the Southern Region small fruit consortium

## Viruses in Caneberries

July 29-30, 2013 Asheville, North Carolina

Table of Contents


2 .....Agenda
4 .....Welcome
19 .....Berry viruses: movement and diseases
91 .....Identifying key pathogen vectors in small fruits
124 .....Nematode Vectors of Plant Viruses
141 .....Virus identification and detection
193 .....NCPN Program Update Early Summer FY 2013
208 .....Virus-Host Interactions
249 .....Arthropod vector management strategies in small fruits

**An Update on Viruses in Caneberries:**

**What We Know and Still Need to Learn**

**County Extension Agent Training Co-Sponsored by the SRSFC and**

**SCRI Grant on Management of Virus Complexes in Rubus**

**July 29-30, 2013**

**Asheville, North Carolina**

**Lodging** Hampton Inn and Suites, Asheville-I-26, 18 Rockwood Rd., Fletcher, NC 28732(828-687-0806)

**Objectives:** Educate agents on how viruses are transmitted in Rubus sp.; how they move; and management practices

**July 29, 2013**

**Basics of virus movements and transmissions-classroom instruction at the MHCREC Assembly Room, Mills River, NC**

**Moderator: Elena Garcia-University of Arkansas**

1:00 p.m. Welcome - Tom Monaco, Coordinator, SRSFC, NC State University

1:15 p.m. General Overview of How Viruses Move - Ioannis (Yannis) Tzanetakis, University of Arkansas

2:30 p.m. Insect Vectors - Hannah Burrack, NC State University and Donn Johnson, University of Arkansas

3:30 p.m. Break

4:00 p.m. Nematode Vectors - Terry Kirkpatrick, University of Arkansas

4:30 p.m. Collecting and Handling Nematode Samples (field activity) - Terry Kirkpatrick, University of Arkansas

**July 30, 2013**

7:30 a.m. Bus Pickup at Hampton Inn for Field Tour of Blackberry Grower Locations - Marvin Owings, Henderson County Cooperative Extension and Gina Fernandez, NC State University

1st Stop 8:30 AM Steve Dalton, Sugar Loaf Mtn.

2nd Stop 9:45 Don Justus (Trucking Don) Edneyville

3rd Stop 11:00 Andy Brownlee, Reiter and Rickie Holness, Driscoll's Edneyville

4th Stop Lunch, 12:30 p.m. Henderson County Cooperative Extension Office

These are approximated times, with approximately one hour stops.

**Plant Issues and Virus Management-classroom instruction at Henderson County Cooperative Extension Office Assembly Room**

**Moderator: Gina Fernandez-NC State University**

1:30 p.m. Recognizing Virus Symptoms – Ioannis (Yannis) Tzanetakis, University of Arkansas

2:00 p.m. Getting Clean Plant Material – Erich S. Rudyj, APHIS

2:45 p.m. Break

3:15 p.m. Plant Responses - Elena Garcia, University of Arkansas

4:00 p.m. Management Practices - Donn Johnson, University of Arkansas and Hannah Burrack, NC State University

5:00 p.m. Depart

the
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small fruit consortium

# History

- 1999 Meeting
- MOU 2000 NC State, Clemson, Univ of GA
- 2002 Univ of TN
- 2005 VA Tech
- 2008 Univ of Arkansas
- Six Member Institution
- Annual Budget $210,000

# Objectives

- Pool expertise
- County Agent Training
- Promote Research
- Education
- Web site

# Grant Program

- Goal-provide funding for applied research and extension activities
- Competitive
- Seed Grants-maximum of $5000
- Total amount awarded 2001-2013= $1.12 million
- Reports posted on SRSFC website
- http://www.smallfruits.org/SRSFCReserchFunding/index.htm

# County Extension Agent Training

- Enhance expertise in small fruit production
- Twenty three trainings since 1999
- A total of 575 agents from the member states have attended
- Scholarships awarded to cover cost of training, 4 per member state
- Events held in the member states

# Blueberry Workshop June 2007

# Muscadine Workshop 2006

# Pruning Workshop 2006 Walterboro, South Carolina

# Strawberry Training 2008 Charlotte, North Carolina

# Blackberry Training 2009 Lincolnton, North Carolina

# SRSFC Web Site

www.smallfruits.org

Web Master  Brenda Willis UGA

- Hits per day 4,000
- Contents
  - SFN-published quarterly
  - IPM/Production Guides
  - Crops
  - County Agent Trainings
  - Research and Ext Grants

# Other Activities

- Sponsorships of small fruit meetings/conferences
- Travel grants for county agents
- Support of state extension meetings
- Recruiting membership
- Partnering in Specialty Crops Grants
- Grant writing
- Publication sponsorships

# USDA NIFA Award to SRSFC

- The SRSFC received the 2012 NIFA Partnership Award in Multi State efforts. Only one of these presented per year in the US
- Citation-”For successful regionalization focusing on improving the knowledge base and service to small fruit growers in the southern region of the United States”

# July 29-30, 2013 Training

- Reimbursement questions?
- Evaluations
- Other Questions?
- Participant Introductions

# July 29-30, 2013 Training

- Organizers-Elena Garcia, Ioannis(Yannis) Tzanetakis, Tom Monaco

Sponsors

SRSFC

SCRI Specialty Crop Grant-UAR

# Berry viruses: movement and diseases

Ioannis E. Tzanetakis

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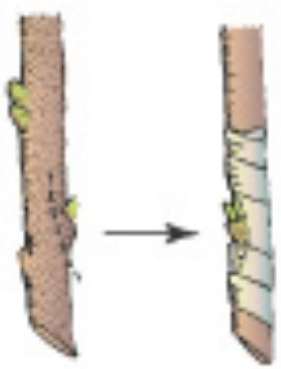
479-575-3180

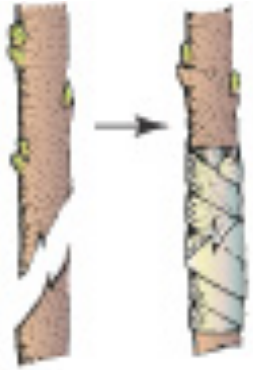
UNIVERSITY OF ARKANSAS
DALE BUMPERS COLLEGE
OF AGRICULTURAL, FOOD & LIFE SCIENCES

U of A
DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

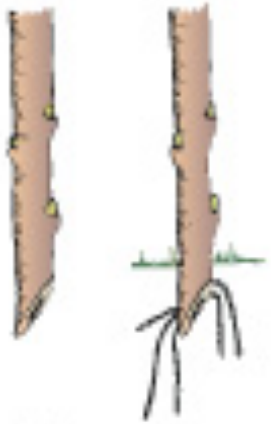
Virus transmission 101



By budding



By grafting

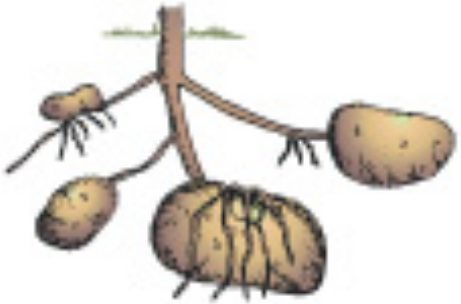


By cuttings

By bulbs

By corms

By rhizomes



By tubers

By runners (stolons)

Through natural root grafts

Through dodder

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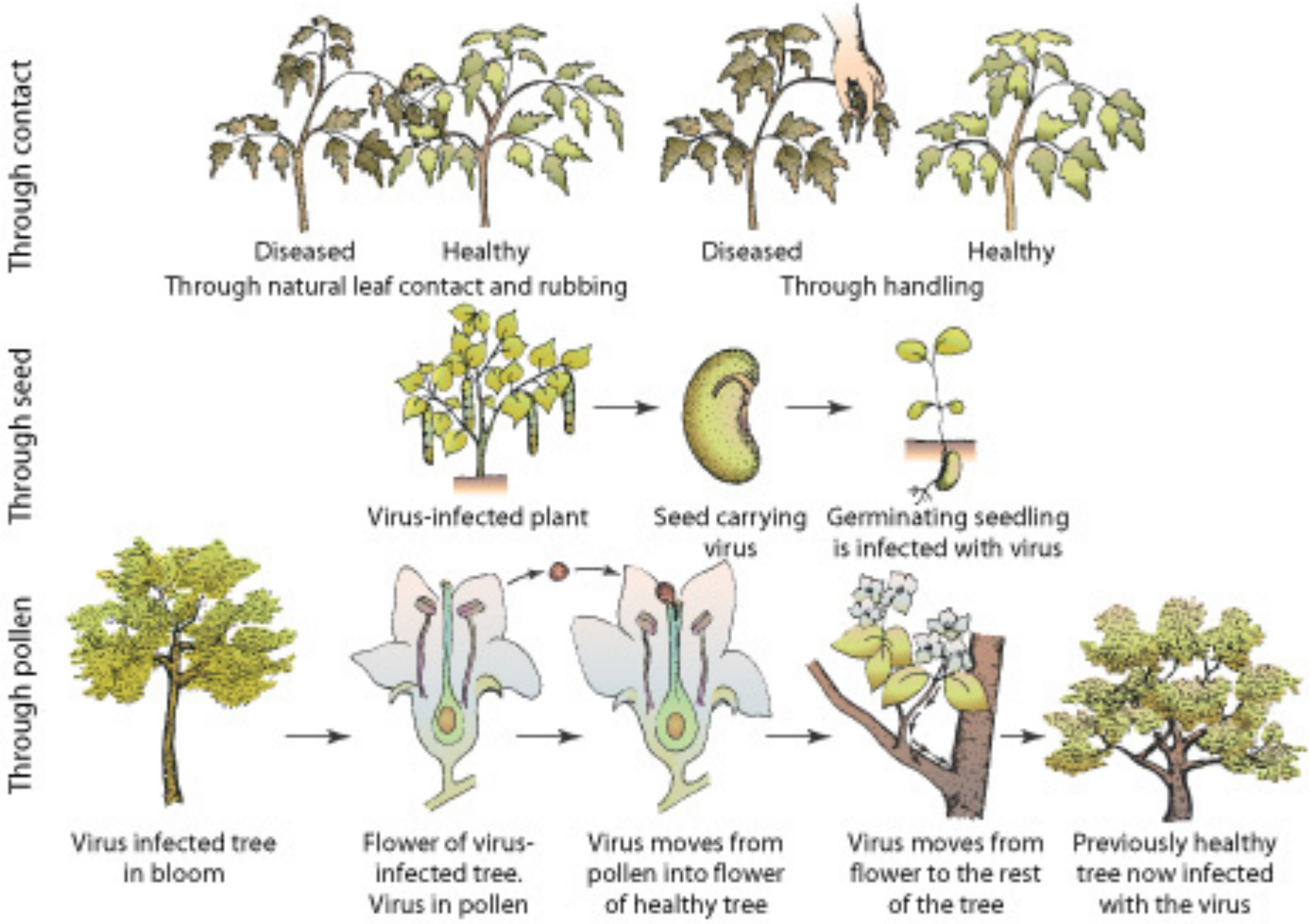


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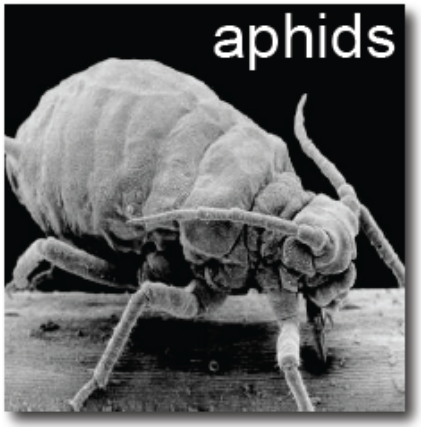
# Phylum *Arthropoda*, Class *Insecta*, Order *Hemiptera*



# Phylum *Arthropoda*, Class *Insecta*

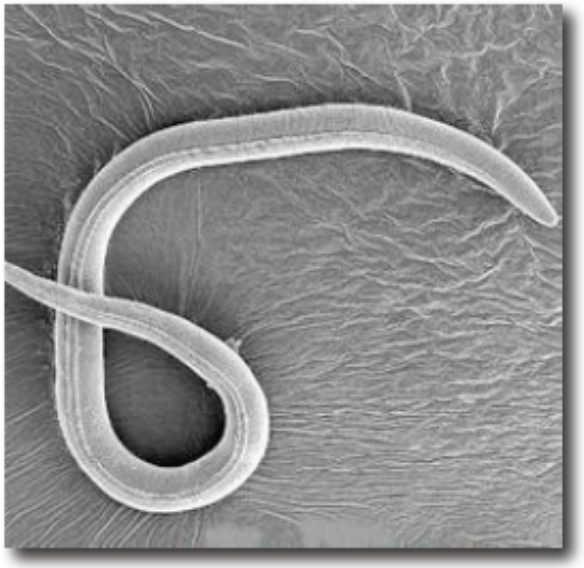
Order *Thysanoptera*

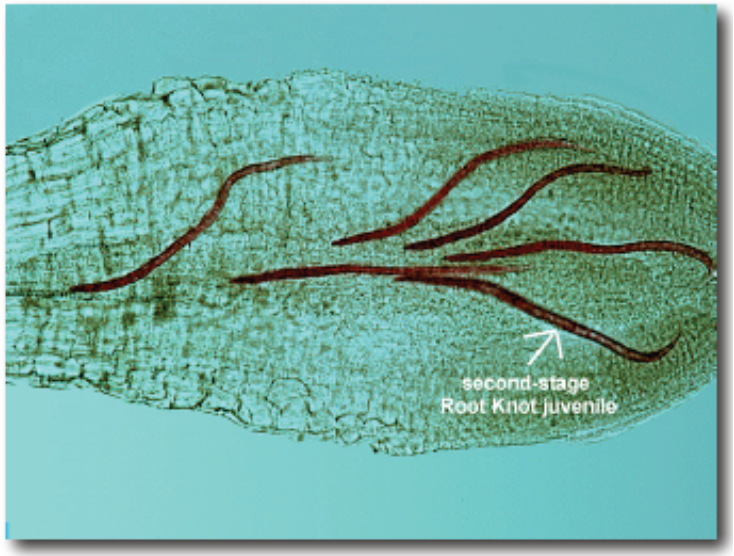
Order *Coleoptera*

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

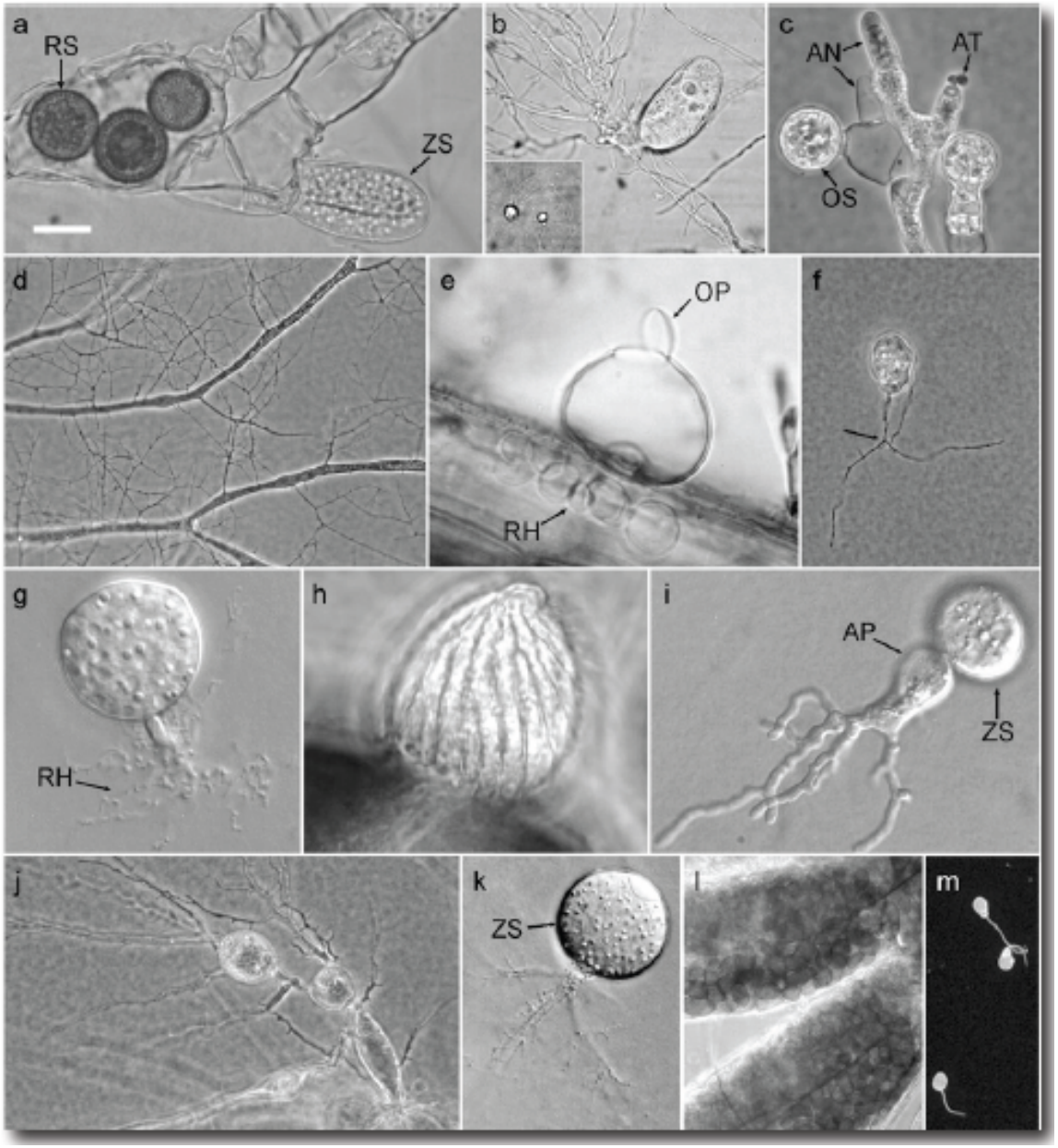
mites

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






Nematodes




Kingdom: *Fungi*

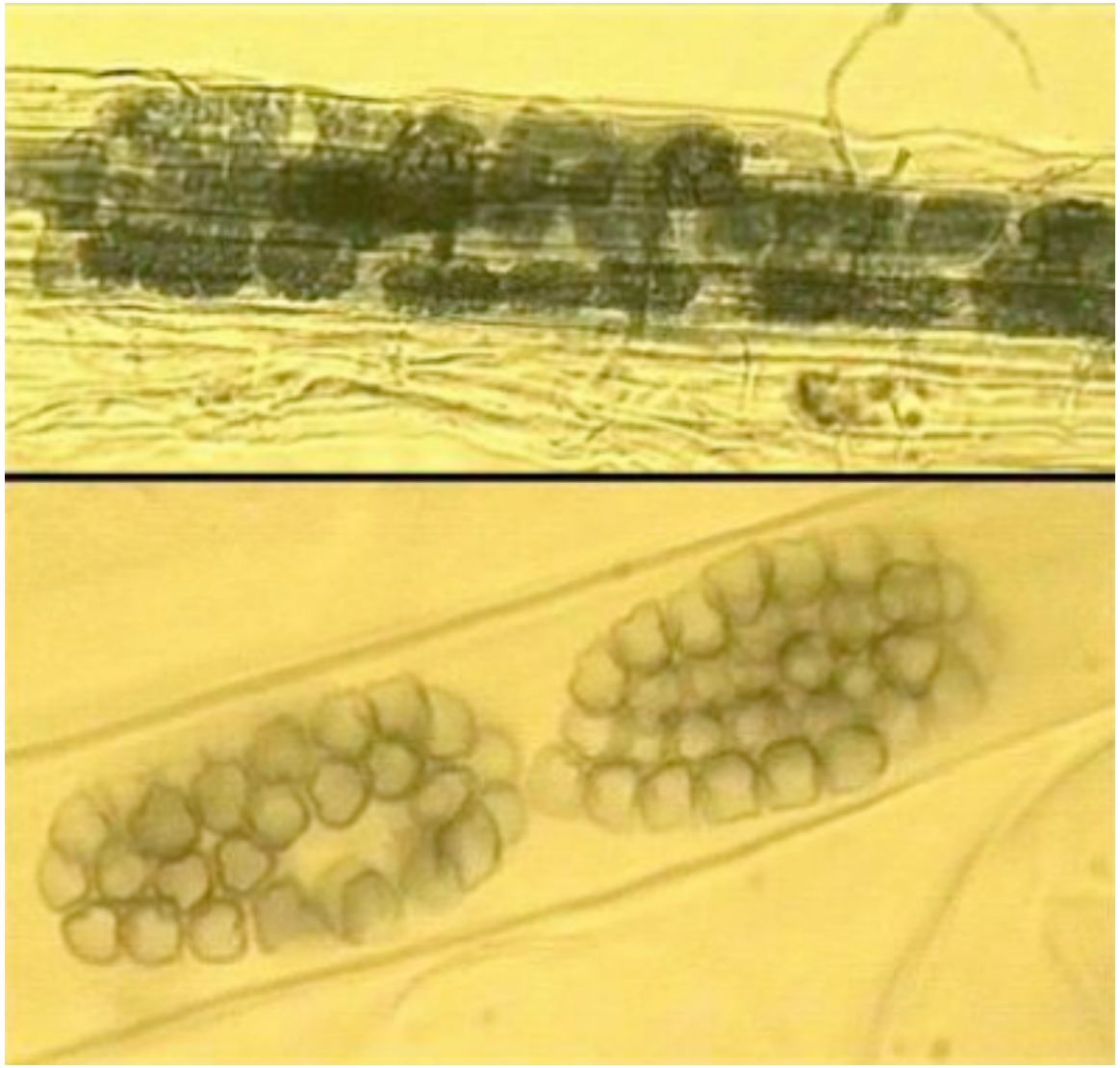
Phylum: *Chytridiomycota*

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

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

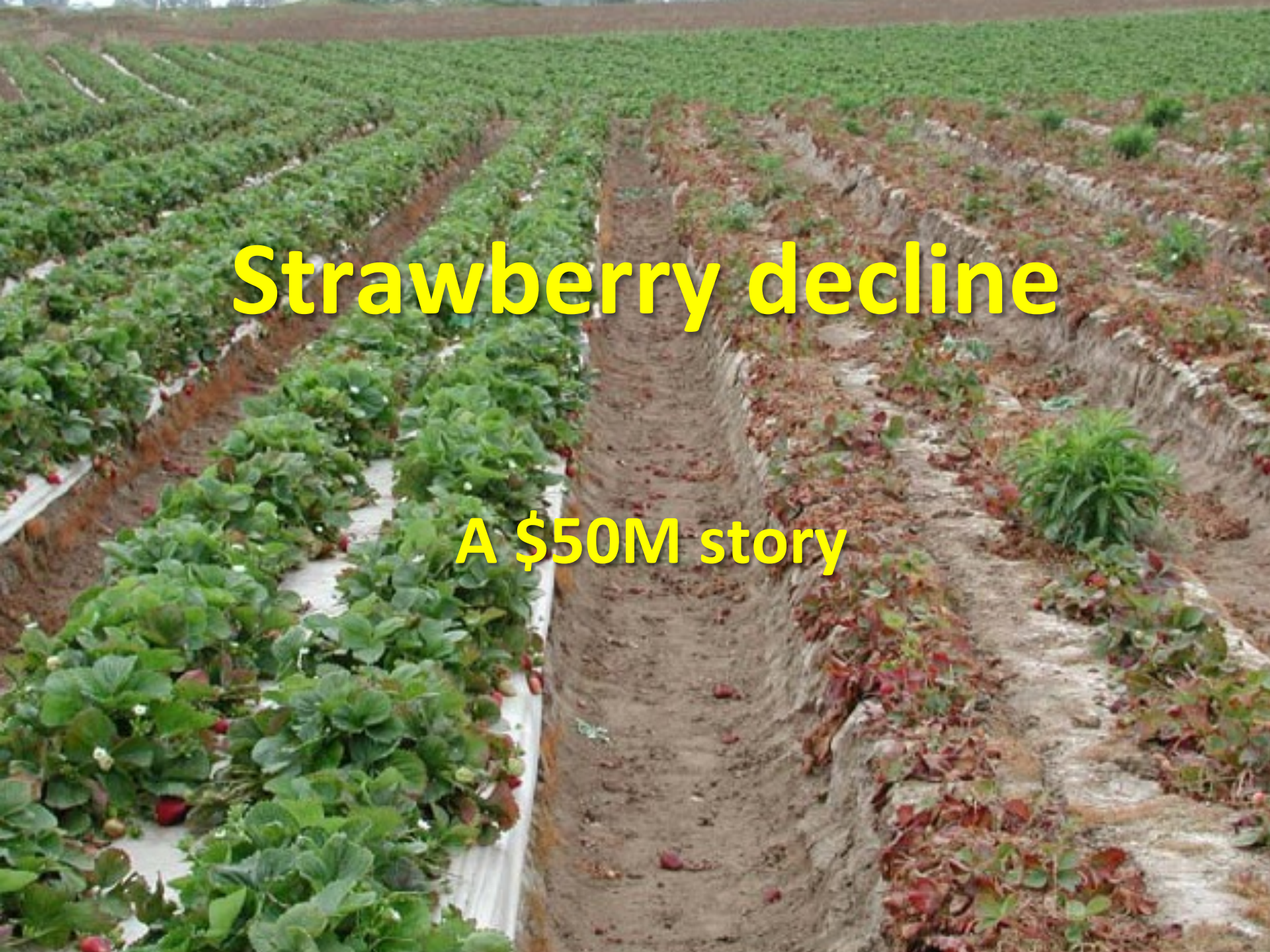
Aphids

Fragaria chiloensis latent – Chile
Tobacco streak*

Pollen

Strawberry pallidosis
Beet pseudo-yellows

Whiteflies*


Strawberry decline
A $50M story

# 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*)

| | Plant species | |
|---|---|---|
| **Virus** | ***Fragaria* × *ananassa*** | ***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 decade

16 viruses & virus-like agents were known to infect *Rubus* before we started looking into *Rubus* complexes – We now have over 45…

**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…..

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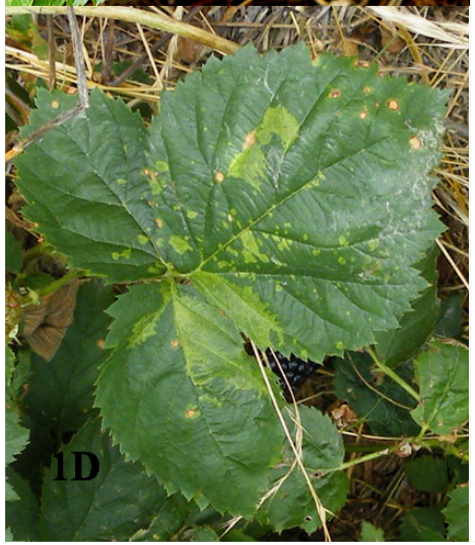
*Raspberry latent virus*
etc…..

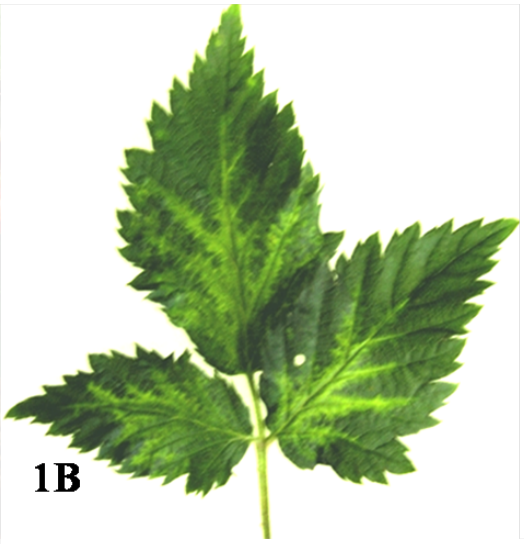
**Tests are available for all the new viruses**

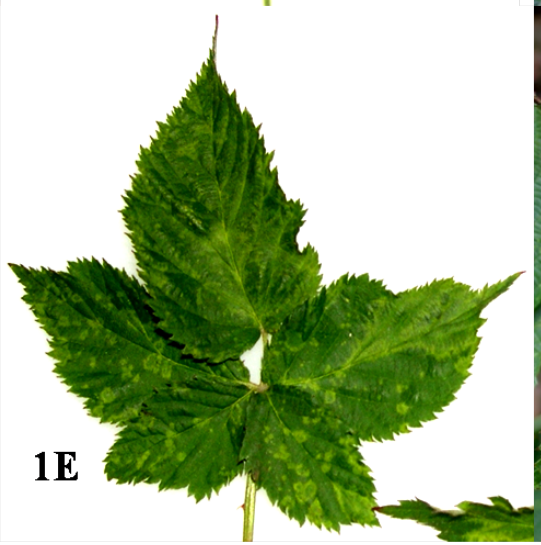
# Same disease-different viruses

## AR

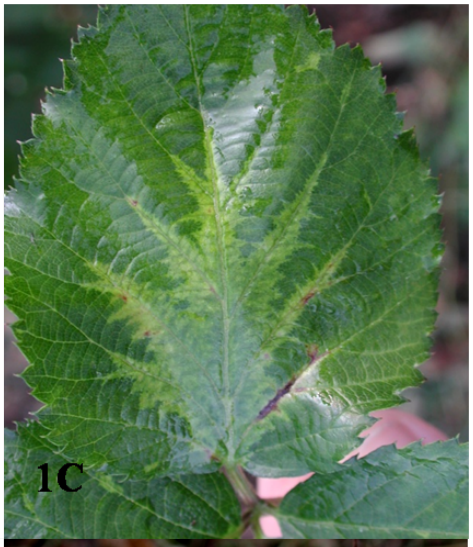



## MS




## 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.

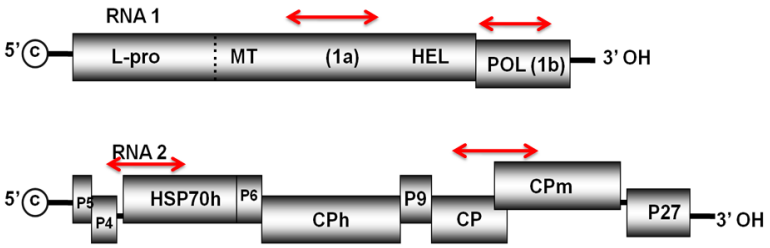
C. Identify virus vectors.

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.

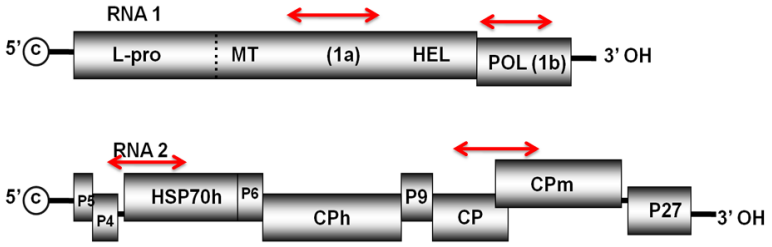
# What are the viruses present in your area? The importance of detection

- BYVaV - Multistate sample collection - 35 isolates

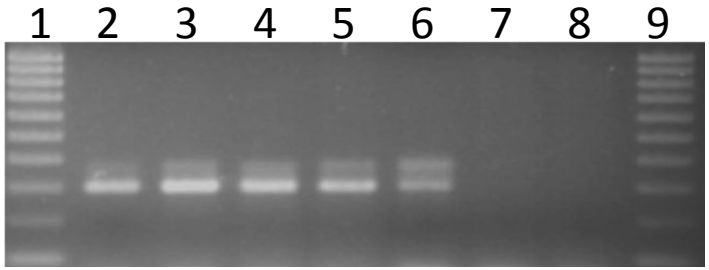


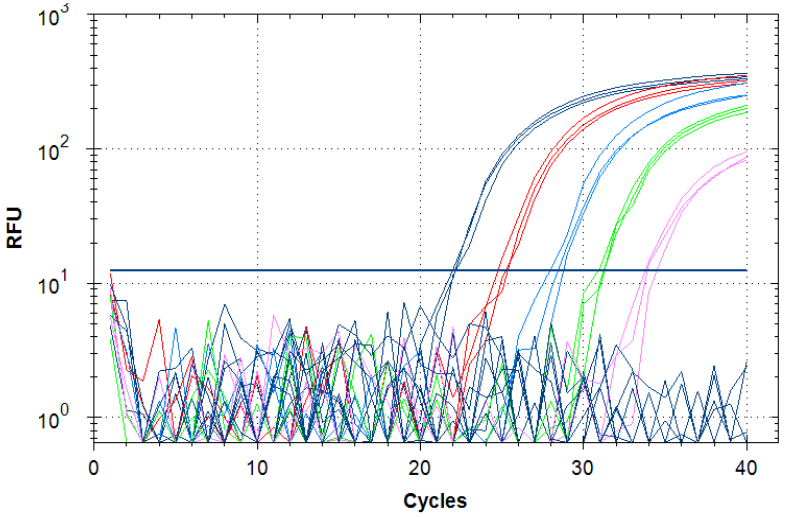
# What are the viruses present in your area? The importance of detection

- 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.

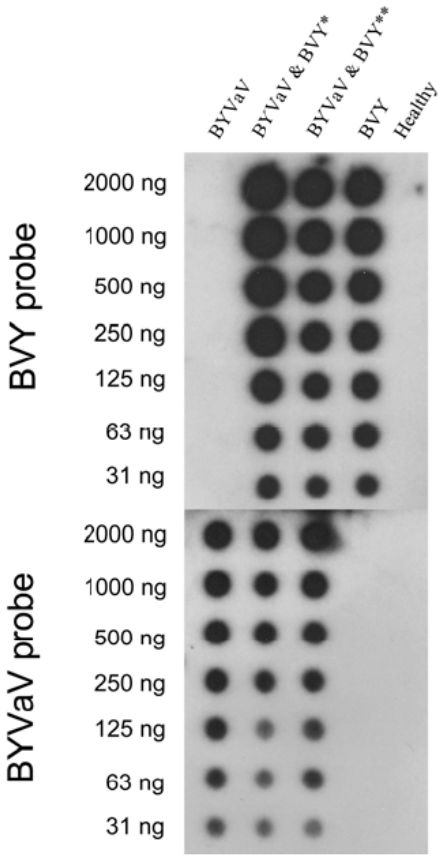



**Fig. 4.** Nucleic acid spot hybridization (NASH) detection of Blackberry virus Y (BVY) and Blackberry yellow vein associated virus (BYVaV) in blackberry. The blot was initially hybridized with a BVY-specific DNA probe and subsequently with a BYVaV-specific probe after stripping the BVY probe. *symptomatic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

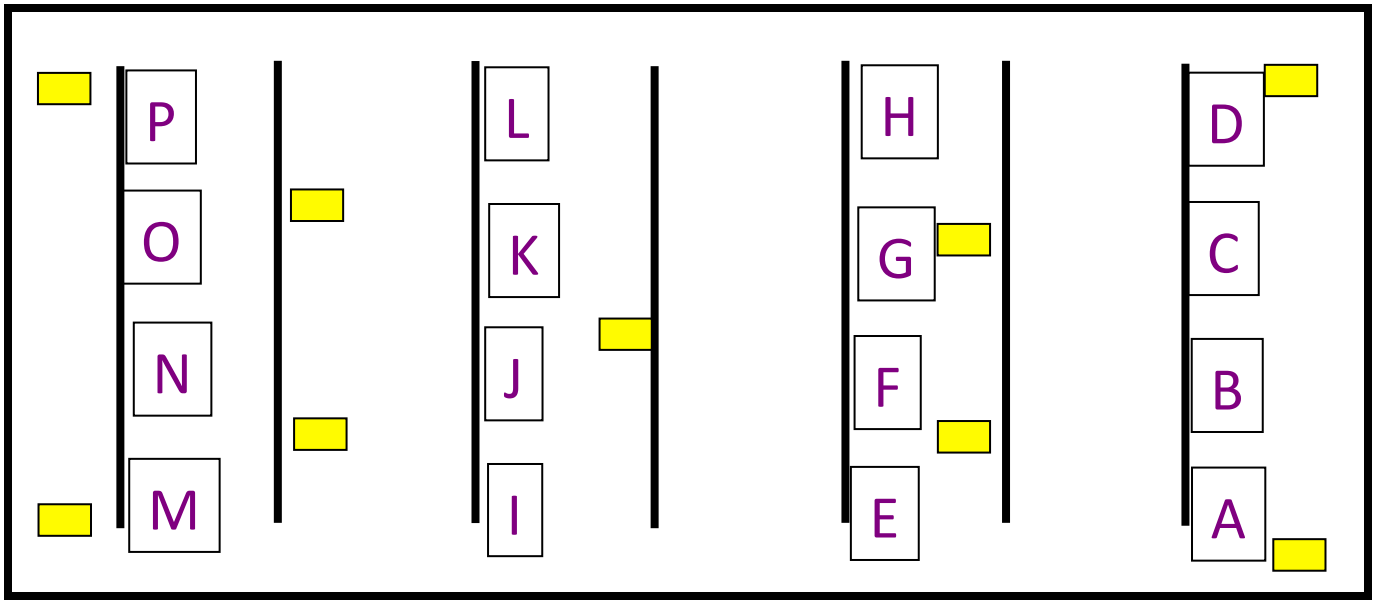
# Alternate hosts

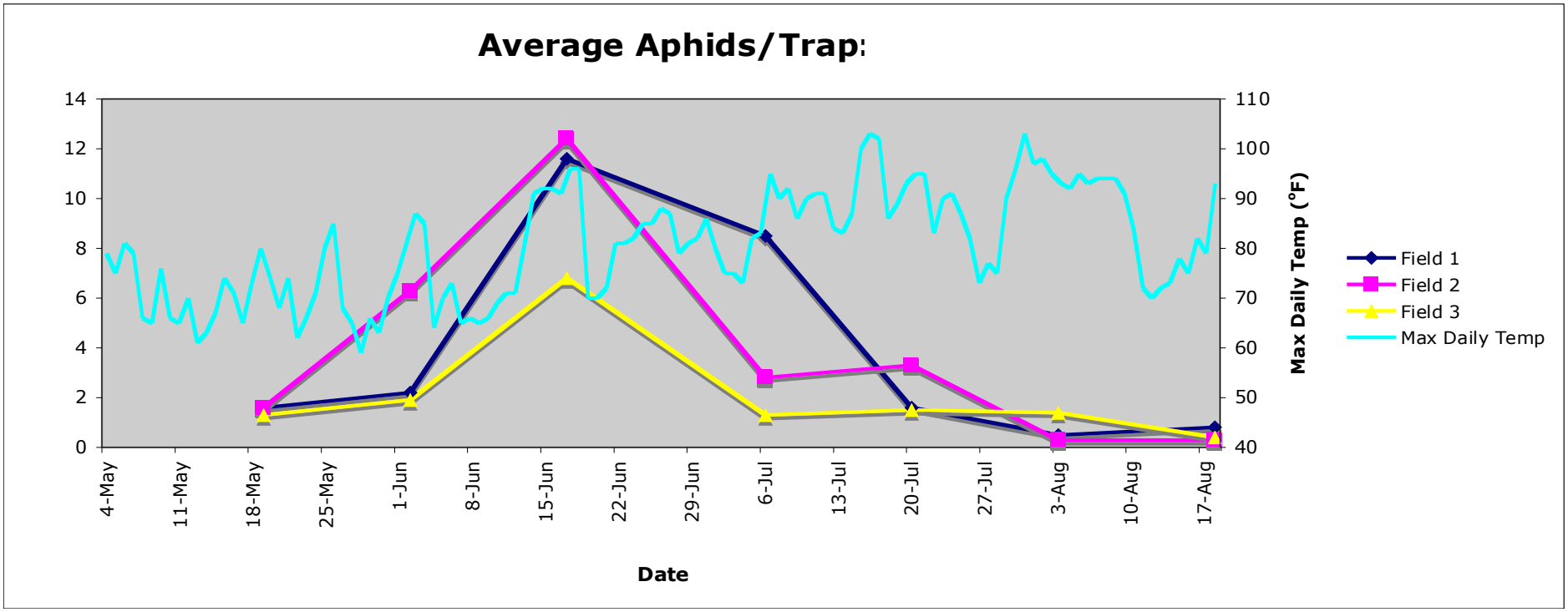
| Plant species | Scientific name | Family | Number of plants tested |
|---|---|---|---|
| Garden vetch | *Vicia sativa* | Fabaceae | 16 |
| Virginia creeper | *Parthenocissus quinquefolia* | Vitaceae | 16 |
| Red clover | *Trifolium pretense* | Fabaceae | 16 |
| Wild garlic | *Allium vineale* | Amaryllidaceae | 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 |

