

NATIONAL PLANT DIAGNOSTIC NETWORK **VIRUS DIAGNOSTICS WORKSHOP** MARCH 2016

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WHY DO WE NEED TO DETECT, IDENTIFY, AND DIFFERENTIATE PLANT VIRUSES?

- VIRUS DISEASES AFFECT YIELD AND QUALITY
- VIRUS-INFECTED PLANTS CANNOT BE 'CURED' BY APPLICATION OF AGROCHEMICALS
- NEED TO KNOW WHICH VIRUS(ES) ARE PRESENT TO CONTROL VECTORS AND PREVENT FURTHER SPREAD
- NEED TO TRACK INFECTION SOURCES TO BREAK INFECTION CYCLE
- VEGETATIVELY-PROPAGATED CROPS CARRY VIRUSES OVER TO NEXT CROP CYCLE

BIOASSAY AND INDICATOR PLANTS

MECHANICAL INOCULATION, GRAFT TRANSMISSION, VECTORED TRANSMISSION

EFFECTS OF TEMPERATURE, LIGHT, BUFFERS, ETC.

COMMONLY USED BIOASSAY PLANTS

- AIZOACEAE
Tetragonia tetragonioides
- AMARANTHACEAE
Gomphrena globosa
- CHENOPODIACEAE
Chenopodium quinoa
C. amaranticolor
SPINACHIA OLERACEA
- CUCURBITACEAE
Cucumis sativus
- POACEAE (GRAMINEAE)
Hordeum vulgare
Triticum aestivum
- FABACEAE
Phaseolus vulgaris
Pisum sativum
Vigna unguiculata
- SOLANACEAE
Capsicum annum
Nicotiana benthamiana
N. clevelandii
N. edwardsonii
N. glutinosa
N. tabacum
Petunia hybrida
Solanum lycopersicum

Note: Depending on the virus, the bioassay plants used and symptoms produced, some combinations of host response may be diagnostic for identification of a particular virus

FACTORS AFFECTING ASSAY PLANT SUSCEPTIBILITY

- LIGHT, HUMIDITY, AND NUTRITION

Plants grown under low light and high humidity are generally 'softer' and more susceptible (more sites of infection, quicker systemic spread) than plants grown under high light and low humidity. Plants shaded for 16-48 hours prior to inoculation (to allow starch transport out of the expanding leaves) are also more susceptible to infection. Avoid nutrient deficiencies, which may cause virus-like symptoms.

- BUFFER CHOICE AND ADDITIVES

Ionic strength and pH can significantly affect infectivity; if the type of virus is not known, low molarity (10-50mM) buffers close to pH 7.0 may be best. However, some isometric viruses are more stable in more acidic buffers, and many rod-shaped viruses will do well in buffers of $> \text{pH } 7$; unbuffered 1% K_2HPO_4 ($\sim \text{pH } 9.1$) works well with most potyviruses and flexiviruses.

Anti-oxidants increase infectivity of many viruses, especially ilarviruses.

Rhabdoviruses may require ice-cold buffers with added Zn, Mg, or Cu salts.

- EXTRACTS OF WOODY HOSTS MAY BENEFIT FROM INCLUSION OF PVP AND/OR NICOTINE

- ABRASIVES ARE TYPICALLY USED TO MAKE MINOR WOUNDS TO ALLOW VIRUS ENTRY

- Carborundum or corundum dusted on leaves to be inoculated, or Celite mixed with the inoculum.

MECHANICAL INOCULATION



Select appropriate source tissue (preferably young, symptomatic tissue)



Grind tissue in ~ 10 volumes of appropriate buffer (often 10-100mM Na phosphate, pH 7.0; for some viruses 100mM Na acetate pH 6.0 is better. 1% K_2HPO_4 in water works for many flexuous or rod-shaped viruses; some viruses may require more complex buffers with Zn, Cu, or Mn, or anti-oxidants). Adding Celite to the tissue extract, or dusting leaves with carborundum, typically increases efficiency of infection. Pre-shading of plants for ≥ 24 h or growth under low light prior to inoculation also helps.

