Clean Plants

for the future of the Eastern Wine and Grape Industry

Viral Diseases in the East: Leafroll, tomato ringspot and red blotch

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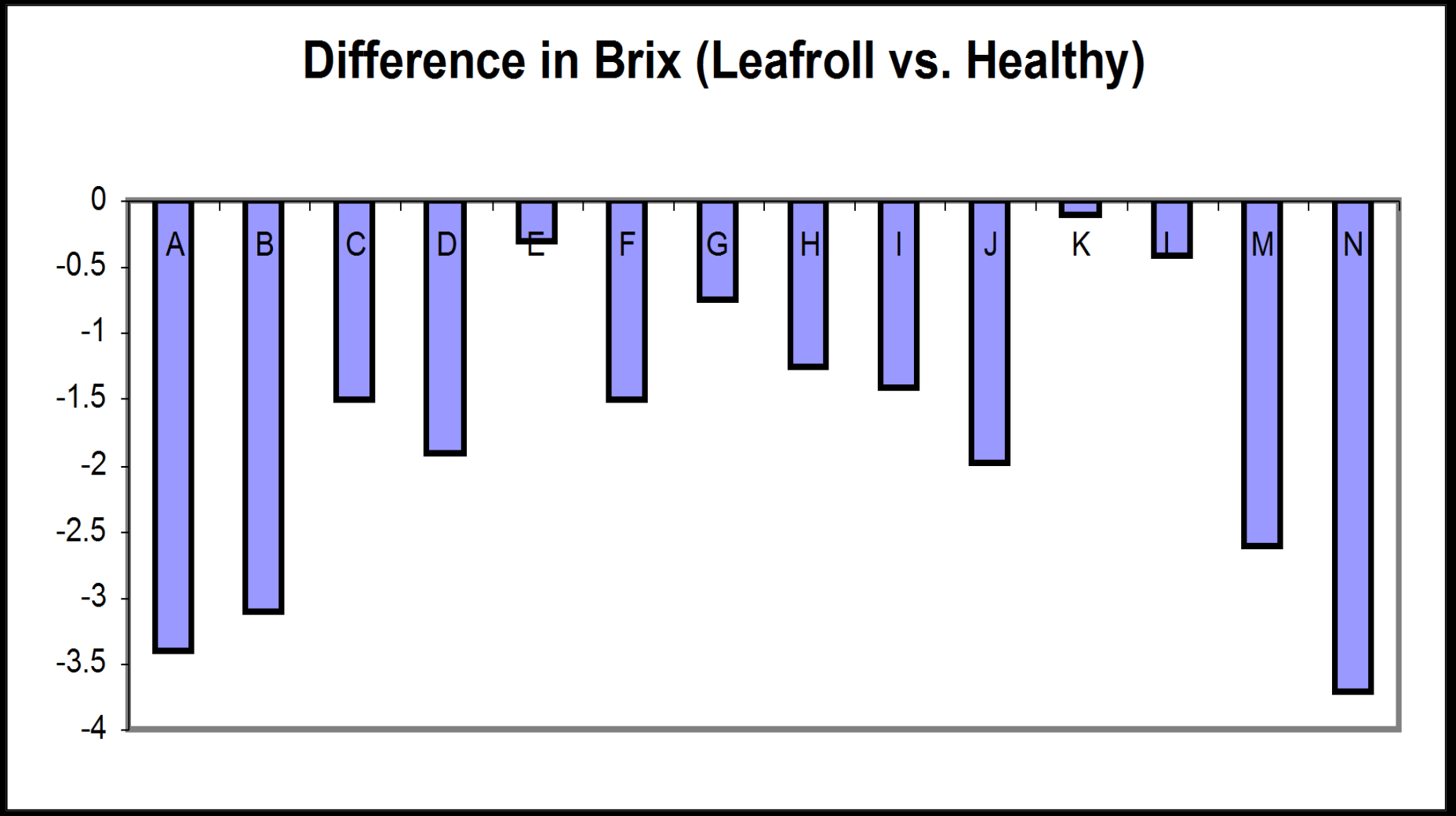
Leafroll Disease


Pinot noir

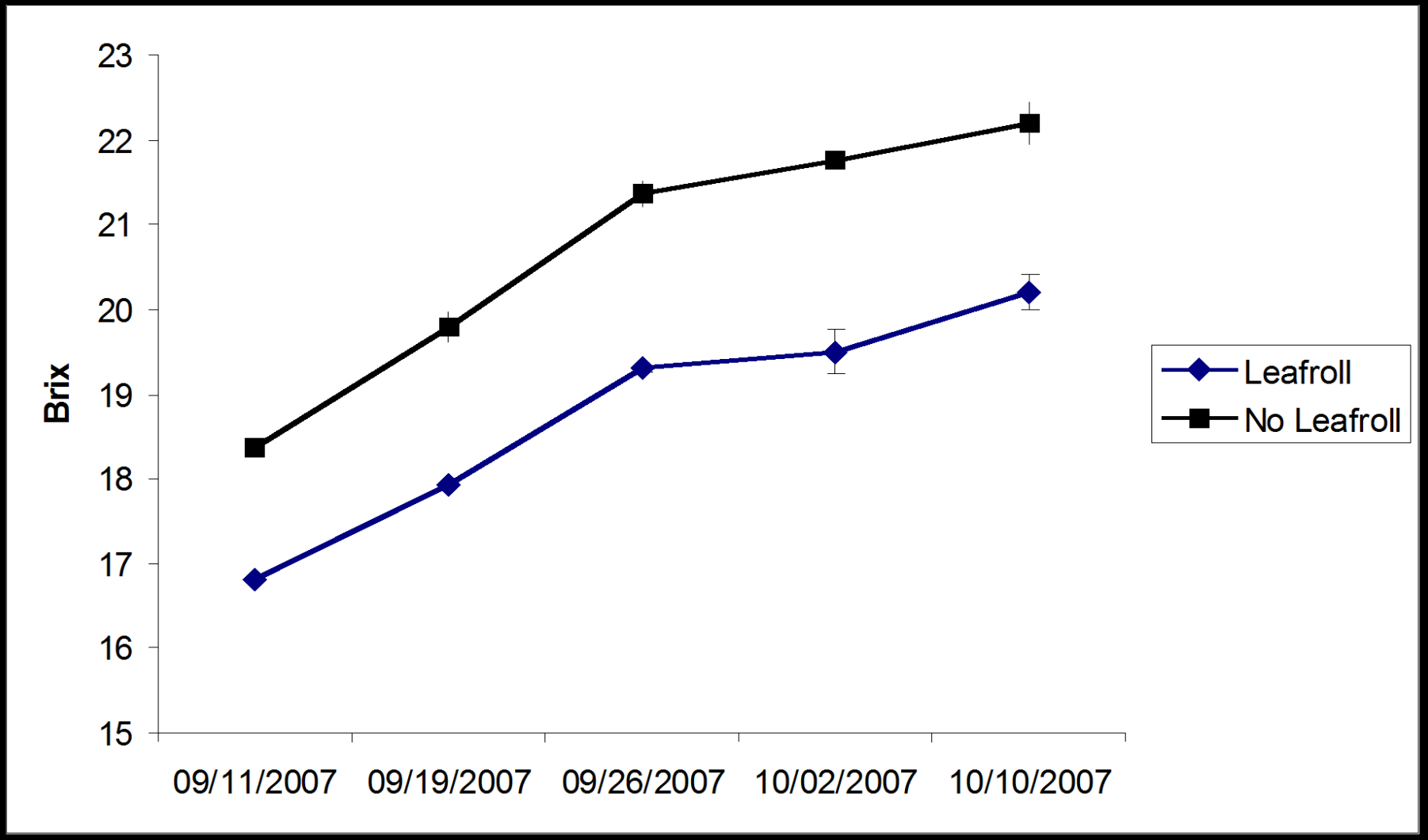

Chardonnay

Leafroll on Cabernet franc

Leafroll Impact on Fruit Juice Quality (Brix°)



