## Sustainable production: Start with clean plants and keep them clean





# Implementing new technologies for plant cleanliness validation





## Clean plants: the cornerstone of a productive farm





Nursery 'Virologist's paradise'

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Nursery 'Virologist's paradise'

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Nursery ' In clean plants we trust

#### Past:

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- Approach: Comparative study using plants with 'known' virome.
- High Throughput Sequencing (HTS) vs conventional diagnostics (PCR/ELISA) vs grafting

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Melon severe mosaic virus Recently discovered in the US

#### Setup

Crop		Sam			
NCGR	Spring	Fall	Spring	Fall	Total #
Fragaria (strawberry)	12	12	12	12	48
Rubus (black- /raspberry)	10	10	10	10	40
Vaccinium (blueberry)	10	8	11	9	38

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#### Setup

Crop		Sam	pling			Input files			
NCGR	Spring	Fall	Spring	Fall	Total #	fasta fastq sff			
Fragaria (strawberry)	12	12	12	12	48	trim both ends			
Rubus (black- /raspberry)	10	10	10	10	40	map to reference genome       Mapped_reads_host.fna         de novo assembly       Blastn_NON_VIRUS_reads.fna         Blastn_NON_VIRUS_report.tab			
Vaccinium (blueberry)	10	8	11	9	38	Blastn to GenBank nt Blastn_VIRUS_reads.fna Blastn_VIRUS_report.tab			

Collected all above ground tissue and ground in liquid N<sub>2</sub> in fine powder before shared between labs

HTS data analysis by VirFind (Ho and Tzanetakis 2014)

Blastx to GenBank virus proteins

Blastx\_VIRUS\_reads.fna

Reads with NO Blastn NO Blastx.fna

Reads\_with\_NO\_Blastn\_NO\_Blastx.faa

Conserved domain search report.txt

translate to amino acid

conserved domain search

Blastx\_VIRUS\_report.tab

		Standard protocol						rotocol <sup>c</sup>			
			H	ГS <sup>b</sup>			RT-	PCR			
Selection	Virus	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Biologic	al indexing
number	prome	2017	2017	2018	2018	2017	2017	2018	2018	2017	2018
Fragaria											
F1	SMYEV	-	-	5,945	5,950	-	-	SMYEV	SMYEV	No symptoms	No symptoms
	SVBV	-	-	1,824	-	-	-	SVBV	-		
	ToRSV	6,287	5,882	6,639	5,168	ToRSV	ToRSV	ToRSV	ToRSV		
F2	SPaV	1,158	1.730	7,932	7,962	SPaV	SPaV	SPaV	SPaV	SPaV or BPYV symptoms	No symptoms
	SVBV	_	_	639	_	-	-	SVBV	-		
	SMYEV	_	_	5,952	5.561	-	_	SMYEV	SMYEV		
	ToRSV	1.583	2.663	7.028	687	inc <sup>b</sup>	inc	ToRSV	inc		
	SCrV-3	1,453	1.253	2.421	2.573	nt <sup>c</sup>	nt	nt	nt		
	SCrV-4	1,256	2,554	2,844	2,505	nt	nt	nt	nt		
F3	BPYV	2.027	964	2,262	7.975	BPYV	BPYV	BPYV	BPYV	BPYV or SPaV and SNSV	SNSV
	SNSV	3,430	3 394	3 463	3 450	SNSV	SNSV	SNSV	SNSV		
	ToRSV	982	651	577	-	inc	inc	inc	_		
	TSV	-	-	1 477	222	nt	nt	nt	nt		
	FCILV	_	_	1,477	_	nt	nt	nt	nt		
F4	BPYV	1 386	3 800	2 200	8 054	BPYV	BPYV	BPYV	BPYV	SNSV symptoms	SNSV symptoms
	SNSV	1 091	1 266	-		SNSV	SNSV	_	_	<i>y</i> 1	
	SMoV	1,901	1,200	7.015	7.015	_	_	SMoV	SMoV		
	SVBV	_	_	477	7,015	_	_	SVBV	_		
	ToRSV	1 241	266	4//	_	inc	_	_	_		
	TSV	1,541	300	1,293	1 707	nt	nt	nt	nt		
	FCILV	_	_	2 400	1,787	nt	nt	nt	nt		
F5	SMYEV	2 457	2566	5,400	5.042	SMYEV	SMYEV	SMYEV	SMYEV	SMVEV symptoms	SMYEV symptoms
10	SNSV	3,457	2,500	5,990	5,943	SNSV	SNSV	_		Shire v Symptoms	Shirist symptoms
	ToRSV	190	120	161	_	ToRSV	inc	_	_		
	TSV	438	332	101	-	nt	nt	nt	nt		
E6	SVBV	2,886	3,450	3,466	3,495	SVBV	SVBV	SVBV	SVBV	SVRV symptoms	SVBV symptoms
10	SNSV	7,902	7,900	8,408	8,631	SNSV	SNSV	3101	3101	SVDV symptoms	SVDV Symptoms
	ToPSV	159	346	-	-	2142 4	2142.4	_	Topev		
F7	DDVV	510	302	216	3,741	- DDVV	- PDVV	- PDVV	10K3V	BDVV or SDaV symptoms	No symptoms
F/	SNEV	4,064	2,143	7,955	_	SNEW	SNEV	DF I V	-	briv of Srav symptoms	No symptoms
	SDeV	3,430	3,478	-	-	21/2 /	2142 4	SD-V	CD-V		
	SPAV	-	-	5,780	8,017	-	-	SPav	SPav		
	2ARA	-	-	-	598	_	_	-	2ARA		
	Nucleorhabdovirus- like	-	-	-	14,427	nt	nt	nt	nt		