TYPES OF SYMPTOMS

INOCULATED LEAVES

LOCAL LESIONS

- Chlorotic
- Reddish
- Necrotic
- Papery

RINGSPOTS

VEINAL/PETIOLE NECROSIS

FOLIAR COLLAPSE OR ABSCISSION

STREAKING (IN MONOCOTS)

SYSTEMIC INFECTION

VEIN CLEARING

MOTTLE

MOSAIC

VEIN BANDING

VEINAL NECROSIS

RINGSPOTS

LINE PATTERNS (e.g. oakleaf pattern)

GENERAL CHLOROSIS

BRONZING

DWARFING

VEIN NETTING

APICAL NECROSIS

LEAF DISTORTION

LEAFROLL

EPINASTY

FLOWER BREAK

FRUIT DISTORTION

SEED COLORATION

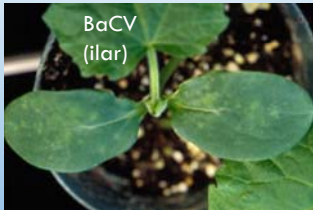
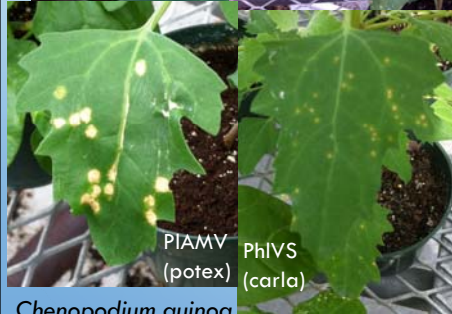
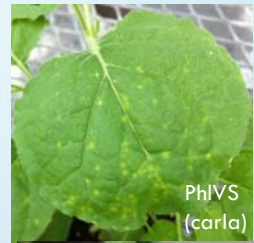
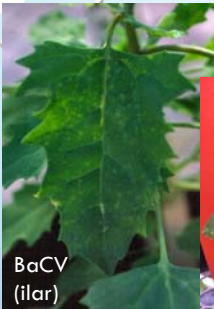
STEM PITTING

ENATIONS

Local lesions (if induced) will typically become apparent in herbaceous hosts in 3-21 days

Systemic symptoms may be visible as early as 7 days in fast-growing plants; or after many weeks or months in woody plants or plants that produce few new leaves, such as many orchids