Sugar Content prior to Harvest



Cabernet franc

Pinot noir

Leafroll

Healthy

Dr. P. Gugerli, RAC, Changins, Switzerland

Pinot noir

Leafroll

Healthy

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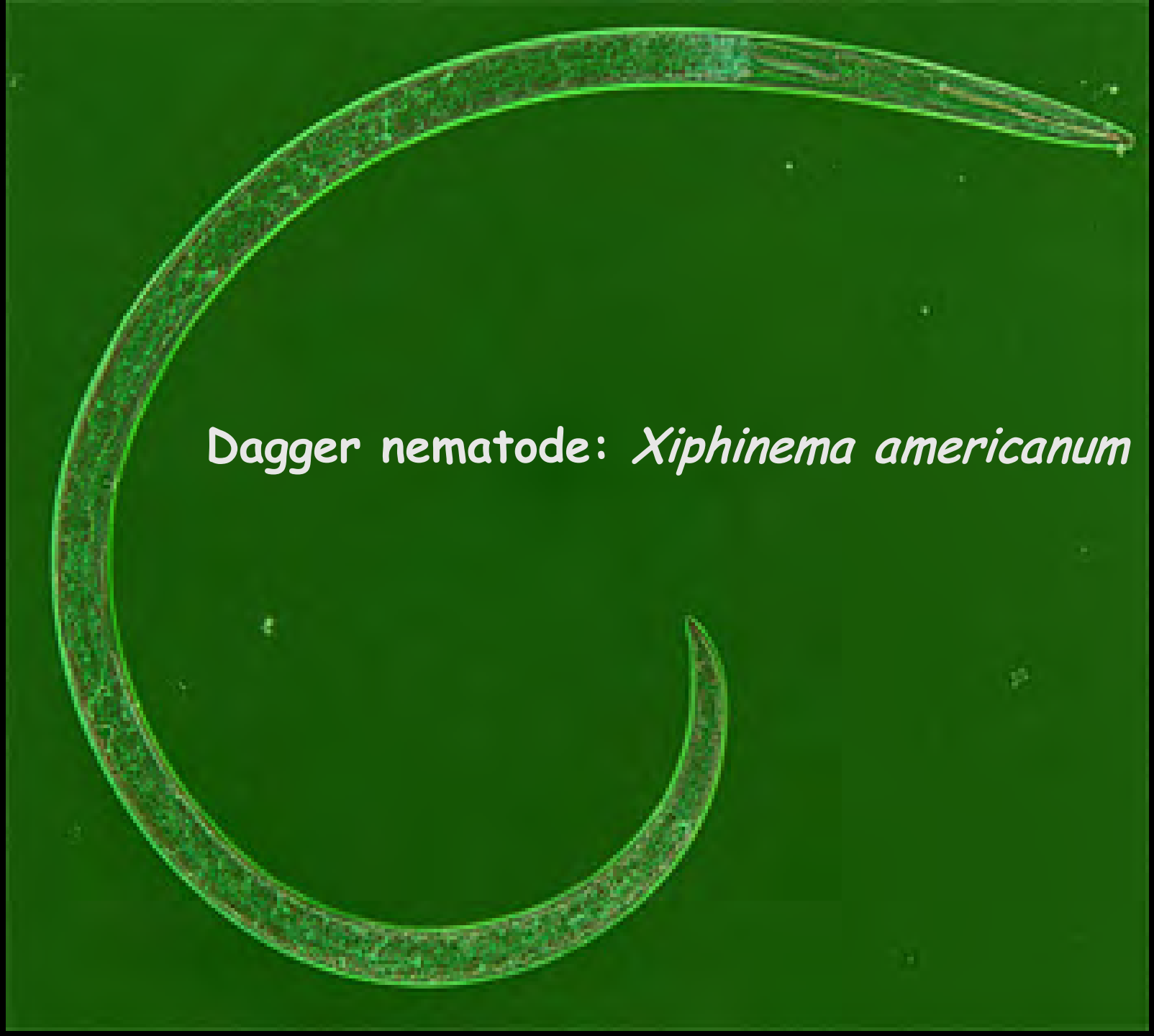
Leafroll: Insect Vector Species

- Brown scale (*Parthenolecanium corni*): GLRaV-1
- Cottony maple scale (*Neopulvinaria innumerabilis*): GLRaV-1 and GLRaV-3

Grape mealybug (*Pseudococcus maritimus*): GLRaV-3

Tomato ringspot virus


Dagger nematode: *Xiphinema americanum*

Tomato ringspot virus on Vidal

Tomato ringspot virus on Chelois

Tomato ringspot virus on Vidal

Red Blotch Disease

Cabernet franc

Pinot noir


Chardonnay

Red blotch on Pinot noir

Three cornered alfalfa treehopper

Grapevine Virus Diseases: Summary

Leafroll	Mealybugs/Soft Scales
Grapevine leafroll-associated virus 1 (GLRaV-1)	~10 different species
Grapevine leafroll-associated virus 2 (GLRaV-2)	none
Grapevine leafroll-associated virus 3 (GLRaV-3)	~20 different species
Grapevine leafroll-associated virus 4 (GLRaV-4)	~3 different species
Grapevine leafroll-associated virus 7 (GLRaV-7)	none

Ringspot	Dagger nematode
Tomato ringspot virus	~3 different species

Red Blotch	Alfalfa Treehopper
Grapevine red blotch-associated virus (GRBaV)	

Conclusions

- Viruses can have severe effects on vigor, yield, fruit quality, and productive lifespan of vineyards

Economic Analyses

Leafroll:

$9,384 per acre (30% yield reduction, no quality penalty) to $16,013 per acre (50% yield reduction and 10% penalty for poor quality)

Red Blotch:

$5,468 per acre (25% quality penalty) to $39,140 per acre (60% penalty for poor quality)

Atallah et al. (2012) AJEV 63:73-79

Ricketts et al. (2016) AJEV, submitted

Conclusions

- Viruses can have severe effects on vigor, yield, fruit quality, and productive lifespan of vineyards
- No mechanical transmission in vineyards
- Symptoms are not always reliable for diagnosis
- No cure in infected vineyards; chemical control of virus vectors is costly, often not efficient, and of environmental concern
- Certification programs limit the presence and dissemination of viruses in propagation material

Occurrence of Grapevine leafroll associated virus-2, -3 and Grapevine fleck virus in Virginia, and factors affecting virus infected vines

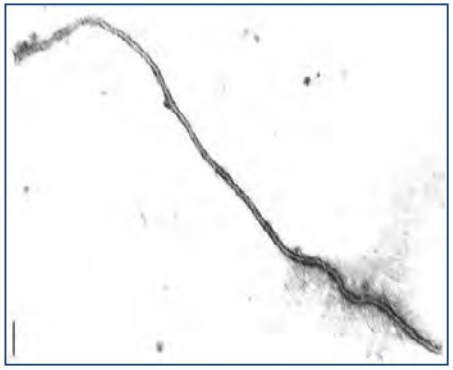
Mizuho Nita, Taylor Jones, (Virginia Tech, AHS AREC) and Naidu Rayapati (WSU)

VirginiaTech
Invent the Future®

Grapevine Leafroll-associate Viruses (GLRaVs)

Grapevines are hosts to over 60 different viruses

- Found in all major wine growing areas
  - 36+ countries
- Group of viruses
  - Grapevine Leafroll Associated Viruses, GLRaV-#
- Family: Closteroviridae, alpha-like
  - (+)ssRNA, non-enveloped, filamentous
  - 1400-2200nm in length, ~12nm width



Fuchs, M.F. (2007). Grape leafroll disease: Grapevine leafroll-associtaed viruses.

Transmission

- Grafting (but not natural field root grafting)
- Vectors
    - Unknown: GLRaV-2 (*Closterovirus)*
    - Mealybugs and scale insects: GLRaV-1 and 3

Mealybugs

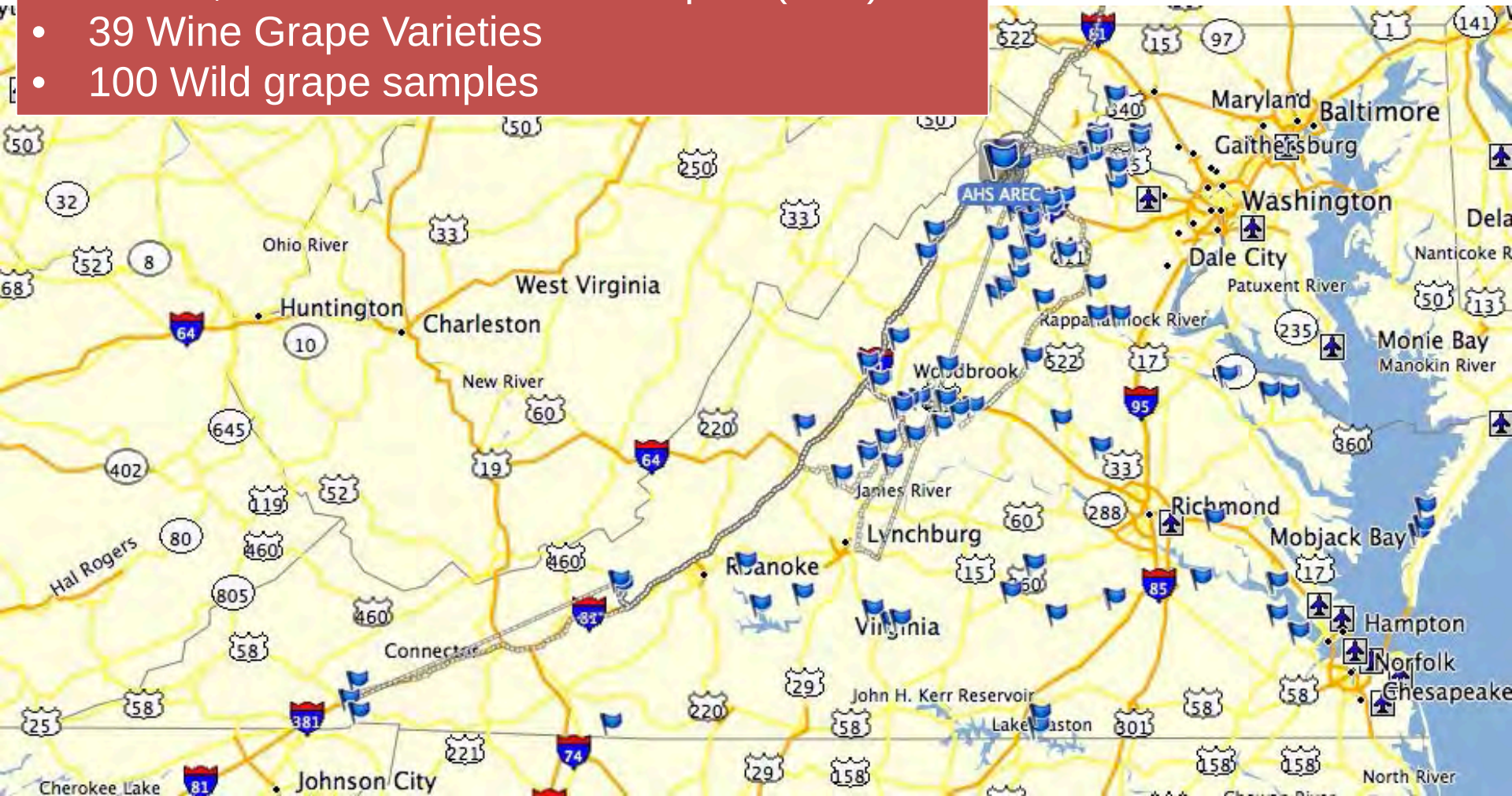
- Family: Pseudococcidae
- Semi-persistent transmitters (15min-12hr to acquire; 12hr-5days to transmit)
- ~2-3 or more generations/year (species dependent)
  - Grape mealybugs (*Pseudococcus maritimus)*
  - Gill's mealybugs (*Ferrisia gilli*)
- First instars can be brown by wind
- Can crawl too
- Males does not feed

