Viruses of Grapes: separating the bad from the not so bad

James E. Schoelz
Dean Volenberg
Division of Plant Sciences
University of Missouri
Columbia MO

The 2017 virus survey: Missouri vineyards tested for the presence of 26 different viruses

25 hybrid grape cultivars tested

400 samples collected in July through a prearranged pattern to avoid bias towards selection of virus-infected plants

Each sample was a composite of 4 vines (for a total of 1600 vines sampled)

Each sample tested for 26 different viruses

Which viruses could potentially cause the greatest problems for grape production in Missouri?

Virus	Incidence in Survey
Grapevine rupestris stem pitting associated virus	58.7%
Grapevine leafroll associated virus 3	52.7%
Grapevine red blotch virus	35.0%
Grapevine virus E	31.0%
Grapevine leafroll associated virus 2	19.0%
Grapevine virus B	17.2%
Grapevine fleck virus	13.5%
Grapevine leafroll associated virus 2RG	9.2%
Grapevine vein clearing virus	8.2%
Grapevine virus A	0.5%
Grapevine leafroll associated virus 5	0.2%

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What does the literature say about the impact of GRBV and GLRaV-3 on yield and juice quality in *Vitis vinifera*?

GRBV reduces yield in *Vitis* vinifera

Cultivar/Location	Yield (Kg of grapes per vine)
Cabernet franc/ British Columbia	Two-year study Reductions of 22% and 30%
Chardonnay/California	Three-year study Reductions between 19 and 27%

Cabernet franc – Bowen et al., 2020
Chardonnay - Girardello et al. 2020

GLRaV-3 reduces yield in *Vitis* vinifera

Cultivar/Location	Yield (Kg of grapes per vine)
Cabernet franc/Michigan	One-year study Reduction of 30%
Merlot/California	Three-year study Reductions between 16 and 21%

Cabernet franc – Endeshaw et al., 2014
Merlot - Alabi et al. 2016

GRBV and GLRaV-3 reduce yield in *Vitis* vinifera

Reductions also observed in several yield components

- Cluster numbers per vine
- Berries per cluster
- Pruning weight

Only exception

- Individual berry weight increased
  - 109% up to 145%, depending on the year/cultivar

GRBV and GLRaV-3 slow or prematurely halt the maturation process of *Vitis vinifera* grapes

- Brix ↓
- pH ↓
- TA ↑

Studies with Cabernet franc, Cabernet Sauvignon, Merlot, Chardonnay

How do Grapevine red blotch virus and Grapevine leafroll-associated virus 3 affect the chemical composition of wine?

Lower levels of ethanol[1,2,3,4]

Higher pH[1,2]

Alterations in volatiles[1]

Alterations in phenolics (anthocyanins and tannins)[1,2,3,4]

Grapevine red blotch virus

[1]Chardonnay - Girardello et al. 2020. J. Sci Food Agric. 100, 14361447

[2]Cabernet and Merlot – Girardello et al., 2020. Molecules 25, 3299; doi:10.3390/molecules25143299

[3]Cabernet franc - Bowen et al. 2020. Am J Enol Vitic 71, 308-318.

Grapevine leafroll-associated virus 3

[4]Merlot Alabi et al. 2016. PLOS One. e0149666.doi:10.1371/journal.pone.0149666

Differences in Sensory Attributes of Chardonnay made from GRBV- and GRBV+ Grapes

2014

- ↑ Yeasty
- ↓ Astringency

2015

- ↑ Apple Juice
- ↓ Acetone
- ↓ Hot
- ↓ Spicy
- ↓ Citrus
- ↓ Apple

2016

- ↑ Sweet
- ↑ Salty
- ↓ Carbonated

Judges Panels
2014 – 12 judges
2015 – 13 judges
2016 – 12 judges

Girardello et al. 2020. J. Sci Food Agric. 100, 14361447

Estimated Economic Impact of GRBV and GLRaV-3 in *Vitis vinifera*

- GRBV causes estimated loses of:
  - $2.2k - $68.5k per hectare over a 25-year life cycle of Cabernet Sauvignon or Merlot

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- GLRaV-3 causes estimated loses of:
  - $25k - $40k per hectare in Cabernet Franc in New York
  - $29k - $225k per hectare in Cabernet Sauvignon in California

How do GLRaV-3 and GRBV affect American and French-American hybrids?

Impact of GLRaV-3 on French-American hybrids relative to *Vitis vinifera*

- *Vitis vinifera*
  - Brix ↓
  - pH ↓
  - TA ↑
  - Berry weight ↑
  - Pruning weight ↓
  - Clusters/vine ↓
  - Berries/cluster ↓
- St. Vincent & Vidal blanc
  - Brix ↓
  - pH ↔
  - TA ↑
  - Berry weight ↓
  - Pruning weight ↔
  - Clusters/vine ↔
  - Berries/cluster ↔

Kovacs et al., 2001, Am. J. Enol. Vitic. 52, 254-259

Comparison of virus titers in Norton (*Vitis aestivalis*) to Kishmish Vatkana (*Vitis vinifera*)

Overall virus load is lower in Norton than in Kishmish Vatkana

The titer of the leafroll viruses (GLRaV-1, GLRaV-2, GLRaV3) was significantly lower in Norton than in Kishmish Vatkana

Howard et al., 2021, Phytobiomes 5, 432-441

What viruses were found in Norton in the 2017 survey?

Virus	Incidence in Survey
Grapevine rupestris stem pitting associated virus	0.0%
Grapevine leafroll associated virus 3	85.0%
Grapevine red blotch virus	77.5%
Grapevine virus E	30.0%
Grapevine leafroll associated virus 2	0.0%
Grapevine virus B	22.5%
Grapevine fleck virus	15.0%
Grapevine leafroll associated virus 2RG	72.5%
Grapevine vein clearing virus	0.0%
Grapevine virus A	2.5%
Grapevine leafroll associated virus 5	0.0%

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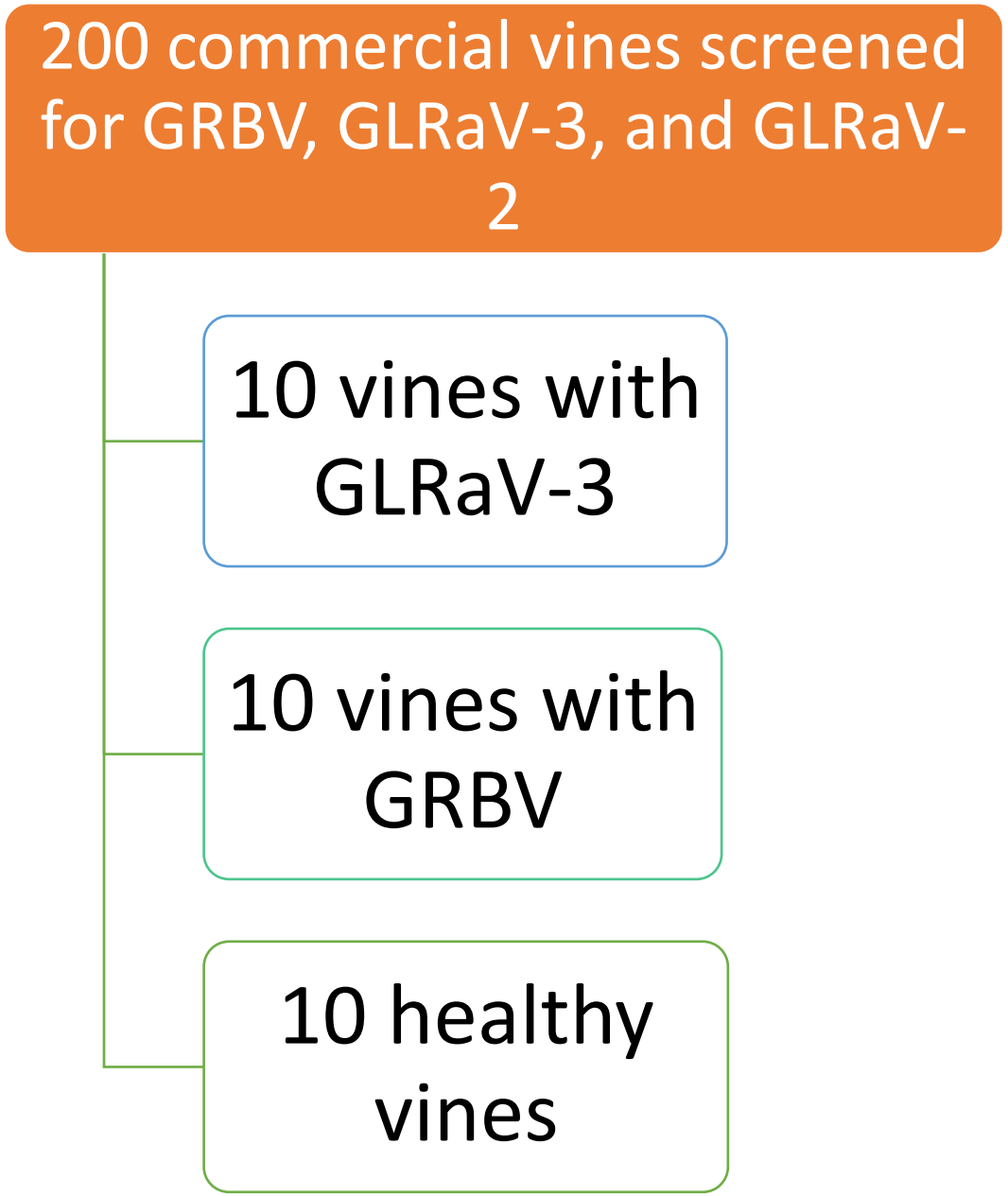
Grapevine leafroll associated virus 3	85.0%
Grapevine red blotch virus	77.5%
Grapevine virus E	30.0%
Grapevine leafroll associated virus 2	0.0%
Grapevine virus B	22.5%
Grapevine fleck virus	15.0%
Grapevine leafroll associated virus 2RG	72.5%
Grapevine vein clearing virus	0.0%
Grapevine virus A	2.5%
Grapevine leafroll associated virus 5	0.0%

How do GRBV and GLRaV-3 affect Norton yield and berry juice quality?



