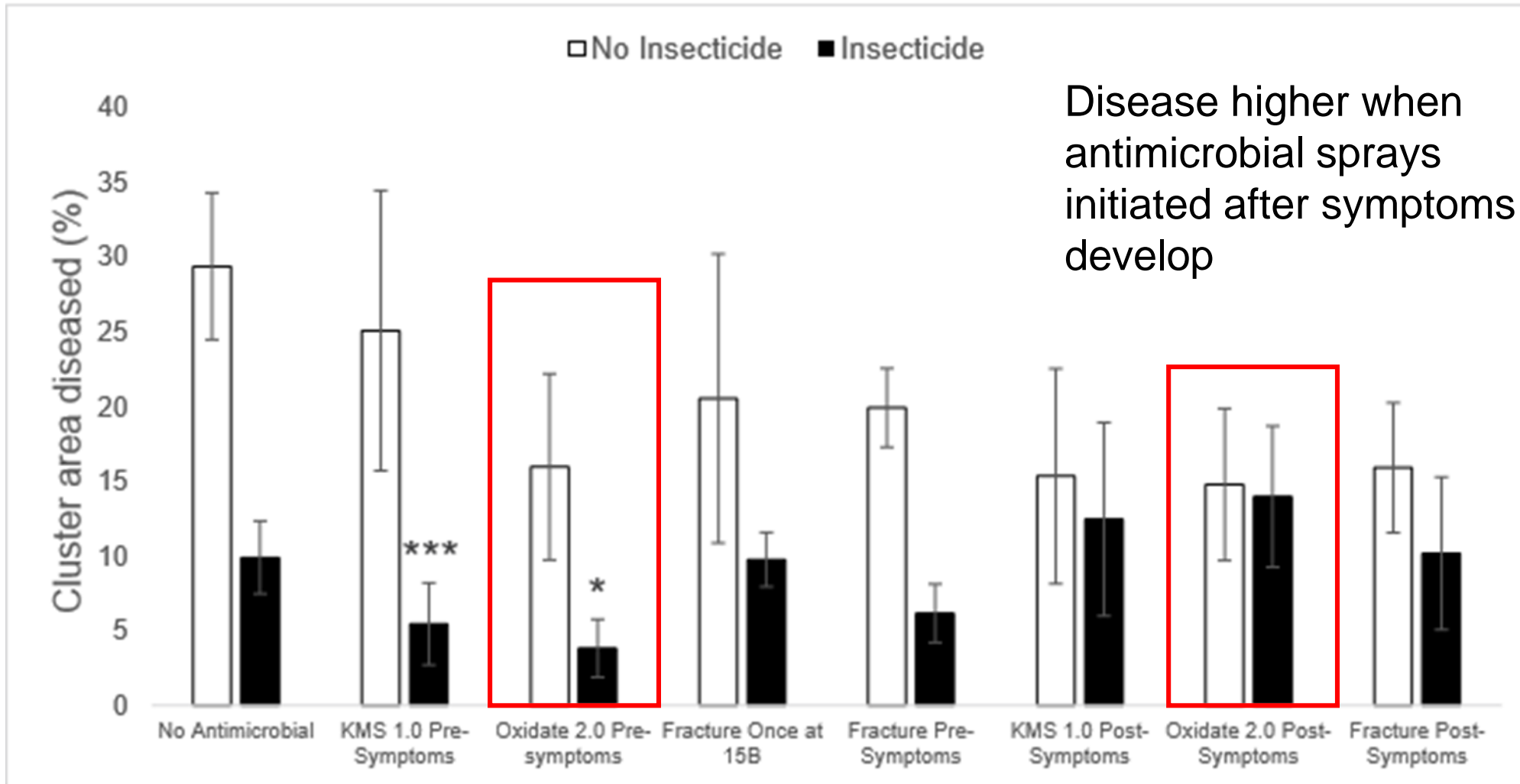


Less control in treatments that did not receive insecticide

Hall, M. E., Loeb, G. M., & Wilcox, W. F. (2018). Control of sour rot using chemical and canopy management techniques. *American Journal of Enology and Viticulture*, 69(4), 342-350.



