Guidelines for Diagnostic Work in Plant Virology

S.K. Green

Asian Vegetable Research and Development Center

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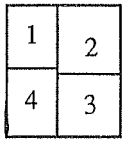

1 D.E. Lesemann
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FOREWORD

Because of popular demand, a second revised edition of "Guidelines for Diagnostic Work in Plant Virology" has been prepared. More recent information on viruses has been included and some chapters, such as those on the identification of viruses and control of virus diseases, have been expanded. Up to date references have also been included.

This volume aims to provide basic understanding of useful practical approaches for the identification of viruses and control measures. The booklet is intended as a general guide to diagnostic work and is meant to give only the most basic background. Those seeking more details are referred to the further reading section at the end.

We wish to thank Dr. D.E. Lesemann of the Federal Institute of Biological Research in Agriculture and Forestry (BBA), Germany, and Prof. Dr. C. Wetter of the University of Saarbrücken, Germany, for critical comments and useful suggestions to improve the previous version of this book.

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Emil Q. Javier
Director General
AVRDC

I. VIRUS STRUCTURE AND GENERAL CHARACTERISTICS

Viruses are extremely small agents that multiply only in living cells. They are potentially pathogenic and can be seen only through an electron microscope. Virus particles are measured in nanometer (nm) or millimicron.

1 nm (nanometer = millimicron mμ)	= 0.001 μm
	= 0.000001 mm (millimeter)
1 μm (micrometer = micron μ)	= 0.001 mm

Viruses consist of nucleic acid, normally enclosed within a protective coat of protein or lipoprotein. They are able to organize their own replication only within suitable living host cells. The nucleic acid is the infectious component and carries the genetic information necessary for the replication of the virus. The nucleic acid of most plant viruses is ribonucleic acid (RNA); in some plant viruses it is deoxyribonucleic acid (DNA).

Plant viruses have different shapes and sizes. They may be isometric (spherical), elongated (helically constructed), or have other shapes (e.g. bacilliform, bullet-shaped or geminate). Among the elongated viruses, one can distinguish between rigid rods and flexuous filamentous particles. Isometric particles vary from 17 to 85 nm in diameter. Bacilliform viruses are between 36 and 150 nm long and 18 to 30 nm wide. Bullet-shaped particles are between 95 and 380 nm long and 45 to 95 nm wide. Rod-shaped viruses range from approximately 100 to 300 nm long and 13 to 23 nm wide, whereas filamentous particles range from approximately 470 to 2000 nm in length and 10 to 13 nm in width.

Viruses are classified into 35 groups based on the type of nucleic acid, particle size and shape.

Plant viruses cannot penetrate the cuticle of their host. Entry takes place through wounds. In nature, this process is usually achieved by another organism which introduces the virus from an infected into a healthy plant. The organism carrying the virus, called a vector, may be an insect, a nematode or a fungus.

Some viruses do not have vectors and they enter the hosts following natural mechanical damage as a result of abrasion of tissues as roots grow through the soil or of leaves rubbing together due to the wind. They may also enter plants through broken leaf hairs, following the manipulation of plants with tools or hands.

Many viruses are transmitted through seed and pollen and by vegetatively propagated plant parts, such as cuttings, tubers, runners and bulbs.

II. SYMPTOMS OF VIRUS DISEASES

A. General

Symptoms are of particular importance since viruses are ordinarily invisible and can be recognized only by their effect on their hosts. However, field diagnosis based on symptoms alone should only serve as a guide. Symptoms can provide only a partial diagnosis, because:

- Similar symptoms can be produced by different viruses.
- Symptoms may be extremely variable; the same virus can produce a range of symptoms, depending on environment and host genotype.
- A lack of symptoms does not necessarily mean that no viruses are present. It may simply mean that the infection is latent or masked.
- Mixed infection with several viruses may have an additive effect on the host, resulting in more severe symptoms.