Impact of GLRaV-3 and GRBV infection on Norton

Count # of clusters per vine in late August

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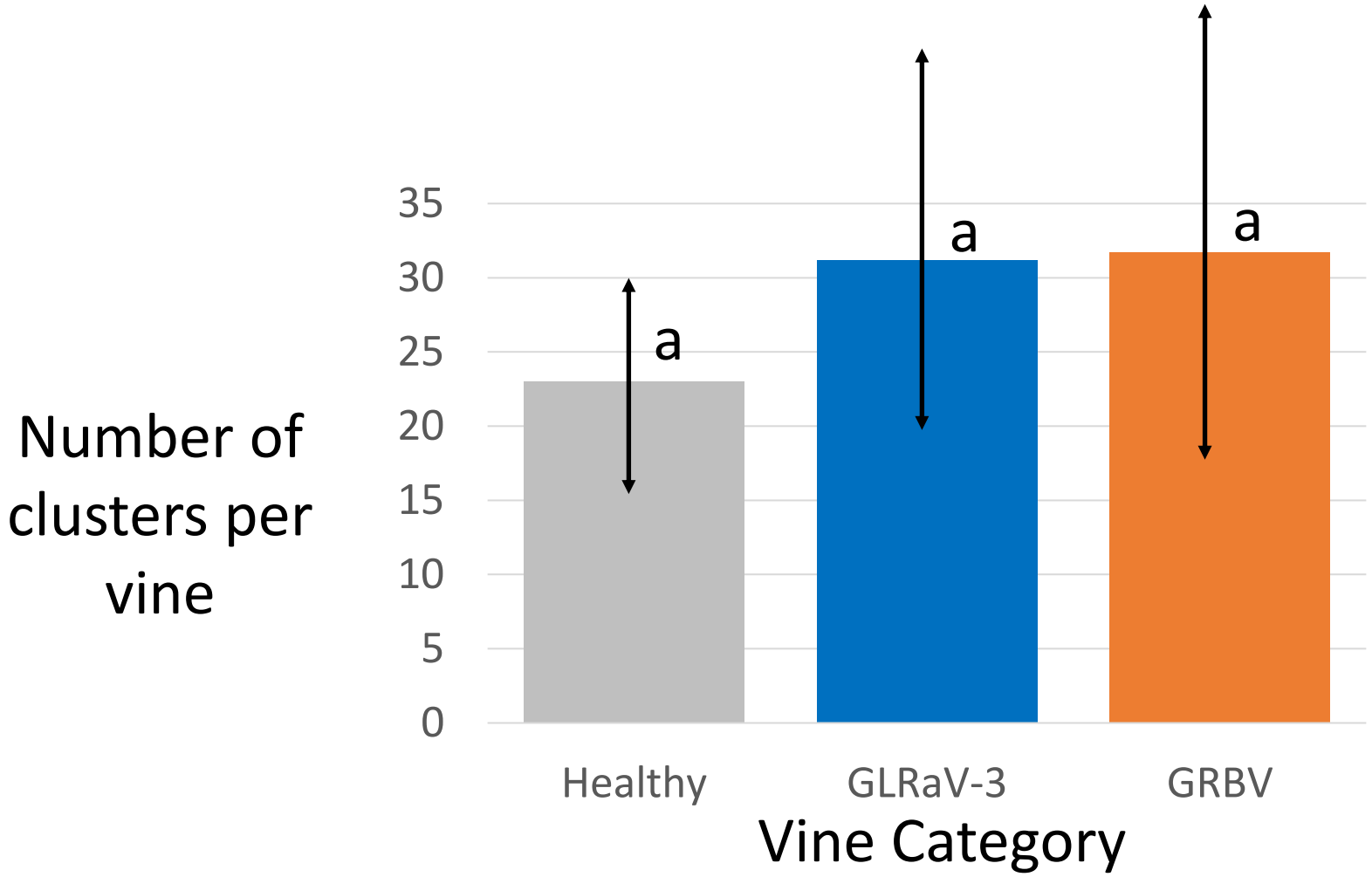
Collect 25 berries per vine weekly, beginning in August until harvest – assess berry weight, Brix, pH and TA

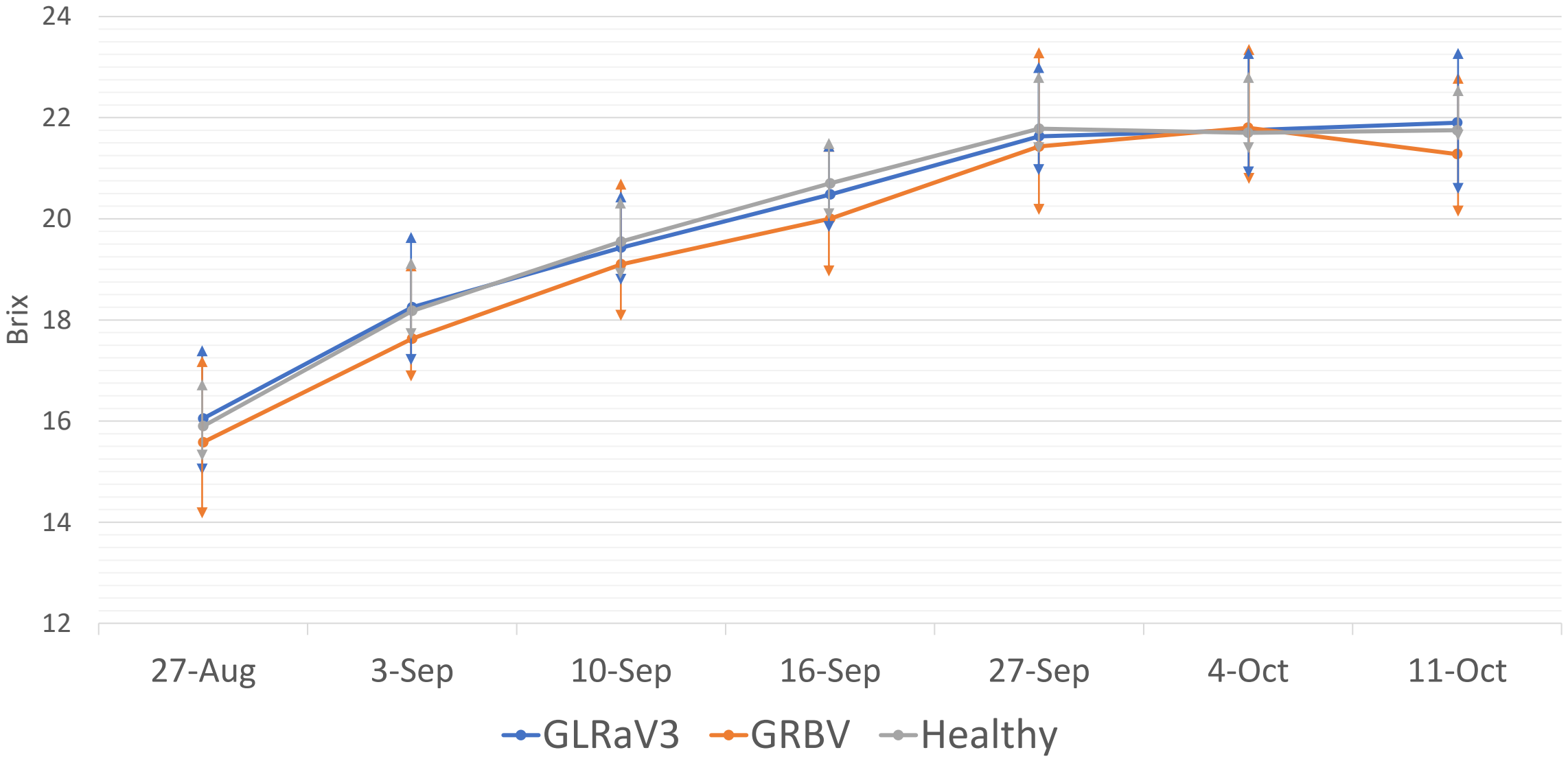
Pruning weights determined in December

GRLaV-3 and GRBV infection had no effect on number of clusters per vine in Norton

