

Prevalence of Grapevine Vein Clearing Virus in Grape Aphid *Aphis illinoisensis*

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Grapevine vein clearing virus (GVCV) Symptoms

- Translucent vein clearing and berry deformation
- Stunting and eventual death of the vine
- Has caused the removal of several vineyard blocks



Grapevine vein clearing virus

- Prevalence
 - ~10% in *Vitis* spp.
 - ~34% in *Ampelopsis cordata*
- Vector
 - *Aphis illinoisensis* (Grape aphid)



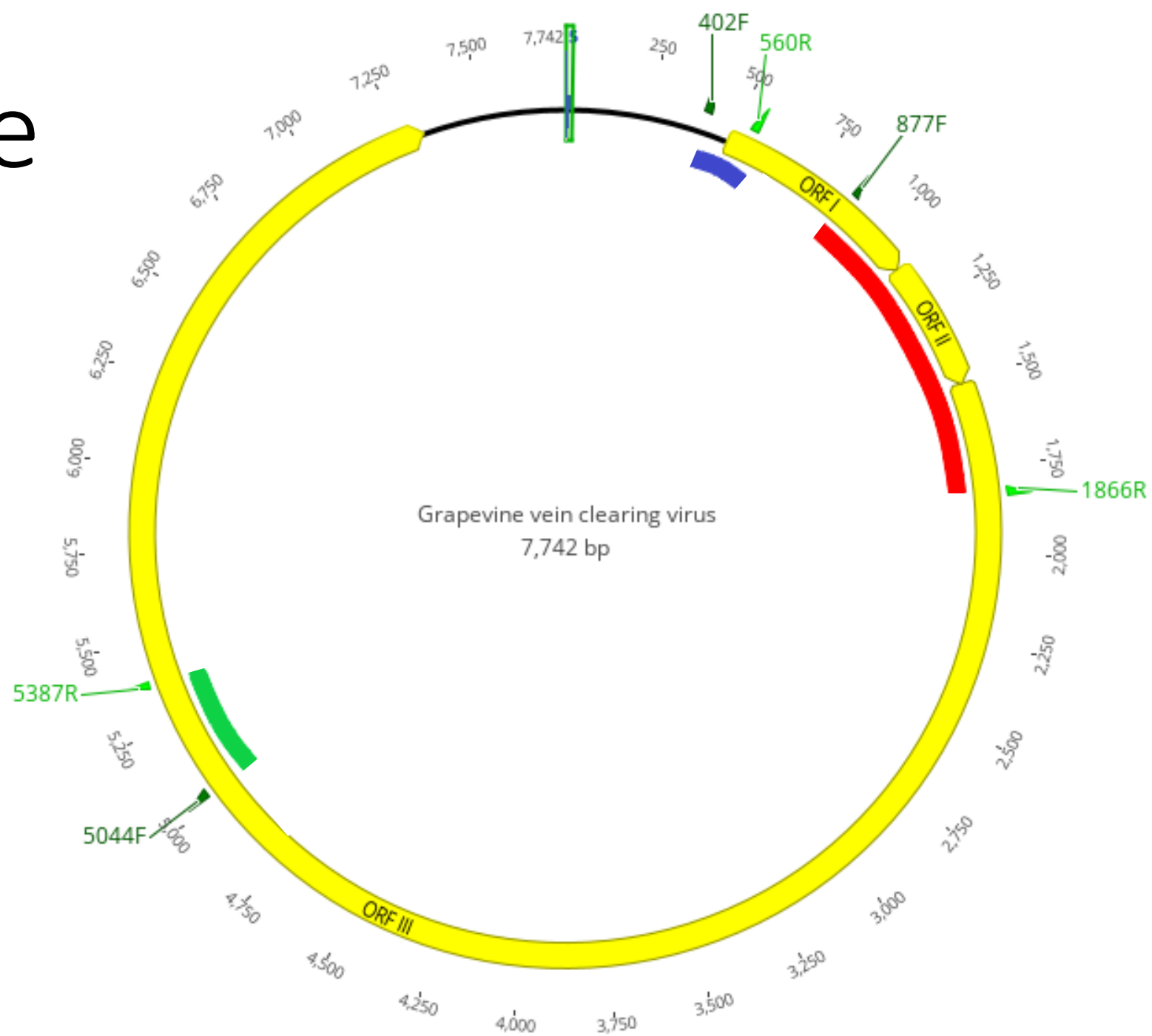
Aphid Transmitted Plant Viruses



- Aphids account for 50% of all insect vectored plant viruses (Brunt et al., 1996; Nault, 1997).
- Piercing-sucking mouthparts aid in delivering virions without much plant cell damage.
- Aphids can travel hundreds of miles with the aid of the Jetstream.