Table 1. Summary of virus detection by high throughput sequencing and standard protocol

		Standard protocol <sup>e</sup>									
	-		H	ГS <sup>b</sup>			RT-	PCR			
Selection	Virus	Spring	Fall	Spring	Fall	Spring	Fall 2017	Spring	Fall	Biologic	al indexing
number	prome	2017	2017	2018	2010	2017	2017	2010	2010	2017	2018
Fragaria	$\frown$										
F1	SMYEV	-	-	5,945	5,950	-	-	SMYEV	SMYEV	No symptoms	No symptoms
	SVBV	-	-	1,824	-	-	-	SVBV	-		
	ToRSV	6,287	5,882	6,639	5,168	ToRSV	ToRSV	ToRSV	ToRSV		
F2	SPaV	1,158	1,730	7,932	7,962	SPaV	SPaV	SPaV	SPaV	SPaV or BPYV symptoms	No symptoms
	SVBV	-	-	639	-	-	-	SVBV	-		
	SMYEV	-	-	5,952	5,561	-	-	SMYEV	SMYEV		
	ToRSV	1,583	2,663	7,028	687	inc <sup>b</sup>	inc	ToRSV	inc		
	SCrV-3	1,453	1,253	2,421	2,573	nt <sup>c</sup>	nt	nt	nt		
	SCrV-4	1.256	2.554	2.844	2.505	nt	nt	nt	nt		
F3	BPYV	2.027	964	2.262	7.975	BPYV	BPYV	BPYV	BPYV	BPYV or SPaV and SNSV	SNSV
	SNSV	3,430	3.394	3.463	3,450	SNSV	SNSV	SNSV	SNSV		
	ToRSV	982	651	577	-	inc	inc	inc	_		
	TSV	-	-	1 477	222	nt	nt	nt	nt		
	FCILV	_	-	1,477	_	nt	nt	nt	nt		
F4	BPYV	4 386	3 800	2 200	8 054	BPYV	BPYV	BPYV	BPYV	SNSV symptoms	SNSV symptoms
	SNSV	1 081	1 266	-		SNSV	SNSV	_	_		71
	SMoV	1,901	1,200	7.015	7.015	_	_	SMoV	SMoV		
	SVBV	_	_	7,015	7,015	_	_	SVBV	_		
	ToRSV	1 241	266	4//	_	inc	_	_	_		
	TSV	1,541	300	1,293	1 707	nt	nt	nt	nt		
	FCII V			2 400	1,787	nt	nt	nt	nt		
E5	SMVEV	2 4 5 7		3,400		SMVEV	SMVEV	SMVEV	SMVEV	SMVEV symptoms	SMVEV symptoms
15	SNEV	3,457	2,566	5,996	5,943	SNSV	SNSV	SMIL	SMILY	Sivi i E V Symptoms	SWITE V Symptoms
	ToPSV	190	126	-	_	ToDEV	inc	_	_		
	TONSY	438	332	161	_	TORSV	me	-	-		
Eć	SVDV	2,886	3,450	3,466	3,495	evov	CUDU	CUDV	EVDV	SVDV	CVDV comptone
1.0	SVBV	7,902	7,900	8,408	8,631	SVBV	SVBV	2484	SVBV	SVBV symptoms	SVBV symptoms
	5N5V	159	346	-	-	2N2A	2N2A	-	-		
127	TORSV	510	302	216	3,741	-	-	-	TORSV	DDVU OD V	
F7	BPYV	4,064	2,143	7,955	-	BPYV	BPYV	BPYV	-	BPYV or SPaV symptoms	No symptoms
	SNSV	3,430	3,478	-	-	SNSV	SNSV	-	-		
	SPaV	-	-	5,780	8,017	-	-	SPaV	SPaV		
	SVBV	-	-	-	598	-	-	-	SVBV		
	Nucleorhabdovirus- like	-	-	-	14,427	nt	nt	nt	nt		