Ants herding mealybugs

Honeydews from mealybugs can promote sooty mold development on clusters

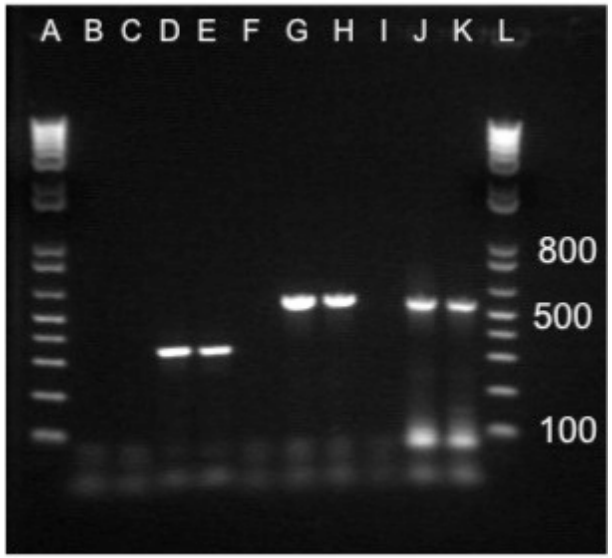
To determine the level of infestation in VA, a state-wide survey was conducted in 2009-2012

- 77 vineyards (415 sampling sites)
  - ~1,300 Cultivated Vine Samples (total)
- 39 Wine Grape Varieties
- 100 Wild grape samples



High level of infestation was found from the survey

- 8% infected with GLRaV-2
- 25% infected with GLRaV-3
- 1% infected with GfkV
- 64% of vineyards contained at least one infected vine sample
- **No wild grapevines tested positive**



Jones, Naidu, and Nita (2015) EJPP (DOI 10.1007/s10658-015-0605-z)

We have differences, but why is it?

- At the time of sampling, we have obtained as much information as possible
  - Variety, age, GPS location, mealybugs, etc.

Variety effect was not very clear
GLRaV-3 can be found from every variety…

Variety	Number GLRaV-2 (+) Vines	Number GLRaV-3 (+) Vines	Number GFkV (+) Vines	Mixed Infection Cases	Total number of samples	Percent of vines positive for at least one virus
Cabernet Sauvignon[X+]	13	15	0	4	81	31
Cabernet franc[X+]	2	6	0	0	54	15
Chardonnay[X*]	4	16	1	5	36	47
Petit Verdot[X+]	0	7	0	0	36	19
Merlot[X+]	0	7	0	0	33	21
Viognier[X*]	0	8	0	0	27	30
Traminette[Y+]	1	1	0	1	15	7
Petit Manseng[X*]	0	1	0	0	14	7
Chambourcin[Y+]	1	2	0	1	12	17
Malbec[X+]	0	2	0	0	12	17
Norton[Y+]	0	6	0	0	11	55
Vidal blanc[Y*]	7	5	1	4	11	82
Syrah[X+]	0	4	0	0	8	50
Riesling[X*]	1	3	0	1	6	50
Chardonel[Y*]	0	0	0	0	5	0
Pinot gris[X*]	0	2	1	1	5	40
Seyval blanc[Y*]	1	2	0	0	5	60

The age of vine has some effects

Age group	Vineyards (n)	GLRaV-2 LSMean[z]		GLRaV-3 LSMean[z]	
Pre-1990	49	18.4%	A	71.4%	A
1990's	88	9.1%	B	38.6%	B
2000's	278	5.0%	B	12.2%	C

- Recent planting are most likely using certified vines
  - Through the efforts of National Clean Plant Network and Foundation Planting Service
  - Grapevine certification itself has been getting better over the years
- Longer the years, the higher chance of being spread by vectors (GLRaV-3)

There are some evidence of spread of the virus within a field by mealybugs

Condition	Virus	χ^2	*P*-value
Symptoms	GLRaV-2	0.99	0.32
	GLRaV-3	0.03	0.85
Mealybugs	GLRaV-2	0.23	0.63
	GLRaV-3	16.2	< 0.0001

- Symptoms do not tell much about infection
  - Logistic regression results showed that probability of isolating GLRaV-3 from vine were not affected by visual symptoms
  - Others had symptoms that are most likely caused by other diseases (Red Bloch virus) or disorders (Nutrient deficiency)
- Higher prob. of finding GLRaV-3, with a vineyard with mealybug, but not for GLRaV-2 which is not vectored by mealybugs.

Jones and Nita (2016) EJPP

When the effect of spatial scale was evaluated, no regional effect was found, and the model fits better (explain the source of variation) as we go down in the spatial scale

Vineyard < Cultivar < Site

Virus	R^a	V^b	C^b	S^b
GLRaV-2	0.59	89.9* (0.02)	63.0* (0.01)	58.3* (0.04)
GLRaV-3	0.89	485.8* (0.06)	397.7* (0.08)	349.9* (0.06)

- The numbers in the table is difference in log-likelihood values between models = larger number means the model with another factor
- The numbers in parentheses are variance

- Better fit of the model with lower spatial scale suggests the source of variation probably resides at the sampling site

  → transmission by mealybugs

At newer vineyards, there were tendencies of aggregation of GLRaV-3 infected vines

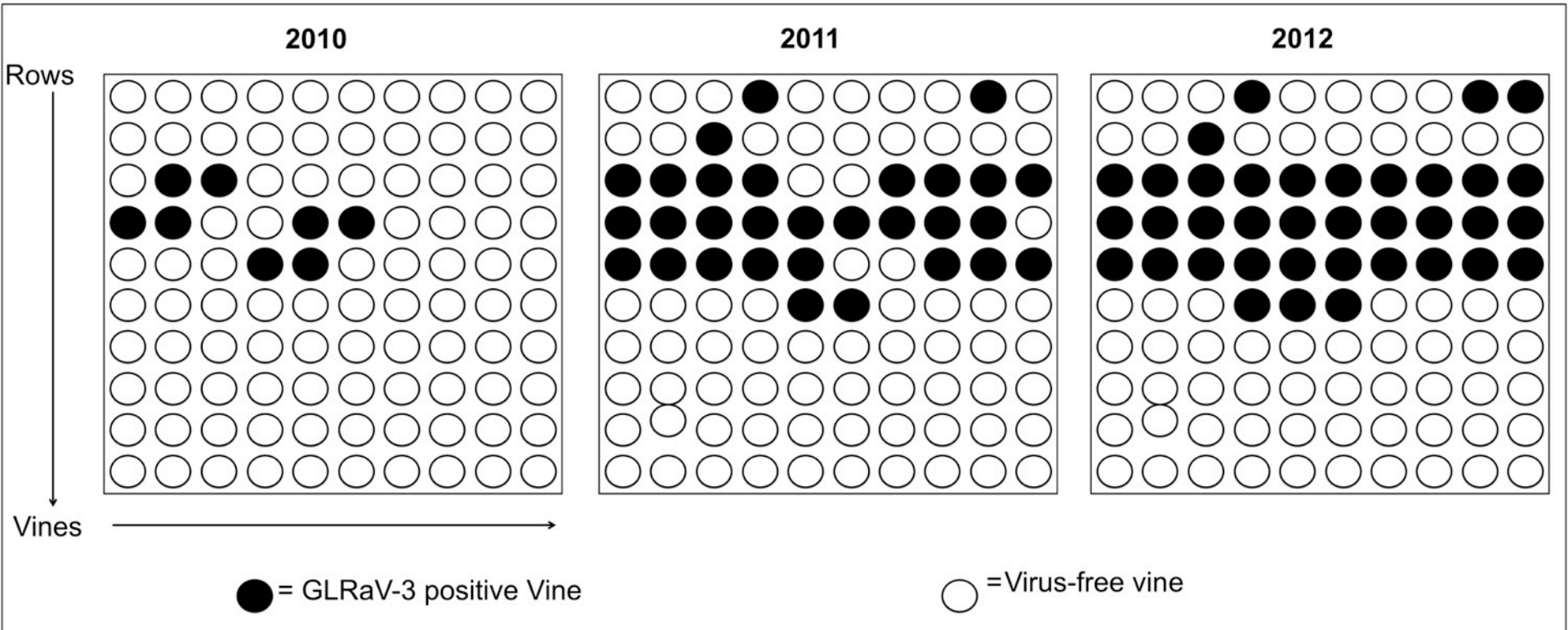
- Another evidence of vector-based transmission of GLRaV-3
- As oppose to more uniform or random distribution which may be due to introduction of GLRaV-3 through compromised nursery stocks.