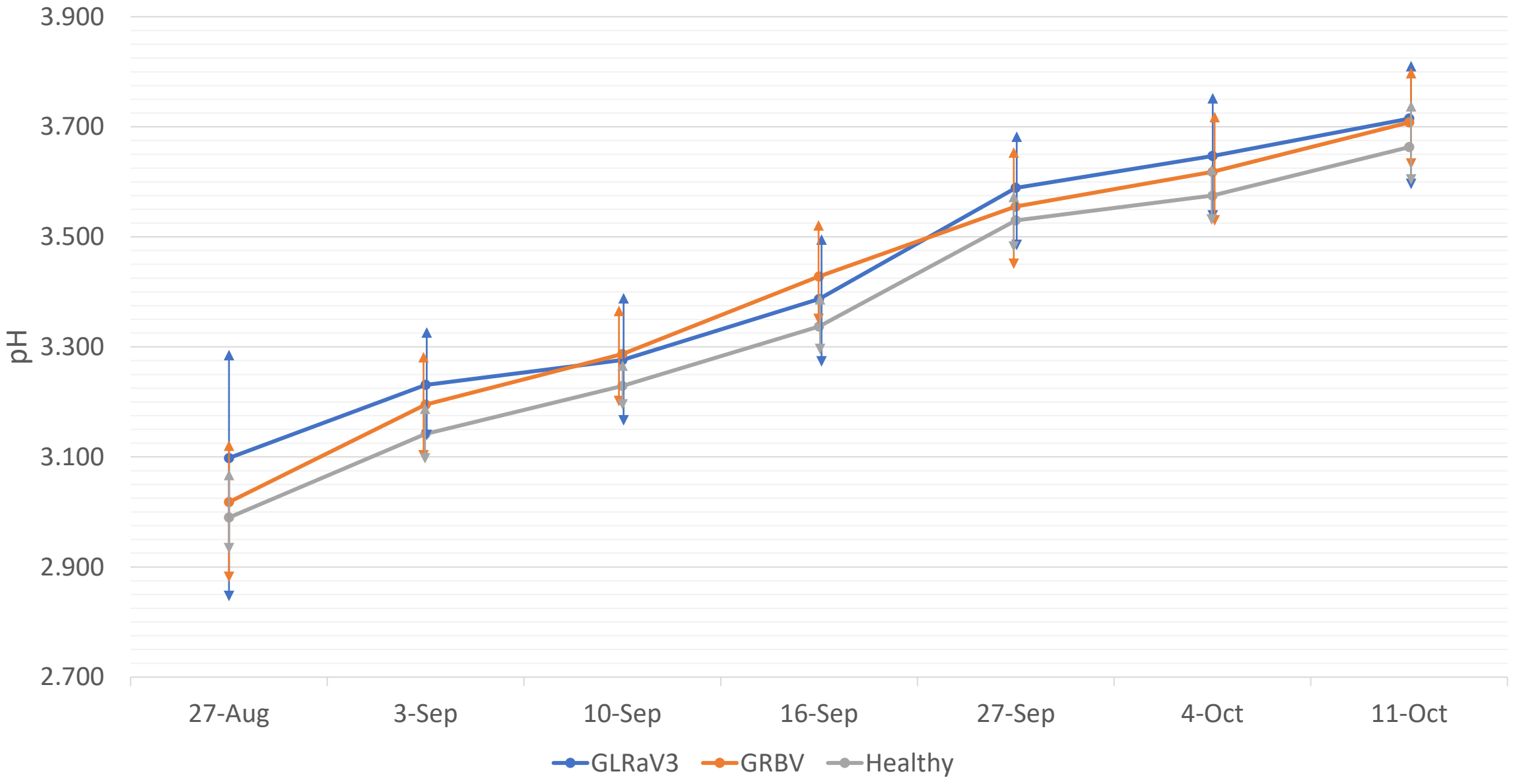


*No statistical difference observed at p < .05

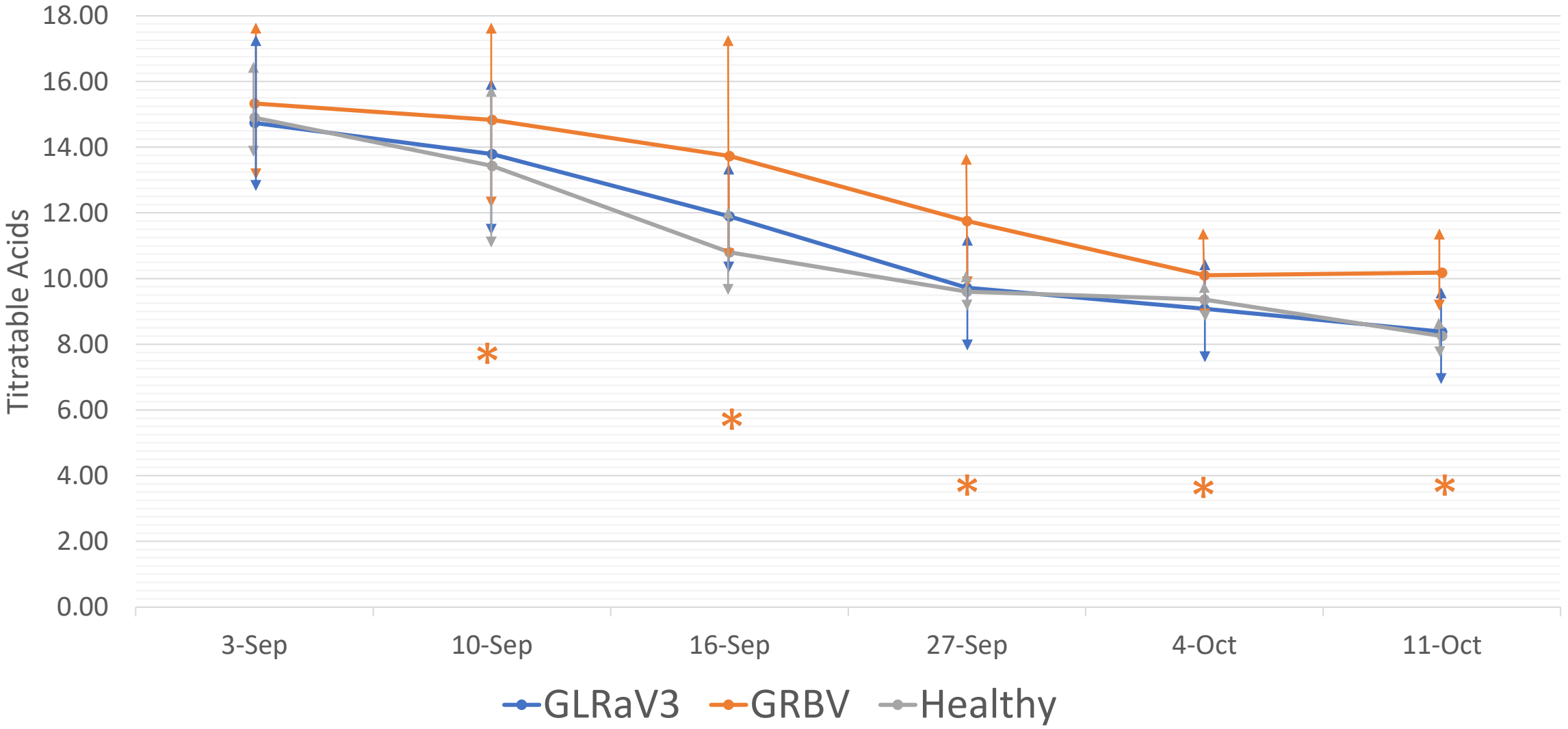
GRBV and GLRaV3 infections have no effect on Brix in Norton berry juice



GRBV and GLRaV-3 infection have no effect on pH in Norton berry juice

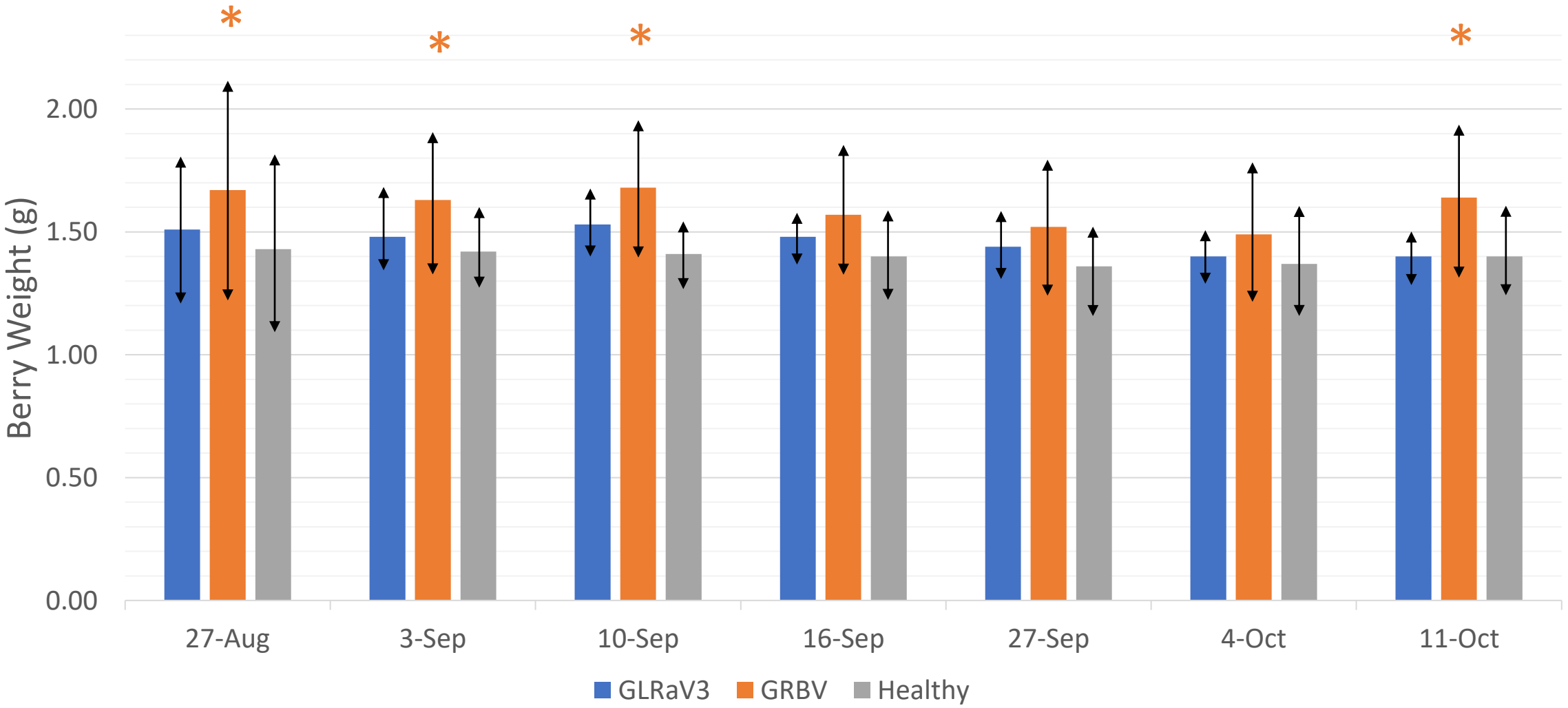


Titratable acids from berries infected with GRBV are higher than those from virus-free berries



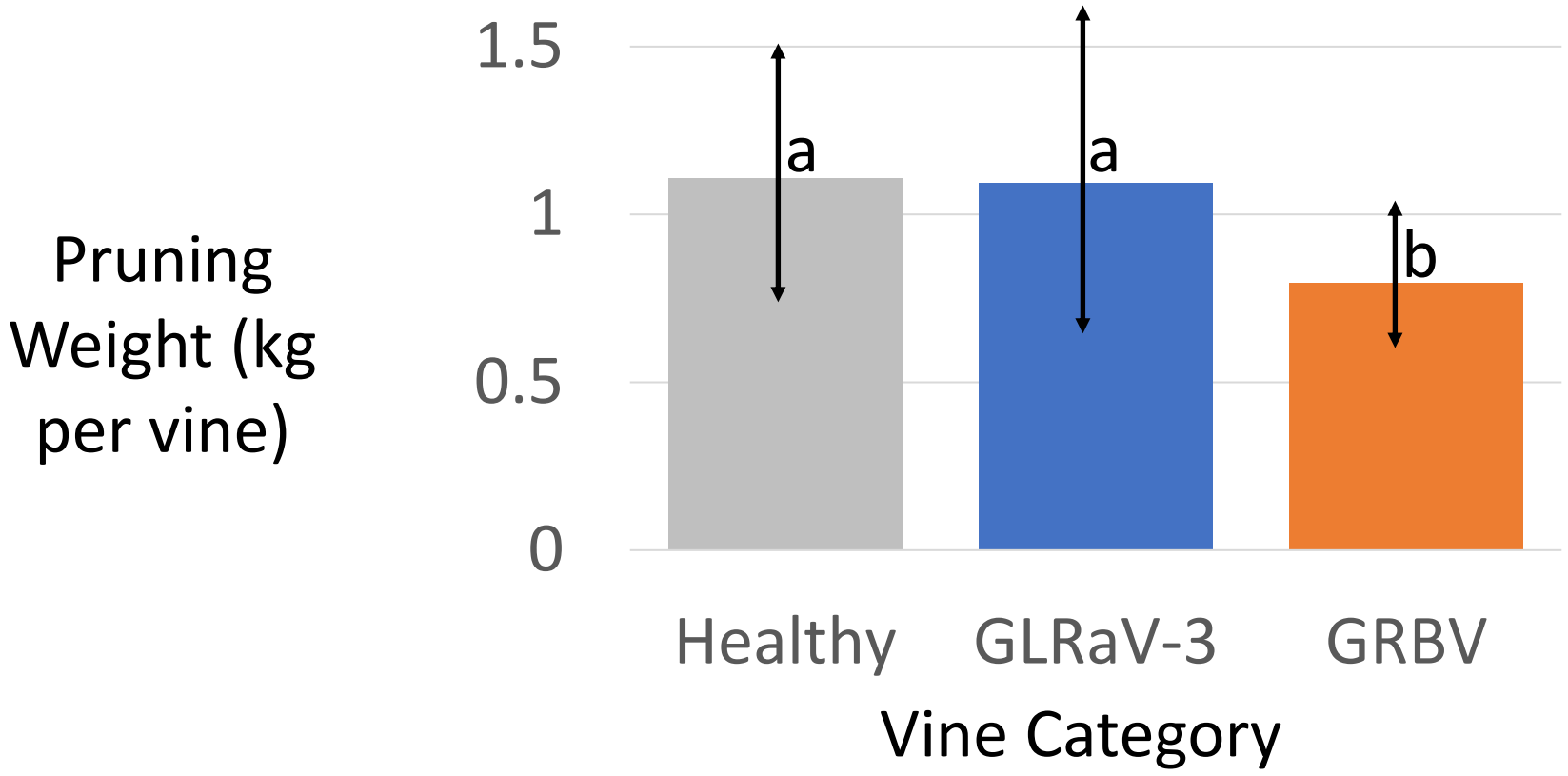
* Notes a significant difference at p < .05