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							St	rotocol <sup>c</sup>				
	-		H	ITS <sup>b</sup> RT-PCR								
Selection	Virus	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Biological indexing		
number	profile <sup>a</sup>	2017	2017	2018	2018	2017	2017	2018	2018	2017	2018	
R4	RBDV	2,810	ns	1,950	5,487	RBDV	ns <sup>c</sup>	RBDV	RBDV	No symptoms	No symptoms	
R5	RLMV	1,076	2,538	16,280	2,381	RLMV	RLMV	RLMV	RLMV	ToRSV symptoms on	ToRSV symptoms on source	
	ToRSV	2,970	2,772	7,448	7,640	ToRSV	ToRSV	ToRSV	ToRSV	source plant Willamette, mottle on Munger	on Munger	
	RBDV	200	191	3,259	-	RBDV	RBDV	inc	-	monte en manger	en manger	
	Alphaflexivirus-like	-	578	4,148	2,732	nt	nt	nt	nt			
R6	ToRSV	413	930	-	-	ToRSV	ToRSV	-	-	No symptoms	No symptoms	
	RBDV	4,492	393	772	5,459	RBDV	inc	RBDV	inc			
R7	ToRSV	428	2,482	-	290	ToRSV	ToRSV	-	ToRSV	No symptoms	No symptoms	
	RBDV	234	150	255	-	-	RBDV	RBDV	-			
R8	BYVaV	2,394	634	7,894	7,955	BYVaV	BYVaV	BYVaV	BYVaV	No symptoms	No symptoms	
	RBDV	285	-	961	_	RBDV	-	RBDV	-			
	ToRSV	208	127	420	623	inc	inc	ToRSV	ToRSV			
	Coguvirus-like	6,663	6,654	6.686	6,667	nt	nt	nt	nt			
Vaccinium		-,			-,							
V1	BSSV	4.075	3.802	3.055	2.876	BSSV	BSSV	BSSV	BSSV	n/a <sup>c</sup>	n/a	
	BBLV	210	130	1,165	1.873	BBLV	BBLV	BBLV	BBLV			
V2	BSSV	1.340	ns	129	2.874	BSSV	ns	BSSV	BSSV	n/a	n/a	
	BlMaV	1.445	ns	=	=	BIMaV	ns	-	-			
	BBLV	394	ns	887	1.236	BBLV	ns	BBLV	BBLV			
	Carlavirus-like	8.524	ns	8.520	8.536	nt	ns	nt	nt			
	Luteovirus-like	917	ns	-	-	nt	ns	nt	nt			
V3	BFDaV	(9%)	ns	327	426	BFDaV	ns	BFDaV	BFDaV	n/a	n/a	
V4	BlMaV	4 708	1 527	7 940	7 966	BlMaV	BlMaV	BlMaV	BlMaV	n/a	n/a	
V5	BBLV	-	-	219	289	-	_	BBLV	BBLV	n/a	n/a	
	BSSV	514	645	161	351	BSSV	BSSV	BSSV	BSSV			
V6	BFDaV	9.839	135	- 101	9777	BFDaV	BFDaV	_	BFDaV	n/a	n/a	
	BlMaV	4 409	7 954	_	7 983	BIMaV	BlMaV	_	BlMaV			
	BlScV	-	-	8 596	939	_	_	_	_			
	BBLV	_	_	406	522	_	_	BBLV	BBLV			
	BGMaV	_	_	813	1 314	nt	nt	nt	nt			
	Ampelovirus-like	_	_	766	454	nt	nt	nt	nt			
V7	BlShV	3,240	ns	3,334	3,009	Missed shock	ns	BlShV	BlShV	n/a	n/a	
	Luteovirus-like	_	ns	_	628	nt	ns	nt	nt			
V8	BlScV	_	_	771	-	_	_	_	_	n/a	n/a	
	BBLV	_	200	1 201	405	_	BBLV	BBLV	BBLV			
	BGMaV	767	-	-		nt	nt	nt	nt			
V9	BRRSV	(32%)	8 312	8 435	8 306	BRRSV	BRRSV	BRRSV	BRRSV	n/a	n/a	
	BlShV	1 040	-	-	-	nt	nt	nt	nt			
V10	BlScV	203	ns	8 974	50		ns	_	ns	n/a	n/a	
		293		0,9/4	ns							