Location	Year	Total DI[z]	I_z[y]
AHS-AREC	2010	0.08	1.517*
AHS-AREC	2011	0.3	1.896*
AHS-AREC	2012	0.37	2.111*
Commercial Vineyard A	2009	1	1.369
Commercial Vineyard B	2009	0.3	2.419*
Commercial Vineyard C	2009	0.99	1.328
Commercial Vineyard D	2009	0.52	1.058
Commercial Vineyard E	2009	0.64	0.943

I_z > 1.5 = Significant levels of aggregation
A and C are Old (20 yr+) vineyards with whole vineyard infestations
AHS and E = new vineyard planted in 2009 and 2007, respectively

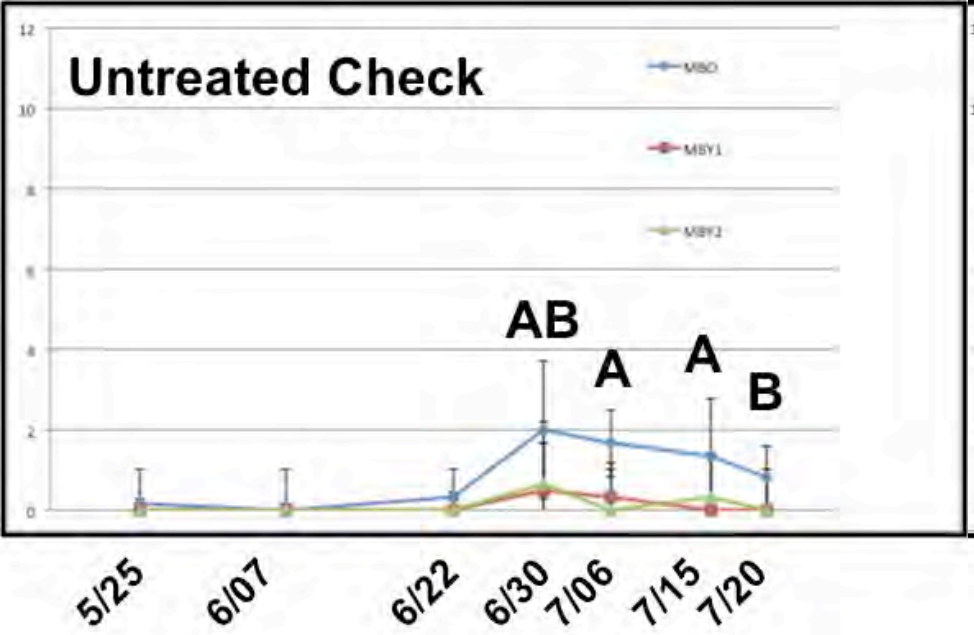
Spatio-temporal association of GLRaV-3 infected vines was found at a young planting site

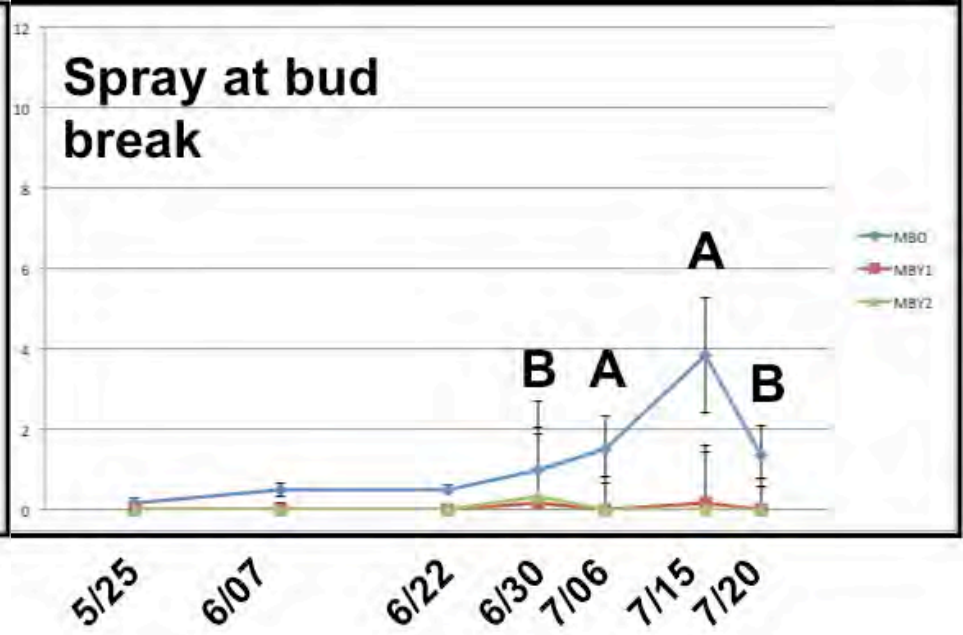
- For the 2010-2011 season, SADIE's overall index of association (χ_i) between two years was 0.7283 ($P < 0.0001$) in 2010-2011 and 0.9176 ($P < 0.0001$) in 2011-2012
- Strong spatio-temporal association, as expected

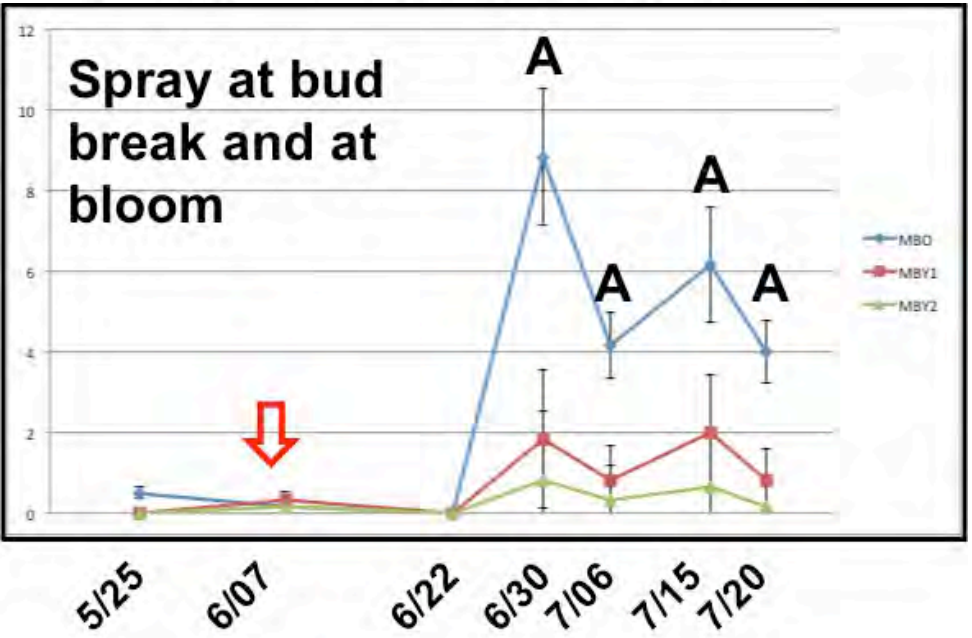


We conducted two types of field trials to examine the efficacy of current insecticide options in the Eastern US

- First trial was prevention trial
- Inter-planted vines (new Cab. franc vines planted at 5 and 10 ft from a 20 yr., infected Cab. Sauv)
  - Assail (acetamiprid) at pre-bud break (2.5 oz/A)
  - Assail (acetamiprid) at pre-bud break (2.5 oz/A) and Baythroid XL (pyrethroid) at bloom (3 oz/A)
  - Untreated Check



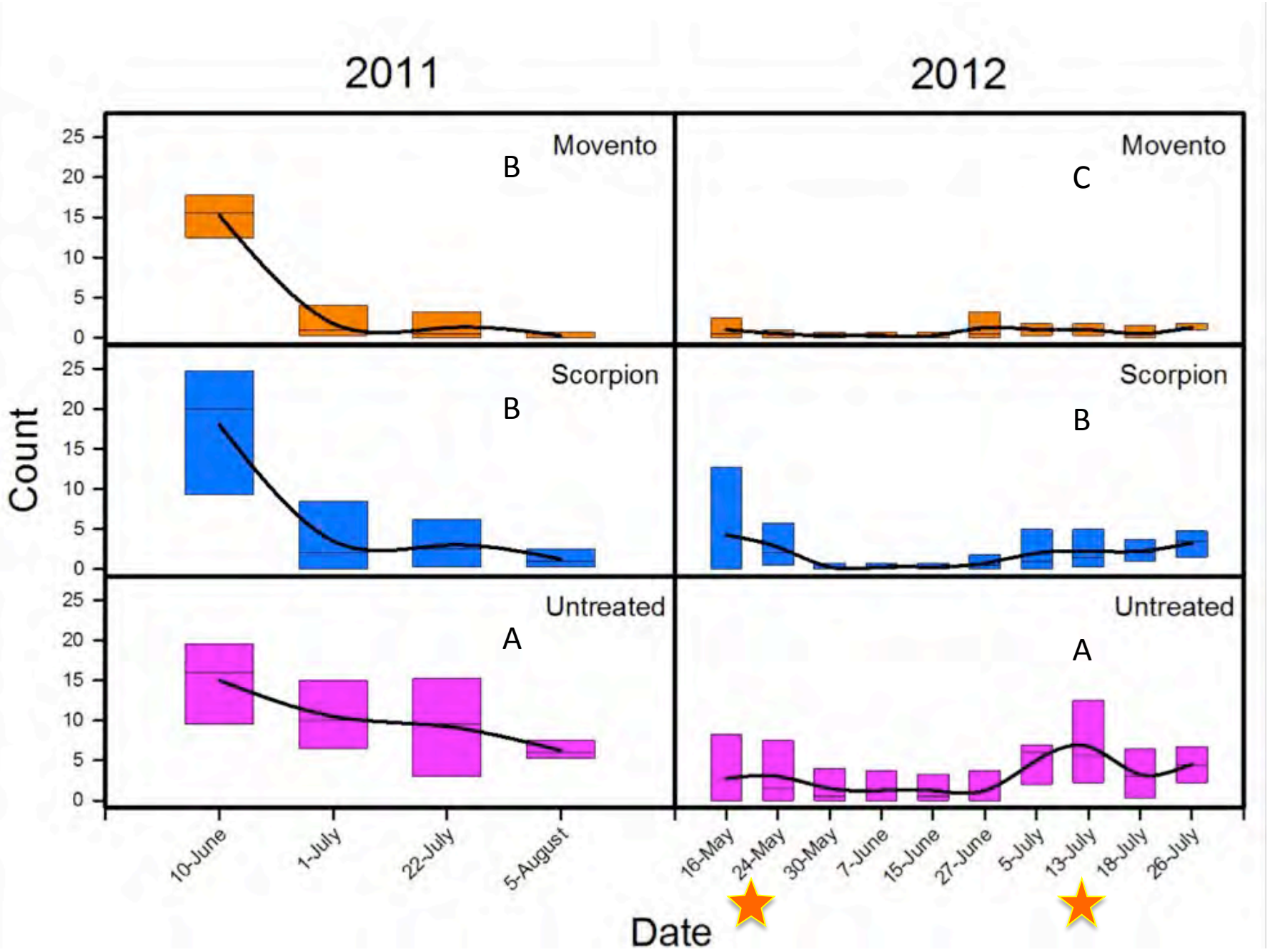




- 2009: 1 new vine positive for GLRaV-3
- 2010: Mealybugs found moving to young vines
- 2011: Significant difference between age of vine and treatment. Significantly higher number of mealybugs on **twice sprayed vines**