Pre-symptom sprays more effective



Hall, M. E., Loeb, G. M., & Wilcox, W. F. (2018). Control of sour rot using chemical and canopy management techniques. *American Journal of Enology and Viticulture*, 69(4), 342-350.



Previous Spray Trials Have Shown

- Antimicrobial + Insecticide sprays applied before symptoms provide the best control of sour rot
- Antimicrobials alone do not reduce sour rot
- Insecticide applications target just adult fruit flies
- ❖ Vineyards commonly wait for symptoms to develop before initiating applications



Objective I

Reduce sprays at different phenological timings after symptoms develop



Spray Trial Experiment

Research Question: Can we reduce the number of sprays after symptoms develop around 15 Brix?

Two commercial vineyards of Vignoles





Spray Trial Experiment

- Oxidate (antimicrobial) and Mustang Maxx (broad spectrum insecticide)
- Applied to full rows based on Brix measurements taken each week
- Rated at harvest for severity and incidence



❖ Compare sour rot using three different treatments

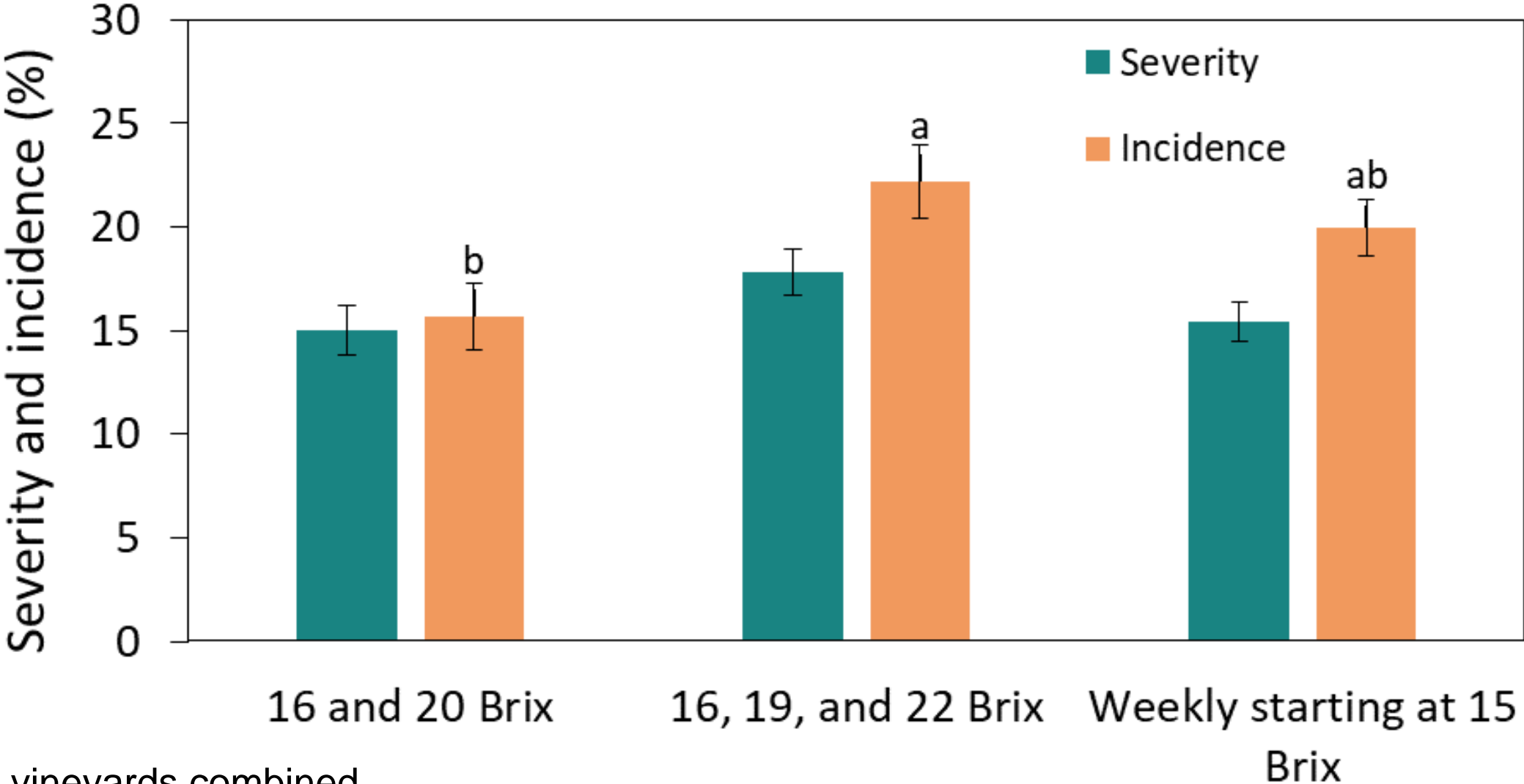


2018 Spray Trial

Treatment Applications:

1. 16 and 20 Brix (2 total)
2. 16, 19, and 22 Brix (3 total)
3. Weekly starting at 15 Brix (4-5 total)
*Industry standard used as control treatment

No significant difference compared to weekly sprays



Two vineyards combined
n= 105 vines



2019 Spray Trial

Treatment Applications:

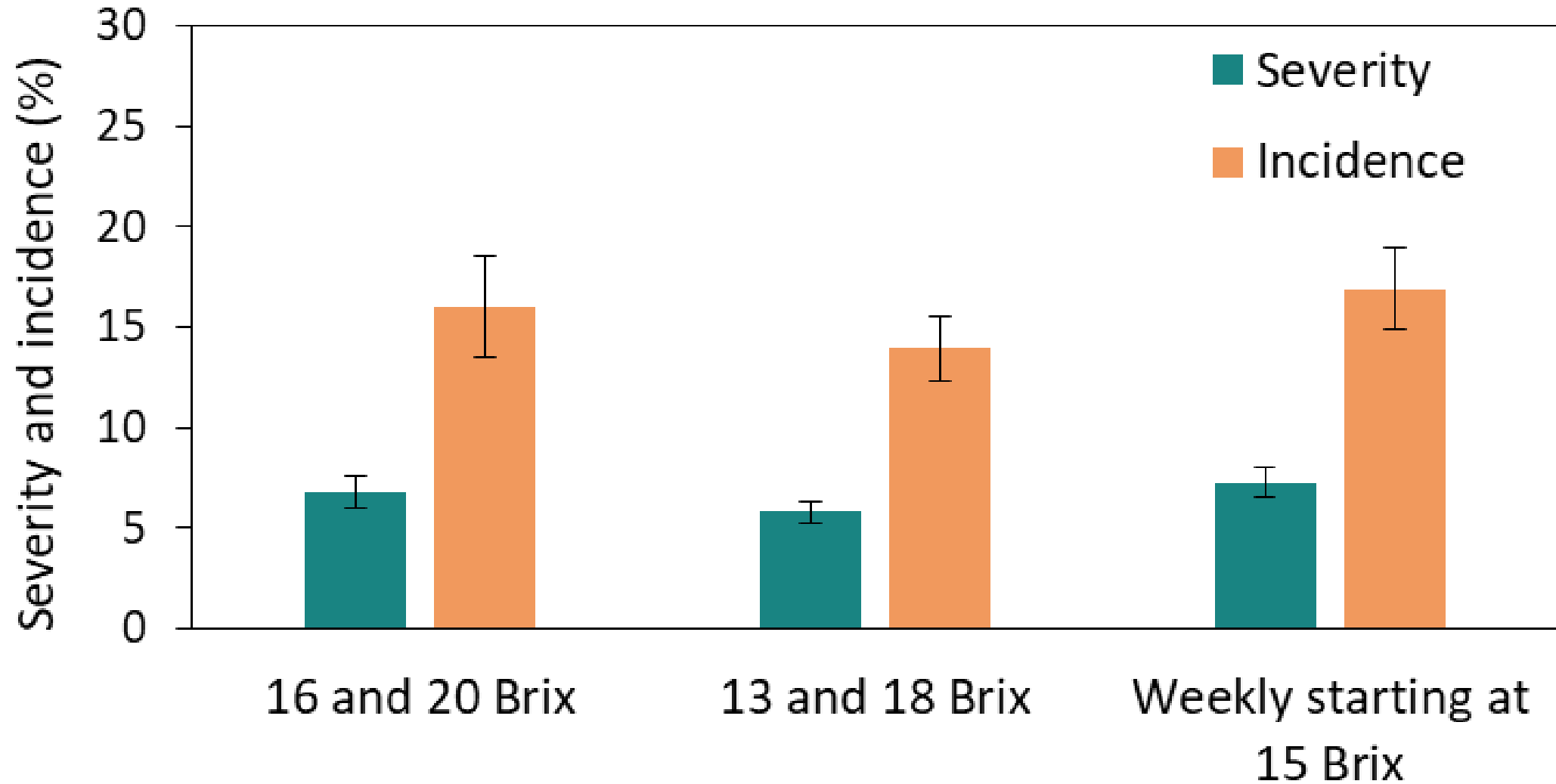
1. 16 and 20 Brix (2 total)

