# Mealybug research - from pesticide movement in the vine to their possible role as vectors of plant viruses

UCCE Sonoma Grape Day
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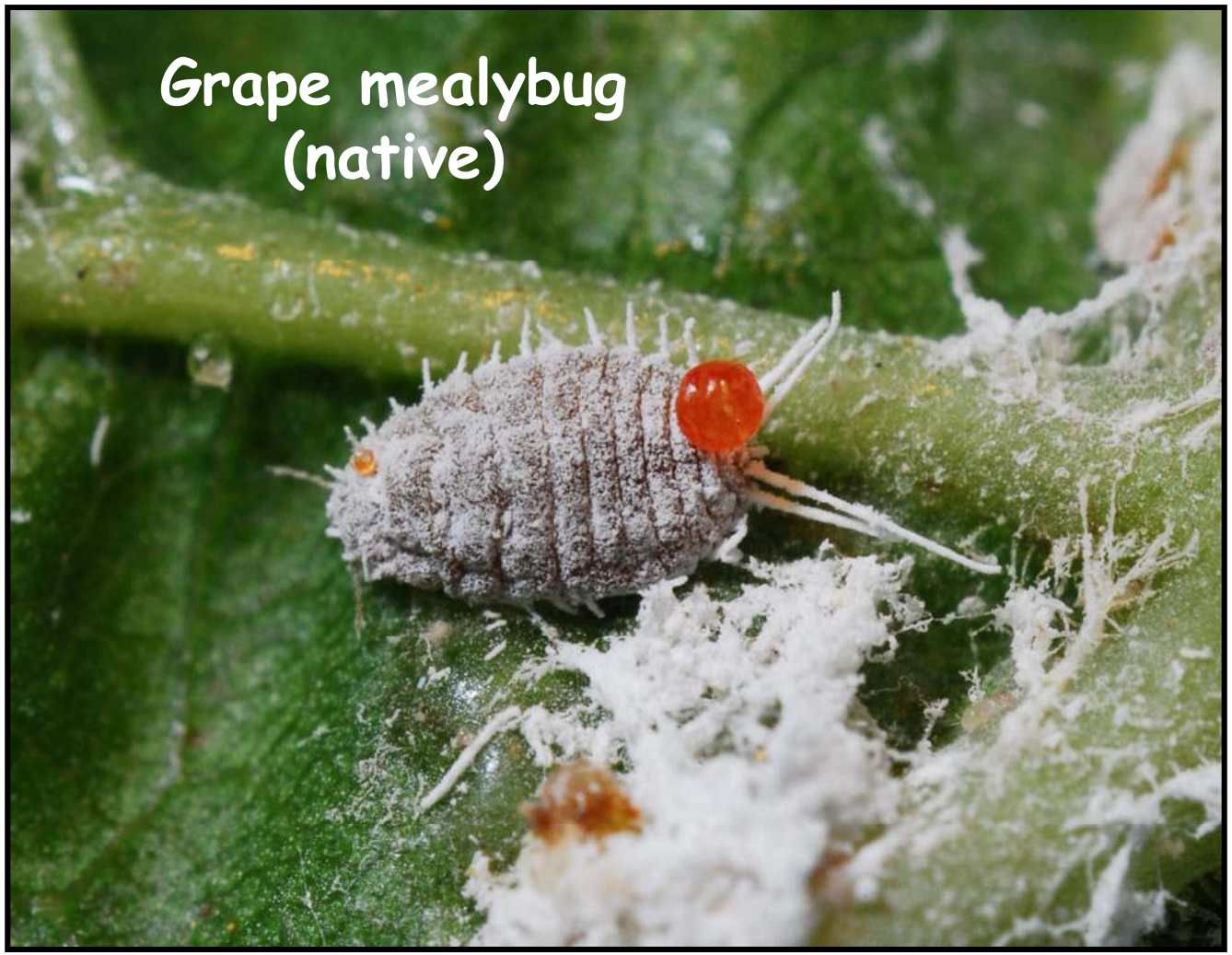
# Current Research on Vine Mealybug

1. Mealybug Controls
    a) damage – a review
    b) insecticides – current studies
    c) biological controls – a review
    d) mating disruption – final comments

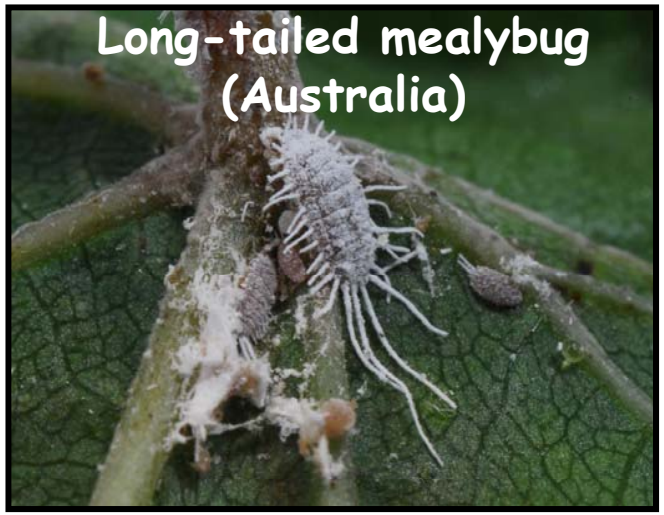
2. GLRaV Control Programs
    a) mealybug-leafroll epidemiology
    b) areawide controls