B. General Appearance

- Abnormal color
- Stunting (often one-sided)
- Rosetting (shortening of the internodes which produces a bunched appearance)
- Witches' broom (excessive budding and branching, stunting and shortening of internodes)
- Decline (loss of vigor of the whole plant or of parts of the plants)
- Necrosis and plant death

C. Color Deviation

1. Leaves

Discoloration evenly distributed:

- chlorosis (weakening of the green color; can also be caused by mineral deficiencies)
- bleaching (disappearance of all color; white appearance)
- yellowing (chlorosis and dominance of yellow pigments)
- reddening (abnormal anthocyanin formation; can also be caused by mineral deficiencies)
- browning and blackening (production of dark melanin-like substances)
- bronzing (necrosis and collapse of epidermal cells covering the still green and apparently healthy mesophyll; can also be caused by mites)

Discoloration irregularly distributed:

- mosaic (pale green, yellow or chlorotic areas, sharply bordered by small veins that are often angular in appearance)
- mottle (discolored areas of various rounded shapes, often diffusely bordered)
- local lesions ranging from small pinpoint-size chlorotic or necrotic areas to large irregular patches
- ringspots (single or concentric rings of chlorotic or necrotic tissue separated by normal green tissue)
- streaking (elongated, sharply defined chlorotic patches)

Certain parts of the leaf uniformly discolored:

- vein yellowing (yellow discoloration of the veins due to lack of chlorophyll, accented color of carotenes and xanthophylls)
- vein clearing (veins appear translucent rather than chlorotic or yellow)
- vein banding (discolored areas along the veins)
- vein necrosis (death of vascular tissues resulting in browning)

2. Flowers

- color deviation (intensification, weakening or change of pigments in the epidermal layer of the petals)
- breaking (usually consists of flecks, streaks or sectors of abnormally colored tissue; may be confused with genetic variegation)

3. Fruits

- discoloration of the whole fruit
- discoloration of parts of the fruit (marbling, mottling, spotting)

4. Roots

- lesions
- necrosis

D. Malformation

1. Leaves

- distortion (crinkling, curling, twisting)
- epinasty (curling downwards)
- narrowing (reduction of laminar tissue; vein growth remains almost normal)
- reduction in size
- thickening of all or part of the lamina or veins
- enations (outgrowths of the leaf blade, often resulting in curling of leaves)

2. Flowers

- various kinds of distortions
- abnormal flower parts
- reduction in size

3. Fruits

- reduction in size
- deformation and irregular shapes
- tumorous swellings
- abortive seeds

4. Stems

- distortion
- shortening of internodes

5. Roots

- decay and dieback
- tumors (can also be caused by certain bacteria)
- proliferation of side roots

E. Other Symptoms

- wilting
- defoliation
- premature leaf drop
- deviation in flower number
- premature or delayed flowering
- abnormal fruit flavor
- woodiness of the fruit
- abnormal secretion
- gummosis
- bark scaling
- wood pitting
- shoot swelling
- graft incompatibility

F. Masking of Symptoms

Under certain environmental conditions no visible symptoms are produced even though a virus is present in the plant. This temporarily latent infection is generally due to environmental factors such

as temperature, light and nutrient excesses or deficiencies. Certain viruses never induce symptoms in certain hosts and are, therefore, permanently latent.

G. Tolerance

Due to the genetic disposition of the plant, no visible symptoms are produced by the presence of the virus.

H. Mixed Infections

When several viruses infect one plant, effects may be additive, synergistic or antagonistic.

I. Phenomena Causing Viruslike Symptoms

- genetic abnormalities
- nutritional deficiencies
- herbicide toxemia
- insect or mite feeding damage
- air pollution damage

The agents that cause these symptoms are neither sap- nor graft-transmissible, and recovery of plants is common (except for genetic abnormalities).

III. TRANSMISSION OF VIRUSES IN NATURE

Plant viruses cannot enter their hosts by themselves. They can only enter host tissue through wounds or with the help of other organisms which can acquire them from an infected plant and then introduce them into a healthy plant. Such a virus-carrying organism is called a vector.

A. Insect-transmitted Viruses

The major characteristics of the individual insect groups are described below. Special emphasis is given to their relationship to viruses.

