Berry viruses complexes and what we can do about it

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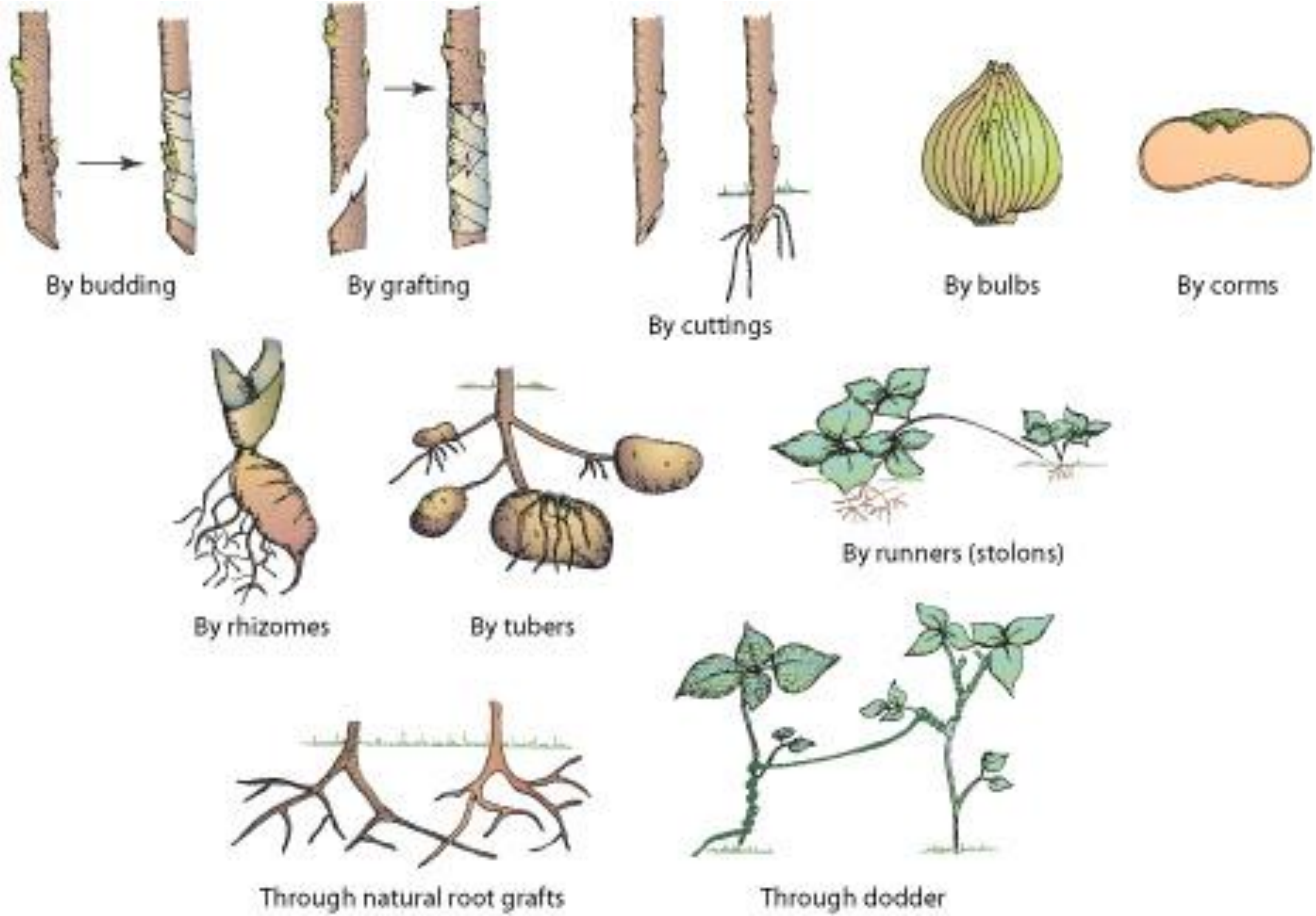
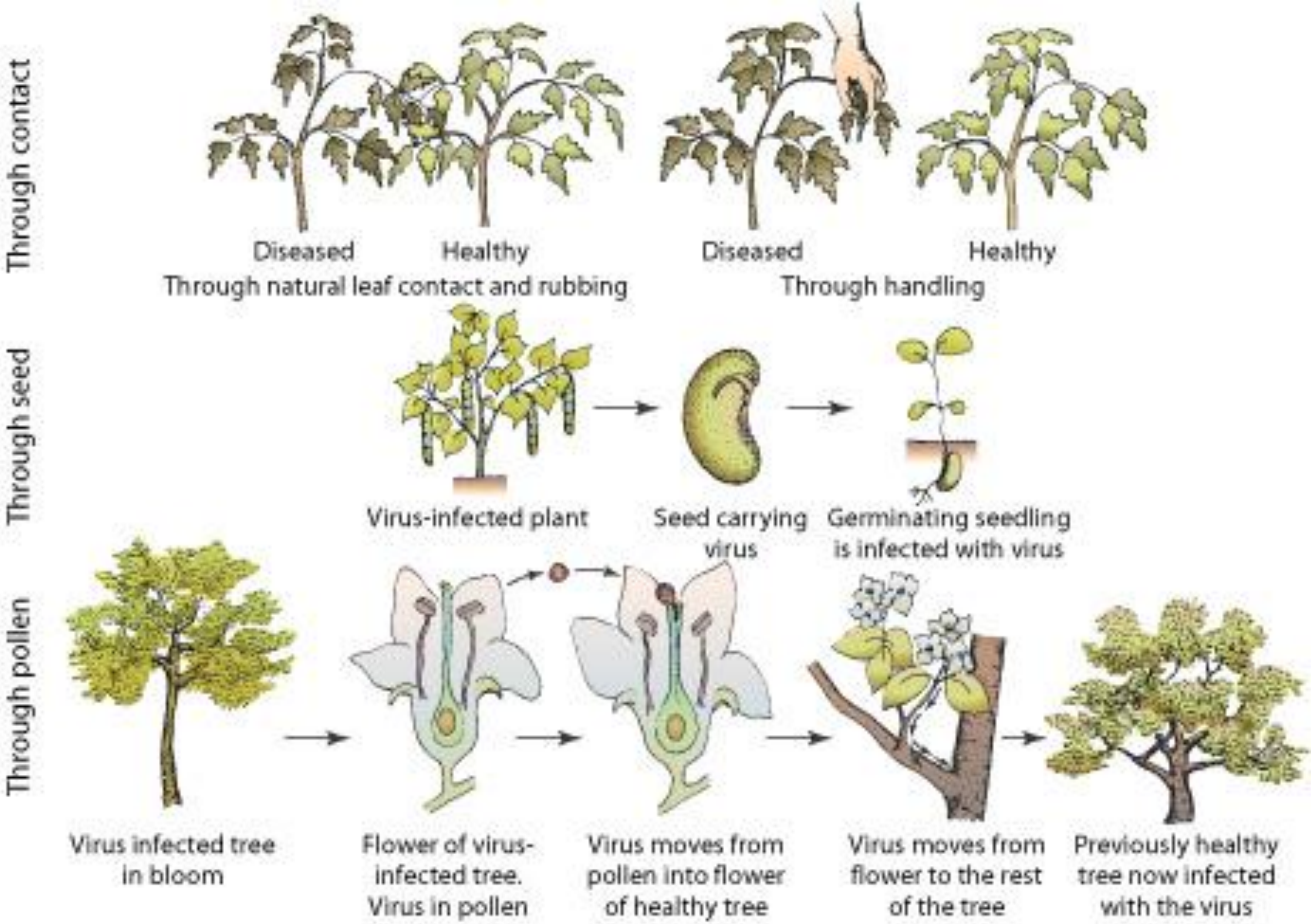
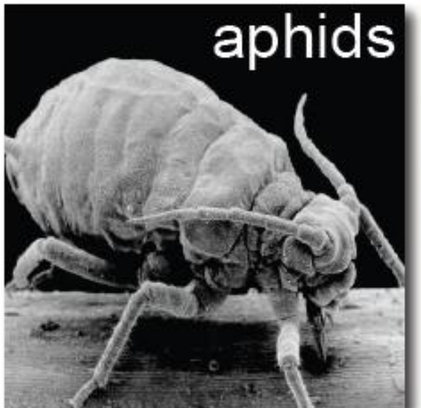
Virus transmission 101


By budding
By grafting
By cuttings
By bulbs
By corms
By rhizomes
By tubers
By runners (stolons)
Through natural root grafts
Through dodder


Through contact
Diseased
Healthy
Through natural leaf contact and rubbing
Diseased
Healthy
Through handling
Through seed
Virus-infected plant
Seed carrying virus
Germinating seedling is infected with virus
Through pollen
Virus infected tree in bloom
Flower of virus-infected tree. Virus in pollen
Virus moves from pollen into flower of healthy tree
Virus moves from flower to the rest of the tree
Previously healthy tree now infected with the virus