3. Red Blotch
    a) are mealybugs or any insects vectors

# Which mealybug species do you have?

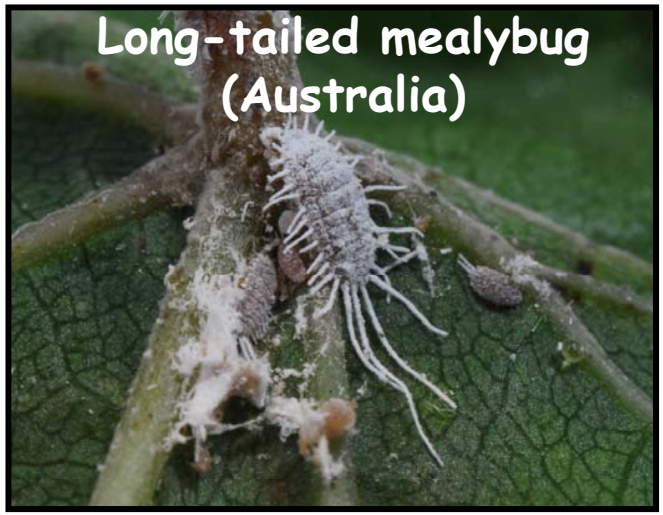




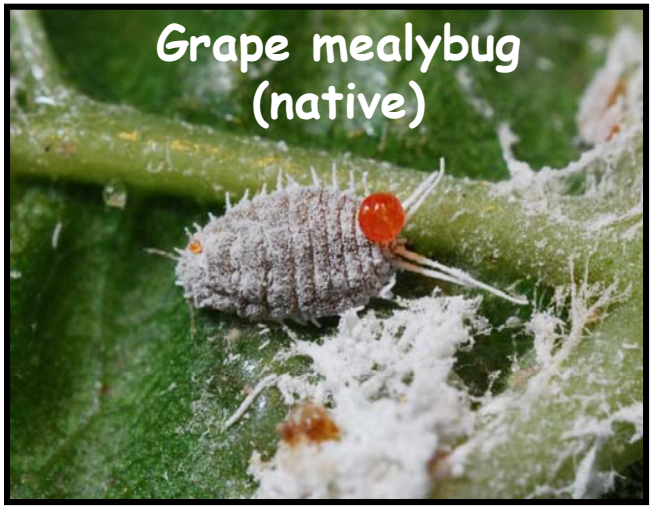



# Vine MB is 1 of 4 important invasive mealybug species (sets us apart)

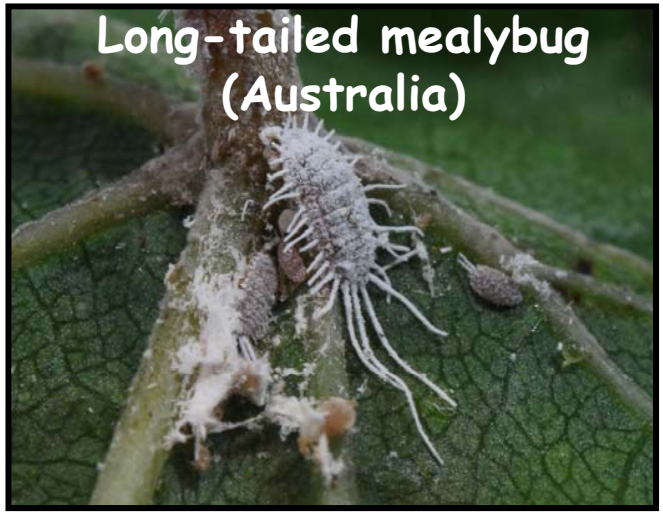




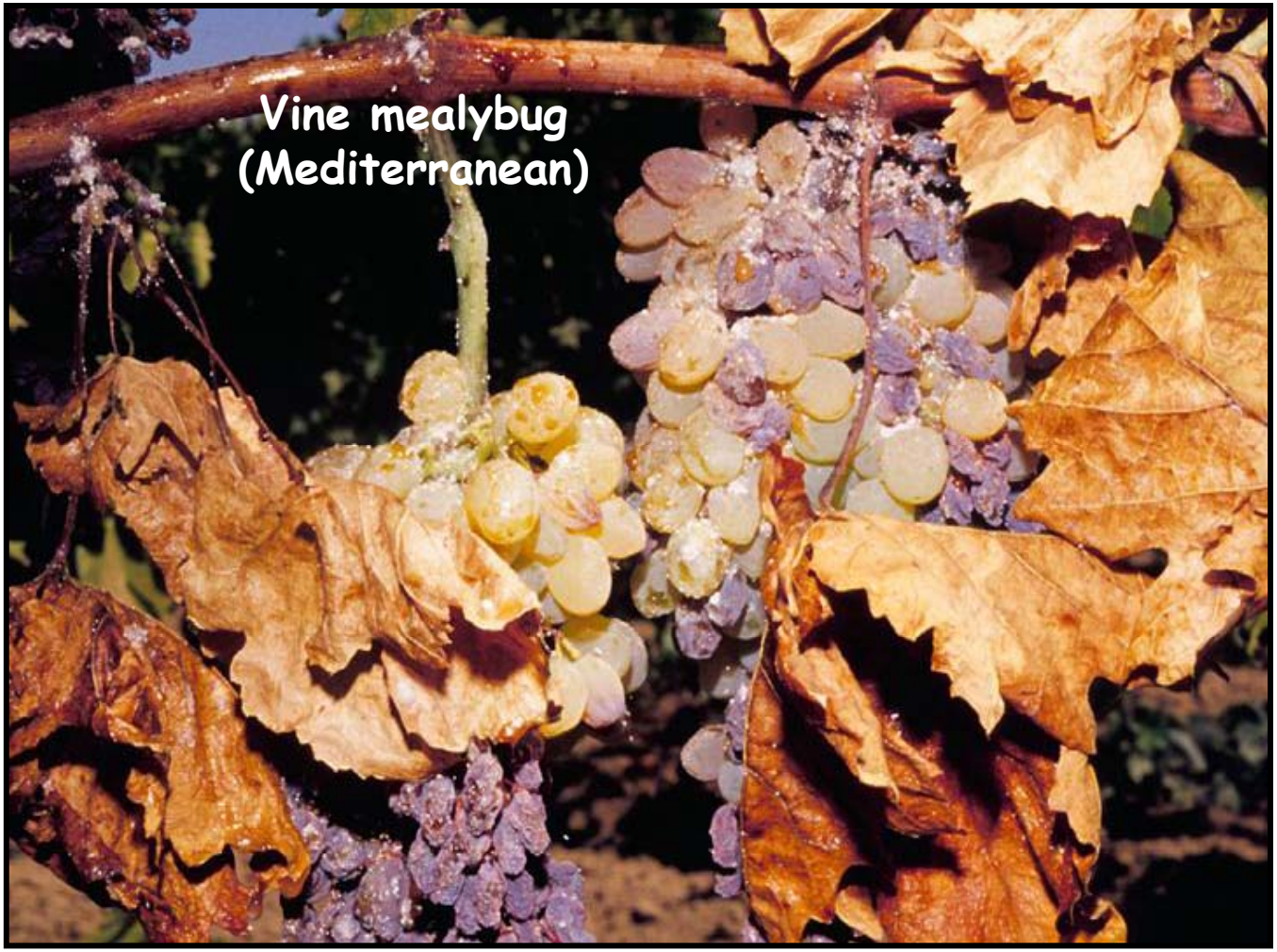

# Vine MB causes more damage
# 1) more eggs (higher fecundity)
# 2) feeds on leaves

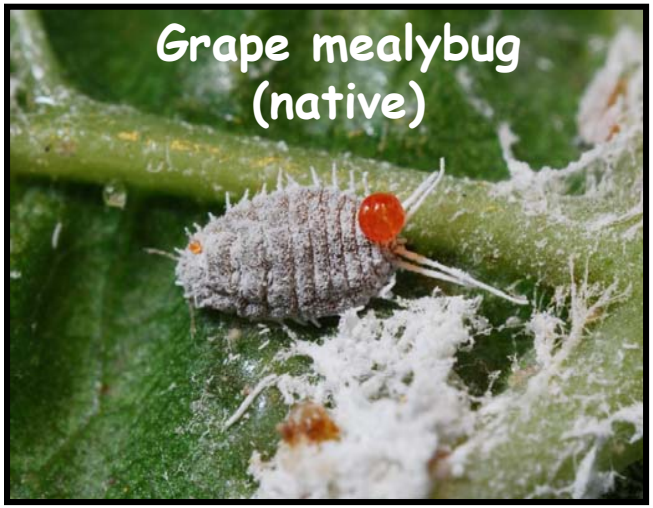




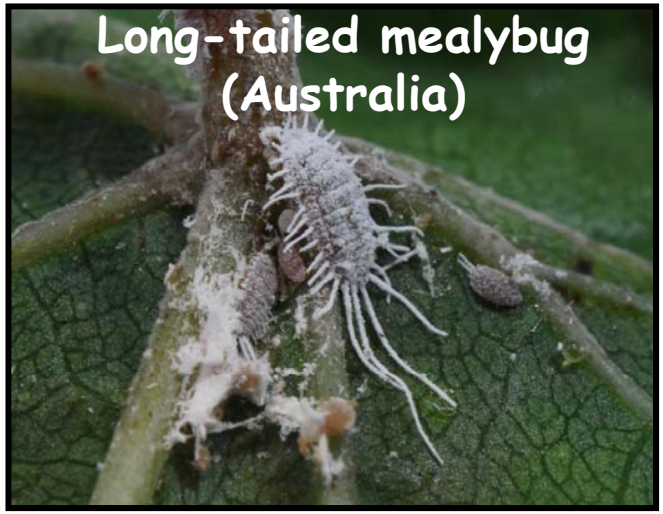



# Vine MB causes more damage
# 1) more eggs (higher fecundity)
# 2) feeds on leaves
# 3) more honeydew excretion

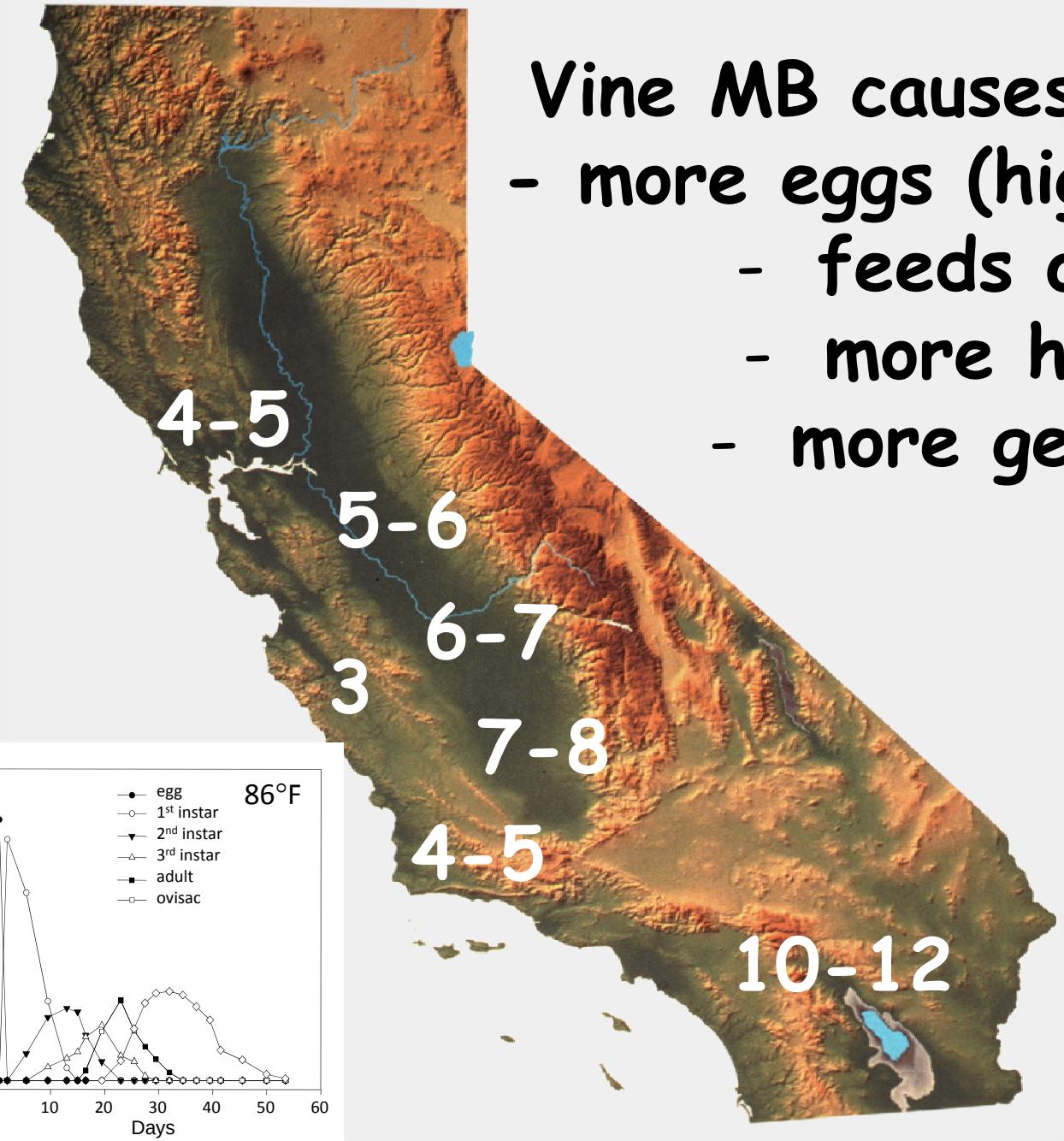






# Vine MB causes more damage
- more eggs (higher fecundity)
- feeds on leaves
- more honeydew
- more generations







Daane, Walton, Daugherty, submitted J Econ Entomol, based on temperature cabinet studies, field validation in some areas.





photo courtesy of Deborah Golino

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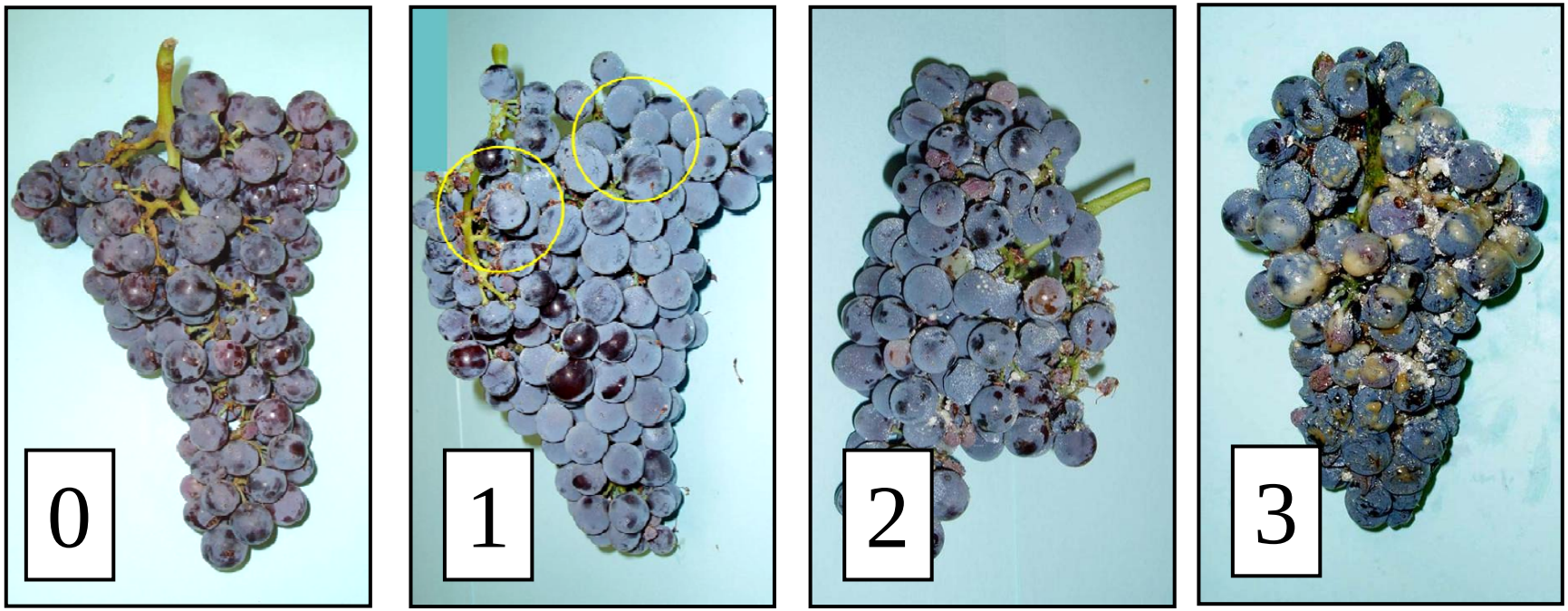
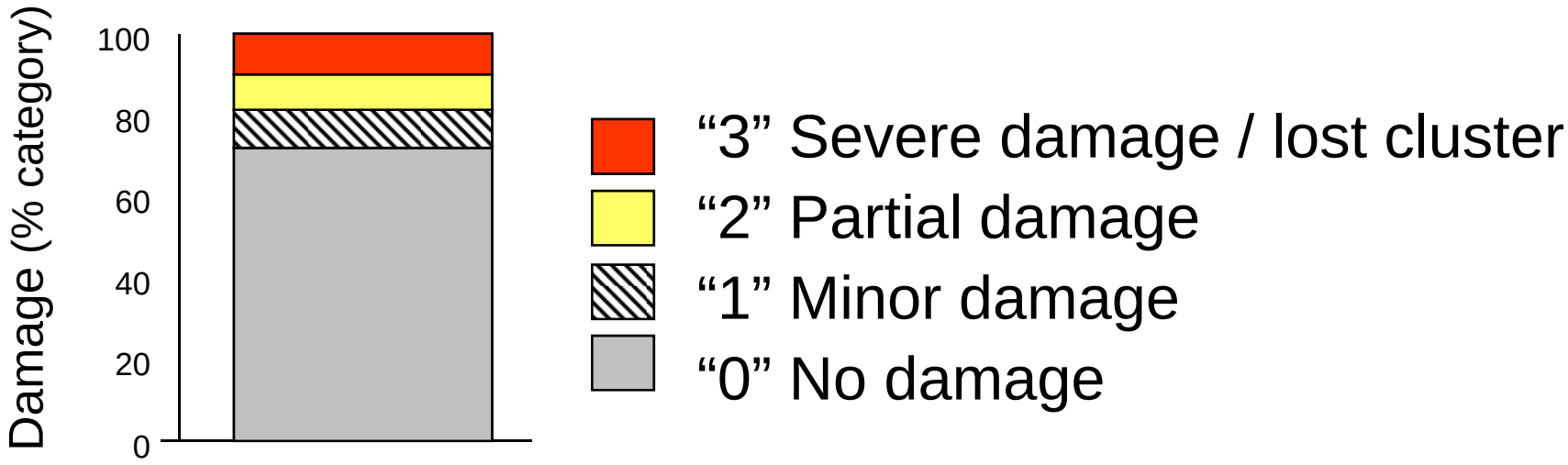
   - a) are mealybugs or any insects vectors

# Previous studies with insecticides

- Movento is typically the best product



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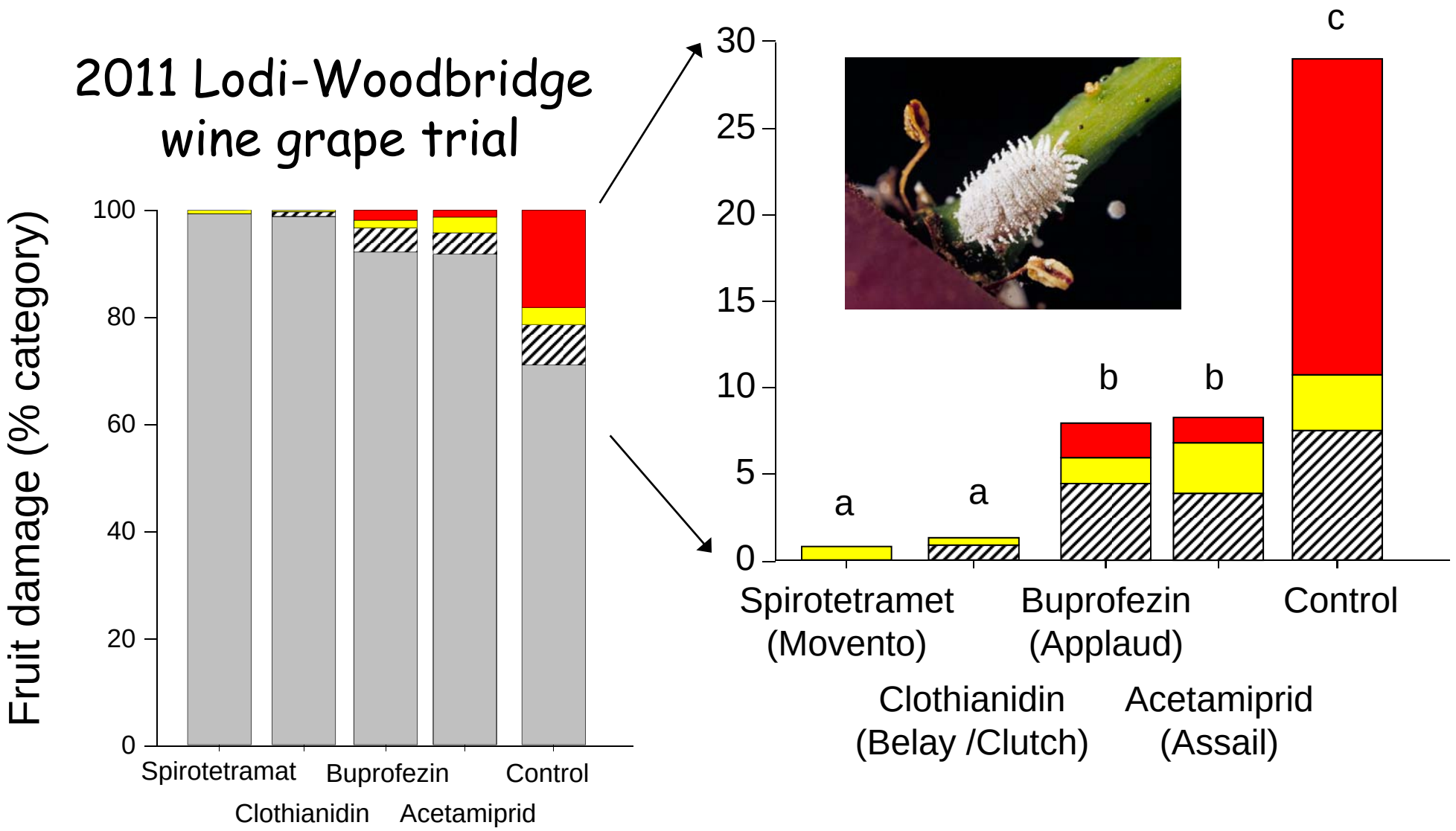
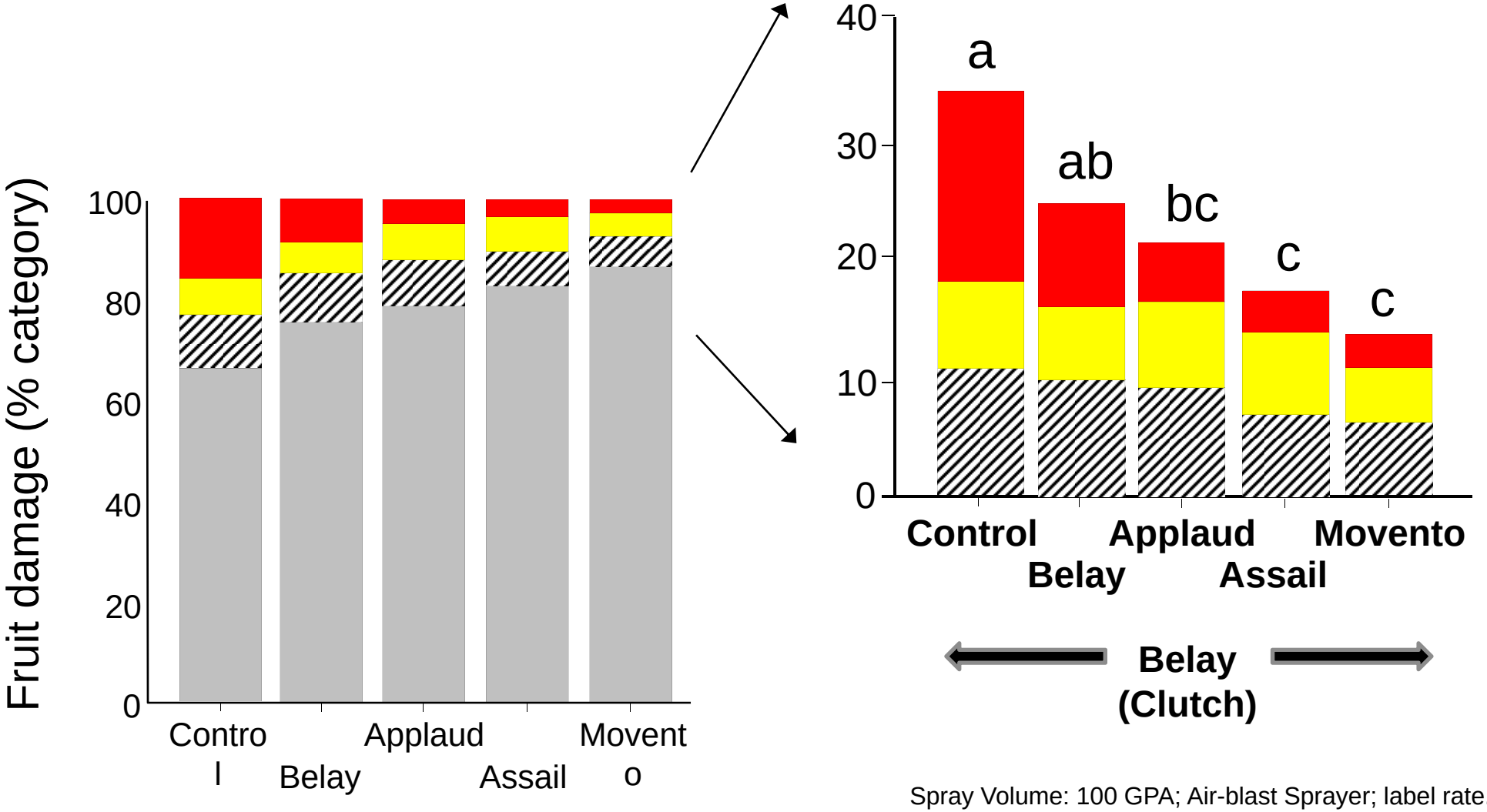