Field Experiment 2 (rescue operation)

- Single row of Chardonnay at commercial vineyard
- Three completely randomized treatments (2011-2012)
  - Movento (spirotetramet), 6 oz/A
  - Scorpion (dinotefuran), 4 oz/A
  - Untreated Check


2011
2012
Movento
B
Movento
C
Scorpion
B
Scorpion
B
Untreated
A
Untreated
A
Count
25
20
15
10
5
0
10-June
1-July
22-July
5-August
16-May
24-May
30-May
7-June
15-June
27-June
5-July
13-July
18-July
26-July
Date

Summary of Insecticide Trials

- Baythroid XL (= broad spectrum contact insecticide) treatment probably decreased beneficial insect populations, allowing mealybug populations to rebound
- Movento and Scorpion were effective
  - Movento might have some residual effects on the following years population levels
    - First counts of the season (before application of treatment) showed lower number of MB with Movento than Scorpion
- Mealybug population numbers changed over time and through years
  - Mid-June spike (~1 month post-bloom)
  - Late July decline

Take Home Messages

- GLRaV-2, GLRaV-3, and GfkV are all present in VA
  - High level of infestation by GLRaV-3
- Clean plant materials seem to be the key to the management
- The vectors (at least for GLRaV-3) are common in VA
  - There are evidences of movement in vineyards
    - We recently completed an infection assay with Gill's mealybugs
  - GLRaV-3 can spread rapidly throughout a field, and previously infected vines become most likely a source of next round of infections
- We also conducted insecticide trials and found out some of systemic materials (Movento, Scorpion, and Lorsban) provided a good control, yet, we were not able to completely stop the movement of GLRaV-3

Virus testing beyond GLRaV-2 and -3

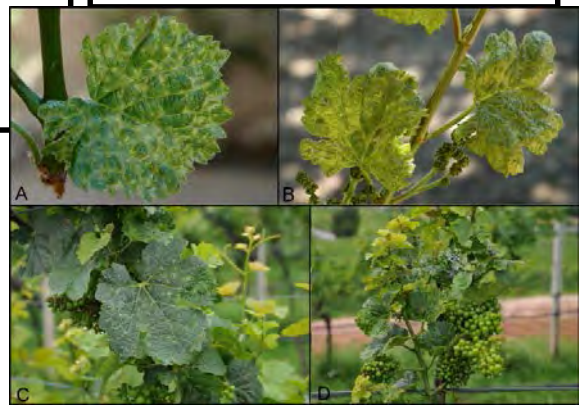
- Testing random samples via RT-PCR and PCR, verified by sequencing for:
  - GLRaV-1, -2, -3, and -4
  - Rugose wood complex
    - Rupestris stem pitting associated virus-1, Grapevine virus A and B
  - Grapevine fleck virus
  - Newly found Viruses

Grapevine vein clearing virus
-Found first in Missouri
-Mealybugs? Mites?
-DNA virus!

Grapevine red blotch
-Very similar to leafroll
-Virginia creeper leafhopper
-DNA virus!

Grapevine Pinot gris virus
-Newly discovered
-Characterized by chlorotic mottling and leaf deformations
-Similar to frost

Tomato Ringspot Virus
-Could be common in VA, we know it is common in apples and dandelions
-Dagger Nematode



Current results of virus survey out of 722 grapevines

Virus	Number of Positive Vines	% Positive	Number of those that are involved in mixed infections
GLRaV-1	15	2.07%*	5
GLRaV-2	64	8.86%*	36
GLRaV-3	**166**	**22.99%***	**79**
GLRaV-4	6	0.83%*	6
GLRaV-4s5	3	0.41%*	3
GLRaV-4s9	3	0.41%*	3
RSPaV-1	**372**	**51.52%***	**91**
GVA	29	4.01%*	25
GVB	13	1.80%*	11
GFkV	6	0.83%*	4

Current results of virus survey out of 574 grapevines

Virus	Number of Positive Vines	% Positive	Number of those that are involved in mixed infections
ToRSV	9	1.57%	7
GpgV	0	--	--
GVCV	0	--	--
GRBaV	**125**	**21.78%**	**78**

- ToRSV results surprising
- Red Blotch incidence almost as high as leafroll-3 (~22%)
  - 62.4% involved in mixed infections, primarily with Rupestris

Diagnosis and impact of grape viruses in Michigan

Annemiek Schilder

Dept. Plant, Soil and Microbial Sciences

Samples from 90 vineyards on 47 farms

Virus	# samples positive	
Grapevine leafroll assoc. virus 1	2	68%
Grapevine leafroll assoc. virus 2	18	
Grapevine leafroll assoc. virus 3	81	
Grapevine leafroll assoc. virus 4-9	11	
Tomato ringspot virus	5	18%
Tobacco ringspot virus	24	
Peach rosette mosaic virus	5	
Grapevine fleck virus	36	
Grapevine fanleaf virus	1?	