1. Aphid-transmitted Viruses

- More than 190 aphid species are known to transmit virus diseases. The most common virus-transmitting genera are:

Aphis	*Myzus*
Brevicoryne	*Rophalosiphum*
Macrosiphum	*Toxoptera*

- Aphids are responsible for the transmission of more than 160 different viruses.
- Most aphid-transmitted viruses induce mosaic diseases. Some also produce a yellows-type disease.
- Aphid-transmitted viruses are rarely transmitted transovarially in the insect (i.e., through the egg stage). Thus, newly hatched aphids are nearly always virus-free.
- Aphids are attracted to the color yellow.
- Aphid-transmitted viruses can be categorized into nonpersistent, semipersistent and persistent categories. However, some aphid-transmitted viruses are transmitted atypically and do not fall in any of the above categories. They can be transmitted both nonpersistently and semipersistently, e.g. cauliflower mosaic and dahlia mosaic viruses.

a. Nonpersistent (stylet-borne) viruses

- Most aphid-transmitted viruses belong to this category.
- The virus is acquired by the insect during superficial probing.
- The virus is carried on the mouth parts (stylet) of the insect.
- The virus is usually retained on the mouth parts for less than one hour and is not ingested.
- The virus is acquired by the insect after a short feeding time (from a few seconds to a few minutes).
- There is no latent period in the vector and the virus can be transmitted immediately after acquisition feeding.
- Inoculation feeding time is short (from a few seconds to a few minutes).
- Insects which have fasted prior to acquisition feeding can transmit viruses more effectively.

- Nonpersistent viruses are sap-transmissible.
- Nonpersistent viruses often have a wide host range and a low host specificity.
- The viruses are of considerable economic importance.
- Examples of nonpersistent viruses:

  alfalfa mosaic virus
  bean common mosaic virus
  bean yellow mosaic virus
  broad bean wilt virus
  chili veinal mottle virus
  cowpea aphid-borne mosaic virus
  cucumber mosaic virus
  lettuce mosaic virus
  onion yellow dwarf virus
  papaya ringspot virus
  pea seed-borne mosaic virus
  pea streak virus
  peanut mottle virus
  peanut stripe virus
  pepper mottle virus
  pepper veinal mottle virus
  potato virus Y
  soybean mosaic virus
  sugarcane mosaic virus
  tobacco etch virus
  turnip mosaic virus
  watermelon mosaic virus-2

b. Semipersistent viruses

- The virus is ingested into the alimentary canal of the insect.
- Acquisition feeding time is somewhat longer than for nonpersistent viruses but shorter than for persistent viruses (from several minutes to one or two hours).
- Transmission improves with increased acquisition feeding time.
- There is no latent period in the vector.
- Inoculation feeding is longer than for nonpersistent viruses (from several minutes to a few hours).
- Retention in the insect is longer than for nonpersistent viruses (12 to 24 hours and sometimes several days).
- The virus can only be sap-transmitted with great difficulty.
- Examples of semipersistent viruses:

  beet yellows virus
  citrus tristeza virus
  clover yellows virus

c. Persistent (circulative) viruses

- The virus is carried in the hemolymph and in the salivary and alimentary ducts of the insect.
- Acquisition time varies from 30 minutes to several hours.
- There is a delay (latent period) before aphids can transmit the virus.

- The efficiency of transmission depends on the amount of virus ingested during acquisition feeding.
- Transmission only occurs when the inoculation feeding lasts for at least a few hours.
- Fasting has no effect on virus transmission.
- The insect retains the virus for a long period, frequently for life. The virus is transstadial, i.e., it is retained through molting of the insect.
- Persistent viruses often multiply in the vector.
- Persistent viruses are generally phloem-associated.
- Persistent viruses often have a narrow host range and may be extremely host-specific.
- Persistent viruses generally cannot be sap-transmitted.
- Examples of persistent viruses:
  barley yellow dwarf virus
  beet western yellows virus
  carrot mottle virus
  lettuce necrotic yellows virus
  maize mosaic virus
  pea enation mosaic virus
  potato leafroll virus
  potato yellow dwarf virus
  rice transitory yellowing virus
  wheat striate mosaic virus