# Previous studies with insecticides

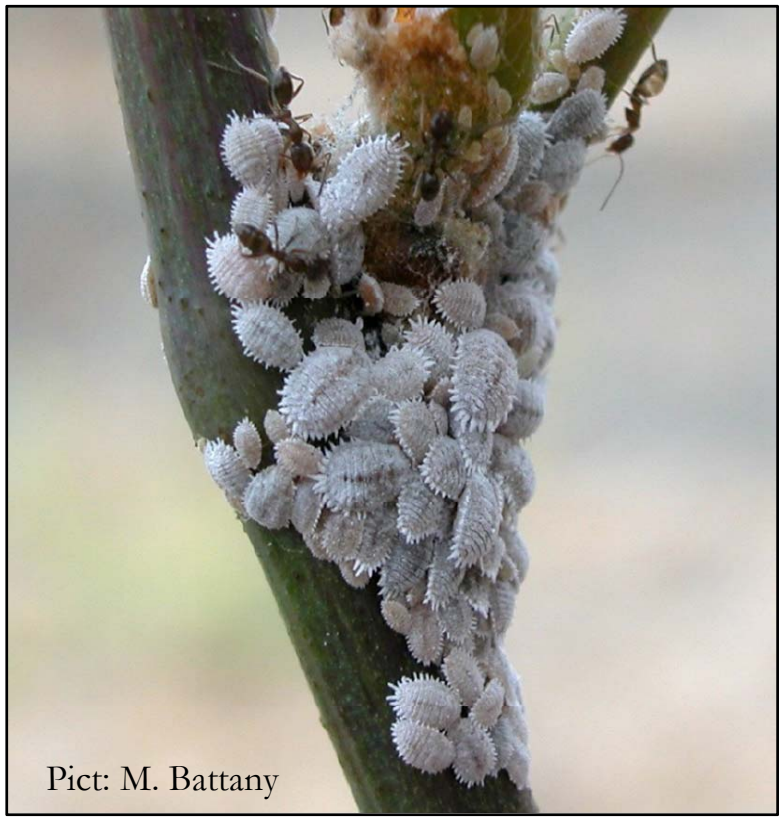
- we are working to find out how Movento moves




Spray Volume: 100 GPA; Air-blast Sprayer; label rate.
Vine mealybug (*P. ficus*) was the target pest; Lodi, CA.
Movento and Belay applied May 29, 2012
Applaud and Assail applied June 20, 2012
Belay additionally applied to all plots July 20, 2012

## Why are VMB sometimes hard to kill?

- population on the leaves can be controlled
- fruit generally remain clean
- population on the trunks and roots harder to kill

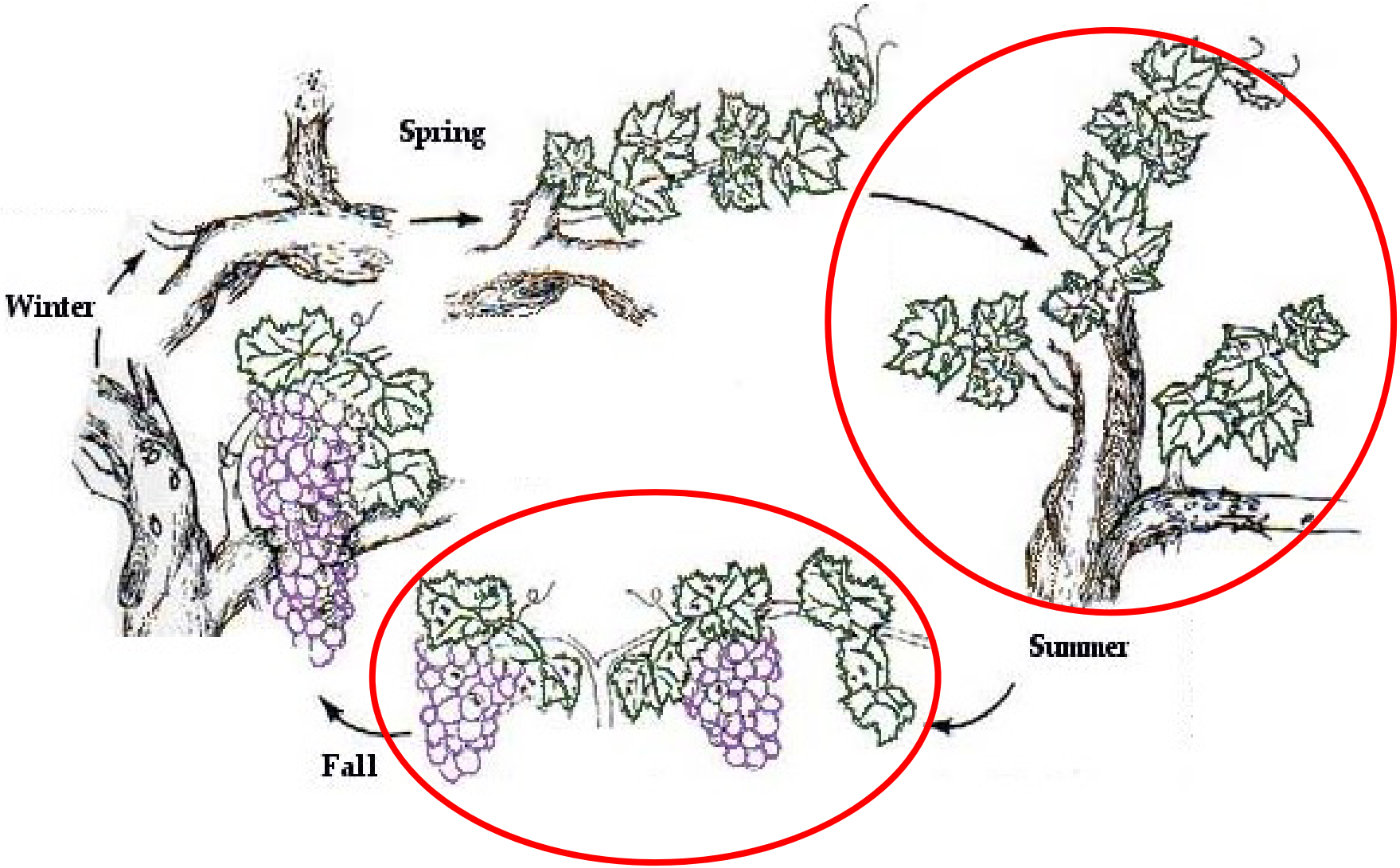

Pict: M. Battany


Daane

**some failures are not failures – but a delay in kill**

# Our Goals: better VMB on the trunk

- test applications in the spring and early fall
- different vineyards, regions & cultural practices




Modified after : http://www.ipm.ucdavis.edu/PMG/GARDEN/IMAGES/INVERT/lcgrapemealy.jpg

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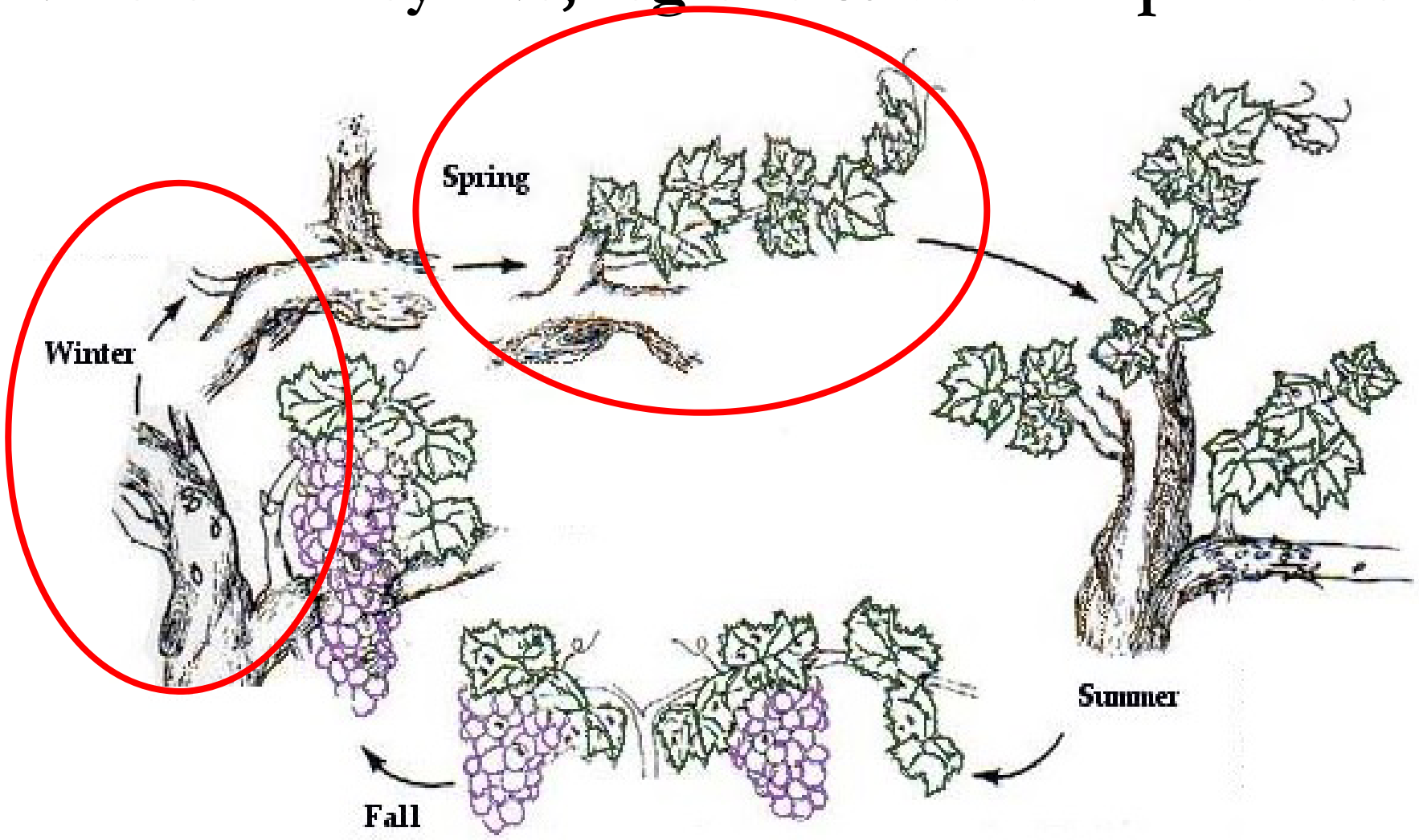
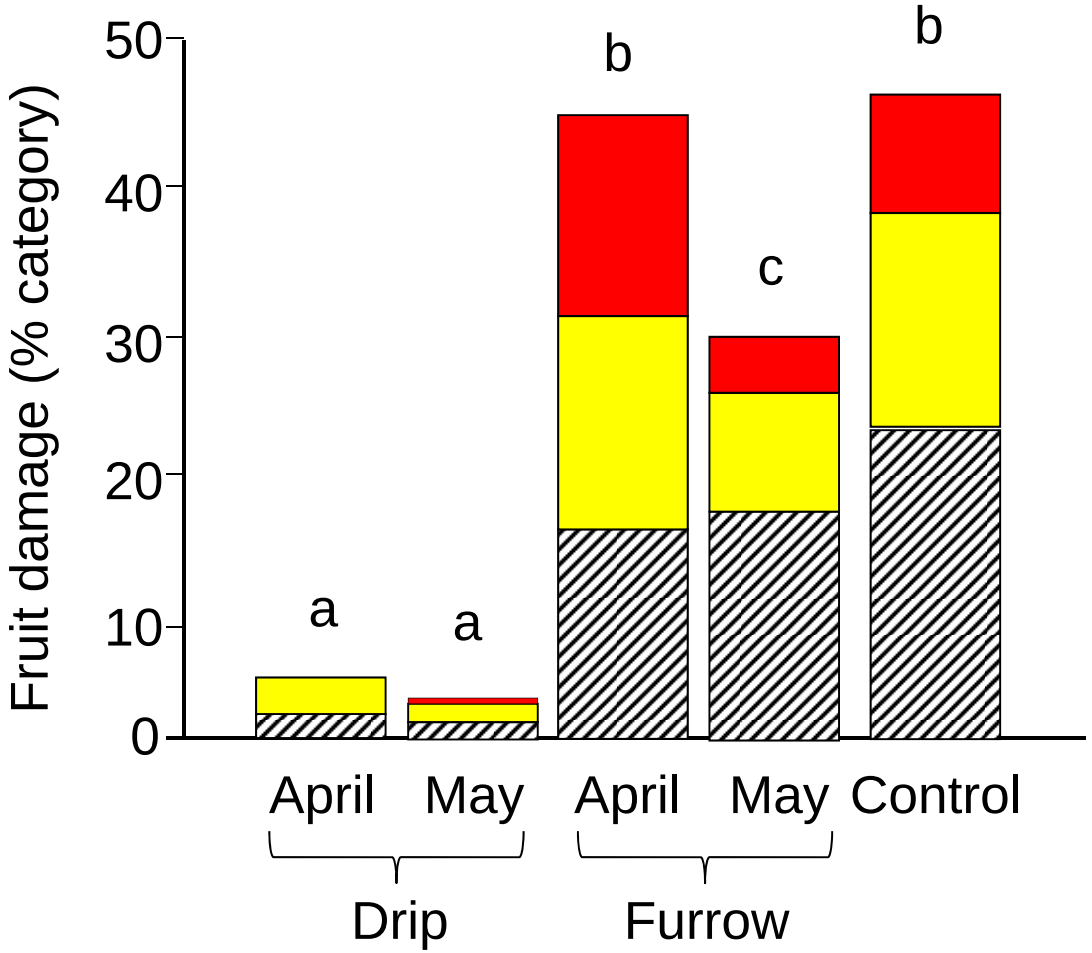



Modified after : http://www.ipm.ucdavis.edu/PMG/GARDEN/IMAGES/INVERT/lcgrapemealy.jpg