Norton Berry weights from vines infected with GRBV were significantly higher than those from healthy vines



* Notes a significant difference at p < .05

GRLaV-3 infection in Norton had no effect on pruning weight, whereas vines infected with GRBV had a lower pruning weight



*differences in letters above columns denote a significant difference at p < .06

Norton is tolerant to GLRaV-3

- *Vitis vinifera*
  - Brix ↓
  - pH ↓
  - TA ↑
  - Berry weight ↑
  - Pruning weight ↓
  - Clusters/vine ↓
  - Berries/cluster ↓
- Norton (*Vitis aestivalis*)
  - Brix ↔
  - pH ↔
  - TA ↔
  - Berry weight ↔
  - Pruning weight ↔
  - Clusters/vine ↔
  - Berries/cluster?

A lower titer of GLRaV3 in Norton could explain why this virus has no apparent effect on yield and berry quality

Overall virus load is lower in Norton than in Kishmish Vatkana

The titer of the leafroll viruses (GLRaV-1, GLRaV-2, GLRaV3) was significantly lower in Norton than in Kishmish Vatkana

Howard et al., 2021, Phytobiomes 5, 432-441

Norton may have some degree of tolerance to GRBV

- *Vitis vinifera*
  - Brix ↓
  - pH ↓
  - TA ↑
  - Berry weight ↑
  - Pruning weight ↓
  - Clusters/vine ↓
  - Berries/cluster
- Norton (*Vitis aestivalis*)
  - Brix ↔
  - pH ↔
  - TA ↑
  - Berry weight ↑
  - Pruning weight ↓
  - Clusters/vine ↔
  - Berries/cluster?

Remaining questions that need to be addressed

Does the apparent resistance in Norton stand up to further scrutiny? Repeat field studies for GLRaV3 & GRBV

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What about other the French-American hybrids? One study indicates some resistance – needs more investigation – vector management should be emphasized

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What about other the French-American hybrids? One study indicates some resistance – needs more investigation – vector management should be emphasized

What about vines infected with both GRBV and GLRaV-3? This combination commonly found in Norton - Synergism

How do GRBV, and GVCV affect French-American hybrids?

What viruses were found in Chardonel in the 2017 survey?

Virus	Incidence in Survey
Grapevine rupestris stem pitting associated virus	46.7%
Grapevine leafroll associated virus 3	33.3%
Grapevine red blotch virus	75.5%
Grapevine virus E	8.9%
Grapevine leafroll associated virus 2	6.7%
Grapevine virus B	0.0%
Grapevine fleck virus	0.0%
Grapevine leafroll associated virus 2RG	0.0%
Grapevine vein clearing virus	24.4%
Grapevine virus A	0.0%
Grapevine leafroll associated virus 5	2.5%

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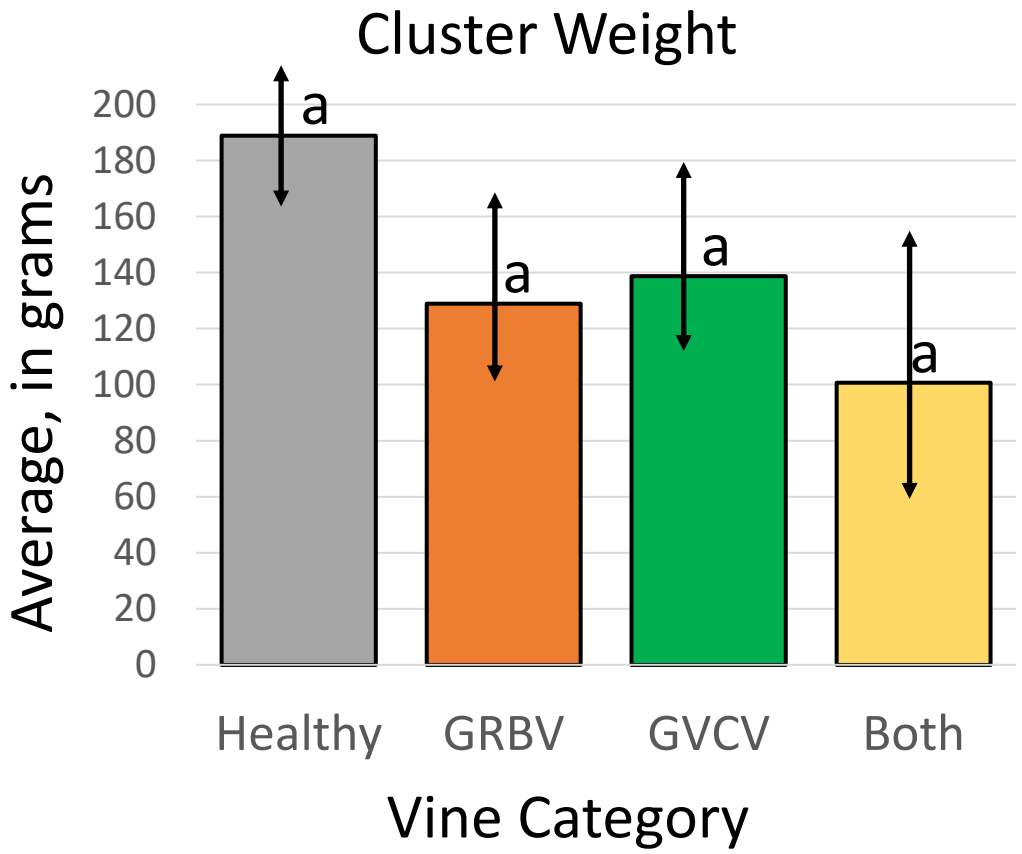
Grapevine vein clearing virus	24.4%
Grapevine virus A	0.0%
Grapevine leafroll associated virus 5	2.5%

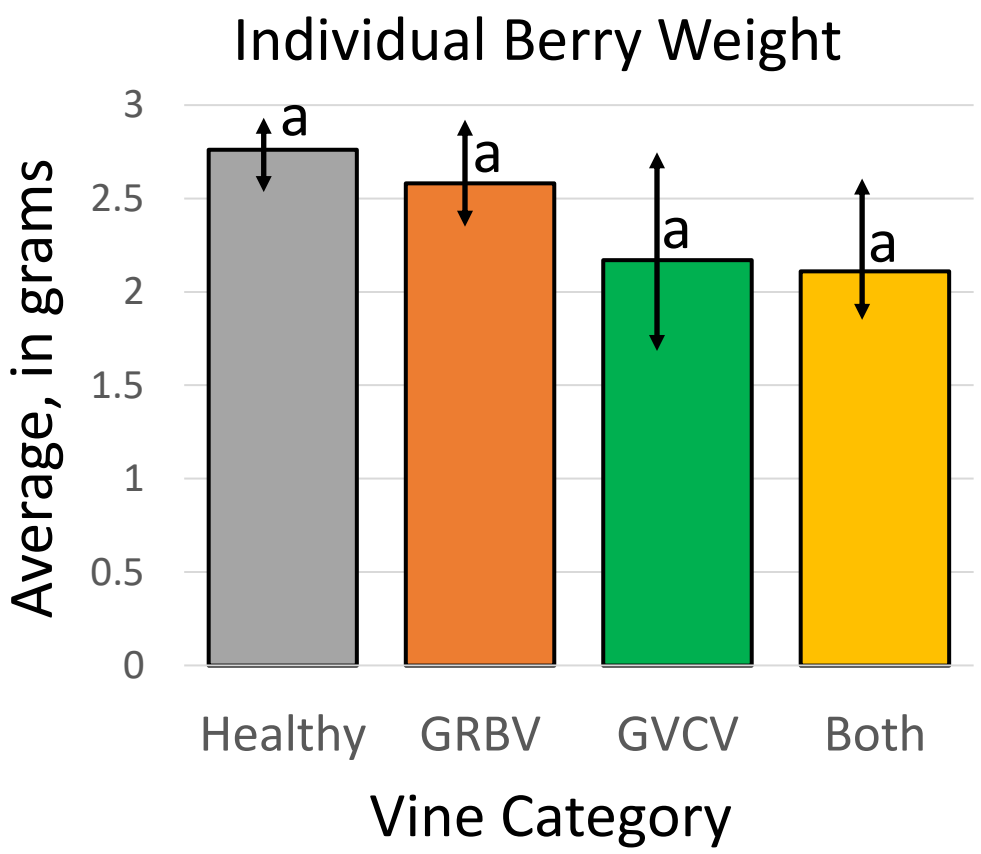
Impact of GVCV and GRBV infection on Chardonel