		Standard protocol							rotocol <sup>c</sup>		
	-		H	ГS <sup>b</sup>		RT-PCR					
Selection	Virus	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Biologic	al indexing
number	profile <sup>a</sup>	2017	2017	2018	2018	2017	2017	2018	2018	2017	2018
R4	RBDV	2,810	ns	1,950	5.487	RBDV	ns <sup>c</sup>	RBDV	RBDV	No symptoms	No symptoms
R5	RLMV	1.076	2.538	16.280	2.381	RLMV	RLMV	RLMV	RLMV	ToRSV symptoms on	ToRSV symptoms on source
	ToRSV	2,970	2,772	7,448	7.640	ToRSV	ToRSV	ToRSV	ToRSV	source plant Willamette,	plant Willamette, mottle
	RBDV	200	191	3,259	_	RBDV	RBDV	inc	-	mode on Munger	on Munger
	Alphaflexivirus-like	_	578	4.148	2.732	nt	nt	nt	nt		
.6	ToRSV	413	930	=		ToRSV	ToRSV	-	-	No symptoms	No symptoms
	RBDV	4.492	393	772	5,459	RBDV	inc	RBDV	inc		
7	ToRSV	428	2.482	_	290	ToRSV	ToRSV	-	ToRSV	No symptoms	No symptoms
	RBDV	234	150	255	-	-	RBDV	RBDV	-		
8	BYVaV	2.394	634	7.894	7.955	BYVaV	BYVaV	BYVaV	BYVaV	No symptoms	No symptoms
	RBDV	285	-	961	-	RBDV	_	RBDV	_ `		
	ToRSV	205	127	420	623	inc	inc	ToRSV	ToRSV	$\mathbf{X}$	
	Coguvirus-like	6.663	6.654	6.686	6.667	nt	nt	nt	nt		
accinium		0,000	0,004	0,000	0,007						
1	BSSV	4.075	3 802	3.055	2 876	BSSV	BSSV	BSSV	BSSV	n/a <sup>c</sup>	n/a
	BBLV	210	130	1 165	1 873	BBLV	BBLV	BBLV	BBLV		
2	BSSV	1 340	ns	1,105	2 874	BSSV	ns	BSSV	BSSV	n/a	n/a
	BlMaV	1,540	ns		2,074	BlMaV	ns	-	_		
	BBLV	204	ns	007	1 226	BBLV	ns	BBLV	BBLV		
	Carlavirus-like	8 524	ns	8 520	9 536	nt	ns	nt	nt		
	Luteovirus-like	0,524	ns	0,520	a,530 -	nt	ns	nt	nt		
3	BFDaV	(0%)	ns	327	126	BFDaV	ns	BFDaV	BFDaV	n/a	n/a
4	BlMaV	(9%)	1 527	7.940	7 066	BlMaV	BlMaV	BIMaV	BlMaV	n/a	n/a
5	BBLV	4,708	1,527	210	7,900	_	_	BBLV	BBLV	n/a	n/a
	BSSV	514	615	219	209	BSSV	BSSV	BSSV	BSSV		
6	BFDaV	0.820	125	101	0 777	BFDaV	BFDaV	_	BFDaV	n/a	n/a
-	BIMaV	9,639	7 054	_	7,092	BIMaV	BIMaV	_	BlMaV		
	BIScV	4,409	7,954	9 506	7,985	_	_	_	_		
	BBLV	_	_	8,590	939	_	_	BBLV	BBLV		
	BGMaV	_	_	400	1 214	nt	nt	nt	nt		
	Ampelovirus-like	_	_	815	1,514	nt	nt	nt	nt		
7	BIShV	2.240	ns	2 2 2 4	454	Missed shock	ns	BIShV	BIShV	n/a	n/a
,	DIDIT	5,240	115	3,334	3,009	testing	115	DIDITY	DIDIT	10 4	10.00
	Luteovirus-like	-	ns	-	628	nt	ns	nt	nt		
8	BIScV	-	_	771	_	_	-	-	-	n/a	n/a
	BBLV	-	200	1,201	495	-	BBLV	BBLV	BBLV		
	BGMaV	767	-	-	-	nt	nt	nt	nt		
/9	BRRSV	(32%)	8,312	8,435	8,396	BRRSV	BRRSV	BRRSV	BRRSV	n/a	n/a
	BlShV	1,949	-	-	-	nt	nt	nt	nt		
10	BlScV	293	ns	8,974	ns	-	ns	-	ns	n/a	n/a

#### Is a single HTS test the solution?

 Table 2. Parallel detection of known berry viruses by high throughput sequencing and reverse transcription PCR

