Survey and discovery of viruses in blackberry and wild *Rubus* in South Carolina

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Photo: Daniela Negrete Moreno

Blackberry production in South Carolina

IPMdata


Blackberry virus disease
symptoms are diverse

There are diverse viruses associated with blackberry yellow vein disease

With numerous vectors...

Wind-dispersed mites

Pollen-borne viruses spread by pollinators

Sap-feeding aphids, mealybugs, and whiteflies

Soil-borne dagger nematodes

Effective management strategies for virus diseases

1. Starting with clean plants.

2. Preventing viruses from invading plants by **controlling vectors** and **removing alternative sources of virus inoculum.**

The *reality* of management strategies for virus diseases in blackberry

Starting with clean plants.

- Which viruses are there?
- Are the vectors there?
- When are the vectors there?
- Are the vectors pests?
- Are there weeds or wild Rubus contributing viruses?

→ Will the investments in clean plants and planting new blocks pay off?

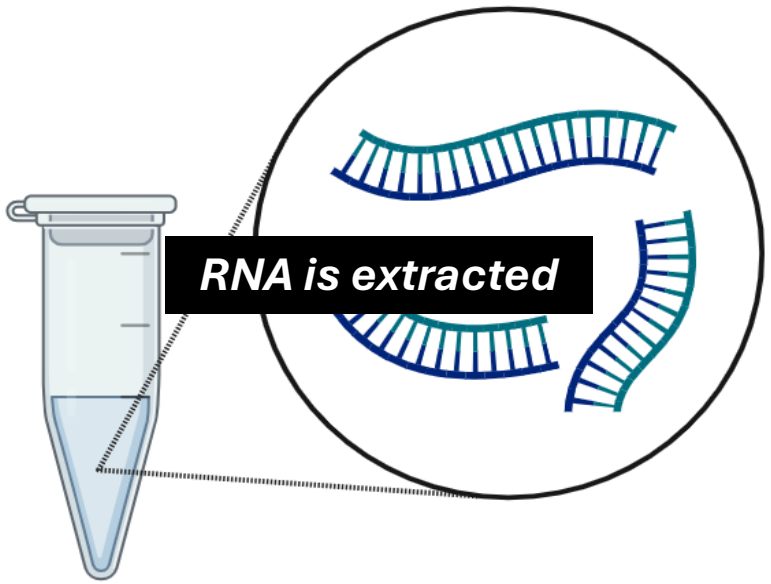
Virus Survey: Sample collection methodology