2. 13 and 18 Brix (2 total)

3. Weekly starting at 15 Brix (4-5 total)

*Industry standard used as control treatment

No significant difference between treatments



Two vineyards combined
n= 75 vines



More sprays \neq better control

- Reduced sprays did not differ from industry standard
- Spraying 4-5 times to control fruit flies does not offer more disease control (similar severity/incidence for 2 sprays)
- Heavy applications are costly to growers and the environment
 - Create resistant fruit fly populations
- ❖ If you wait until symptoms to develop, 1-2 sprays just as effective



Why?

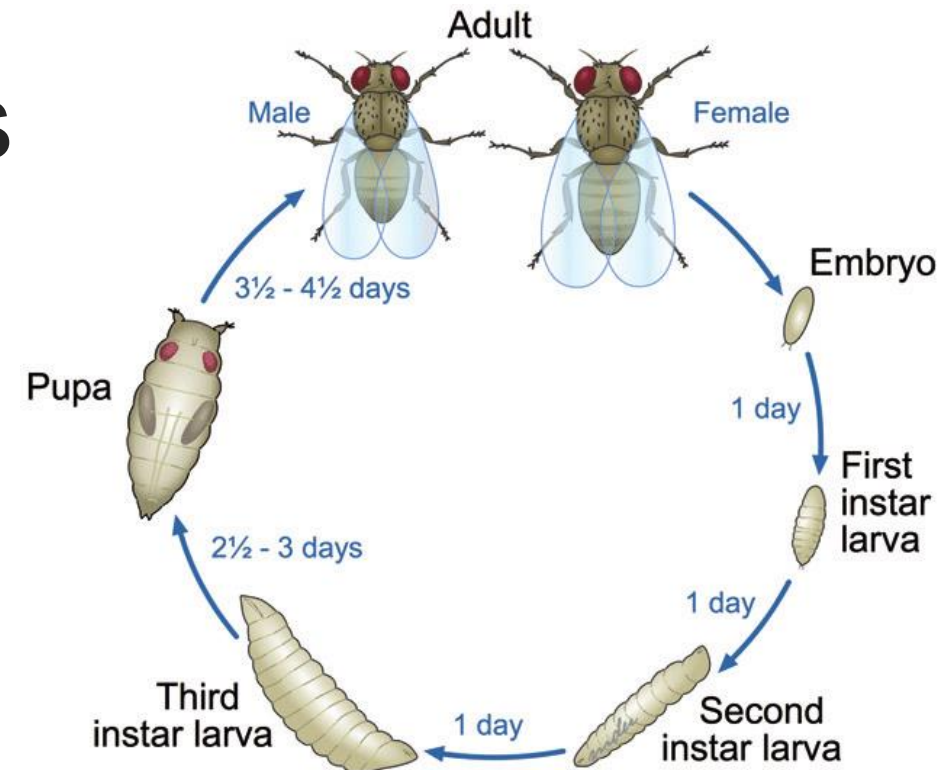
- Post symptom sprays target fruit fly adults
- Weekly sprays after symptoms develop does not change disease at harvest
- ❖ Why do more sprays not reduce sour rot?