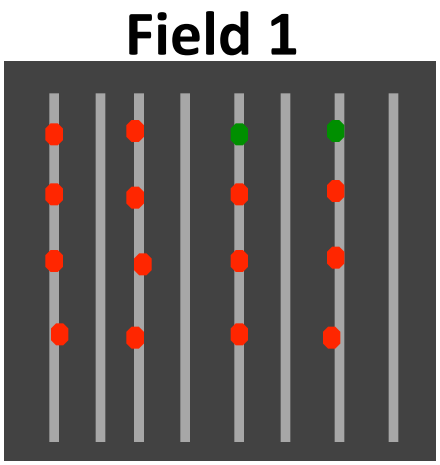
# Vector elimination
# The BRNV paradigm

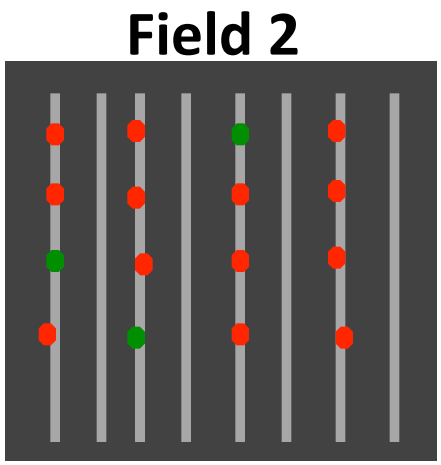
**New field monitoring**
Permanent tagged plants

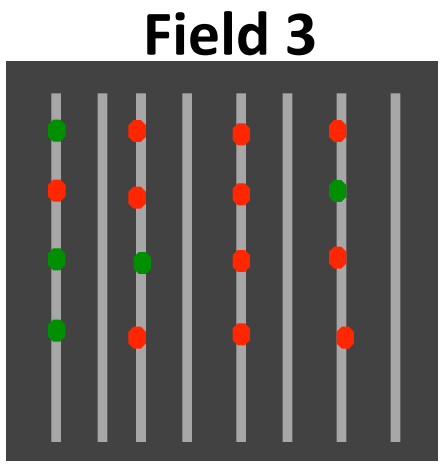
**Time of transmission**
High incidence of virus
Potted plants
Rotated every month

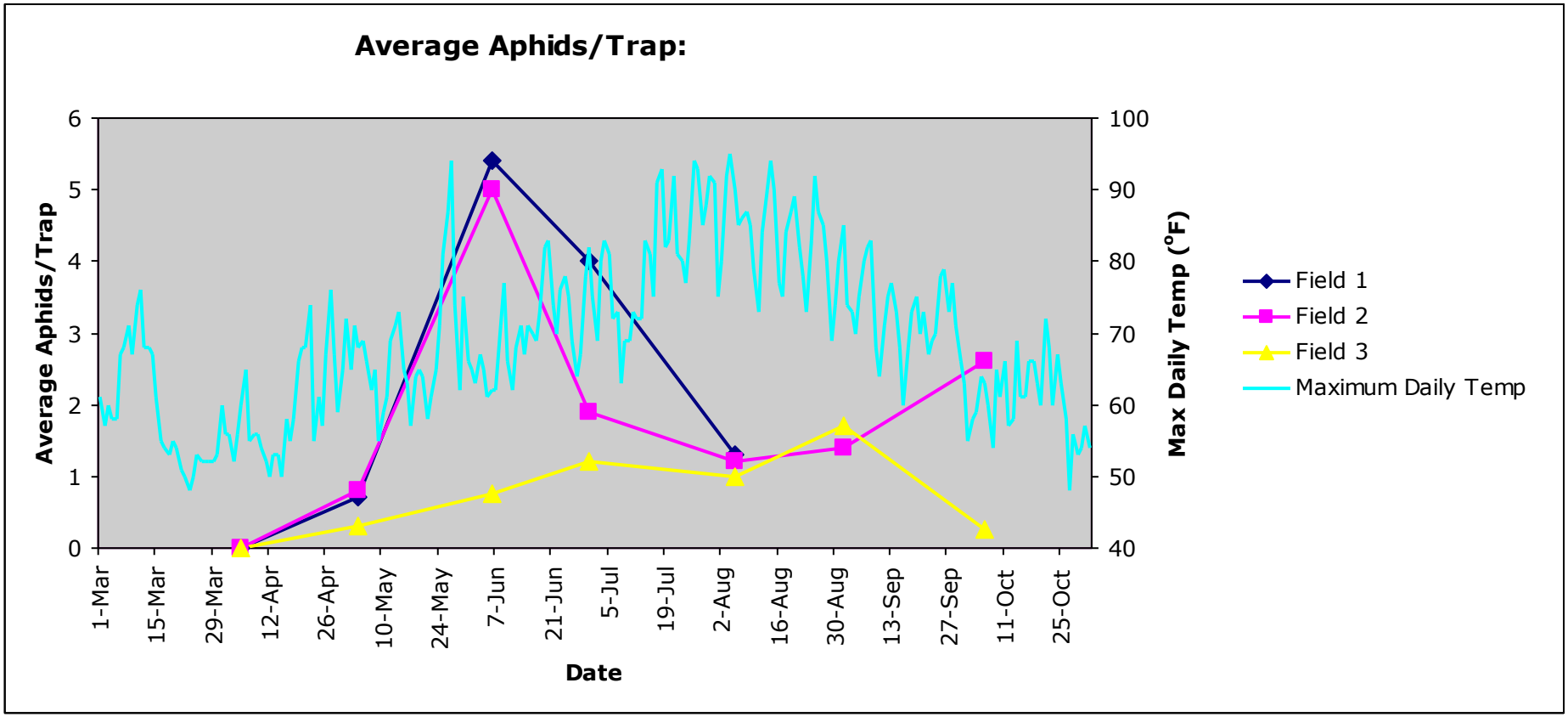


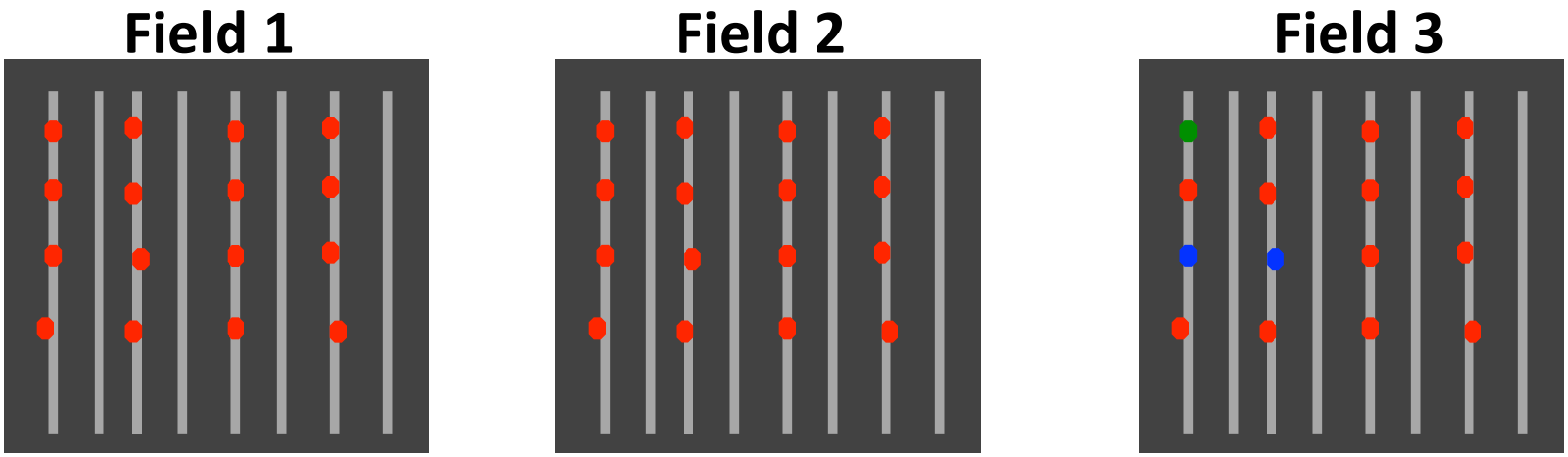

Average Aphids/Trap:
14
12
10
8
6
4
2
0
110
100
90
80
70
60
50
40
Max Daily Temp (°F)
4-May
11-May
18-May
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
6-Jul
13-Jul
20-Jul
27-Jul
3-Aug
10-Aug
17-Aug
Date
Field 1
Field 2
Field 3
Max Daily Temp


Field 1


Field 2

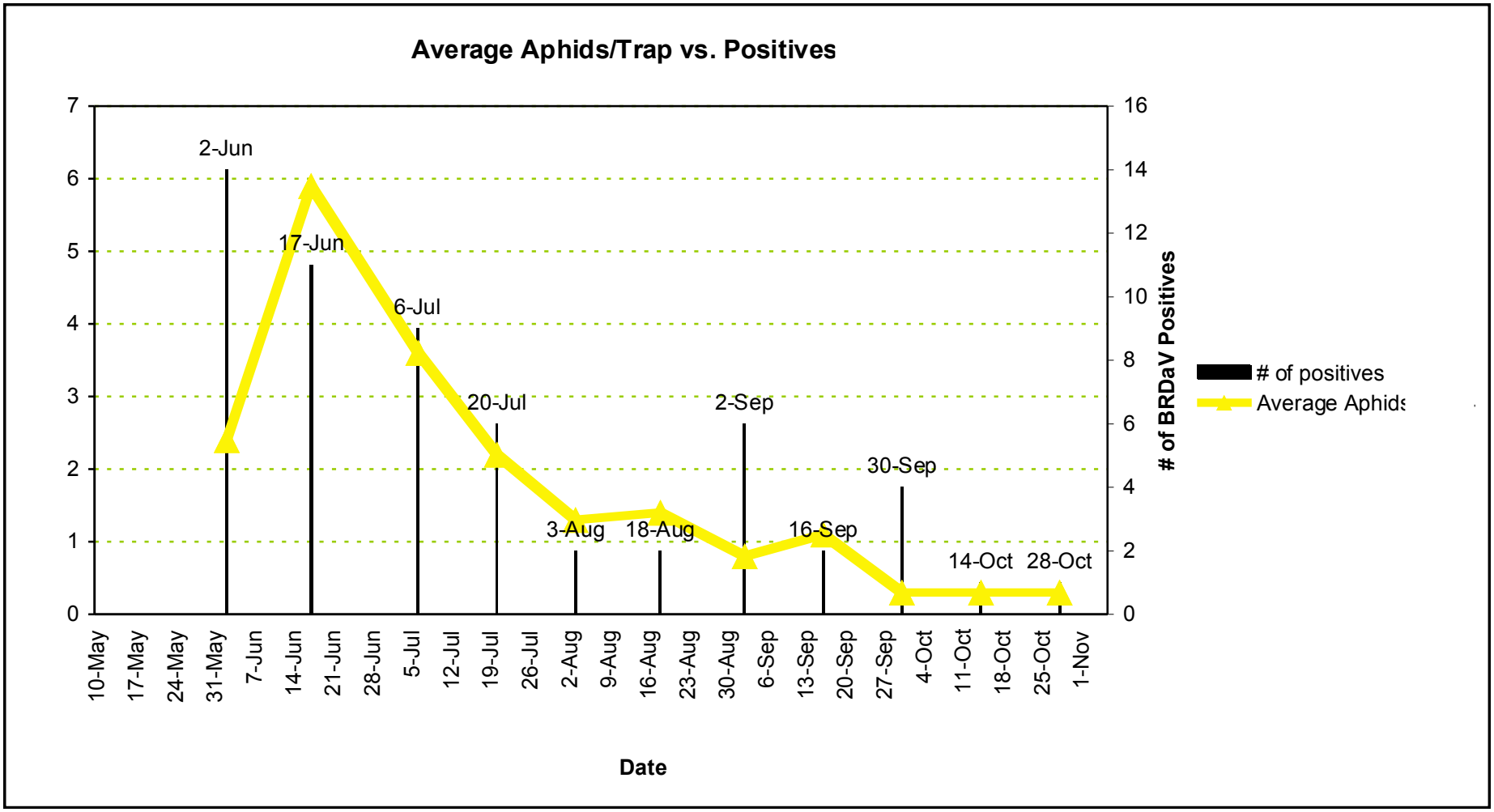

Field 3




**Nearly 100% transmission in three years!**

# Time of Transmission

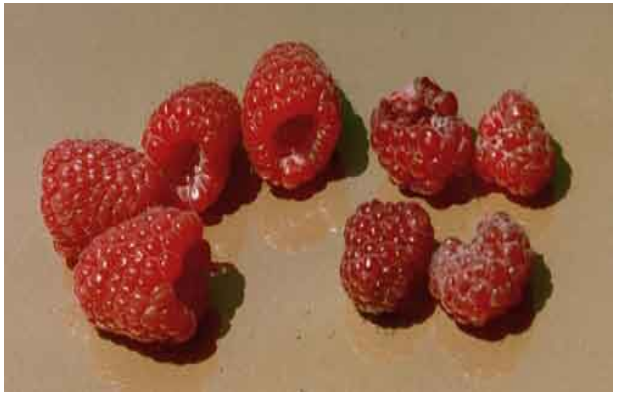


# Raspberry crumbly fruit and decline

- 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*

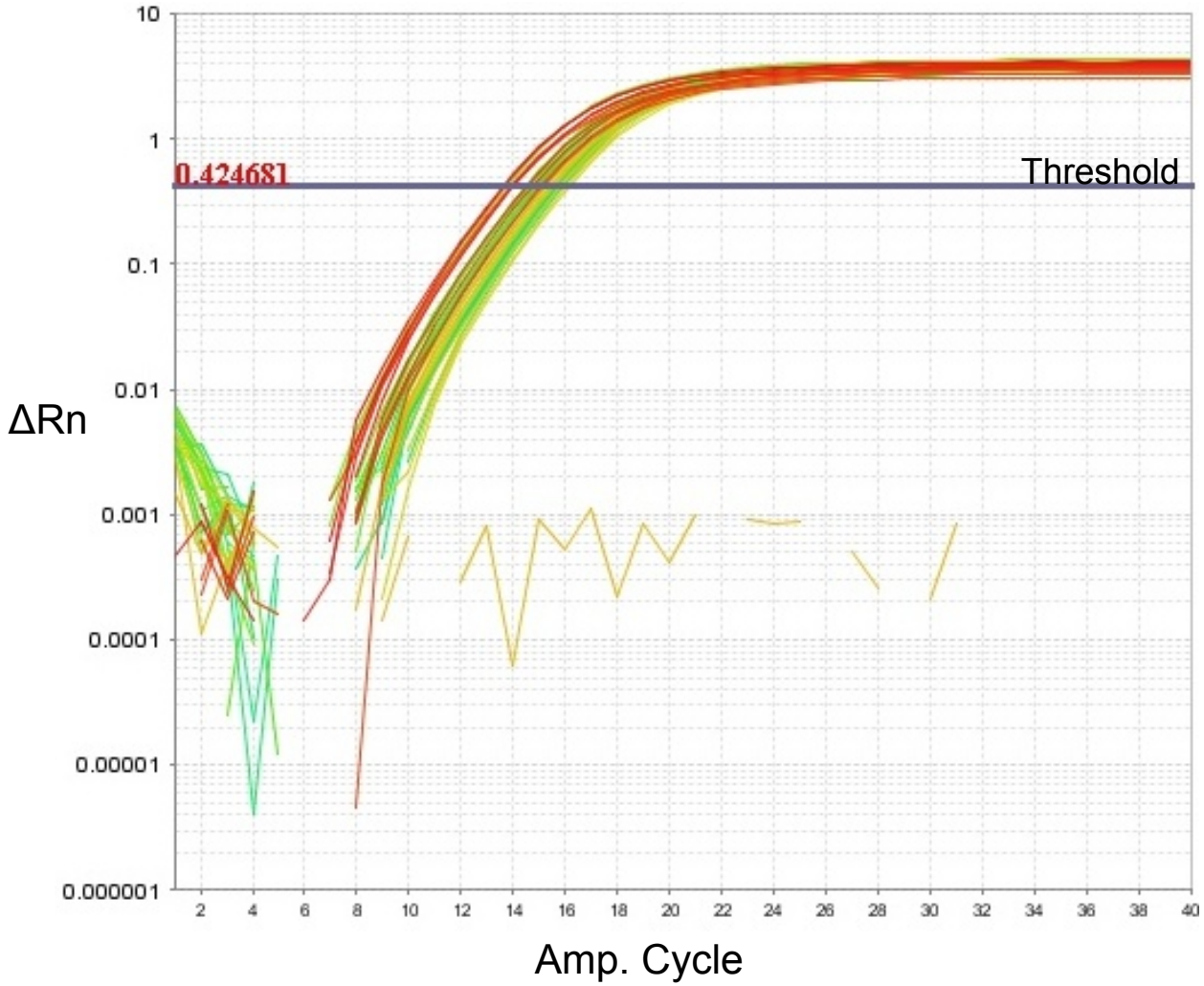
2. Two additional viruses found in severely affected fields,

   **Raspberry leaf mottle (RLMV)** and **Raspberry latent (RpLV)**

# RBDV, RLMV and RpLV interactions

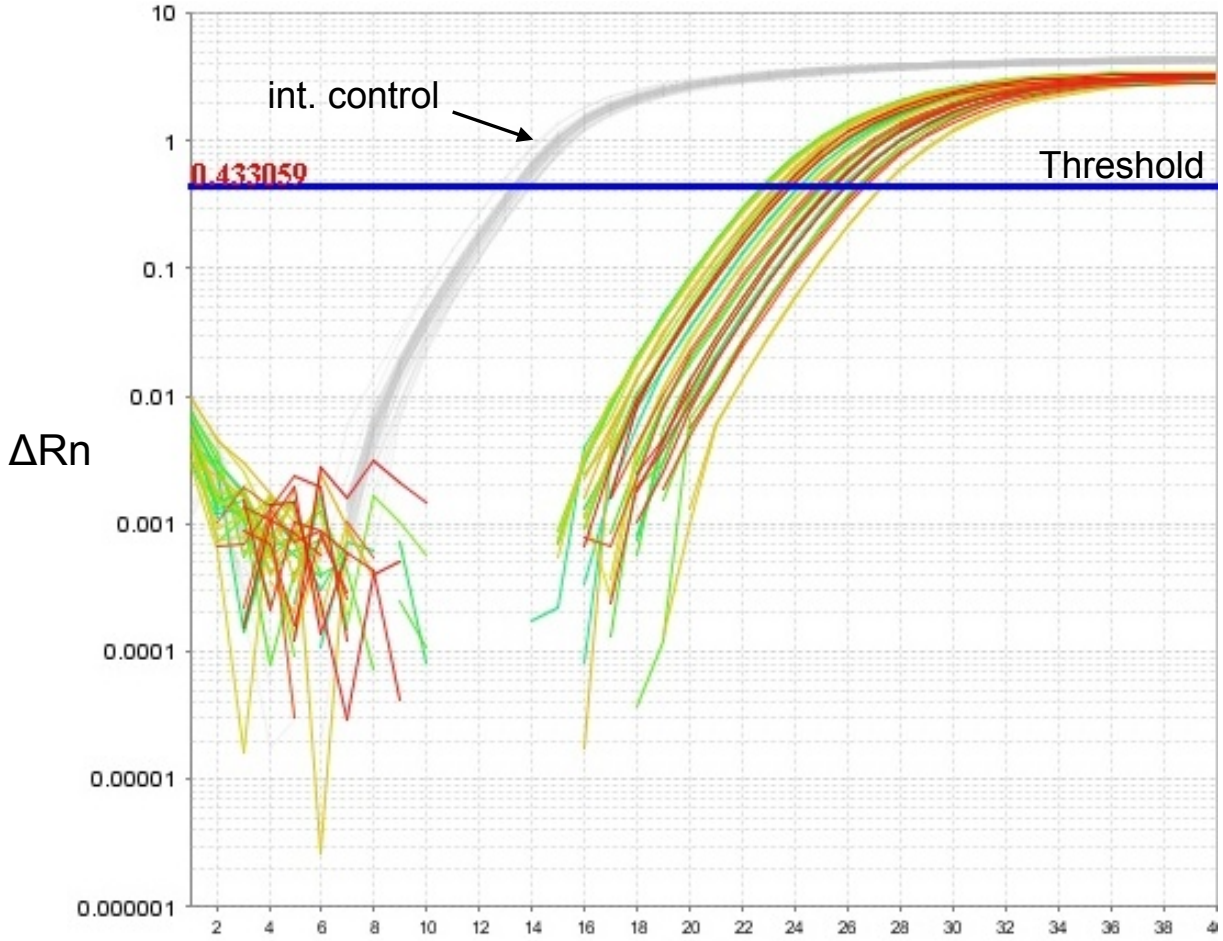
# RLMV qRT-PCR

**RLMV titer in single and mixed infections over time**

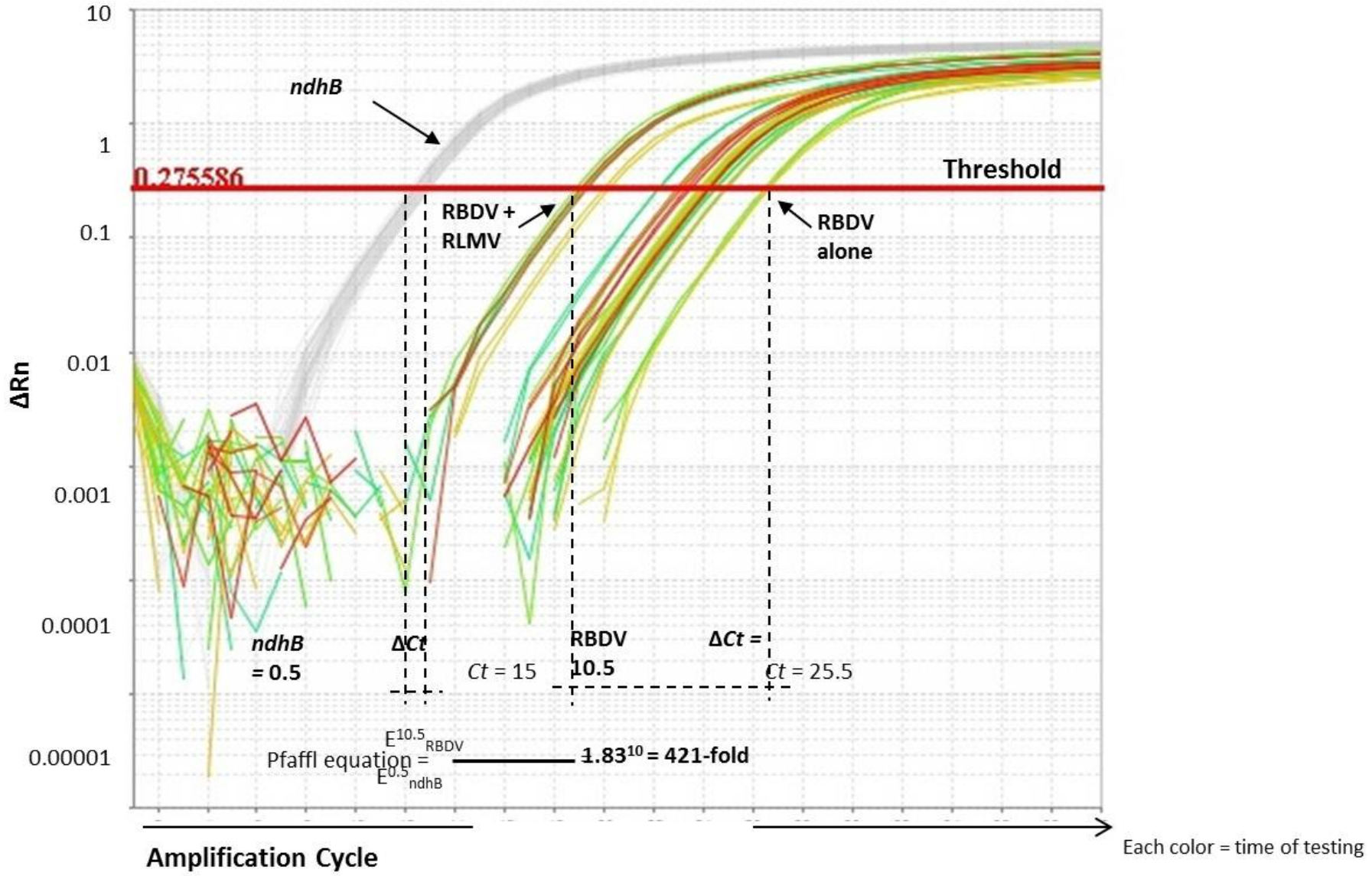


# RpLV qRT-PCR

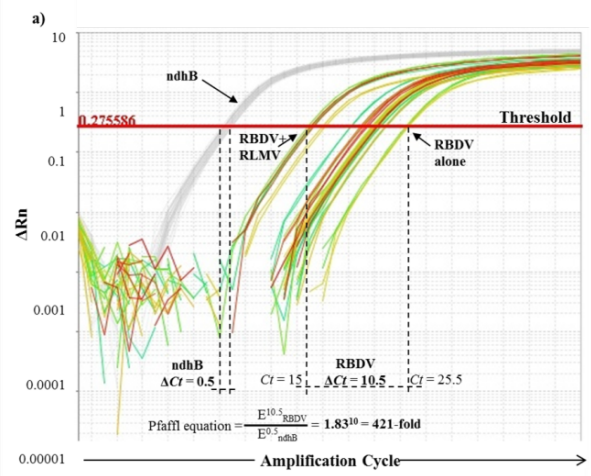
**RpLV titer in single and mixed infections over time**



# RBDV titer enhanced in co-infections with RLMV

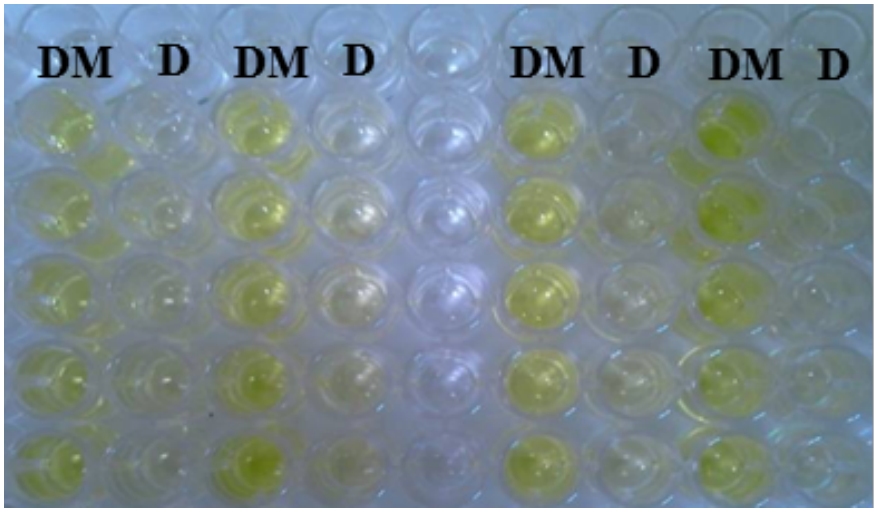


# RBDV titer enhanced in co-infections with RLMV

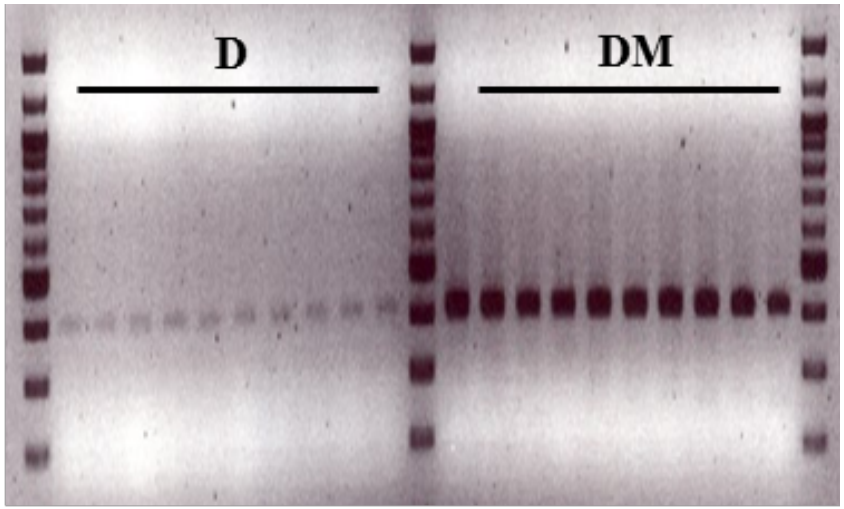



RBDV titer increase verified by conventional methods

ELISA

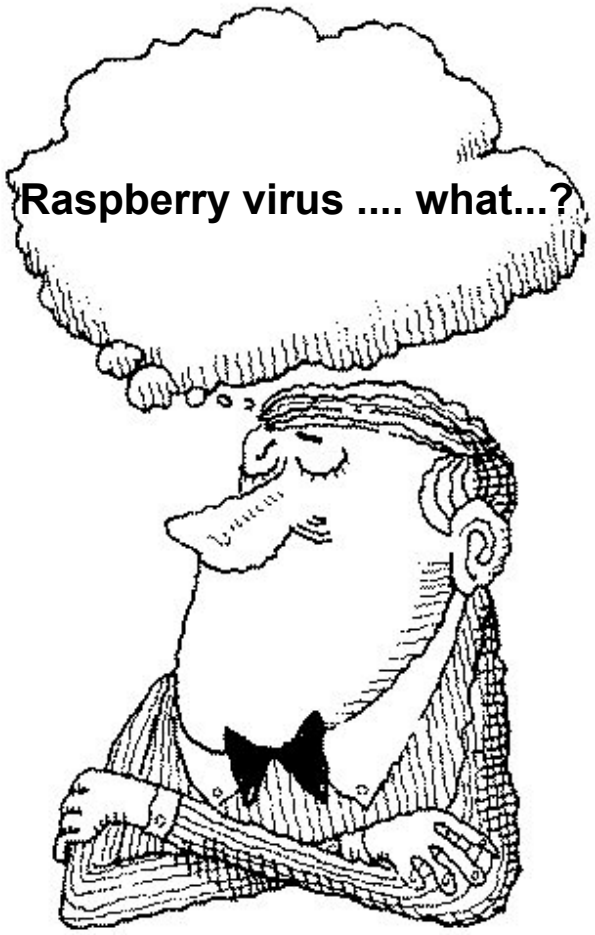



RT-PCR (20 cycles)

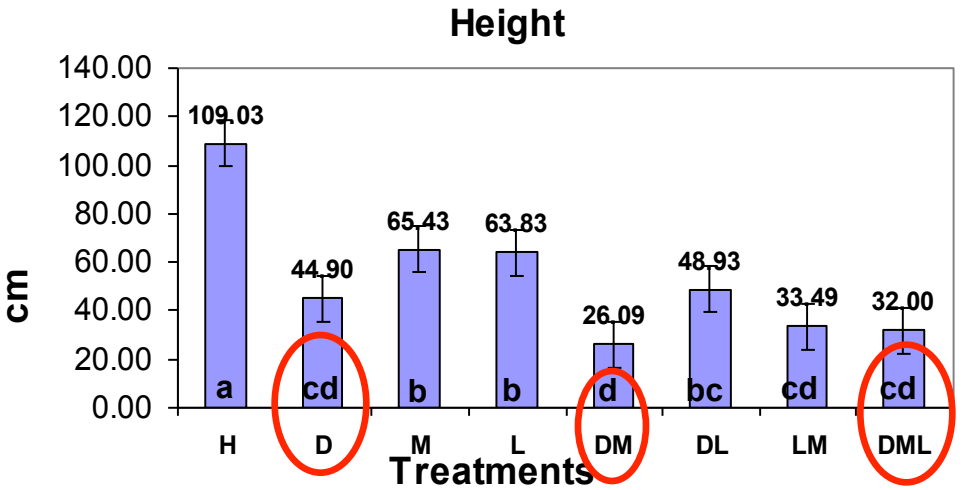


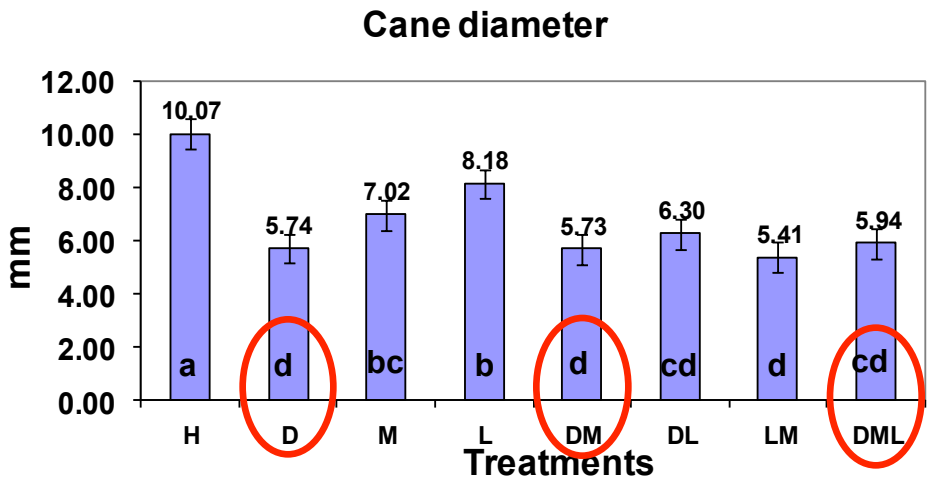
# Mixed virus infections affect on plant growth and fruit crumbliness

- H Control
- **D** RBDV - Dwarf
- **M** RLMV - Mottle
- **L** RpLV - Latent
- **DM** RBDV + RLMV
- **DL** RBDV + RpLV
- **ML** RLMV + RpLV
- **DML** RBDV + RLMV + RpLV

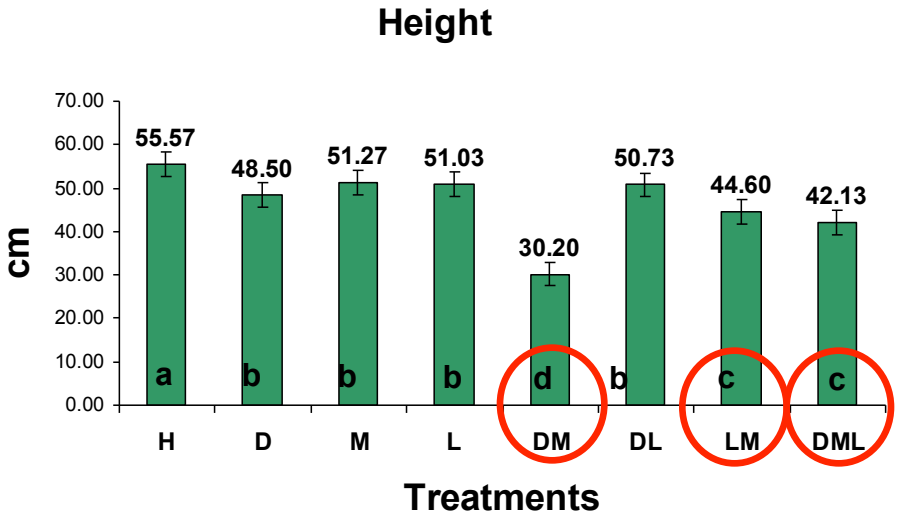


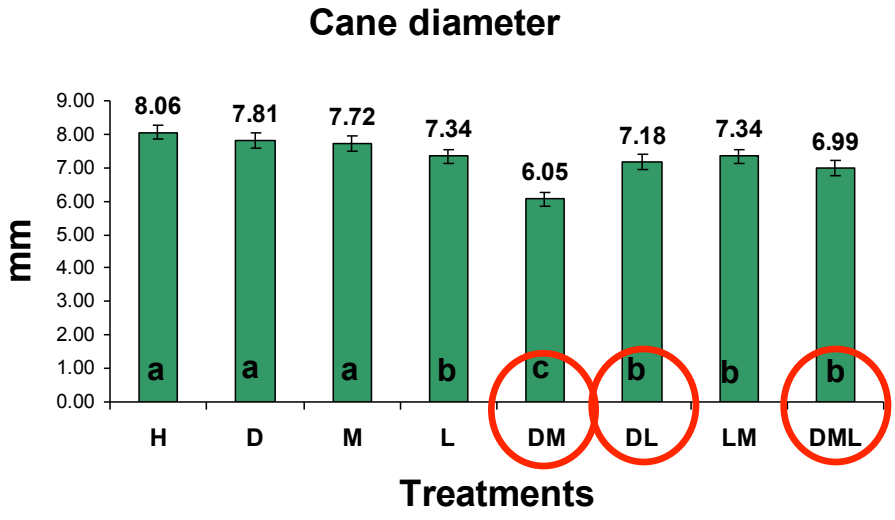
# Plant Growth Establishment (2010)