1. Symptomatic blackberry
2. Asymptomatic blackberry
3. Wild *Rubus*

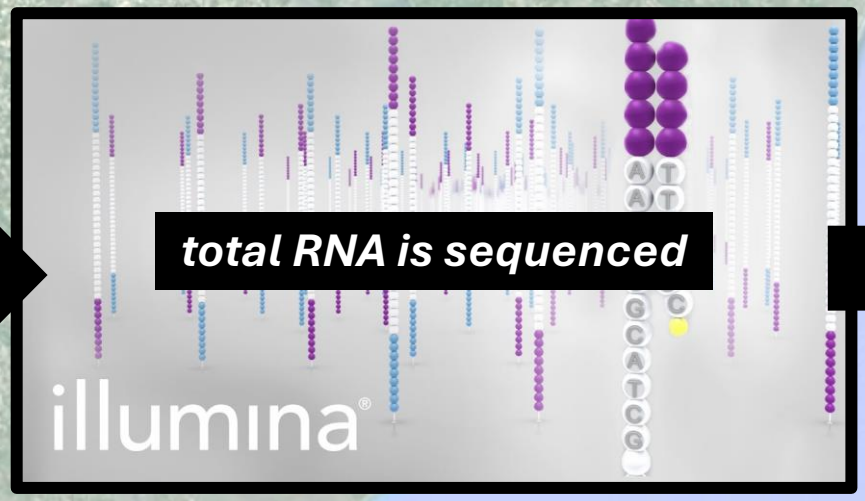
Sampling Location

Virus Survey: High throughput sequencing

samples are pooled



RNA is extracted



total RNA is sequenced

known and novel viruses are revealed

HTS Survey Results: *No particular virus is always associated with symptoms*

			Coo		Col			Jo			ML			Do		Cox		BV			IP			Number of samples
		Sampling location ID →	Coo		Col			Jo			ML			Do		Cox		BV			IP			
		Symptomatic/ Asymptomatic/ Wild *Rubus*	A	S	A	S	W	A	S	W	A	S	W	A	W	A	W	A	S	W	A	S	W	21
		Number of plants in the composite sample →	20	20	14	14	10	13	13	10	20	19	10	11	10	29	10	30	19	10	15	15	10	
		Number of viruses detected →	1	1	4	7	5	5	6	12	8	6	8	5	2	0	2	0	3	6	5	10	11	
Ilarvirus	**blackberry chlorotic ringspot virus**	**BCRV**					**W**	**A**	**S**	**W**														**4**
Ampelovirus	**blackberry vein banding-associated virus**	**BVBaV**	**A**				**W**	**A**	**S**	**W**	**A**	**S**					**W**		**S**	**W**		**S**	**W**	**12**
Crinivirus	**blackberry yellow vein-associated virus**	**BYVaV**			**A**	**S**		**A**		**W**	**A**	**S**	**W**	**A**	**W**					**W**	**A**	**S**	**W**	**13**
Idaeovirus	**raspberry bushy dwarf virus**	**RBDV**			**A**	**S**			**S**	**W**														**4**
Brambyvirus	**blackberry virus Y**	**BVY**									**A**	**S**							**S**				**W**	**4**
Nepovirus	**tobacco ringspot virus**	**TRSV**												**A**							**A**			**2**
Nepovirus	**tomato ringspot virus**	**ToRSV**								**W**														**1**
Allexivirus	**blackberry virus E**	**BVE**				**S**		**A**	**S**	**W**	**A**	**S**	**W**							**W**		**S**	**W**	**10**
Emaravirus	**blackberry leaf mottle-associated virus**	**BLMaV**		**S**		**S**						**S**	**W**									**S**	**W**	**6**
Coguvirus	**blackberry line pattern virus**	**BlaLPV**				**S**	**W**	**A**	**S**	**W**	**A**		**W**	**A**					**S**	**W**	**A**	**S**	**W**	**13**
Ilarvirus	Solanum nigrum ilarvirus 1	SnIV1																					W	1
Ilarvirus	tobacco streak virus	TSV								W														1
Ilarvirus	lilac leaf chlorosis virus	LLCV									A		W											2
Tobamovirus	turnip vein clearing virus	TVCV																				S		1
Badnavirus	blackberry virus F	BVF				S	W		S	W			W	A	W		W			W	A		W	11
unclassified	blackberry virus X	BVX			A					W	A	S	W	A						W		S	W	9
Carlavirus	rose virus A	RoVA								W														1
Roymovirus	blackberry roymovirus 1	BRV1			A	S																		2
unclassified	blackberry tombus-like virus 1	BTV1																			A	S		2
unclassified	blackberry tombus-like virus 2	BTV2																				S		1
Badnavirus	Rubus badnavirus 1	RBV1					W			W														2
Cytorhabdovirus	Rubus trirhavirus 1	RTV1									A		W										W	3
Varicosavirus	blackberry varicosavirus 1	BVV1																				S	W	2

Some of the novel viruses were detected in symptomatic pooled samples.

Association with disease is unclear.

- **BVE**, **BLMaV**, **BVBaV**, and **BlaLPV** found in 4/6 symptomatic pools
- **BYVaV** found in 3/6 symptomatic pools