INOCULATED LEAVES

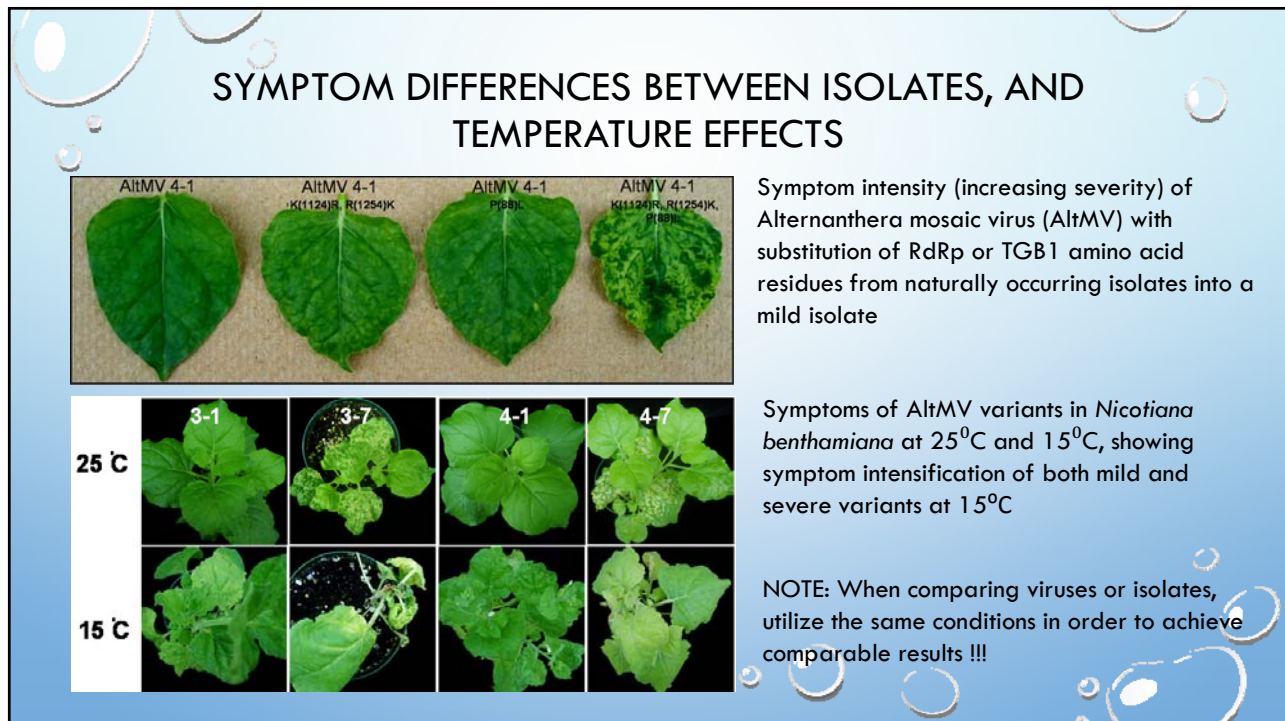
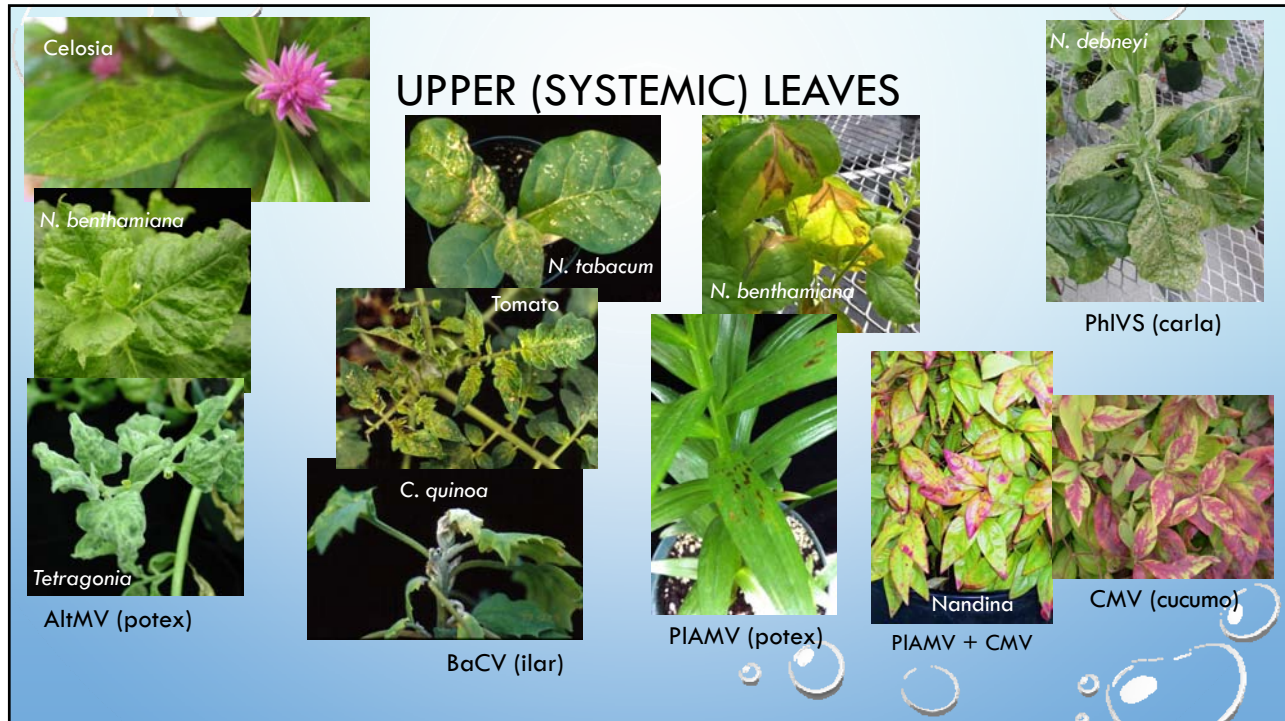