2. Whitefly-transmitted Viruses

- *Bemisia tabaci* is the most important and widespread vector.
- Whitefly-transmitted viruses generally cause yellowing, leaf curling and some mosaic diseases.
- The viruses and their vectors are found primarily in tropical and subtropical areas.
- Whitefly-transmitted viruses are persistent in the vector (exception: cucumber vein yellowing virus and cowpea mild mottle virus).
- Whiteflies are carried by wind and hence can spread viruses over long distances.
- The virus is carried in the hemolymph.
- An acquisition feeding period of 24 to 48 hours on a diseased plant is generally enough to make most whiteflies infective.
- The virus has a variable latent period in the whitefly of 4 to 20 hours.
- The whitefly remains infective for anywhere from a few days to 35 days or longer.
- The virus can be acquired by whitefly nymphs. It persists through pupation and can be immediately transmitted by the newly emerged adult.
- There is no evidence of the virus being passed to the egg.
- Whiteflies are phloem feeders and thus whitefly-transmitted viruses are also generally found in the phloem.
- Whiteflies prefer to feed on young tissues and on the lower surface of the leaves.
- Whiteflies are attracted to blue/ultraviolet light and yellow colors.
- Whitefly-transmitted viruses are usually not transmitted by mechanical means (exceptions: bean golden mosaic virus, tomato golden yellow mosaic virus and cowpea mild mottle virus).

- Exposure to the viruliferous whitefly vector is considered the most reliable method of screening for resistance to whitefly-transmitted viruses.
- Examples of whitefly-transmitted viruses:

  abutilon mosaic virus
  bean golden mosaic virus
  cassava mosaic virus
  cowpea mild mottle virus
  chili leaf curl virus
  cotton leaf curl virus
  cucumber vein yellowing virus
  euphorbia mosaic virus
  mungbean yellow mosaic virus
  okra leaf curl virus
  sweet potato leaf curl virus
  sweet potato mild mottle virus
  tobacco leaf curl virus
  tomato yellow leaf curl virus
  tomato golden mosaic virus
  tomato yellow dwarf virus

3. Leafhopper- and Planthopper-transmitted Viruses

- More than 30 species of leafhoppers have been reported to transmit 30 different viruses. The most common genera of virus-transmitting leafhoppers are:

Aceratagallia	*Dalbulus*	*Javesella*
Agallia	*Empoasca*	*Macrosteles*
Cicadulina	*Eutettix*	*Nephotettix*
Circulifer	*Graminella*	

- About 22 species of planthoppers are reported to be virus vectors. The two most important virus-transmitting genera are:

  Laodelphax *Peregrinus*

- Leafhoppers and planthoppers are phloem feeders; they mainly transmit phloem-associated viruses.
- The viruses are generally transmitted in a persistent (circulative) manner:
  - Their acquisition time varies from 30 minutes to several hours.
  - They have a latent period in the vector.
  - They can only be acquired after an inoculation feeding of several hours.
  - They are retained throughout life (exception: rice tungro virus).
  - They are carried in the gut and hemolymph of the vector
- Some viruses appear to multiply in the vector (exception: beet curly top virus).
- Transovarial passage occurs with some viruses.
- The viruses have a high vector specificity.
- The viruses have a limited host range.
- Many of the viruses cause yellowing, leaf rolling and leaf curling types of disease.
- The viruses are generally not sap-transmissible.

- Examples of leafhopper-transmitted viruses:
  beet curly top virus
  maize chlorotic dwarf virus
  maize streak virus
  maize stripe virus
  oat striate mosaic virus
  potato yellow dwarf virus
  rice tungro spherical virus
  rice dwarf virus
  rice transitory yellowing virus
  rice bunchy top virus
  soybean rosette virus
  wheat chlorotic streak virus
  wound tumor virus
- Examples of planthopper-transmitted viruses:
  northern cereal mosaic virus
  oat sterile dwarf virus
  pangola stunt virus
  rice black streaked dwarf virus
  rice grassy stunt virus
  rice hoja blanca virus
  rice stripe virus