What we still don't know

- Many unknowns about how fruit flies contribute to disease
- What fly life stage(s) cause sour rot?
- This will help us understand how we manage

❖ Developmental Stages

- Adults
- Larvae
- Eggs



What we still don't know

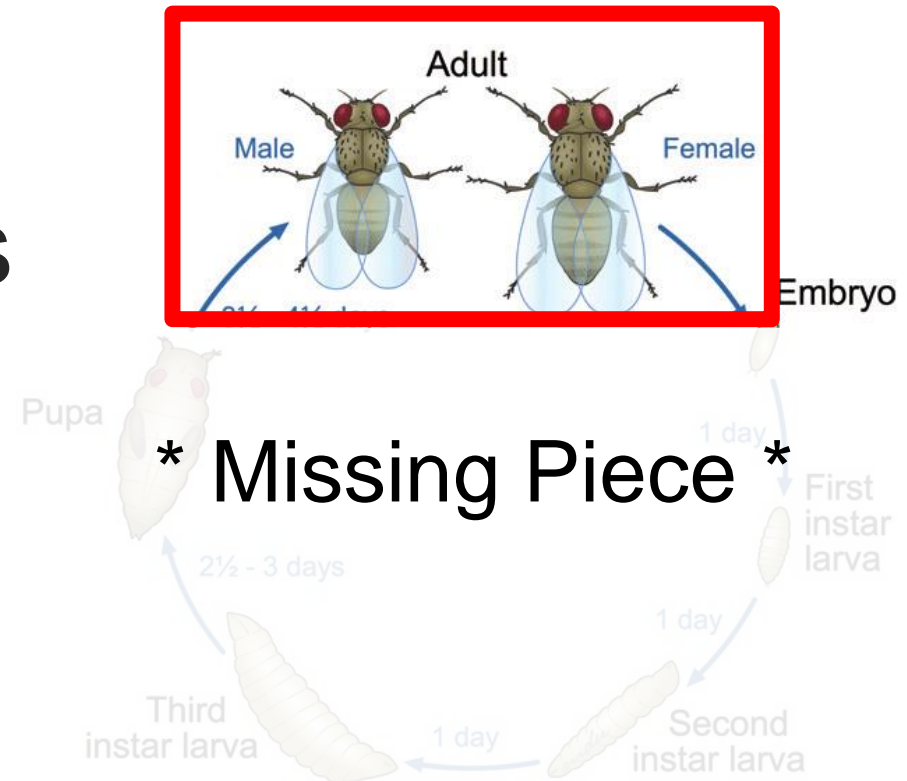


- Yeast
- Acetic Acid Bacteria
- Wounds

What fly life stage(s) cause sour rot?

❖ Developmental Stages

- **Adults**
- Larvae
- Eggs



Objective II

Effects of fruit fly life stage

What life stage(s) cause sour rot?