Nicotiana tabacum

Nicotiana benthamiana

Chenopodium quinoa

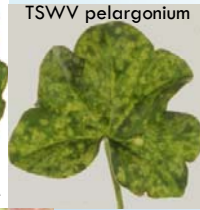
Cucumis sativus



SOME VIRUS GENERA INDUCE TYPICAL SYMPTOM TYPES IN MULTIPLE HOSTS



INSV Kalanchoe



TSWV pelargonium



TSWV gloxinia

Tospoviruses – ringspots,
concentric lines

Chazz Hesselein
Alabama Cooperative
Extension System,
Bugwood.org



CIGMCNV Salvia

Begomoviruses – yellow
vein-netting, often with
leaf curling



BYDV

BYDV images:
University of
Saskatchewan

Luteoviruses – yellowing or reddening,
often with dwarfing and/or leaf
rolling

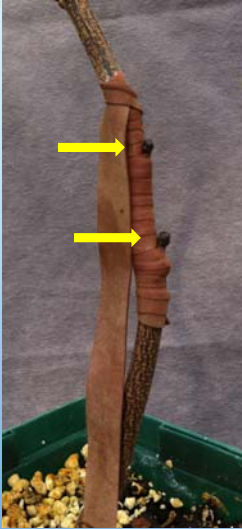
GRAFTING AND DODDER TRANSMISSION

- Some viruses are difficult to transmit mechanically
- especially phloem-restricted viruses (e.g. members of the Closteroviridae)
- Other viruses have no identified vector, or the vector may not be readily cultured or available
- e.g. *Apple stem grooving virus*
- Woody plants often have a high content of phenolic compounds that inhibit mechanical transmission

In these cases graft transmission, or dodder (parasitic *Cuscuta* spp.) may be an alternative for transmission. Either method can be used between different species or even genera – but grafting is most successful between related species. Grafting of woody plants may require months for symptom development.

Dodder seeds can be germinated in the soil around the infected plant, and then shoots trailed from the infected plant to the test plants to serve as a biological conduit.

GRAFTS AND DODDER



There are many types of graft that can be used; shown here are bud grafts as commonly used on fruit trees