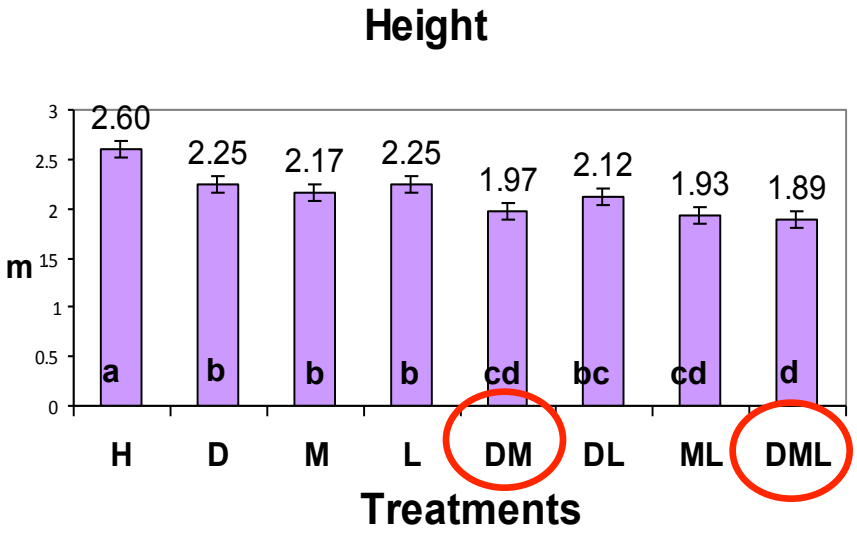


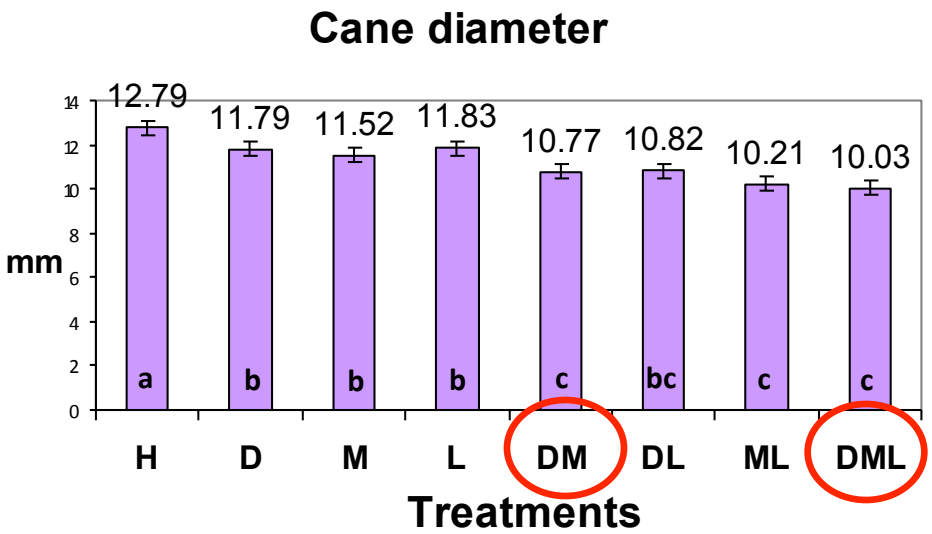
# Plant Growth (2011)



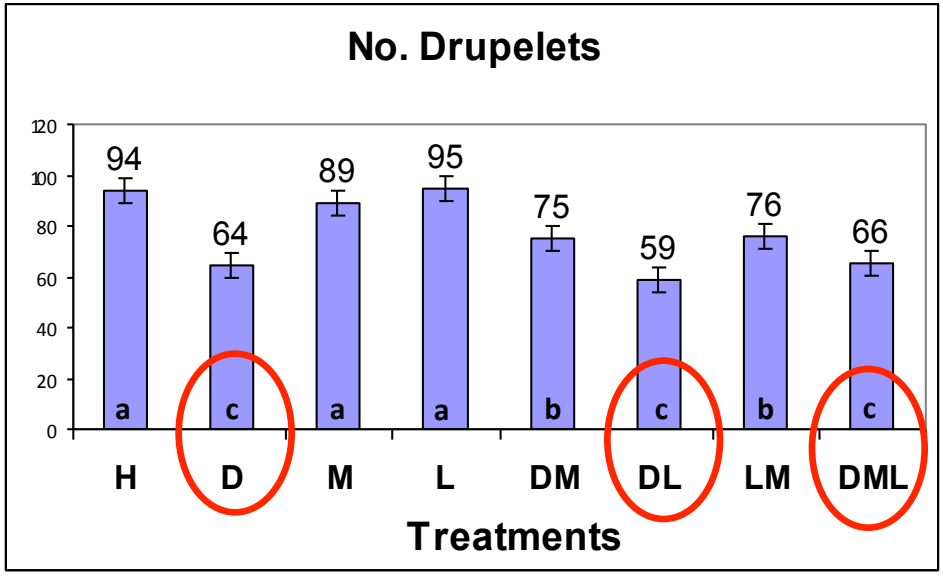



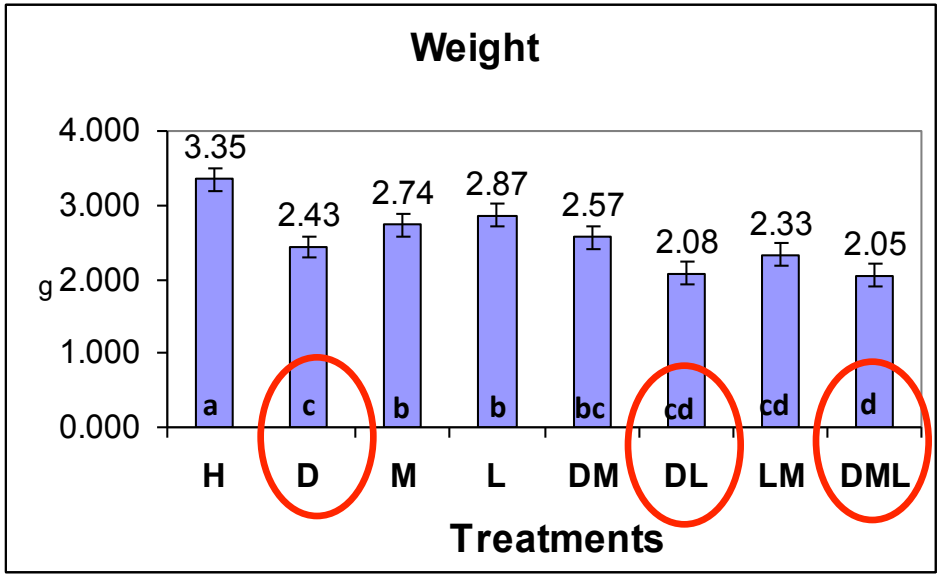
# Plant Growth (2011)



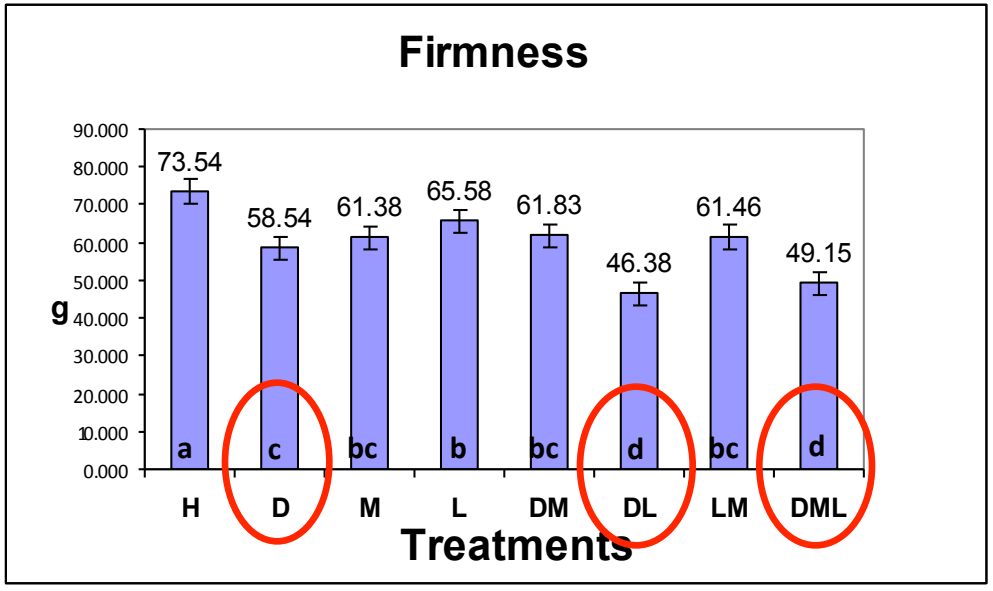



# Crumbly Fruit



# 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

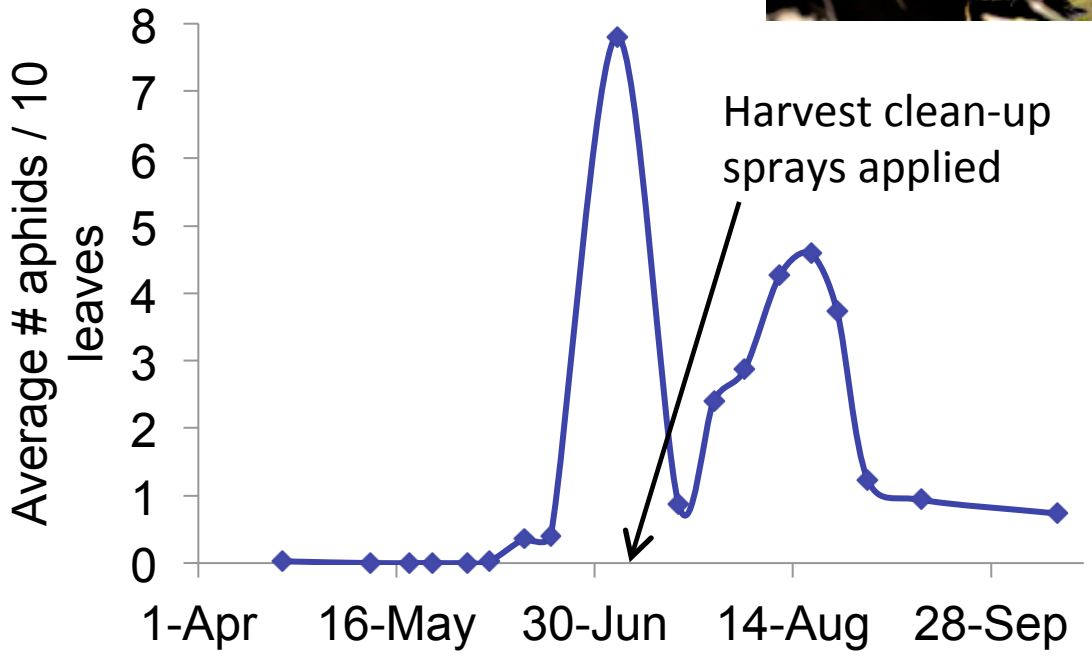
| Field Age | Crumbliness 0: normal 3: severe | Virus incidence % | | |
|---|---|---|---|---|
| | 0 1 2 3 | RBDV | RLMV | RpLV |
| 4 | 1 | 44 | 25 | 0 |
| 4 | 2 | 100 | 100 | 0 |
| 4 | 3 | 93 | 100 | 7 |
| 5 | **3** | **100** | **100** | **40** |
| 6 | **3** | **92** | **96** | **40** |

# Insects in Traps (2011)

*Empoasca fabae* was sporadic

Few numbers *of* *Macropsis fuscula*

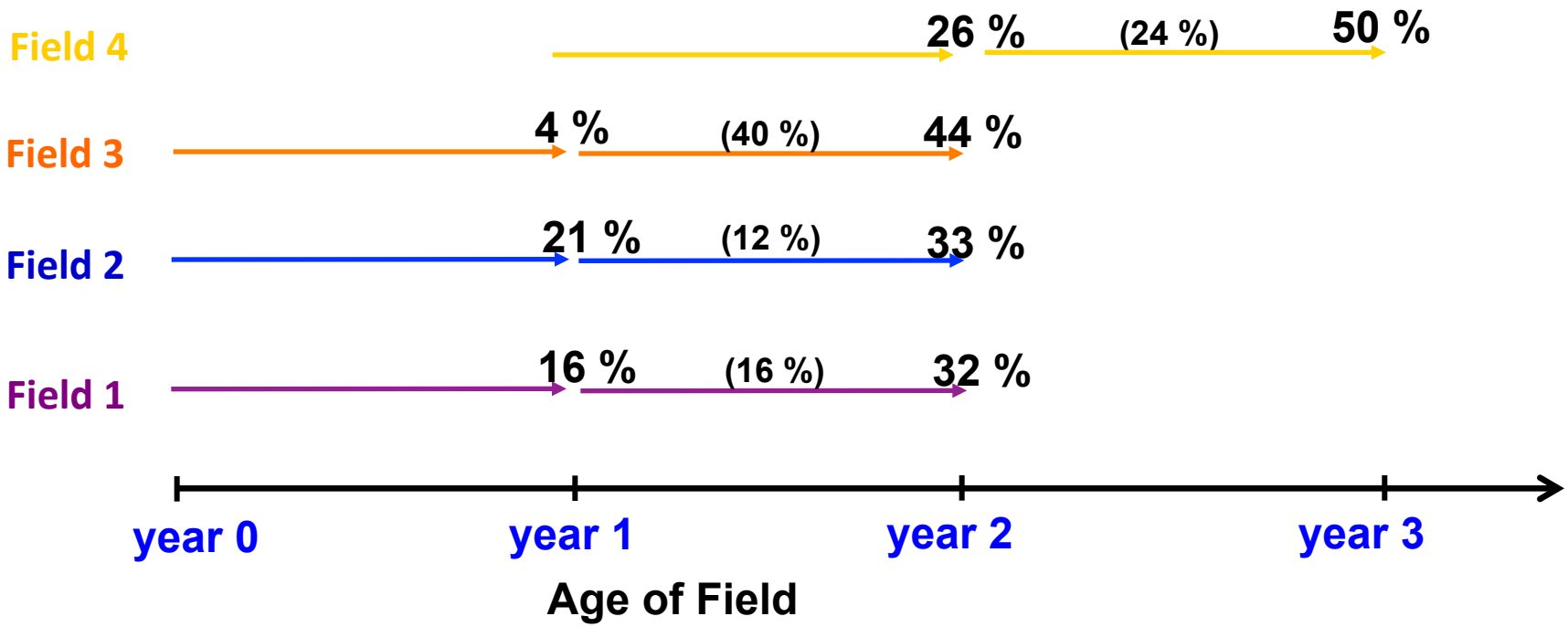
Raspberry aphid *A. agathonica* predominant insect




**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

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

# Identifying key pathogen vectors in small fruits

Hannah Burrack, NC State University and
Donn Johnson, University of Arkansas

# What is a vector?

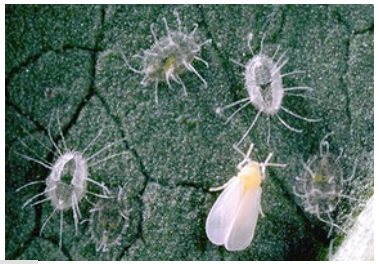
An organism that spreads pathogens

# What arthropod groups vector plant pathogens in small fruits?

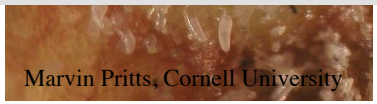
- Whiteflies
- Aphids
- Leafhoppers
- Mites
- Psyllids
- Mealybugs
- Thrips

# What arthropod groups vector plant pathogens in small fruits?

- Whiteflies
- Aphi
- Leaf
- Mite
- Psyllids
- Mealybugs
- Thrips



**What do these groups have in common?**



Marvin Pritts, Cornell University

# How do arthropods vector plant viruses?

| Virus taxon(s) | Main vector group | Virus taxon(s) | Main vector group |
|---|---|---|---|
| **Noncirculative, nonpersistent** | | **Circulative, nonpropagative** | |
| Caulimovirus, Fabavirus, Potyvirus, Carlavirus, Cucumovirus, Alfamovirus | Aphids | Enamovirus, Luteovirus, Nanavirus, Umbravirus | Aphids |
| Macluravirus | Thrips, Beetles | Geminivirus | Whiteflies, treehoppers, leafhoppers |
| Potexvirus | Aphids, mites, also some mechanical | Bromovirus, Carmovirus, Comovirus, Sobemovirus, Tymovirus | Beetles |
| **Noncirculatative, semipersistant** | | Rymovirus | Mites |
| Badnavirus | Mealybugs, leafhoppers | **Circulative, propagative** | |
| Closterovirus | Aphids, whiteflies, mealybugs | Tosopovirus | Thrips |
| Sequivirus | Aphids | Marafivirus, Phytoveovirus | Leafhoppers |
| Trichovirus | Aphids, mealybugs, mites | Fijivirus, Oryzavirus, Tenuivirus | Planthoppers |
| Waikavirus | Aphids, leafhoppers | Phytorhabdovirus, Nucleorhabdovirus | Aphids, leafhoppers, planthoppers |
| | | Cytorhabdovirus | Aphids, planthoppers |

**Adapted from**
**Gray S M, and Banerjee N Microbiol. Mol. Biol. Rev. 1999; 63:128-148**

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| | | Cytorhabdovirus | **Aphids**, planthoppers |

**Adapted from**
**Gray S M, and Banerjee N Microbiol. Mol. Biol. Rev. 1999; 63:128-148**

# How do arthropods vector plant viruses?

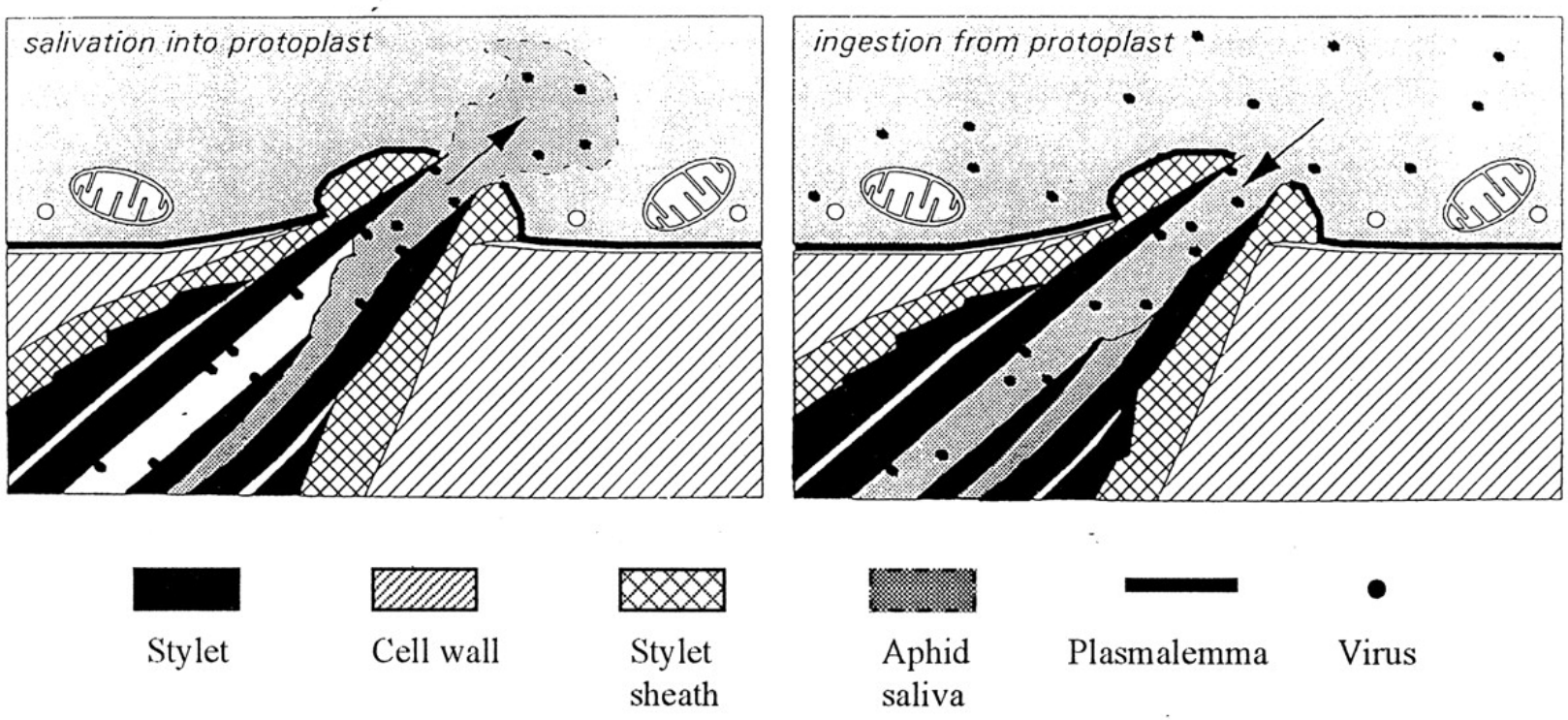
- Circulative

  Moves systemically within arthropod's body

- Propagative

  Replicates within arthropod body

## Model of the ingestion-salivation mechanism of noncirculative, nonpersistent transmission




Gray S M, and Banerjee N Microbiol. Mol. Biol. Rev. 1999; 63:128-148

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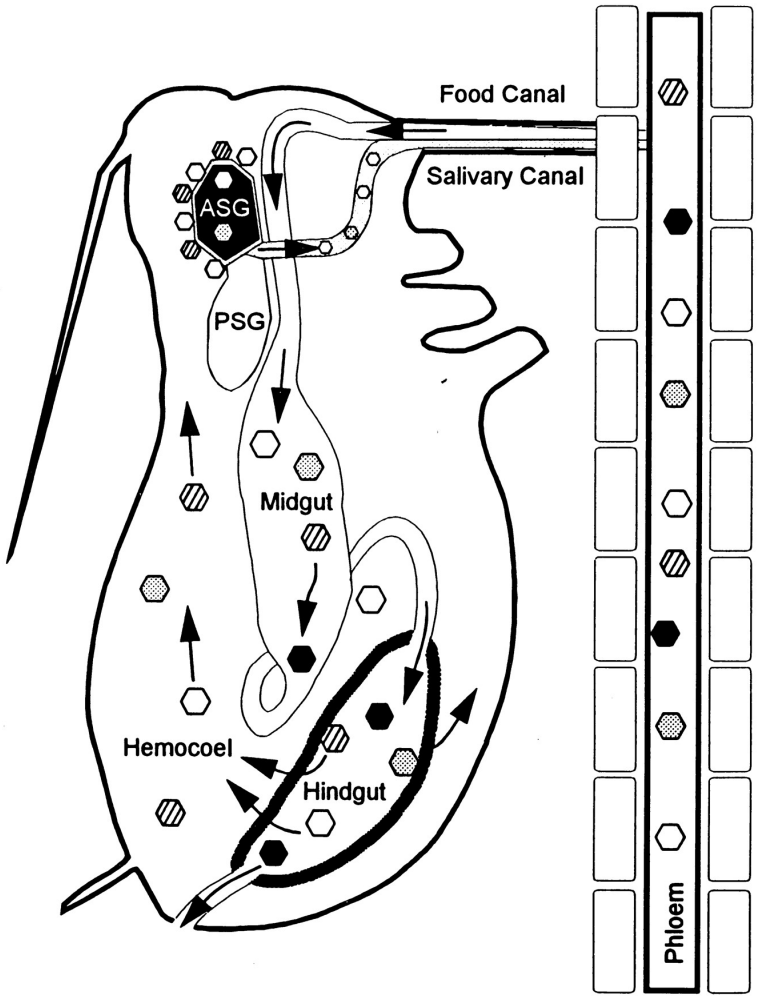
# Circulative route of barley yellow dwarf luteoviruses (BYDVs) through aphids




Gray S M, and Banerjee N Microbiol. Mol. Biol. Rev. 1999; 63:128-148

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# Nonpersistent transmission

- Acquisition time

  Seconds
- Inoculation time

  Seconds
- Latent period (time from acquisition to ability to transmit)

  Zero
- Retention time (time after acquisition a vector remains capable of transmission)

  Minutes to hours

Adapted from "Management of Insect Transmitted Plant Diseases, R. Groves. University of Wisconsin, Department of Entomology. http://bit.ly/12a44SB

# Persistent transmission

- Acquisition time

  Minutes
- Inoculation time

  Minutes

  *Longer feeding positively related to acquisition and inoculation*
- Latent period (time from acquisition to ability to transmit)

  1 or more days
- Retention time (time after acquisition a vector remains capable of transmission)

  Through molt or life of insect

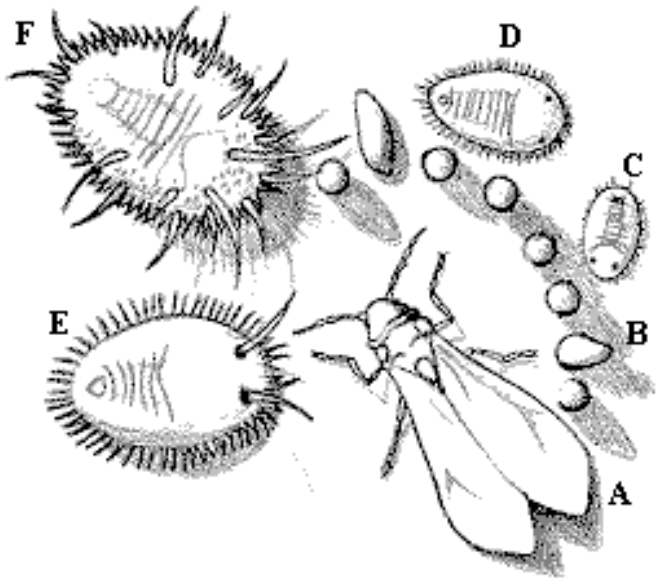
**Adapted from "Management of Insect Transmitted Plant Diseases, R. Groves. University of Wisconsin, Department of Entomology. http://bit.ly/12a44SB**

# Biology of arthropod vectors in small fruits

- **Whiteflies**
- **Aphids**
- **Leafhoppers**
- **Mites**
- Psyllids
- Mealybugs
- **Thrips**

# Whitefly biology

- Can be found on strawberries, blueberries, and caneberries in the southeast
- Known vectors of strawberry viruses




Greenhouse whitefly. A, Adult. B, Eggs. C-E, Nymphs. F, Pupa.

# Whitefly biology

- Microscopic eggs laid on undersides of leaves
- Fourth instar larvae ("pupae") are diagnostic stage

# Whitefly biology

- Parasitoid wasps attack “pupae”
- Species with white pupae will turn black if parasitized
- Not all black “pupae” are parasitized!

# Whiteflies as vectors

- Known vector species
  *Bemesia tabaci*, *Trialeurodes vaporaiorum*, *T. abutilonea*, *T. ricini* (none in US)

# Whiteflies found on small fruits

**Greenhouse whitefly**
*Trialeurodes vaporariorum*
Strawberry (in CA)

**Silverleaf whitefly**
*Bemesia tabaci*
Grape

**Bearberry whitefly**
*Tetraleurodes ursorum*
**Non vector**
Blueberry

**Iris whitefly**
*Aleyrodes spiroeoides*
**Non vector**
Strawberry (in CA)

Unidentified whitefly species also found in blackberries

# Aphid biology

**Aphids**

1/8” long, soft-bodied, pear-shaped, two cornicles, and adults are wingless or winged

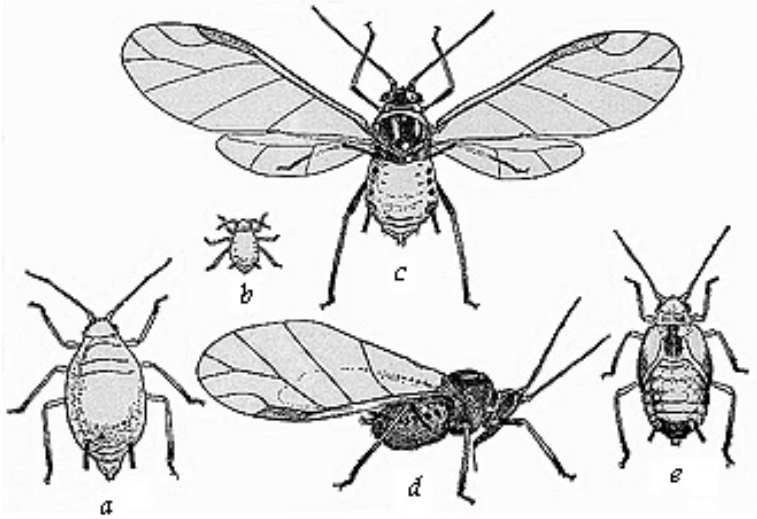
**Biology**

In fall, develop male and female aphids, mate and lay overwintering eggs

In spring, eggs hatch into females that produce live young (parthenogenesis), densities build up quickly over multiple generations, suck on plant juice and excrete honeydew

Some young develop wings and disperse to other plants

***In the southeast, many aphid species DO NOT reproduce sexually!***




Aphids: a, wingless; b, newborn nymph; c and d, winged; e, nymph

# Aphids as vectors

- Most common vector arthropod vectors of plant viruses
- Many known vector species
  *Chaetosiphon fraegaefolii* (strawberry aphid) and *Aphis gossypii* (melon aphid) the most significant for strawberries
  *Amphorophora agathonica* (large raspberry aphid) vectors Raspberry Latent Virus in the Pacific Northwest but does not appear to be an issue in the eastern US

# Aphids found on small fruits

**Strawberry aphid**
*Chaetosiphon fraegaefolii*
Strawberry, uncommon in southeast
Vectors SMYEV (persistent, circulative), SMoV (semi-persistent)

**Melon aphid**
*Aphis gossypii*
Strawberry
Vectors SMYEV (persistent, circulative), SMoV (semi-persistent)

# Aphids found on small fruits

Photo: Tom Peerbolt

**Large raspberry aphid**
*Amphorophora agathonica*
Raspberry, unknown from southeast

# Leafhopper biology

Adults are 1/8” ½”, wedge shaped and triangular in cross-section. Bodies are colored yellow, green, red, brown, gray or with color patterns. They jump and fly off readily.

Nymphs resemble adults but are wingless and molt leaving whitish cast skins on leaf. They can run rapidly, occasionally sideways, and hop.

# Leafhoppers as vectors

- Two Marafiviruses found in *Rubus* in Mississippi:
  - Blackberry virus S (B1VS) (Sabanadzovic &Ghanem-Sanadzovic 2009)
  - Grapevine Q virus (GVQ) (Sabanadzovic et al. 2009)
- **Marafiviruses** are persistent, circulative, transmitted by leafhoppers, and retained during molts (Burnt et al. 1996)
- Leafhoppers transmit virus within 1 to 4 weeks (Conti 1985)
- Transmission rates are higher if the leafhopper acquires the virus as a nymph rather than an adult

# Leafhoppers in small fruits

- 32 genera of leafhoppers collected in AR blackberries
- The predominant leafhopper species are pictured below

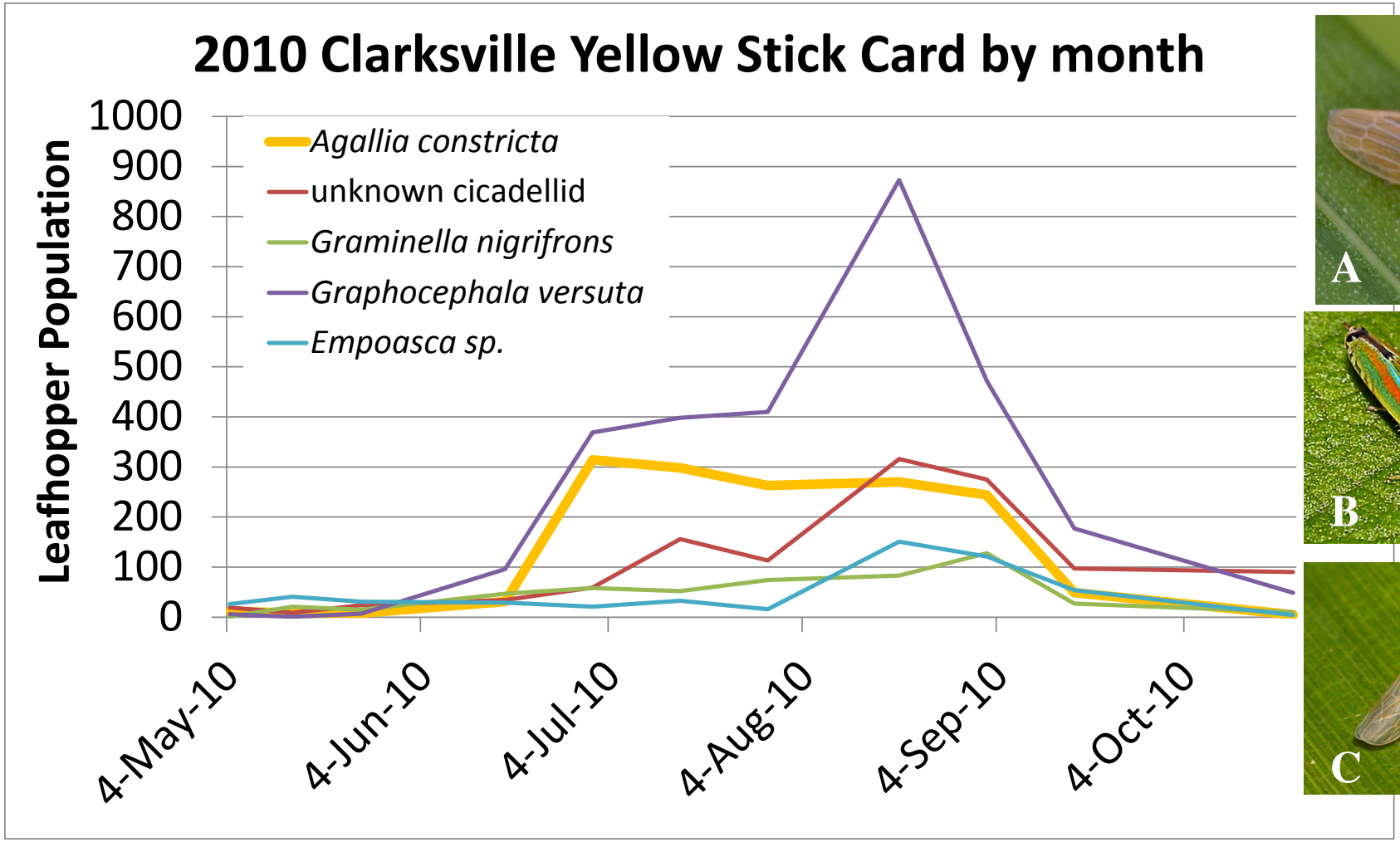
*Deltacephalus flavocostatus*

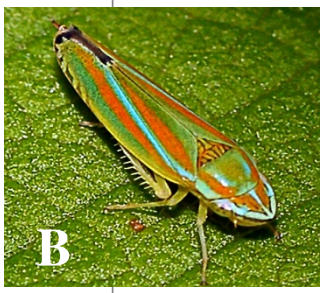
*Exitianus exitiosus*

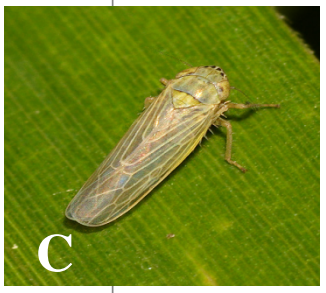
*Graminella nigrifrons*

*Graphocephala versuta*

*Agallia constricta*



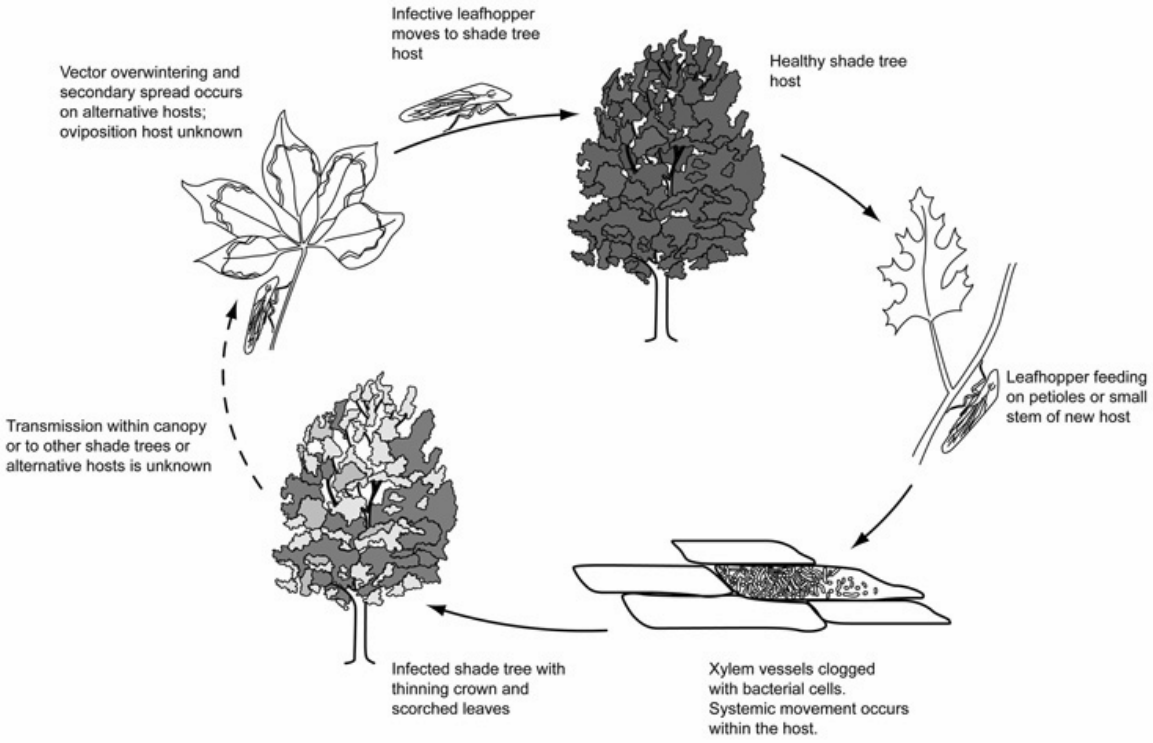



Three potential vector leafhoppers: *A) Agallia constricta*, B) *Graphocephala versuta*, and C) *Graminella nigrifrons;* Photos: www.bugguide.net

# Leafhoppers as vectors

- Leafhoppers are most commonly associated with another **bacterial** plant pathogen in small fruits, *Xyllela fastidiosa*
- Causes Pierce's Disease in grape



*Graphocephala versuta*

NC STATE UNIVERSITY

# Leafhoppers as vectors

TABLE 1. Number of leafhoppers trapped in four North Carolina vineyards in 2004 and 2005 and the percent composition of the most abundant species

| | 2004 | | | 2005 | | |
|---|---|---|---|---|---|---|
| Leafhopper species | Vineyard[y] | Number trapped | Percent | Vineyard[y] | Number trapped | Percent |
| *Graphocephala versuta* | 1 | 2,206 | 55 | 1 | 1,848 | 50 |
| | 2 | 2,240 | 64 | 2 | 2,198 | 63 |
| | 3 | 5,076 | 51 | 3 | 4,560 | 40 |
| | 4 | 138 | 16 | 4 | 113 | 18 |
| *Oncometopia orbona* | 1 | 264 | 6 | 1 | 142 | 4 |
| | 2 | 56 | 2 | 2 | 102 | 3 |
| | 3 | 50 | 1 | 3 | 161 | 1 |
| | 4 | 20 | 2 | 4 | 58 | 9 |
| *Paraphelpsius irroratus* | 1 | 291 | 7 | 1 | 452 | 12 |
| | 2 | 165 | 5 | 2 | 102 | 3 |
| | 3 | 252 | 3 | 3 | 380 | 3 |
| | 4 | 74 | 9 | 4 | 88 | 14 |
| *Agalliota constricta* | 1 | 1,142 | 28 | 1 | 1,068 | 29 |
| | 2 | 965 | 27 | 2 | 1,027 | 29 |
| | 3 | 4,433 | 45 | 3 | 6,213 | 54 |
| | 4 | 535 | 64 | 4 | 290 | 47 |
| Other species[z] | 1 | 128 | 3 | 1 | 167 | 5 |
| | 2 | 98 | 3 | 2 | 72 | 2 |
| | 3 | 127 | 1 | 3 | 113 | 1 |
| | 4 | 74 | 9 | 4 | 72 | 12 |

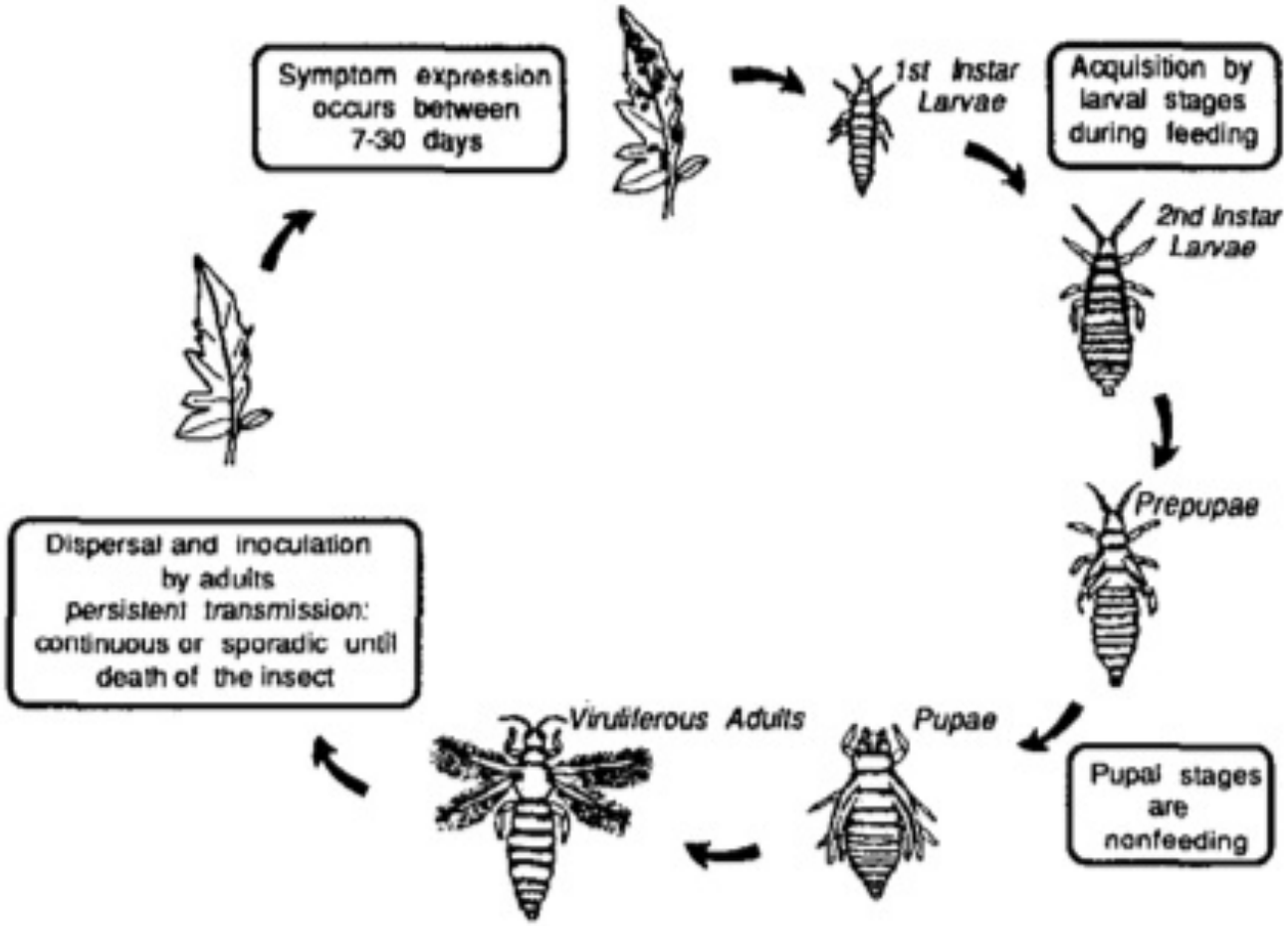
[y] Vineyards 1, 2, and 3 were located in central North Carolina. Vineyard 4 was located in the northeastern Coastal Plain of North Carolina.
[z] Five leafhopper and one spittlebug species making up <2% relative abundance were grouped as other species.