Phylum *Arthropoda*, Class *Insecta*, Order *Hemiptera*



Phylum *Arthropoda*, Class *Insecta*

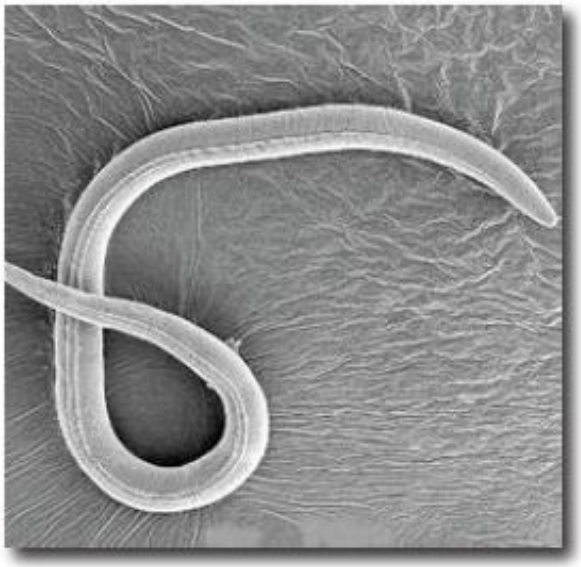
Order *Thysanoptera*

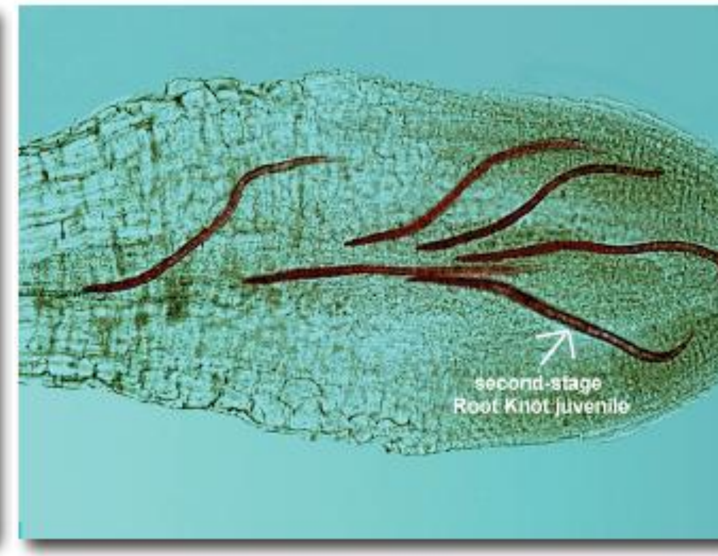
Order *Coleoptera*

Phylum *Arthropoda*, Class *Arachnida*, Order *Acariformes*

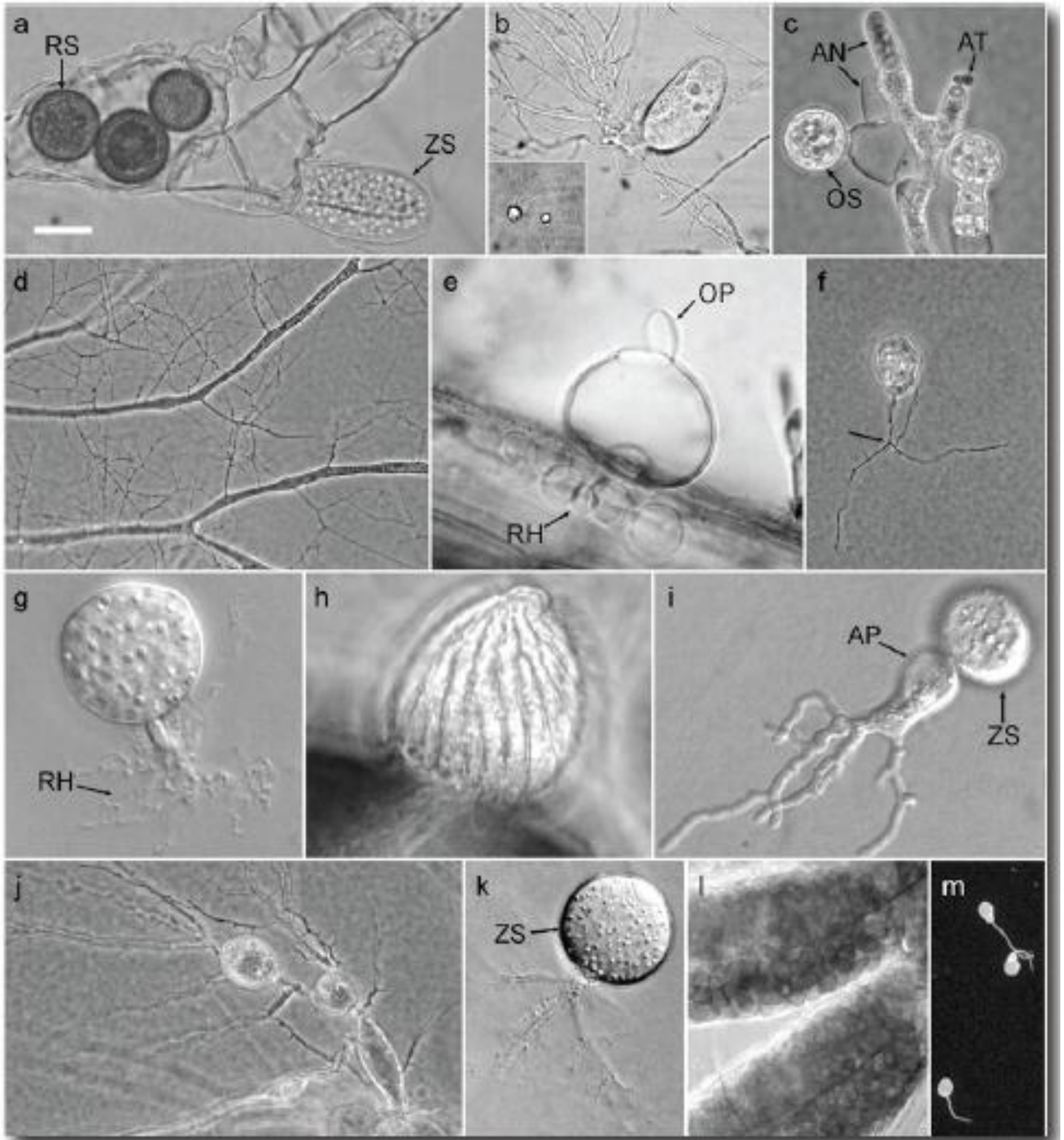
mites

Phylum *Nematoda*, Class *Secernentea*, Order *Tylenchida*





Nematodes



Kingdom: *Fungi*

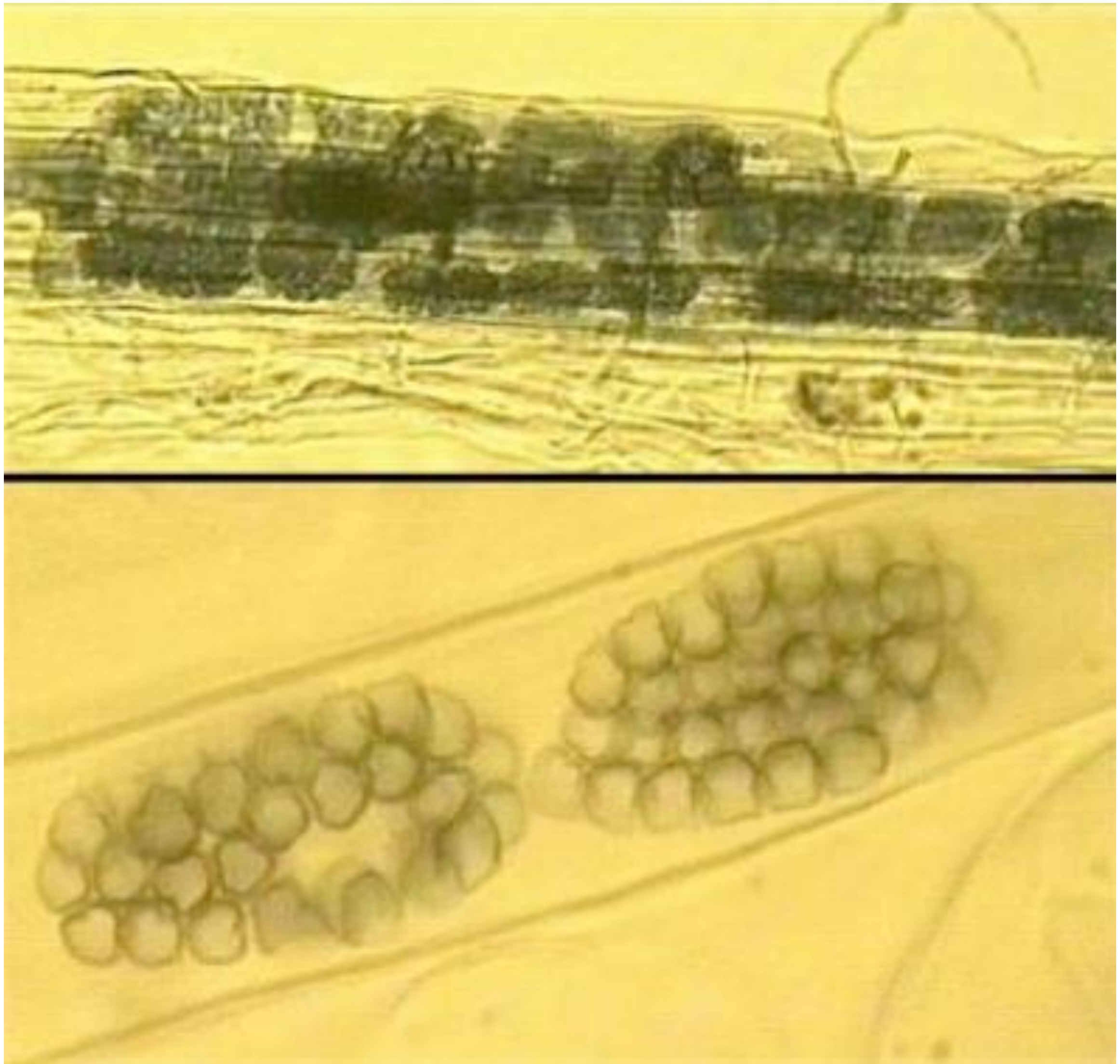
Phylum: *Chytridiomycota*

Class: *Chytridiomycetes*

Order: *Incertae sedis*

Family: *Olpidiaceae*

Genus: ***Olpidium***



Kingdom: *Rhizaria*

Phylum: *Cercozoa*

Class: *Plasmodiophorea*

Order: *Plasmodiophorida*

Family: *Plasmodiophoridae*

Genus: ***Polymyxa***

Vectors and plant viruses they transmit

		Virus groups					
Vector taxa	Vector group	Icosahedral particles RNA genome	Rod-shaped particles RNA genome	DNA genome	Enveloped particles RNA genome	Total	%
Hemiptera	Aphids	26	153[a]	13	5	197	28
	Whiteflies	–	13	115[b]	–	128	18
	Leafhoppers	8	–	15	3	26	4
	Planthoppers	10	4[c]	–	4	18	3
	Other hemiptera	–	8	5	–	13	2
Thysanoptera	Thrips	2	–	–	14	16	2
Coleoptera	Beetles	50	1	–	–	51	7
Acari	Mites	10	9	–	–	10	1
Nematoda	Nematodes	45	3	–	–	48	7
Mycota	Fungi	8	16	–	–	24	3
	No identified vectors	84	60	19	3[d]	166	24
	Total	233	268	167	30	697	
	%	33	39	24			

[a]Includes 110 virus species of the genus *Potyvirus*, family *Potyviridae*;

[b]Virus species of the genus *Begomovirus*, family *Geminiviridae*;

[c]These are all tenuiviruses that have multiple shapes;

[d]These viruses probably have insect vectors.

Four modes of virus transmission

Biological characteristic	Nonpersistent stylet-borne	Semipersistent foregut-borne[b]	Persistent circulative	Persistent propagative
AAP and IAP[a]	Seconds, minutes[c]	Minutes, hours[d]	Hours, days[d]	Hours, days[d]
Latent period	None	None	Hours, days	Days, weeks
Retention time in vector	Minutes, lost after molting	Hours, lost after molting	Days, weeks	Lifespan of insect
Presence in vector's hemolymph	No	No	Yes	Yes
Multiplication in vector	No	No	No[e]	Yes
Transovarial transmission	No	No	No	Often

[a]AAP, Acquisition access period; IAP, Inoculation access period;

[b]A recent publication revealed that the semi-persistent virus *Cauliflower mosaic virus* (CaMV) is retained in the stylet (178);

[c]The time period during which virus can be acquired from and inoculated into plant epidermal cells;

[d]AAP and IAP times depend on the location of the virus in the plant, i.e., acquisition of the virus from the plant phloem takes longer than acquisition from the epidermis or mesophyll cells;

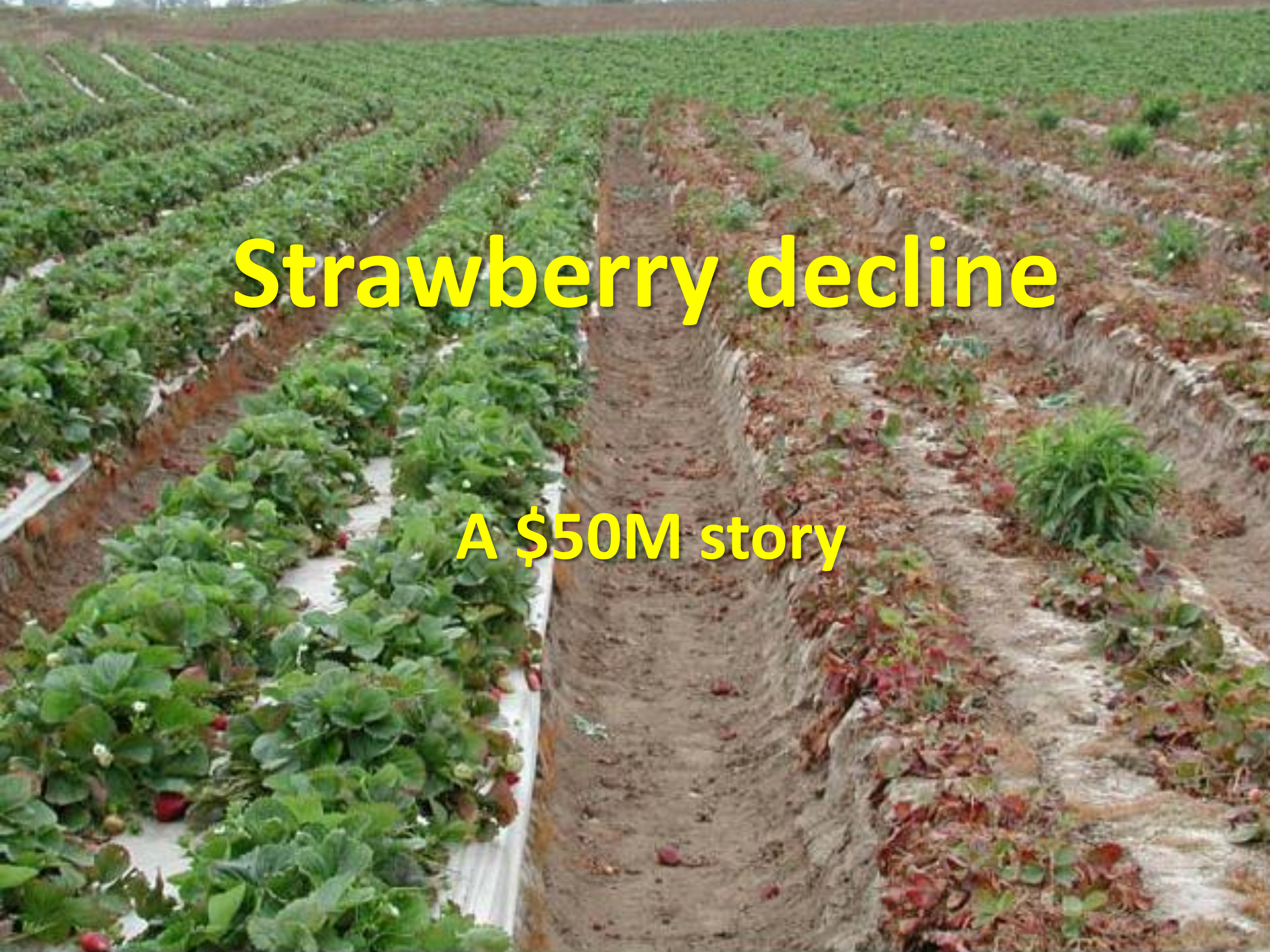
[e]Except for TYLCV for which there is evidence that it replicates in its whitefly vector.