HTS detected numerous viruses in all sample groups

			Sampling location ID →	Coo		Col			Jo			ML			Do		Cox		BV			IP			Number of samples
			Symptomatic/ Asymptomatic/ Wild *Rubus*	A	S	A	S	W	A	S	W	A	S	W	A	W	A	W	A	S	W	A	S	W	21
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Crinivirus	**blackberry yellow vein-associated virus**	**BYVaV**			**A**	**S**		**A**		**W**	**A**	**S**	**W**	**A**	**W**					**W**	**A**	**S**	**W**	**13**	
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Nepovirus	**tomato ringspot virus**	**ToRSV**								**W**														**1**	
Allexivirus	**blackberry virus E**	**BVE**				**S**		**A**	**S**	**W**	**A**	**S**	**W**							**W**		**S**	**W**	**10**	
Emaravirus	**blackberry leaf mottle-associated virus**	**BLMaV**		**S**		**S**						**S**	**W**									**S**	**W**	**6**	
Coguvirus	**blackberry line pattern virus**	**BlaLPV**				**S**	**W**	**A**	**S**	**W**	**A**		**W**	**A**					**S**	**W**	**A**	**S**	**W**	**13**	
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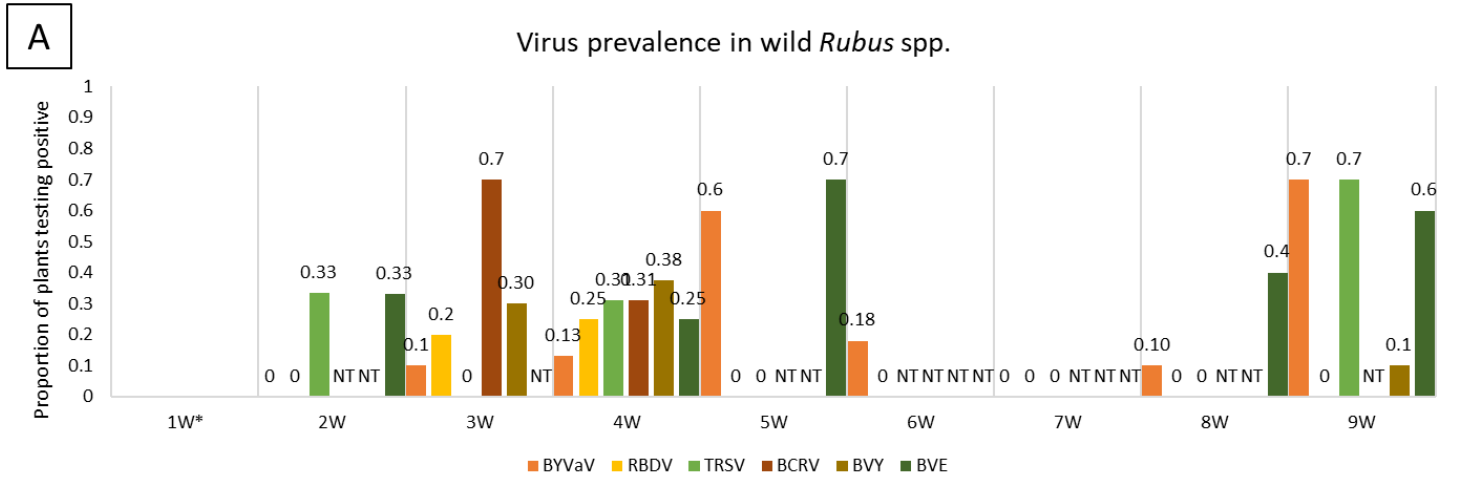
Cytorhabdovirus	Rubus trirhavirus 1	RTV1										A		W									W	3	
Varicosavirus	blackberry varicosavirus 1	BVV1																				S	W	2	

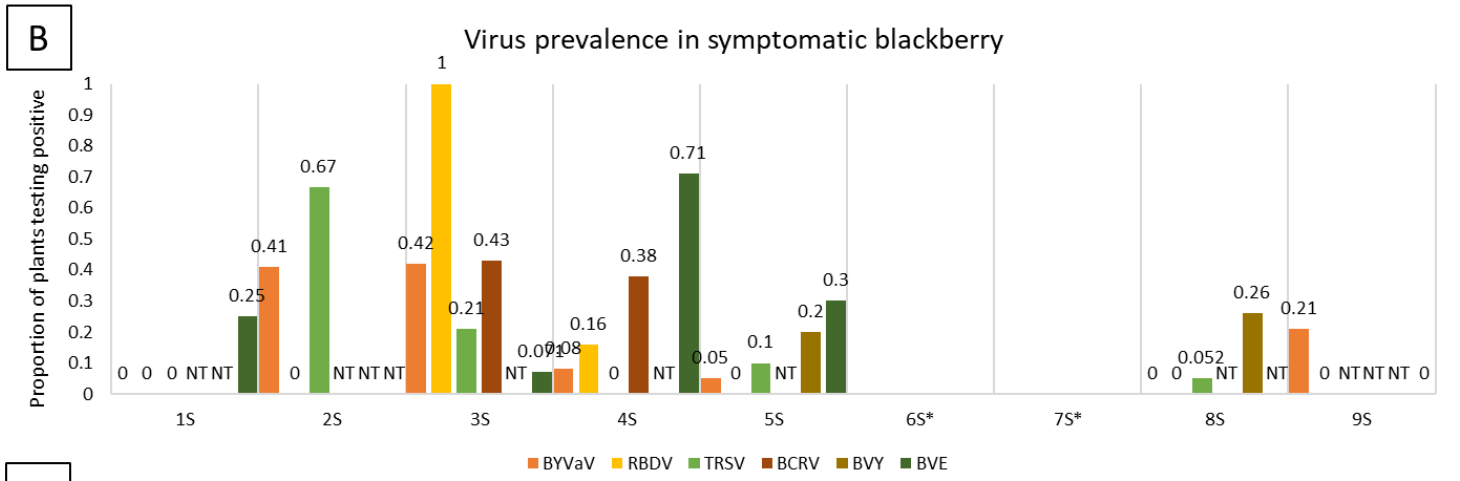
Asymptomatic: viruses detected in 6/8 sites