Identify infected vines early in the season

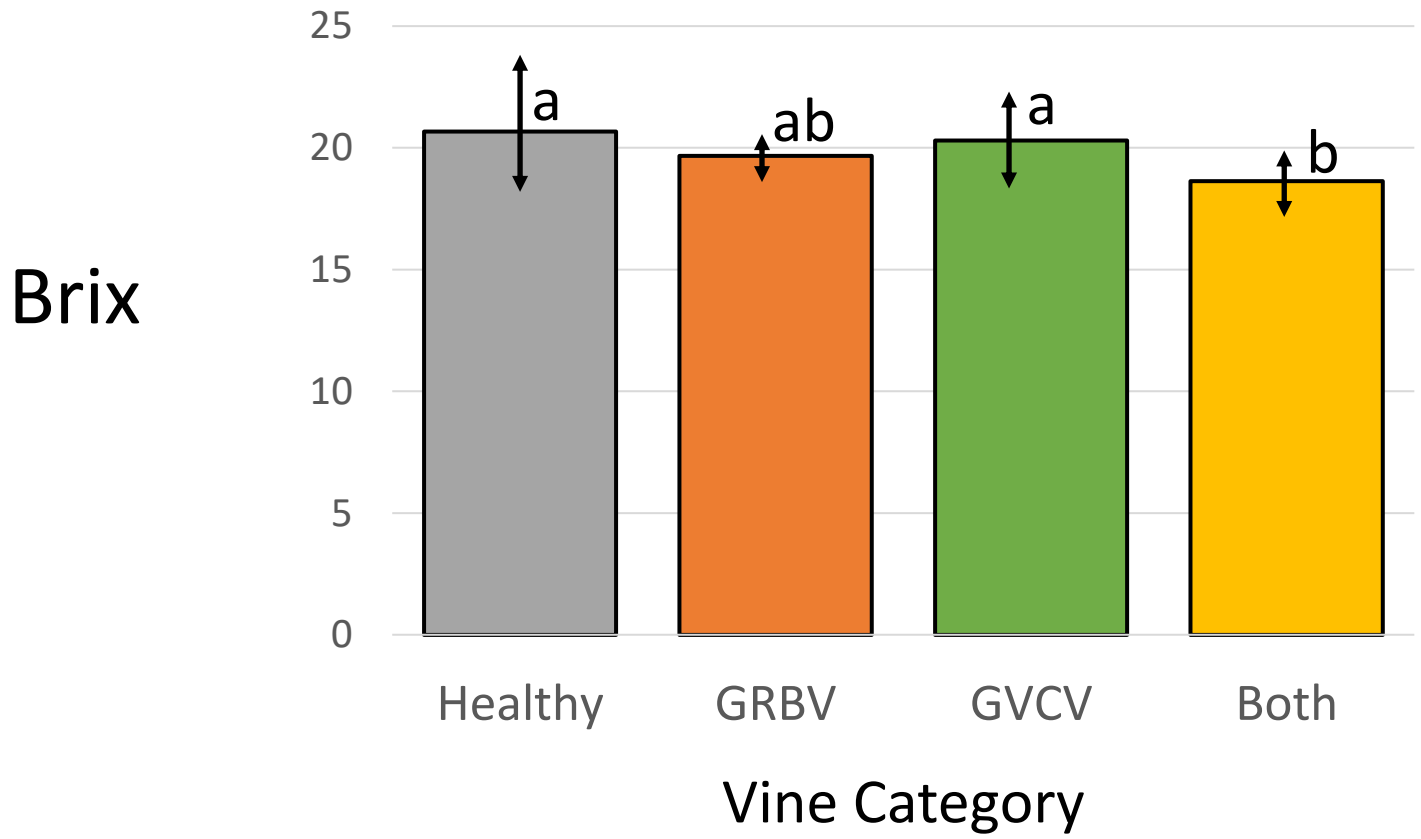
Collect 25 berries per vine – assess berry weight, Brix, pH and TA

Influence of GRBV and GVCV on cluster weight and in individual berry weight in Chardonel - 2020





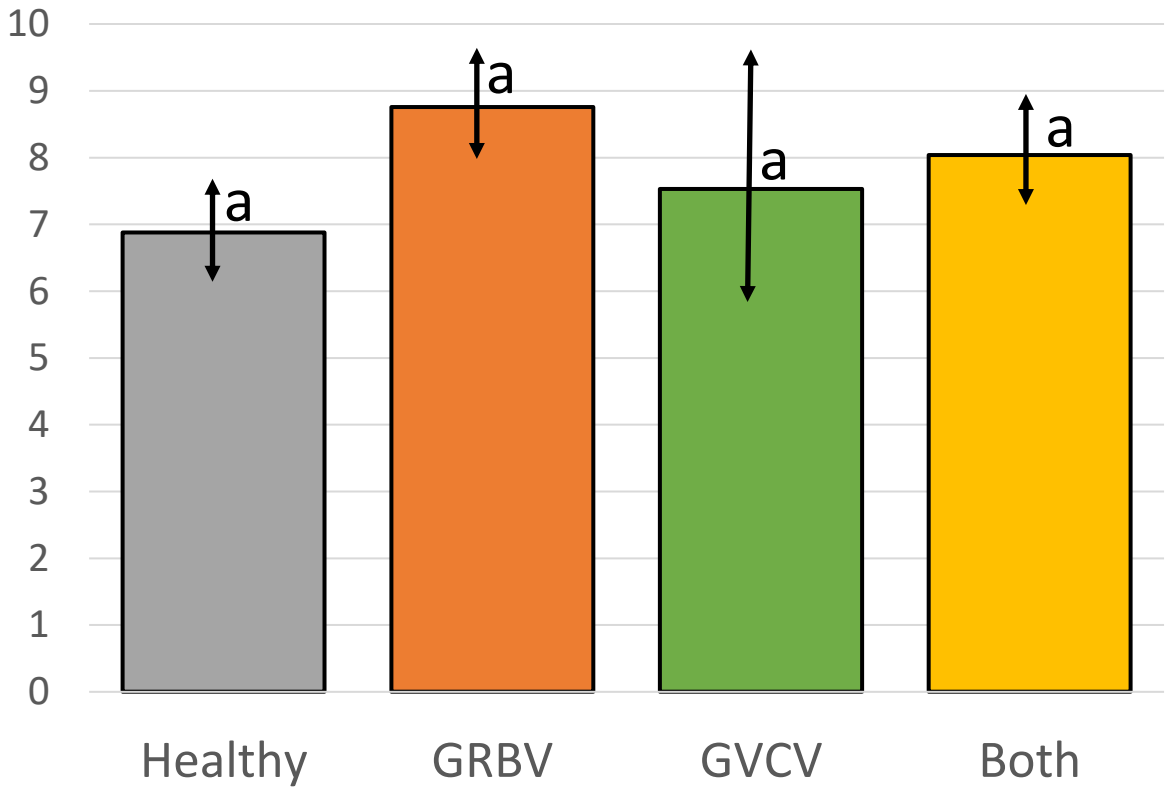
Influence of GRBV and GVCV on Brix in Chardonel - 2020



Brix significantly lower only in berries infected with both GRBV and GVCV

Influence of GRBV and GVCV on TA in Chardonel - 2020

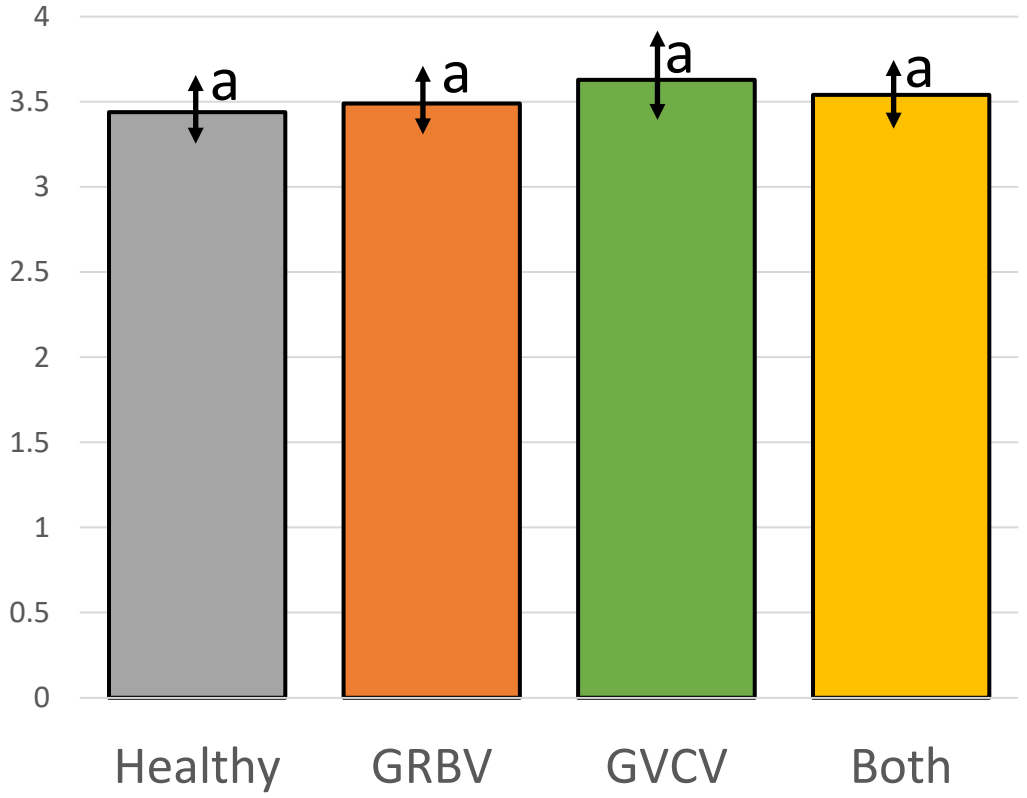
TA



No significant differences in TA

Influence of GRBV and GVCV on pH in Chardonel - 2020

pH



No significant differences in pH

Remaining questions that need to be addressed

What are the baseline effects of GRBV, GLRaV-3 and GVCV in French-American hybrids?

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Kovacs – some tolerance to GLRaV3?

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How are vines affected by multiple viruses?

- GLRaV3/GVCV
- GRLaV3/GRBV
- GVCV/GRBV

Problem

Almost all of the studies that have examined the impact of virus infections on grapes have only examined vines infected with one virus.

However, composite samples from the 2017 survey indicate that vines may be infected with multiple viruses

2017 Survey Results for Vidal blanc
Virus present in composite samples

No Target Virus*	GVCV	GLRaV-3	GRBV	GVCV GLRaV-3	GRBV GLRaV-3	GRBV GVCV GLRaV-3
3	1	20	0	9	6	6

45 composite samples in total

*Did not contain GVCV, GLRaV-3 or GRBV

2017 Survey Results for Norton
Virus present in composite samples

No Target Virus*	GRBV	GLRaV-3	GRBV GLRaV-3
1	5	8	26

40 composite samples in total

*Did not contain GLRaV-3 or GRBV

Planning for future studies

We plan to establish vines infected with different combinations of viruses at a single location, which will be essential for examining the long-term impact of the viruses on vine health and berry quality.