Strawberry viruses circa 2003

Viruses	Vector
Arabis mosaic – Europe Tomato ringspot Raspberry ringspot – Europe Strawberry latent ringspot-Europe Tomato black ring –Europe	Nematodes
Strawberry crinkle Strawberry mild yellow edge Strawberry mottle Strawberry vein banding	Aphids
Fragaria chiloensis latent – Chile Tobacco streak*	Pollen
Strawberry pallidosis Beet pseudo-yellows	Whiteflies*

Strawberry decline

A $50M story







VENTANA
PLTD 8/27
CAMAROSA
PLTD 9/10
CAMAROSA
PLTD 8/27

The whitefly viruses

Table 2. Transmission frequency of Strawberry pallidosis-associated virus (SPaV) and *Beet pseudo yellows virus* (BPYV) with the greenhouse whitefly (*Trialeurodes vaporariorum*)

Virus	Plant species: *Fragaria × ananassa*	Plant species: *Nicotiana benthamiana*
BPYV	8/21 (38%)	16/20 (80%)
SPaV	3/21 (14%)	3/20 (15%)

Table 3. Host range of Strawberry pallidosis-associated virus (SPaV) utilizing *Trialeurodes vaporariorum* for transmission

Plant species tested	Infected/ inoculated
Fragaria × ananassa	3/10
Sibbaldia procumbens	3/8
Duchesnea indica	0/3
Nicotiana benthamiana	6/10
N. glutinosa	0/5
N. clevelandii	2/5
N. tabacum	0/5
Physalis wrightii	5/6
P. floridana	0/5
► *Malva parviflora*	2/5
Citrullus spp.	0/5
Chenopodium murale	0/5
C. capitatum	0/5
C. amaranticolor	0/5
Gomphrena globosa	0/5
Capsella bursa-pastoris	0/5
Brassica oleracea var. *italica*	0/5
Lycopersicon esculentum	0/5
Beta vulgaris	0/5
B. maritima subsp. *macrocarpa*	0/5
Datura stramonium	0/5
Urtica urens[a]	0/5

► [a] SPaV has also been found in field isolates of nettle (*Urtica* sp.) associated with high field populations of greenhouse whitefly. It is not known if field isolates were *U. urens* or another *Urtica* species.

BPYV-SPaV double infections

Other strawberry viruses?

Revisited strawberry virus-like diseases.

Chlorotic fleck

Leafroll

Goal: Identify unknown viruses that may contribute to the decline.

Identification, characterization and development of detection techniques for strawberry viruses

Virus name	Acronym	Mode of transmission	Genus	Laboratory detection[b]
* *Apple mosaic*	ApMV	Pollen, seed	*Ilarvirus*	ELISA, RT-PCR
Arabis mosaic	ArMV	Nematode, seed	*Nepovirus*	ELISA, RT-PCR
* *Beet pseudo-yellows*	BPYV	Whitefly	*Crinivirus*	RT-PCR
* Fragaria chiloensis cryptic	FClCV	Unknown	Unknown	RT-PCR
* *Fragaria chiloensis latent*	FClLV	Pollen, seed	*Ilarvirus*	ELISA, RT-PCR
Raspberry ringspot	RpRSV	Nematode, seed	*Nepovirus*	ELISA, RT-PCR
* *Strawberry chlorotic fleck*	StCFV	Aphid	*Closterovirus*	RT-PCR
Strawberry crinkle	SCV	Aphid	*Cytorhabdovirus*	RT-PCR
Strawberry feather leaf	NA	Unknown	Unknown	NA
* Strawberry latent	StLV	Unknown	*Cripavirus*	RT-PCR
Strawberry latent C	SLCV	Aphid	*Nucleorhabdovirus*	NA
* *Strawberry latent ringspot*	SLRSV	Nematode, seed	*Sadwavirus*	ELISA, RT-PCR
Strawberry mild yellow edge	SMYEV	Aphid	*Potexvirus*	ELISA, RT-PCR
Strawberry mottle	SMoV	Aphid	*Sadwavirus*	RT-PCR
* Strawberry necrotic shock	SNSV	Thrips, pollen, seed	*Ilarvirus*	ELISA, RT-PCR
* Strawberry pallidosis associated	SPaV	Whitefly	*Crinivirus*	RT-PCR
Strawberry pseudo mild yellow edge	SPMYEV	Aphid	*Carlavirus*	ELISA
Strawberry vein banding	SVBV	Aphid	*Caulimovirus*	PCR
Tobacco necrosis	TNV	Oomycete	*Necrovirus*	ELISA, RT-PCR
Tomato black ring	TBRV	Nematode, seed	*Nepovirus*	ELISA, RT-PCR
Tomato ringspot	ToRSV	Nematode, seed	*Nepovirus*	ELISA, RT-PCR

Tobacco streak virus

* Strawberry crinivirus 3

* Strawberry crinivirus 4


No vector control

Vector control

Blackberry yellow vein disease

First observed in 2000 in the Carolinas.

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

Tobacco ringspot virus and BYVD

BYVD is very similar to what people thought as being TRSV symptoms

TRSV textbook symptoms

Single TRSV-infection

Are symptoms cv. dependent? The **majority** of plants infected with TRSV are symptomless

New viruses in *Rubus* in the last 7 yrs

16 viruses & virus-like agents were known to infect *Rubus* before we started looking into *Rubus* complexes – We now have over 40…

New *Rubus* viruses

Blackberry yellow vein associated virus
Blackberry virus E
Blackberry virus X
Blackberry virus Y
Blackberry virus Z
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
etc…..

New viruses in *Rubus* in the last 8 yrs

16 viruses & virus-like agents were known to infect *Rubus* before we started looking into *Rubus* complexes – We now have over 40…

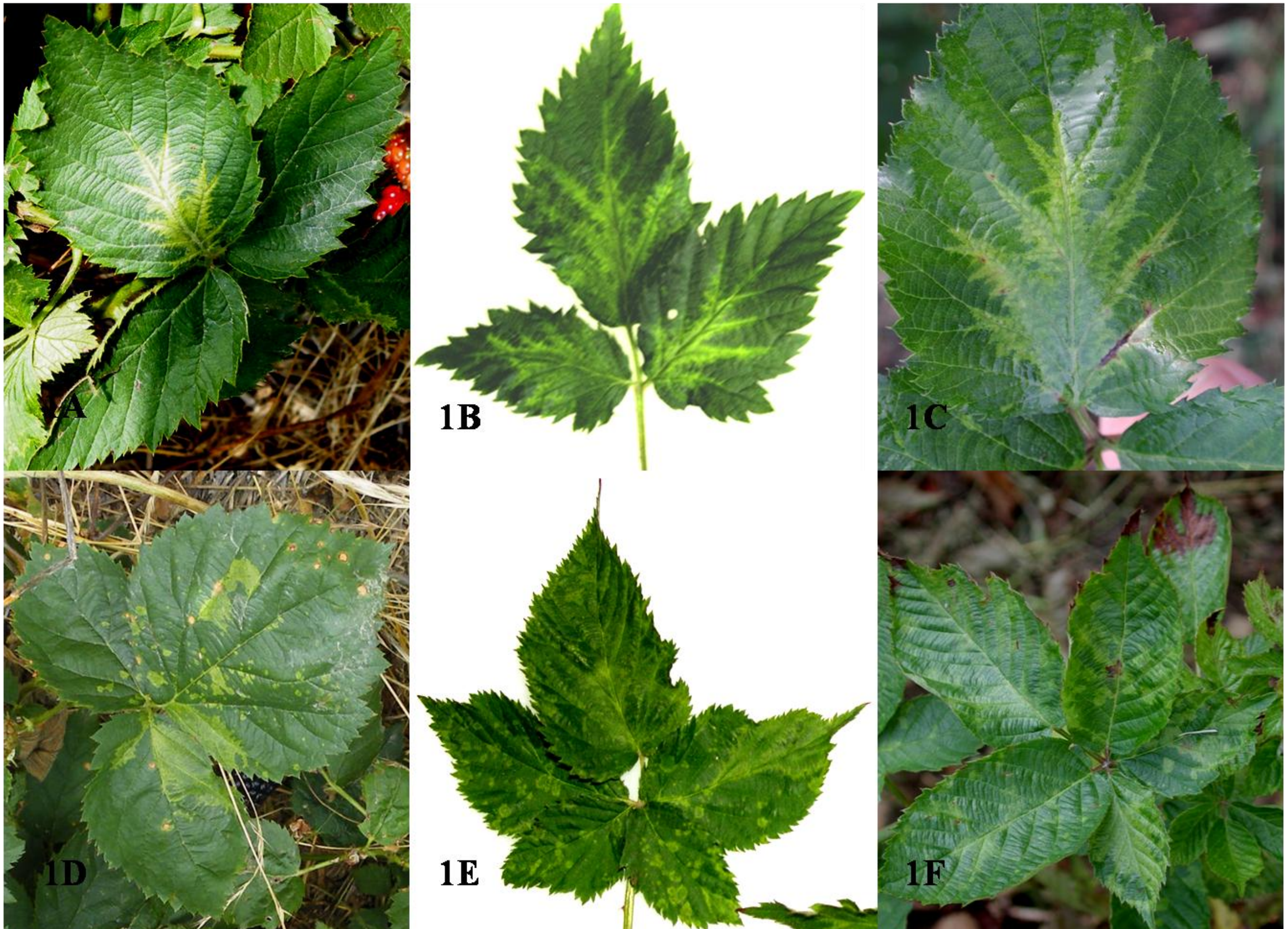
New *Rubus* viruses

Blackberry yellow vein associated virus
Blackberry virus E
Blackberry virus X
Blackberry virus Y
Blackberry virus Z
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
etc…..

Tests are available for all the new viruses

Same disease-different viruses

AR **MS** **NC**



Arkansas

BYVaV
BVY
TRSV

Carolinas

BYVaV
BVX
BPYV
INSV
TRSV

Mississippi

BYVaV
TRSV
BVE

How do we tackle BYVD?

After identification of all (or almost all) viruses that are involved in the disease we need to:

A. **Make sure that mother plants are being tested for the new viruses before they are propagated.**

B. Identify virus combinations that can cause BYVD.

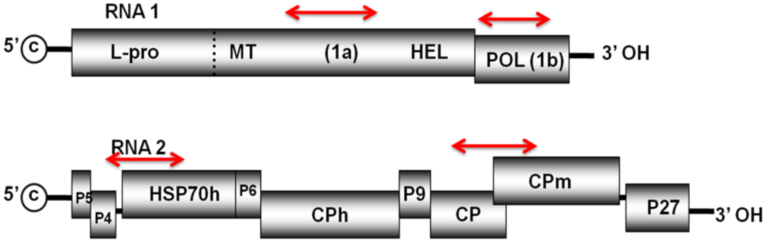
C. Identify virus vectors.

D. Find alternative hosts of the viruses in the field.

E. Minimize or eliminate BYVD by eliminating the weakest link, the virus vector(s) that is the easiest to control.

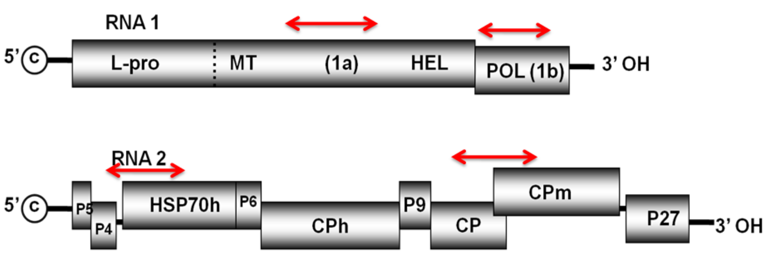
What are the viruses present in your area? The importance of detection

- BYVaV - Multistate sample collection - 35 isolates

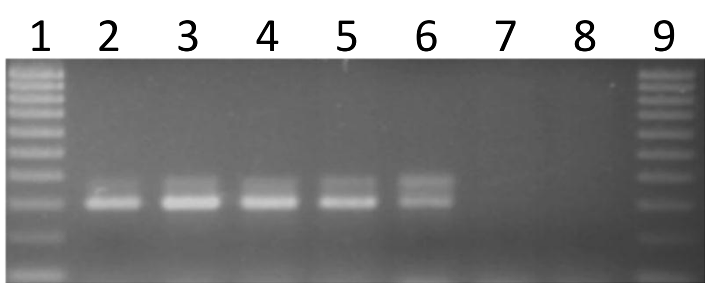
- 

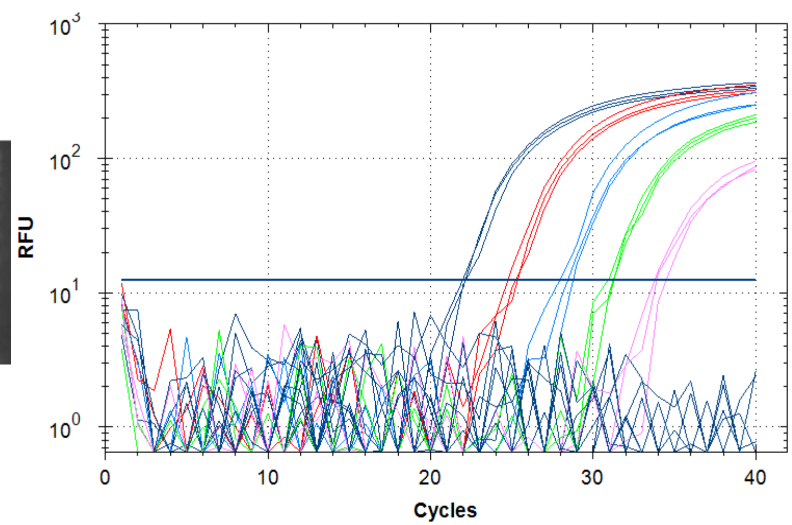
What are the viruses present in your area? The importance of detection

- BYVaV - Multistate sample collection - 35 isolates

- 

- Detection 100% identity





Virus interactions: The BYVaV/BVY story

BVY did not cause symptoms in single infections but together with BYVaV they cause BYVD.

In mixed infections, BVY knocks down concentration of BYVaV to about 0.1% compared to titer in single infections.

In mixed infections, they can cause death of fruiting canes.