*Agallia constricta*

UofA UNIVERSITY OF ARKANSAS DIVISION OF AGRICULTURE

From Myers, et. al. 2007. Phytopathology.

# Thrips biology




Thrips are minute, cylindrical insects that primarily vector Tospoviruses

Multiple, overlapping generations occur

Photo: D. Johnson

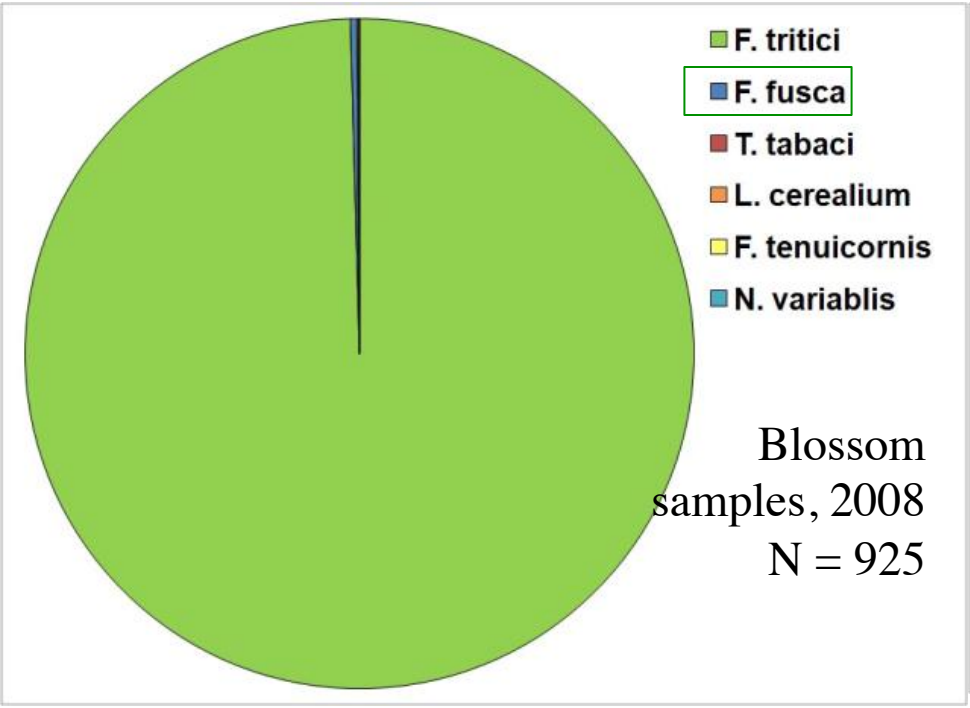
# Thrips as vectors

- 14 thrips species vector Tospoviruses
  ***Frankliniella occidentalis***, *Thrips tabaci*, *Frankliniella schultzei*, ***Frankliniella fusca***, *Thrips palmi*, *Scirtothrips dorsalis*, ***Frankliniella intosa***, *Frankliniella bispinosa*, *Thrips setosus*, *Ceratothripoides claratris*, *Frankliniella zucchini*, *Frankliniella gemina*, *Frankliniella cephalica*, *Dictypthrips betae*
  ***Impatiens necrotic spot virus has been found in southeastern blackberries***

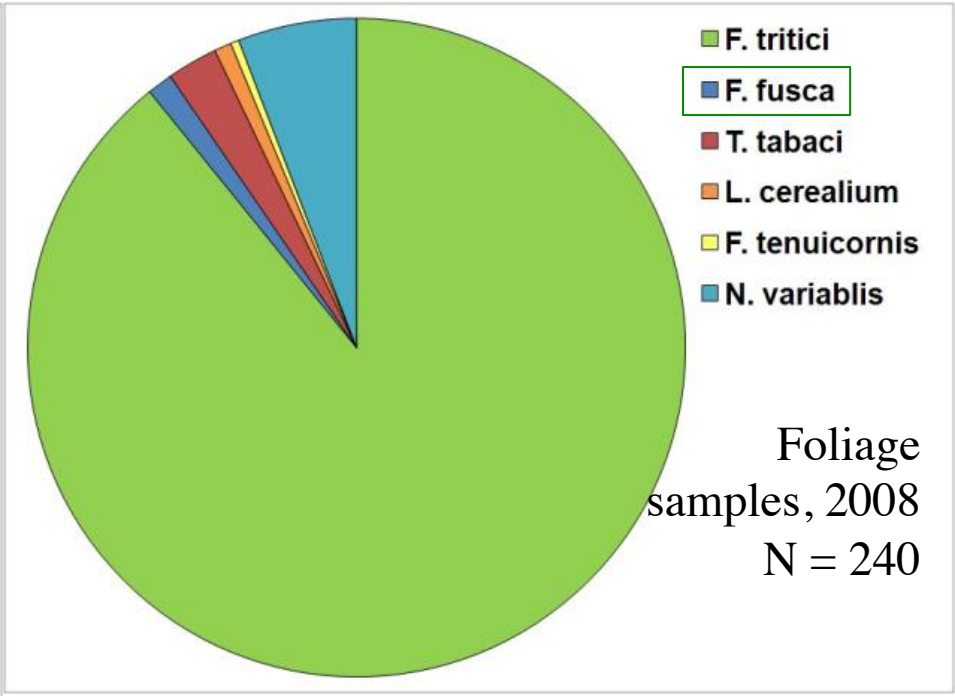
For identification information, see Riley, et al. 2011. Thrips vectors of Tospoviruses. Journal of Integrated Pest Management. http://bit.ly/17wonaW

# Thrips found on small fruit

Thrips species present on North Carolina blackberries




Blossom samples, 2008
N = 925




Foliage samples, 2008
N = 240

# Eriophyid mite biology

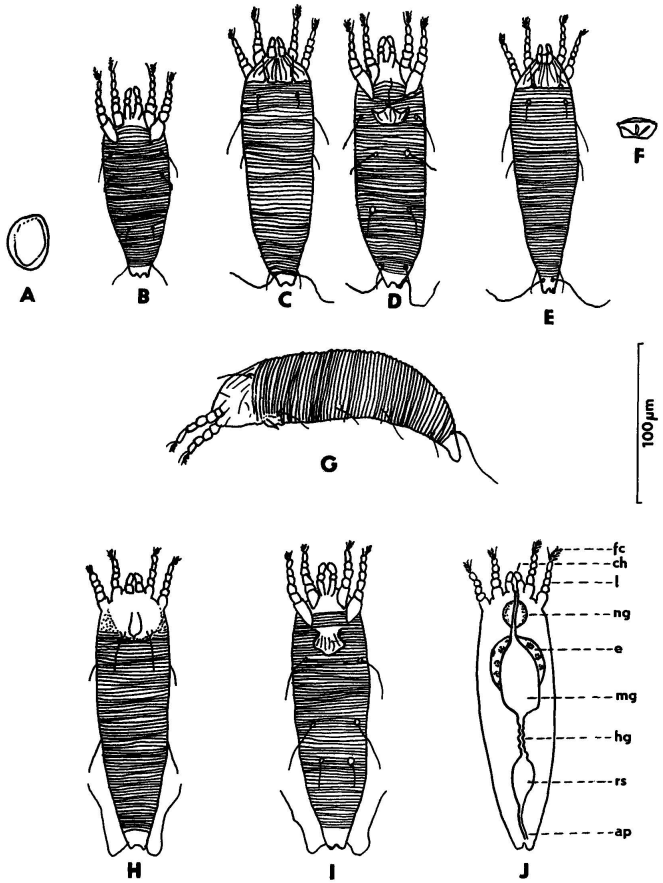
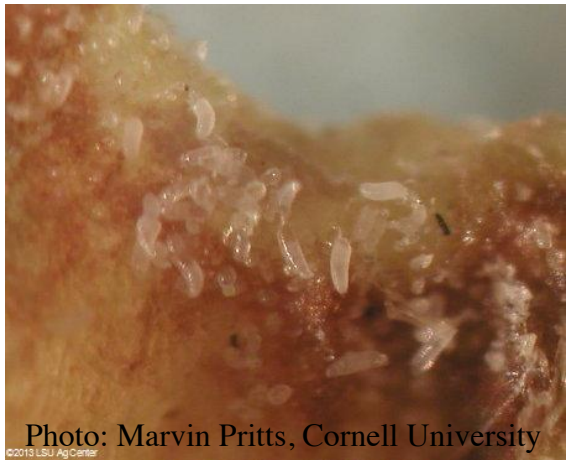



*Fig. 2. A–G: Development stages of* Eriophyes liosoma *(Nal.): A—egg; B—nympha; C—protogyne in dorsal view; D—protogyne in ventral view; E—male; F—genital flap; G—deutogyne in lateral view; H–J: Morphology and anatomy of the female adult of* Eriophyes fraxinivorus *Nal.: H—dorsal view, I—ventral view; J—anatomy shown in longitudinal section; ap—anal part; ch—chelicerae; e—egg; fc—featherclaw; hg—hind gut; l—legs; mg—mid gut; ng—neurosynganglion; rs—rectal sac*

- Small (<1mm)
- Spindle-shaped, with four legs and may be white, yellow or orange
- Not visible without magnification

From Vaneckova-Skuhrava. 1996. Journal of Applied Entomology.

# Eriophyid mites on small fruits



Two known eriophyid mite species on small fruits

Blueberry bud mite

*Acalitus vaccinii*

Blueberries

*Not known pathogen vector*

Redberry mite

*Acalitus essigi*

Caneberries

*Causes abnormal ripening of fruit*

# Virus vector biology in southeastern blackberries

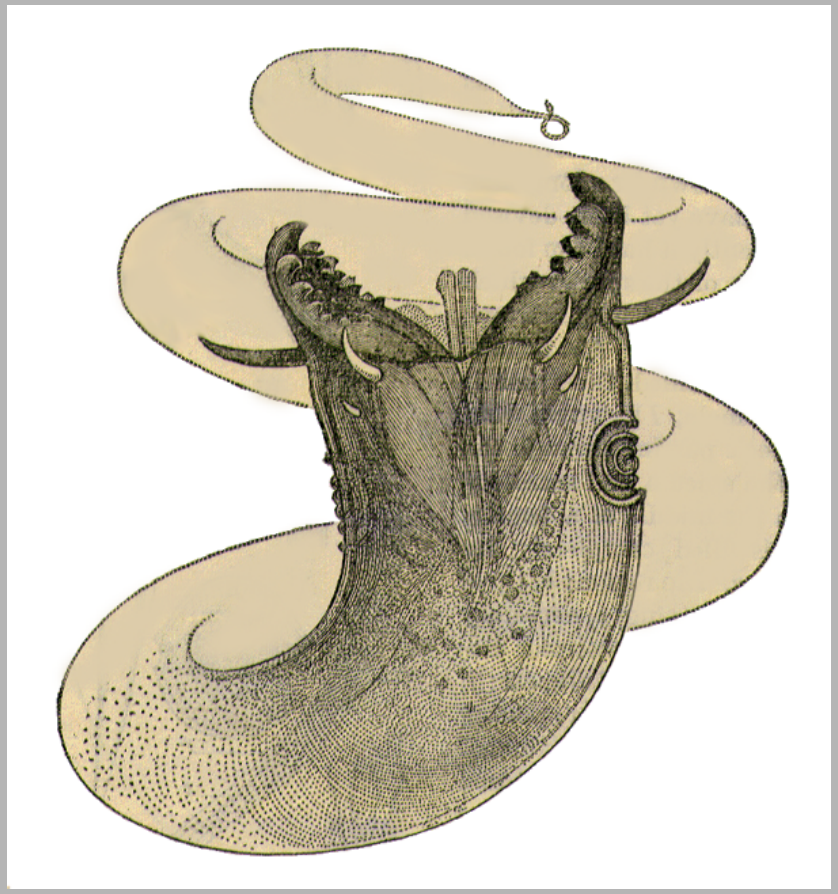
Seasonal capture of potential vector species of leafhoppers, mites, whiteflies and aphids in AR and NC

Correlation with virus infection timing

Identification of potential vector species

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

# Nematode Vectors of Plant Viruses



Terry Kirkpatrick, University of Arkansas Southwest Research & Extension Center
R.J. Bateman, Arkansas Nematode Diagnostic Laboratory

The photos used in this presentation were provided by NemaPix (Volumes I & II), or by R.J. Bateman, and T.L. Kirkpatrick

# Plant Nematology 101

- Microscopic roundworms
- Largest phylum (Nematoda) other than insects
- Cosmopolitan in occurrence; beneficial, parasitic, free-living
- Plant-parasitic ca. 10% of known species
- Reproduce at high rates/short life cycle
- A few genera can vector plant viruses

# Plant-parasitic nematodes, like plant viruses, are obligate parasites:

**It is in their best interest NOT to kill their host!**

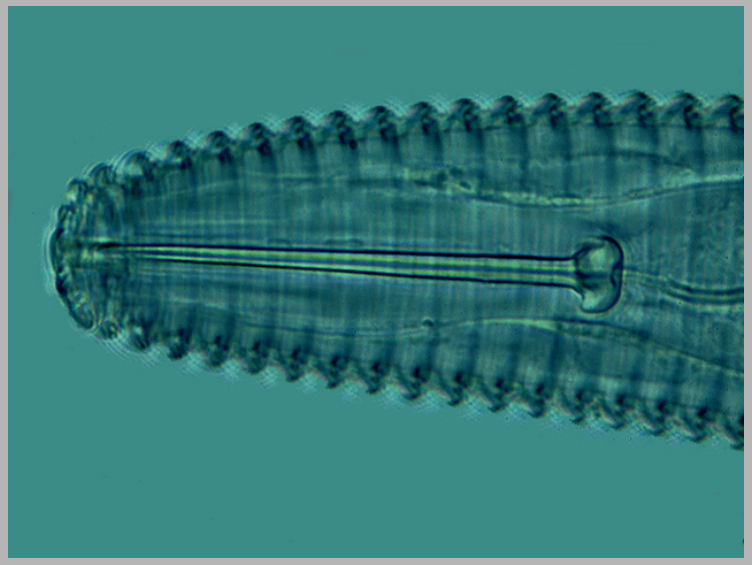
**They may, however, severely limit yield - or they may impair quality (or they may do both).**

# Taxonomy:

## PHYLUM NEMATODA

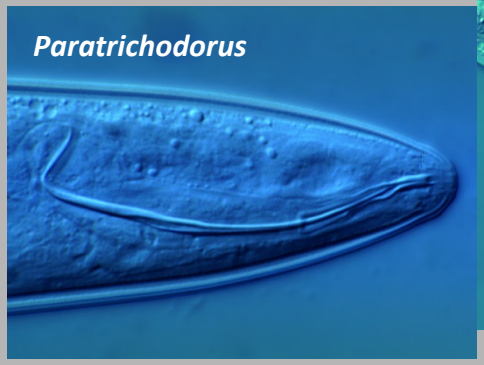
### CLASS:
### CHROMADOREA (SECERNENTIA)

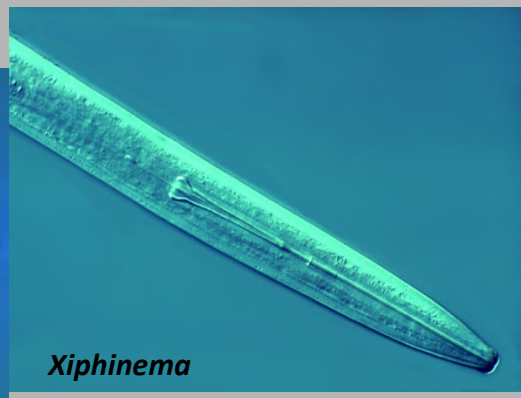
Stylet (stomatostyle) with knobs
Not virus vectors
Examples: root-knot, cyst, lesion, sting, spiral, ring

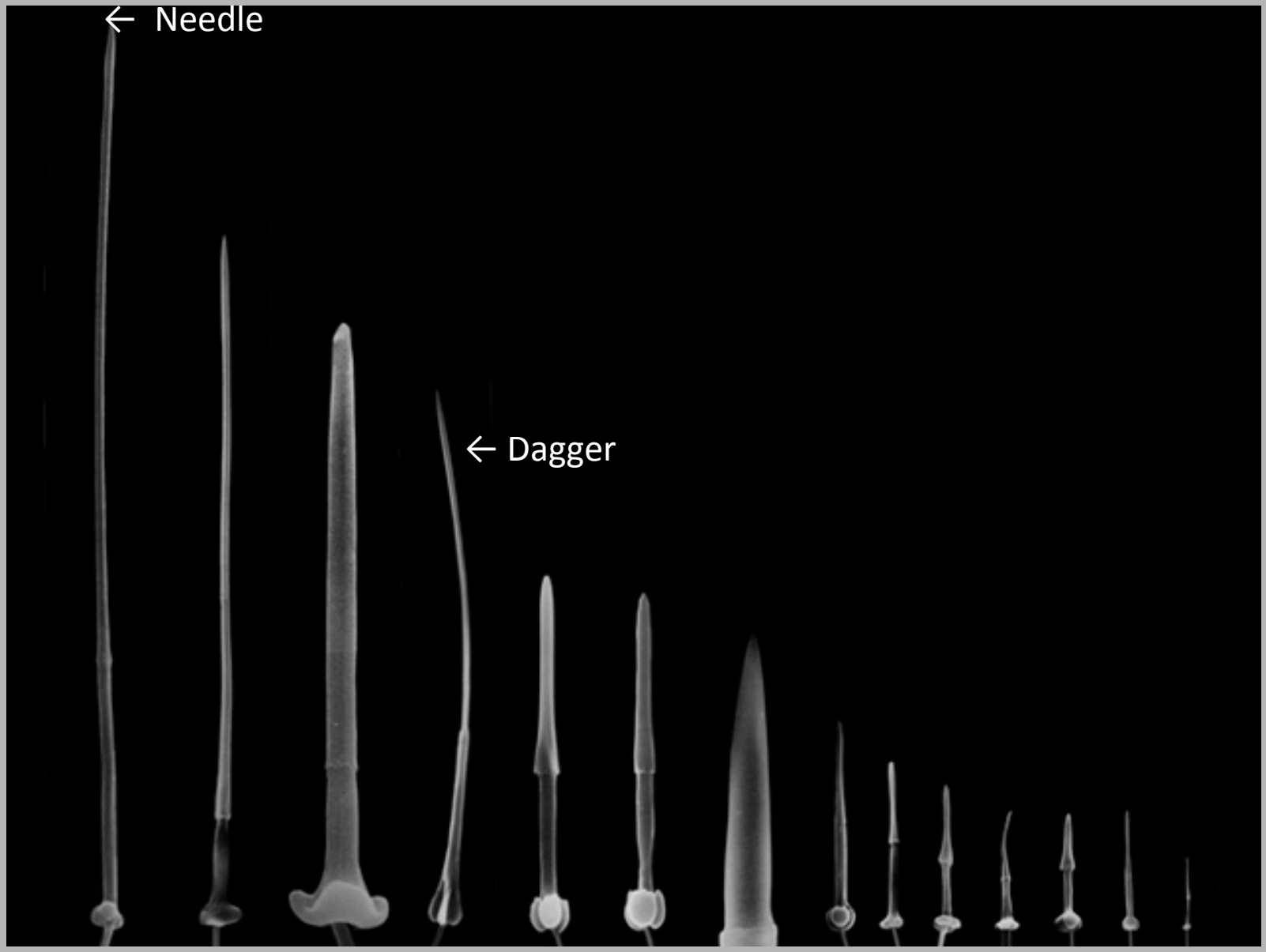


### CLASS:
### ENOPLEA (ADENOPHOREA)

Stylet (odontostyle or onchiostyle) without knobs
Virus vectors
Examples:
- *Xiphinema* (dagger)
- *Longidorus* (needle)
- *Paratrichodorus* (stubby-root)

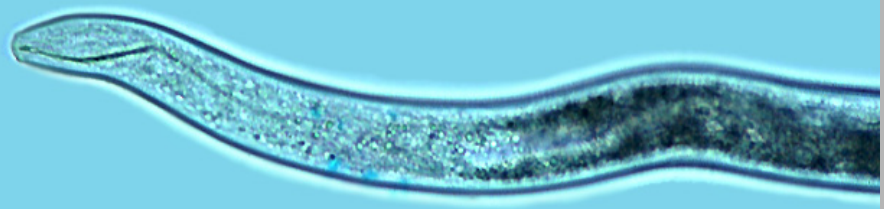


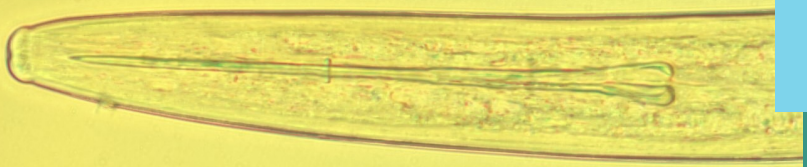

← Needle
← Dagger

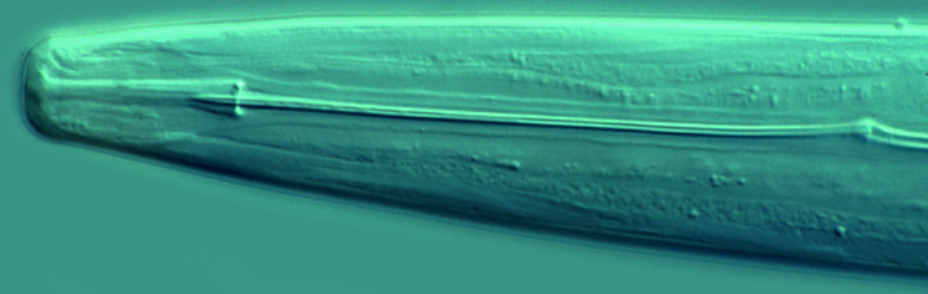
# Nematode Virus Vectors:

***Paratrichodorus; Trichodorus*** **(stubby-root)**



***Xiphinema*** **(dagger)**





***Longidorus*** **(needle)**

# Rubus viruses known to be transmitted by nematodes

| Virus | Vector | Notes/remarks |
|---|---|---|
| arabis mosaic (ArMV) | *Xiphinema* | Importance declined with use of methyl bromide; may increase in incidence in the future |
| cherry leafroll (CLRV) | *Xiphinema*; *Longidorus* | Not found in the U.S. on *Rubus*; mainly Europe |
| cherry rasp leaf (CRL) | *Xiphinema*; *Longidorus* | Not found (yet) in *Rubus* in U.S., but broad host range that includes ornamentals and fruit trees |
| raspberry ringspot (RpRSV) | *Longidorus; Xiphinema; Paratrichodorus* | Found throughout Europe; broad host range |
| strawberry latent ringspot virus (SLRSV) | *Xiphinema* | Detected in *Rubus* only in Europe, but found in strawberry and mint in U.S. |

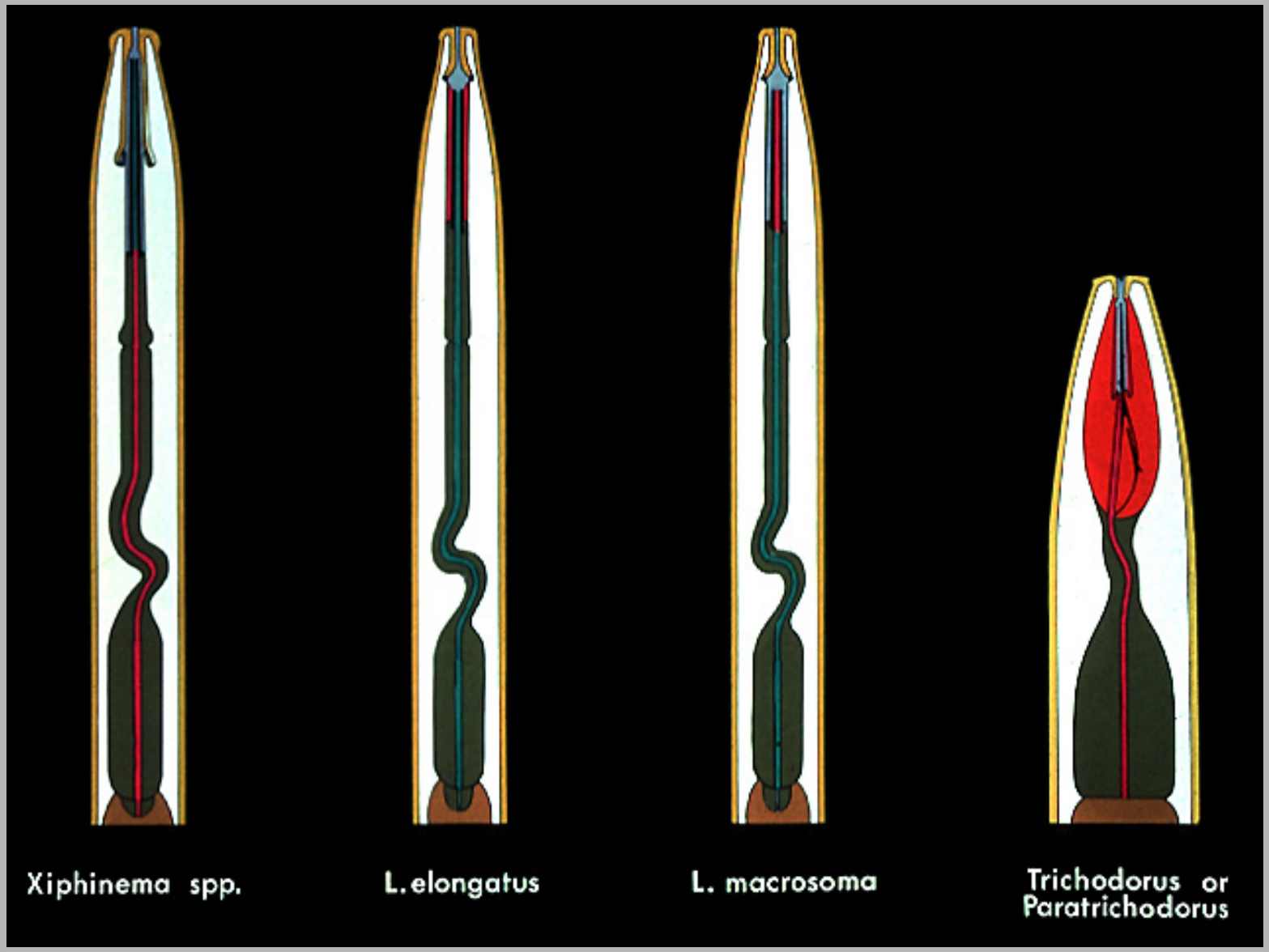
# Rubus viruses known to be transmitted by nematodes (continued)

| Virus | Vector | Notes/remarks |
|---|---|---|
| tobacco ringspot virus (TRSV) | *Xiphinema* | Multiple species transmit; U.S. presence |
| tomato black ring virus (TBRV) | *Longidorus* | *L. elongatus;* mainly Europe |
| tomato ringspot virus ToRSV | *Xiphinema* | Broad host range; *X. americanum;* found in U.S. |

# Points to consider:

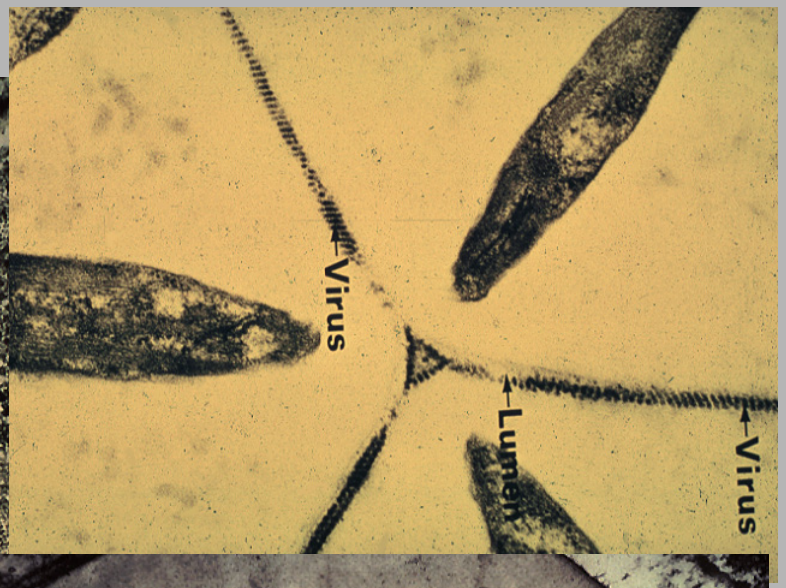
- **The nematode may not be all that damaging alone, but the threshold is "any detectible level" because of virus transmission potential.**
- **Mechanisms of acquisition & release not well understood; acquired and transmitted while feeding, likely transmitted in dorsal gland secretions.**
- **Ability to transmit viruses is lost after a molt; must be re-acquired - all life stages can transmit viruses.**
- **Specificity – generally only one or a few species can transmit a particular virus or strain. Less specificity in U.S. than in Europe. ……BUT……..**
- **Taxonomy of the nematode genera is in flux, so the specificity we now know (or think we know) will likely change.**
- **Virus particles are retained in specific locations in the nematode**
  - **-- *Longidorus* spp.: Either between the guide sheath and stylet or attached to stylet lumen wall.**
  - **-- *Xiphinema* spp. : Stylet lumen wall or the cuticular lining of the esophagus**
  - **-- *Paratrichodorus; Trichodorus* spp.: cuticular lining of esophagus.**

# Virus particle location in nematode vectors

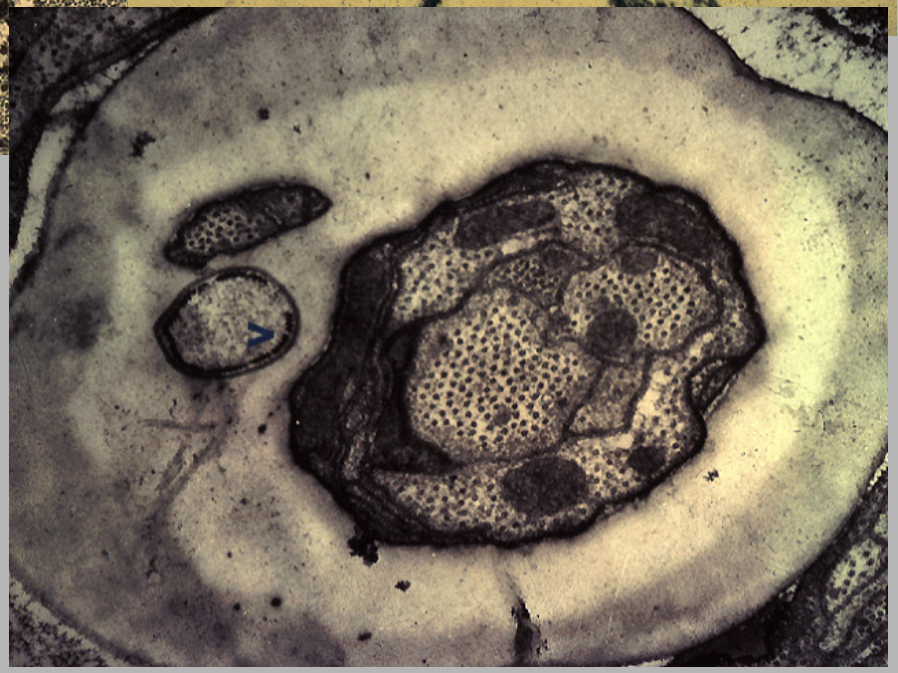


**Raspberry ringspot in *Longidorus elongatus* odontostyle**

**Tobacco rattle virus (TRV) in esophagus of *Paratrichodorus***




**Arabis mosaic virus particles in lumen of *Xiphenema diversicaudatum* food canal**



# Management of Nematode-Transmitted Virus Diseases

**No nematode control**

↓

**Methyl bromide fumigation**

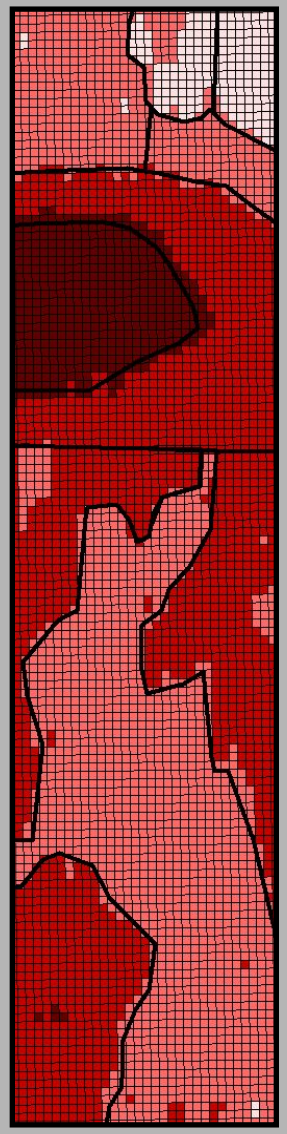
↓

# Avoiding a problem to begin with is the best disease control strategy.

## Proper site selection is essential:

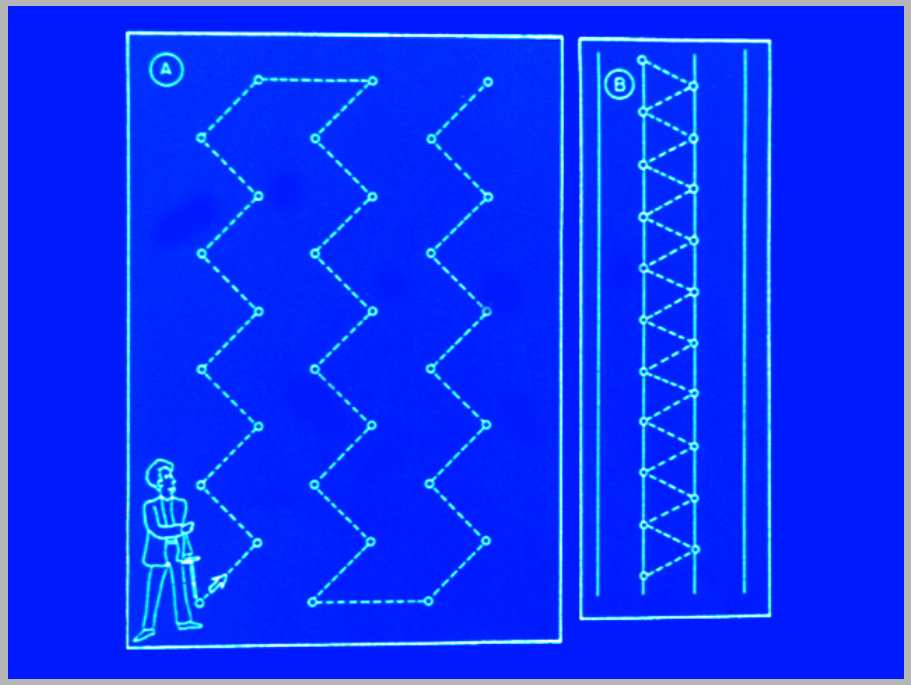
- Nematodes present? Detection/quantification are essential
- Weed hosts of virus present?
- Past crop history – don't follow a known problem

# Nematodes are NOT normally distributed



Detection/quantification depends on:

- nematode species
- time of year
- soil type
- crop & crop history
- how the sample is collected, handled, and assayed



# Management of Nematode-Transmitted Virus Diseases

- **Plant virus-free stocks.**
- **Minimize initial nematode vector population density**
  - **-- Clean fallow (1 year is good, 2 years would be better)**
  - **-- Soil fumigation with Vapam, Kpam, Telone or Basamid. (Paladin??)**
- **Plant virus-resistant cultivars where availability – there are NO nematode-resistant cultivars.**
- **Ongoing weed management to minimize reservoir/alternative hosts.**

# Nematode Diagnostic Services

## Arkansas

Arkansas Nematode Diagnostic Laboratory
362 Highway 174 North
Hope, AR 71801
Phone: (870) 777-9702, Ext. 128 or 119
Email: rbateman@uaex.edu or choward@uaex.edu
For more in-depth info:
http://www.aragriculture.org/nematodes/nematode_clinic.htm
To submit a sample: http://dddi.org/ua/
Services and Fees: General - $10, Soybean - $20, Pinewood - $25, Nursery - $25, Rice Grain - $25, Rice Grain Certified - $75. Out-of-state samples accepted.