4. Beetle-transmitted Viruses

- The most common virus-transmitting beetles are flea beetles *(Phyllotreta* spp.), mustard beetles *(Phaedon* spp.) and cucumber beetles *(Acalymma* spp. and *Diabrotica* spp.) .
- Beetles have been reported to transmit about 45 different viruses.
- The virus is acquired by the vector following acquisition feeding periods of 24 hours or less. Some beetles can acquire the virus in as little as five minutes or after a single bite.
- After feeding on infected plants the beetle remains infectious for at least one day, and often much longer.
- Transmission efficiency increases with longer acquisition feeding times.
- There is no latent period in the vector.
- The virus is usually carried in the hemolymph.
- The viruses are generally stable.
- The viruses can easily be transmitted mechanically.
- Transmission is also possible by macerating the beetle with buffer and inoculating plants with the resulting fluid.
- The viruses are widely distributed and infect economically important crops such as bean, cowpea and soybean in many tropical countries.
- Most of the beetle-transmitted viruses are spherical (about 25-30 nm in diameter).
- Examples of beetle-transmitted viruses:
  Andean potato latent virus
  bean pod mottle virus
  bean rugose mosaic virus

belladonna mottle virus
broad bean mottle virus
broad bean stain virus
broad bean true mosaic virus
cocoa yellow mosaic virus
cowpea chlorotic mottle virus
cowpea mosaic virus
cowpea severe mosaic virus
eggplant mosaic virus
okra mosaic virus
radish mosaic virus
red clover mottle virus
rice yellow mottle virus
southern bean mosaic virus
squash mosaic virus
turnip crinkle virus
turnip rosette virus
turnip yellow mosaic virus
wild cucumber mosaic virus

5. Mealybug-transmitted Viruses

- The main genera of mealybugs known to transmit viruses are:
  Planococcus
  Pseudococcus
  Dysmicoccus
- Mealybugs are often attended by ants. If the ants are controlled, the mealybugs will also be controlled.
- Mealybugs are phloem feeders and feed by sucking.
- Mealybug-transmitted viruses are sap-transmissible.
- The viruses are generally semipersistent, and possibly stylet-borne.
- The probability of infection increases with the length of the acquisition feeding period; 48 to 70 hours gives the best transmission.
- The minimum inoculation feeding time is five to seven hours.
- Fasting prior to acquisition feeding increases the efficiency of the vector.
- There is no latent period.
- The virus persists through the molt.
- Mealybug-transmitted viruses are mechanically transmitted.
- Examples of mealybug-transmitted viruses:
  cacao mottle virus
  cacao swollen shoot virus
  pineapple latent virus

6. Thrips-transmitted Viruses

- Thrips usually feed on very young tissue.

- The known genera of virus-transmitting thrips are:
  Thrips
  Frankliniella
  Scirtothrips
- Tomato spotted wilt virus (TSWV) is the only thrips-transmitted virus.
  - TSWV must be acquired by the nymphs and the adults usually transmit the virus.
  - TSWV has a wide host range and infects more than 400 species of dicotyledons and monocotyledons from more than 50 families.
  - TSWV is persistent in the vector.
  - TSWV is very unstable in plant sap.
  - TSWV is readily sap-transmissible when additives such as mercaptoethanol or sodium sulfite are used in the inoculum preparation.
  - Thrips are difficult to handle and require special rearing techniques.
  - Thrips are attracted by the color blue.

B. Mite-transmitted Viruses

- The most common virus-transmitting mite genera are:
  Aceria
  Brevipalpus
  Eryophyes
- The viruses are carried in the alimentary tract of the insects.
- They are carried over during the molting.
- They are not passed transovarially to the offspring.
- Transmission improves with longer acquisition feedings.
- Mites prefer to feed on very young plant tissue.
- Extreme care must be taken to avoid confusion between symptoms due to feeding (phytotoxemia) and those due to virus infection.
- Mites are extremely difficult to rear and handle. Because of their small size (0.25 mm in length), a hand lens is required to observe them. They can be handled with a single hair. Taffetta or any other very fine meshed material can be used for caging.
- Examples of mite-transmitted viruses:
  agropyron mosaic virus
  citrus leprosis virus
  coffee ringspot virus
  fig mosaic virus
  hordeum mosaic virus
  ryegrass mosaic virus
  wheat streak mosaic virus