			H	ſS <sup>b</sup>		RT-PCR <sup>b</sup>			
Sample	Viruses <sup>a</sup>	Spring 2017	Fall 2017	Spring 2018	Fall 2018	Spring 2017	Fall 2017	Spring 2018	Fall 2018
Detecti	on in all s	ampling	point	s					
F2	SPaV	+	+	+	+	+	+	+	+
F3	BPYV	+	+	+	+	+	+	+	+
	SNSV	+	+	+	+	+	+	+	+
F4	BPYV	+	+	+	+	+	+	+	+
F5	SMYEV	+	+	+	+	+	+	+	+
F6	SVBV	+	+	+	+	+	+	+	+
F8	BPYV	+	+	+	+	+	+	+	+
	SMoV	+	+	+	+	+	+	+	+
F9	BPYV	+	+	+	+	+	+	+	+
R1	RBDV	+	+	+	+	+	+	+	+
	RLMV	+	+	+	+	+	+	+	+
R2	RBDV	+	+	+	+	+	+	+	+
	RLMV	+	+	+	+	+	+	+	+
	RpLV	+	+	+	+	+	+	+	+
R3	BNRV	+	+	+	+	+	+	+	+
	RBDV	+	+	+	+	+	+	+	+
	SNSV	+	+	+	+	+	+	+	+
R4	RBDV	+	nsc	+	+	+	ns	+	+
R5	RLMV	+	+	+	+	+	+	+	+
	ToRSV	+	+	+	+	+	+	+	+
R8	BYVaV	+	+	+	+	+	+	+	+
V1	BSSV	+	+	+	+	+	+	+	+
	BBLV	+	+	+	+	+	+	+	+
V2	BBLV	+	ns	+	+	+	ns	+	+
	BSSV	+	ns	+	+	+	ns	+	+
V4	BlMaV	+	+	+	+	+	+	+	+
V5	BSSV	+	+	+	+	+	+	+	+
V9	BRRSV	+	+	+	+	+	+	+	+

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Sample	Viruses <sup>a</sup>	Spring 2017	Fall 2017	Spring 2018	Fall 2018	Spring 2017	Fall 2017	Spring 2018	Fall 2018
Detecti	on in all s	ampling	point	s					
F2	SPaV	+	+	+	+	+	+	+	+
F3	BPYV	+	+	+	+	+	+	+	+
	SNSV	+	+	+	+	+	+	+	+
<b>F</b> 4	BPYV	+	+	+	+	+	+	+	+
F5	SMYEV	+	+	+	+	+	+	+	+
F6	SVBV	+	+	+	+	+	+	+	+
F8	BPYV	+	+	+	+	+	+	+	+
	SMoV	+	+	+	+	+	+	+	+
F9	BPYV	+	+	+	+	+	+	+	+
R1	RBDV	+	+	+	+	+	+	+	+
	RLMV	+	+	+	+	+	+	+	+
R2	RBDV	+	+	+	+	+	+	+	+
	RLMV	+	+	+	+	+	+	+	+
	RpLV	+	+	+	+	+	+	+	+
R3	BNRV	+	+	+	+	+	+	+	+
	RBDV	+	+	+	+	+	+	+	+
	SNSV	+	+	+	+	+	+	+	+
R4	RBDV	+	nsc	+	+	+	ns	+	+
R5	RLMV	+	+	+	+	+	+	+	+
	ToRSV	+	+	+	+	+	+	+	+
R8	BYVaV	+	+	+	+	+	+	+	+
V1	BSSV	+	+	+	+	+	+	+	+
	BBLV	+	+	+	+	+	+	+	+
V2	BBLV	+	ns	+	+	+	ns	+	+
	BSSV	+	ns	+	+	+	ns	+	+
V4	BlMaV	+	+	+	+	+	+	+	+
V5	BSSV	+	+	+	+	+	+	+	+
V9	BRRSV	+	+	+	+	+	+	+	+

			HI	'S <sup>b</sup>		RT-PCR <sup>b</sup>			
Sample	Viruses <sup>a</sup>	Spring 2017	Fall 2017	Spring 2018	Fall 2018	Spring 2017	Fall 2017	Spring 2018	Fall 2018
Detec	ction in so	ne sam	pling	points	-	-	-	-	
FI	SMYEV	-	P	+	+	_	_	. +	
	SVBV	_	_	+	_	_	_	. +	
F2	SMYEV	_	_	+	+	_	_	. +	
	SVBV	_	_	+	_	_	_	+	
F4	SNSV	+	+	_	_	+	+		
	SMoV	_	_	+	+	_	-	+	
	SVBV	-	_	+	-	-	-	+	
F5	SNSV	+	+	-	-	+	+	· _	
F6	SNSV	+	+		_	+	+	· _	
F7	BPYV	+	+	+	_	+	+	• +	
	SNSV	+	+	-	-	+	+	• –	
	SPaV	-	-	+	+	-	-	+	· ·
	SVBV	-	-	-	+	-	-	· _	
F8	SNSV	+	+	-	+	+	+	· –	
F9	SMoV	+	+	+	-	+	+	• +	
F10	SVBV	-	-	+	+	-	-	+	-
R6	ToRSV	+	+	-	-	+	+	• –	
R7	ToRSV	+	+	-	+	+	+	· –	
R8	RBDV	+	-	+	-	+	-	+	
V2	BlMaV	+	ns	s –	-	+	n	s –	
V5	BBLV	-	-	+	+	-	-	+	
V6	BlMaV	+	+	-	+	+	+		
	BBLV	-	-	+	+	-	-	• +	
	BFDaV	+	+	-	+	+	+		-
V8	BBLV	-	+	+	+	-	+	• +	