Effects of fruit fly life stage

Lab Experiment

Ratings: 2

4

- Inoculate with microbes
- Fruit fly life stages that are clean (Axenic)
- Rate on a scale of 0-4



4 = inner pulp liquefies and skin is completely discolored, berry not intact (acetic acid)

Yeast and
Bacteria
Alone

+ *Drosophila melanogaster*

Experiment Procedure

I.

Surface sterilize berries



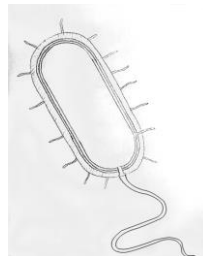
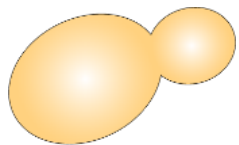
II.

Wound with toothpick



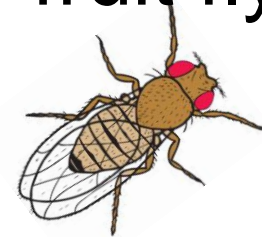
III.

Inoculate with yeast +
acetic acid bacteria



IV.

Add axenic
fruit fly life stage



Creating Axenic Flies

- ❖ Axenic flies lack microorganisms associated with gut and surface
 - Collected eggs are washed with bleach, killing symbionts associated with fruit flies



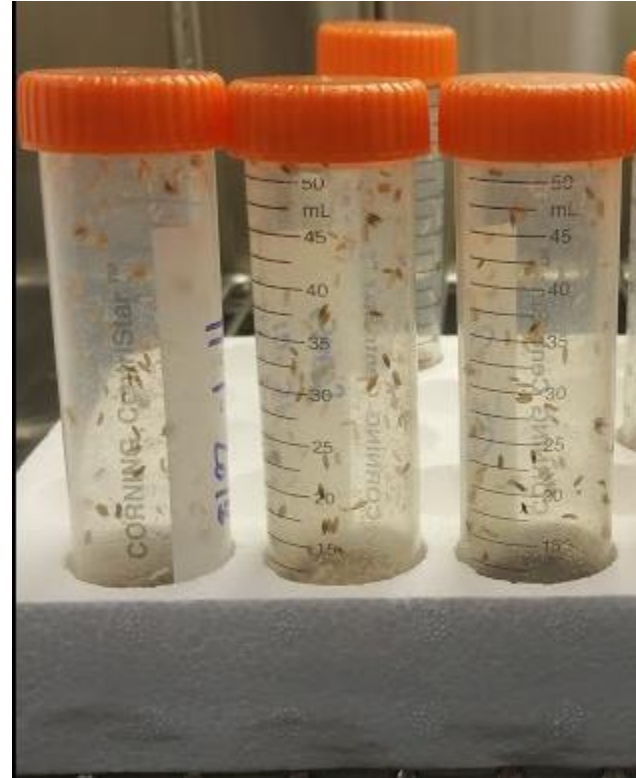
Dechorionation-
Removal of outer
chorion layer and
microorganisms



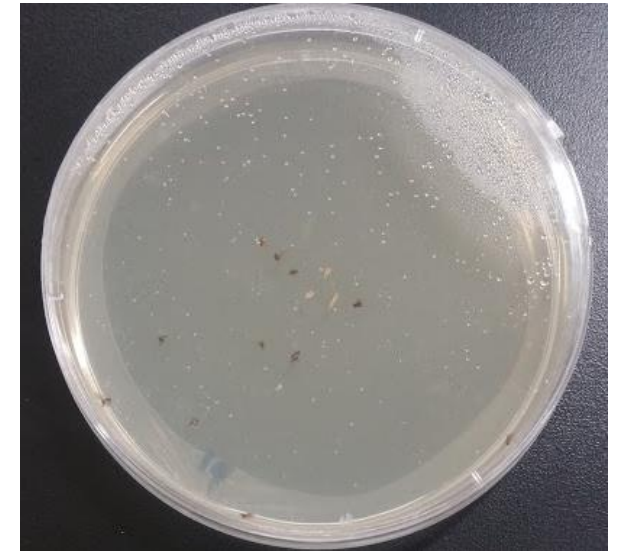
Creating Axenic Flies



Sterilize eggs
with bleach



Eggs are added to
sterile media and
develop into adults



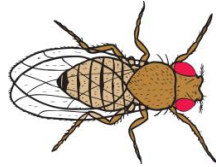
Life stages are
added to plates for
48 hrs. to ensure
they are clean