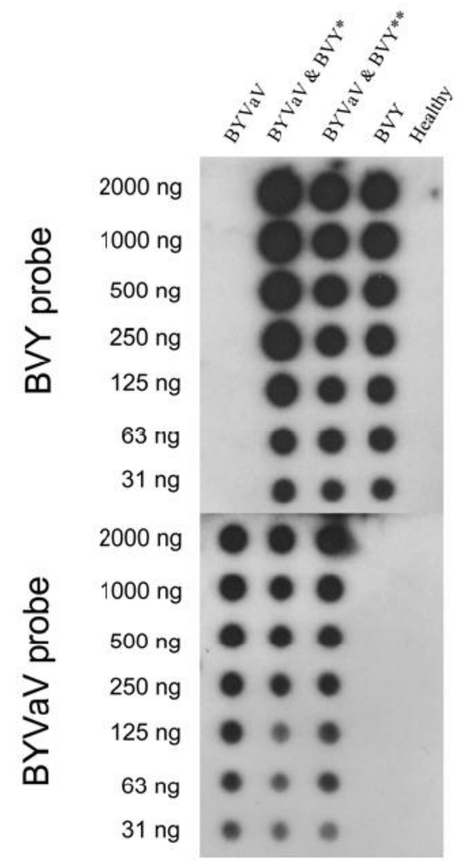


Fig. 4. Nucleic acid spot hybridization (NASH) detection of Blackberry virus Y (BVY) and Blackberry yellow vein associated virus (BYVaV) in blackberry. The blot was initially hybridized with a BVY-specific DNA probe and subsequently with a BYVaV-specific probe after stripping the BVY probe. *symptomatic mixed infection and **asymptomatic mixed infection.

Susaimuthu et al.,2008. Plant Disease 92:1288-1292

Transmission

Experiment	*Trialeurodes abutilonea*	*Trialeurodes vaporariorum*
Experiment 1	4/7	3/9
Experiment 2	5/8	1/8
Experiment 3	3/10	3/10
Total	12/25	7/27

www.apsnet.org
Greenhouse whitefly

- Both whitefly species transmitted the virus at a rate >30%

Alternate hosts

Plant species	Scientific name	Family	Number of plants tested
Garden vetch	*Vicia sativa*	Fabaceae	16
Virginia creeper	*Parthenocissus quinquefolia*	Vitaceae	16
Red clover	*Trifolium pretense*	Fabaceae	16
Wild garlic	*Allium vineale*	Amaryllidaceae	16
Creeping woodsorrel	*Oxalis corniculata*	Oxalidaceae	16
Carolina geranium	*Geranium carolinianum*	Geraniaceae	16
Curly dock	*Rumex crispus*	Polygonaceae	16
Dandelion	*Taraxacum officinale*	Asteraceae	16
Tall fescue	*Festuca arundinacea*	Poaceae	16
Wild wheat	*Avena fatua*	Poaceae	16
Grapes	*Vitis vinifera*	Vitaceae	16
Peach	*Prunus persica*	Rosaceae	16
Blueberry	*Vaccinium* spp.	Ericaceae	16
Shepherd's purse	*Capsella bursa-pastoris*	Brassicaceae	16
Nutsedge	*Cyperus* spp.	Cyperaceae	16
Horsenettle	*Solanum carolinense*	Solanaceae	16
Common ragweed	*Ambrosia artemisiifolia*	Asteraceae	16
Tree of heaven	*Ailanthus altissima*	Simaroubaceae	16
Apple	*Malus* spp.	Rosaceae	200
Rose	*Rosa multiflora*	Rosaceae	40
Carpetweed	*Mollugo verticillata*	Molluginaceae	16
Amaranthus	*Amaranthus* spp.	Amaranthaceae	16
Poor joe	*Diodia teres*	Rubiaceae	16
Ground cherry	*Physalis* spp.	Solanaceae	16
Sorghum	*Sorghum* spp.	Poaceae	16