# Understanding the systemic movement of pesticides

- **Example from the early work with Admire**

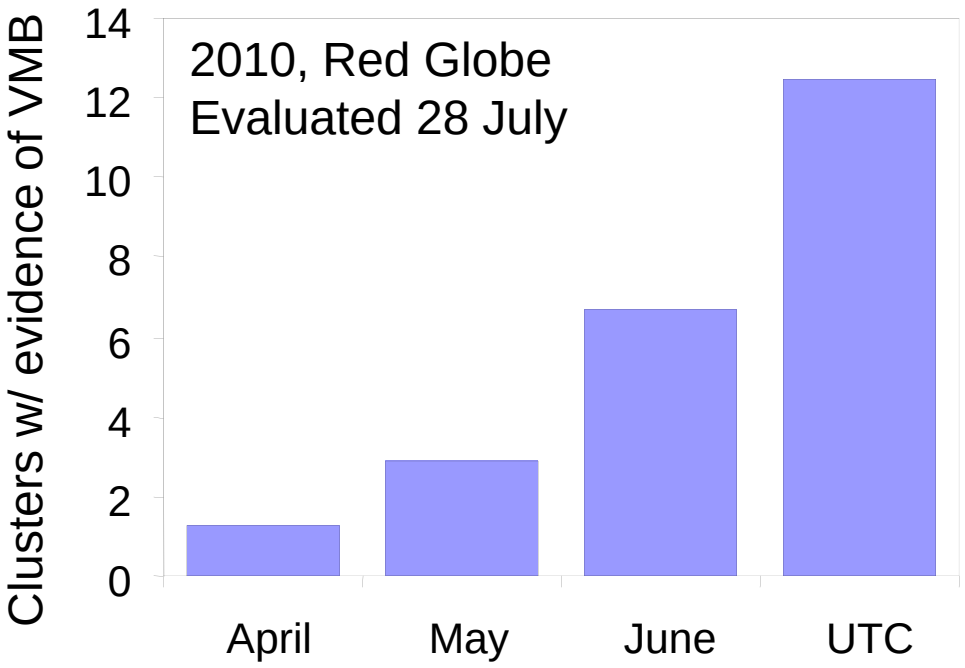



Several factors might affect the **pesticide uptake rate**

Efficiency to kill the pest

# For most materials – timing and coverage is critical

## Courtesy of Haviland & Rill (Kern County):
## Timing of Movento may impact levels of control




**In most trials the earlier application (April) had better control in Kern County table grapes trials.**

# For most materials – timing and coverage is critical

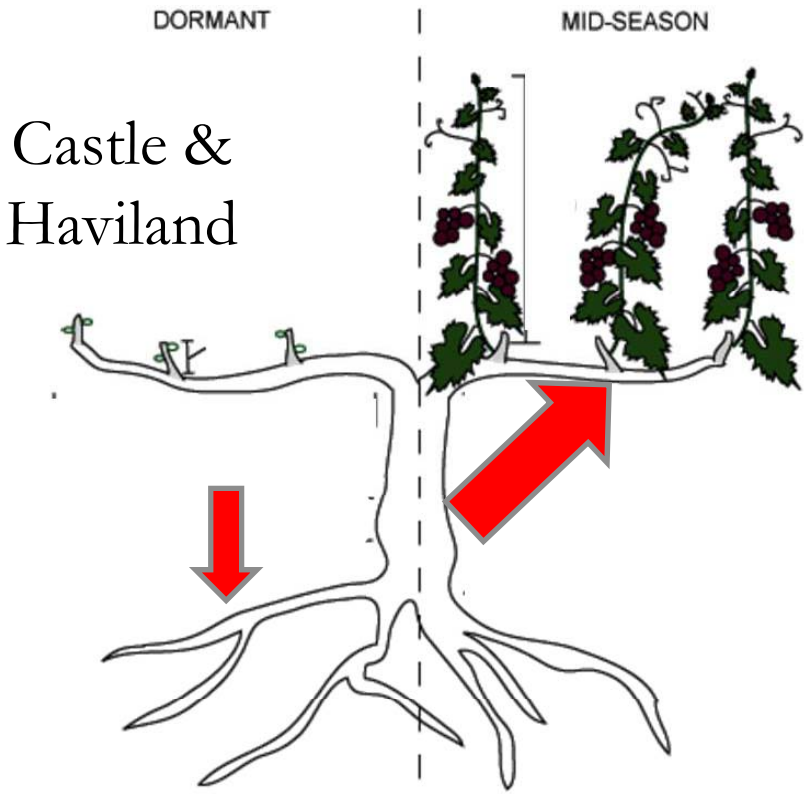
## Courtesy of Haviland & Rill (Kern County):
## Timing of Movento may impact levels of control



**Work by Bentley (2010) & Daane (2012) in Fresno Co. showed applications from April - May were equally effective**

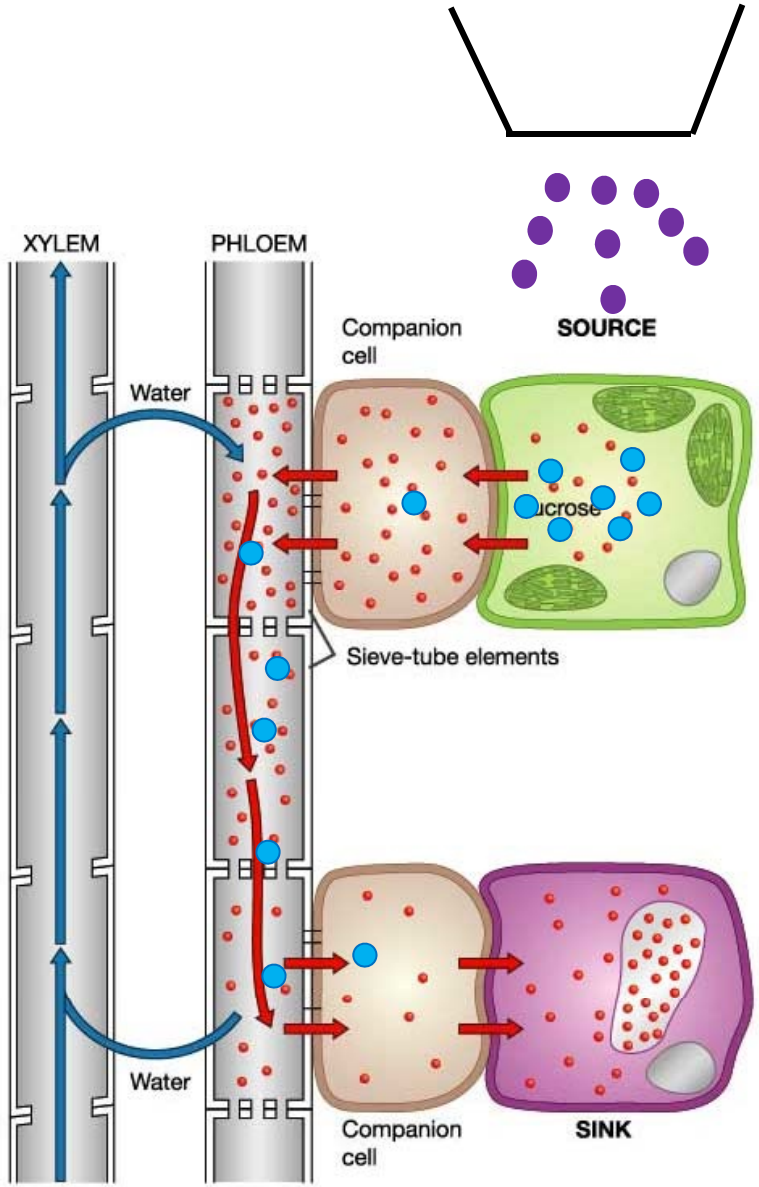
# Understanding the systematic uptake of pesticide

- Timing of application
- Location of pest population
- Vine factors (e.g., vine age)
- Pest population stage




Castle & Haviland

# Understanding the systematic uptake of pesticide




Spirotetramat (SPTA)

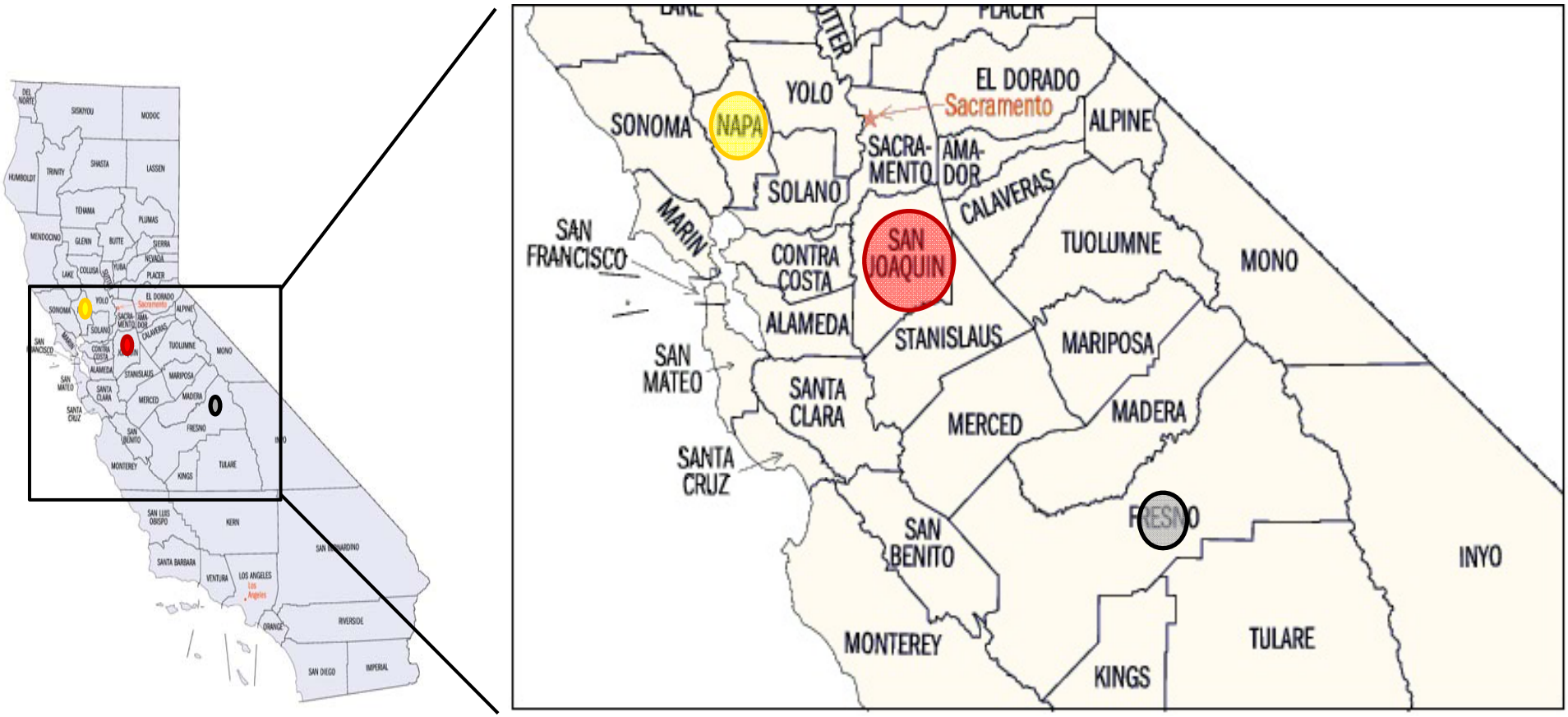
Spirotetramat metabolite (SPTA Enol)

Modified after: http://www.uic.edu/classes/bios/bios100/lectf03am/translocation.jpg

# Factors

- Age of vineyards: 6 to 25 years old vines
- Irrigation type: drip vs. flood
- Type of vines:
  - Table grapes (Crimson, Thompson)
  - Raisin grapes (Selma Pete, Thompson)
  - Wine grapes ( 9 varieties)
- Grafted vs. non-grafted
- Different rootstocks
- Presence of girdle
- Different pesticide application rates
- Geographical area
- Level of VMB infestation and location on the vine