#### Our conventional diagnostics need help...

RT-PCR<sup>b</sup> HTSb **Current primers** New primers Spring Fall Spring Fall Spring Fall Fall Spring Fall Fall Spring Spring Viruses<sup>a</sup> 2017 2018 2017 2018 2017 2018 2017 2018 2018 2017 2017 2018 Sample BIScV V6 + + + + -\_ \_ **V8** + + ---\_ \_ nsb V10 ns + ns + ns ns + ns + -\_ incc RBDV R5 + + + + + + + + -**R6** inc inc + + + + + + + + + + **R7** + + + + + + + \_ -\_ RYNV **R1** + + + + + + + \_ \_ R2 + + + + + + + \_ \_ -\_ SCV F9 + + + + + + + + -\_ \_ -ToRSV **R**8 inc + + + inc + + + + + \_ \_ F1<sup>d</sup> + + + + + + + + + + + + F2<sup>d</sup> inc inc inc + + + + + + + + + F3<sup>d</sup> inc + + inc inc + + + + -\_ \_ F4<sup>d</sup> inc + + + + + + -\_ \_ F5<sup>d</sup> + + inc + + + + + -\_ F6<sup>d</sup> + + + + + + + + + \_ \_ F8<sup>d</sup> + + + + + + + + \_ \_ \_ \_ F9<sup>d</sup> + + inc + + + + + -\_ \_  $F10^d$ + + + inc inc inc + + + + +

Table 3. Comparative detection of known viruses by high throughput sequencing and reverse transcription PCR using current and new primers

#### Our conventional diagnostics need help...

RT-PCR<sup>b</sup> HTSb **Current primers** New primers Fall Fall Spring Fall Spring Spring Fall Spring Fall Fall Spring Spring Viruses<sup>a</sup> 2017 2018 2017 2018 2017 2018 2018 2017 2017 2018 2017 2018 Sample BIScV V6 + + + + -\_ \_ **V8** + + ---\_ \_ ns<sup>b</sup> V10 ns + ns + ns ns + ns + incc RBDV R5 + + + ++ + + + -**R6** inc inc + + + + ++ + + + + **R7** + + + + + + \_ -RYNV **R1** + + + + + + + \_ \_ R2 + + + + + + + \_ \_\_\_\_ SCV F9 + + + + + + + + ToRSV **R**8 inc inc + + + ++ + + + \_ \_ F1<sup>d</sup> + ++ + + ++++ + + + F2<sup>d</sup> inc inc inc + + + + ++ + + + F3<sup>d</sup> inc + + inc + + inc + + -\_ F4<sup>d</sup> inc + + + + + + -F5<sup>d</sup> + + +inc + + + + -F6<sup>d</sup> + + + + + + + + + F8<sup>d</sup> + + + ++ + + + \_ \_ \_ F9<sup>d</sup> inc + + + + + + + \_  $F10^d$ + + + inc inc inc + + + + +

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Table 3. Comparative detection of known viruses by high throughput sequencing and reverse transcription PCR using current and new primers