GVCV Genome

- qPCR primers
- Sequencing primers
ORF II
- GVCV detection primers



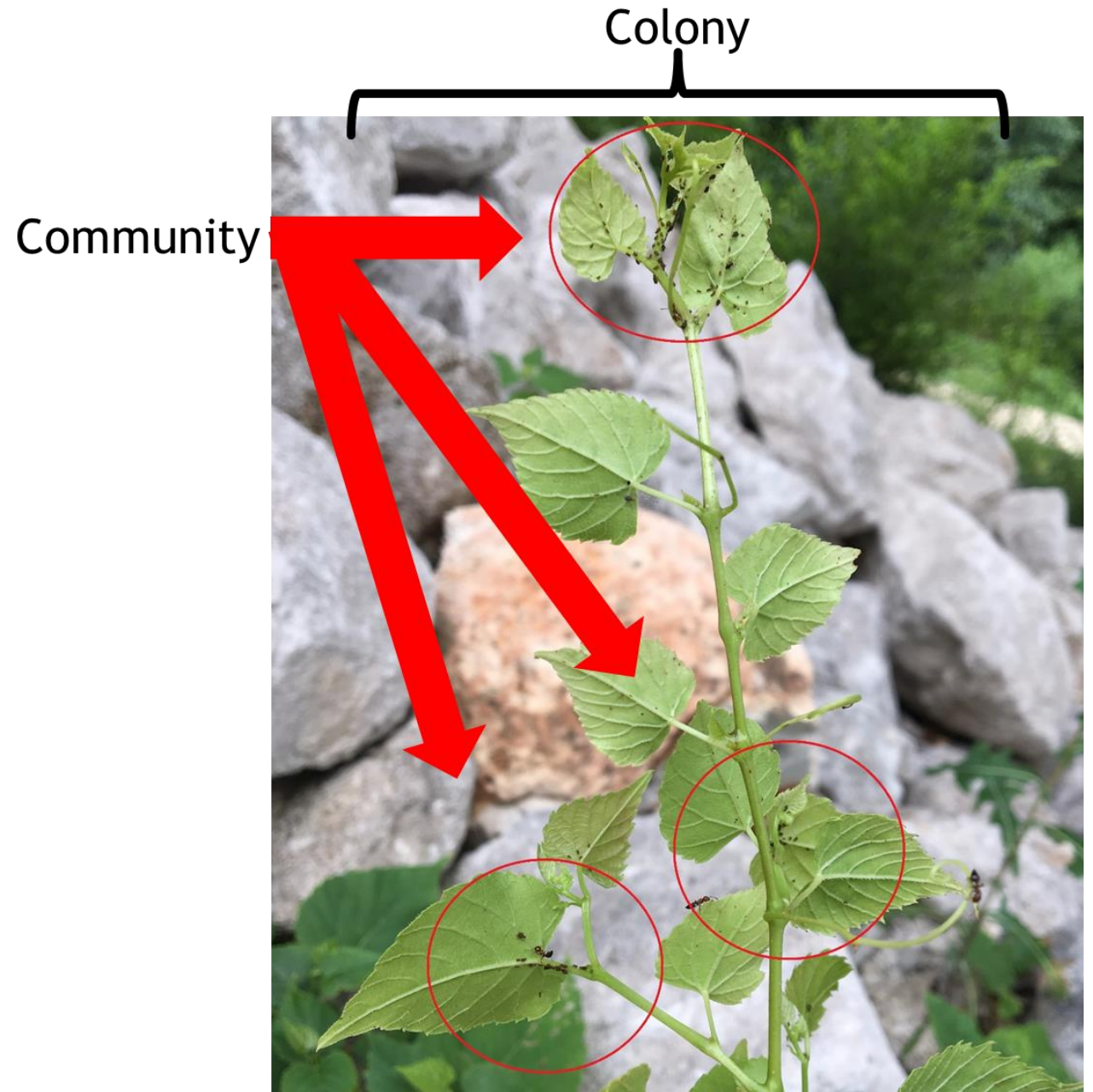
Questions

- What is the incidence of GVCV in grape aphids?
- What is the evidence supporting that grape aphids can transmit GVCV from wild Vitaceae plants to grape cultivars?
- What is the genetic structure of GVCV among plants and aphids?



Experimental design

- Collected grape aphids from wild Vitaceae plants.
- Tested for GVCV using PCR and qPCR.
- Conducted genetic analysis of GVCV based on the ORF II sequences.