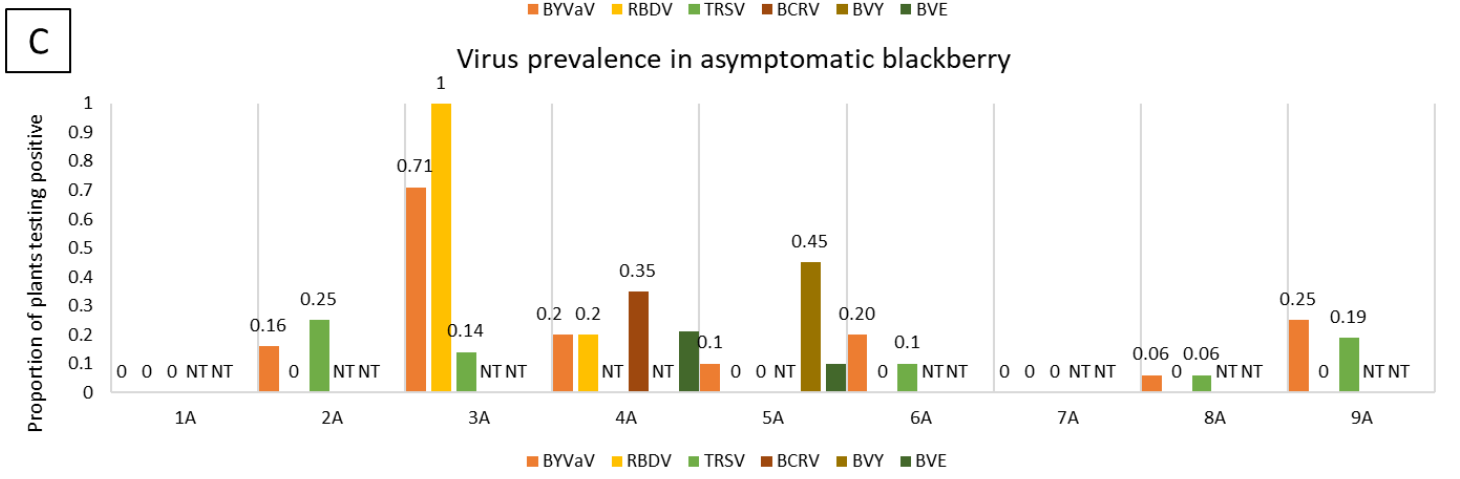
Symptomatic: viruses detected at all sites where symptoms were apparent

Wild Rubus: viruses detected at all sites where wild samples were collected

PCR testing for specific viruses in individual plants is consistent with HTS data on pooled samples.







Blackberry yellow vein associated virus (BYVaV)

Suggested to be the central virus in the BYVD complex
(Martin et al. 2013)

	Coo		Col			Jo			ML			Do		Cox		BV			IP		
	A	S	A	S	W	A	S	W	A	S	W	A	W	A	W	A	S	W	A	S	W
BYVaV			A	S		A		W	A	S	W	A	W					W	A	S	W

In our HTS data: BYVaV was only detected in **3/6** symptomatic blackberry sample pools

In our PCR data: BYVaV was detected in:

- **16/113** symptomatic blackberry
- **24/166** asymptomatic blackberry
- **19/192** wild Rubus samples

BYVaV is unlikely to be the major driving force of yellow vein disease symptoms at South Carolina blackberry farms