#### Why PCR is not always reliable



#### Why PCR is not always reliable



Consensus CTTGAAGGAGCACGAAAAGCACCTGAGCATCATGCTTGGGATATGTCGA



RYNV-6R (Diaz-Lara et al., 2020): GGAAGAAATCTGCGCTTACG

RYNV\_NC\_026238.1 GACTCAGCAAGCAAGGAAGAAATCTGCGCTTACGCCAGTGGTAAATTCC

Consensus GACTCAGCTGGCAAGGAAGAAATCTGCGCTTACGCAAGCGGTAAGTTC(



M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10
R5: RBDV current primers M 1 2 3 4 5 6 7 8 9 10	R5: RBDV new primers M 1 2 3 4 5 6 7 8 9 10	F5: ToRSV current primers M 1 2 3 4 5 7 8 9 10	F5: ToRSV new primers M 1  2  3  4  5  7  8  9  10	V6: BIScV new primers M 1 2 3 4 5 6 7 8 9 10
R6: RBDV current primers M 1 2 3 4 5 6 7 8 9 10	R6: RBDV new primers M 1 2 3 4 5 6 7 8 9 10	F6: ToRSV current primers	F6: ToRSV new primers	V8: BIScV new primers M 1 3 7 9 10
R7: RBDV current primers	R7: RBDV new primers	F8: ToRSV current primers	F8: ToRSV new primers	V10: BIScV new primers
F2: ToRSV current primers	F2: ToRSV new primers	F9: ToRSV current primers	F9: ToRSV new primers	R1: RYNV new primers
F3: ToRSV current primers	F3: ToRSV new primers	F10: ToRSV current primers	F10: ToRSV new primers	R2: RYNV new primers
F4: ToRSV current primers	F4: ToRSV new primers	R8. ToRSV current primers	R8: ToRSV new primers	F9: SCV new primers

#### Why PCR is not always reliable





Consensus GACTCAGCTGGCAAGGAAGAAATCTGCGCTTACGCAAGCGGTAAGTTCC



Ν	A 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10
	R5: RBDV current primers	R5: RBDV new primers	F5: ToRSV current primers	F5: ToRSV new primers	V6: BIScV new primers
Ν	A 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 7 8 9 10	M 1 2 3 4 5 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10
	R6: RBDV current primers	R6: RBDV new primers	F6: ToRSV current primers	F6: ToRSV new primers	V8: BIScV new primers
	VI 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 3 7 9 10
1		ㅋㅋㅋㅋ ㅋㅋ ㅋㅋ ㅋㅋ		Ξ 및 및 및 이 및 및 및 및 및 및 및 및 및 및 및 및 및 및 및	-
	R7: RBDV current primers	R7: RBDV new primers M 1 2 3 4 5 6 7 8 9 10	F8: ToRSV current primers	F8: ToRSV new primers	V10: BIScV new primers
	F2: ToRSV current primers	F2: ToRSV new primers	EQ: ToRSV current primers	EQ: ToRSV new primers	P1: PVNV pow primors
Ν	И 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 9 10	M 1 2 3 4 5 6 7 8 10 10
1111	=	seel Hell H			
				三下可使规定出现的目的	
	F3: ToRSV current primers	F3: ToRSV new primers	F10: ToRSV current primers	F10: ToRSV new primers	R2: RYNV new primers
Ĺ					
			=		
		그렇던 병을 한 명령 모두 물고			
- 18	4: TOKSV current primers	F4: LORSV new primers	R8: TORSV current primers	R8: TORSV new primers	F9: SCV new primers





#### Comparative study using plants with 'known' virome

			Percent identity	HTS <sup>a</sup>	longes len	t virus co gth	ontig		RT-I	PCR <sup>a</sup>	
Selectio	on BLASTX virus	Virus genus	(BLASTX e-value of longest contig)	Spring 2017	Fall 2017	Spring 2018	Fall 2018	Spring 2017	Fall 2017	Spring 2018	Fall 2018
R5	Euonymus yellow vein associated virus	Unclassified alphaflexivirus	56 (0)	_ <sup>b</sup>	578	4,148	2,732	+ <sup>b</sup>	+	+	+
V6	Grapevine leafroll- associated virus 3	Ampelovirus	58 (3e-16)	-	-	766	454	-	-	+	+
V2	Blueberry scorch virus	Carlavirus	$69^{\rm c}(0)$	8,524	ns <sup>a</sup>	8,520	8,536	+	ns	+	+
R8	Citrus concave gum- associated virus	Coguvirus	58 (0)	6,683	6,654	6,686	6,667	+	+	+	+
V2	Nectarine stem pitting- associated virus	Luteovirus	71 (4e-143)	917	ns	_b	-	+	ns	+ <sup>b</sup>	-
V7	Nectarine stem pitting- associated virus	Luteovirus	76 (3 e-72)	-	-	-	628	-	-	-	+
F7	Apple rootstock virus A	Unclassified nucleorhabdovirus	35 (0)	-	-	-	14,427	-	-	-	+

Table 4. Novel virus discovery by high throughput sequencing and confirmation by reverse transcription PCR

 Comprehensive analysis: Simultaneously tests for multiple pathogens, including newly emerging or unknown ones.

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- Unbiased: Pathogen **diversity** is not important
- Speed and efficiency: Processes samples quickly, providing faster results than traditional techniques.
- Accuracy and sensitivity: Can detect low levels of pathogen presence, identifying diseases before symptoms appear.