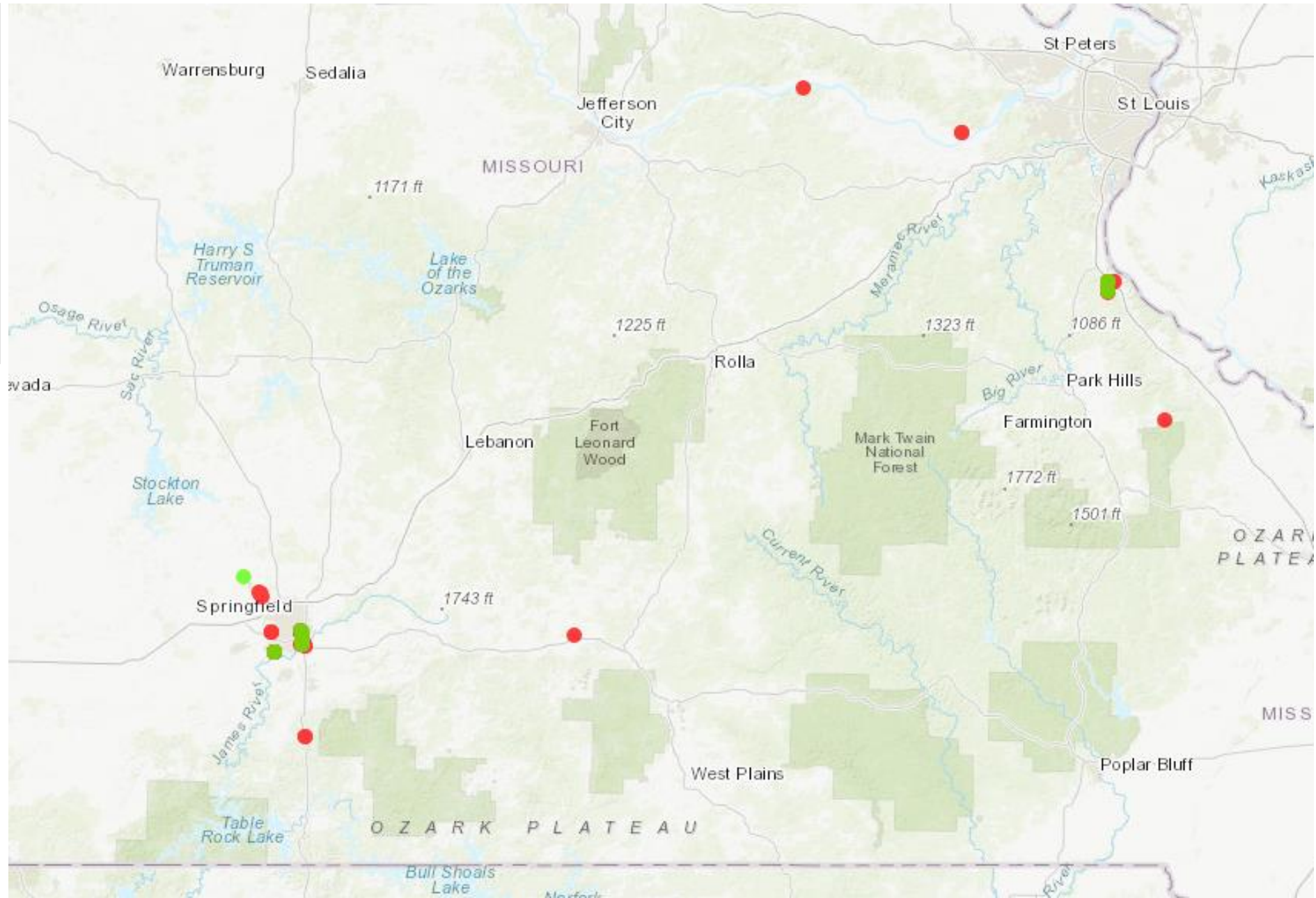
Collection sites

Legend

GVCV Negative



GVCV Positive



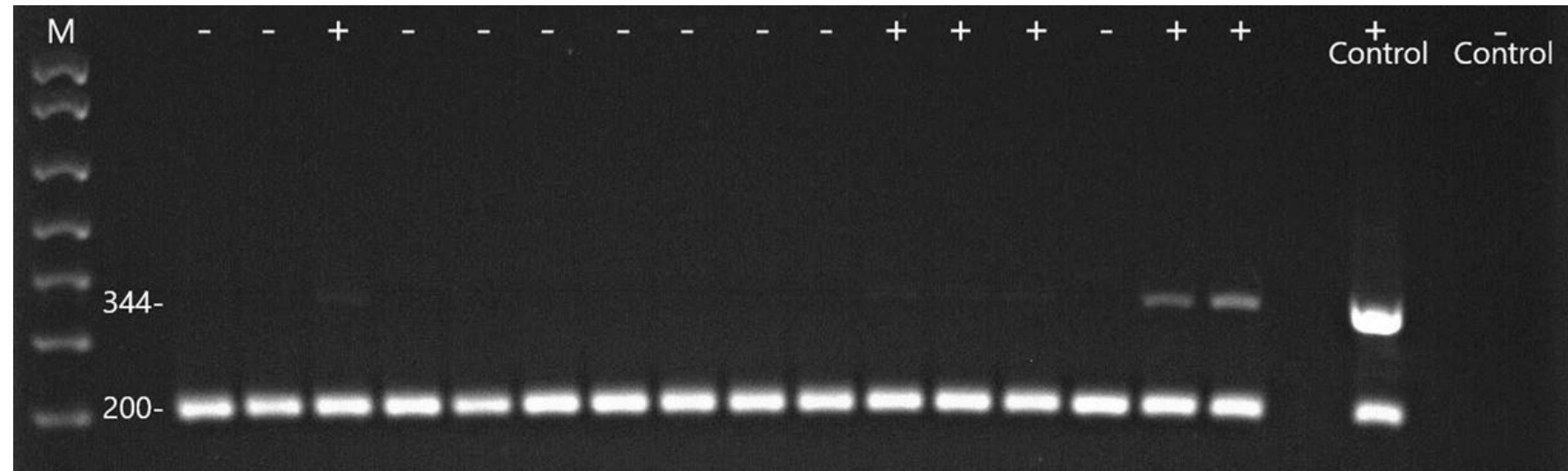
GVCV in aphid community

Missouri location (town)	Number of samples	GVCV Positive	Percentage
Springfield	52	43	83%
Battlefield	26	23	88%
Willard	2	0	0%
Saddlebook	2	2	100%
Mountain Grove	1	1	100%
Plattin	22	16	73%
Total	105	85	81%

Detection of GVCV in single grape aphids

- 50 ng of DNA used.