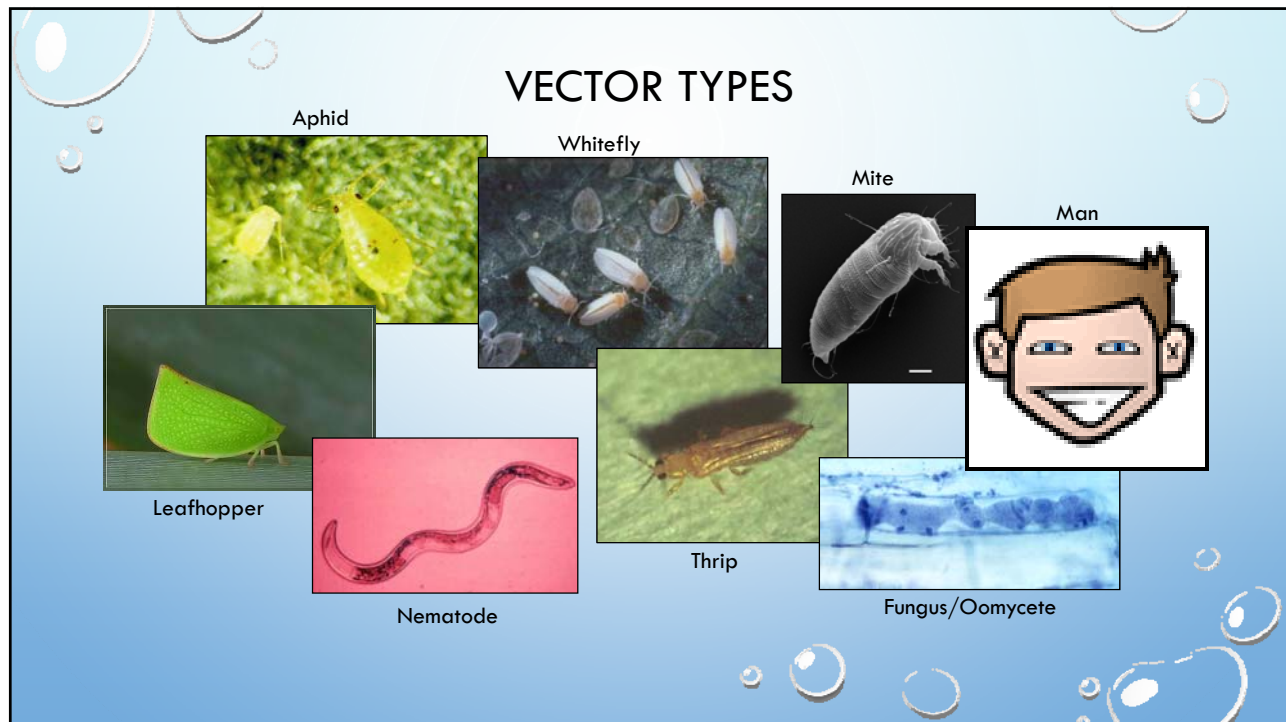


Dodder (*Cuscuta* spp.) are used for transmission of viruses, phytoplasma, and fastidious bacteria; shown here on citrus (image - John Hartung)

INSECT OR OTHER VECTORED TRANSMISSION

Many viruses are naturally transmitted by specific vectors:

- APHIDS – e.g. genera Carlavirus, Closterovirus, Cucumovirus, Potyvirus
- WHITEFLIES – e.g. Begomovirus, Crinivirus, Ipomovirus
- LEAFHOPPERS/PLANTHOPPERS – e.g. Curtovirus, Mastrevirus, Marafivirus, Tenuivirus, Waikavirus
- THRIPS – e.g. Tospovirus, (Ilarvirus), (Carmovirus), (Machlomovirus), (Sobemovirus)
- MITES – e.g. Allexivirus, Emaravirus, Tritimovirus
- NEMATODES – e.g. Nepovirus, Tobravirus
- FUNGI/OOMYCETES
 - Olpidium* spp. - e.g. genera Necrovirus, Varicosavirus, Ophiovirus
 - Polymyxa* spp. - e.g. genera Benyvirus, Furovirus, Pecluvirus, Bymovirus



PRACTICALITIES OF EXPERIMENTAL VECTOR TRANSMISSION

- Need to maintain colonies of virus-free vectors on suitable healthy host plants
- Need for containment to prevent vector escape (including clip-on cages)
- Need for controlled transfer of vectors to virus source plants, and then to healthy test plants (and potentially to then kill vectors to prevent escape or vector-induced damage to test plants)
- Differences between virus genera in:
 - acquisition access time for vector to become viruliferous
 - possible latent period for virus to pass through vector back to mouthparts
 - inoculation access time for vector to transmit virus to test plant
- Possible to cage infected and healthy plants together (aerial vectors) or plant in the same container (soil-borne vectors)