C. Nematode-transmitted Viruses

- The three main genera of nematodes known to transmit viruses are:
  Trichodorus
  Xiphinema
  Longidorus

- Nematode-transmitted viruses are sap-transmitted, host-specific and lost in molting.
- Viruses are retained in nematodes from a few weeks to several months. They persist for about two weeks in *Trichodorus* and *Longidorus* and for about eight months in *Xiphinema*.
- The probability of transmission increases with the length of acquisition feeding; 48 hours is considered optimal.
- Nematode-borne virus diseases often occur in slowly spreading patches in the field.
- Some examples of nematode-transmitted viruses are:

pea early browning virus tobacco rattle virus	*Trichodorus* spp.
raspberry ringspot virus tomato black ring virus	*Longidorus* spp. (the virus persists in the vector for about two weeks)
arabis mosaic virus cherry leaf roll virus cherry rasp leaf virus grapevine fanleaf virus peach rosette mosaic virus strawberry latent ringspot virus tobacco ringspot virus tomato ringspot virus	*Xiphinema* spp. (the virus persists in the vector for about eight months)

D. Fungus-transmitted Viruses

- Soil-inhabiting fungi belonging to the genera *Olpidium*, *Polymyxa* and *Spongospora*, are also vectors of viruses. These fungi are obligate parasites, commonly infecting the roots of crop plants.
- The viruses they transmit are spread by movement of soil, root debris and drainage water. They can be carried long distances by transplanting of infected plant materials and by movement of soil particles in the wind.
- Examples of fungus-transmitted viruses are:
  - barley yellow mosaic virus
  - cucumber necrosis virus
  - oat mosaic virus
  - potato mop top virus
  - rice necrosis mosaic virus
  - tobacco necrosis virus
  - wheat spindle streak mosaic virus

E. Seed-transmitted Viruses

- Seed infection plays an important role in the transmission and survival of many plant viruses.
- Evidence of seed transmission is the scattered infection within a planting and the infection of young seedlings.
- More than 60 viruses are seed-transmitted.
- Seed transmission depends on the host species, the strain of the virus, and the temperature at which the plant is grown.

- Examples of common seed-transmitted viruses are:
  - alfalfa mosaic virus
  - bean common mosaic virus
  - cowpea mild mottle virus
  - cucumber green mottle mosaic virus
  - lettuce mosaic virus
  - pea seed-borne mosaic virus
  - peanut clump virus
  - peanut stripe virus
  - soybean mosaic virus
  - squash mosaic virus
  - tobacco mosaic virus

F. Pollen-transmitted Viruses

- Transmission of viruses through pollen is not common in nature.
- Examples of viruses which can be transmitted by pollen are:
  - bean common mosaic virus
  - prunus necrotic ringspot virus
  - tomato aspermy virus

G. Contact-transmitted Viruses

- Contact transmission can occur by using contaminated tools and implements for pruning, weeding and hoeing, and also by animals and humans.
- This type of transmission is rare and occurs only with very stable viruses that are present in high concentrations within the plant tissue.
- Examples of contact-transmitted viruses are:
  - all potexviruses
  - all tobamoviruses
  - all tombusviruses

IV. TRANSMISSION OF VIRUSES IN THE LABORATORY

Transmission of viruses in the laboratory is necessary for their isolation from diseased field plants, for their identification and sometimes for their separation from plants infected with several viruses.

A. Sap Transmission

Sap transmission or mechanical inoculation is the application of virus-containing plant extracts (i.e. inoculum) to the leaf surface of healthy plants.

For virus particles to penetrate the cuticle and epidermis of a healthy leaf, leaf surfaces must be artificially wounded. When the inoculated plant is susceptible, the following reactions may occur:

- Virus movement is restricted; symptoms usually appear as local lesions on the inoculated leaves.
- The virus spreads systemically to other areas of the host. Systemic symptoms appear, such as mottle, mosaic, leaf deformation, lesions, necroses, etc. which are usually distributed throughout the plant.
- There are no symptoms:

Although the virus has invaded the plant and is multiplying, no host reaction is visible. The plant is either tolerant of the virus or the symptoms are masked by environmental conditions.