- Aphid EF and GVCV primer sets.



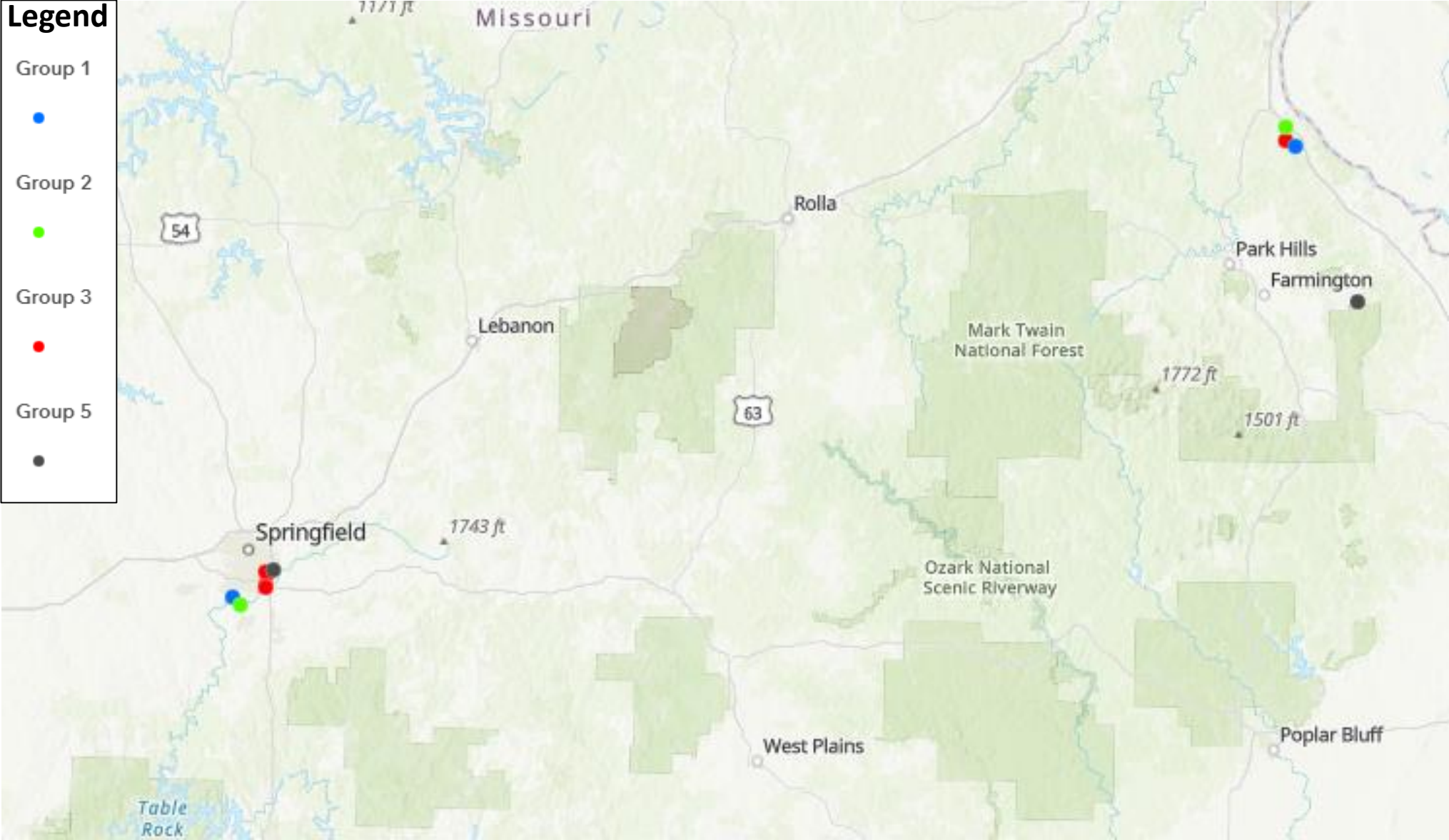
GVCV in single aphids

Missouri location (region, town)	Number of samples	GVCV Positive	Percentage
II, Springfield	194	79	41%
II, Battlefield	26	13	50%
II, Willard	10	3	30%
II, Saddlebook	1	1	100%
II, Mountain Grove	5	3	60%
II, Plattin	106	36	34%
<i>III, Herman</i>	12	5	42%
<i>I, Augusta</i>	26	8	31%
<i>II, Coffman</i>	75	29	39%
Total	455	177	39%

Percent identity matrix of 25 GVCV ORF II sequences from grape aphids

1. 18Aph023-3	100																								
2. 18Aph046-7	99.49	100																							
3. 18Aph001-1	87.02	87.02	100																						
4. 18Aph040-1	86.77	86.77	96.95	100																					
5. 18Aph023-2	89.82	90.33	89.31	89.82	100																				
6. 18Aphs006	92.03	92.03	91.26	91.52	92.29	100																			
7. 18Aph006-4	91.35	91.35	92.11	91.35	92.11	97.94	100																		
8. 17Aphs002	89.31	89.31	89.06	89.57	88.3	89.2	89.06	100																	
9. 18Aph032-2	88.28	88.28	91.93	92.45	89.58	92.63	93.23	87.76	100																
10. 19Aph18-12	88.54	88.54	90.89	91.67	89.58	91.84	92.19	87.5	95.83	100															
11. 19Aph18-13	88.54	88.54	90.89	91.67	89.58	91.84	92.19	87.5	95.83	100	100														
12. 19Aph18-19	88.54	88.54	90.89	91.67	89.58	91.84	92.19	87.5	95.83	100	100	100													
13. 19Aph18-25	88.54	88.54	90.89	91.67	89.58	91.84	92.19	87.5	95.83	100	100	100	100												
14. 18Aph015-2	90.89	90.89	90.1	89.84	88.54	89.21	89.58	90.62	92.45	92.71	92.71	92.71	92.71	100											
15. 18Aph032-4	90.36	90.36	89.58	89.32	89.06	90	89.32	90.62	91.41	91.67	91.67	91.67	91.67	98.44	100										