## South Carolina

Clemson University Nematode Assay Laboratory
511 Westinghouse Road
Pendleton, SC 29670
Phone: (864) 646-2133; Fax: 864-646-2178
nemalab@clemson.edu
http://www.clemson.edu/plantclinic
Fees: In-State - $10, Out-of-State - $30.

## North Carolina

North Carolina Department of Agriculture and Consumer Services Agronomic Division
Nematode Assay Section
4300 Reedy Creek Road
Raleigh, NC
Phone: 919.733.2655
Fax: 919.733.2837
WEB URL: http://www.ncagr.com/agronomi/index.htm
Services and fees: In-state - $3, Out-of-State, Pinewood, Research, PCR - $10.

## Georgia

Extension Nematology Laboratory
2350 College Station Rd.
Athens, GA 30602
Contact Information: Dr. Ganpati Jagdale, gbjagdal@uga.edu
Ms. Judy Cross, jcross@uga.edu [706-542-9144]
http://plantpath.caes.uga.edu/extension/clinic.html
Services and Fees: All samples submitted through GA County Extension Office - $12, All samples not submitted through County Office and Out-of-State - $25, Root-knot Nematode Species - $35, Free-Living at Genus Level - $45.

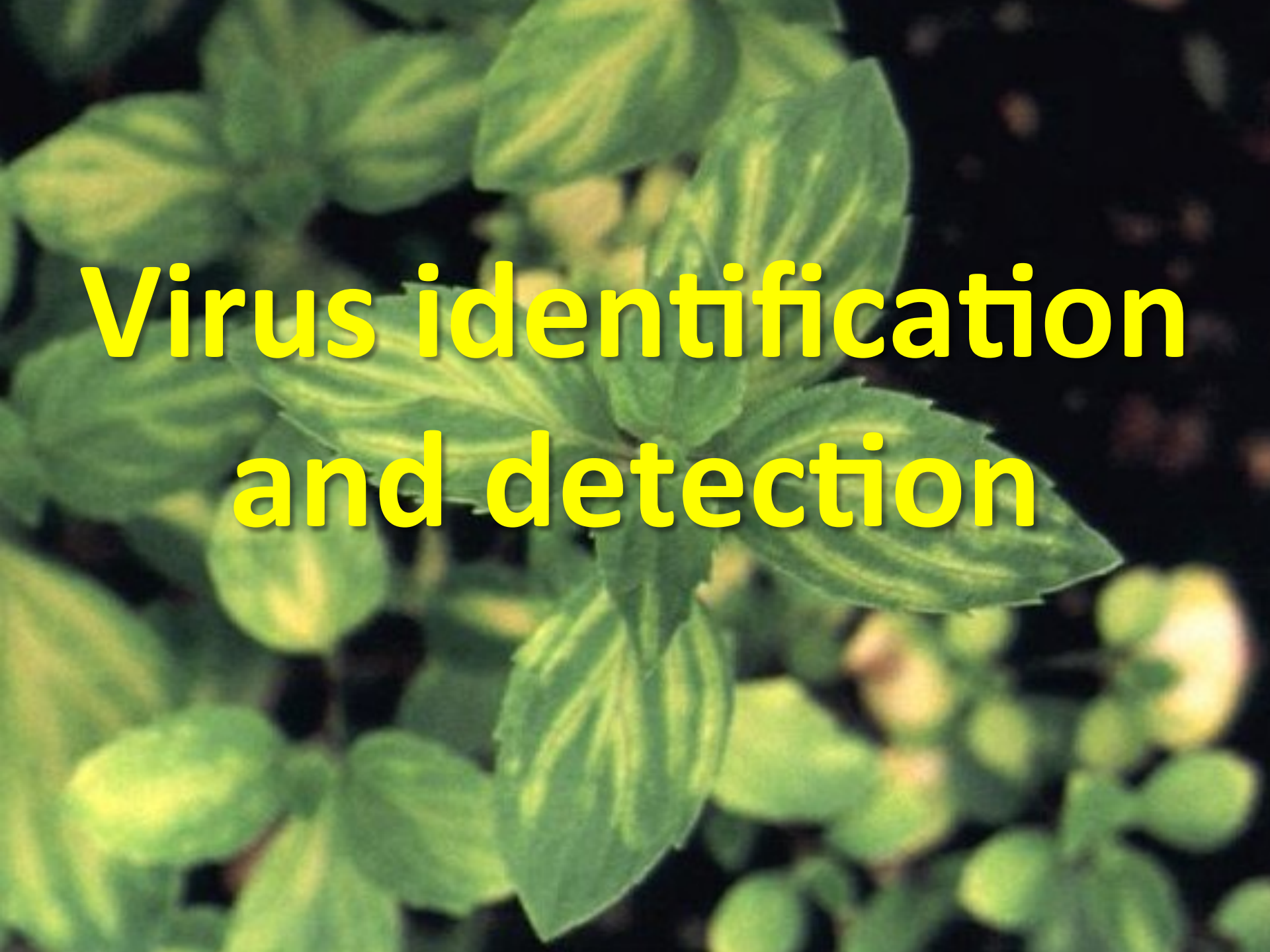
# Nematode Diagnostic Services

## Virginia

**Nematode Assay Laboratory**
**115 Price Hall**
**Virginia Tech**
**Blacksburg, VA 24061-0331**
**Phone: (540) 231-4650**
**Fax: (540) 231-7477**
**Email: jon@vt.edu**
**Services and Fees: Routine assays - $11,**
**Routine plus cysts (usually for soybeans and tobacco) - $19.00**

## Tennessee

**UT Plant & Pest Diagnostic Center**
**5201 Marchant Drive**
**Nashville, TN 37211-5112**
**Phone: (615) 832-6802**
**Email: tstebbin@utk.edu**
**Services and Fees: Basic - $5, Out-of-State $15,**
**Soybean Cyst Nematode Race Determination - $15**


Virus identification
and detection

# The first step: Is it a virus?

Symptoms

Transmission

Movement

Detection

# Symptoms

No fruiting bodies, no oozing

Viruses change the host physiology and adapt cell machinery to facilitate optimal replication

Some plants are more tolerant than others to virus infections giving different types of symptoms when infected with the same virus

# Types of symptoms

Mosaic

Chlorosis

Discoloration
and leafroll

Ringspot

# Mottle

Vein-Banding, Chlorosis, Clearing

Oak-leaf

# Necrosis

Epinasty-malformation, enation

# Fruit deformation

## Pitting and grooving

Cambium tissue in the grooved region disappears

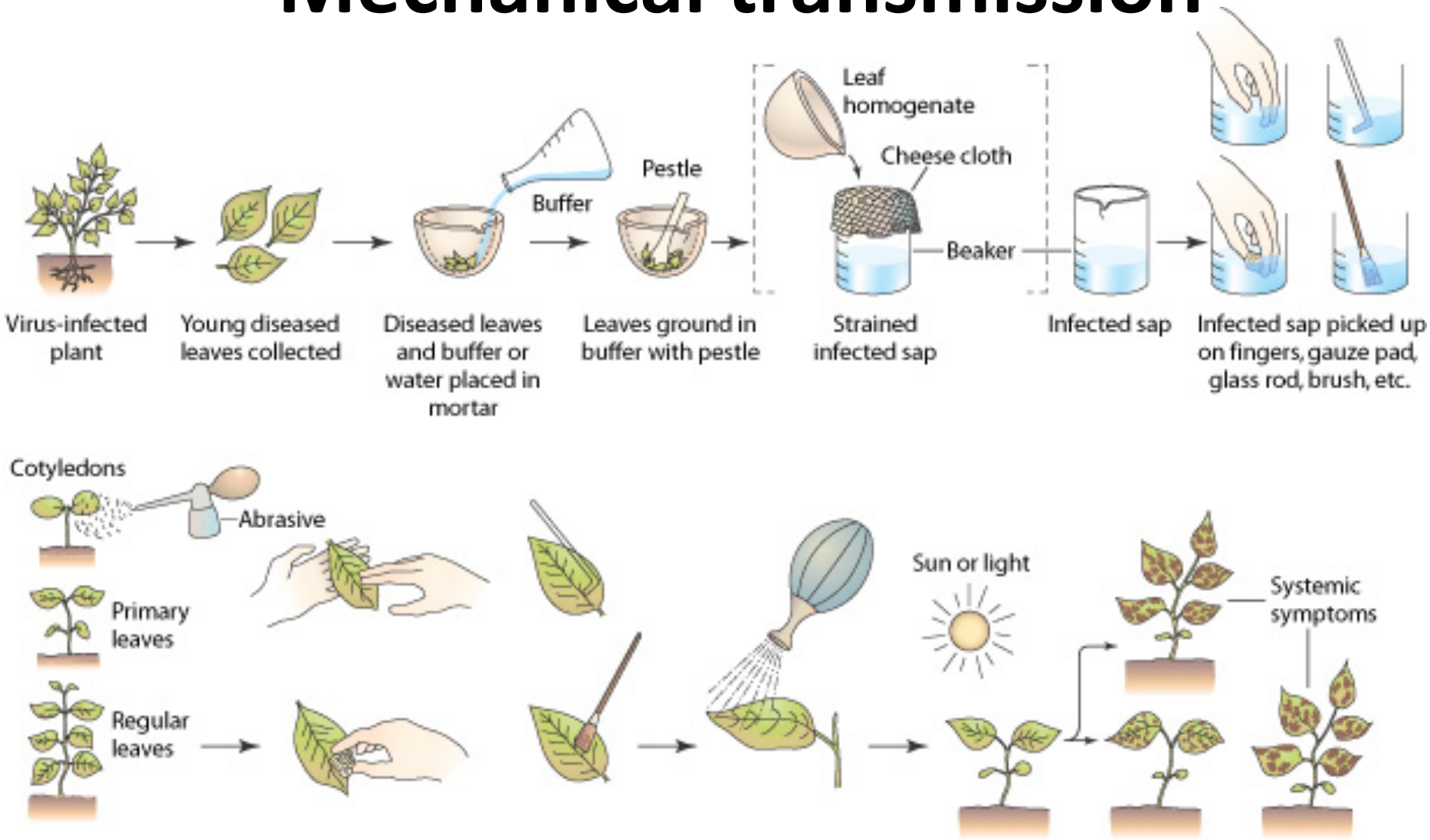
# Graft incompatibility

***Apple mosaic virus***
Apple vs. strawberry

***Tulip virus X***
Lemon balm vs. gomphrena and tobacco

***Tobacco ringspot virus***
Blackberry ‘Apache’ vs. ‘Ouachita’

# Mechanical transmission




Agrios, 2005

# Inoculation

Infected sap rubbed on healthy plants

Inoculated plants must be rinsed and watered soon after inoculation

**More often than not inoculations do not result in infection.**

Symptoms develop
in 3 – 21 days

Systemic

Local lesions

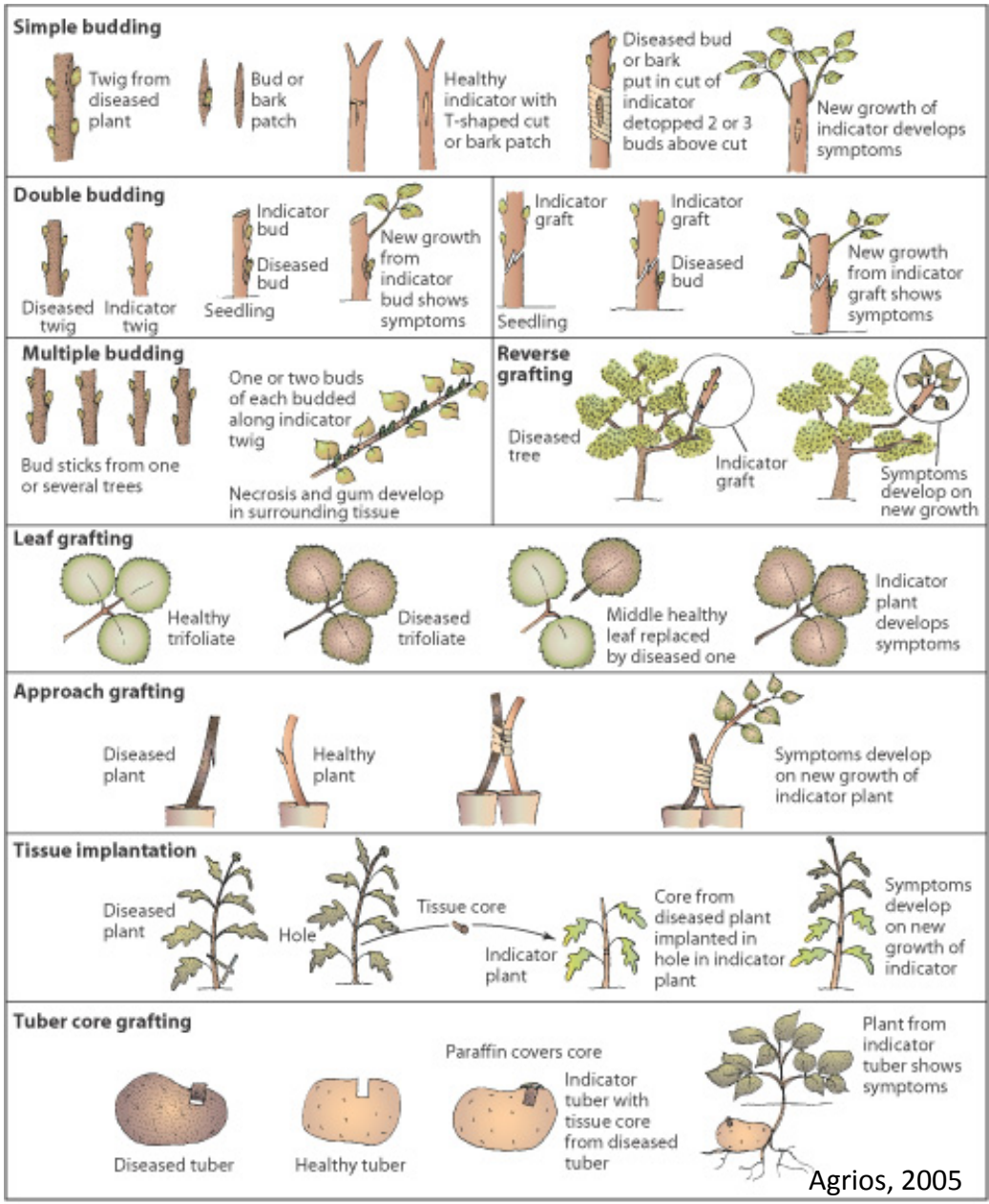
***Cucumber mosaic virus***
Transmission from
Anemone to Nicotiana

**Fig leaf mottle associated virus-4**
Transmission from Fig to Soybean

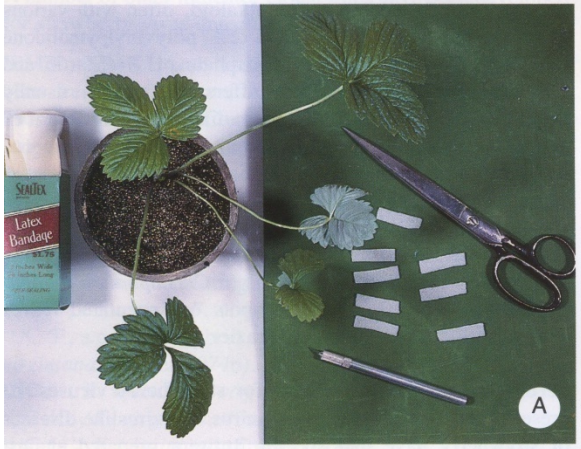
# Grafting

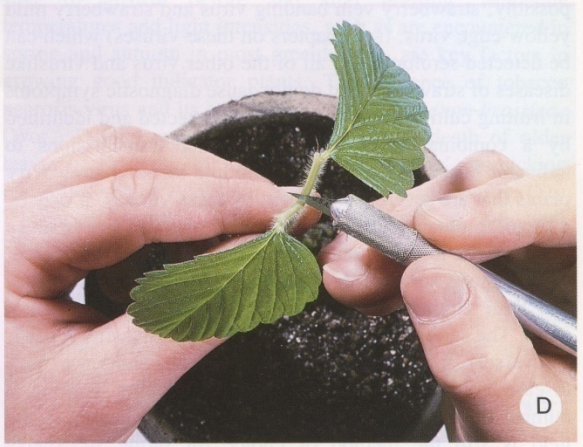
The technique of choice for woody plants

**An extremely valuable tool for agents yet to be characterized**




Agrios, 2005


SEALTEX
Latex
Bandage
A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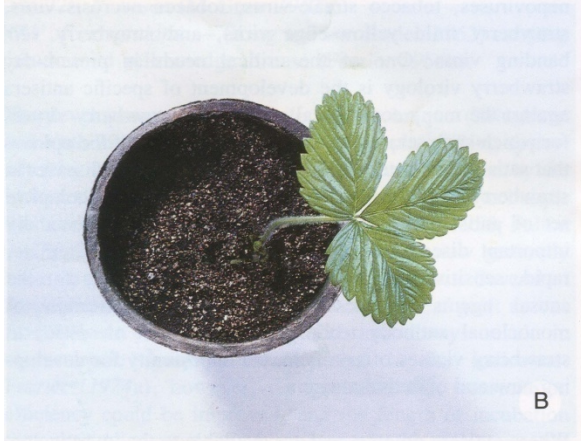
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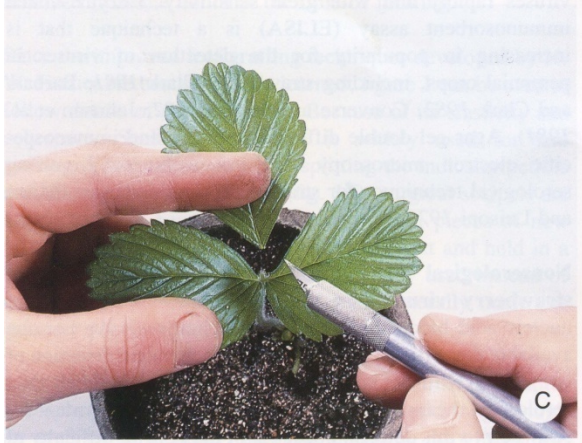
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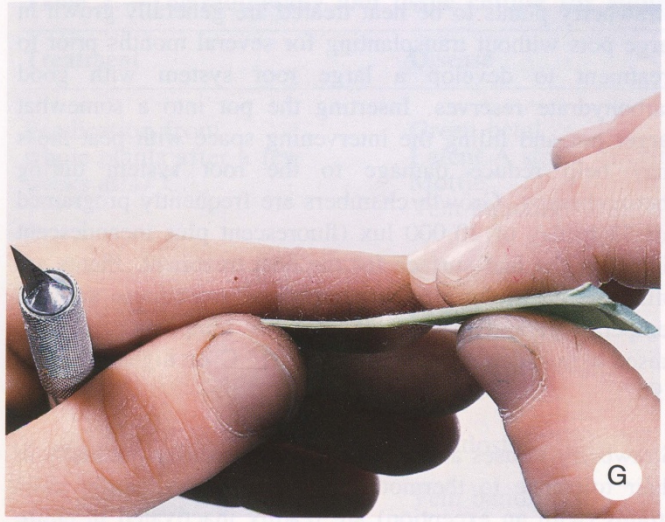
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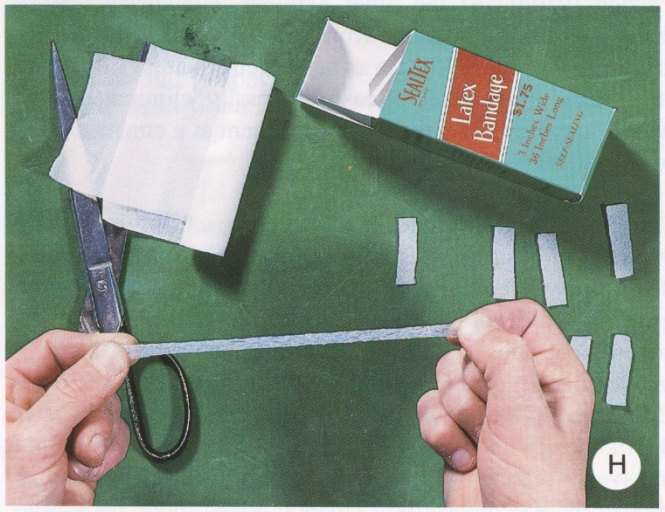
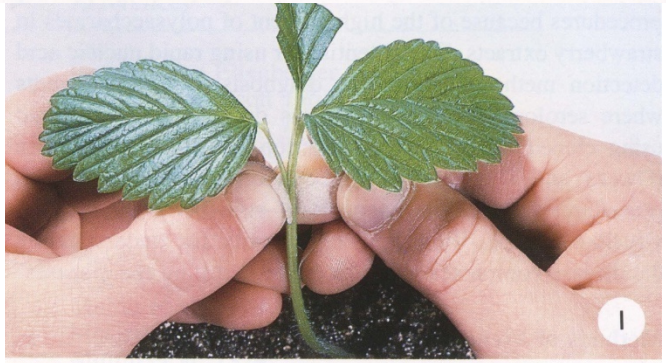

SAMTEX
Latex Bandage
$1.75
H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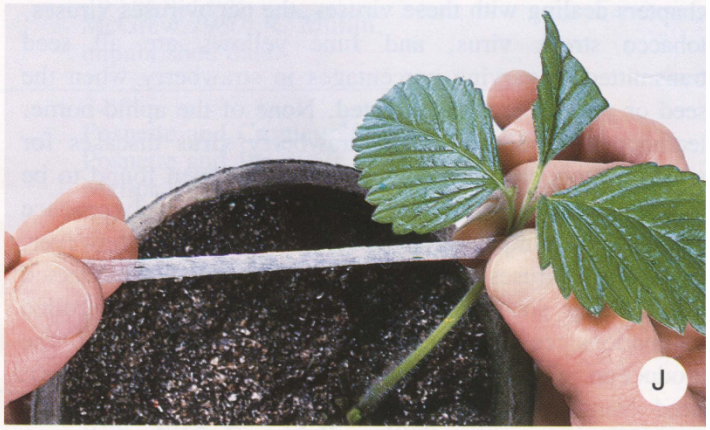
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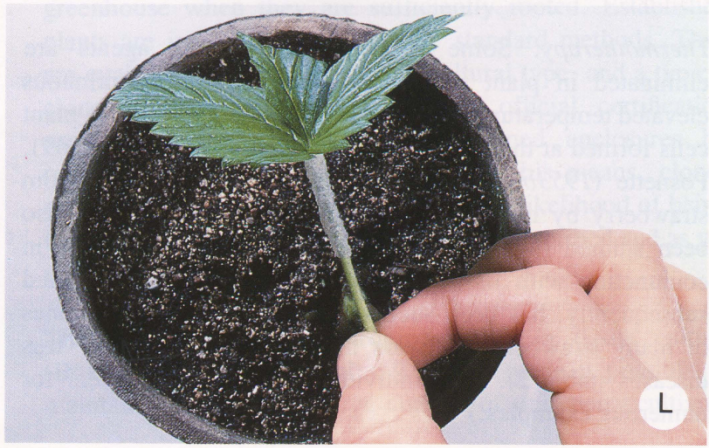
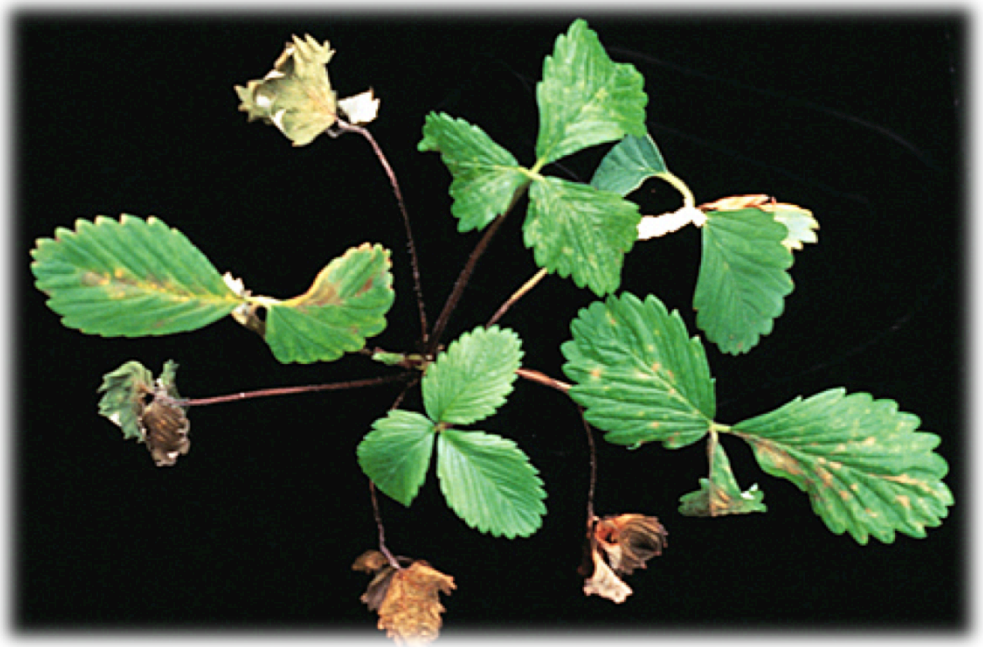
K


L

**Leaf grafting**
Strawberry



**Tissue transplantation**
Blueberry onto tobacco, raspberry and chenopodium

# Strengths and Weaknesses

**Transmission by Grafting or Mechanically:**

- Detects wide range of organisms even if we lack information on the causal agent
- Often considered the standard to which other tests must be compared
- Can potentially detect a range of pathogens in a single test

- Requires considerable field and/or greenhouse space
- Labor intensive
- Can take several years to get results with some diseases of woody hosts

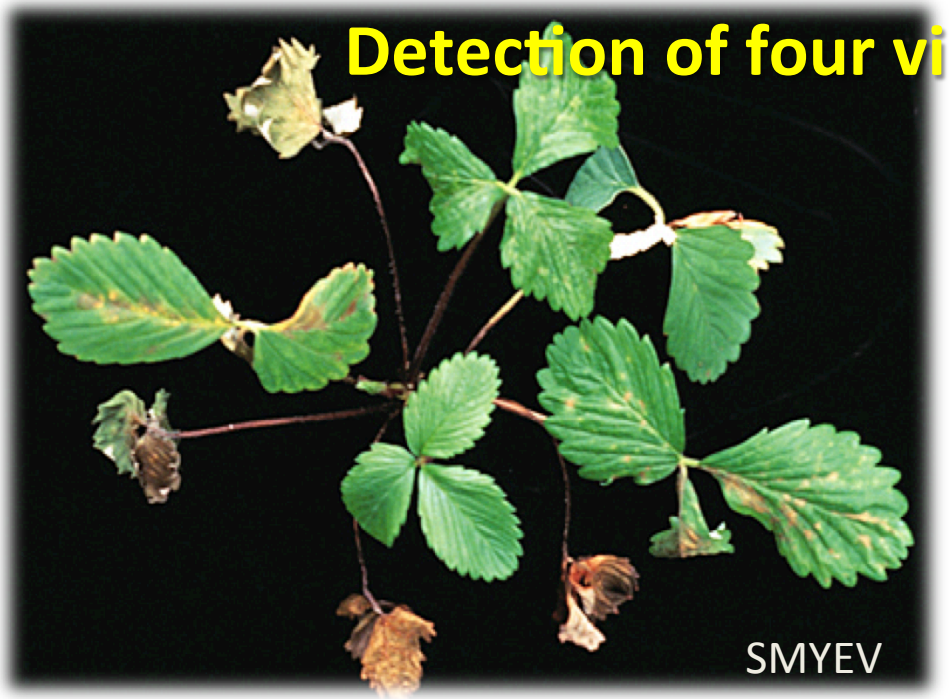
# Cases where  grafting has made a difference

Strawberry pallidosis disease

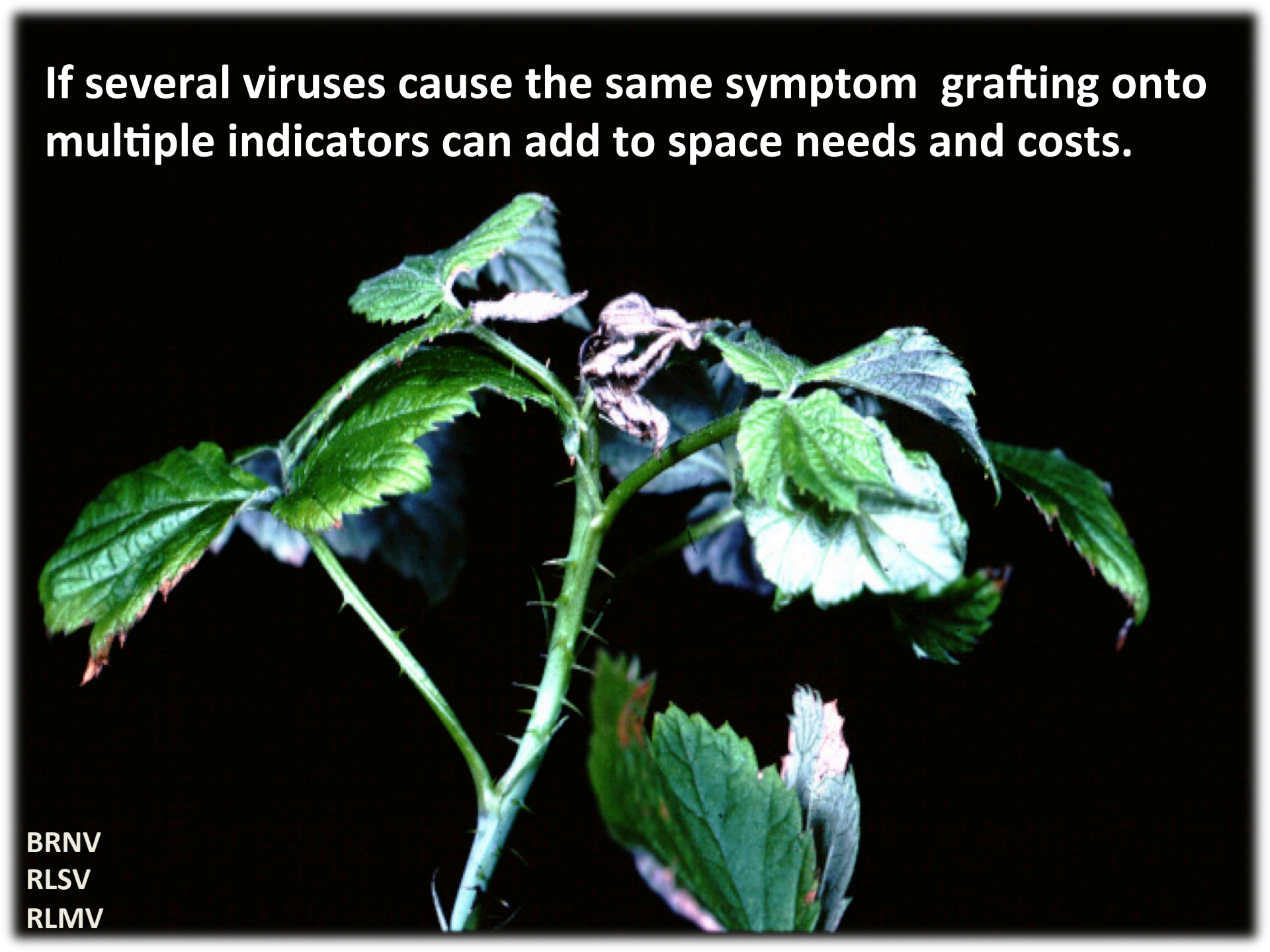
Identified in 1958, virus discovered in 2001

**Blackberry yellow vein disease is not caused by ~~BYVV~~ BYVaV**

# Detection of four viruses on a single indicator



**If several viruses cause the same symptom grafting onto multiple indicators can add to space needs and costs.**



Difficult to sort out a
complex by grafting.

# Immunology

Viruses are made of nucleic acids and proteins

Proteins trigger antibody (Ab) production. Abs are used by the vertebrate adaptive immune system to neutralize invading organisms including viruses

So we can use plant viruses and develop Abs against the coat protein(s)?

Yes, and the Ab can be used for sensitive detection of viruses

# ELISA

Enzyme-linked immunosorbent assay (ELISA) uses Abs developed against plant viruses for detection.

Sensitivity can reach pg of virus.

Major variants:

Direct (Double and triple

Antibody sandwich ) ELISA

Indirect ELISA

**DAS ELISA**

Antibody binding to matrix

# ELISA

Enzyme-linked immunosorbent assay (ELISA) uses Abs developed against plant viruses for detection.

Sensitivity can reach pg of virus.

Major variants:

Direct (Double and triple

Antibody sandwich ) ELISA

Indirect ELISA

**DAS ELISA**

Addition of plant sap/virus

# ELISA

Enzyme-linked immunosorbent assay (ELISA) uses Abs developed against plant viruses for detection.

Sensitivity can reach pg of virus.

Major variants:

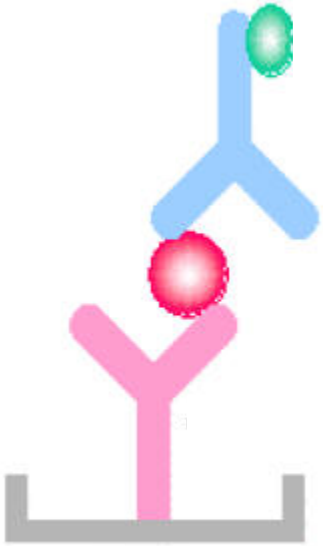
Direct (Double and triple

Antibody sandwich ) ELISA

Indirect ELISA

**DAS ELISA**

Addition of secondary antibody, conjugate



# ELISA

Enzyme-linked immunosorbent assay (ELISA) uses Abs developed against plant viruses for detection.

Sensitivity can reach pg of virus.