Vector elimination
The BRNV paradigm

New field monitoring

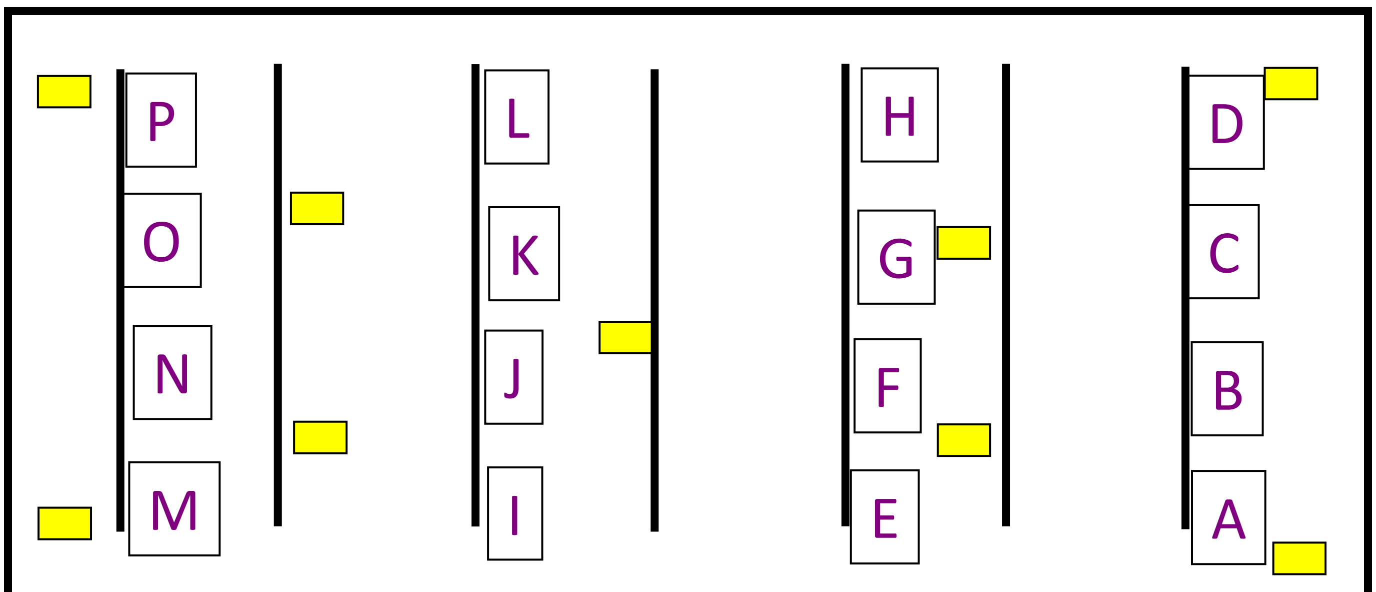
Permanent tagged plants

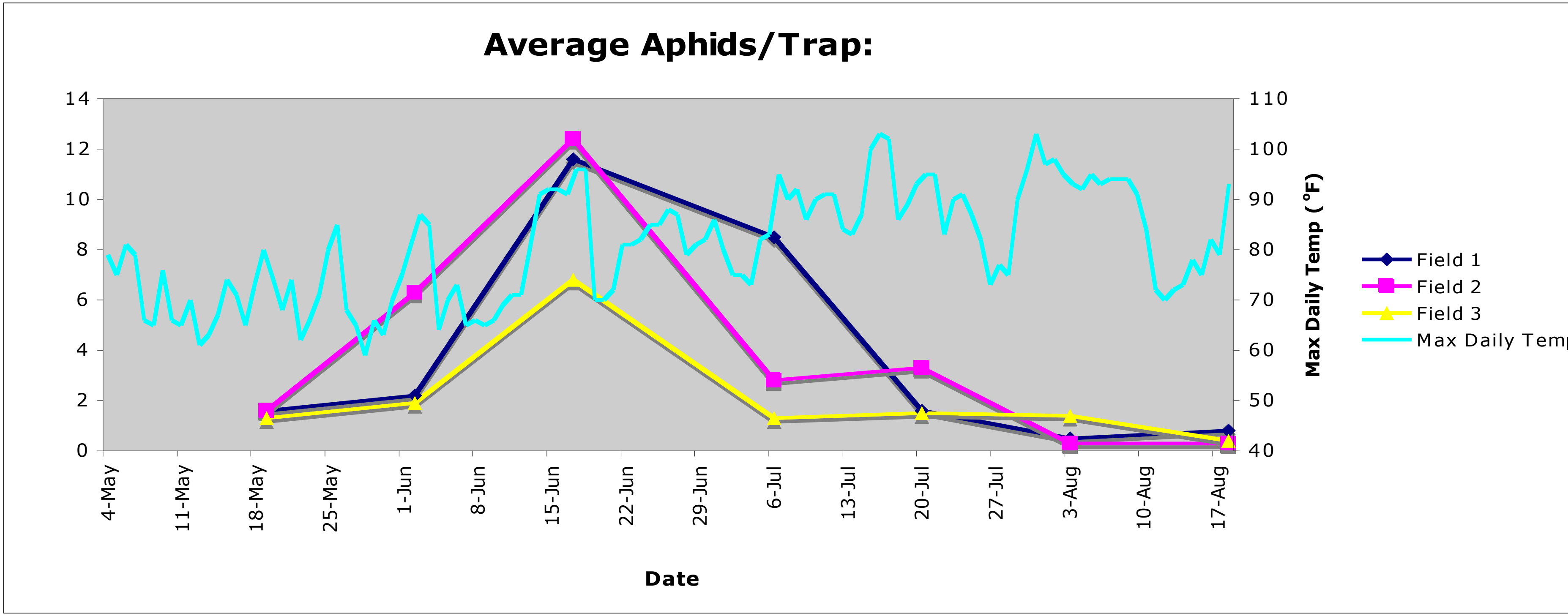
Time of transmission

High incidence of virus

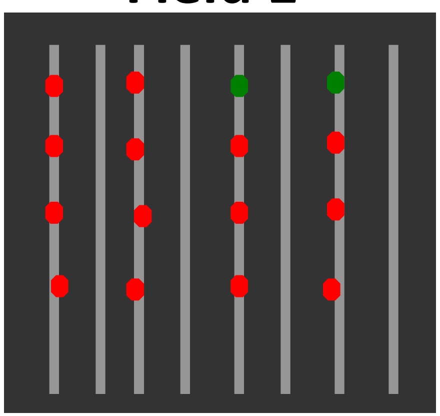
Potted plants

Rotated every month

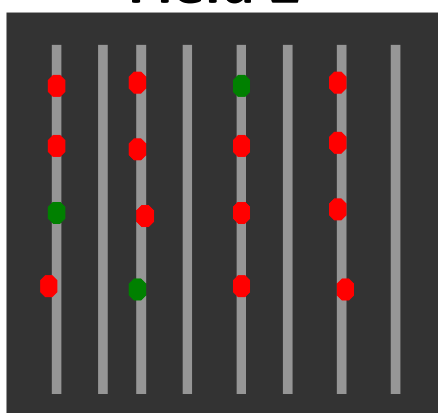


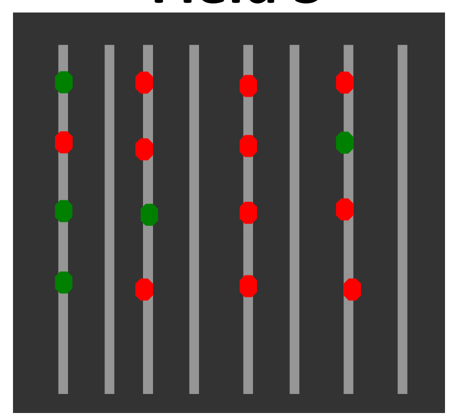
Field 1

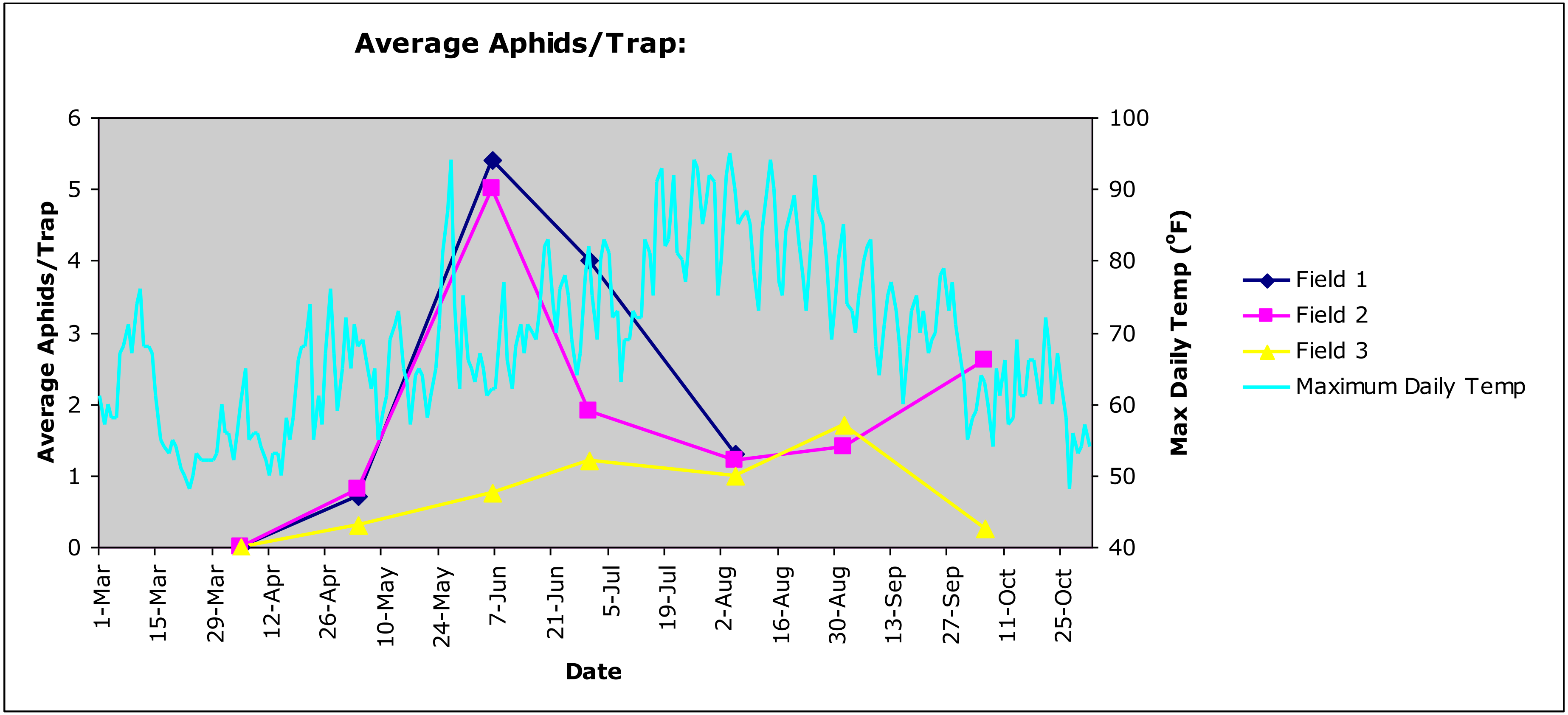


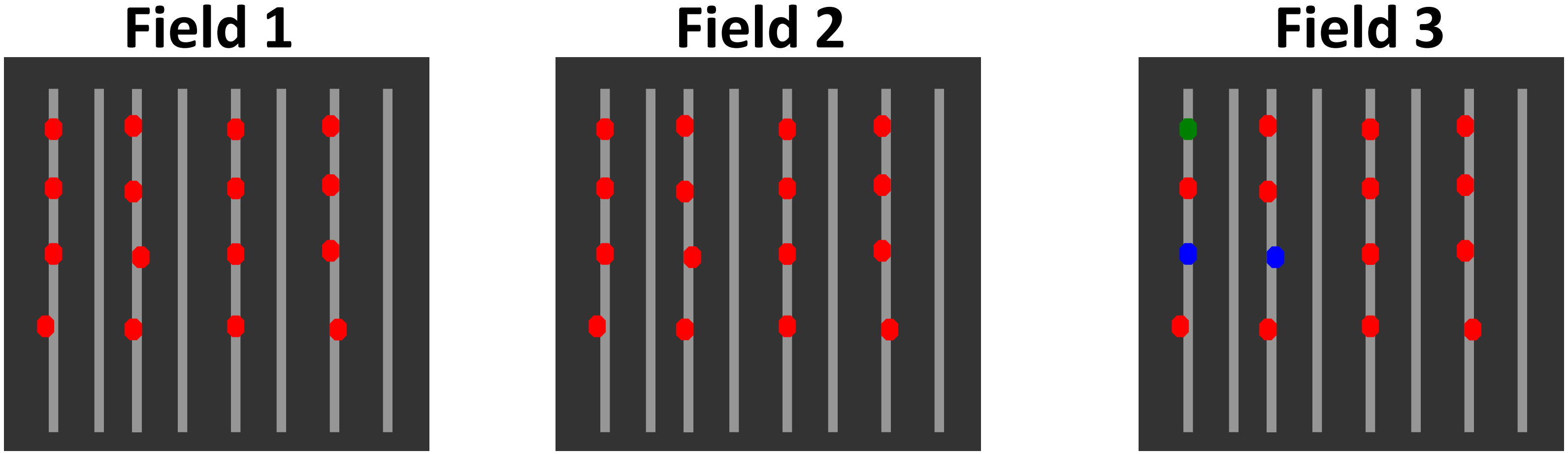
Field 2



Field 3

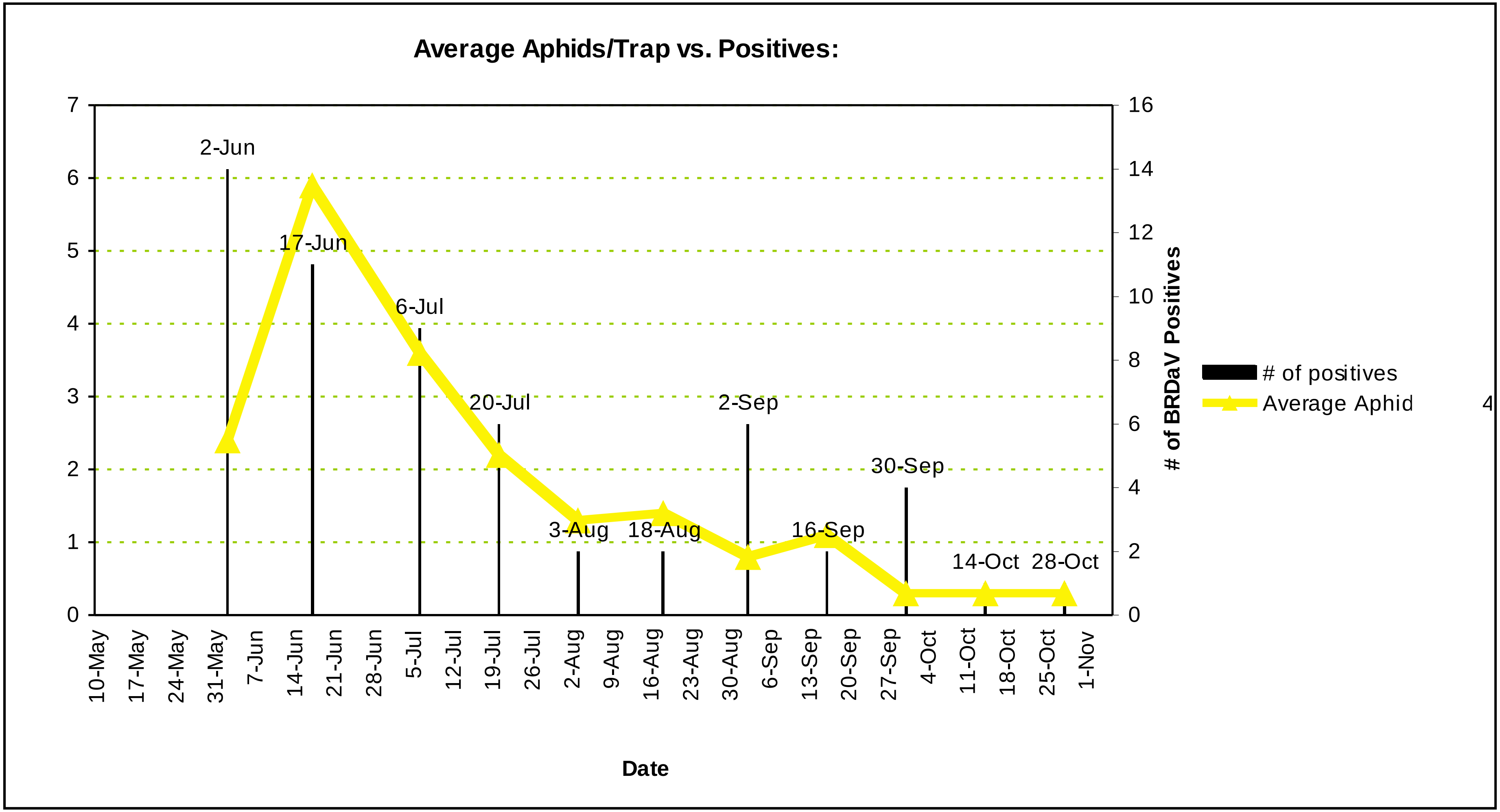






Nearly 100% transmission in three years!

Time of Transmission

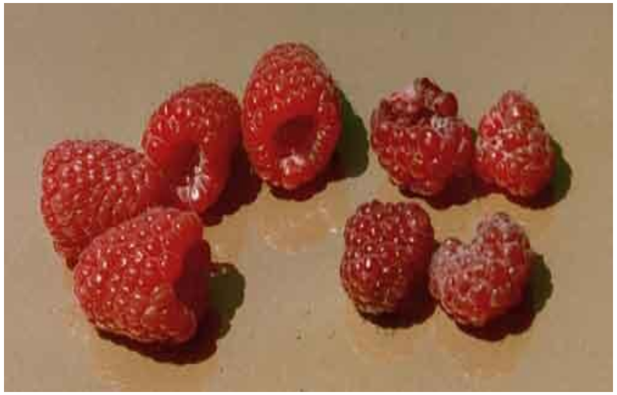


Raspberry crumbly fruit and decline

- The Pacific Northwest (PNW) is a primary producer of red raspberries

Raspberry crumbly fruit and decline

- ‘Several cultivars are susceptible to crumbly fruit disease (drupelets abortion)



- *Raspberry bushy dwarf virus* (RBDV), a pollen-borne idaeovirus was considered the causal agent of crumbly fruit

- Still, in many cases RBDV single infections did not cause symptoms

Another virus complex?

Important observations suggested that crumbly fruit symptoms may be increased by additional viruses:

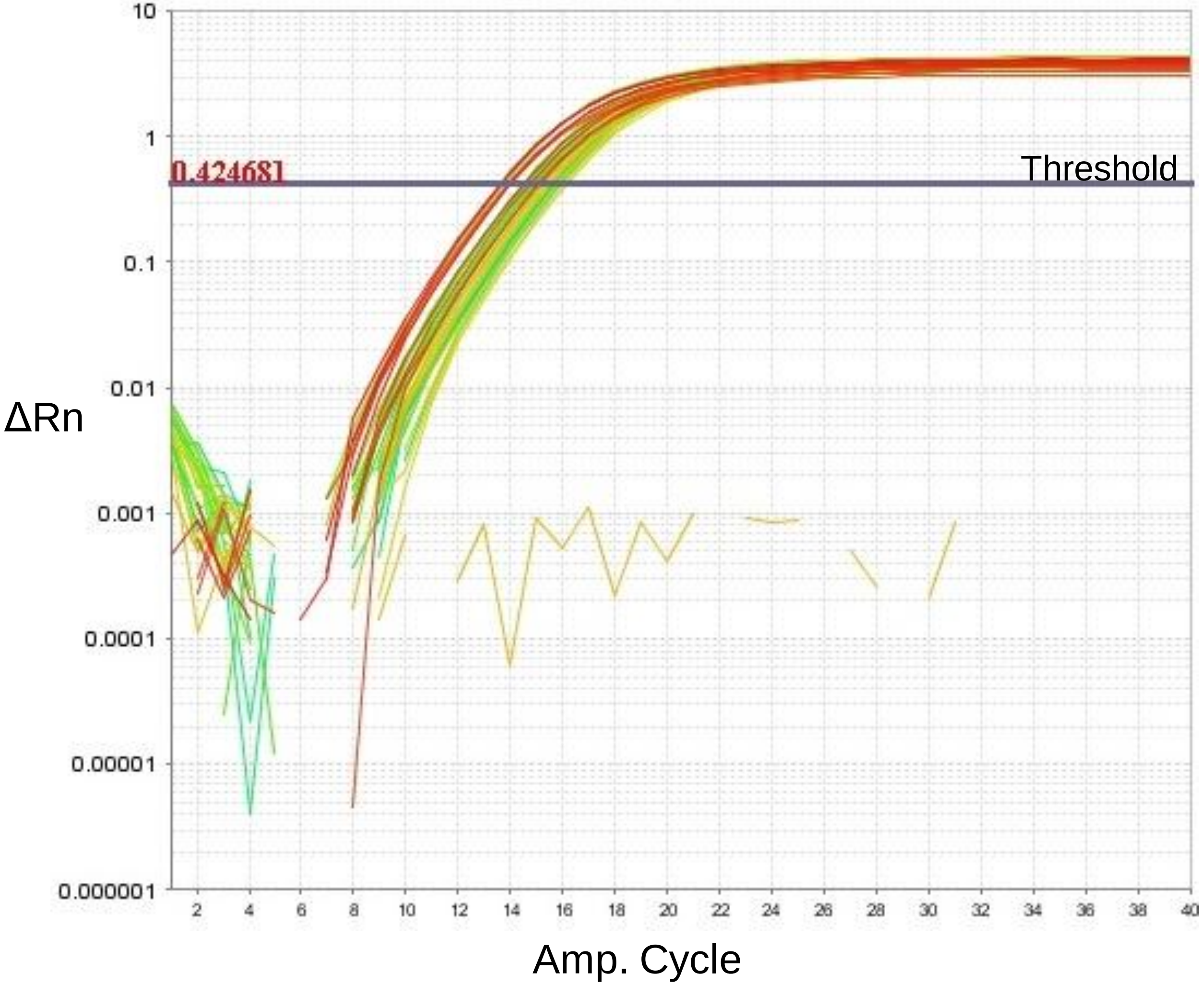
1. The disorder is more severe in cool areas with high populations of the large raspberry aphid *Amphorophora agathonica*

2. Two additional viruses found in severely affected fields, **Raspberry leaf mottle (RLMV)** and **Raspberry latent (RpLV)**