Goal of our study

Identify vines infected with individual viruses as well as specific virus combinations.

We have sampled hundreds of Norton, Chardonel and Vidal blanc vines for GRBV, GLRaV-3, GLRaV-2, GLRaV-2RG, and GVCV

Each vine was tagged to allow for collection of canes during the dormant season and retesting in subsequent years.

Cuttings in the greenhouse in early spring, 2021

Specific Virus Combinations Planted at HARC

Planted in Groups of 4, replicated 3 times, previously screened for viruses.

Norton

- Healthy
- GRBV
- GLRaV-3
- Certified Virus
- GLRaV-3, GLRaV-2
- GRBV, GLRaV-3
- GLRaV-2, GLRaV-2RG, GLRaV-3
- GLRaV-2, GLRaV-2RG

Chardonel

- Healthy
- GRBV
- GRBV, GVCV
- GLRaV-3
- GVCV

Vidal Blanc

- Healthy
- ToRSV
- GVCV
- GLRaV-3, GLRaV-2
- ToRSV, GLRaV-3, GLRaV-2
- GVCV, GLRaV-3, GLRaV-2
- GVCV, ToRSV, GLRaV-3, GLRaV-2

Column 1 Border	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Gaps are left in white
Border vine	Border vine	Border vine	Border vine	Border vine	Border vine	Border vine	Border vine	Border vine			Norton vines to be added
	N-H 152.1	N-V2.2RG.V3 154.1	N-PCW 19.1	N-RB.V3 369.5	N-V2.V3 ***	C-H 115.1	C-RB.GV ****	C-GV 114.18			
	N-H 152.3	N-V2.2RG.V3 154.2	N-PCW 26.2	N-RB.V3 369.6	N-V2.V3 ***	C-H 115.2	C-RB.GV 119.11	C-GV 114.19			C=Chardonel
	N-H 155.3	N-V2.2RG.V3 154.3	N-PCW 29.3	N-RB.V3 ***	N-V2.V3 ***	C-H 115.3	C-RB.GV 119.12	C-GV ****			N= Norton
	N-H 155.4	N-V2.2RG.V3 156.2	N-PCW 29.4	N-RB.V3 ***	N-V2.V3 ***	C-H 115.9	C-RB.GV 120.1	C-GV ****			PCW -Pre Civil War
	N-RB 165.1	N-V2.2RG 155.1	N-C 05	N-H 161.2	N-RB 369.2	C-RB 103.5	C-V3 219.4	C-H 145.3			V = Vidal blanc
	N-RB 177.1	N-V2.2RG 157.2	N-C 06	N-H 372.1	N-RB 165.4	C-RB 103.6	C-V3 219.6	C-H 145.6			0 - no viruses
	N-RB 183.1	N-V2.2RG 159.1	N-C 07	N-H 373.1	N-RB 165.5	C-RB 103.9	C-V3 219.7	C-H 145.7			C-virus certified
	N-RB 351.1	N-V2.2RG 166.1	N-C 08	N-H 372.2	N-RB 165.6	C-RB 104.2	C-V3 219.8	C-H 145.11			RB-GRBV
Norton	N-V3 151.2	N-PCW 6.3	N-V3 158.1	N-V2.2RG.V3 161.1	N-V2.2RG ***		C-GV 114.13	C-RB 104.9			GV-GVCV
	N-V3 151.3	N-PCW 13.2	N-V3 158.3	N-V2.2RG.V3 164.1	N-V2.2RG ***		C-GV 114.15	C-RB 104.11			G3-GLRaV3
	N-V3 152.2	N-PCW 13.3	N-V3 165.2	N-V2.2RG.V3 164.2	N-V2.2RG ***		C-GV 114.16	C-RB 104.13			G2-GRLaV2
	N-V3 152.4	N-PCW 16.2	N-V3 167.3	N-V2.2RG.V3 167.1	N-V2.2RG ***		C-GV 114.17	C-RB 104.14			G2RG-GLRaV2RG
	N-C 01	N-RB 351.2	N-V2.V3 158.6	N-RB.V3 ***		C-RB.GV 119.2	C-H 115.10	C-V3 219.9			T-ToRSV
	N-C 02	N-RB 354.2	N-V2.V3 161.3	N-RB.V3 ***		C-RB.GV 119.3	C-H 115.11	C-V3 219.10			***-Added later
	N-C 03	N-RB 361.1	N-V2.V3 ***	N-RB.V3 ***		C-RB.GV 119.4	C-H 115.12	C-V3 ****			
	N-C 04	N-RB 364.5	N-GV.V3 ***	N-RB.V3 ***		C-RB.GV 119.8	C-H 115.14	C-V3 ****			
	N-V2.V3 151.1	N-V3 371.1	N-H 375.1	N-PCW 32.2		C-V3 217.5 (219.5)					
	N-V2.V3 153.1	N-V3 371.2	N-H 375.2	N-PCW 32.3		C-V3 219.1					
	N-V2.V3 154.4	N-V3 371.3	N-H 375.3	N-PCW 36.1		C-V3 219.2					
	N-V2.V3 158.4	N-V3 374.1	N-H 375.4	N-PCW 36.4		C-V3 219.3					
	N-RB.V3 352.1	N-V2.2RG.V3 156.3	N-V2.2RG 166.2	N-C 09		C-GV 114.5	C-RB 104.3	C-RB.GV 120.3			
	N-RB.V3 364.2	N-V2.2RG.V3 156.4	N-V2.2RG 166.3	N-C 10		C-GV 114.7	C-RB 104.4	C-RB.GV 120.8			
	N-RB.V3 369.1	N-V2.2RG.V3 157.1	N-V2.2RG 362.1	N-C 11		C-GV 114.8	C-RB 104.6	C-RB.GV 120.9			
	N-RB.V3 369.4	N-V2.2RG.V3 159.2	N-V2.2RG 362.2	N-C 12		C-GV 114.10	C-RB 104.7	C-RB.GV 120.10			
V-GV	V-H 506.5	V-T.V2.V3 536.1	V-GV.T.V2.V3 540.1	V-V2.V3. 550.6	V-T 533.5	V-GV.V2.V3 549A7	V-GV 534.4	V-H 507.1	V-T.V2.V3 536.13	V-GV.T.V2.V3 540.9	
V-GV	V-H 506.7	V-T.V2.V3 536.2	V-GV.T.V2.V3 540.2	V-V2.V3 550.7	V-T 533.6	V-GV.V2.V3 549A8	V-GV 534.6	V-H 507.4	V-T.G2.G3 536.14	V-GV.T.V2.V3 539.4	
V-GV	V-H 506.8	V-T.V2.V3 536.3	V-GV.T.V2.V3 540.3	V-V2.V3 550.9	V-T 533.7	V-GV.V2.V3 549A9	V-GV 534.11	V-H 507.10	V-T.G2.G3 537.1	V-GV.T.V2.V3 539.5	
V-GV	V-H 506.12	V-T.V2.V3 536.6	V-GV.T.V2.V3 540.4	V-V2.V3 550.10	V-T 534.7	V-GV.V2.V3 549A12	V-GV 534.12	V-H 507.12	V-T.G2.G3 538.1	V-GV.T.V2.V3 ****	
V-V2.V3	V-T 533.1	V-GV.V2.V3 549A1	V-GV	V-H 506.14	V-T.V2.V3 536.7	V-GV.T.V2.V3 540.5	V-V2.V3	V-T ****	V-GV.V2.V3 549B1		
V-V2.V3	V-T 533.2	V-GV.V2.V3 549A2	V-GV	V-H 506.15	V-T.V2.V3 536.8	V-GV.V2.V3 540.6	V-V2.V3	V-T ****	V-GV.V2.V3 549B2		
V-V2.V3	V-T 533.4	V-GV.V2.V3 549A3	V-GV	V-H 506.16	V-T.V2.V3 536.9	V-GV.T.V2.V3 540.7	V-V2.V3	V-T ****	V-GV.V2.V3 549B7		
V-V2.V3	V-T 533.5	V-GV.V2.V3 549A6	V-GV	V-H 506.18	V-T.V2.V3 536.12	V-GV.T.V2.V3 540.8	V-V2.V3	V-T ****	V-GV.V2.V3 549B13		
33	33	33	31	27	12	29	28	21	8	1	

Chardonel

Vidal Blanc


HARC (as of 8-3-21)

Final Questions

What viruses are present in your grapes?