Although the virus has entered the plant, it is not multiplying and invading other parts of the plant, and no systemic symptoms are produced. The plant is highly resistant or hypersensitive to the virus.

When the virus has not entered the plant, it is immune to the virus.

Not all viruses can be transmitted mechanically. Viruses that in nature persist in the vector such as semipersistent and persistent aphid-transmitted viruses and many of the leafhopper- and whitefly-transmitted viruses are not usually transmitted by sap.

1. Indicator Host Plants

Indicator hosts react diagnostically to certain viruses. They can be used to distinguish among these viruses, usually by showing immunity to one and susceptibility to the other.

The most commonly used indicator plants are:

Chenopodium amaranticolor (susceptible to more than 40 different viruses)
Chenopodium quinoa
Cucumis sativus
Datura stramonium
Gomphrena globosa
Nicotiana benthamiana
Nicotiana glutinosa
Nicotiana tabacum 'Xanthi'
Nicotiana tabacum 'Samsun'
Phaseolus vulgaris 'Pinto'

Vicia faba
Vigna unguiculata

a. Seeds

Seeds of indicator plants can be obtained from:

Plant Introduction
Germplasm Resources Laboratory
Agricultural Research Center
Beltsville, MD 20705
USA

or from institutions actively engaged in applied virus research. If only a small amount of seeds is sent initially, first propagate the seeds in an insect-proof greenhouse.

b. Greenhouse

- Keep indicator plants in an insect-free greenhouse or screenhouse to ensure sufficient light for optimal growth.
- Maintain healthy stock plants separately from inoculated plants, preferably in a separate room.
- Spray the greenhouse regularly with an insecticide to avoid buildup of insect populations. Use insecticides of varying nature to avoid development of resistance. The use of biological control agents, including predators or sticky traps has also become popular. They are commercially available for the control of thrips, whiteflies and aphids.

  Fungicides should be applied as necessary.

c. Soil

To inactivate microbial pathogens and soil-inhabiting viruses and virus vectors, steam-sterilize the soil at about 90°C for 30 minutes.

d. Illumination

Reduced light intensity is known to increase the susceptibility of some plants to certain viruses. Keep indicator plants in the dark for several hours or days prior to inoculation to increase their susceptibility.

2. Inoculum Preparation

Inoculum is the sap extracted from diseased plants used in transmitting the virus.

The following points should be kept in mind when choosing virus-infected leaf tissue for inoculum preparation:

- Virus content often but not always correlates with the severity of the symptoms.
- Young tissues often have the highest virus content.
- Some viruses can only be transmitted at certain times of the year.
- Roots or petals may sometimes be good sources of inoculum.

a. Maceration of virus-infected tissue

Grind one part virus-infected tissue in a small sterilized (autoclaved for 30 minutes at 120°C or boiled in water for two hours) mortar with 2 to 5 parts buffer (generally 0.01 M phosphate buffer, pH 7.0).

Keep the inoculum cool and use it immediately.

Prepare the buffer in the following way:

Solution A: 1.36 g KH_2PO in 1000 ml H_2O
Solution B: 1.78 g $Na_2HPO^-2H_2O$ in 1000 ml H_2O

Mix 51.0 ml of solution B with 49.0 ml of solution A to get 100 ml of 0.01 M phosphate buffer solution pH 7.0.

b. Inoculum additives

- Abrasives

  Abrasives are necessary for successful inoculation; their use increases infection by providing wounds for the entry of virus particles. The most commonly used are carborundum (silicon carbide, 400-600 mesh) and Celite (diatomaceous earth). Dust the abrasive over the leaf surface before inoculation or suspend in the inoculum (0.5-1% w/v).

- Stabilizing additives

  Many plants contain inhibitors that may inactivate the virus, decrease or inhibit its infectivity or interfere with its transmission. The following compounds, when added to the inoculum, are known to have a stabilizing effect on viruses in plant extracts containing such inhibitors. They also have a stabilizing effect on unstable viruses.