# Area and level of infestation



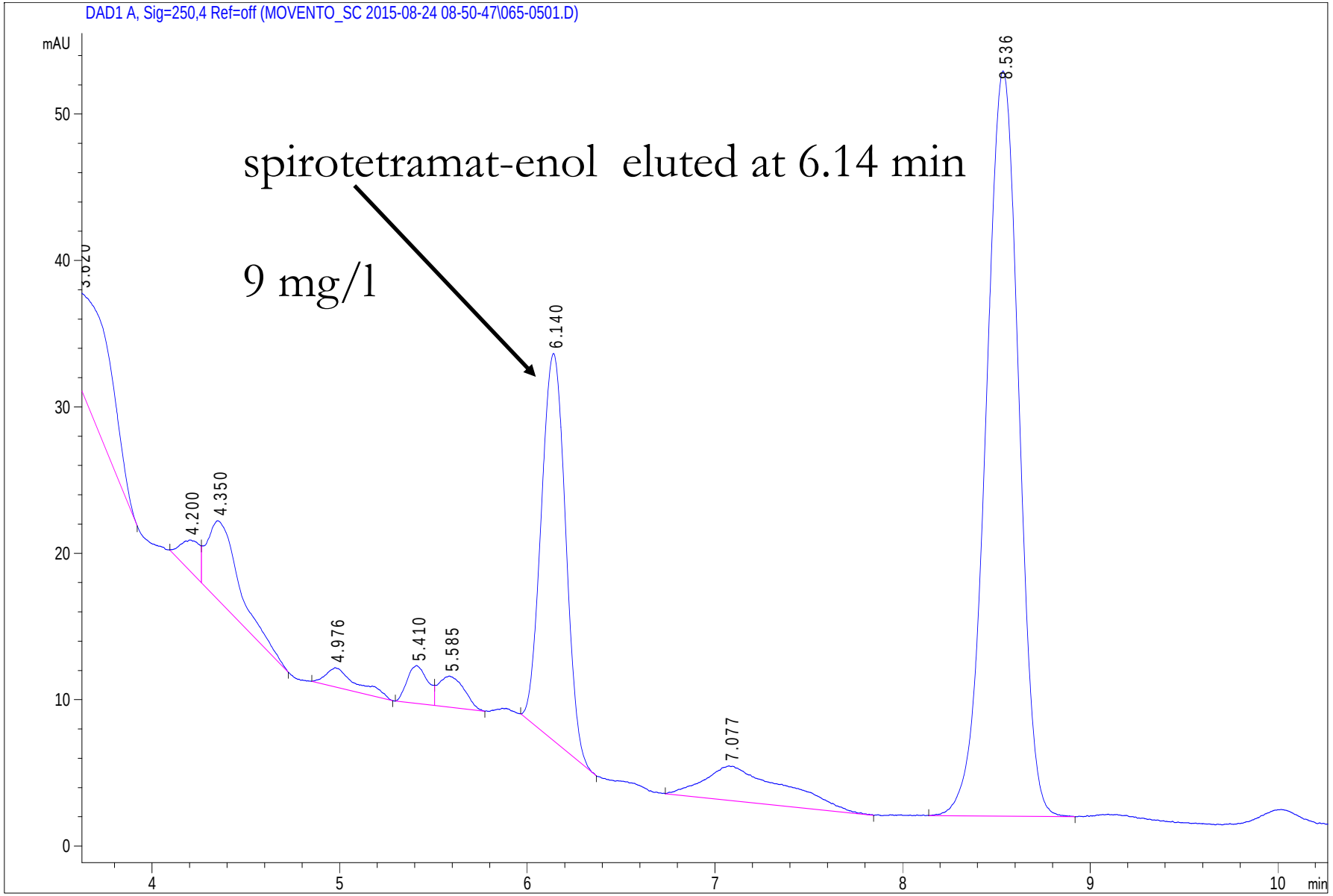

Lodi, heavy infestation

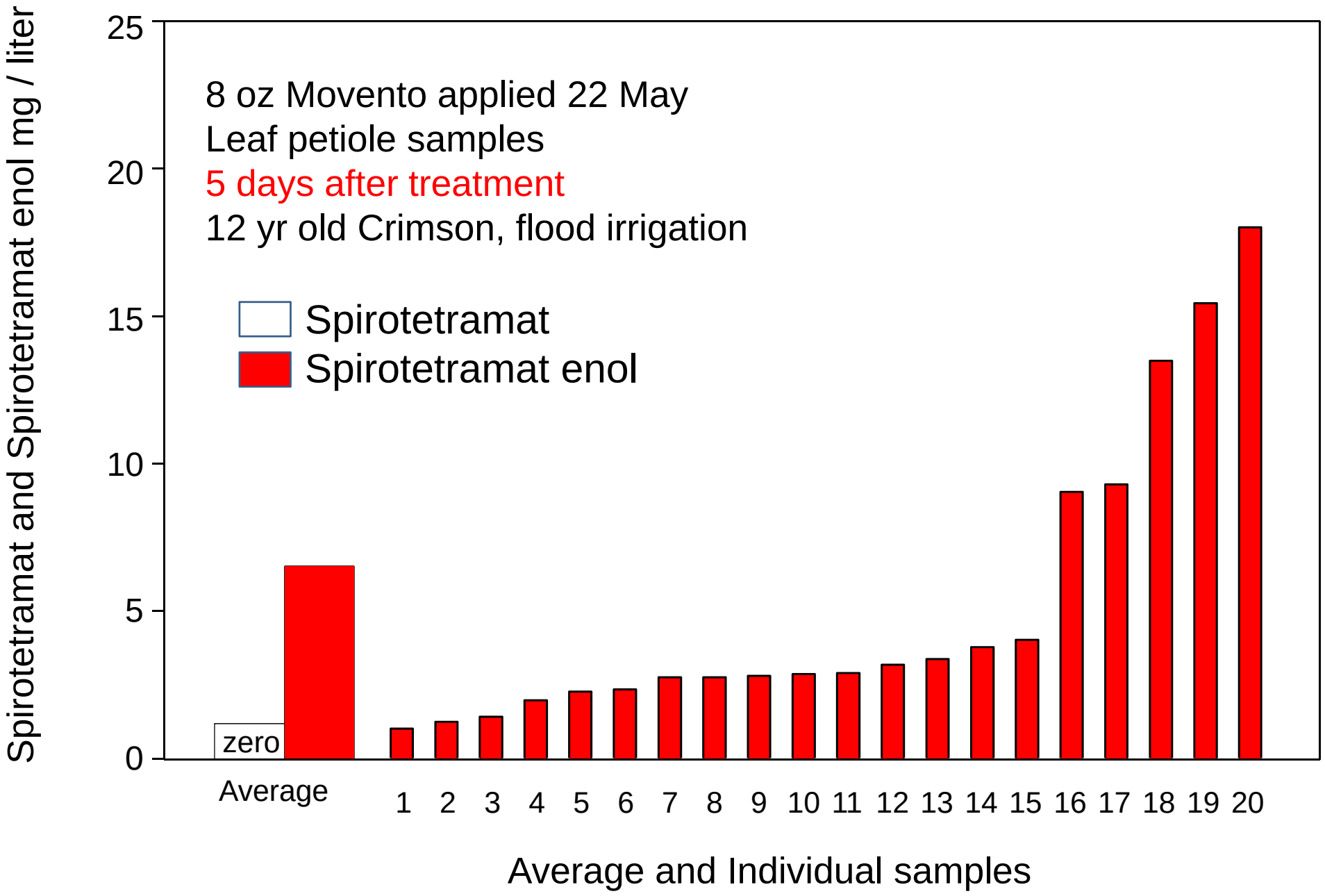
Napa, moderate infestation

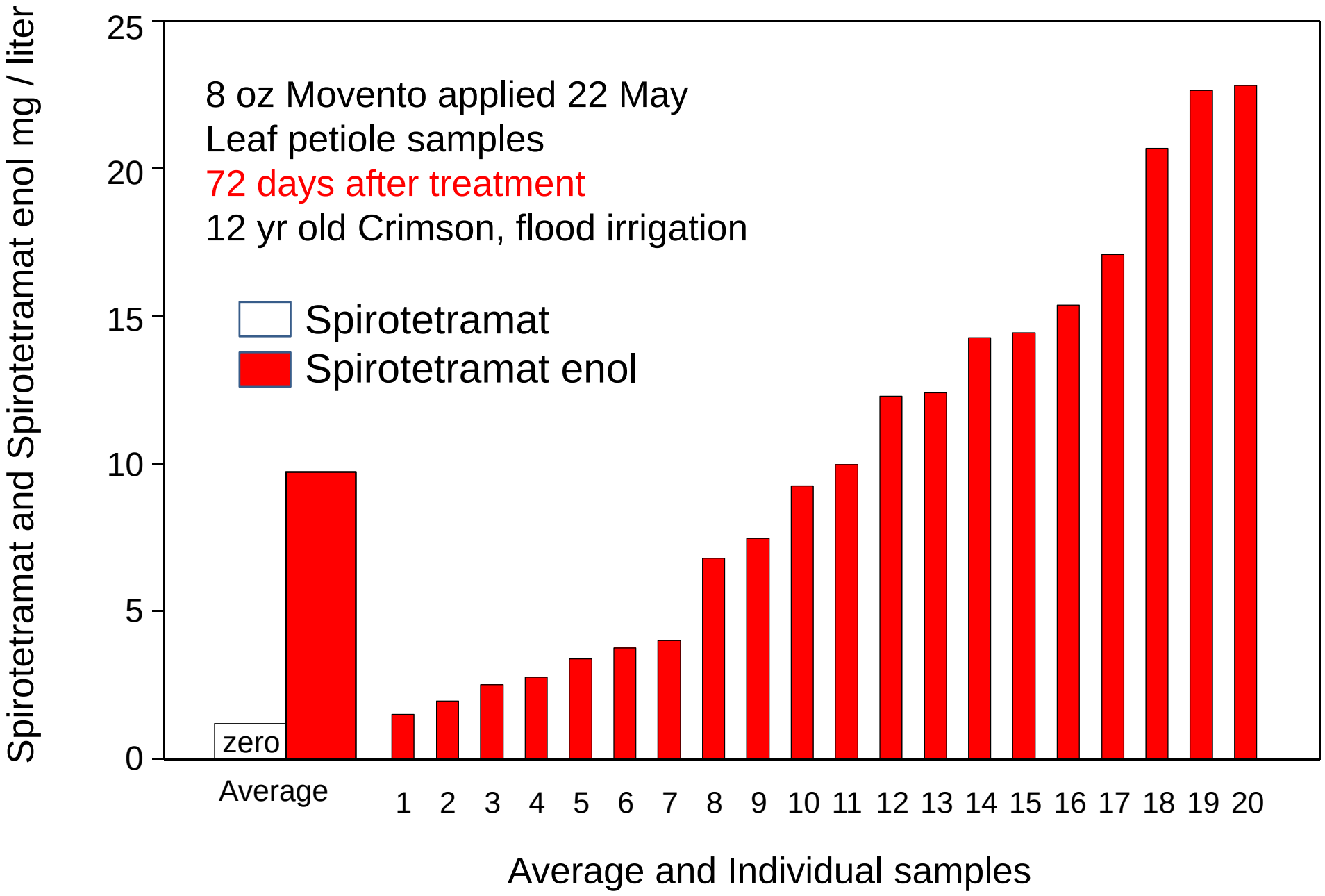
Fresno, light infestation

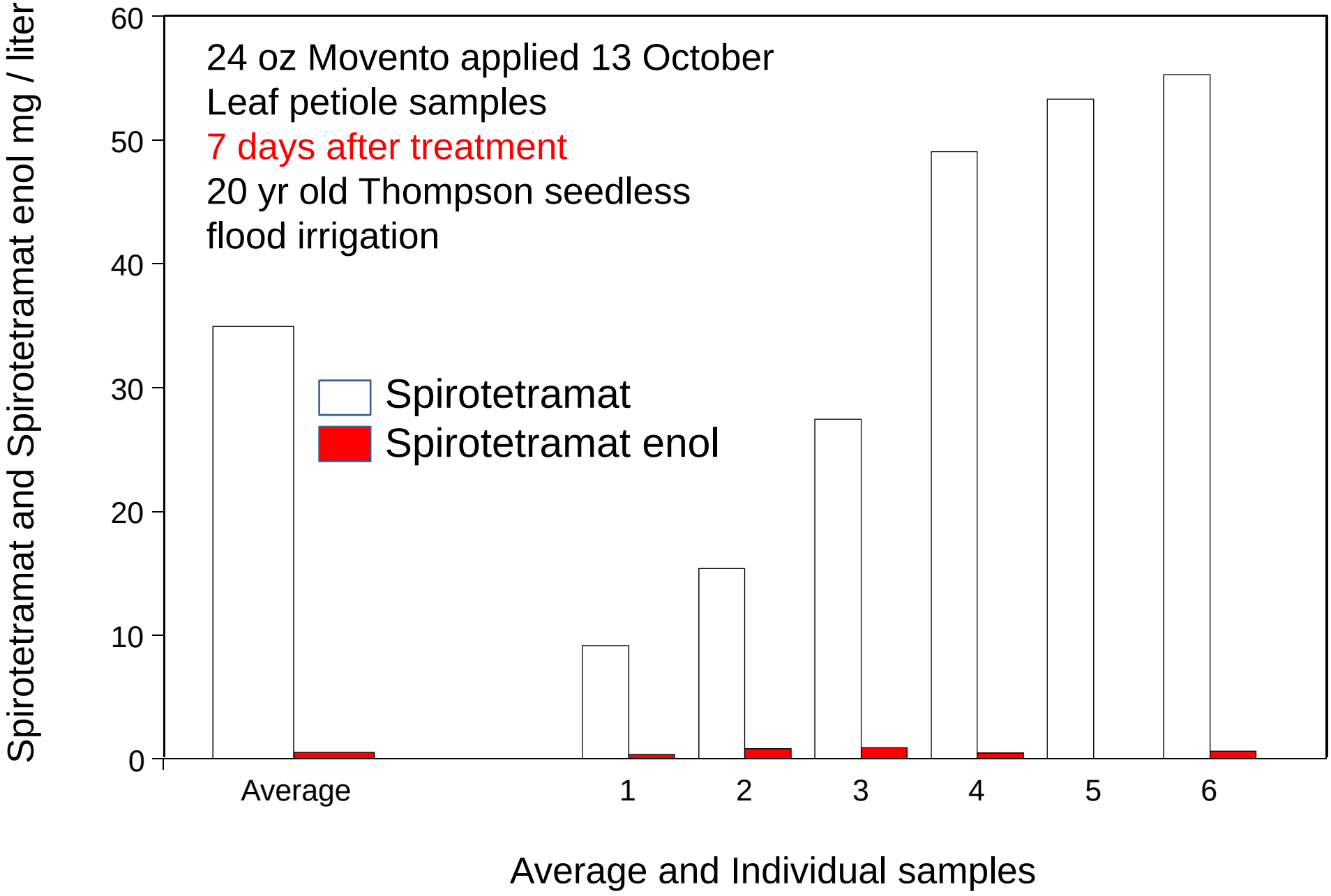

Leaf & petiole
Cane
Arm/Cordon
Trunk
(above & below girdle)
Roots

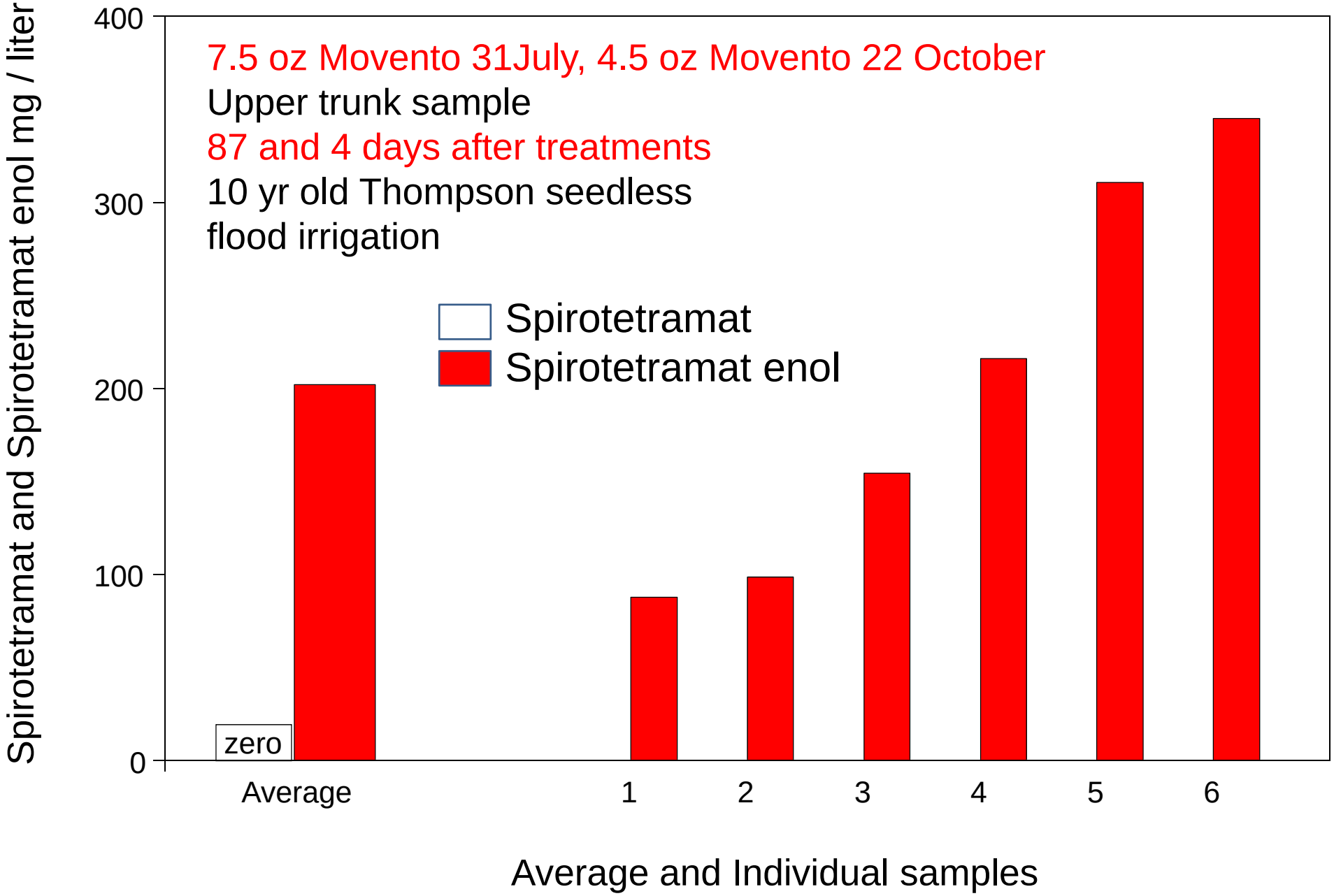
# Testing the extraction QuEChERS method for leaves




Spirotetramat and Spirotetramat enol mg / liter
25
20
15
10
5
0
8 oz Movento applied 22 May
Leaf petiole samples
5 days after treatment
12 yr old Crimson, flood irrigation
Spirotetramat
Spirotetramat enol
zero
Average
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Average and Individual samples


Spirotetramat and Spirotetramat enol mg / liter
25
20
15
10
5
0
8 oz Movento applied 22 May
Leaf petiole samples
72 days after treatment
12 yr old Crimson, flood irrigation
Spirotetramat
Spirotetramat enol
zero
Average
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Average and Individual samples


24 oz Movento applied 13 October
Leaf petiole samples
7 days after treatment
20 yr old Thompson seedless
flood irrigation
Spirotetramat
Spirotetramat enol
Spirotetramat and Spirotetramat enol mg / liter
60
50
40
30
20
10
0
Average
1
2
3
4
5
6
Average and Individual samples


7.5 oz Movento 31July, 4.5 oz Movento 22 October
Upper trunk sample
87 and 4 days after treatments
10 yr old Thompson seedless
flood irrigation
Spirotetramat
Spirotetramat enol
zero
Spirotetramat and Spirotetramat enol mg / liter
400
300
200
100
0
Average
1
2
3
4
5
6
Average and Individual samples