165/394 = 42%

Grapevine leaf roll

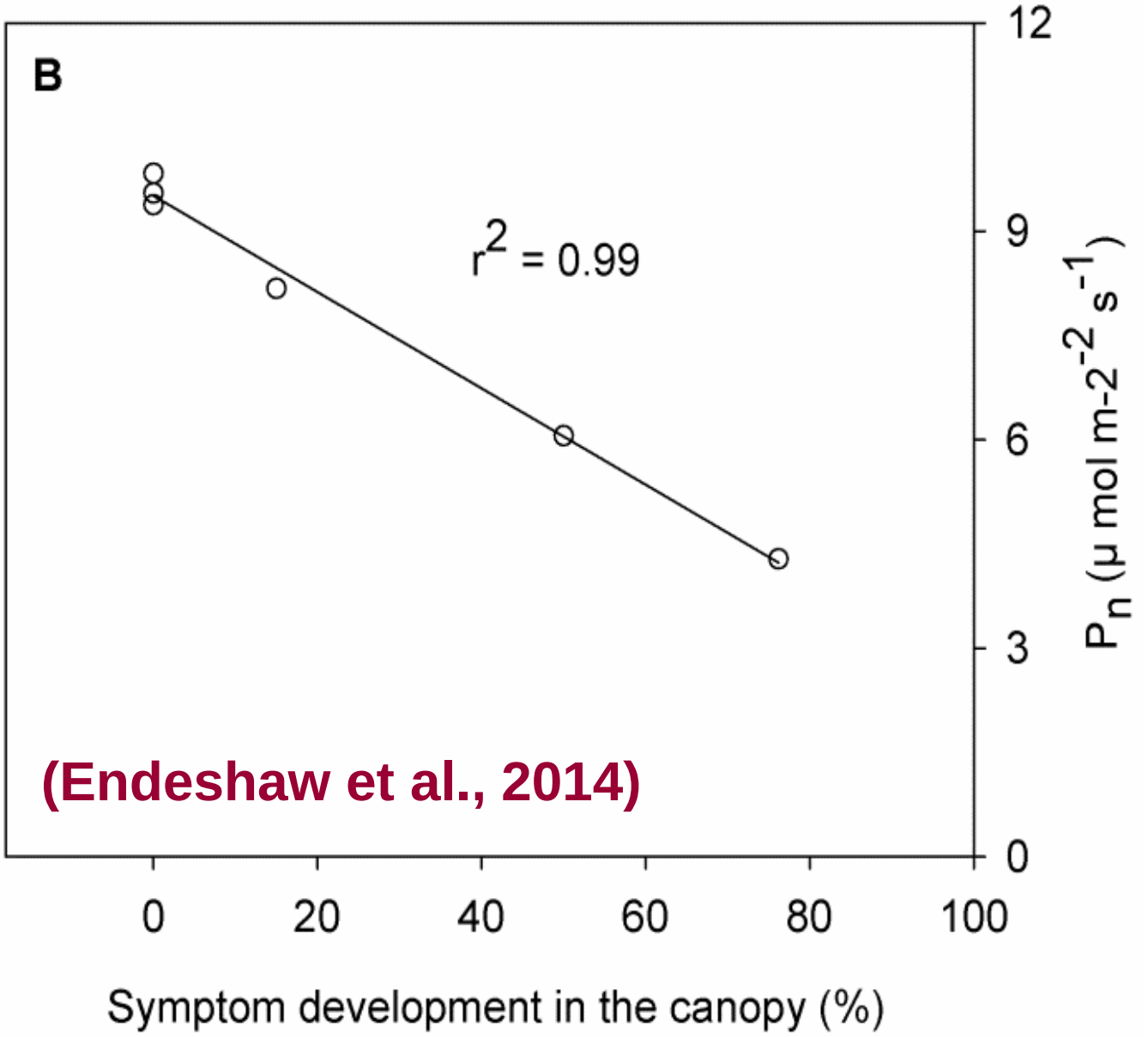
Ringspot decline

Effect of GLRaV-3 infection on fruit quality parameters in Cabernet Franc, 2012

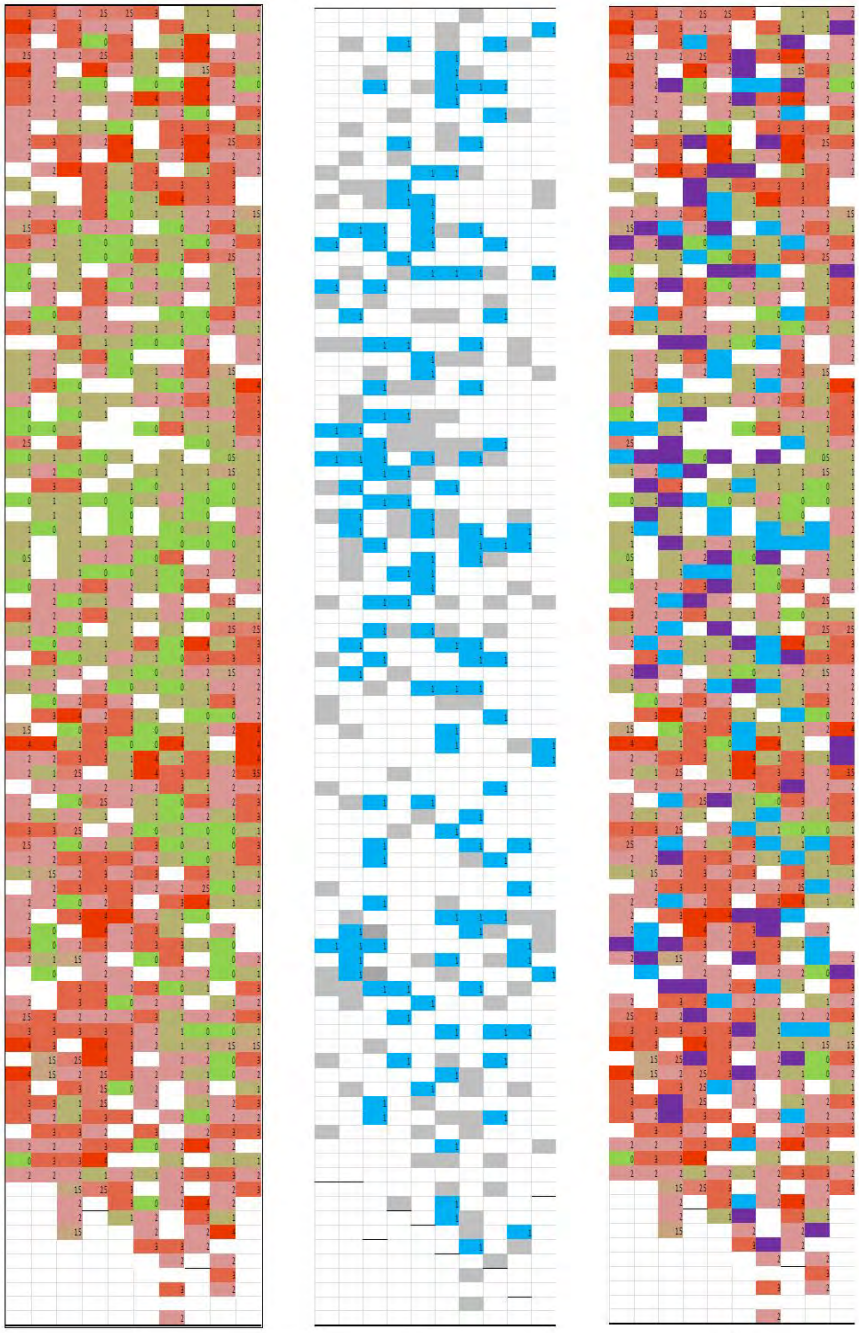
Parameter[x]	**HG**	**SG**
Brix (°Bx)	22.4 a	20.7 b
pH	3.47 a	3.43 a
Titratable acidity (g l^{-1})	6.1 a	7.3 a
Sugar per vine (g l^{-1} $vine^{-1}$)	1657 a	925 b
Anthocyanin (mg g^{-1})	0.76 a	0.75 a
Phenolic (a.u g^{-1})	1.11 a	1.10 a

Endeshaw et al., 2014, Scientia Horticulturae

Net photosynthesis in relation to symptoms



(Endeshaw et al., 2014)



1

0 No leafroll symptoms
1 Mild leafroll symptoms
2 Moderate leafroll symptoms
3 Severe leafroll symptoms
4 Very severe leafroll symptoms
Dead or missing vine