Lab experiment: fruit fly life stages



➤ Different fruit fly developmental stages

- (10) ADULT FLIES



- (15-20) 24HR OLD LARVAE



- (15-20) EGGS



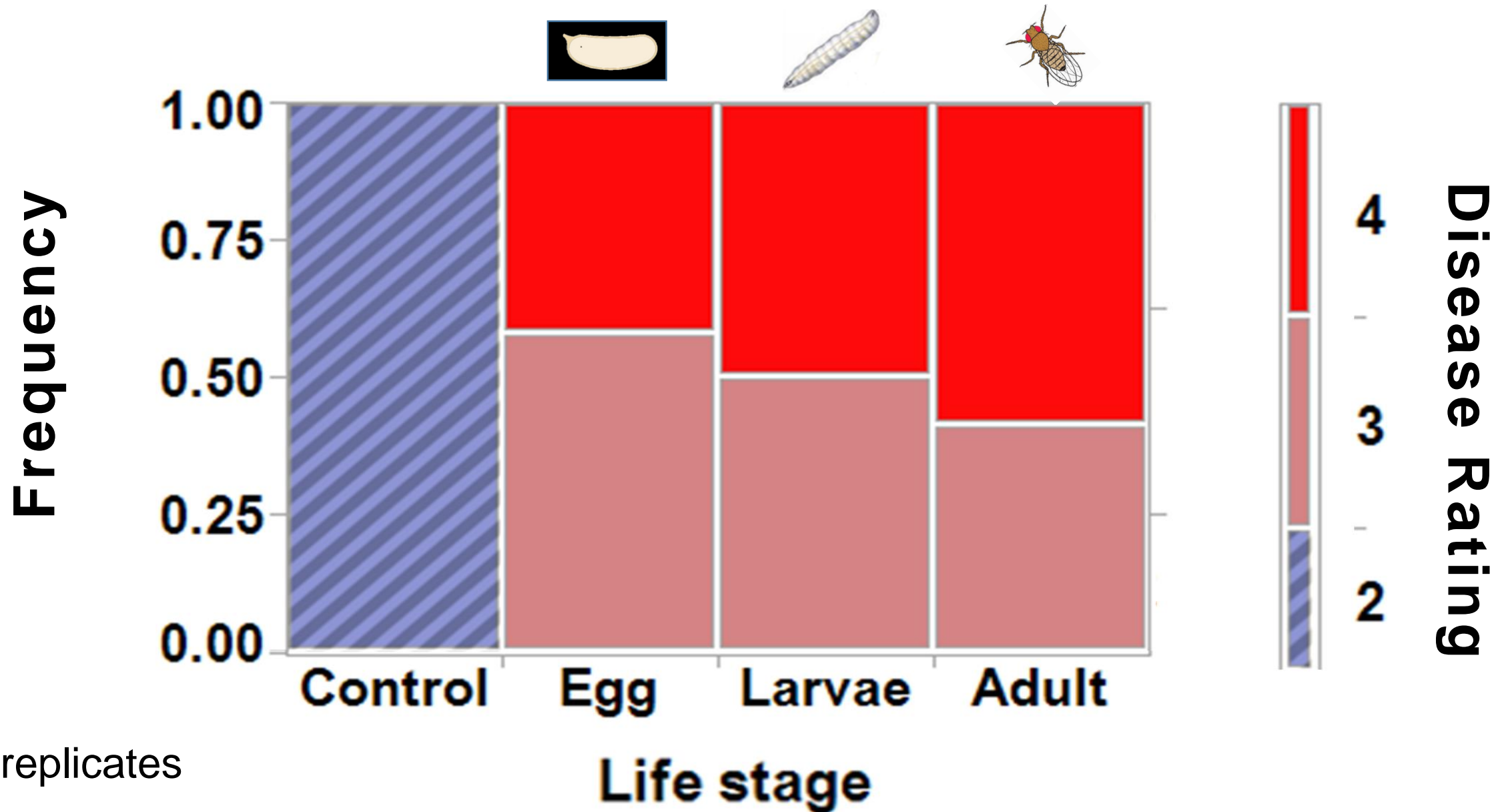
3 Chambourcin berries added to each cup



➤ Does sour rot develop under different life stages?

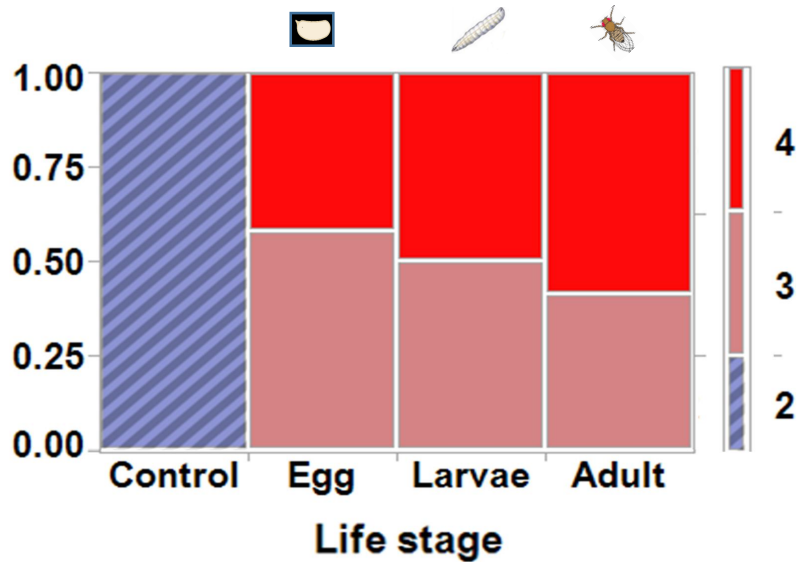
➤ Rated each day for a total of 9 days

Larval stages cause disease at the same rate as adults



*4 replicates

Life Stage Effects



- Eggs hatch into larvae within 24 hours of being collected
- Larvae cups just contain larval stage
- Adults have the advantage of laying eggs that become larvae throughout experiment

Average larvae
at day 9:

Egg	Larvae	Adult
17	15	102

*Power in numbers



Significance of Results

The larval and adult life stages of *Drosophila melanogaster* were **equally successful** at causing sour rot symptoms when grapes were wounded and inoculated.



So What?

- We now understand that the larvae are capable of producing sour rot symptoms
- Weekly sprays after symptoms develop does not change disease at harvest (spray less)

Future Work



- What are the larvae actually doing?
 - ❖ Understand possible enzymatic role
- Current management strategies only address emerging and existing adults
- What are management strategies that incorporates all life stages of fruit fly?



Acknowledgements

Thanks to: Missouri Wine and Grape Board Research Committee Grant and Millikan Endowment Fund for funding my research!

Thanks to vineyards that allowed me to sample

Thanks to my committee members:

Megan Hall

Debbie Finke

Misha Kwasniewski

Greg Loeb

Jim Schoelz

Lab Techs: Zhiwei Fang

Connie Liu (Misha)



MISSOURI
WINES™

Thank You





Berry Damage Caused By:

- Birds
- Insects
- Berry splitting (thin skin & tight clusters)



Separation from pedicle



Yellowjackets

*Fruit flies need wound site to establish