# Current Research on Vine Mealybug

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   - a) mealybug-leafroll epidemiology
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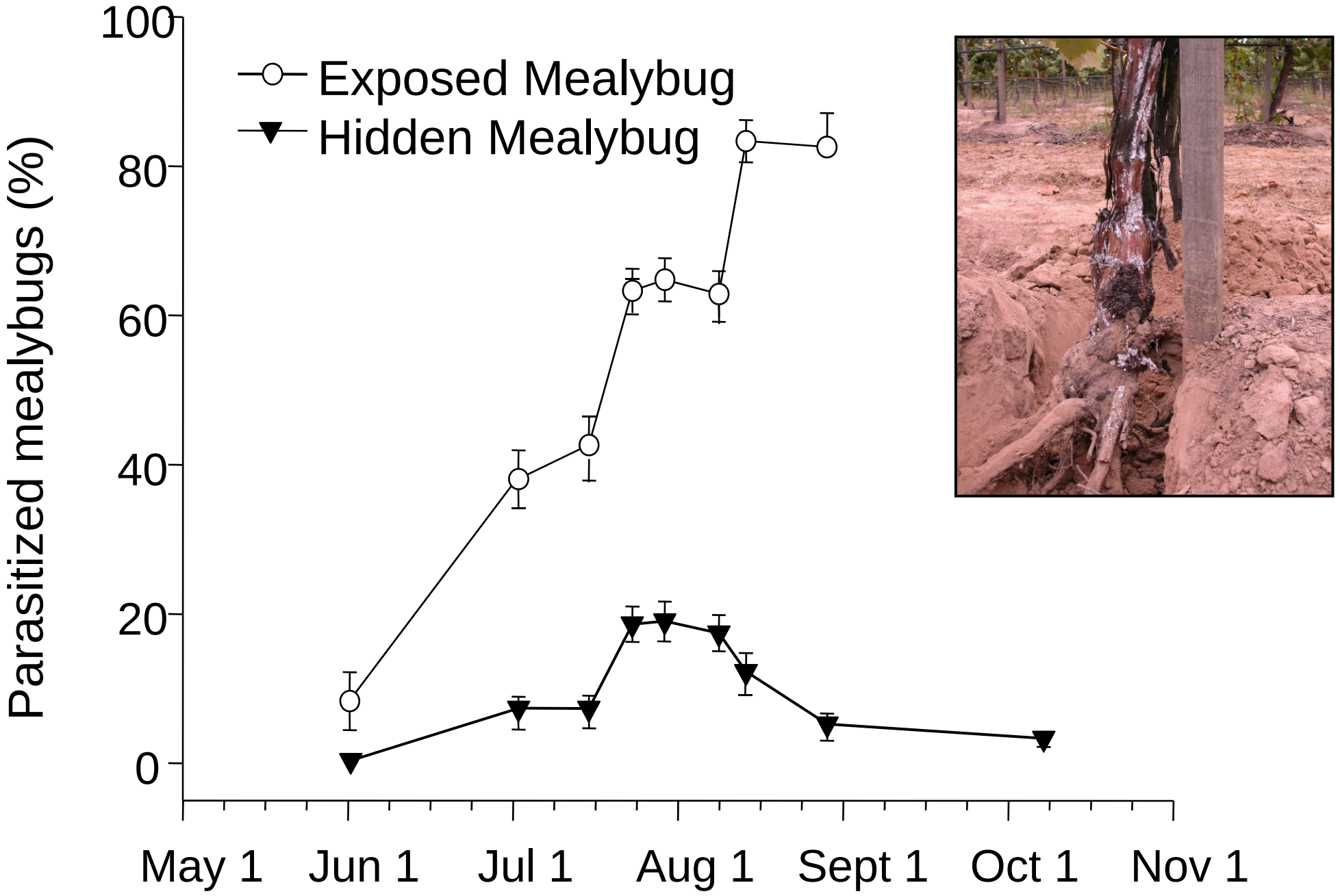
3. Red Blotch
   - a) are mealybugs or any insects vectors


*Anagyrus pseudococci* - female


Anagyrus pseudococci - male

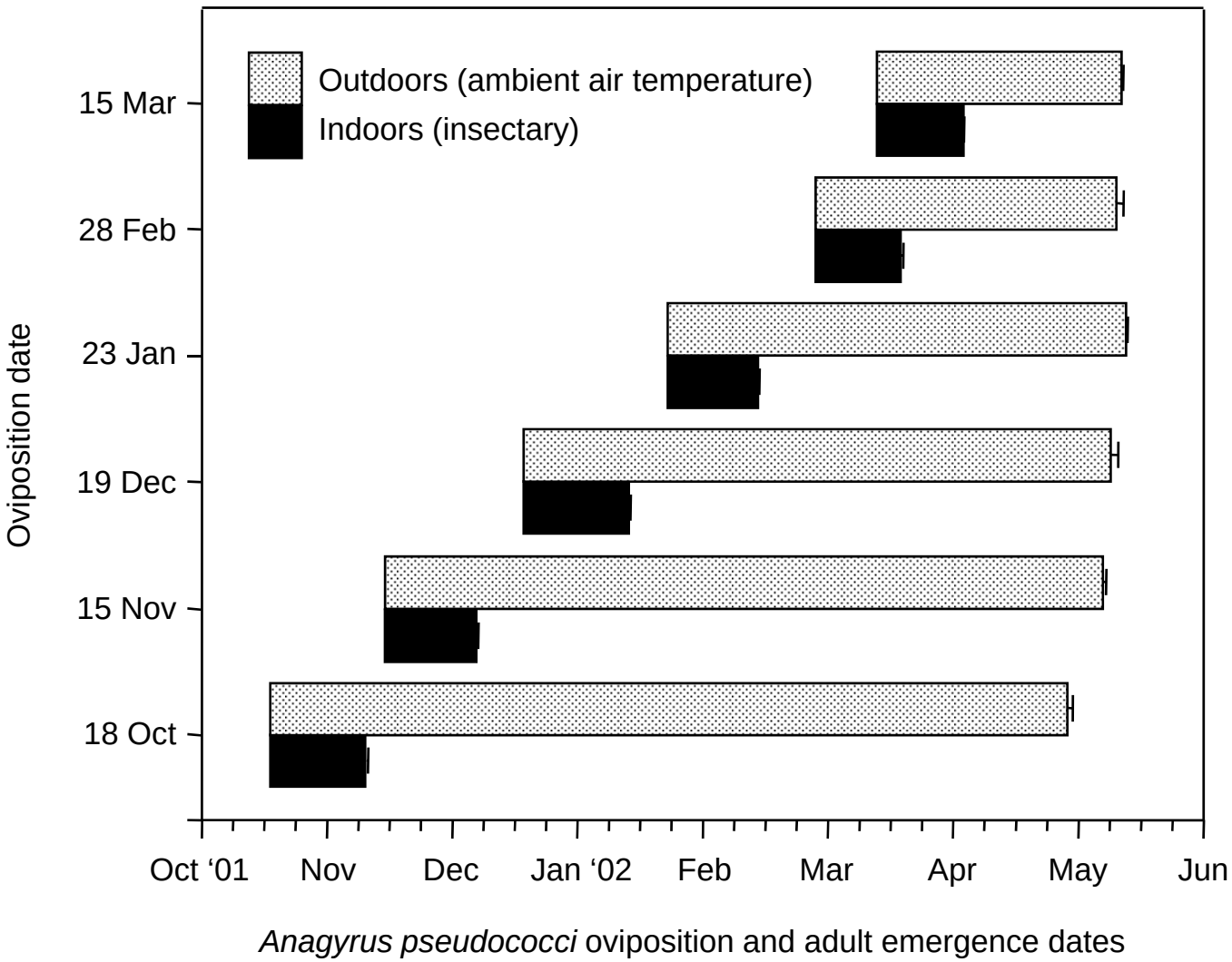
# Problem I: Mealybug Location






Daane et al, unpublished da

# Problem II. Parasitoid Overwintering Biology

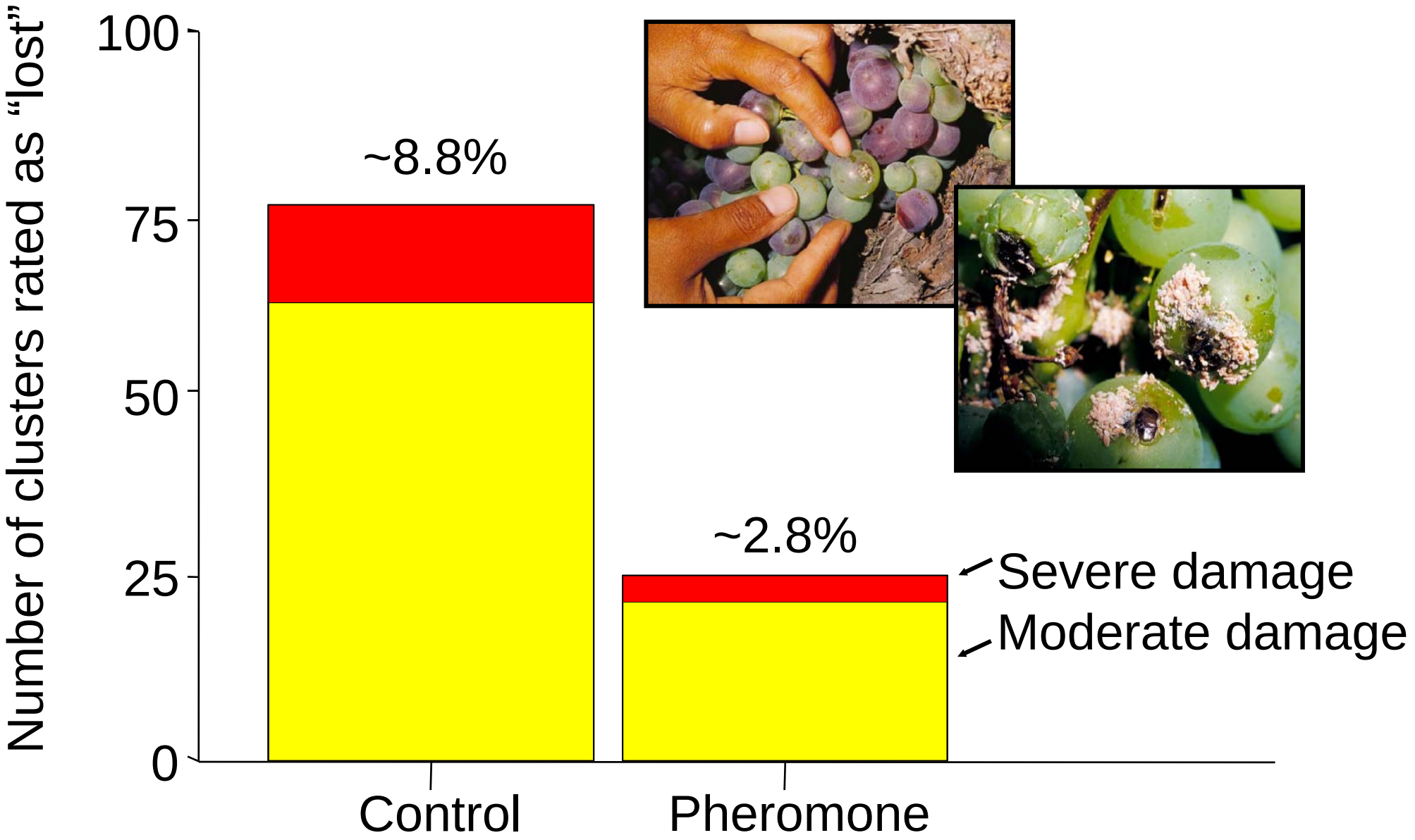



*Anagyrus pseudococci* oviposition and adult emergence dates

Daane et al. 2005. *Biol. Control*

# Mating disruption - synthesized sex pheromone

# Mating disruption - raisin block in Del Rey



# Insecticides for 'high density'
# Mating disruption to prevent spread

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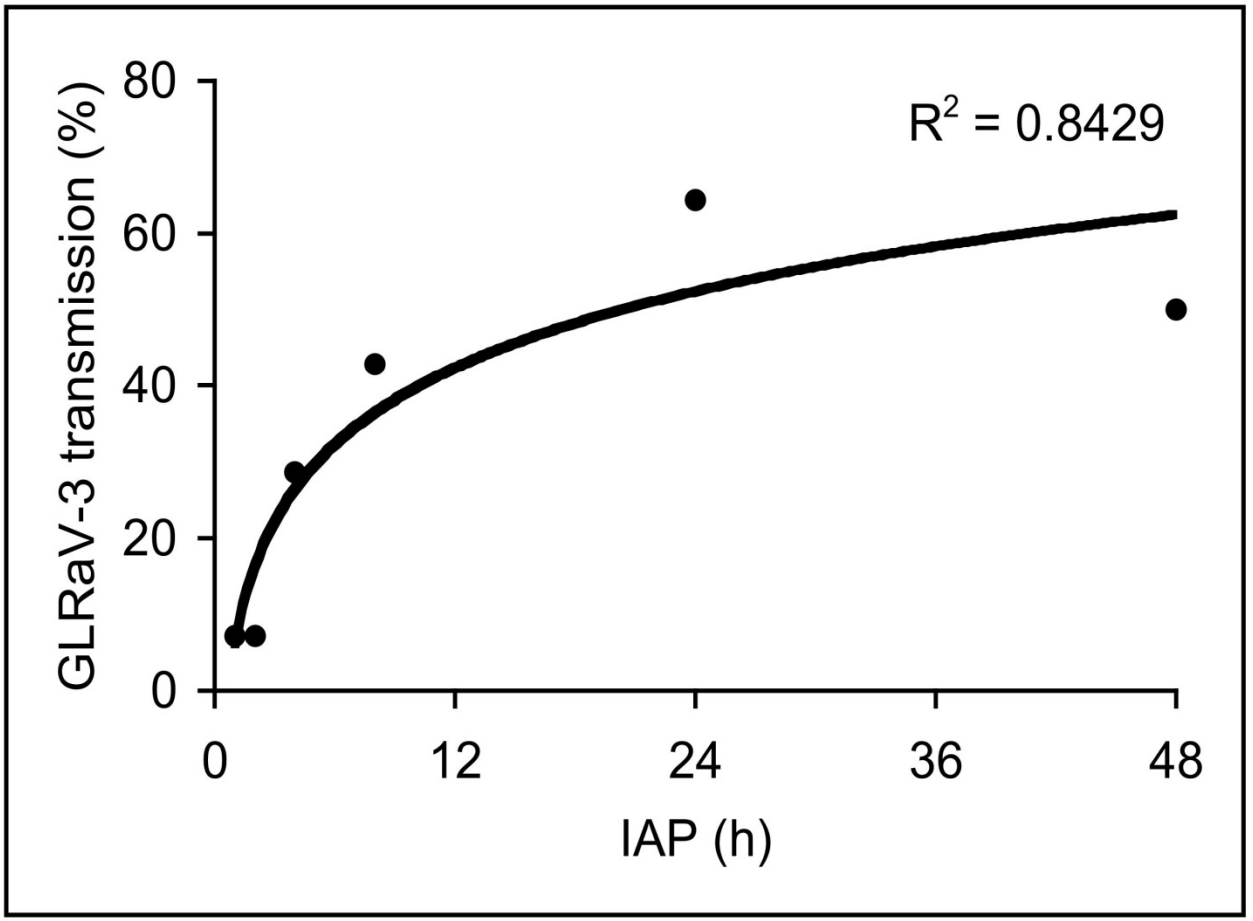
# Key Transmission Facts – Acquisition

- Crawlers **acquired** virus w/in 1 hr
- Peak at 24 hr

Tsai, Almeida et al. *Phytopath.* (2008)