Raspberry bushy dwarf virus

	Coo		Col			Jo			ML			Do		Cox		BV			IP		
	A	S	A	S	W	A	S	W	A	S	W	A	W	A	W	A	S	W	A	S	W
RBDV			A	S			S	W													

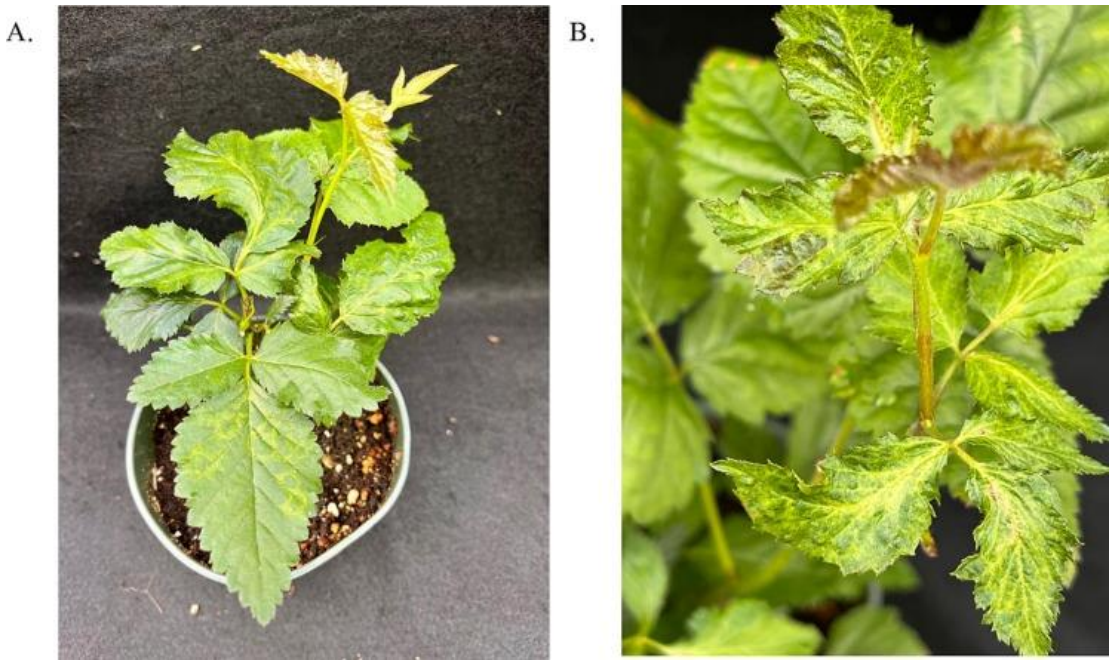
- Pollen and seed-transmitted
- Detected in high amounts at two farms
  - Farm “Col” had it in both symptomatic and asymptomatic blackberry
  - Farm “Jo” had it in the symptomatic blackberry and wild Rubus
- RBDV is often latent, but it has been associated with reduced yields and berry sizes *(Strik et al. 2003)*

Blackberry leaf mottle virus

	Coo		Col			Jo			ML			Do		Cox		BV			IP		
	A	S	A	S	W	A	S	W	A	S	W	A	W	A	W	A	S	W	A	S	W
BLMaV		S		S						S	W									S	W

- BLMV was detected in 4/6 symptomatic blackberry groups in our study and 2 wild Rubus groups, no asymptomatic
- BLMV causes symptoms on its own *(Druciarek et al. 2024)*
- Vectored by eriophyid mites including *Phyllocoptes parviflora*

→ May be one of the major drivers of BYVD in South Carolina



(Druciarek et al. 2024)

Blackberry vein banding associated virus

	Coo		Col			Jo			ML			Do		Cox		BV			IP		
	A	S	A	S	W	A	S	W	A	S	W	A	W	A	W	A	S	W	A	S	W
BVBaV	A				W	A	S	W	A	S					W		S	W		S	W

- BVBaV was detected in 4/6 symptomatic sample groups, and also frequently in the asymptomatic and wild Rubus.
- BVBaV is vectored by mealybugs *(Martin and Tzanetakis, 2015)*
- Often found in mixed infections in declining plants.