16. 18Aph076-5	89.84	89.84	89.58	89.32	89.06	89.47	88.8	90.1	90.89	91.15	91.15	91.15	91.15	97.92	98.96	100									
17. 18Aph018-1	88.28	88.28	85.42	86.98	85.94	88.68	87.5	85.94	88.02	88.28	88.28	88.28	88.28	91.15	91.67	91.67	100								
18. 18Aph011-1	89.06	89.06	86.2	87.76	86.72	89.47	88.28	86.72	88.8	89.06	89.06	89.06	89.06	91.93	92.45	92.45	99.22	100							
19. 18Aph026-1	89.06	89.06	86.2	87.76	86.72	89.47	88.28	86.72	88.8	89.06	89.06	89.06	89.06	91.93	92.45	92.45	99.22	100	100						
20. 18Aphs055	89.06	89.06	86.2	87.76	86.72	89.47	88.28	86.72	88.8	89.06	89.06	89.06	89.06	91.93	92.45	92.45	99.22	100	100	100					
21. 18Aph015-7	88.8	88.8	85.94	87.5	86.46	89.21	88.02	86.46	88.54	88.8	88.8	88.8	88.8	91.67	92.19	92.19	98.96	99.74	99.74	99.74	100				
22. 17Aphs001	89.32	89.32	88.54	89.06	86.2	89.47	88.28	88.28	90.1	89.84	89.84	89.84	89.84	92.19	92.19	92.19	92.97	93.75	93.75	93.75	93.49	100			
23. 18Aph056-4	88.8	88.8	87.5	88.28	85.42	89.47	87.5	87.5	90.1	89.84	89.84	89.84	89.84	92.97	92.97	92.97	92.71	93.49	93.49	93.49	93.23	94.53	100		
24. 18Aph105-7	89.32	89.32	88.02	89.06	87.76	90.79	89.06	88.02	89.84	90.62	90.62	90.62	90.62	92.71	93.23	93.23	93.49	94.27	94.27	94.27	94.01	93.49	94.79	100	
25. 19Aph18-21	89.06	89.06	87.76	88.8	87.5	90.53	88.8	88.02	89.58	90.36	90.36	90.36	90.36	92.45	92.97	92.97	93.23	94.01	94.01	94.01	93.75	93.23	94.53	99.74	100
	1. 18Aph023-3	2. 18Aph046-7	3. 18Aph001-1	4. 18Aph040-1	5. 18Aph023-2	6. 18Aphs006	7. 18Aph006-4	8. 17Aphs002	9. 18Aph032-2	10. 19Aph18-12	11. 19Aph18-13	12. 19Aph18-19	13. 19Aph18-25	14. 18Aph015-2	15. 18Aph032-4	16. 18Aph076-5	17. 18Aph018-1	18. 18Aph011-1	19. 18Aph026-1	20. 18Aphs055	21. 18Aph015-7	22. 17Aphs001	23. 18Aph056-4	24. 18Aph105-7	25. 19Aph18-21