Schoelzj@missouri.edu

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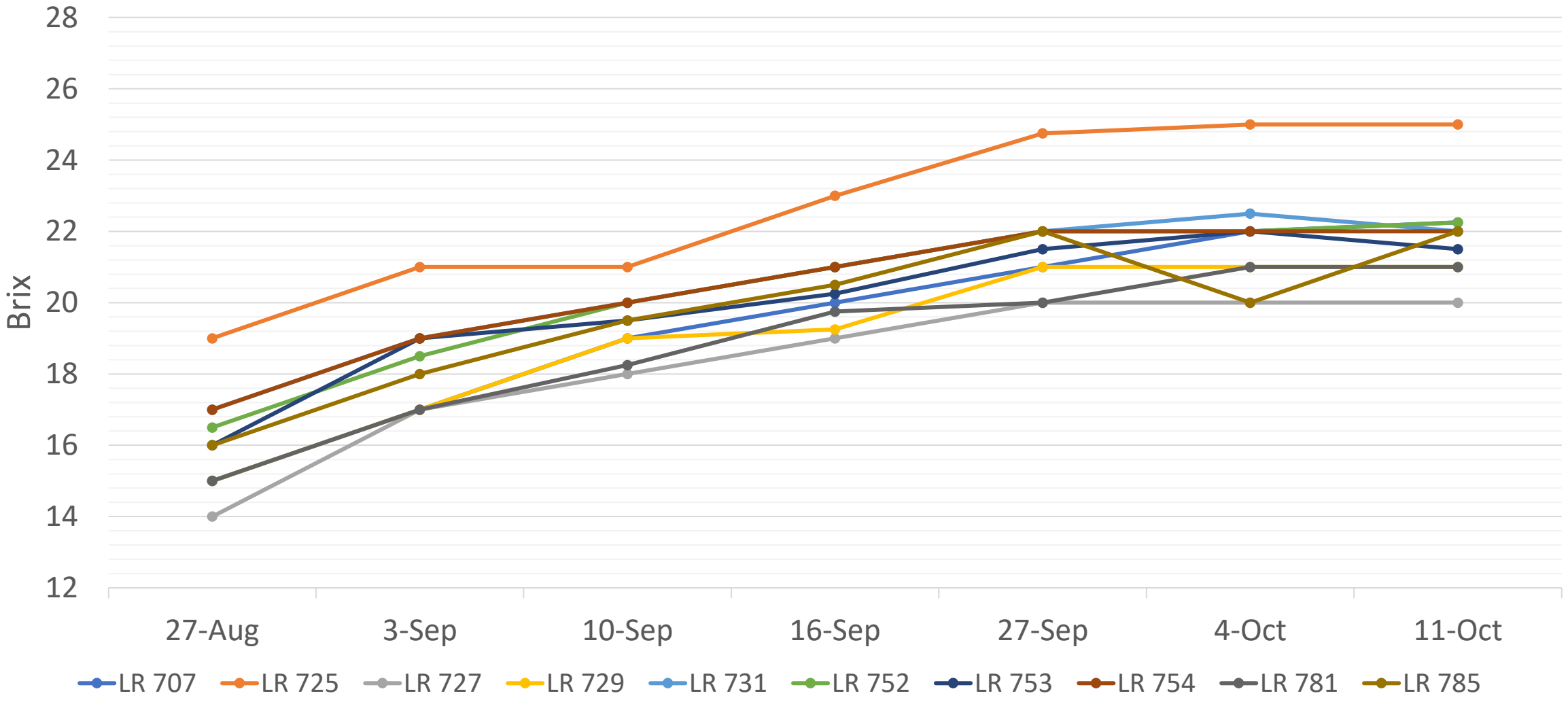
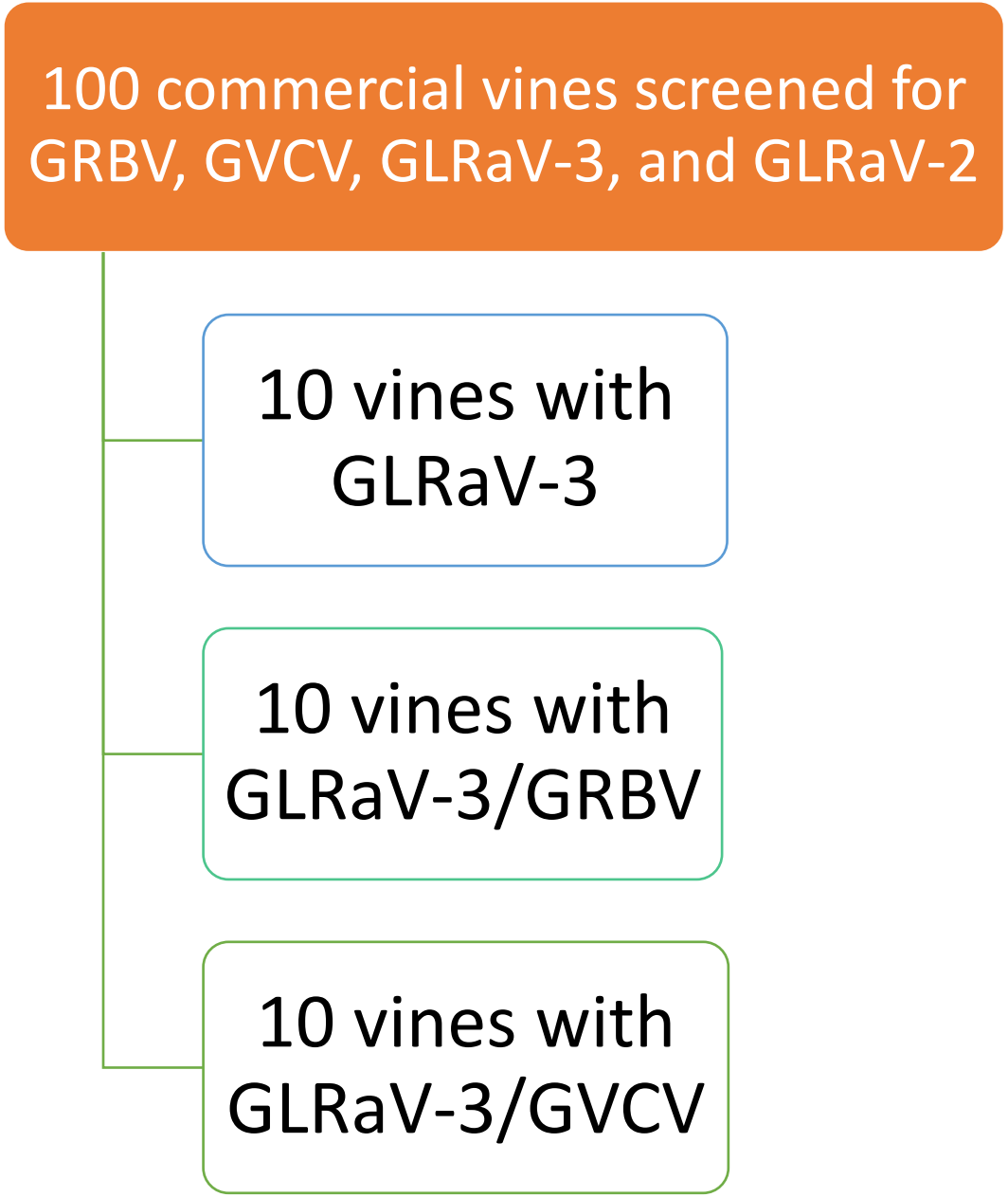
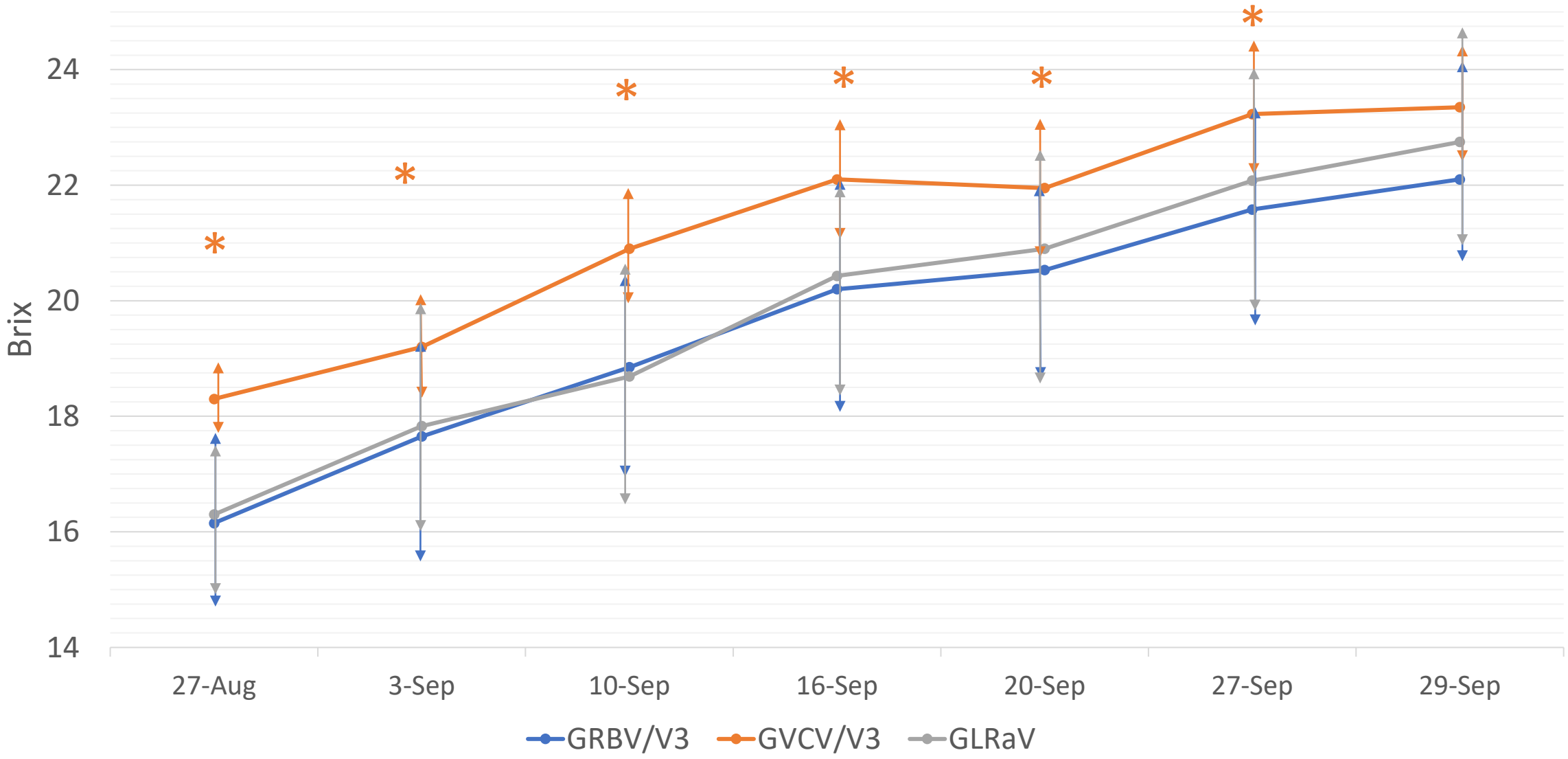
Brix Progression in individual Norton vines infected with GLRaV-3



Year 2 – What influence does GLRaV-3 have on GVCV and GRBV infections in Chardonel?