→ May be one of the major drivers of BYVD in South Carolina

These plants are infected with BVBaV and at least two other viruses *(Thekke-Veetil et al. 2013)*

Blackberry virus Y and blackberry virus E

	Coo		Col			Jo			ML			Do		Cox		BV			IP		
	A	S	A	S	W	A	S	W	A	S	W	A	W	A	W	A	S	W	A	S	W
BVY									A	S							S				W
BVE				S		A	S	W	A	S	W							W		S	W

- BVY and BVE are typically asymptomatic in single infections
- No confirmed vectors of BVY and BVE, potentially mites?
- Often detected in mixed infections in diseased plants

Blackberry line pattern virus

	Coo		Col			Jo			ML			Do		Cox		BV			IP		
	A	S	A	S	W	A	S	W	A	S	W	A	W	A	W	A	S	W	A	S	W
BlaLPV				S	W	A	S	W	A		W	A					S	W	A	S	W

- BLaLPV is a new virus, in a new genus (*Coguvirus*)
- Detected in all sample groups at multiple farms
- Unknown vector(s)
- Related viruses associated with disease symptoms in citrus and apple

→ May be one of the major drivers of BYVD in South Carolina

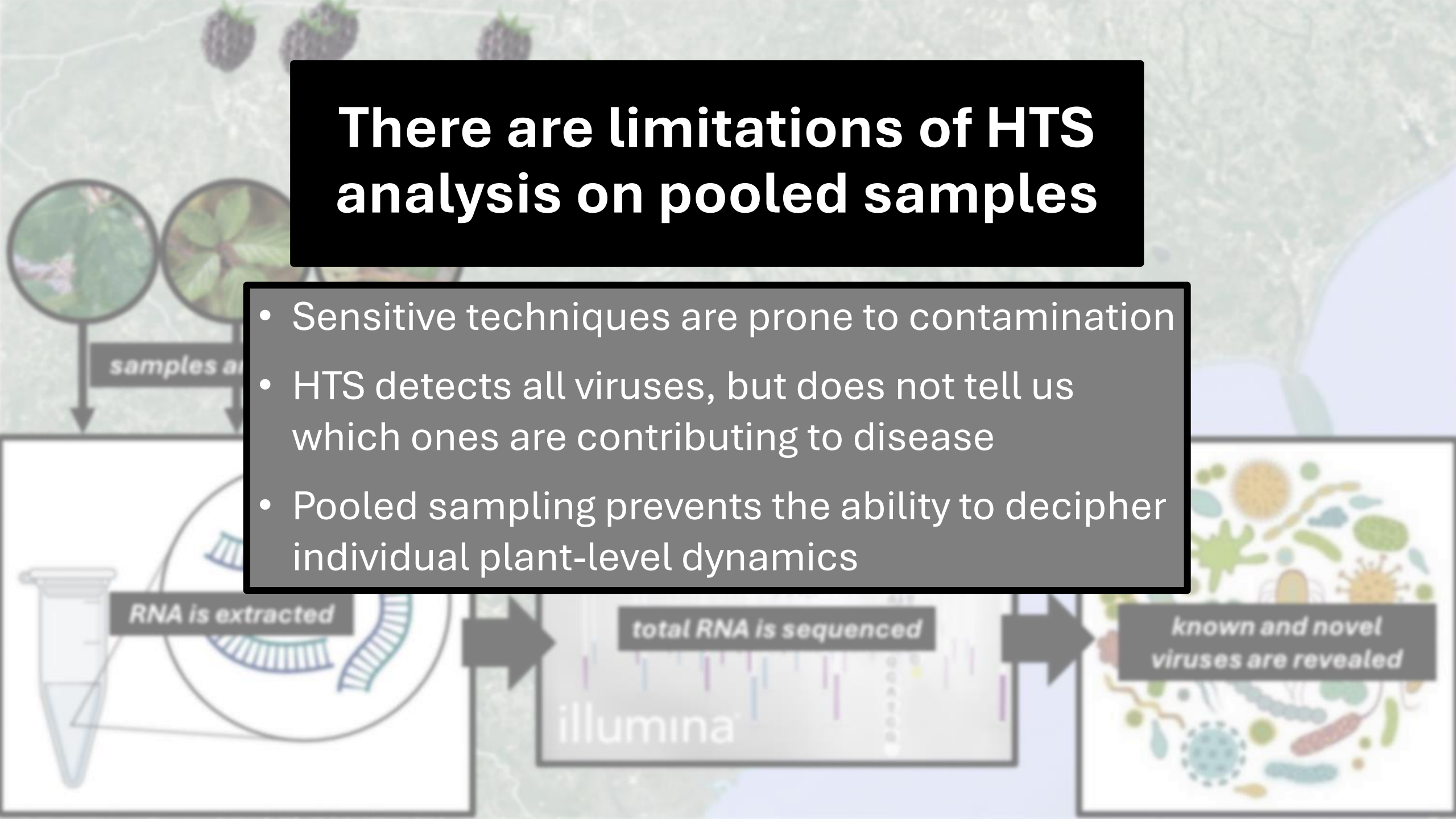
Other new viruses

		Coo		Col			Jo			ML			Do		Cox		BV			IP		
		A	S	A	S	W	A	S	W	A	S	W	A	W	A	W	A	S	W	A	S	W
blackberry roymovirus 1	BRV1			A	S																	
blackberry tombus-like virus 1	BTV1																			A	S	
blackberry tombus-like virus 2	BTV2																				S	
Rubus badnavirus 1	RBV1					W			W													
Rubus trirhavirus 1	RTV1									A		W										W
blackberry varicosavirus 1	BVV1																				S	W

- Several other novel viruses were detected
- No reason to be concerned about these viruses at this time