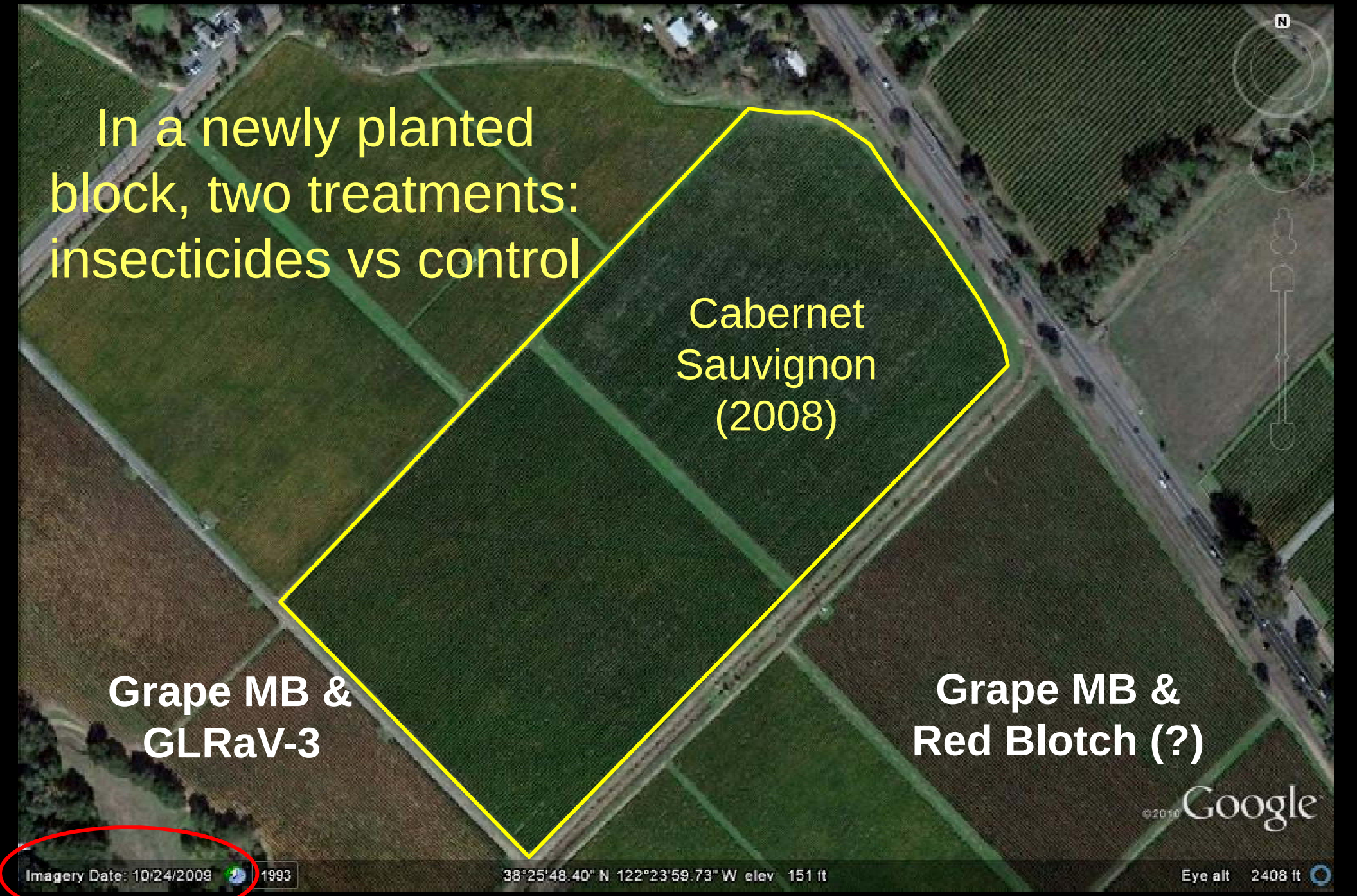
# Key Transmission Facts – Inoculation

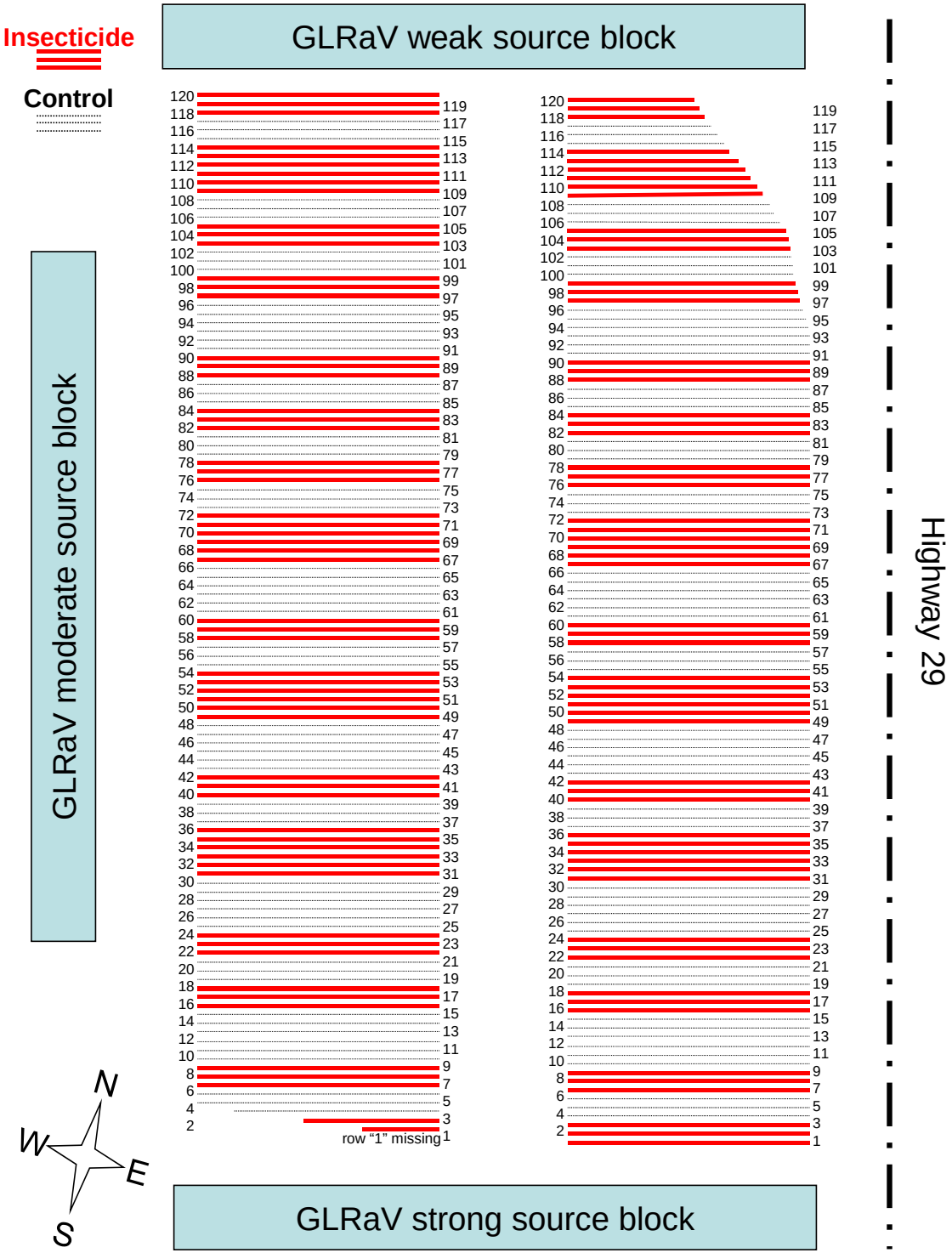
- Crawlers **inoculated** virus w/in 1 hr
- Peak at 24 hr




Tsai, Almeida et al. *Phytopath.* (2008)

# Can we simply kill all mealybugs for GLRaV control?

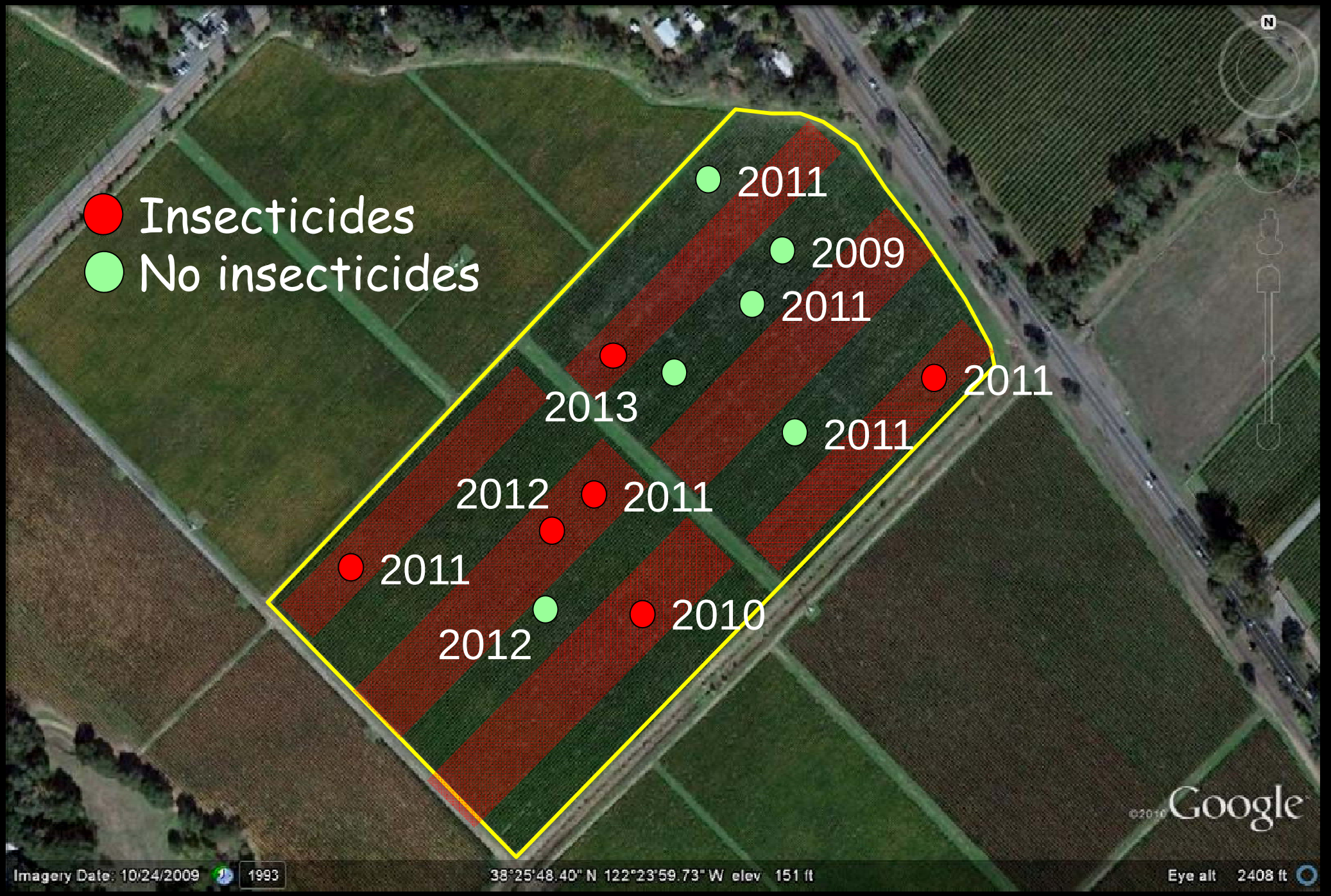





Highway 29

# Two annual applications of a combination of either Applaud, Admire, Clutch or Movento

# Where did the GLRaV-infected vines appear?



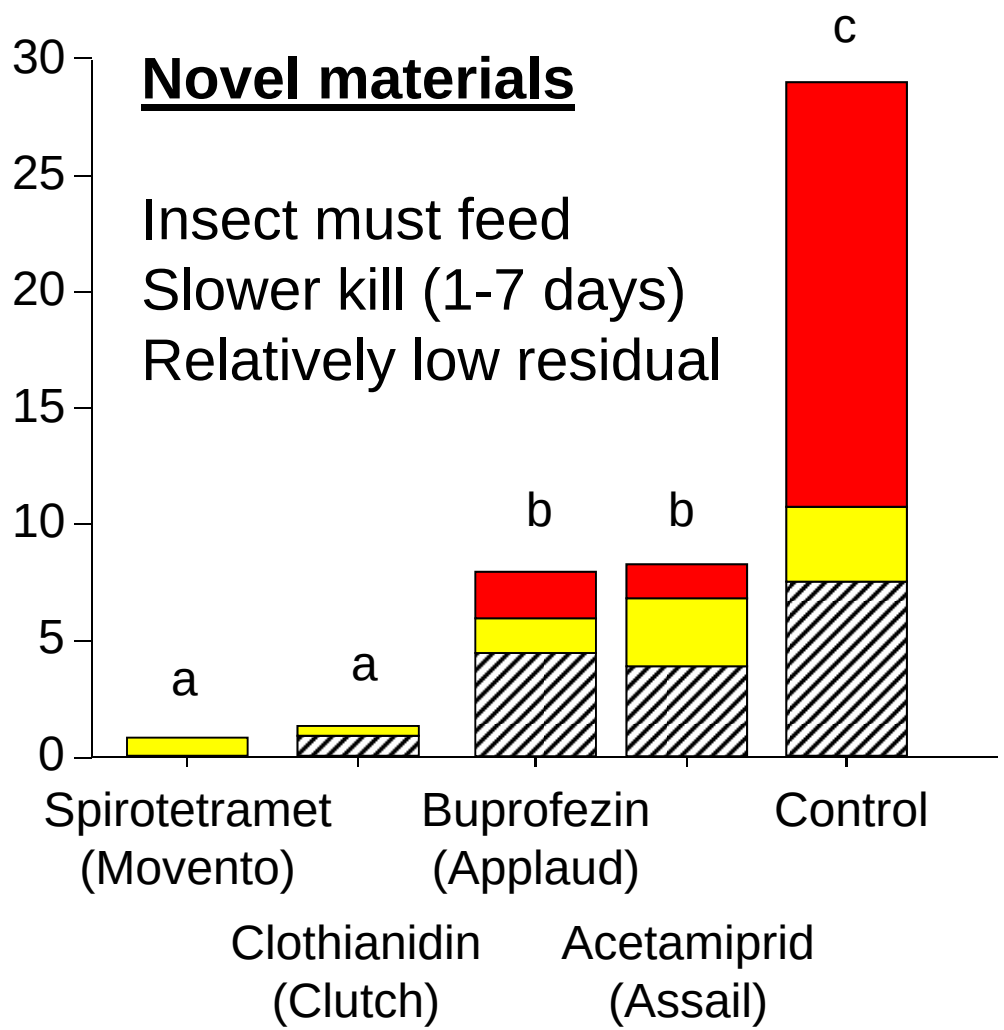
**Insect growth regulator**
Applaud (Buprofezin)

**Neonicotenoids**
Admire (Imidacloprid)
Clutch (Clothianidin)
Assail (Acetamiprid)

**Biosynthesis inhibitor**
Movento (Spirotetramet)

**OPs and Carbamates**
Lorsban (Chlorpyrifos)*
Lannate (Methomyl)*
Dimethoate*

*Listing here materials still effective and still registered (leafhoppers)




Spray Volume: 100 GPA; Air-blast Sprayer; label rate (Applaud 12 oz per ac)
Clutch & Movento on 21 June 2011, Applaud & Assail on 7 July 2011
*Planococcus ficus*, Lodi-Woodbridge wine grapes, Lodi, CA

# Vectors of Viral Pathogens in Vineyards

1. GLRaV Factors that Impact Control
   a) mealybug and leafroll species
   b) facts about mealybug-leafroll epidemiology

2. GLRaV Control Programs
   a) mealybug insecticides: which are best
   b) vineyard/areawide resistance management

3. What do We Know about Red Blotch
   a) grape damage
   b) insects as vectors

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PLOS | ONE

# A Leafhopper-Transmissible DNA Virus with Novel Evolutionary Lineage in the Family *Geminiviridae* Implicated in Grapevine Redleaf Disease by Next-Generation Sequencing

**Sudarsana Poojari[1], Olufemi J. Alabi[1], Viacheslav Y. Fofanov[2], Rayapati A. Naidu[1]***

**1** Department of Plant Pathology, Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, Washington, United States of America, **2** Eureka Genomics, Sugar Land, Texas, United States of America

## Abstract

A graft-transmissible disease displaying red veins, red blotches and total reddening of leaves in red-berried wine grape (*Vitis vinifera* L.) cultivars was observed in commercial vineyards. Next-generation sequencing technology was used to identify etiological agent(s) associated with this emerging disease, designated as grapevine redleaf disease (GRD). High quality RNA extracted from leaves of grape cultivars Merlot and Cabernet Franc with and without GRD symptoms was used to prepare cDNA libraries. Assembly of highly informative sequence reads generated from Illumina sequencing of cDNA libraries, followed by bioinformatic analyses of sequence contigs resulted in specific identification of taxonomically disparate viruses and viroids in samples with and without GRD symptoms. A single-stranded DNA virus, tentatively named Grapevine redleaf-associated virus (GRLaV), and *Grapevine fanleaf virus* were detected only in grapevines showing GRD symptoms. In contrast, *Grapevine rupestris stem pitting-associated virus*, *Hop stunt viroid*, *Grapevine yellow speckle viroid 1*, *Citrus exocortis viroid* and *Citrus exocortis Yucatan viroid* were present in both symptomatic and non-symptomatic grapevines. GRLaV was transmitted by the Virginia creeper leafhopper (*Erythroneura ziczac* Walsh) from grapevine-to-grapevine under greenhouse conditions. Molecular and phylogenetic analyses indicated that GRLaV, almost identical to recently reported Grapevine Cabernet Franc-associated virus from New York and Grapevine red blotch-associated virus from California, represents an evolutionarily distinct lineage in the family *Geminiviridae* with genome characteristics distinct from other leafhopper-transmitted geminiviruses. GRD significantly reduced fruit yield and affected berry quality parameters demonstrating negative impacts of the disease. Higher quantities of carbohydrates were present in symptomatic leaves suggesting their possible role in the expression of redleaf symptoms.