Major variants:

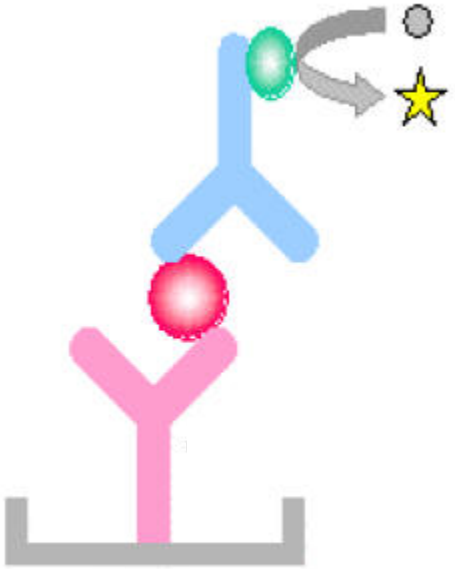
Direct (Double and triple

Antibody sandwich ) ELISA

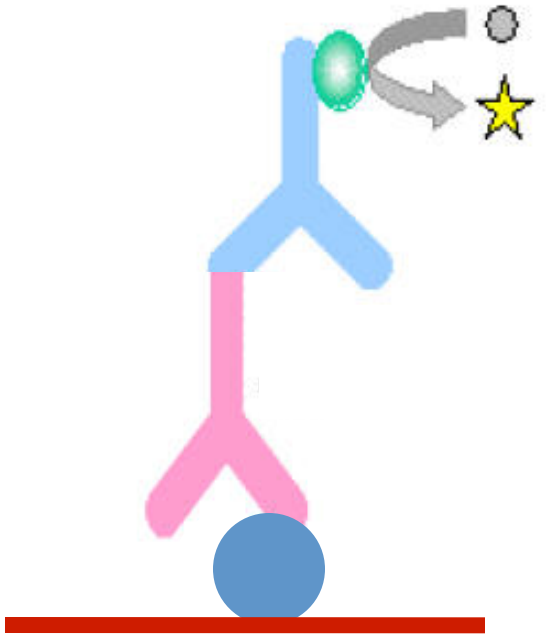
Indirect ELISA

**DAS ELISA**

Addition of substrate, color change



# Membrane-based immunoassays

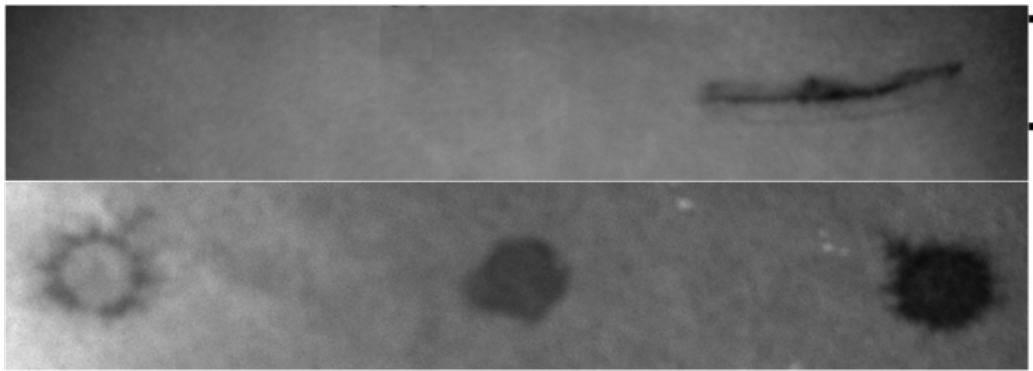


Tissue-blot

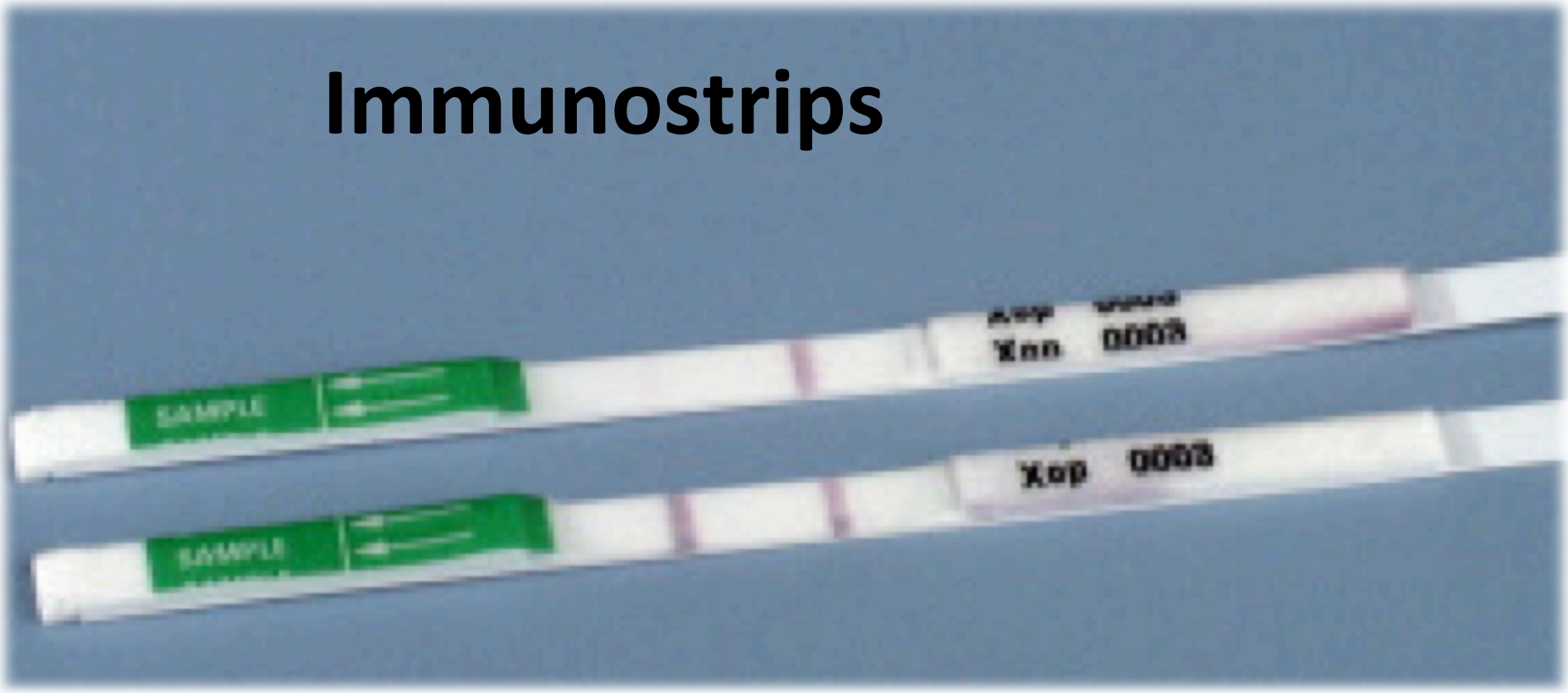
Control TEV VVY

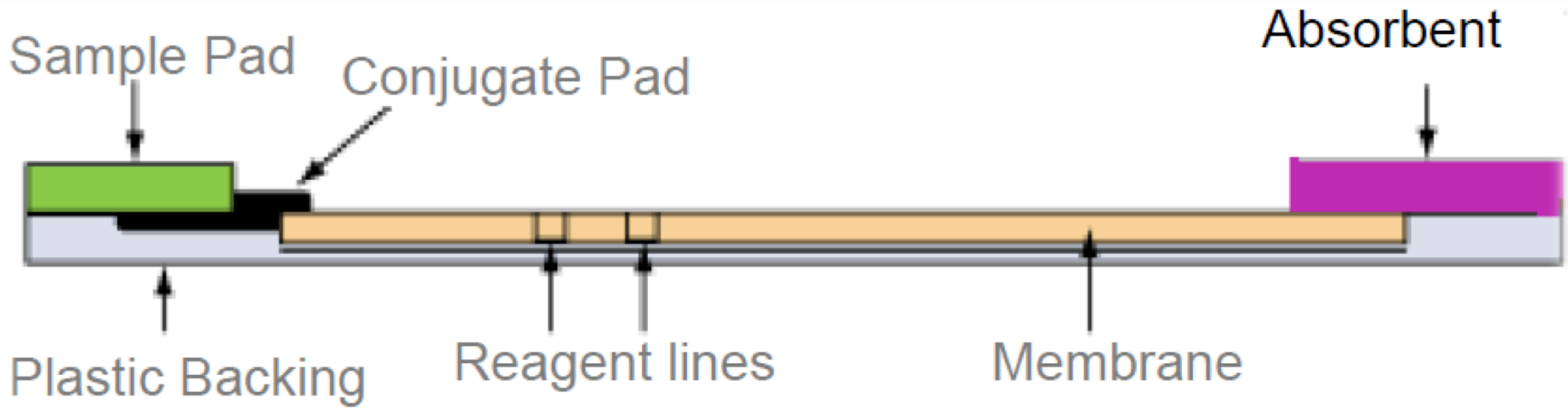
Western blot
*Potato virus Y* polyclonal

Dot blot
Universal Poty monoclonal



# Immunostrips

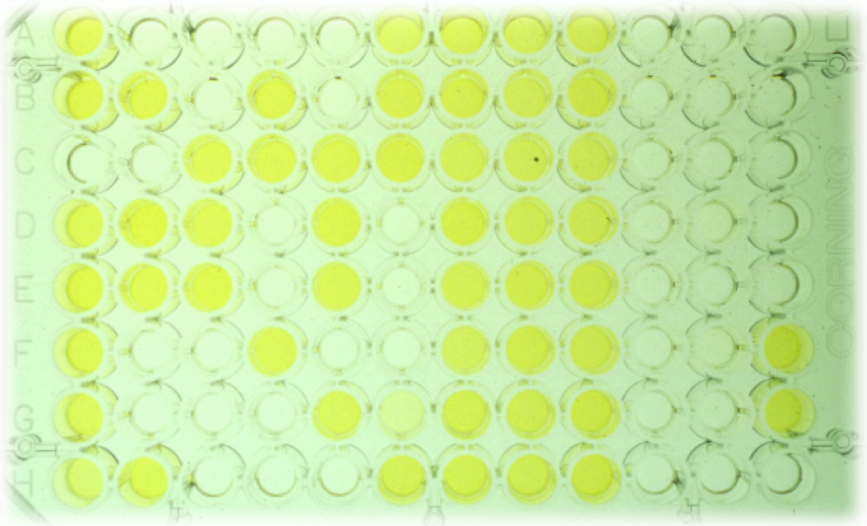


# ELISA advantages/disadvantages

Rapid, sensitive (in most cases), low cost, easy, many excellent means of sample extraction, suitable for large scale testing and automation

Inconsistent supply of antibodies, some hosts or pathogens require special extraction buffers, specificity of antibodies can be a problem - may only detect some strains of a pathogen



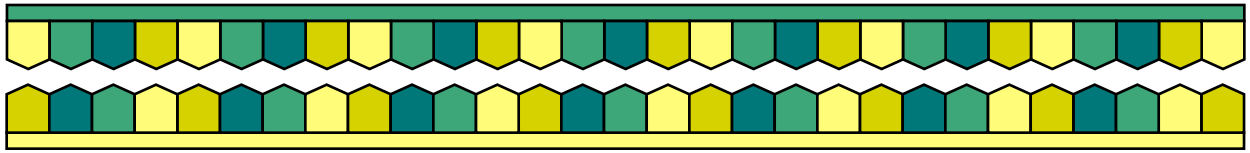
# PCR and RT-PCR

- Virus sequence needed to design primers
- When working with RNA viruses a reverse transcription step is needed to obtain cDNA
- Reverse transcriptase is a RNA-dependent and a DNA dependent DNA polymerase
- **Reverse transcription yields can vary up to 100-fold with the choice of reverse transcriptase and primers and cDNA yield is gene dependent. Be careful in qPCRs**

# PCR

unique sequence of interest

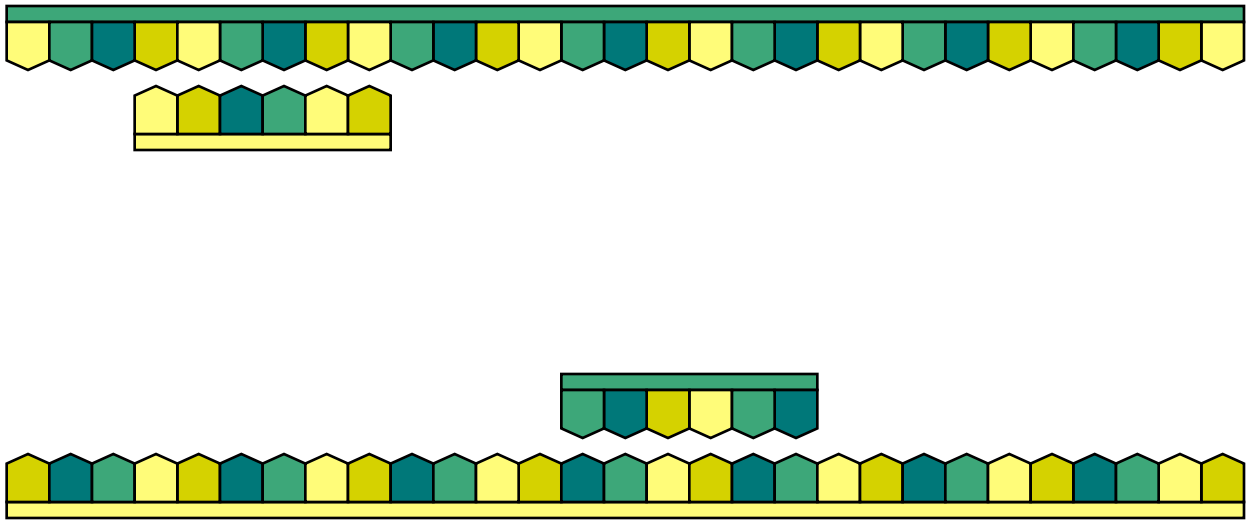
DNA or RNA template



Make a DNA copy if RNA template

Heat to $95^0$C to separate two strands

**unique sequence of interest**

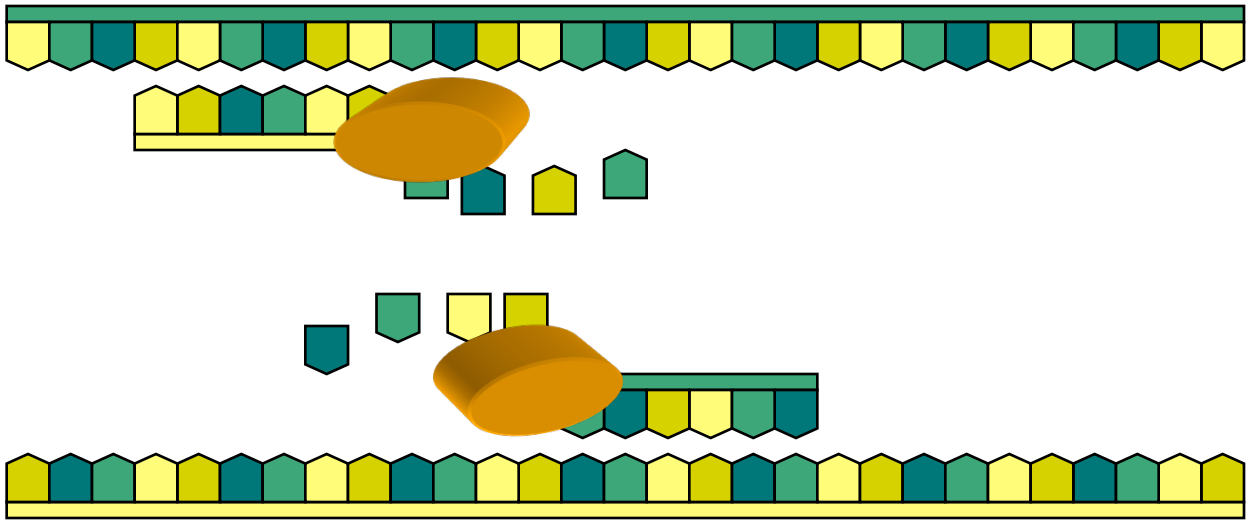


**Anneal primers to the two separated templates**

unique sequence of interest

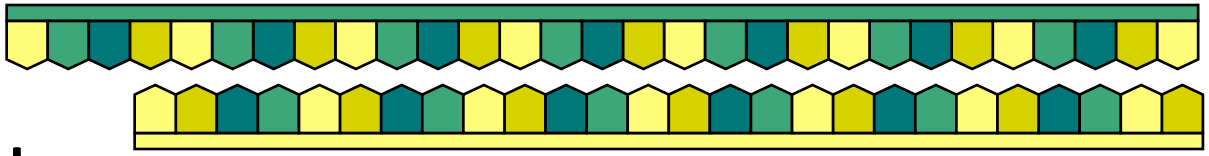
Taq DNA polymerase

adds dNTPs

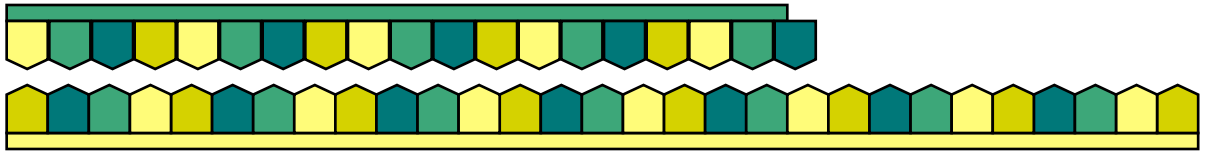


# End of 1st Cycle

unique sequence of interest

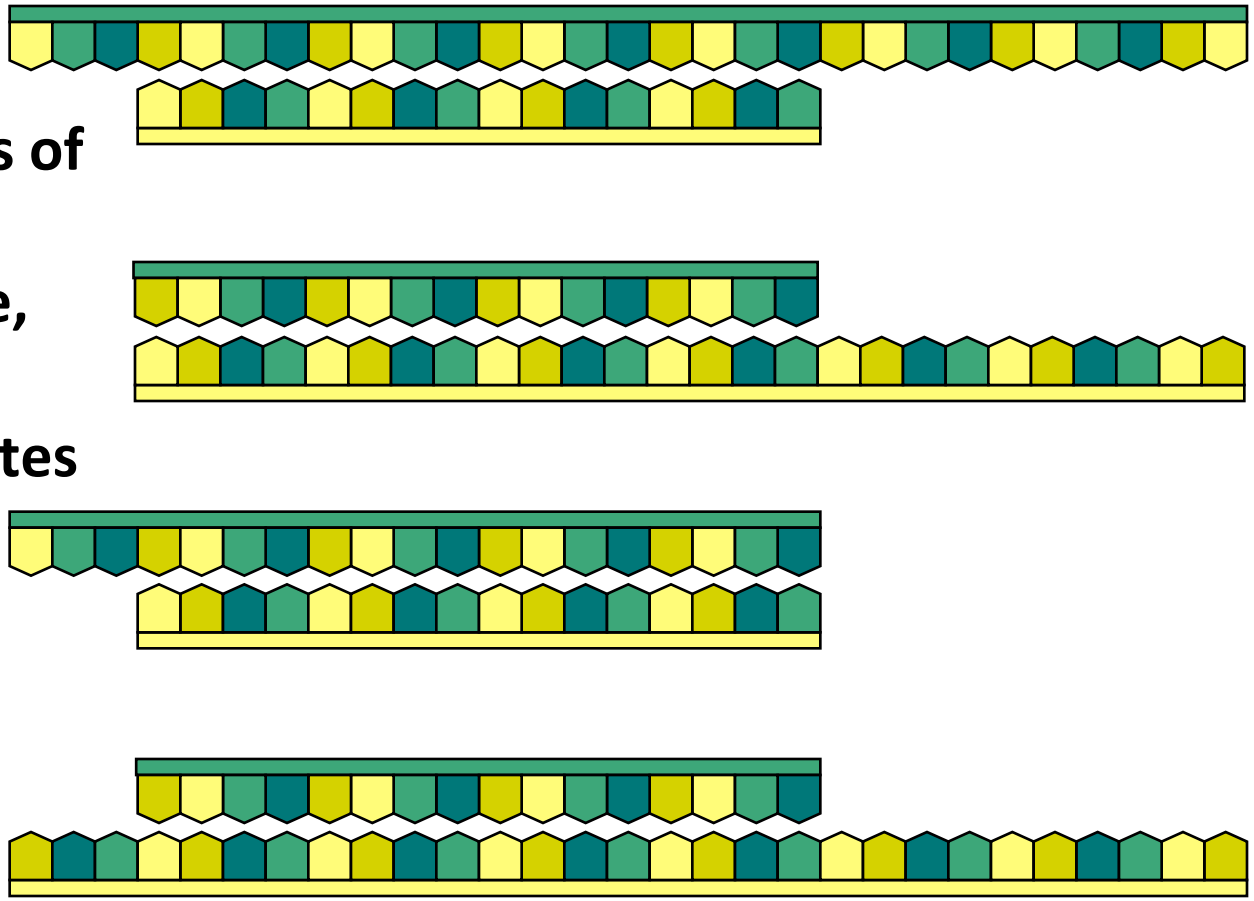


PCR has generated two copies of unique sequence



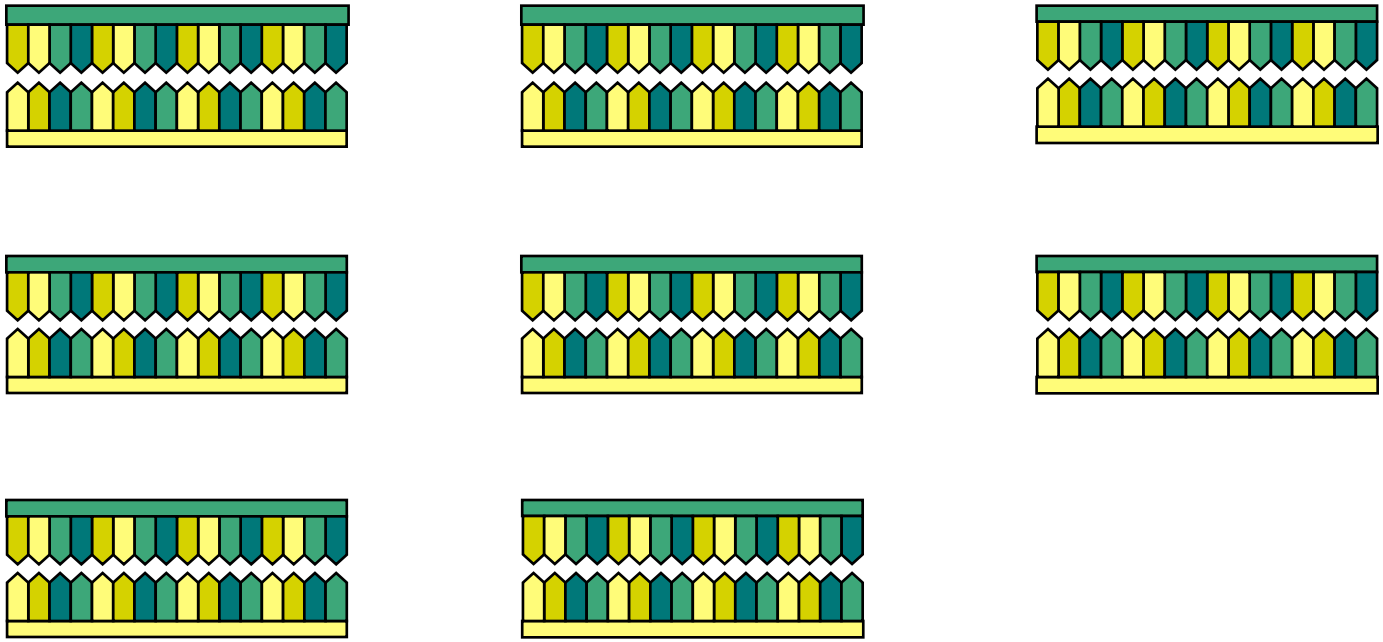
# End of 2nd Cycle

unique sequence of interest

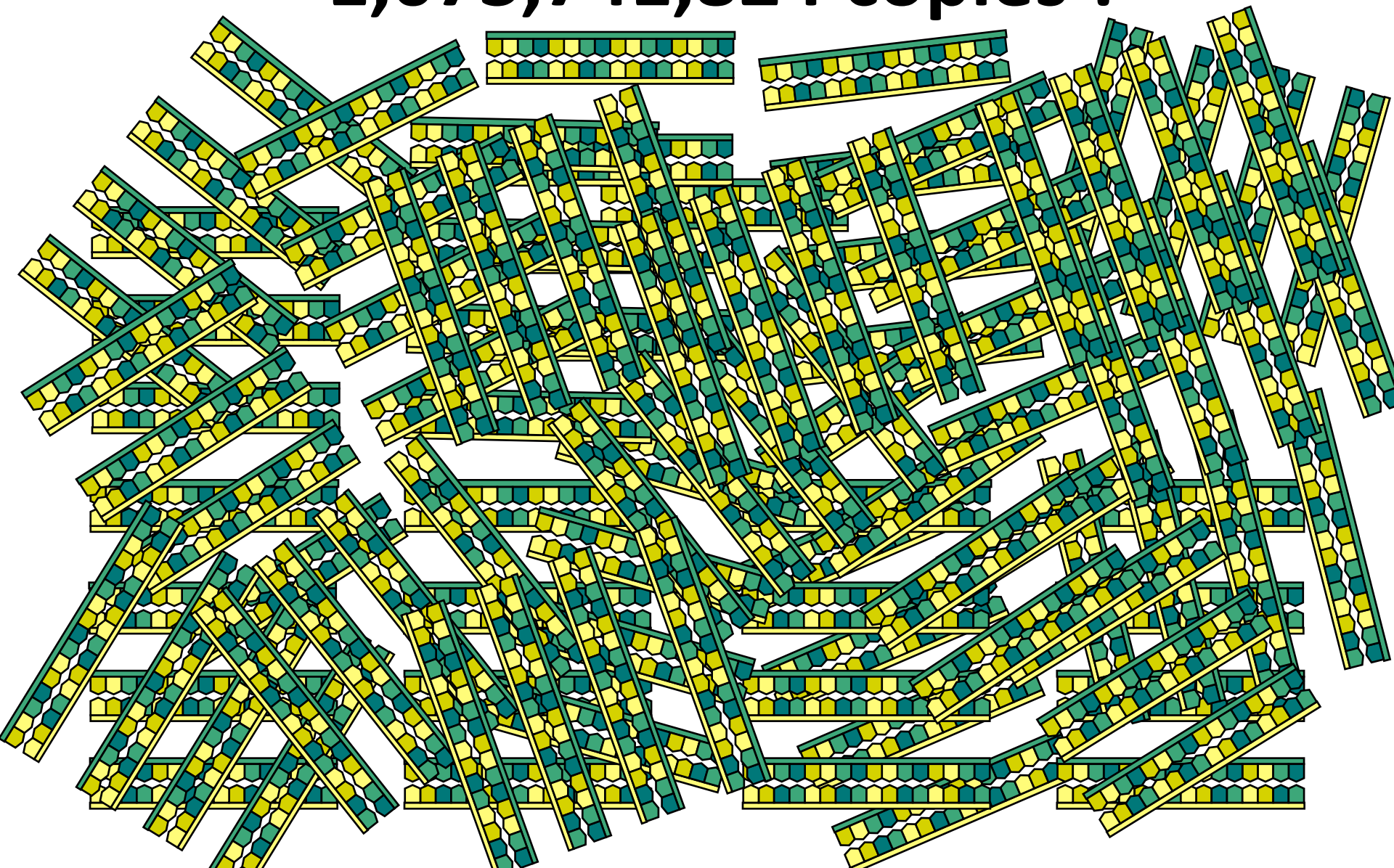


Four additional copies of the unique sequence have now been made, using the original and new copies as templates

# End of 3rd Cycle



# End of 30th Cycles
# =1,073,741,824 copies !



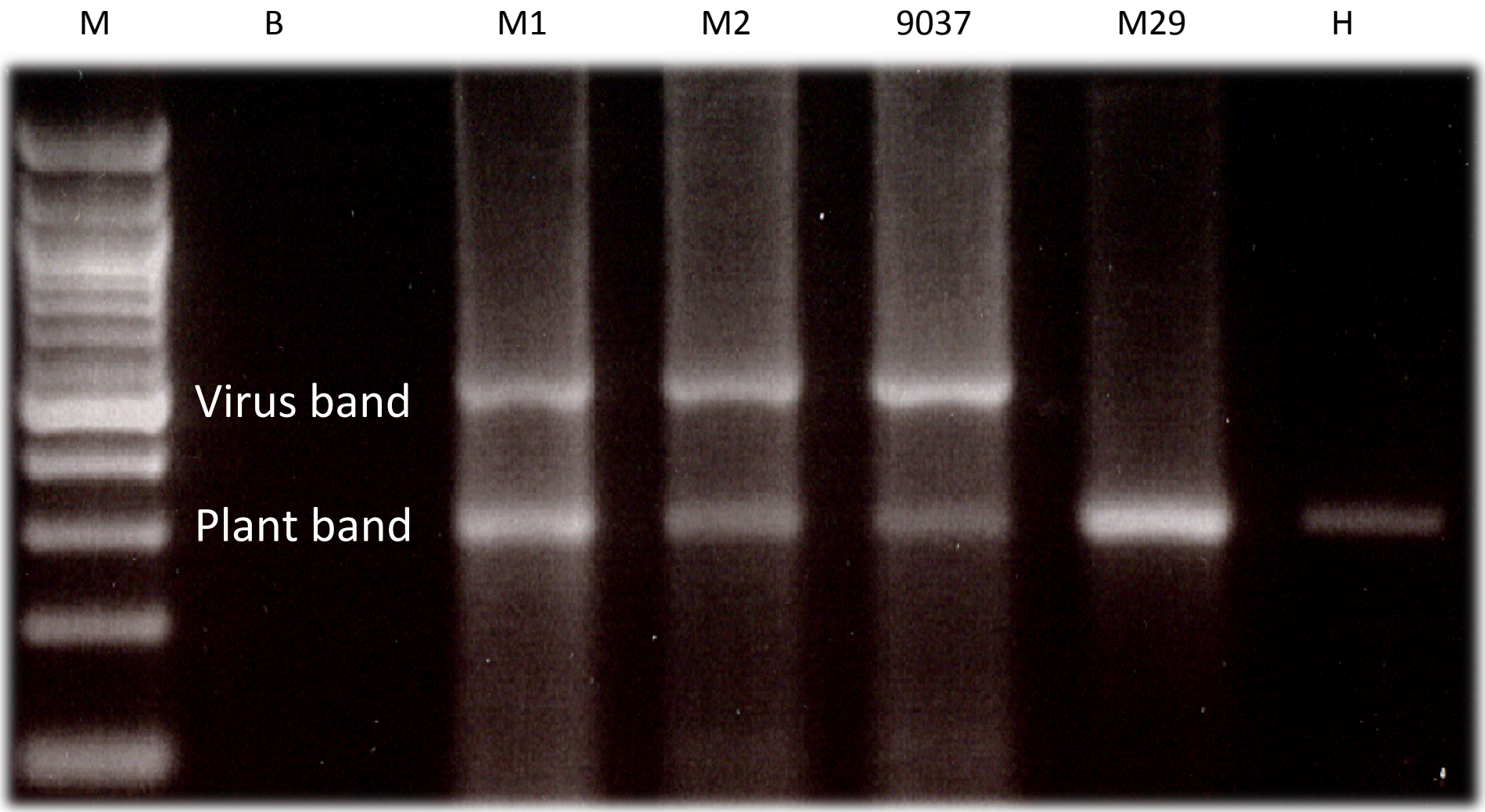
# Problems with RT-PCR

Poor quality RNA

False negatives due to inhibitors, include internal controls

False positives – nonspecific bands on gel sequence several bands to confirm specificity

# Use internal controls to monitor quality of assay



# PCR

Sensitive, conserved sequences can be used to detect range of strains or species, sequencing of amplicons provides information on phylogeny, can detect several pathogens at once.

Requires knowledge of sequence or phylogeny of pathogen, must use internal control to ensure extraction quality. RNA extraction can be difficult from some hosts leading to variable results.

Requires more training than ELISA, must depend on more than just amplicon size to avoid false positives, expensive in terms of time and enzymes**. May get false negatives if there is significant virus diversity.**

# Real-Time PCR

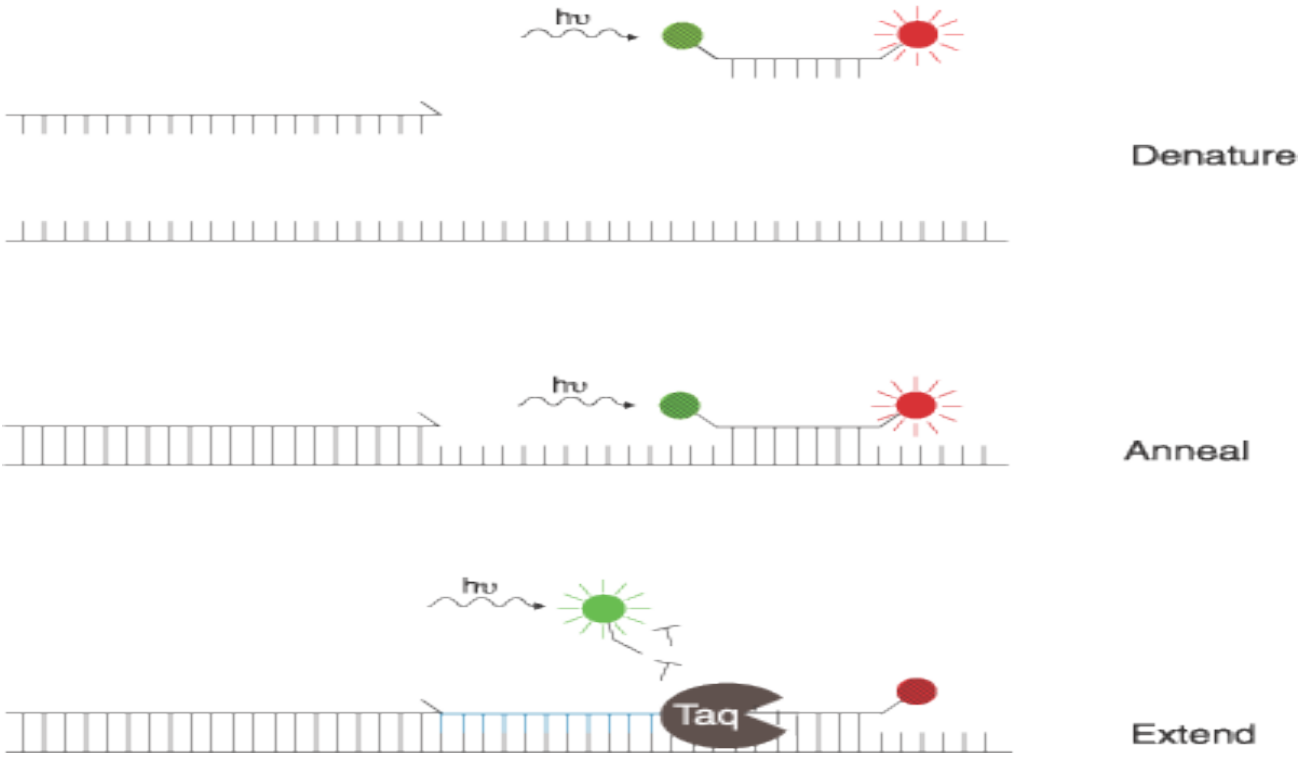
Avoids post amplification analysis, can be quantitative, fast turn around time.

Expensive, requires extreme care in setup to avoid false negatives.

Intercalating dyes such as SYBR green are not suitable for multiplexing.

# Fluorescent reporter (Taqman) Probes

- Primers and probes anneal – polymerization
- The 5’ exonuclease activity of *Taq* digests the probe as it copies and releases the fluor
- Without the quencher, fluorescence increases at each cycle as more probe is digested



# In a nutshell

Viruses change host physiology and thus symptoms

**Detection techniques**

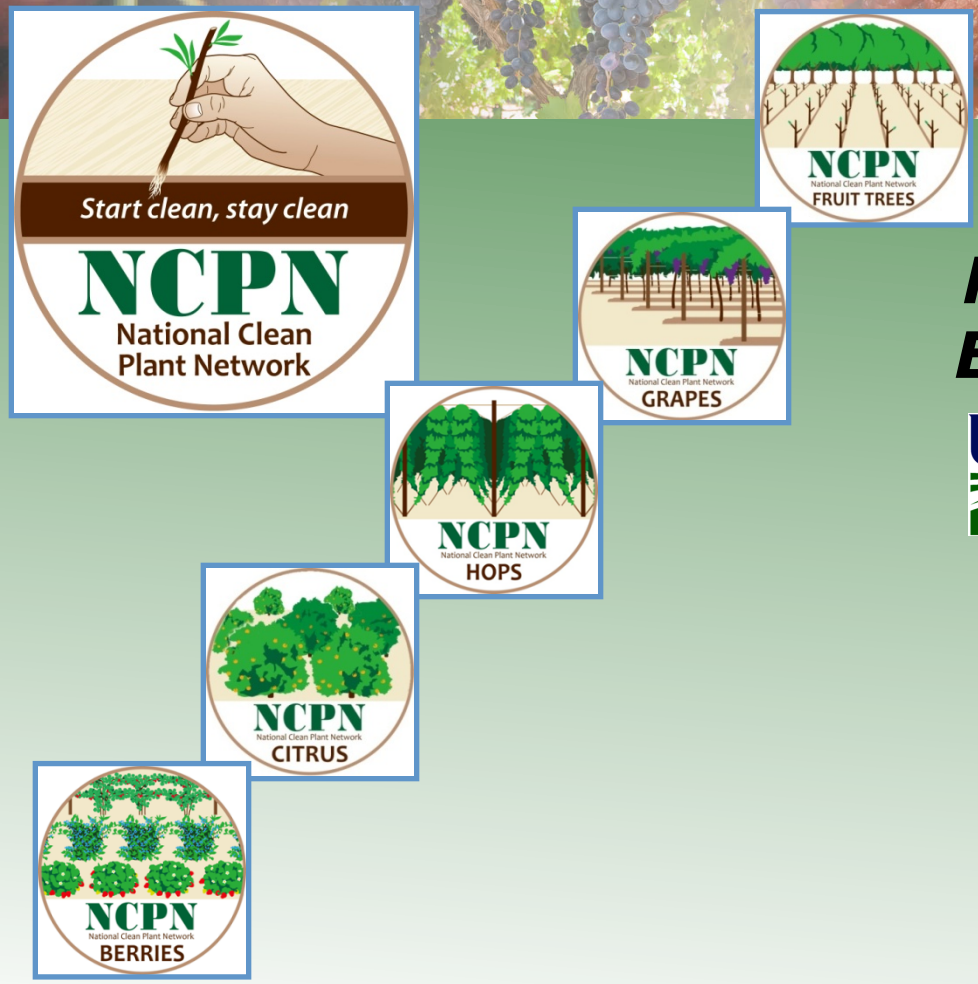
The more broad the technique the less sensitive (in general)

Immunology: Great for large number of samples, not as sensitive as molecular techniques

Molecular: Virus diversity can lead to false negatives

Next generation sequencing: Instant diagnosis of any pathogen???

# National Clean Plant Network (NCPN)




## *NCPN Program Update Early Summer FY 2013*

USDA APHIS

*Erich S. Rudyj*
*Program Coordinator*

*USDA/APHIS/PPQ*
*Science and Technology*
*National Clean Plant Network (NCPN)*

July 2013

# FARM BILL 2008

***Food, Conservation, and Energy Act of 2008 Sec. 10202***
***National Clean Plant Network (NCPN) – Program Background***

- ***Establishment*** - USDA shall establish a 'National Clean Plant Network' for pathogen diagnosis and elimination
- ***Products*** - Clean plant material made available to States for certification programs; and to private nurseries/producers
- ***Consultation*** - Consult w/State Departments of Agriculture, Industry, and Universities; governance
- ***Cooperation*** - Use existing Federal/State clean plant centers
- ***Funding*** - $5m/yr. x 4 years >2009-2012<($20m total) till expended

# Key NCPN Principle

NCPN establishes, administers, and manages
a **network** of collaborating
**clean plant centers** and programs specializing in
**plant pathogen identification and therapy**.

To do this, NCPN identifies and supports
a small but appropriate number of
stakeholder focused, nationally oriented
clean plant centers
that serve as centralized, cost effective hubs
to diagnose and 'clean' plant material and
provide foundational services
to industry and States.