Identical isolates



Identical and near identical GVCV variants

Groups with >99% matching nucleotides	GVCV positive sample	Missouri location (nearest town)	Furthest distance apart (km)	Host plant	Status of GVCV in host plant
1	18Aph46-7	Battlefield	283.1	18Amp21-5	-
	18Aph23-3	Plattin		18Vit035	-
2	18Aph032-4	Plattin	288.8	18Amp016	-
	18Aph076-5	Battlefield		18Vit056	+
3	18Aph011-1	Springfield	277.8	18Vit023	-
	18Aph018-1	Springfield		18Vit030	-
	18Aphs055*	Springfield		18Vit053	+
	18Aph015-7	Springfield		18Amp010	+
	18Aph026-1	Plattin		18Vit038	-
4	19Aph018-12	Coffman	0	19Amp006	+
	19Aph018-13	Coffman		19Amp006	+
	19Aph018-19	Coffman		19Amp006	+
	19Aph018-25	Coffman		19Amp006	+
5	19Aph018-21	Coffman	280.7	19Amp006	+
	18Aph105-7	Springfield		18Amp031	+

Detection of GVCV in grape aphid stylets and bodies

Aphid sample	Viral copies			
	Stylet		Body	
	Actual	Extrapolated	Actual	Extrapolated
1	7.44	42.51	106.60	609.14
2	22.84	130.51	123.70	706.86
3 Winged	73.81	421.77	89.18	509.60
4	28.60	163.43	137.00	782.86
5	*2.46	*14.06	97.46	556.91
6 Winged	7.25	41.43	382.90	2188.00
7 Winged	10.71	61.20	234.20	1338.29
8	126.50	722.86	23380.00	133600.00
9	8993.00	51388.57	299800.00	1713142.86
10	45600.00	260571.43	85620.00	489257.14
11	13.37	133.70	70.51	705.10
12	24.51	245.10	224.50	2245.00
13	76.18	761.80	25.84	258.40
14	7.98	79.80	37.24	372.40
15	46.68	466.80	82.50	825.00
16	14.12	141.20	*1.05	*10.5
17	66.55	665.50	112.70	1127.00
18	66.40	664.00	23.26	232.60
19	7.39	73.90	32.31	323.10
20	19.70	197.00	13.57	135.70
Avg.	2905.95	16682.76	21610.18	123627.16

Summary

- GVCV has been found in 39% of single grape aphids.
- Identical variants of GVCV have been found in grape aphids, wild and cultivated Vitaceae.
- GVCV is highly diverse among grape aphid populations.
- GVCV is found in both stylet and body, its quantity varies largely in individual aphids.



Thank You!

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