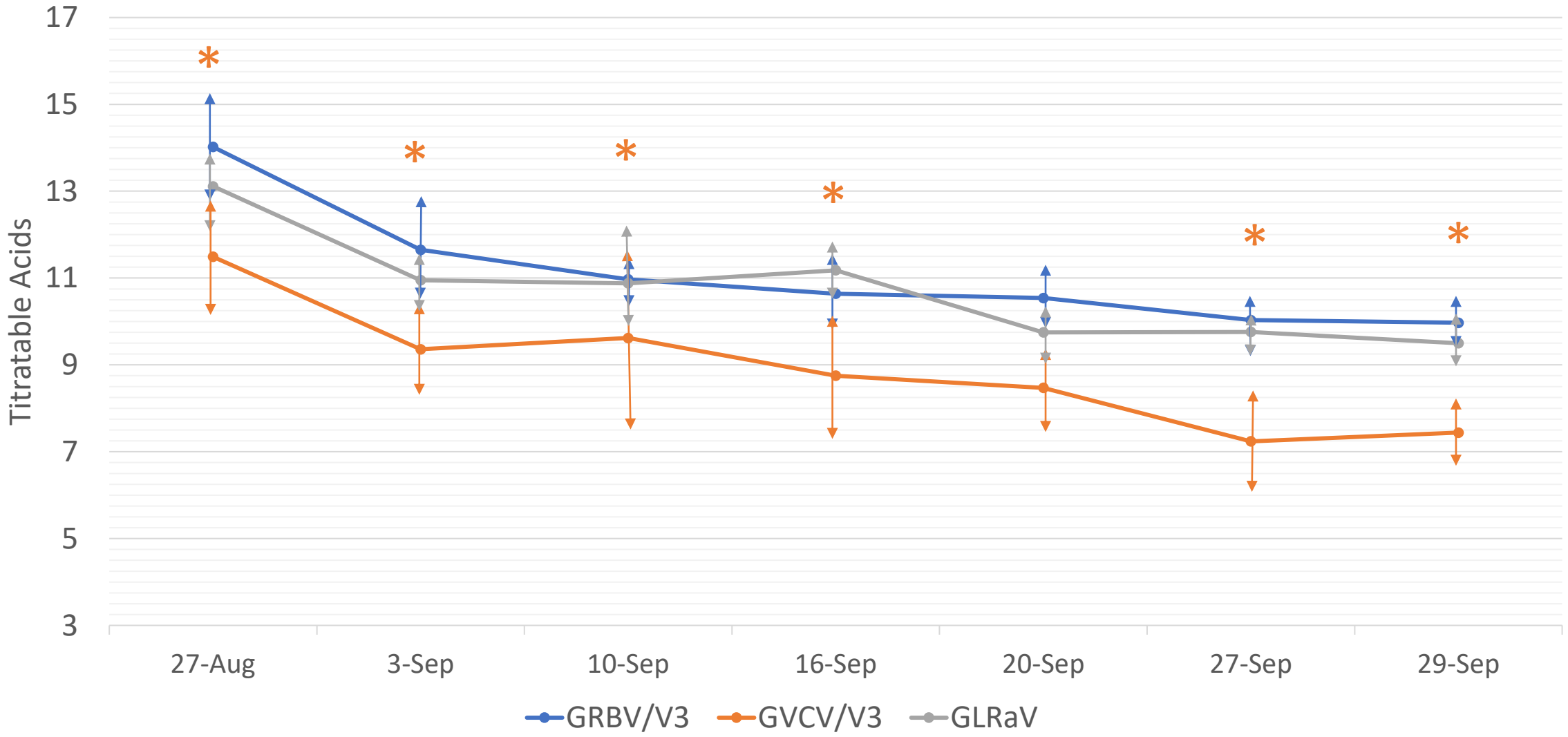


Chardonel Brix values from vines infected with GVCV/V3 were significantly higher than those from vines without GVCV



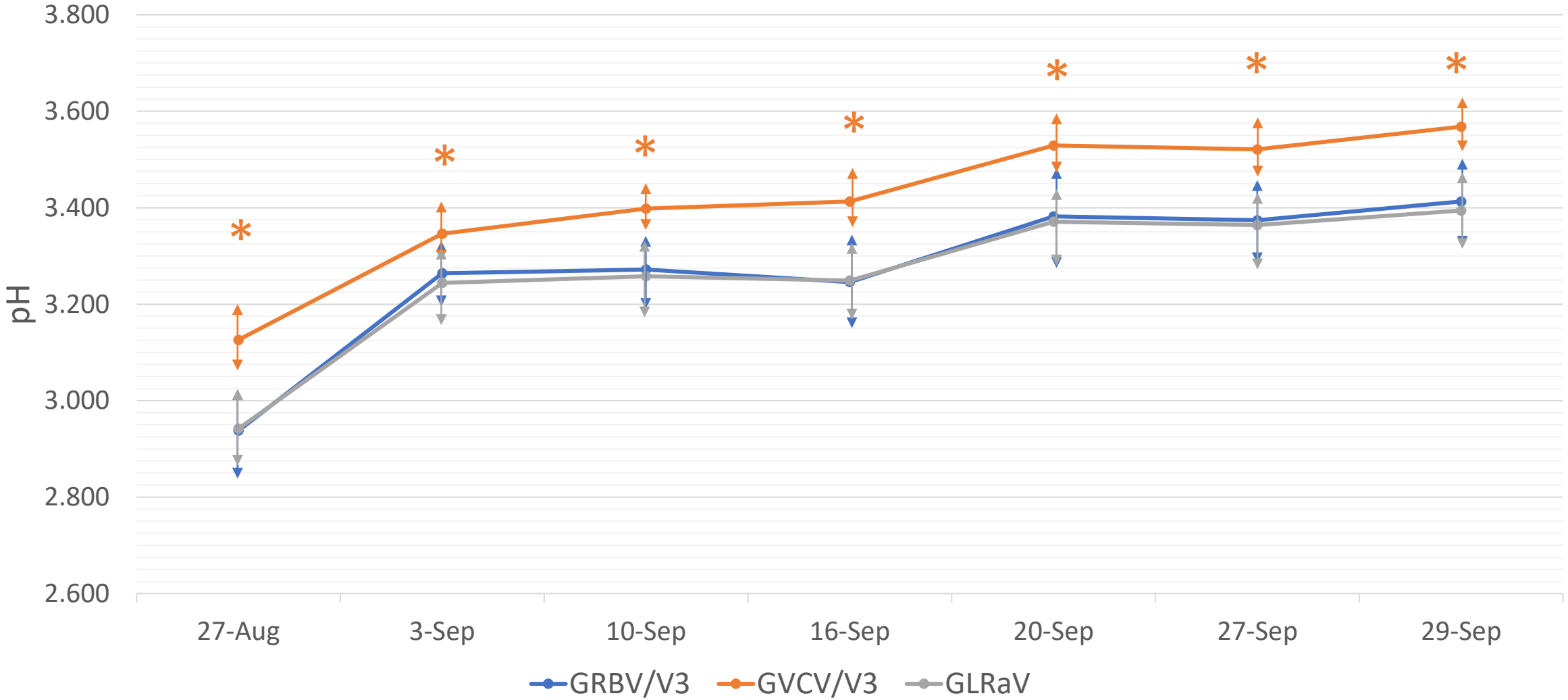
* Notes a significant difference at p < .05

Chardonel Titratable Acids from vines infected with GVCV/V3 were significantly lower than those from vines without GVCV



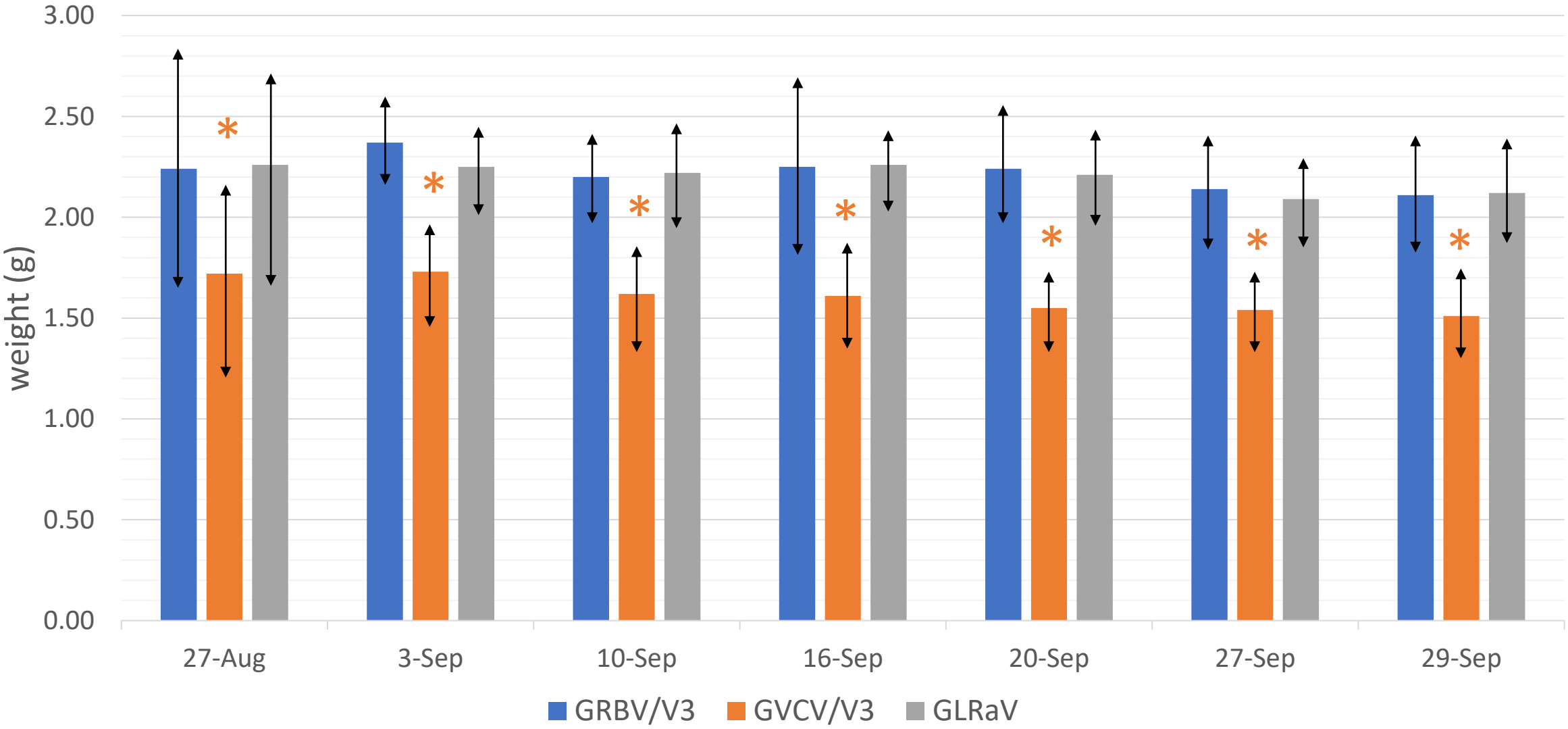
* Notes a significant difference at $p < .05$

Chardonel pH values from vines infected with GVCV/V3 were significantly higher than those from vines without GVCV



* Notes a significant difference at p < .05

Chardonel berry weights from vines infected with GVCV/V3 were significantly lower than those from vines without GVCV



* Notes a significant difference at p < .05