# NCPN Activities – Primary and Related

*NCPN Supports the Primary Service Activities for Clean Plant Center Activities:*

- Diagnostics
- Therapeutics
- Foundation Plantings

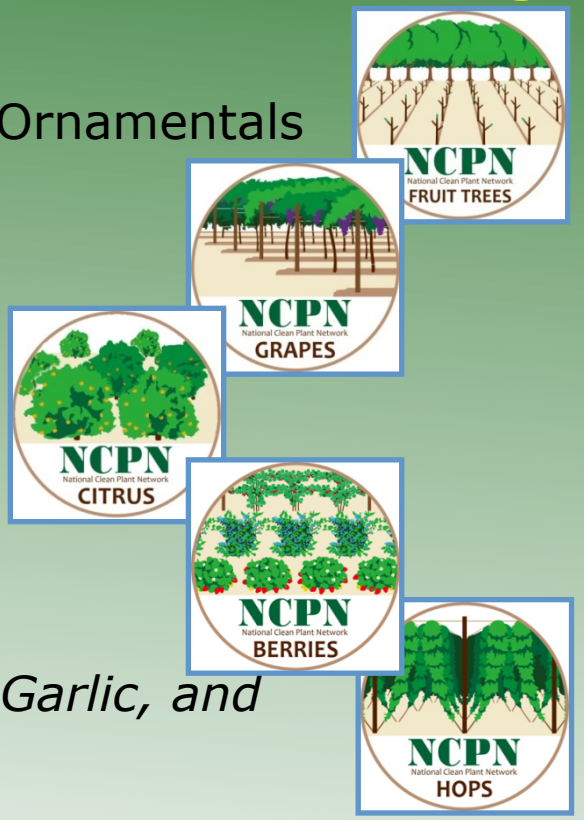
*NCPN also Supports Related Needs for Clean Plant Center Activities, including:*

- Network Governance
- Technology/Methods Development
- Equipment and Supplies
- Education/Outreach/Economics
- Facilities Upgrading
- Clean Nursery Activities

# *What crops does NCPN work on?*

***NCPN supports diagnostics and therapeutics on specialty crops that are generally restricted for entry into the USA, including:***

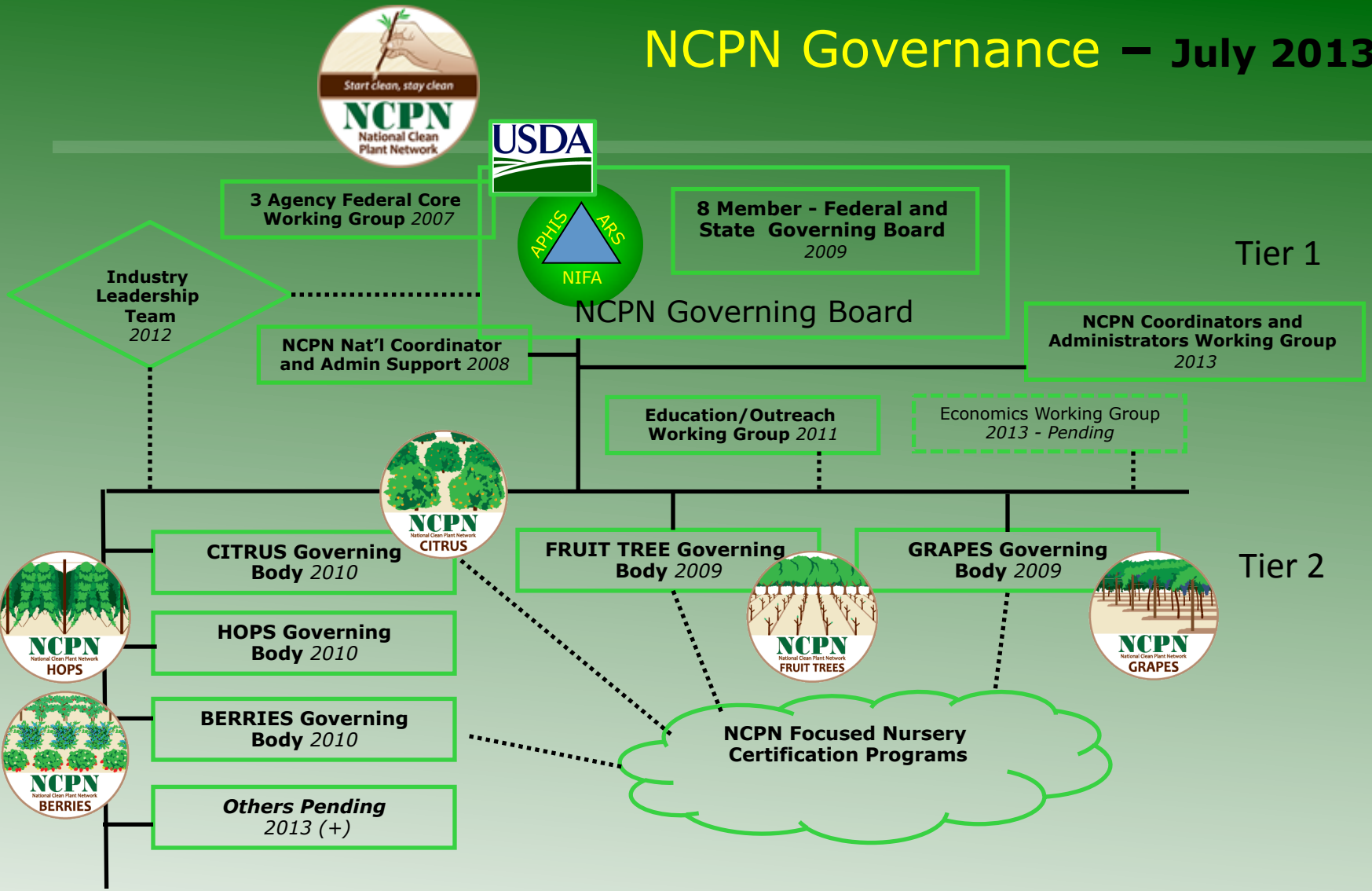
- ***Fruit Trees*** – Stone and Pome Fruits, including Ornamentals
- ***Grapes*** – Table, Raisin, and Wine Grapes
- ***Citrus*** – All Types
- ***Berries*** – *Fragaris, Rubus,* and *Vaccinium*
- ***Hops***




*Other crops considering NCPN entry include:*

*Sweet Potatoes, Roses, Olives, Table Potatoes, Garlic, and Ornamental Nursery Crops.*

# NCPN Governance – July 2013




***http://nationalcleanplantnetwork.org/***

# NCPN Tier 1 Governance and Support

## Core Working Group – Tier 1

- Tom Bewick – NIFA
- M. Watson (R. Johnson) – APHIS
- Gail Wisler - ARS

## NCPN Nat'l Coordination / Support

- Tammy Kolt - APHIS
- Erich Rudyj – APHIS

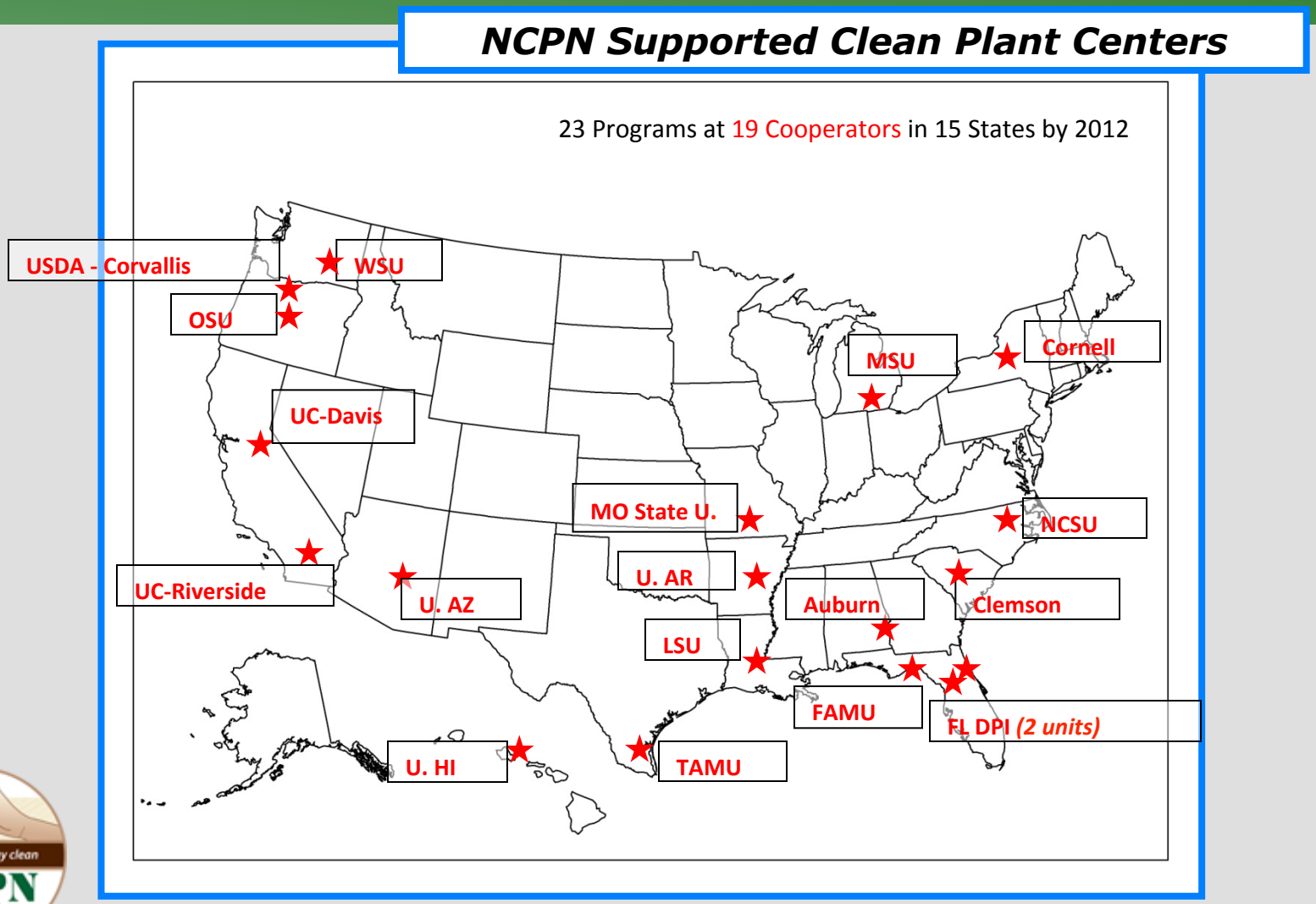
## National Governing Board - Tier 1 - Active

- Wayne Dixon – FL Dept. Agric.
- Joseph Postman – ARS/Germplasm
- Carl Schulz – NJ Dept. Agric.
- Tom Wessels– WA Dept. Agric.
- Geir Friisoe – MN Dept. of Agric.

## *National Governing Board - Tier 1 - Former*

- *Murali Bandla – APHIS (new position)*
- *Bill Dickerson – NC (retired)*
- *Ken Rauscher – MI (retired)*

# What Centers or Programs are in NCPN?




http://nationalcleanplantnetwork.org/

July 2013

# NCPN Program Funding History – FY 2008 - 2012

| Specialty Crop | | NCPN Funding 2008 | NCPN Funding 2009 | NCPN Funding 2010 | NCPN Funding 2011 | NCPN Funding 2012 | Total Funded |
|---|---|---|---|---|---|---|---|
| **Fruit Tree** | | $ 264,980 | $ 1,606,388 | $ 1,205, 232 | $ 985,336 | $1,011,526 | **$ 5,073,462** |
| **Grapes** | | $ 456,150 | $ 1,453,456 | $ 2,151,194 | $ 1,764,681 | $2,155,218 | **$ 7,980,699** |
| **Hops** | | N/A | N/A | $ 146,169 | $ 154,922 | $169,709 | **$ 470,800** |
| **Citrus** | | N/A | N/A | $ 1,337,889 | $ 1,349,891 | $1,170,444 | **$ 3,858,224** |
| **Berries** | | N/A | N/A | $ 742,887 | $ 577,601 | $639,019 | **$ 1,959,507** |
| **Crosscutting** | | N/A | N/A | N/A | $ 125,811 | $137,368 | **$ 263,179** |
| | | $ 721,130 | $ 3,059,844 | $ 5,583,371 | $ 4,958,242 | $5,283,284 | **$ 19,605,871** (2008-2012) |

Start clean, stay clean
NCPN
National Clean Plant Network

***http://nationalcleanplantnetwork.org/***

# NCPN Program Deliverables --- *So What?*

- 19 Laboratories >>> Supported and Improved
- 150,000 Lab Tests / 40,000 Field Tests on High-Value Accessions
- 2,000 Accessions subjected to Diagnostics
- 1,500 Accessions subjected to Therapy
- 1,250 Accessions added to Foundation Plantings
- 4,500 Accessions maintained in Quarantine Foundations

- **350,000 Clean Plant Units supplied to Industry for Increase**
  - **High Value, Pathogen Tested, Cleaned > Buds, Scions, Plantlets, Rootstocks**

**Fruit Trees** | **Grapes** | **Berries** | **Citrus** | **Hops**

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# NCPN Education/Outreach Programs

Specialty Crop Brochures

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July 2013

# NCPN Websites

*More information about NCPN is available at the following sites:*

***http://nationalcleanplantnetwork.org/***

***http://www.aphis.usda.gov/plant_health/ncpn/index.shtml***

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National Clean Plant Network

# Farm Bill 2013/2014 Update and NCPN

*Discussion*

# For more information:


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USDA, APHIS, PPQ

Science and Technology

4700 River Road, Room 4A.03.13

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or

E-Mail >>> NCPN@aphis.usda.gov


***http://nationalcleanplantnetwork.org/***

# THANK YOU

Start clean, stay clean
NCPN
National Clean Plant Network

***http://nationalcleanplantnetwork.org/***

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

# Virus-Host Interactions

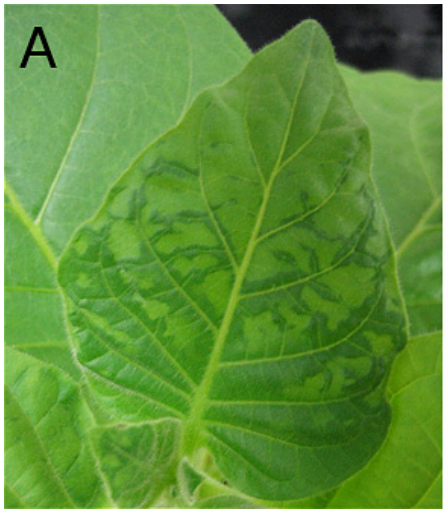
M. Elena Garcia, Ph.D.
Extension Fruit and Nut Specialist

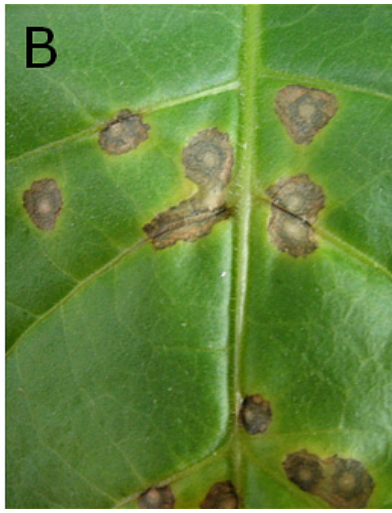
# Viruses

- Infectious pathogens
- Non-cellular
- Not active outside the cell
- Obligate parasites
  - Small piece of nucleic acids surrounded by a protein coat
  - Depend on host's machinery to reproduce
- Infect all types of living organisms, but most infect only one type of host

# History

- 1882- Adolf Mayer: Transmission of tobacco mosaic disease by plant extract
  - Infectious agent could not be isolated
- 1892- Dimitri Ivanofsky: Causative agent of tobacco not retained by filters
  - Cannot be cultivated in vitro
- 1898 Martinus Beijerinck: Causative agent can be cultivated in live tissue



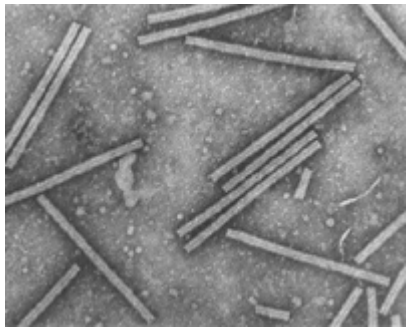



# Morphology

- Composed of two principal parts
  - Genome
    - Nucleic acid (NA)
  - Protective shell (coat)
    - Protein
    - Capsid
  - Some  viruses have an outer membrane
    - Lipoprotein membrane

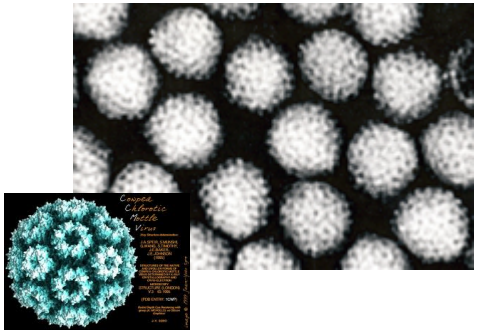
# Plant viruses

- Two protein shell types
  - Helical (elongated)
    - Rigid rods
    - Flexuous filaments
    - NA is highly ordered: helical confirmation (as proteinaceous capsid)
  - Icosahedral
    - Baccilliform
    - Two incomplete icosahedral
    - NA forms a partially ordered ball inside the capsid

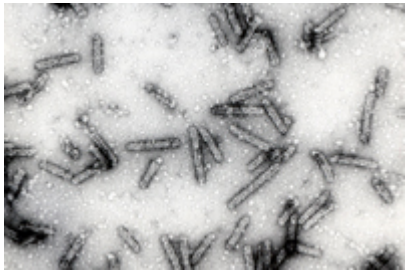


Rigid rods

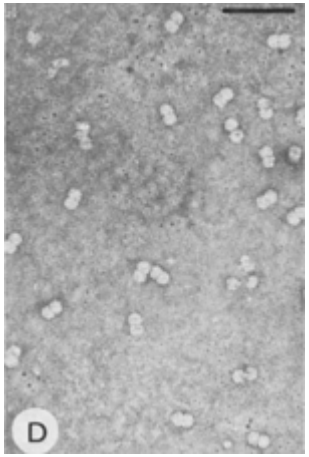
Flexuous filaments



icosahedral



bacilliform




Twin virions composed of two joined incomplete icosahedra

# Virus Types

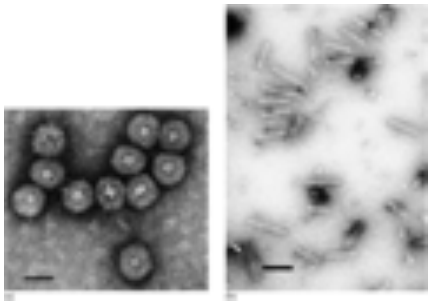
- Helical- the NA is highly ordered: it assumes the same helical conformation as the proteinaceous capsid.
- Icosahedral the genomic NA forms a partially ordered ball inside the proteinaceous capsid.
- Self-assembly in a test tube if the NA and protein subunits are incubated under proper conditions.

# Genome Types

- Single stranded (ss), positive-sense RNA
  - Bromoviridae, Letroviridae
    - Cucumovirus
- Single stranded negative-sense RNA
  - Bunyaviridae
- Double-stranded RNA
  - Reoviridae

# Genome Types

- dsDNA
  - Pararetroviruses
  - Caulimoviridae
  - Replication involves and RNA intermediate
- ssDNA
  - Geminiviridae
  - dsDNA intermidiate

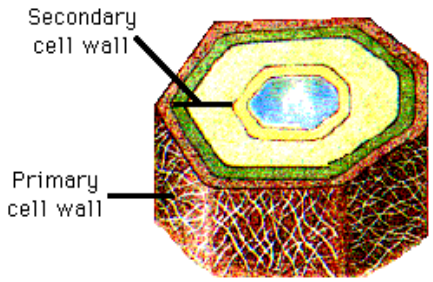


# Systematics

- First plant virus was purified and classified in mid 1930's
- Virus named after host plant and symptom
  - Tobacco mosaic virus- TMV
- Largest subdivisions
  - Chemical form of genome
- Genera and families derived from an important within the family
  - Brome mosaic virus

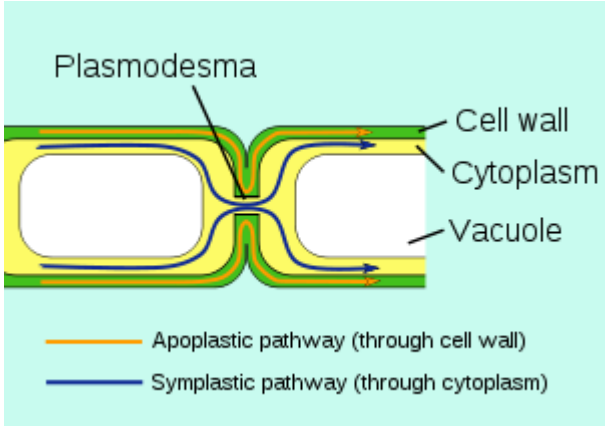
# Plant Viruses- General

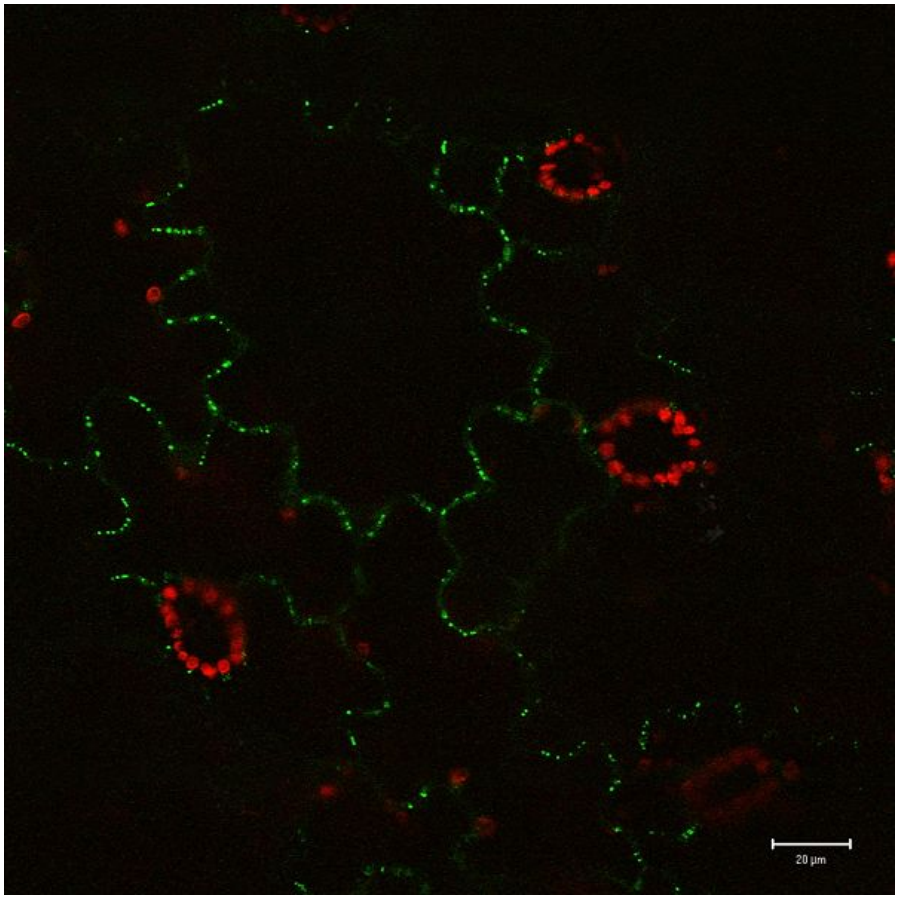
- Approximately 1000 different plant viruses
- Losses due to plant viruses ~ $ 6 x$10^{10}$
- Do not have specific cellular receptors to enter cell
  - Relay on mechanical breaches of cell's integrity
  - Vectors
  - Mechanical damage



# Entrance

- Many plant viruses have evolved specialized movement proteins which modify the plasmodesmata.
  - One of the best known examples of this is the 30k protein of tobacco mosaic virus (TMV). This protein is expressed from a sub-genomic mRNA and its function is to modify plasmodesmata causing genomic RNA coated with 30k protein to be transported from the infected cell to neighboring cells.


Plasmodesma
Cell wall
Cytoplasm
Vacuole
Apoplastic pathway (through cell wall)
Symplastic pathway (through cytoplasm)


20 µm

# Transmission of Plant Viruses

- Seeds
- Vegetative propagation
- Mechanical
- Vectors
  - Bacteria
  - Fungi
  - Nematodes
  - Arthropods
  - Arachnids- mites

# Reproduction

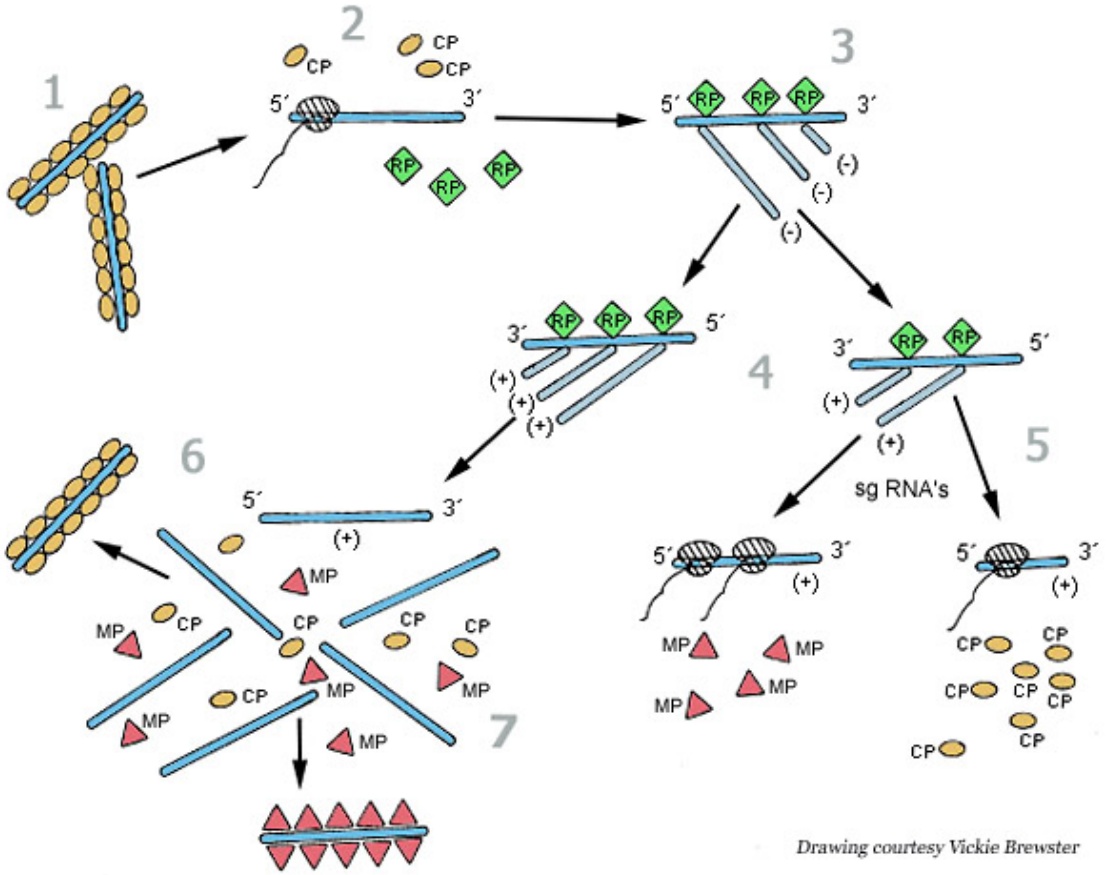
- Passive entrance into cytoplasm
- Removal of protein coat
- Cell mediated expression of viral genome
  - Transcription apparatus (DNA viruses)
  - Translation apparatus (all viruses)
    - Proteins
- DNA viruses must be transported to the nucleus

- Viron enters cytoplasm passively
- Partial or complete removal of coat protein in the cytoplasm
- Mediation of expression of viral genome by providing the transcription apparatus and translation apparatus

# Protein

Three types of proteins

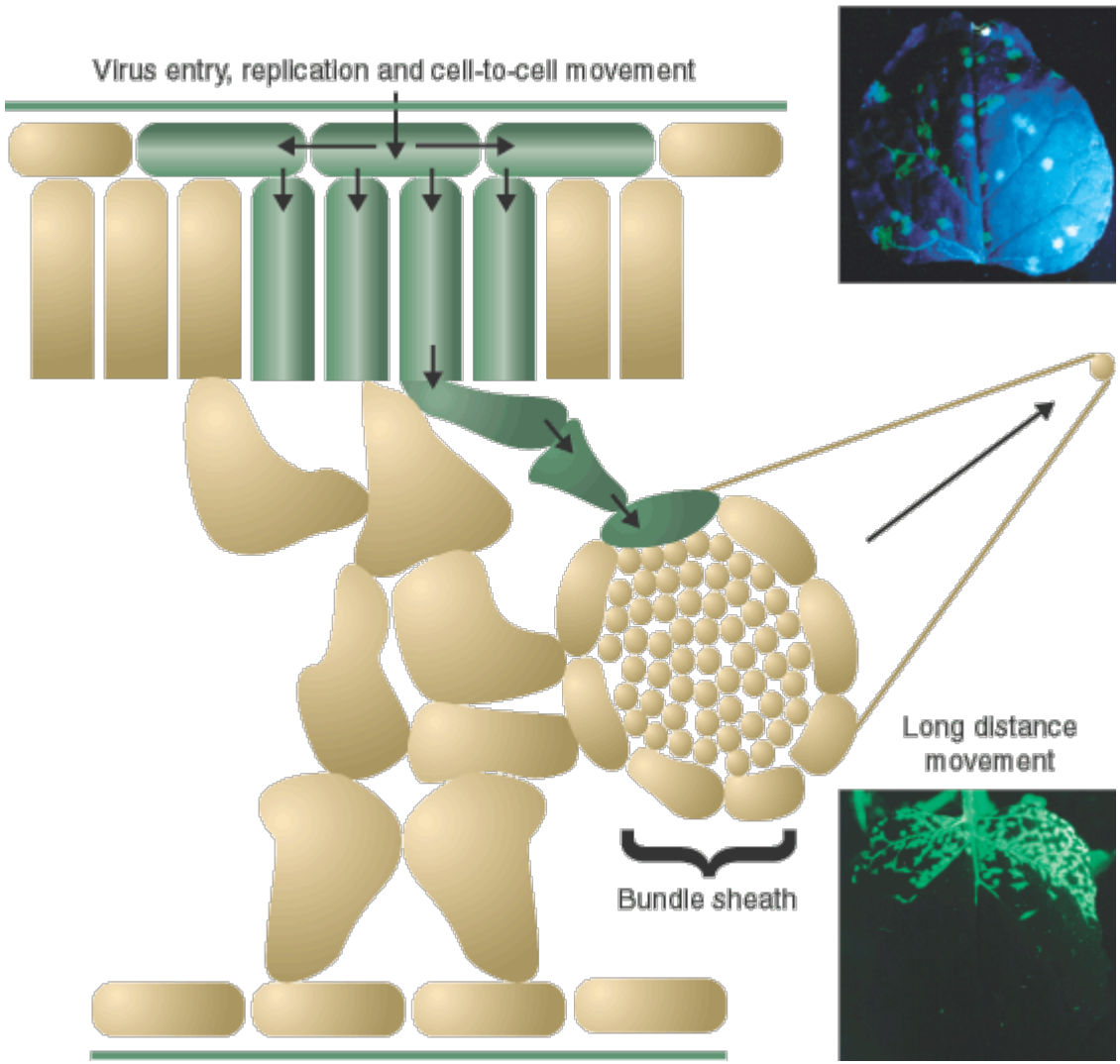
- Replication proteins for nucleic acid production
- Structural protein for protein shell
- Movement protein for transport between cells

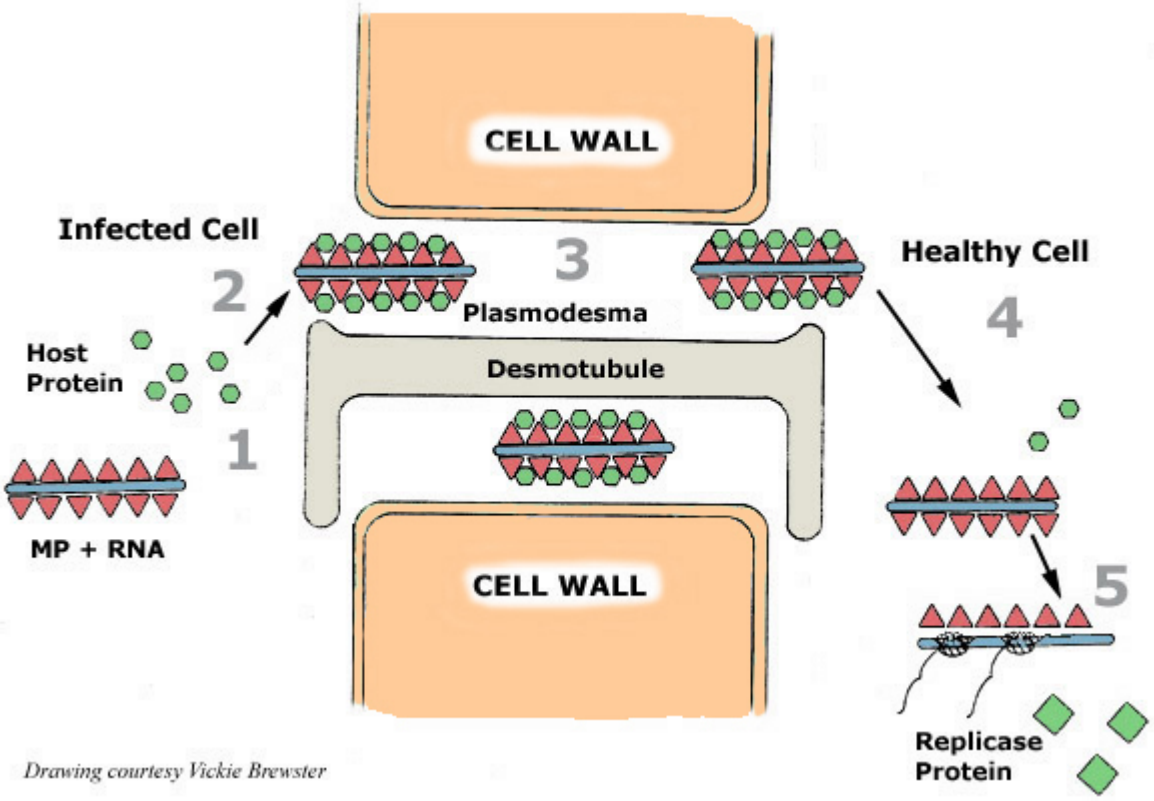



The replication cycle of *Tobacco mosaic virus* (TMV). TMV enters a wounded plant cell to begin the replication cycle [1]. As the coat protein (CP) molecules are stripped away from the RNA [2], host ribosomes begin to translate the two replicase-associated proteins. The replicase proteins (RP) are used to generate a negative-sense (- sense) RNA template from the virus RNA [3]. This - sense RNA is, in turn, used to generate both full-length positive-sense (+ sense) TMV RNA [4] and the + sense subgenomic RNAs (sgRNAs) [5] that are used to express the movement protein (MP) and CP. The + sense TMV RNA is either encapsidated by the CP to form new TMV particles [6] or wrapped with MP [7] to allow it to move to an adjacent cell for another round of replication (Drawing courtesy Vickie Brewster: from K.-B. G. Scholthof 2000).

# Movement in Cell

- Plasmodesmata
- Modification to plasmodesmata
  - Process may take from one to a few hours
  - TMV
- Successful colonization
  - Enter the vascular system
  - Phloem sieve elements
  - Photosynthates flow
- Back into cells


Virus entry, replication and cell-to-cell movement
Long distance movement
Bundle sheath




Cell-to-cell movement of *Tobacco mosaic virus* (TMV). In this model, the movement protein (MP) binds to the viral RNA [1]. Host proteins and/or other virus-encoded proteins may be included in the MP-complex [2]. The MP-complex then moves from cell-to-cell through the plasmodesmata [3]. When the complex is localized to a new cell, the MP (and any host proteins) are presumably released from the TMV RNA [4], allowing for translation of the genomic RNA to express the replicase proteins and to initiate a new round of replication [5]. (Drawing courtesy Vickie Brewster: from K.-B. G. Scholthof 2000).

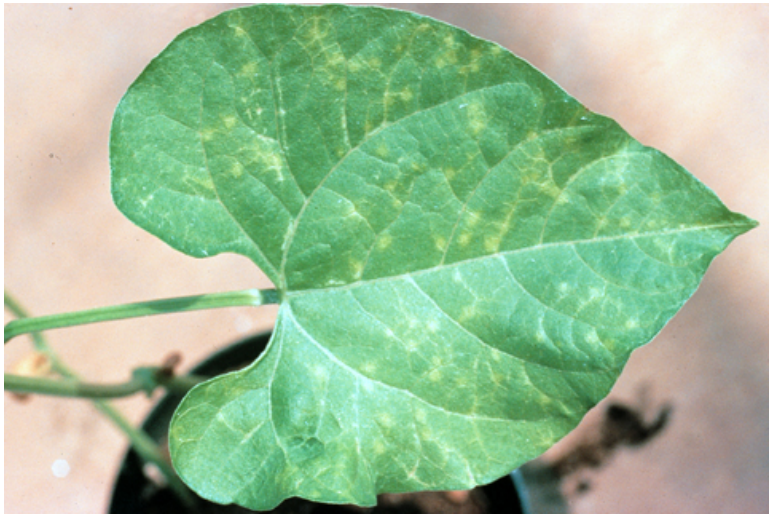
# Morphological Symptoms

- Symptoms range from symptomless conditions to severe disease and plant death
  - Chlorosis and necrosis- direct damage due to virus replication
  - Hypoplasia- localized retarded growth-leads to mosaicism
  - Hyperplasia- excessive cell division- distorted areas

# Chlorosis

- Symptoms associated with disruption of chloroplast structure, function, and/or development
- Yellowed areas in expanded leaves that developed before infection
- Mosaic pattern in leaves that developed after infection

# Chlorosis



*Peanut mottle virus* in bean, chlorotic lesions on leaf
(Courtesy C.W. Kuhn)

*Squash leaf curl virus* in summer squash, vein banding symptom
(Courtesy R. Providenti)

Rubus yellow net virus

# Hyperplasia

- -Excessive cell division- distorted areas

# Necrosis

- Hypersensitive response (HR)
- Different response from bacterial and fungi induced HR
  - Plant recognizes and respond to intracellular gene product
  - Mutations frequently change the phenotype from virulence to avirulence

# Hypersensitive Response

- Synthesis of new proteins- pathogenesis related
- Increased production of cell wall phenolics
- Release of active oxygen species
- Production of phytoalexins
- Accumulation of salicylic acid

# Plant Resistance

Resistance different levels and various mechanisms

- Immunity- complete resistance
  - Prevents viral replication
  - Non-host resistance
  - Extreme resistance
- Subliminal infection
  - Replication, but virus prevented from moving outside the cell

# Plant Resistance

- Hypersensitive response
  - Kills cells within infected area
  - Prevents spreading of infection
  - Systemic acquired resistance (SAR)
    - Pathogen non-specific resistance

# Plant-Virus Interactions

Plants possess active and passive means to prevent infection

Passive

- Failure to host factors necessary for virus reproduction

Active

- Detection and destruction of virus-infected cells
  - Resistance genes
  -

# Symptoms

- Symptoms range from symptomless conditions to severe disease and plant death
  - Chlorosis and necrosis- direct damage due to virus replication
  - Hypoplasia- localized retarded growth-leads to mosaicism
  - Hyperplasia- excessive cell division- distorted areas

# Chlorosis

- Symptoms associated with disruption of chloroplast structure, function, and/or development
- The mosaic pattern that develops in a leaf appears not to depend upon its position, but rather on the stage of leaf development at the time of viral infection
  - Yellowed areas in expanded leaves that developed before infection
  - Mosaic pattern in leaves that developed after infection

# Necrosis

1.- Hypersensitive response (HR)- Usually controlled by single dominant gene

- Different response from bacterial and fungi induced HR
  - Plant recognizes and respond to intracellular gene product
  - Mutations frequently change the phenotype from virulence to avirulence

# Hypersensitive Response

- Synthesis of new proteins- pathogenesis related
- Increased production of cell wall phenolics
- Release of active oxygen species
- Production of phytoalexins
- Accumulation of salicylic acid

# Necrosis

2.- Slower-forming local necrosis that is not related to a single host gene

3.- Various degrees of systemic necrosis

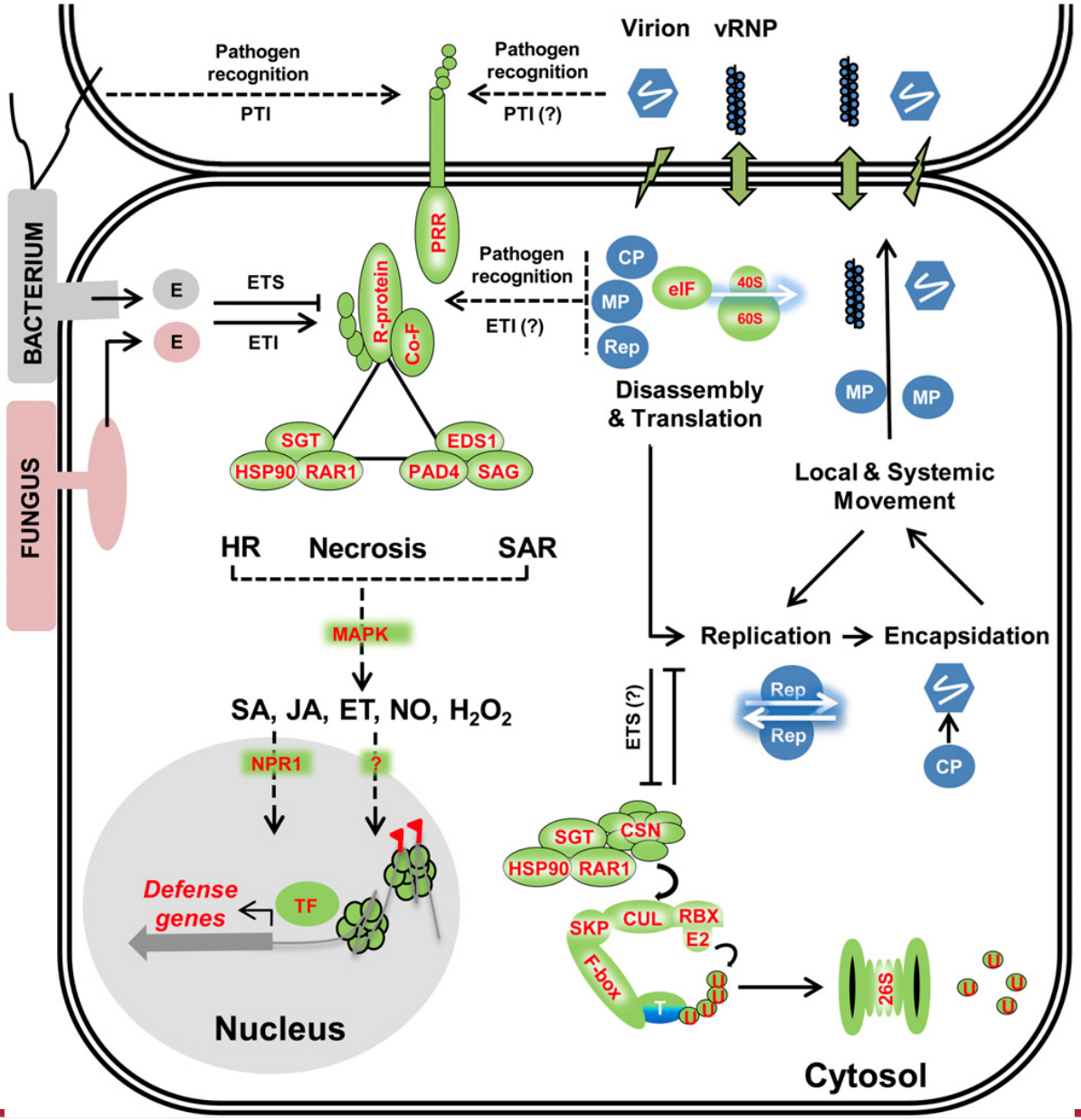
- Not clear if responses are distinctly different or variations of the same process
- Race between how fast the virus replicates and moves and how fast the HR occurs

# Systemic Acquired Resistance (SAR)

- Systemic acquired resistance (SAR) is a plant immune response to pathogen attack.
- SAR is analogous to the innate immune system found in animals, and there is evidence that SAR in plants and innate immunity in animals may be evolutionarily conserved.