There are limitations of HTS analysis on pooled samples

- Sensitive techniques are prone to contamination
- HTS detects all viruses, but does not tell us which ones are contributing to disease
- Pooled sampling prevents the ability to decipher individual plant-level dynamics

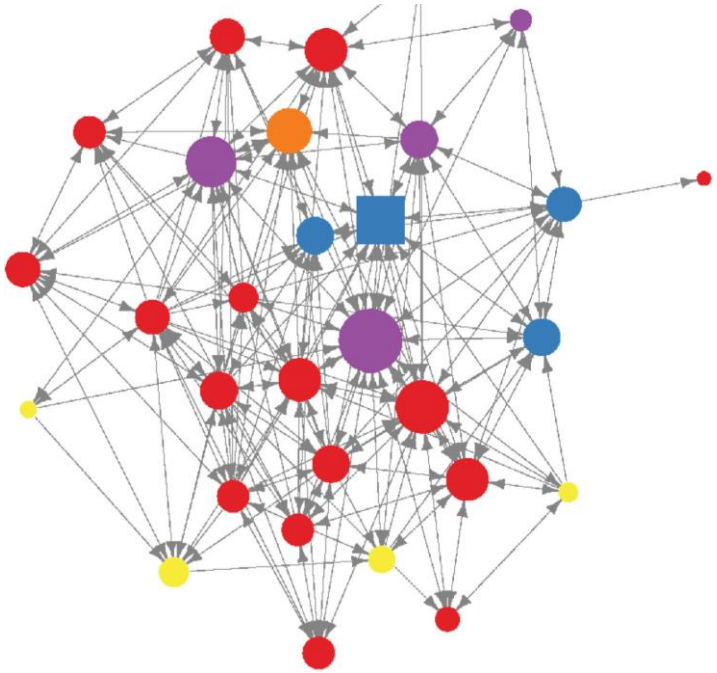


Ongoing research: Resolving relationships using network analysis

Test individual plants for selected viruses.

Catalogue symptoms.

Use network analysis to decipher relationships between virus combinations/ cultivar/ symptoms.



Ongoing study at two farms: epidemiology

Monitoring to see how long it takes
viruses to come into new plantings

What does all of this mean?

We have a lot of work to do.

- improving detection assays
- deciphering disease etiology and consequences of co-infections
- determining vectors and understanding their phenology
- understanding biology and ecology of blackberry viruses

Vectors are diverse and their dynamics are not well understood.

- controlling aphids, whiteflies, thrips, mites?
- which ones need to be managed? how? when?

We need to develop additional strategies to manage viruses in berries.

Starting with clean plants is worthwhile as long as you can ***keep them clean***.

Thank you!

Support and Efforts

Blackberry growers in South Carolina

Clemson Cooperative Extension (Andy Rollins, Bruce McLean, Rob Last)

My lab, especially Wanita Dantes and Elise Schnabel

Collaborators: Anna Whitfield (NCSU) and Pairwise (Durham, NC)

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