RBDV, RLMV and RpLV interactions

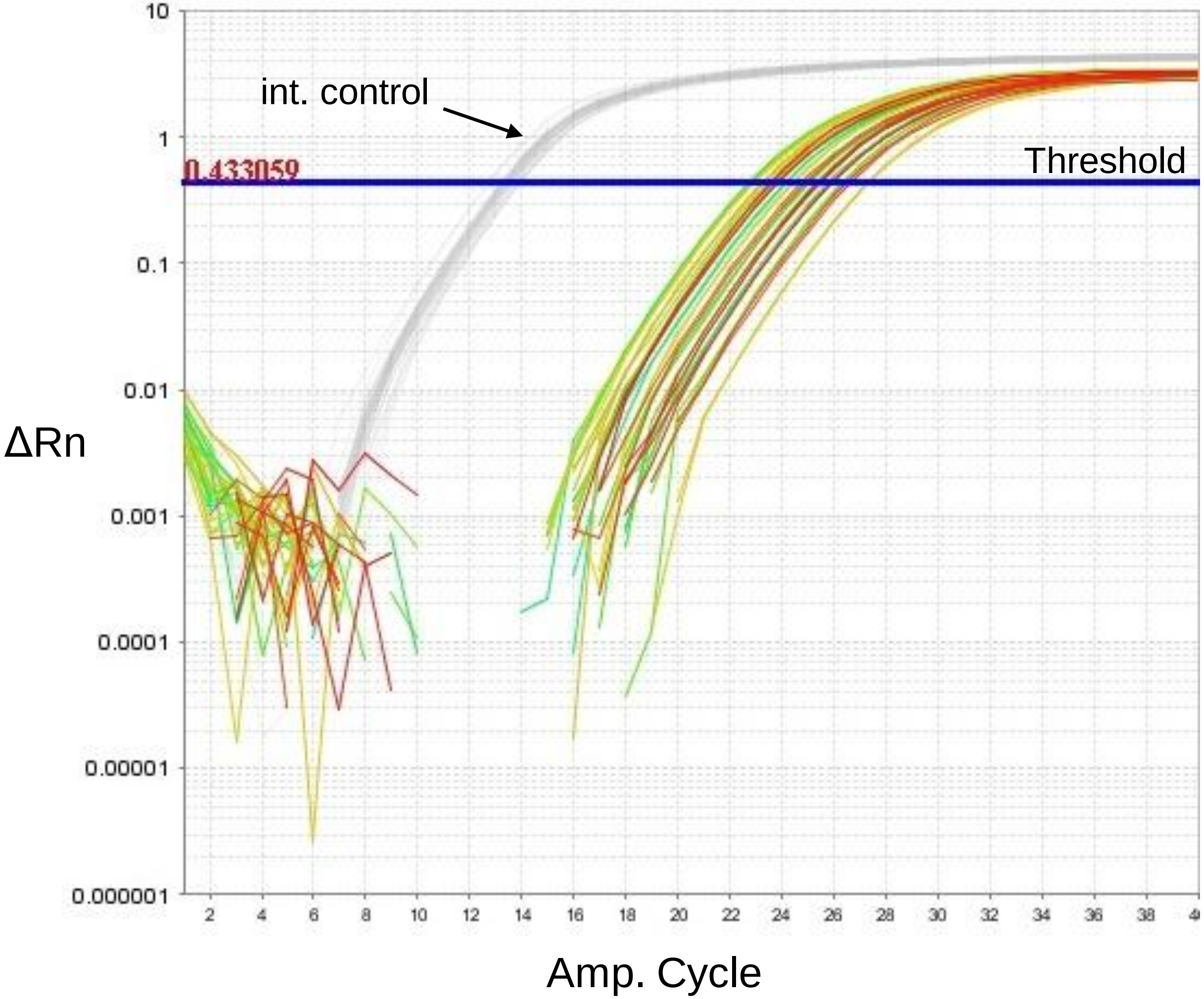
RLMV qRT-PCR

RLMV titer in single and mixed infections over time

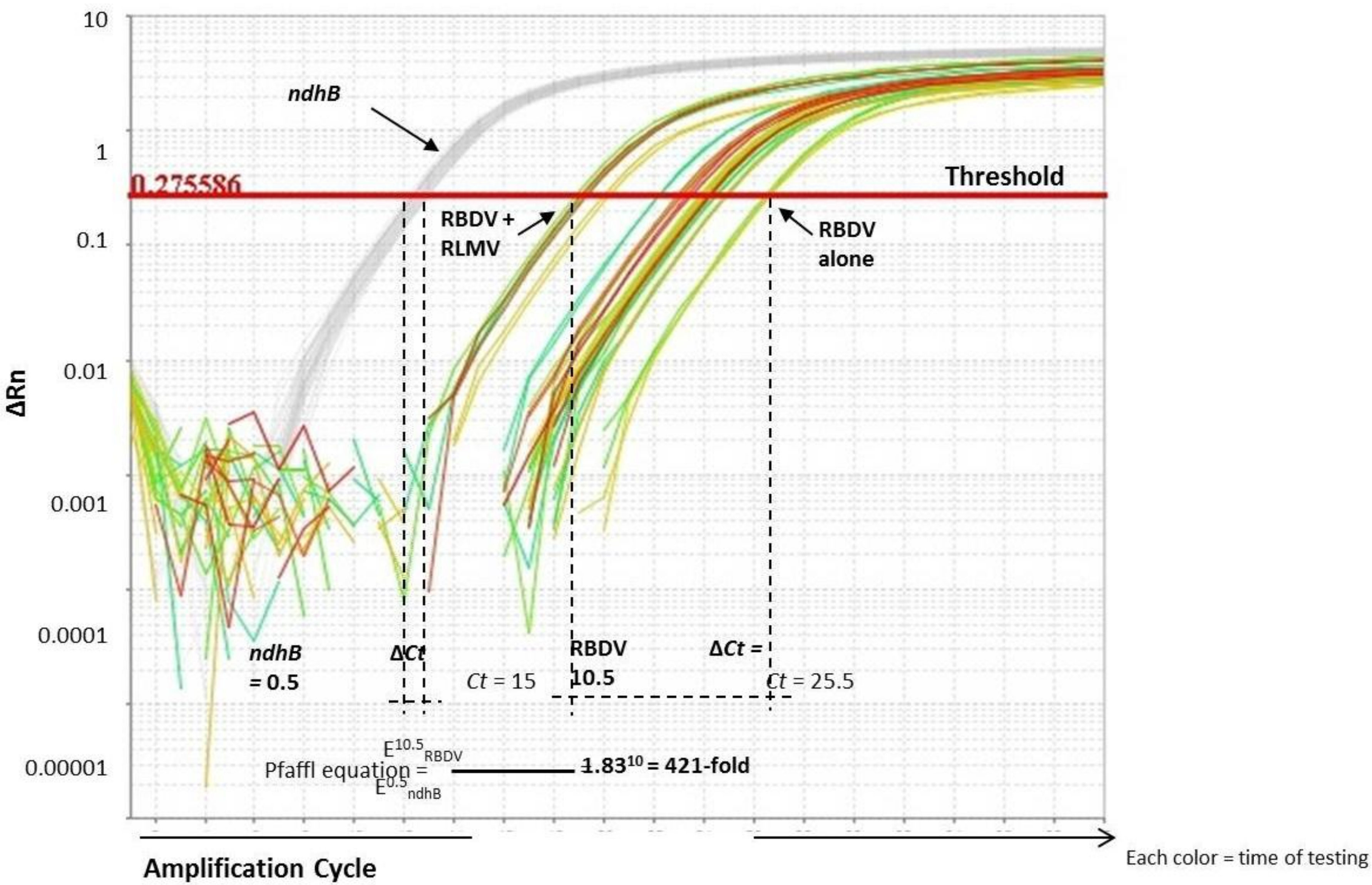


RpLV qRT-PCR

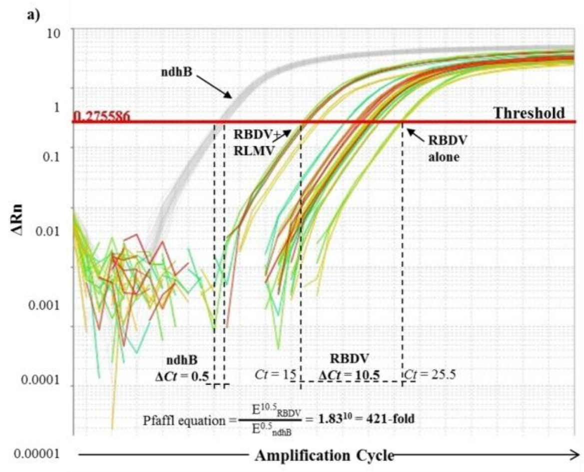
RpLV titer in single and mixed infections over time



RBDV titer enhanced in co-infections with RLMV

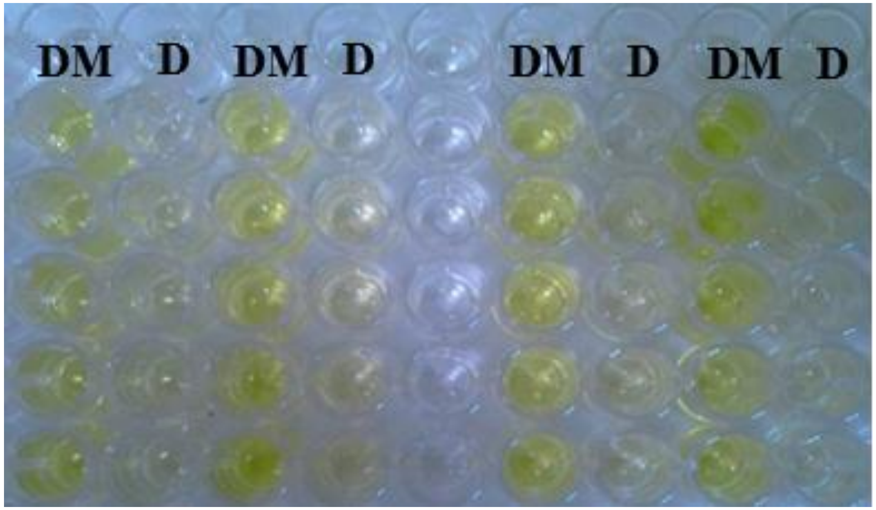


RBDV titer enhanced in co-infections with RLMV

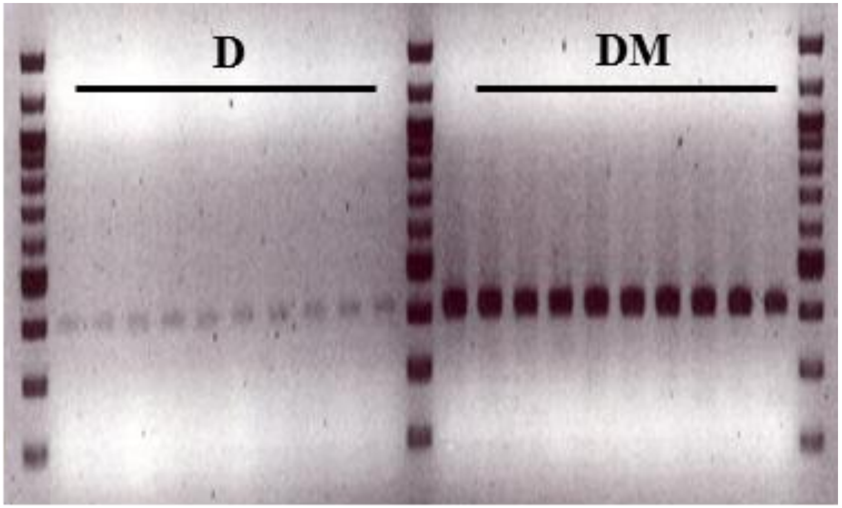


RBDV titer increase verified by conventional methods

ELISA

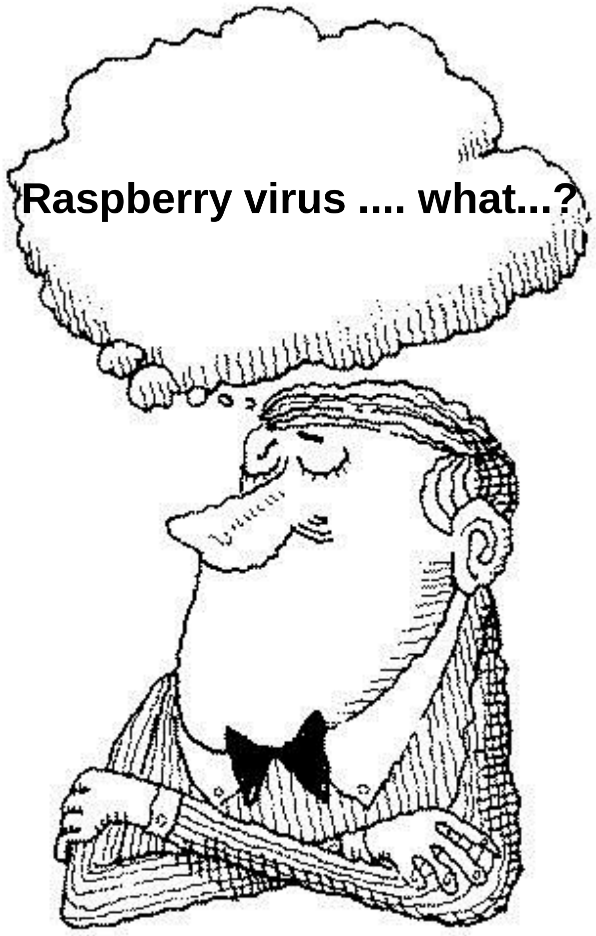


RT-PCR (20 cycles)