# SAR

- Initiated through a local interaction among pattern-recognition receptors to recognize conserved microbial signatures and results in accumulation of phytohormones such as salicylic acid and jasmonic acid, ethylene, $H_2O_2$, at the distant tissues






- Plant Immune Responses Against Viruses: How Does a Virus Cause Disease. Kranthi K. Mandadi and Karen-Beth G. Scholthof1

# Summary

- Plant viruses are diverse and unusual groups of plant pathogens that infect and cause disease in many crop plants.
  - Pathogens depend on the normal cellular machinery of their plant host for reproduction, it is difficult to eliminate them without damaging the host plant.
  - Management strategies for diseases caused by plant viruses and viroids are directed at preventing infection of the plant.

# Questions?

# Thank You

# Arthropod vector management strategies in small fruits

Donn Johnson, University of Arkansas

Hannah Burrack, North Carolina State University

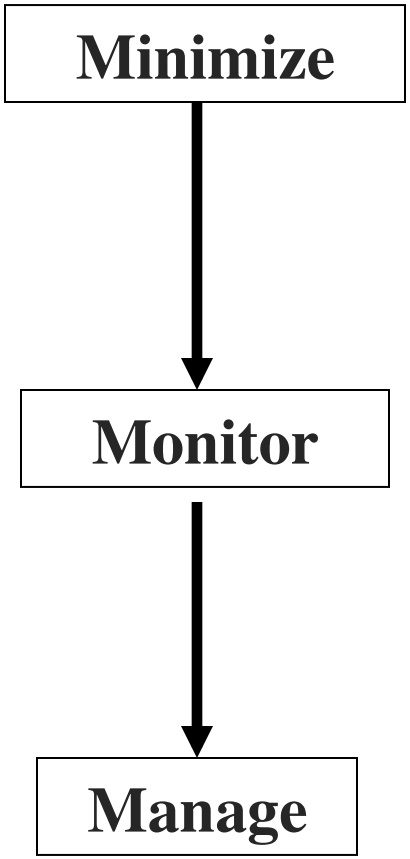
# Virus transmission review

**Virus transmission** involves three time periods - virus acquisition, latent, and inoculation

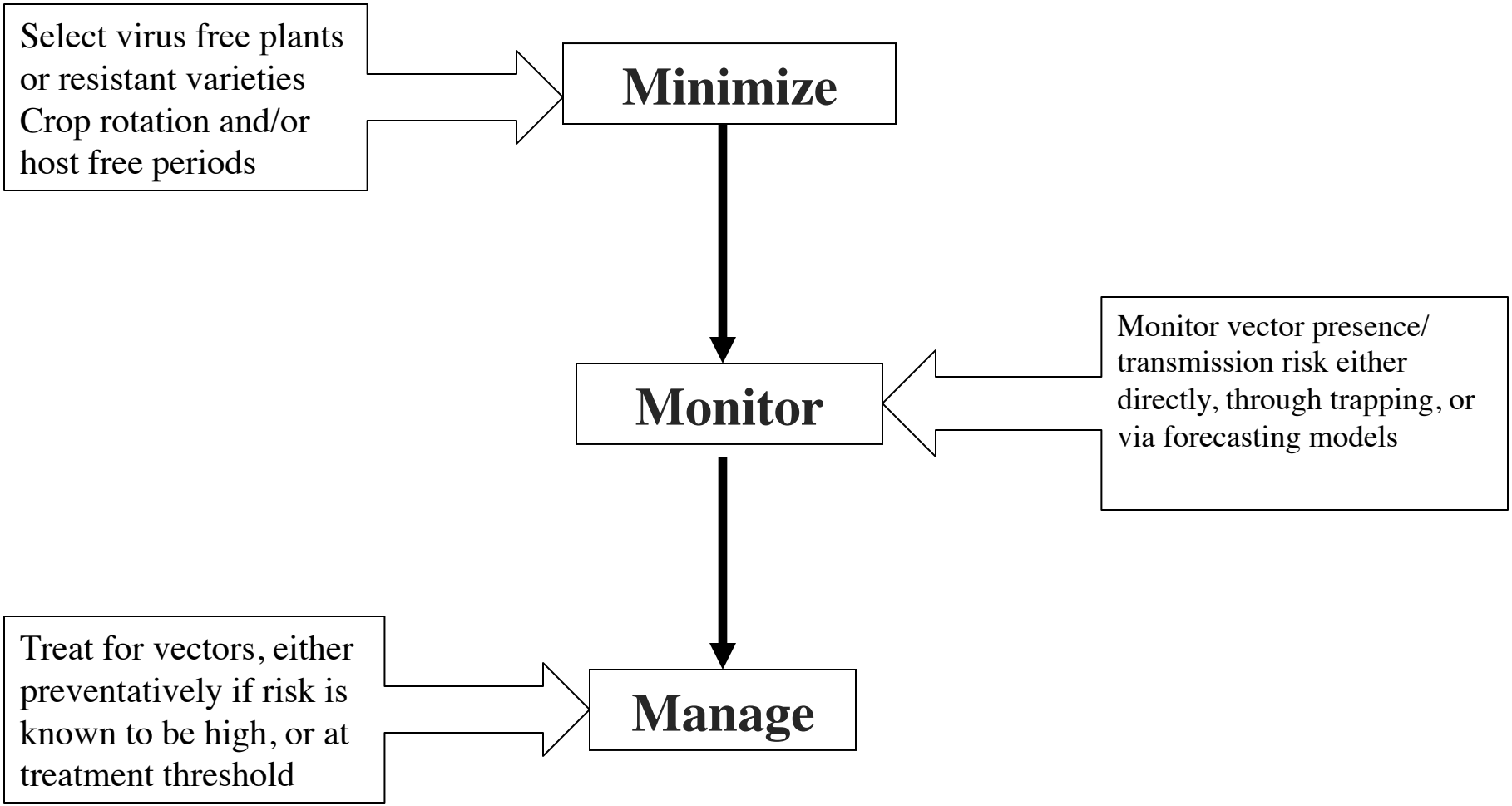
**Types of transmission:**

1. **Non-persistent (mechanical)** – very short probing tine to acquire virus, virus sticks to mouthpart and next probing it transmits virus to healthy plant (no latency period) – hard to control
2. **Semi-persistent –** short acquisition and inoculation period, no latent period and does not retain the virus after it molts
3. **Persistent** - up to 1 week acquisition period, 1 week latent period (propagate virus in vector), then can inoculate virus to plant and retains virus after molt

# Integrated Pest Management



# Integrated Pest Management



# Know Virus Vector & Biology

# Virus transmission case studies

Aphid vectored viruses in southeastern strawberries, 2013

Stunted strawberry plants observed in spring 2013 in FL, GA, SC, NC, and VA

# Virus transmission case studies

Aphid vectored viruses in southeastern strawberries, 2013

Determined that plants were infected with:

SMYEV: Persistent, circulatively transmitted virus spread by *Chaetosiphon fraegolii*, *C. thomasi*, and *C. jacobi*

SMoV: Semi-persistently transmitted by *C. fraegaefolii*, several other *Chsaetosiphon* species, and *Aphis gossypii*

# Virus transmission case studies

Recommended management strategies

For nurseries:

Implement a host free period

# Virus transmission case studies

Recommended management strategies

For nurseries:

Aggressively scout for vectors and consider using systemic insecticides preventatively in high risk years (mild winters, nearby non crop hosts)

# Virus transmission case studies

Recommended management strategies

For growers:

Acquire clean plants

Scout incoming transplants for aphids

Sample for aphids during growing season (10 newly expanded leaflets per acre)

Insecticide treatment is only necessary if viruses have been identified or aphids are at treatment threshold (10+ per leaflet)

# Virus transmission case studies

When scouting, distinguish between live aphids, dead aphids, and parasitized aphids

# Virus Control in Strawberry

1. Start with clean plants
2. Know the high risk viruses for your area
3. Know the risks associated with planting site
4. Isolation from other fields if practical
5. Implement vector control as needed
6. Be sure the problem is viral before taking any action

**(Excerpts from 2011 webinar by Bob Martin, USDA)**

# Virus survey in North America

In progress

| Virus | Symptomless Plants - CA | Declining Plants – CA | Pacific Northwest | Mid west | South east | North east |
|---|---|---|---|---|---|---|
| BPYV | 6/104 | 15/24 | 0/90 | 2/80 | 3/27 | 13/23 |
| FClLV | 0/104 | 0/24 | 0/90 | 5/80 | 0/27 | 4/23 |
| SCV | 7/104 | 5/24 | 33/90 | 3/80 | 2/27 | 0/23 |
| SMoV | 8/104 | 6/24 | 62/90 | 6/80 | 2/27 | 6/23 |
| SMYEV | 12/104 | 8/24 | 48/90 | 5/80 | 2/27 | 4/23 |
| SNSV | 5/104 | 0/24 | 3/90 | 1/80 | 1/27 | 1/23 |
| SPaV | 11/104 | 18/24 | 1/90 | 19/80 | 3/27 | 17/23 |
| SVBV | 11/104 | 4/24 | 43/90 | 3/80 | 2/27 | 1/23 |



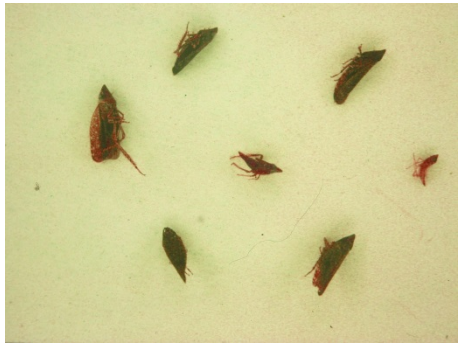
(Excerpts from 2011 webinar by Bob Martin, USDA)

# Whitefly vectored viruses in strawberries (2011 webinar by Bob Martin, USDA)

- Whiteflies vector
  - Strawberry pallidosis associated virus (SPaV)
    - Hosts: Malvaceae (mallow), Cucurbitaceae, Chenopodiaceae (relatives of Lamb's Quarters)
  - Beet pseudo-yellow virus (BPYV)
    - Hosts: Urtica (nettle) and Malva (mallow)
- Symptomatic plants often have a combination of whitefly and aphid vectored viruses in the western US

# Pathogen case studies
# Blueberry stunt

- Plant stunted with downward curled, reddish leaves, sour fruit
- Sharpnosed leafhopper vectors phytoplasma
- Or, spread from cuttings taken from stunt-infected nursery plants



- Adult is 3/16”, pointed head, creamy spots on wings
- Check yellow sticky traps - May, July & Oct.

# Blueberry Stunt management

- **Scouting, eradication or roguing** of any plant showing stunt symptoms but spray infected bush with an insecticide before removal to kill any leafhoppers that may be present.
- **Vector control** - treatment timed to peaks in the insect vector population (May, July and October).
- **Stunt-free propagation** of cuttings

# Pathogen case studies
# Pierce's Disease (PD) or Scorch

- Marginal leaf burn, then leaf blades fall off and petioles remain
- Leafhoppers vector PD bacteria by single probing of grapes or blueberries
- Monitor using yellow sticky cards at perimeter

# PD Cultural Control

- Isolate vineyard from wooded areas
- Keep ditch banks and other weedy strips near vineyard mowed
- Mow vineyard regularly and minimize broadleaf weeds in the vineyard
- If a couple shoots have symptoms of leaf blades dying and/or petioles only remaining on shoot, prune off those infected shoots at the cordon.
- If most shoots on vine are showing above symptoms, dig up the vine and destroy.

# PD Management

(by Dr. Turner Sutton at NCSU)

- Bud break to 15 May: apply soil drench of Clutch, Venom or Admire Pro for systemic control of vectors and apply Surround (kaolin clay) to white-wash perimeter vines (camouflage vines) to reduce dispersal into vineyard and suppress leafhopper feeding
- 15 May to 15 June: apply Danitol and reapply 15 days later
- 15 June: apply Assail which will give you 21 to 30 days protection

# Blackberry Virus Project

Systematic virus infections can significantly reduce the productive lifespan of caneberry plantings

Multiple viral infections are necessary for disease symptoms

Different viruses are likely responsible for infections in raspberry vs. blackberry and in different regions

# Objectives

Identify **what viruses are present** in caneberries and **which combination of viruses result in disease**

Determine **when plants become infected** with important viruses

Identify **what vectors are responsible** for virus transmission

Development **management strategies** for important virus vectors

# Methods

## Virus transmission

# Methods

## Insect monitoring

**25-30 of each trap type placed near the sentinel trap**
**Checked and changed biweekly**

# Methods

## Insect monitoring

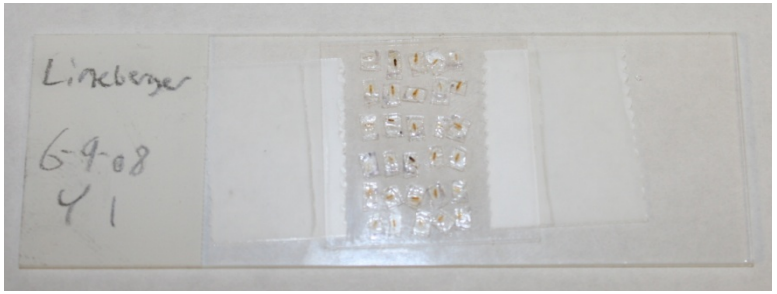
AM (yellow sticky) traps

*Targets: Leafhoppers, aphids, adult whiteflies, beneficial insects*

# Methods

## Insect monitoring

Thrips traps
*Targets: Adult thrips*



# Methods

## Insect monitoring

Blue sticky traps

*Targets: Leafhoppers, aphids, adult whiteflies, some adult thrips*

# Methods

## Insect monitoring

Pan traps

*Targets: Aphids, adult thrips, beneficial insects*

# Methods

## Insect monitoring

Direct observations
Fruit & foliage samples

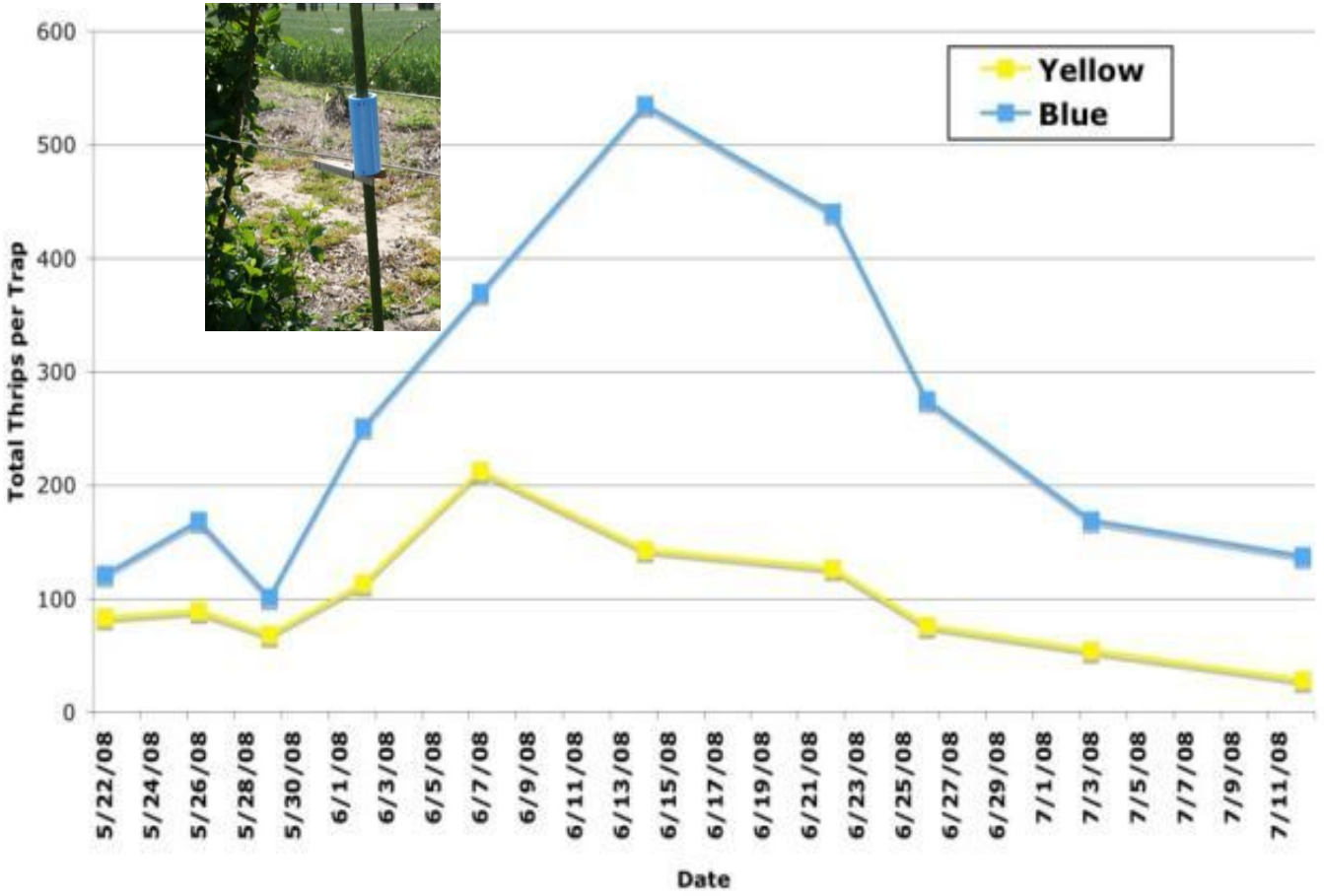
*Targets: Larval thrips, immature whiteflies, eriophyid mites, tetranychid mites, beneficial insects*

# Trapping methods



- Monitoring efficacy blue sticky cylinder and yellow sticky card – Thrips in NC, 2008 & 2009

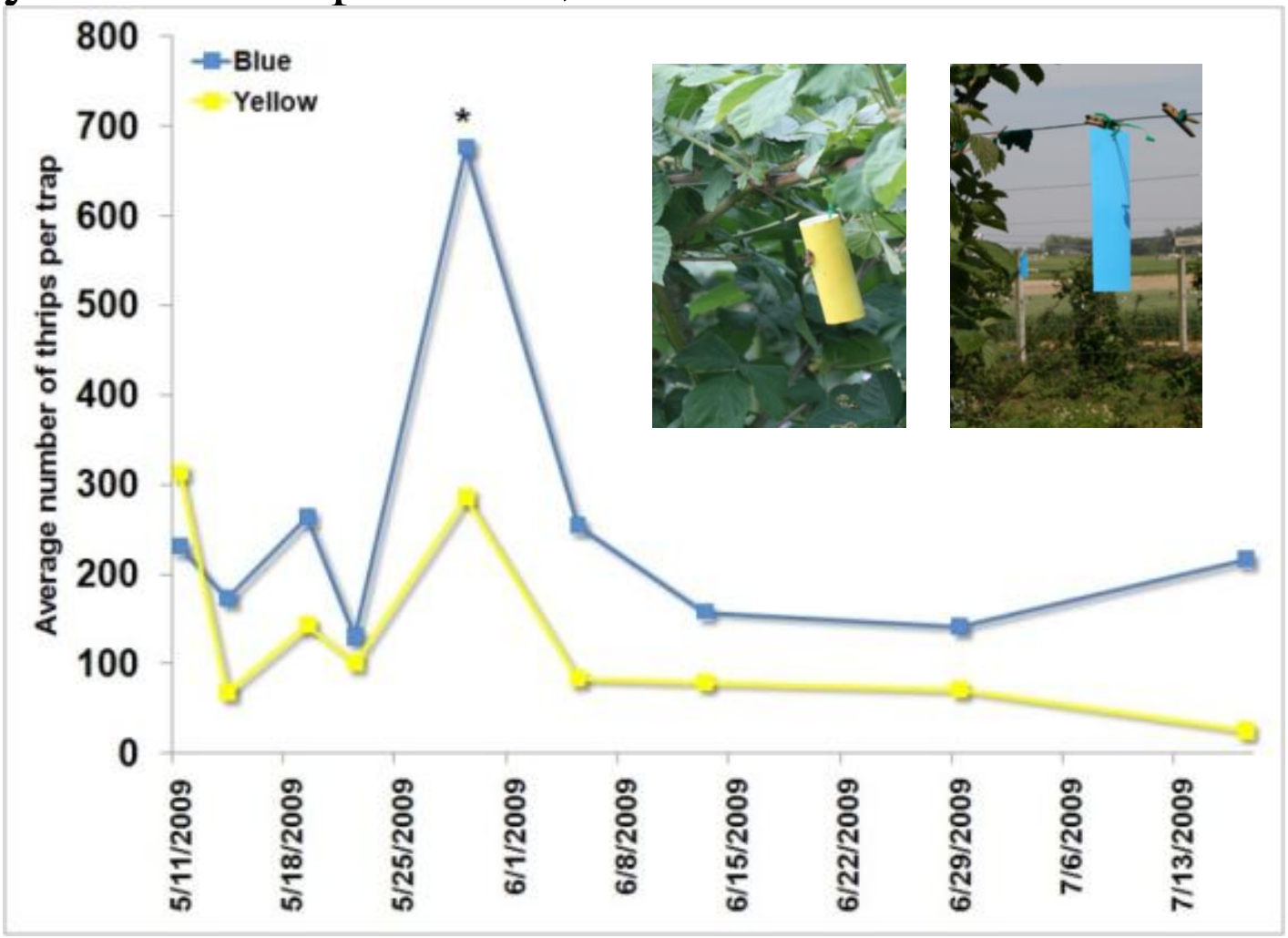




# Trapping methods



- Monitoring efficacy yellow sticky cylinder and blue sticky card – Thrips in NC, 2008 & 2009



# Trapping methods

- Monitoring efficacy – Potential vector groups by trap type in AR, 2010

| | % of total seasonal catch | | | | | |
|---|---|---|---|---|---|---|
| **Family** | **AM Trap** | **Sweep net** | **Blue sticky card** | **Yellow thrips traps** | **Blossoms** | **Yellow pan traps** |
| Cicadellidae | 25.8 | 85.3 | 85.1 | 1.6 | 0 | 7.7 |
| Aphididae | 10.6 | 0.7 | 0.0 | 3.6 | 7.1 | 20.5 |
| Aleyrodidae | 0.1 | 0 | 0.8 | 1.3 | 0 | 0 |
| Delphacidae | 0.14 | 13.5 | | | | |
| Cercopidae | 0.09 | 0.1 | | | | |
| Membracidae | 0.4 | 0.3 | | | | |
| Thripidae | 62.8 | 0 | 8.3 | 94.8 | 92.9 | 71.8 |

# Trapping methods

- Monitoring efficacy – Potential vector groups by trap type in NC, 2010

| Family | % of total seasonal catch | | | | |
|---|---|---|---|---|---|
| | AM Traps | Foliage observation | Blue sticky card | Blossoms | Yellow pan traps |
| Cicadellidae, Delphacidae, Cercopidae, and Membracidae | 8.70 | 3.75 | 1.30 | 0.00 | 3.98 |
| Aphididae | 1.51 | 0.00 | 0.77 | 0.12 | 0.54 |
| Aleyrodidae | 9.80 | 58.40 | 0.00 | 0.00 | 0.80 |
| Thripidae | 79.20 | 37.20 | 97.00 | 80.60 | 56.80 |
| Tetranychidae | | 0.63 | | 15.90 | |
| Psyllidae | 0.74 | | 0.40 | | |
| Eriophyiidae | | | | 3.31 | |

# Trapping methods

- Thrips traps attract numerous other potential vector groups

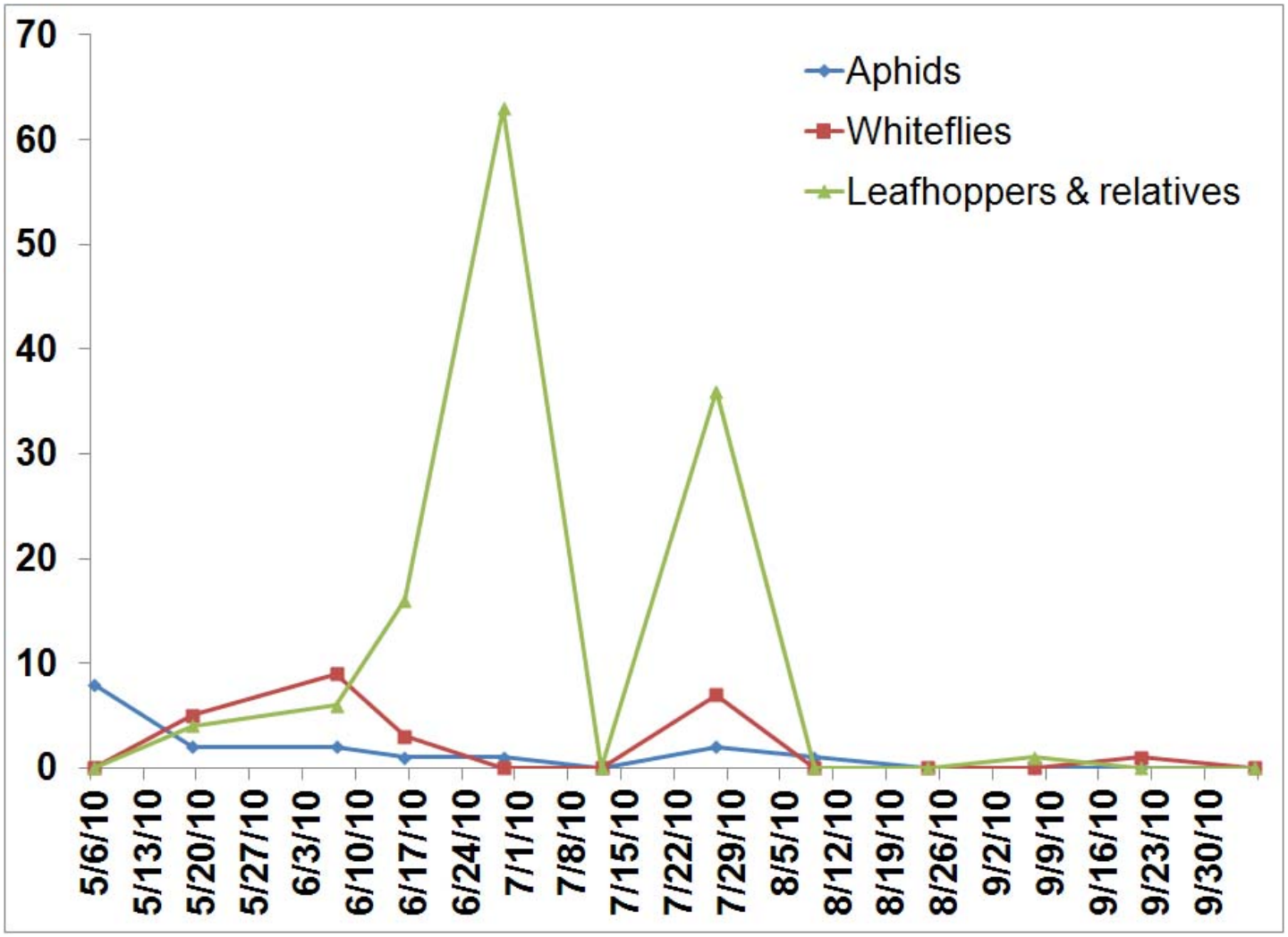
# Potential vector phenology

- Considered by trapping method – thrips considered separately (using 2008 & 2009 data)

| Family | % of total seasonal catch | | | | |
|---|---|---|---|---|---|
| | AM Traps | Foliage observation | Blue sticky card | Blossoms | Yellow pan traps |
| Cicadellidae, Delphacidae, Cercopidae, and Membracidae | 8.70 | 3.75 | 1.30 | 0.00 | 3.98 |
| Aphididae | 1.51 | 0.00 | 0.77 | 0.12 | 0.54 |
| Aleyrodidae | 9.80 | 58.40 | 0.00 | 0.00 | 0.80 |
| **Thripidae** | **79.20** | **37.20** | **97.00** | **80.60** | **56.80** |
| Tetranychidae | | 0.63 | | 15.90 | |
| Psyllidae | 0.74 | | 0.40 | | |
| Eriophyiidae | | | | 3.31 | |

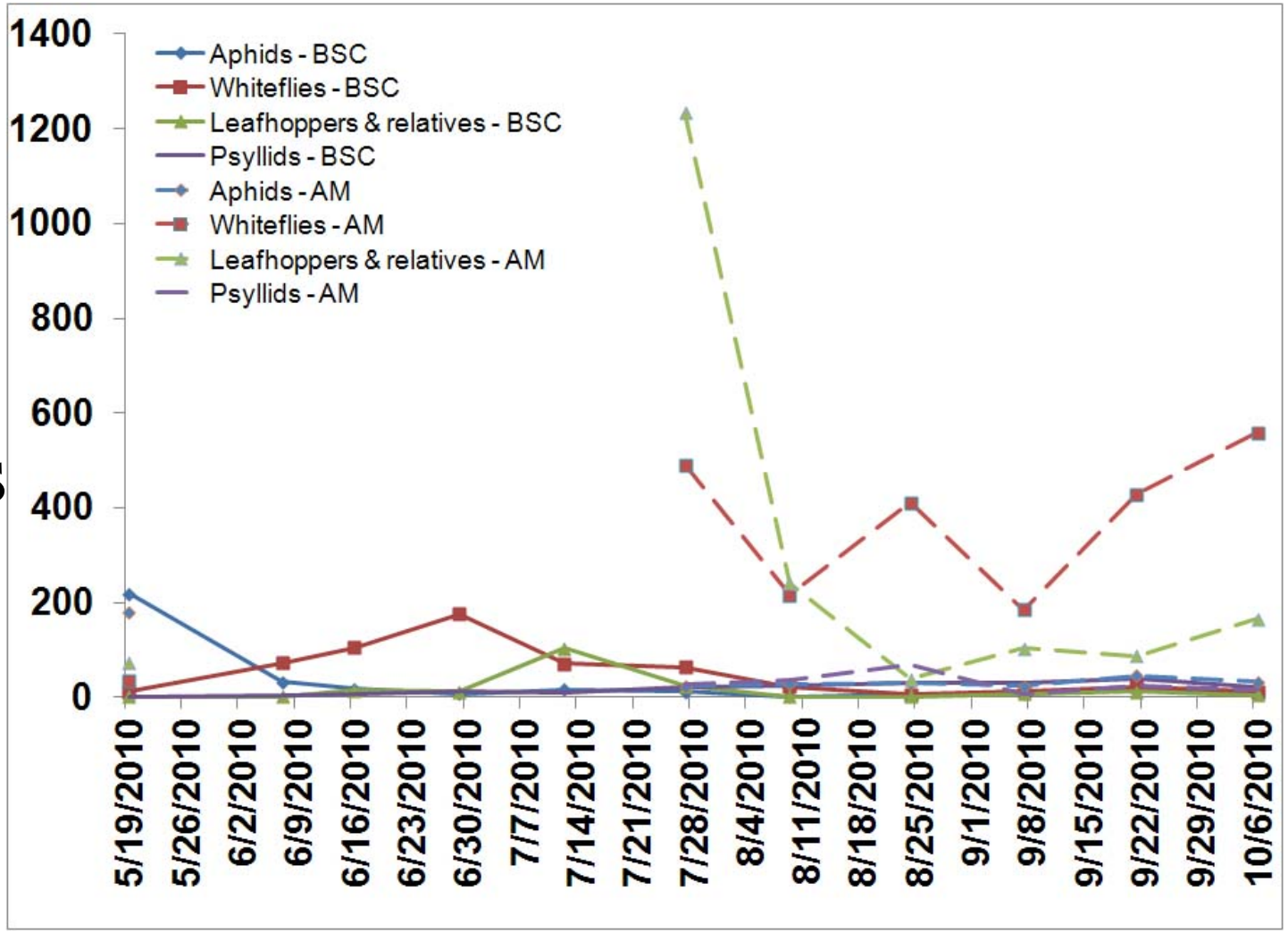
# Potential vector phenology

Pan traps



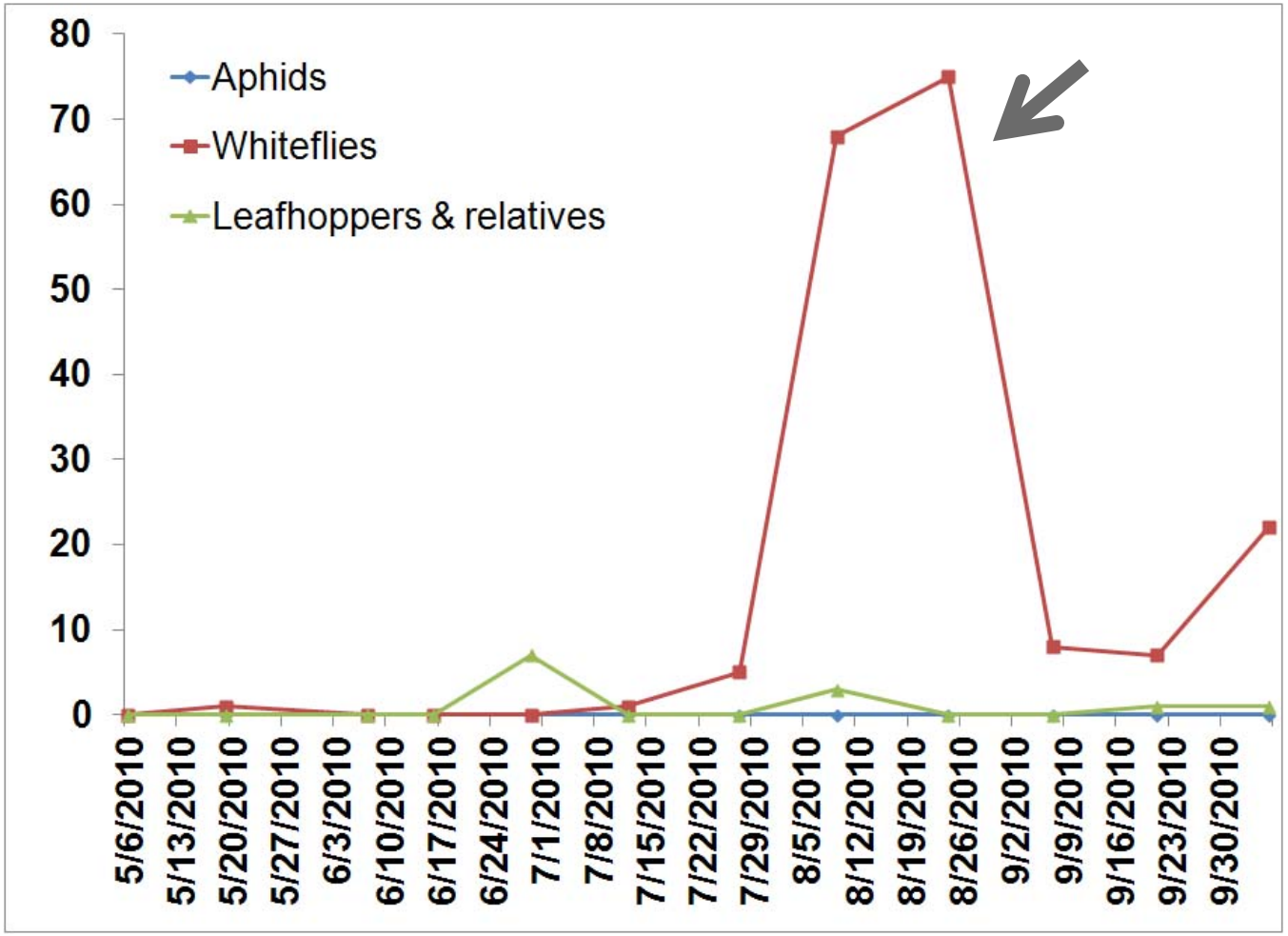
# Potential vector phenology

Blue sticky & yellow sticky cards



# Potential vector phenology

Foliage samples



# Potential vector phenology

Bud, blossom, fruit samples

| Date | Thrips | Whiteflies | Aphids | Eriophyid mites |
|---|---|---|---|---|
| 5/6/2010 | 754 | 0 | 2 | 0 |
| 5/19/2010 | 118 | 0 | 0 | 0 |
| 6/7/2010 | 148 | 0 | 0 | 0 |
| 6/16/2010 | 93 | 0 | 0 | 1 |
| 6/29/2010 | 121 | 0 | 0 | 29 |
| 7/12/2010 | 107 | 0 | 0 | 25 |