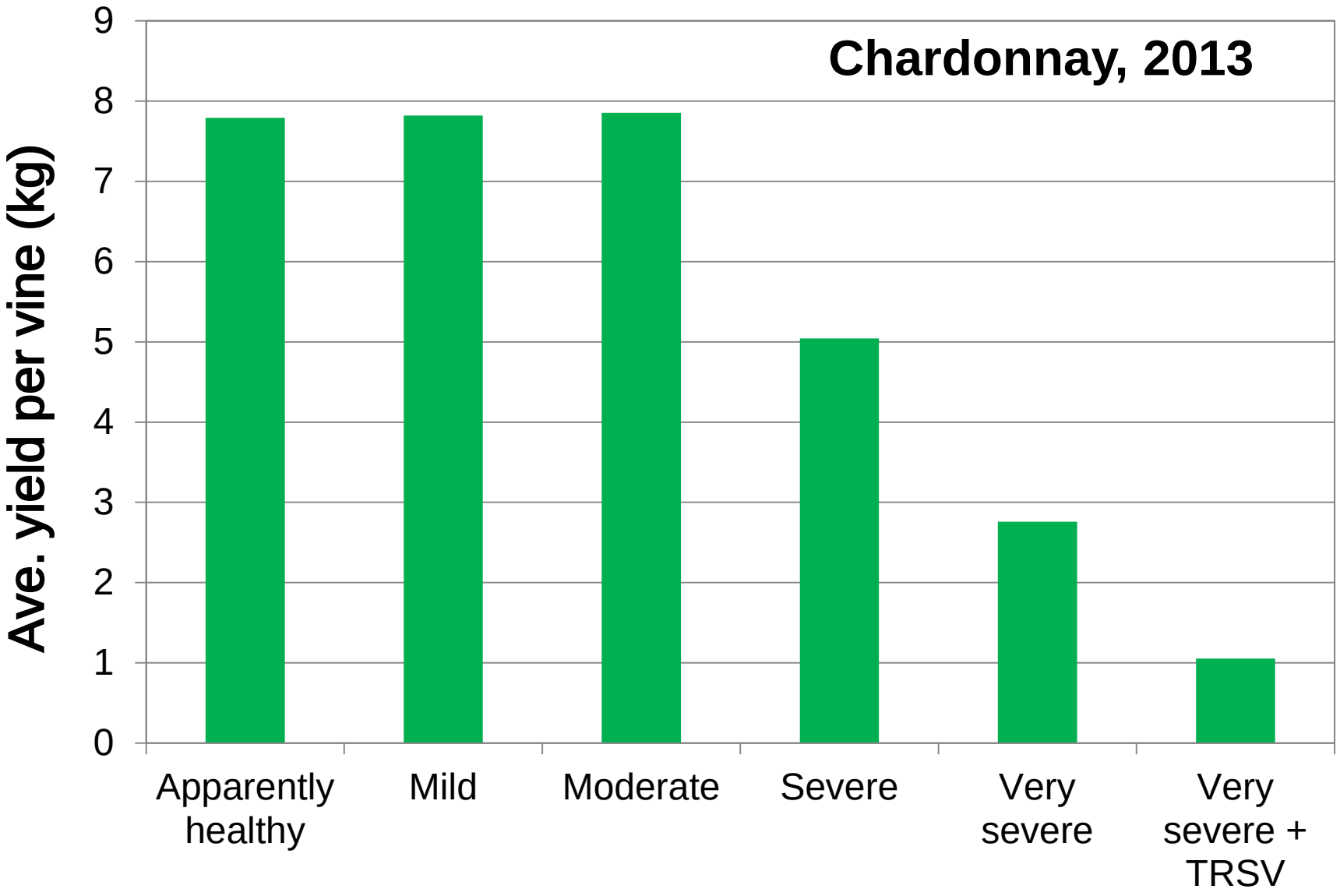
2

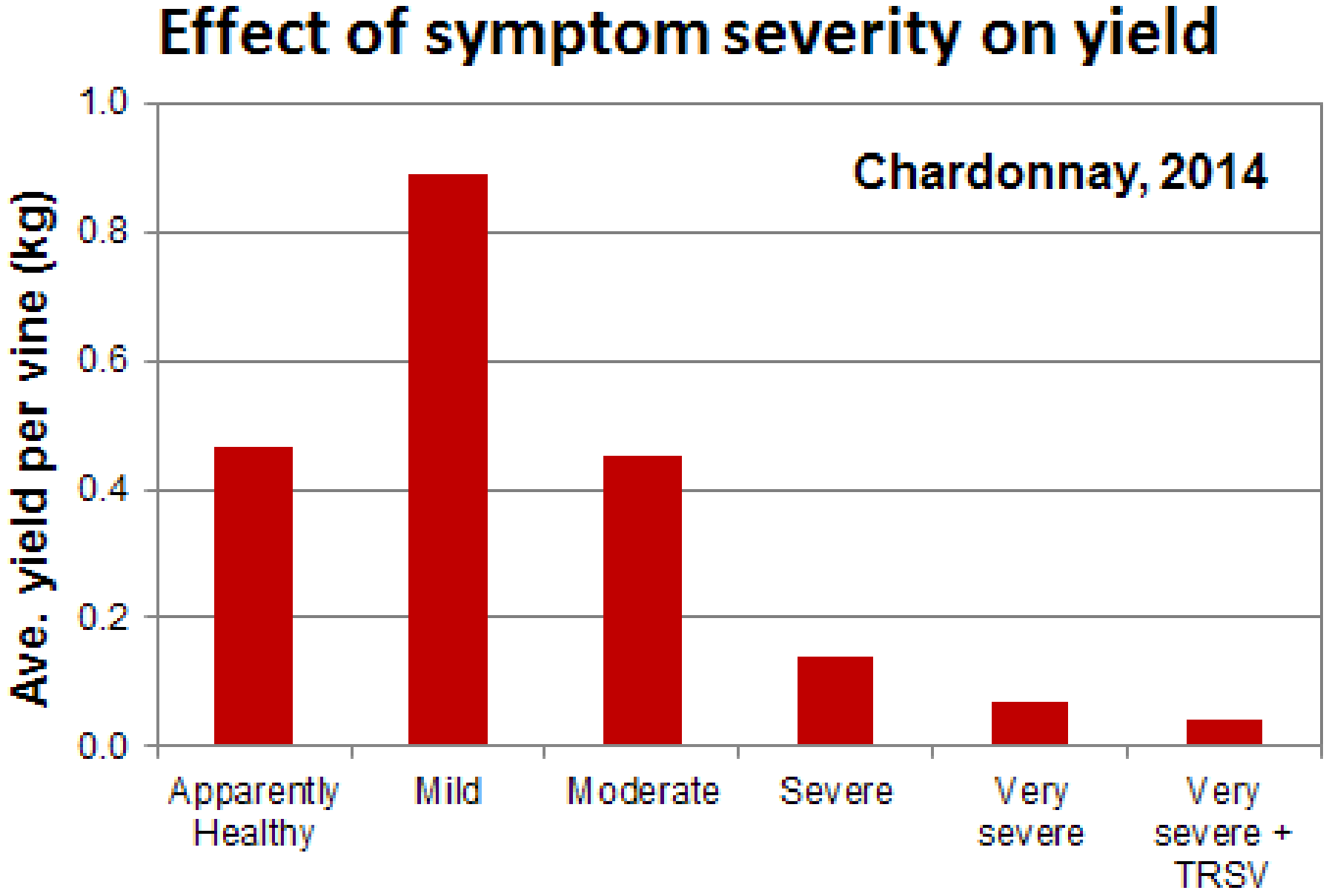
Tobacco ringspot symptoms
No tobacco ringspot symptoms
Dead or missing vine

3

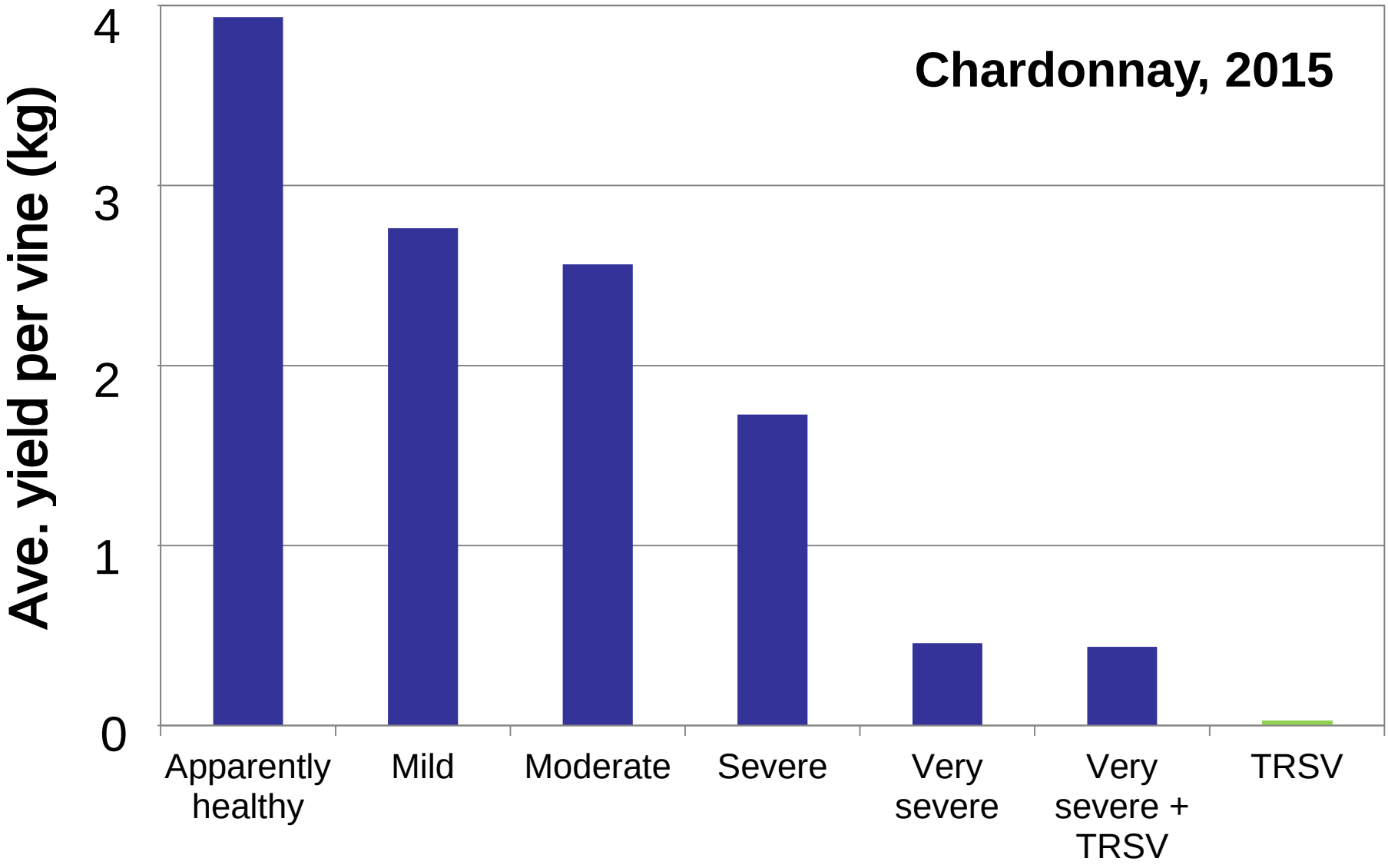
0 No virus symptoms
1 Mild leafroll symptoms
2 Moderate leafroll symptoms
3 Severe leafroll symptoms
4 Very severe leafroll symptoms
Tobacco ringspot symptoms
Leafroll + Tobacco ringspot symptoms
Dead or missing vine