**Citation:** Poojari S, Alabi OJ, Fofanov VY, Naidu RA (2013) A Leafhopper-Transmissible DNA Virus with Novel Evolutionary Lineage in the Family *Geminiviridae* Implicated in Grapevine Redleaf Disease by Next-Generation Sequencing. PLoS ONE 8(6): e64194. doi:10.1371/journal.pone.0064194

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**Competing Interests:** The authors have the following interests to report: Viacheslav Y. Fofanov is employed by Eureka Genomics. There are no patents, products in development or marketed products to declare. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

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## Introduction

Nearly seventy viruses and other infectious sub-cellular obligate parasites, collectively referred to as graft-transmissible agents (GTAs), have been documented in grapevines (*Vitis* spp.) [1], [2]. Among all diseases caused either directly or indirectly by these GTAs, grapevine leafroll disease is considered as the most economically important disease affecting plant vigor and longevity and causing significant losses in fruit yield and impacting berry quality attributes [3], [4], [5]. Other virus diseases, such as rugose wood complex, fanleaf infectious degeneration and fleck complex, represent a group of disorders distributed widely in several grape-growing countries around the world [1], [2]. Besides these 'traditional' virus diseases, which can cause significant problems to grape production, other diseases due to GTAs have limited geographic distribution causing relatively less economic damage to grape production.

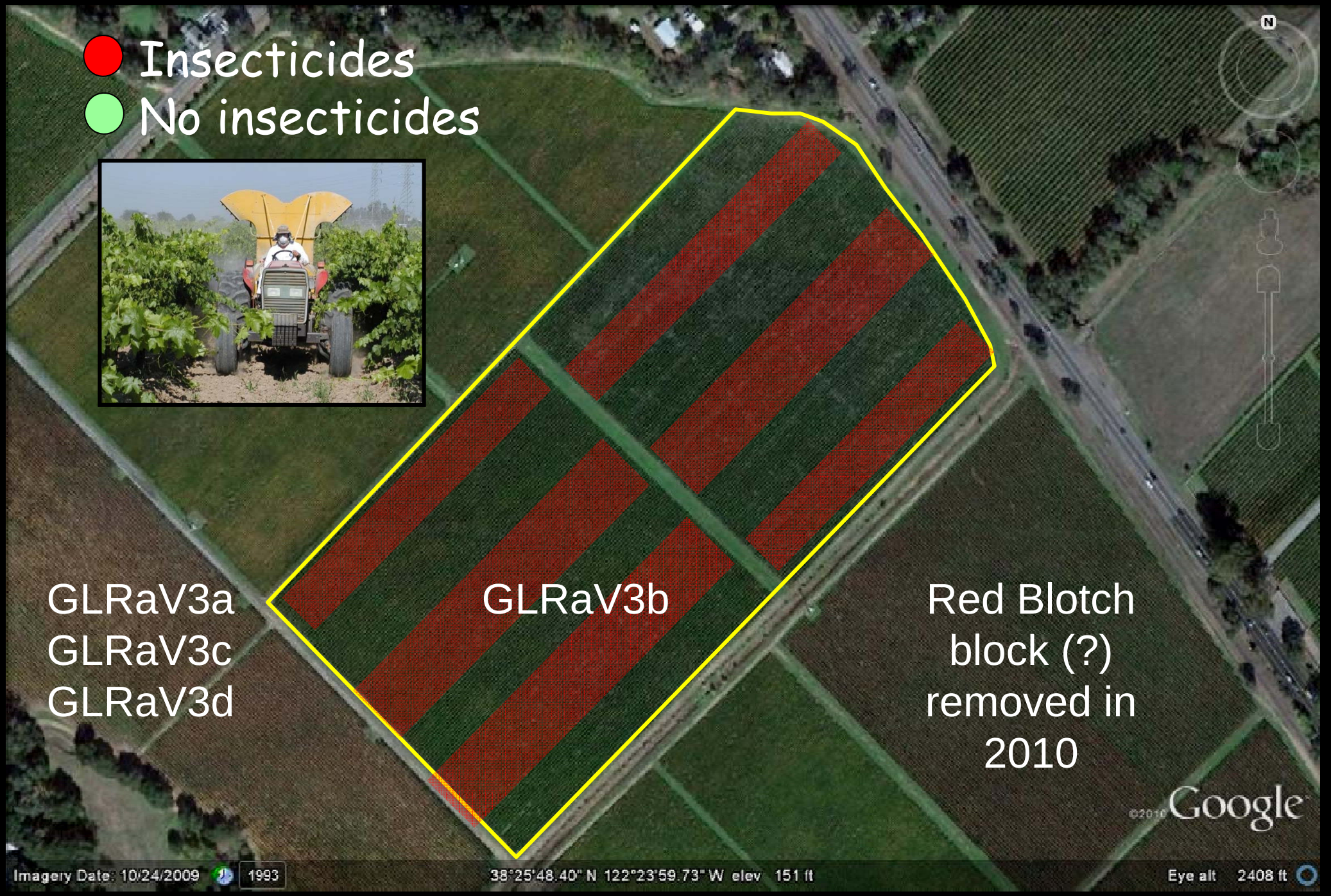
In addition to viruses, several viroids belonging to the family *Pospiviroidae* are ubiquitous in cultivated grapevines [6], [7], [8], [9]. They are *Hop stunt viroid* (HpSVd, genus *Hostuviroid*), *Grapevine yellow speckle viroid 1* (GYSVd-1, genus *Apscaviroid*) and 2 (GYSVd-2, genus *Apscaviroid*), *Citrus exocortis viroid* (CEVd, genus *Pospiviroid*) and *Australian grapevine viroid* (AGVd, genus *Apscaviroid*). Although these viroids are found in symptomless grapevines, GYSVd-1 has been implicated in vein-banding and yellow speckle symptoms, likely due to a synergistic interaction between GYSVd-1 and *Grapevine fanleaf virus* (GFLV, genus *Nepovirus*, family *Comoviridae*) [10], [11].

Besides their negative impacts on yield and quality of grapes, the introduction and subsequent spread of viruses and other GTAs to healthy vineyards is of great concern for sanitation and grapevine

# Insects scheduled for testing

| Common name |
| --- |
| Western grape leafhopper |
| Variegated leafhopper |
| Virginia creeper leafhopper |
| Potato leafhopper |
| Blue-green sharpshooter |
| Vine mealybug |
| Grape mealybug |
| Obscure mealybug |
| European fruit Lecanium scale |
| Grape phylloxera |
| Aphids |
| Grape whitefly |
| Mites |

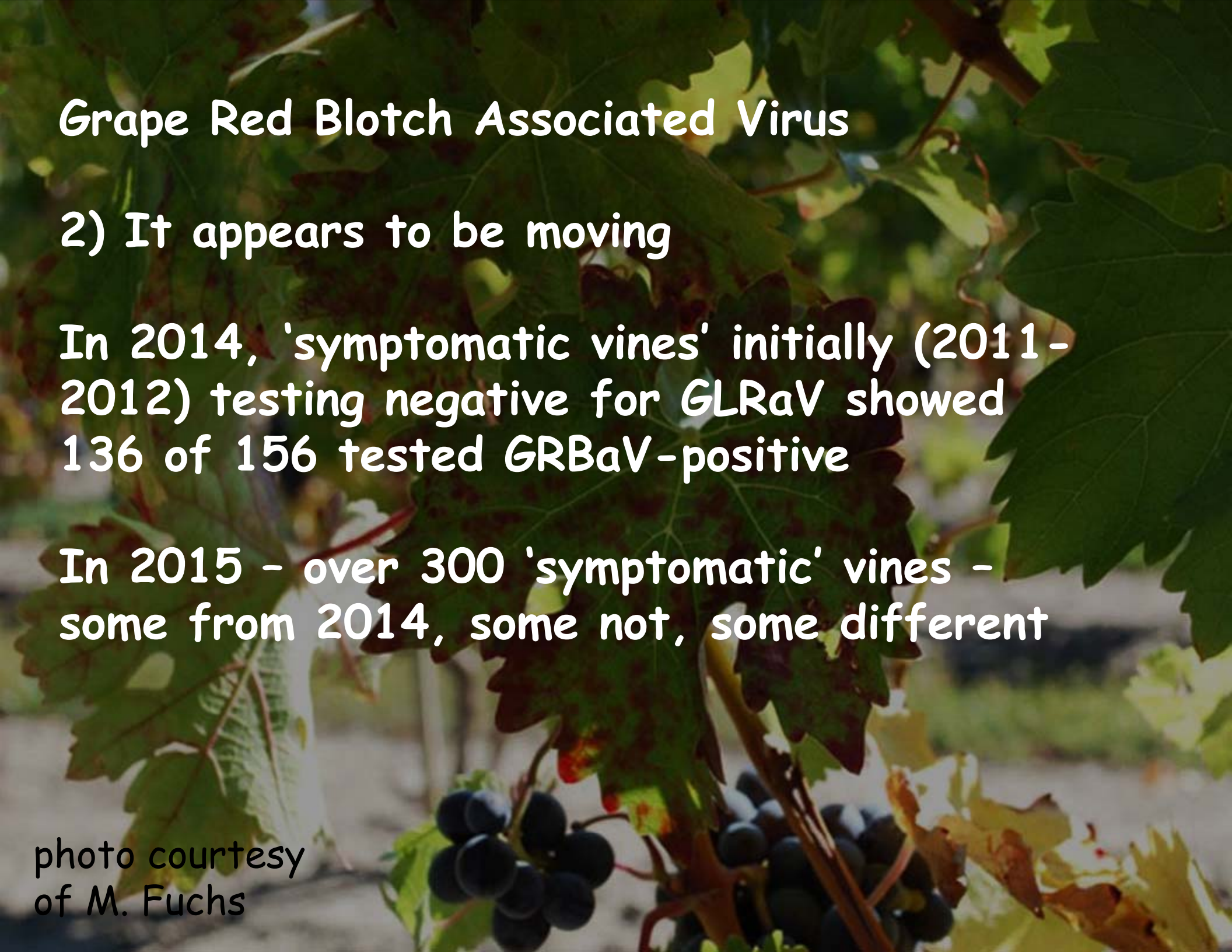
# Red blotch data from a GLRaV trial 2008-2014



# Grape Red Blotch Associated Virus

1) GRBaV has been present since first PCR tests of "false GLRaV" in 2011

photo courtesy of M. Fuchs



## Grape Red Blotch Associated Virus

2) It appears to be moving

In 2014, 'symptomatic vines' initially (2011-2012) testing negative for GLRaV showed 136 of 156 tested GRBaV-positive

In 2015 – over 300 'symptomatic' vines – some from 2014, some not, some different

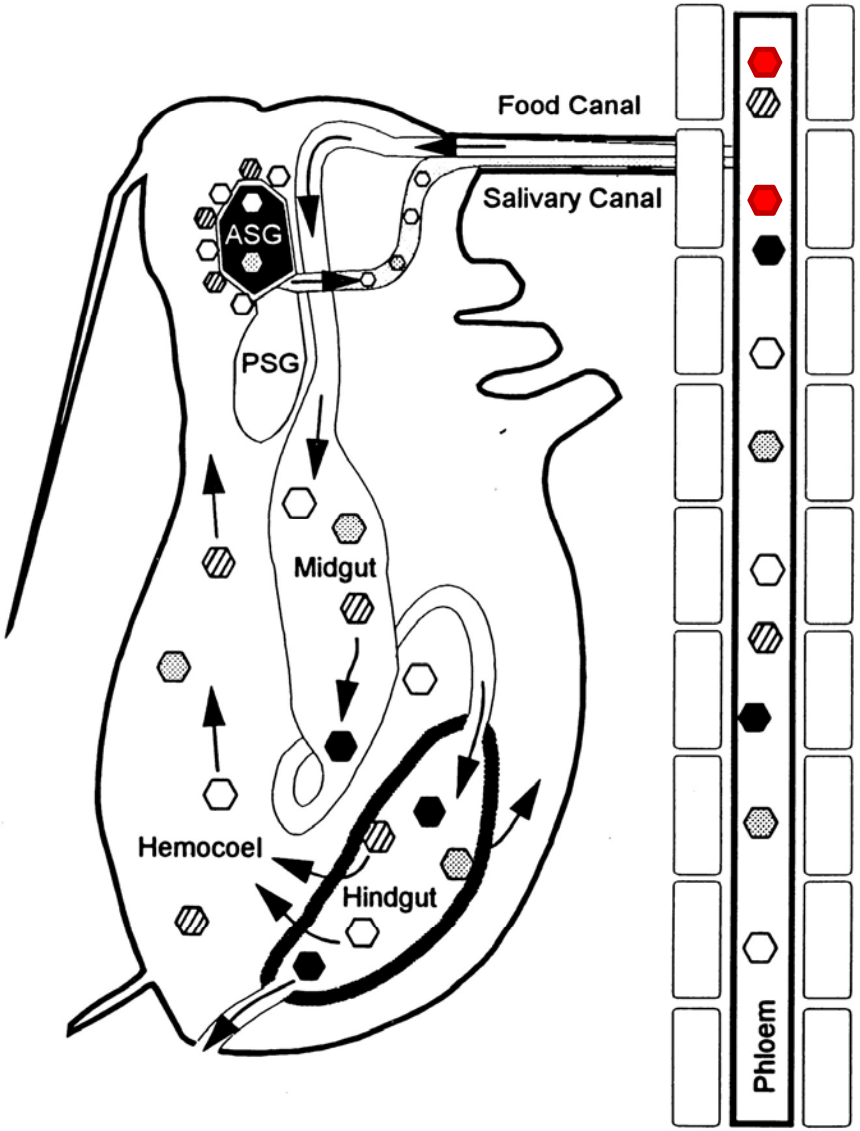
photo courtesy of M. Fuchs

## Grape Red Blotch Associated Virus

3) There was no insecticide impact and…
…there was a random dispersal of GRBaV infected vines – no pattern of insect spread

photo courtesy of M. Fuchs

# 'PCR-positive' does NOT confirm vector!




# Conclusions

1) Movento continues to be the best product for vineyard mealybugs. Application methods (e.g., timing) for optimal performance may vary and we are studying the movement of Movento to better understand this.

2) Biological controls help, but can be incomplete.

3) Mating disruption can help suppress the population , but may best be used in an areawide, annual program.

4) GLRaV movement can be suppressed using rouging and an areawide mealybug control program – but this can be expensive and does not guarantee no future losses

5) We have not shown an insect to be the Red Blotch vector