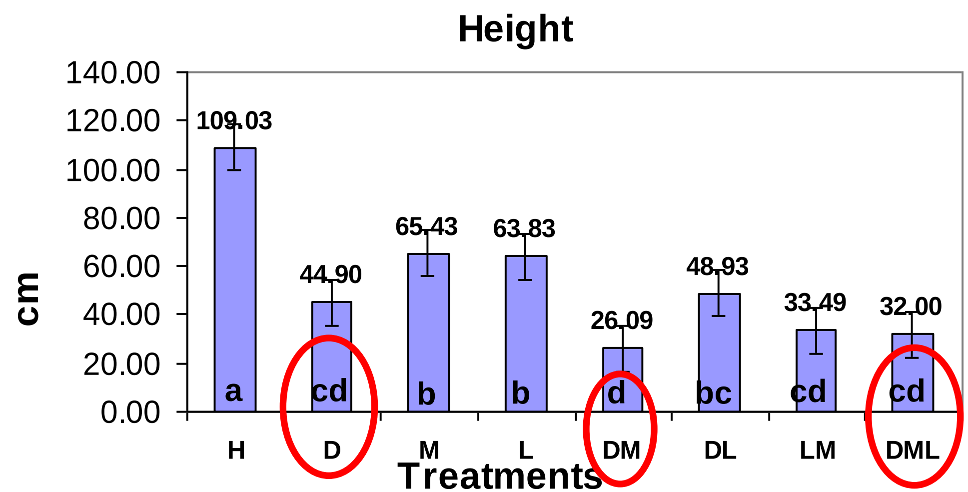


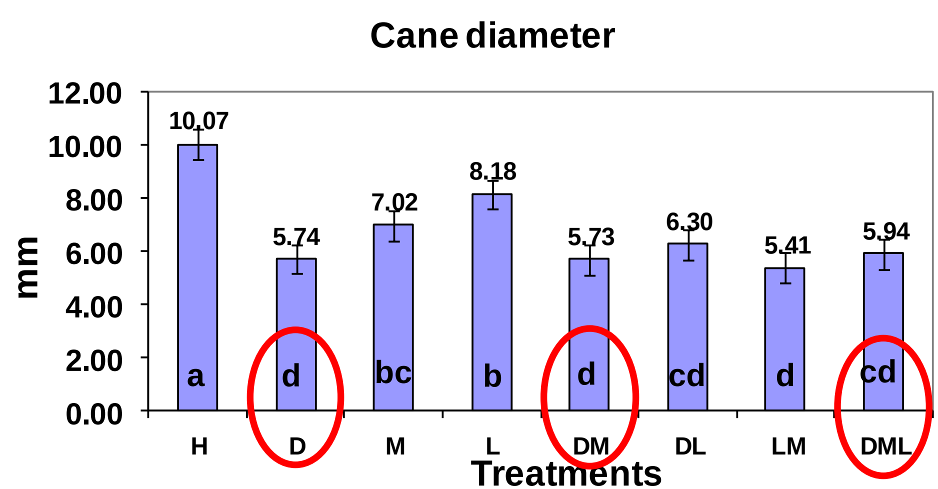
Mixed virus infections affect on plant growth and fruit crumbliness

- H Control
- **D** RB**D**V - **D**warf
- **M** RL**M**V - **M**ottle
- **L** Rp**L**V - **L**atent
- **DM** RB**D**V + RL**M**V
- **DL** RB**D**V + Rp**L**V
- **ML** RL**M**V + Rp**L**V
- **DML** RB**D**V + RL**M**V + Rp**L**V

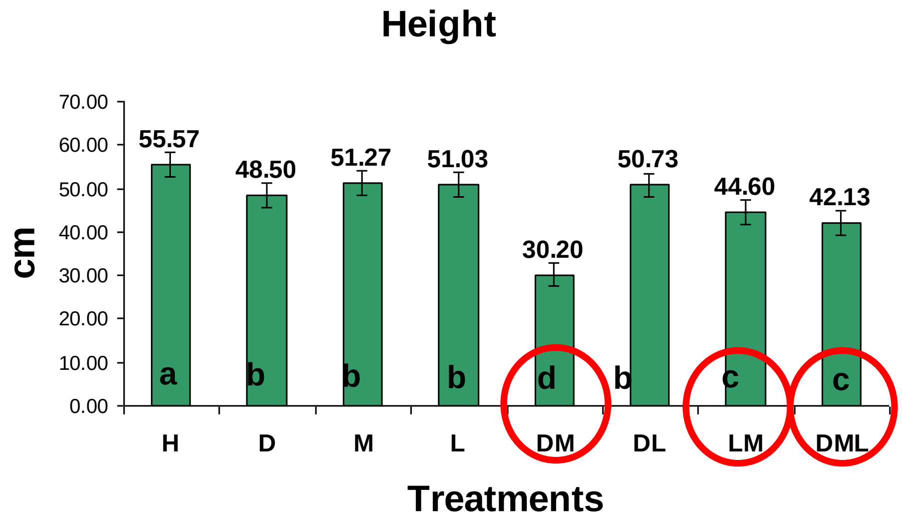


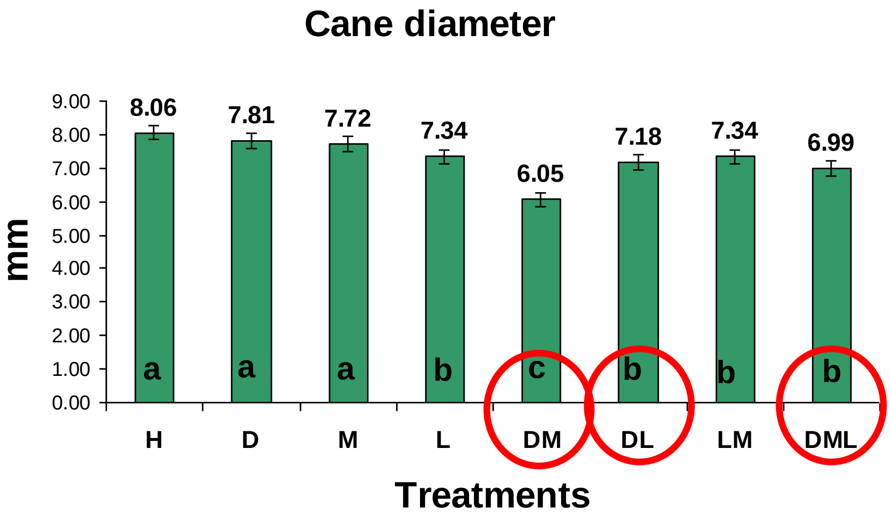
Plant Growth Establishment (2010)





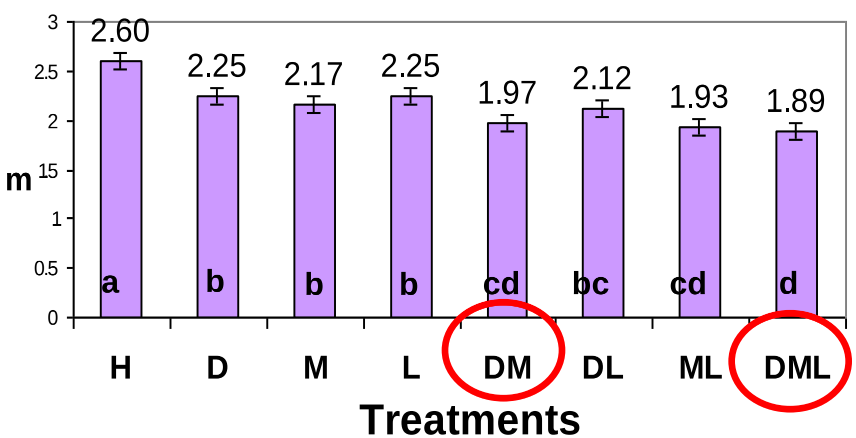
Plant Growth (2011)



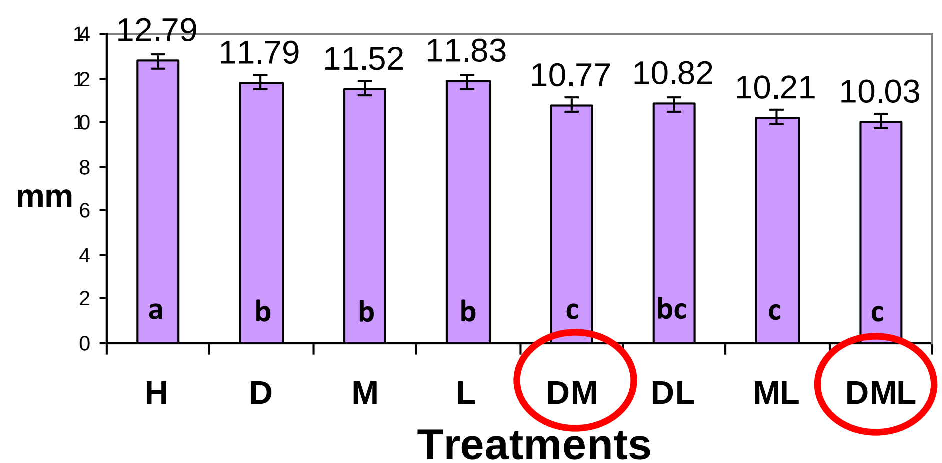


Plant Growth (2011)

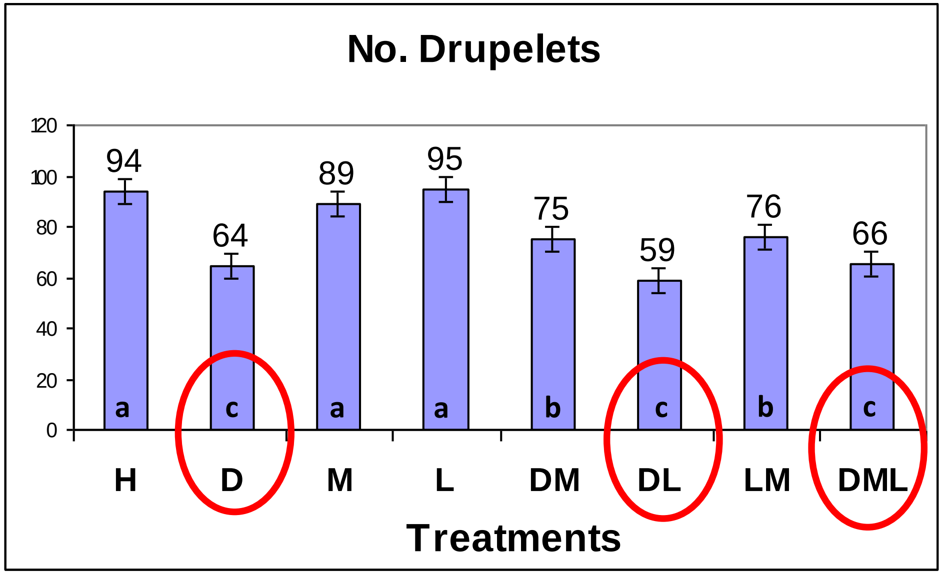
Height

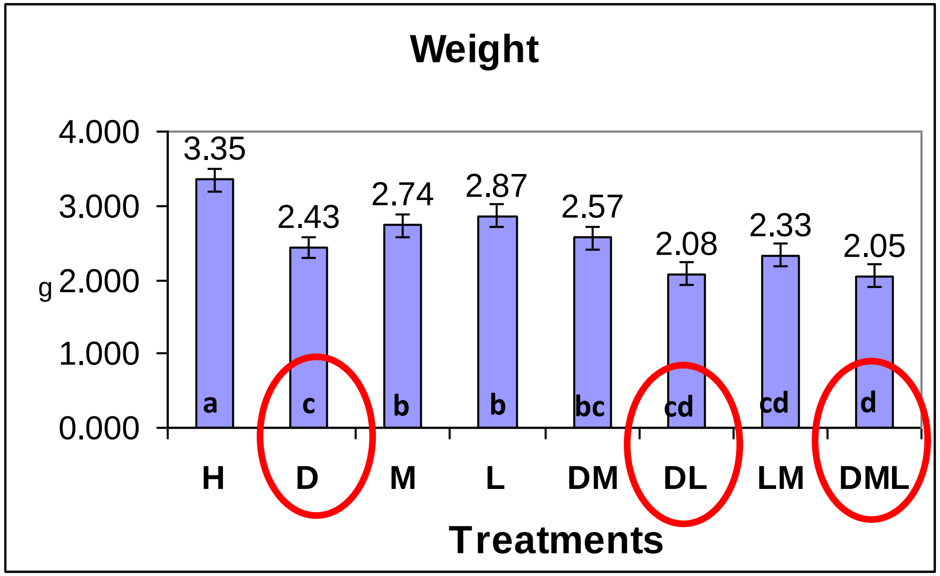


Cane diameter

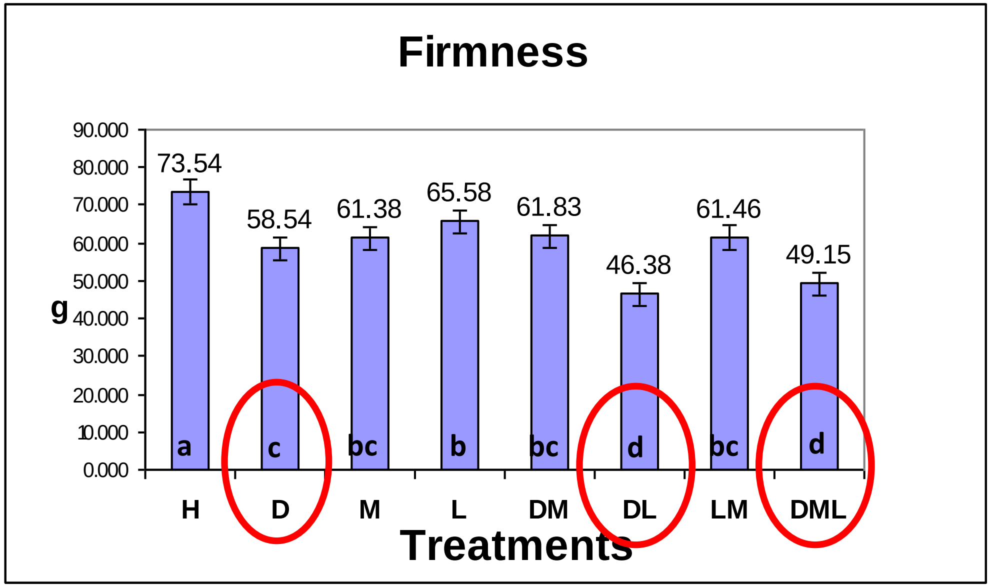


Crumbly Fruit





Crumbly fruit



Virus Incidence in 'Meeker' Fields

Northern Washington		
Field Age (years)	RLMV (%)	RpLV (%)
1	4	0
1	30	0
1	10	0
2	**58**	**21**
2	0	0
2	0	0
2	6	0
2	16	0
3	31	6
3	6	0
3	13	0
3	50	0
4	19	6
4	13	0
5	69	0
5	**90**	**80**
5	**100**	**75**
5	44	6
5	**100**	**17**
6	70	25
6	**100**	**6**
6	**100**	**12**
7	**100**	**6**
8	**100**	**46**