Effect of symptom severity on yield

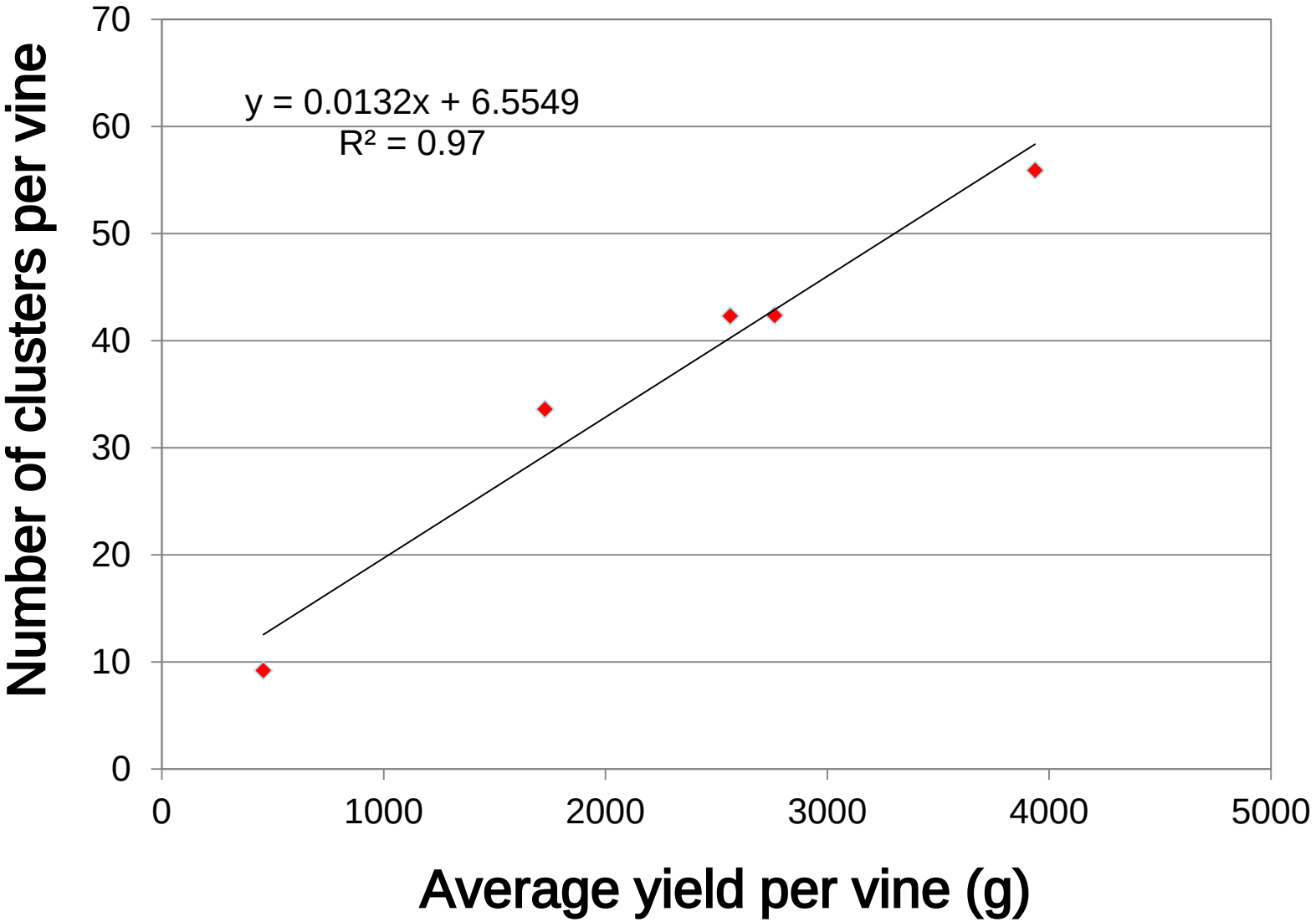



Effect of symptom severity on yield
Chardonnay, 2014
Ave. yield per vine (kg)
1.0
0.8
0.6
0.4
0.2
0.0
Apparently Healthy
Mild
Moderate
Severe
Very severe
Very severe + TRSV

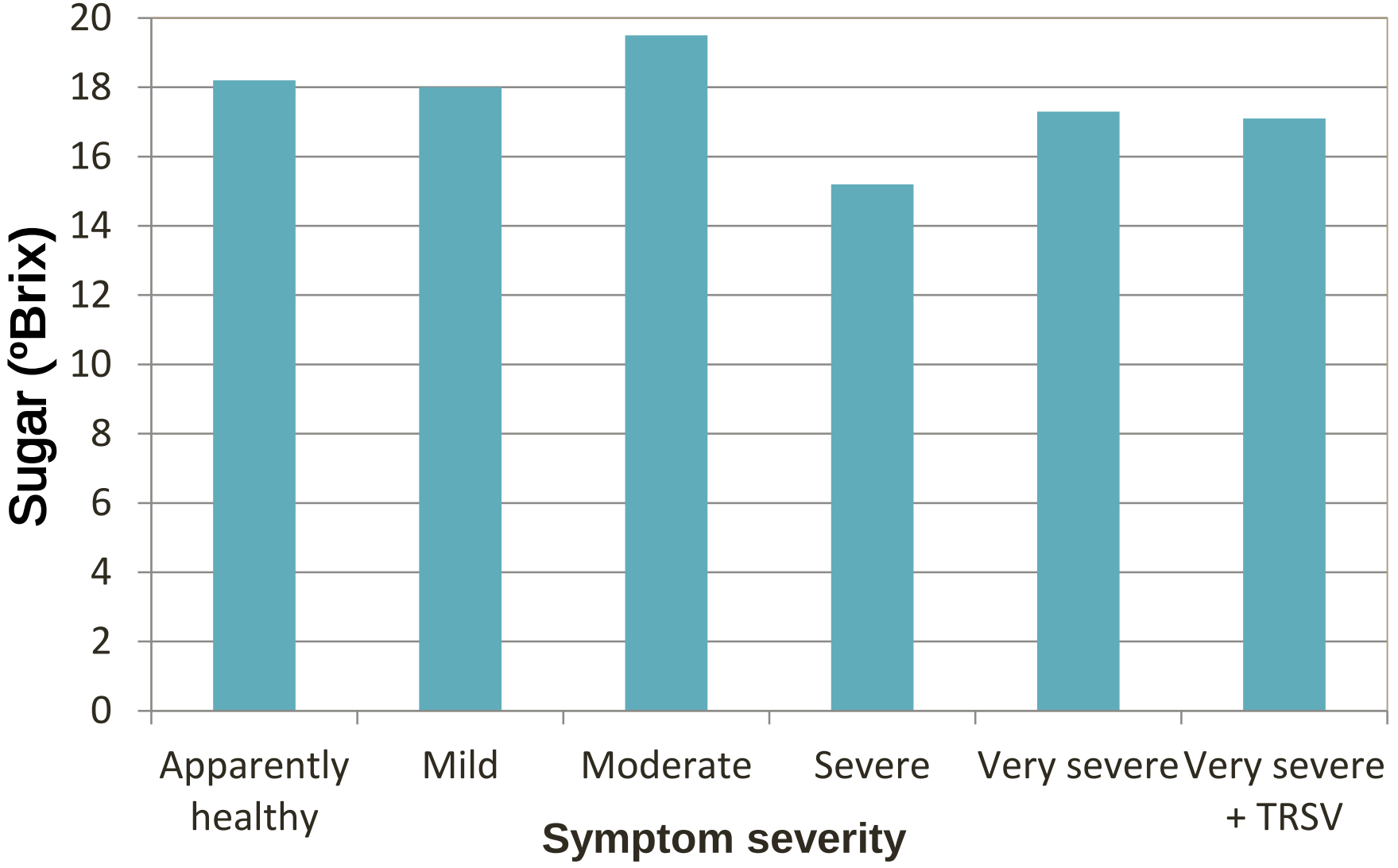
Effect of symptom severity on yield



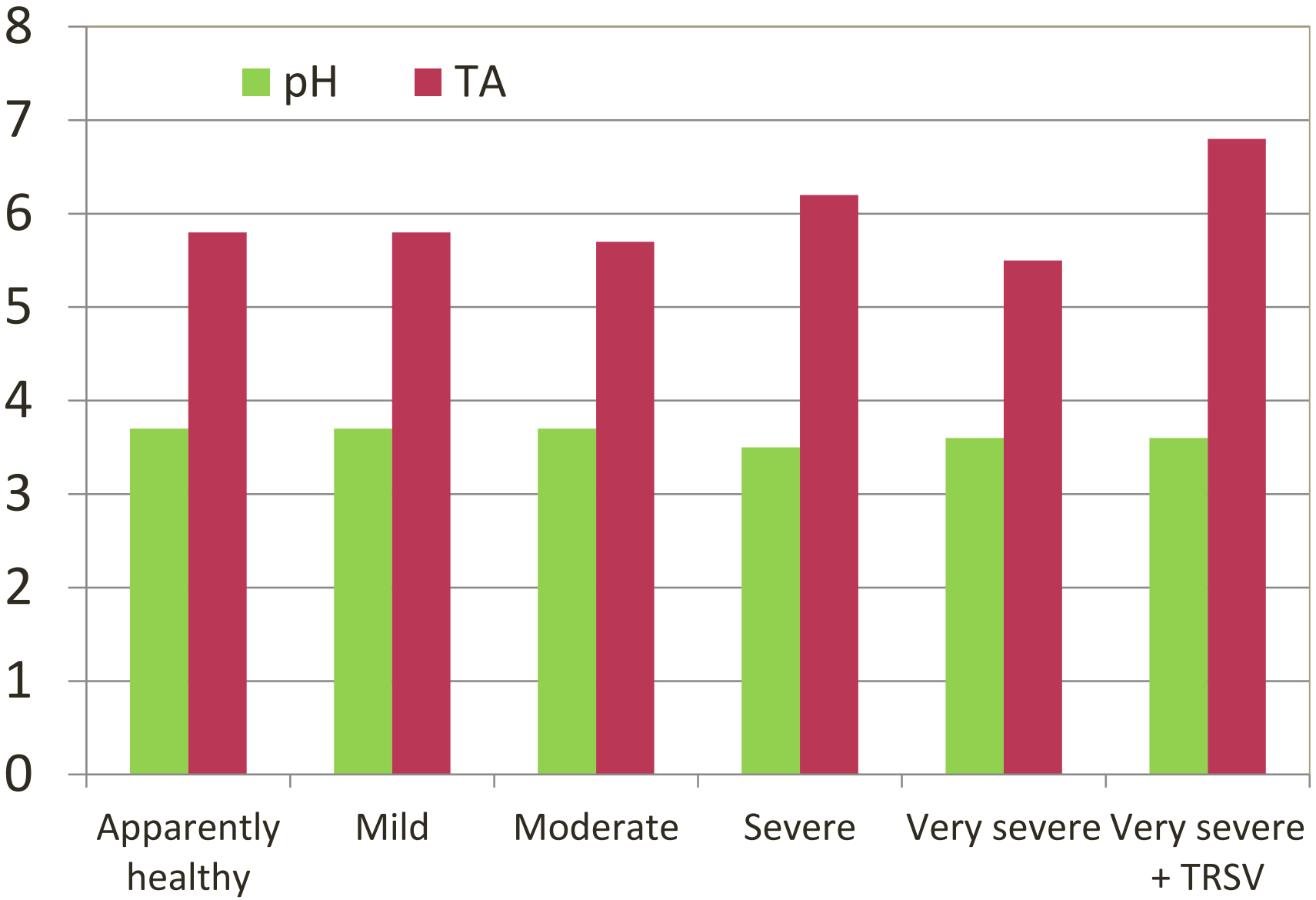
Correlation of yield and number of clusters per vine



Effect of symptom severity on ºBrix



Effect of symptom severity on pH and TA



Conclusions

- Viruses are a threat to Michigan wine grape production
- A comprehensive statewide survey is needed to more accurately assess virus prevalence and risk in Michigan
- Management recommendations needed for grapevine leafroll and ringspot decline
- Increased awareness of risks of virus infection and need for clean planting material