#### Summary

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- HTS provided superior detection compared to RT-PCR on a wide spectrum of virus variants and discovery of novel viruses.
- In most cases where the two protocols showed parallel virus detection, 11 viruses in 16 berry selections were not consistently detected by both methods at all sampling points.
- We now require a four sampling times/two-year testing for berry crops to ensure that no virus remains undetected independent of titer, distribution or other virus/virus or virus/host interactions

# Beware of the eriophyid mites



DIVISION OF AGRICULTURE

#### Blackberry yellow vein disease

• Major issue in the SE United Stated

#### Blackberry yellow vein disease

• Major issue in the SE United Stated





#### Blackberry yellow vein disease

• Major issue in the SE United Stated





 Tested for known viruses (RBDV, TRSV, etc). Several viruses were found but none consistently associated with symptoms



Oak-leaf pattern



**Chlorotic spots** 



Ringspots



Mosaic



Vein banding



Vein banding and chlorosis



#### New viruses in Rubus in last 20 years

Sixteen (16) viruses and virus-like agents were known to infect *Rubus* in 2004 - 2023: **45+** 

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#### New Rubus viruses

blackberry yellow vein virus blackberry virus E blackberry virus X blackberry virus Y blackberry vein banding virus beet pseudo yellows virus blackberry yellow mottle virus blackberry chlorotic ringspot virus strawberry necrotic shock virus black raspberry necrosis virus raspberry leaf mottle virus rubus canadensis virus-1 impatiens necrotic spot virus raspberry latent virus

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#### blackberry leaf mottle associated virus (BLMaV) etc...











#### BLMaV – background info

• Discovered in 2014, genus: *Emaravirus* 

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- Emaraviruses Animal viruses that happen to infect plants...

#### Reference emaravirus





viralzone.expasy.org Swiss Institute of Bioinformatics

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#### BLMaV – background info

- Discovered in 2014, genus: Emaravirus
- Emaraviruses Animal viruses that happen to infect plants...
- Transmitted by eriophyid mites
- Replicate in the vector

### Available knowledge/tools

#### Diversity

Table 1 Analysis of population genetic parameters and neutrality tests on the nucleocapsid protein (NP) and movement protein (MP) of blackberry leaf mottle-associated virus (BLMaV).

Gene	N	S	Hd	π	ENC <sup>a</sup>	dN	dS	dN/dS	Negativ codons <sup>t</sup> SLAC	e FEL	Tajima's D	Fu & Li's <i>D</i> *	Fu & Li's <i>F</i> *
NP	33	146	0.998	0.034	47.48	0.0062	0.141	0.044	31	68	-0.482598	-0.73174	-0.82271
MP	33	271	1.000	0.057	48.33	0.0067	0.349	0.019	103	147	-0.102047	-0.50076	-0.62417

*N*, number of isolates; S, number of segregating sites; Hd, haplotype diversity;  $\pi$ , nucleotide diversity estimated by the average number of differences per site between two sequences; dN, rate of nonsynonymous substitutions per site; dS, rate of synonymous substitutions per site. Tajima's *D*, Fu & Li's *D*<sup>\*</sup> and *P*<sup>\*</sup> are statistical tests of neutrality.

<sup>a</sup>ENC effective number of codons, calculated using DNASP v. 6 (Rozas et al., 2017).

<sup>b</sup>Number of negatively selected codons at 0.05 significance level, obtained using SLAC and FEL algorithms implemented in the Datamonkey server (Weaver *et al.*, 2018).

#### Table 1

Incidence of blackberry leaf mottle-associated virus in cultivated and wild blackberries collected from USA states.

State	infected/tested
Arkansas	25/94 (38%)
Arkansas-W <sup>1</sup>	73/123 (59%)
Florida	2/32 (6%)
Georgia	12/58 (21%)
North Carolina	52/63 (83%)
Maryland	0/20 (0%)
Mississippi	9/42 (21%)
Oklahoma	5/10 (50%)
Oregon	1/9 (11%)
South Carolina	47/73 (64%)
Total	226/524 (43%)

#### Incidence



#### Diagnostics



W<sup>1</sup>: Wild blackberry.





Yes





#### BLMaV transmission studies using 'Ouachita'

**Vector:** *Phyllocoptes parviflori Keifer 30dpi* 

**Experimental design:** 

- 1 mite per plant
  8/20 @ 40%
- 5 mites per plant
   14/20 @ 70%

**Vector:** *Diptacus rubuscolum 30dpi* 

Experimental design:

1 or 5 mites per plant
0/40 @ 0%

Reference point - rose rosette virus <5% transmission using 10 mites/plant



## What are your questions?

in case they come later...

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