Southern Washington/Oregon		
Field Age (years)	RLMV (%)	RpLV (%)
1	0	0
5	40	20
6	0	20
7	8	17
8	**19**	**0**
8	**27**	**0**

Crumbly Fruit Scouting

Crumbly fruit and virus incidence in Washington

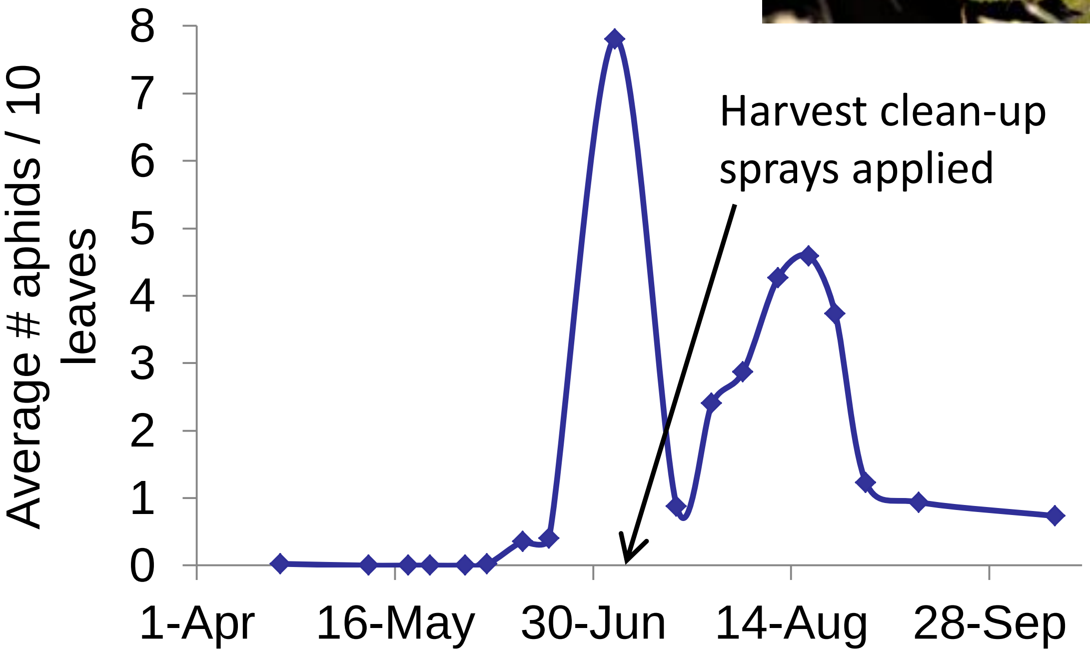
Field Age	Crumbliness 0: normal 3: severe	Virus incidence %		
	0 1 2 3	RBDV	RLMV	RpLV
4	1	44	25	0
4	2	100	100	0
4	3	93	100	7
5	**3**	**100**	**100**	**40**
6	**3**	**92**	**96**	**40**

Insects in Traps (2011)

Empoasca fabae was sporadic

Few numbers *of Macropsis fuscula*

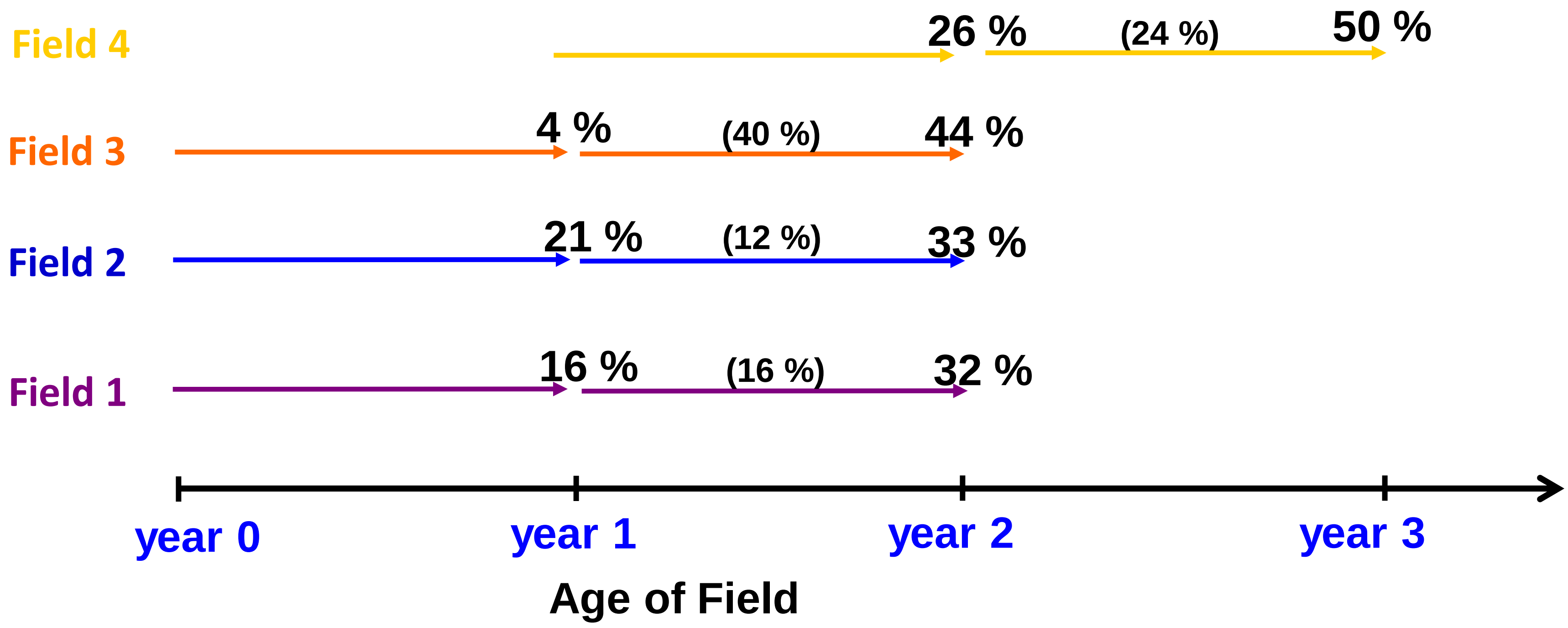
Raspberry aphid *A. agathonica* predominant insect



Lightle, unpublished data

RLMV spread in the field

Four fields being monitored for virus spread



Control Strategies

1. Think long term, identify potential risks of a site

2. **Start with clean plants**

3. Identify and diagnose problems early

4. Implement control strategies ASAP

5. If a virus complex is involved - identify viruses present and which are the easiest to control

The importance of clean plants

- Better establishment

The importance of clean plants

- Better establishment

- Longer life of plantings

The importance of clean plants

- Better establishment
- Longer life of plantings
- Fewer disease problems/Reduce risk of introducing new viruses to a region or field

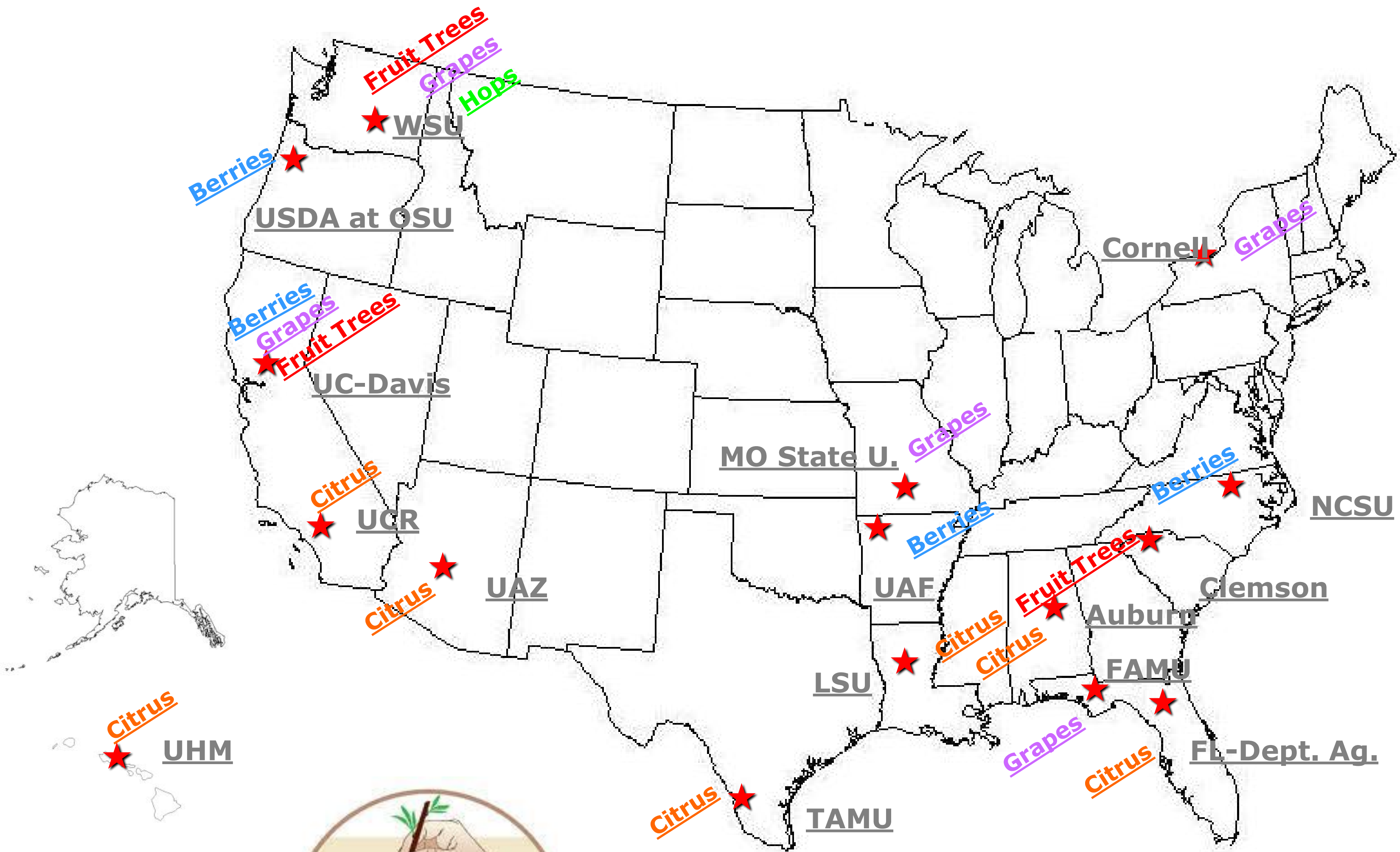
National Clean Plant Network

A federally-coordinated effort to secure high quality virus-tested plants for clonally propagated crops.

<u>NCPN Mission</u>

The NCPN provides high quality asexually propagated plant material free of targeted plant pathogens and pests that cause economic loss to protect the environment and ensure the global competitiveness of specialty crop producers in the United States.

NCPN Supported Clean Plant Centers



Berry Clean Plant Centers


Why care???

The story:

Propagation from an existing plot

10 ton/acre =$30,000/year

Latent infections with Blueberry scorch

The result?

Removal of infected material

Cumulative loss: ~ 100,000/acre

Florida 2013


Start clean!!!!

The team

The berry virus consortium:

16 individuals from UA, NCSU, USDA-ARS, MSU, UGA

Bindu Poudel, Diego Quito, Danielle Lightle, Anne Halgren, James Susaimuthu

NCPN
National Clean Plant Network
BERRIES

USDA
United States Department of Agriculture
National Institute of Food and Agriculture

NARBA
North American
Raspberry & Blackberry
Association