- disodium ethylenediaminetetraacetate (EDTA)	0.0005 - 0.1 M
- sodium diethyl-dithiocarbamate (DIECA)	0.01 - 0.02 M
- ascorbic acid (vitamin C)	0.02 - 0.17 M
- sodium sulfite (Na_2SO_3)	0.1 - 0.3%
- polyvinyl-pyrrolidone (PVP) (MW 10000)	1 - 2%
- thioglycollic acid (TGA)	0.1 - 0.5%
- 2-mercaptoethanol (ME)	0.2 - 1.0%
- bovine serum albumin (BSA)	0.01%

  These compounds are generally added to the inoculum in the concentration range listed. The selection of the compound and concentration depends on the particular virus/host plant system.

3. Mechanical Inoculation

Routine Inoculation Method

Grind approximately 5 g of virus-infected leaves with 10 to 20 ml phosphate buffer (pH 7.0) in a sterilized mortar. Add EDTA or DIECA as a stabilizing agent. Gently rub the suspension on the leaves of healthy indicator plants which have been dusted with carborundum or Celite. Rinse the leaves with water after the inoculation.

a. Indicator plants

Inoculate at least two plants of every species. Set aside one control plant of each species for later comparison of symptoms.

b. Pre-inoculation treatment

Keep indicator plants in the dark for several hours or days prior to inoculation to increase their susceptibility.

c. Plant age

Use young plants since they are generally more susceptible to virus infection than older plants.

d. Time of inoculation

Plants are generally more susceptible to virus infection in the afternoon.

e. Inoculation site

Inoculate the upper leaf surface.

Legumes:	Inoculate the primary leaves.
Cucumber:	Inoculate the cotyledons.
Chenopodium:	Inoculate the fourth to eighth leaf.
Tobacco:	Inoculate any leaf above the third or fourth leaf.
Datura:	Inoculate when the first or second leaf pair has developed.

f. Glassware

Use sterilized glassware; autoclave for 30 minutes at 120°C or boil in water for two hours.

g. Abrasives

Either add to inoculum or apply to leaves prior to inoculation.

h. Application of inoculum

Apply the inoculum gently to the leaf surface with a cotton swab, a pad of cheesecloth, a glass rod with a flattened end or with your finger.

i. Post-inoculation treatments

Rinse the inoculated leaves with water to remove natural toxins in the inoculum which interfere with infection, and to reduce injury from chemicals which have been added to the inoculum. Rinsing also facilitates later observation of symptoms.

- Light reduction

  Keep the plants in the dark for several hours after inoculation to increase the susceptibility of virus indicator plants and to promote better symptom expression.

- Drying

  Quickly dry leaves with an atomizer or with blotting paper.

4. Symptom Development and Recording

- Observe plants daily for several weeks (in some cases for several months, e.g. transmission of viruses from woody plants) and compare them with control plants of the same age.
- Many host plants will develop local lesions, but other symptoms can also appear.
- Distinguish between local reaction on the inoculated leaves and systemic reaction on the non-inoculated leaves.
- Record the symptoms and their sequence.
- Some of the common symbols used for recording symptoms are:

LL	: local lesions
nLL	: necrotic local lesions
cLL	: chlorotic local lesions
Vc	: vein clearing
M	: mosaic
Mo	: mottle
SN	: systemic necrosis
Mal	: malformation
E	: etching
RS	: ringspot

B. Transmission by Grafting

- Most viruses can be transmitted by grafting.
- Materials needed for grafting
  - Sharp razor blade (for soft tissues)
  - Sharp knife (for woody tissues)
    To prevent contamination, flame the knife or razor blade with alcohol before use.
  - Plastic tape (approximately 2 cm wide)

1. Standard Grafting Methods

a. Cleft grafting (Fig. la and lb)

- Top cleft grafting

  This method, also called wedge grafting, is widely used in both herbaceous and woody plants. Cut the top of a diseased plant and make a slit axially through the middle of its stem. After cutting its end into a wedge shape, tightly insert the top scion from a healthy plant into this slit and wrap the joint with plastic tape. Observe symptoms of systemic infection in the new growth of the originally healthy plant parts. The growing tip of the plants may need to be cut back to promote lateral buds with obvious virus symptoms.

- Side cleft grafting

  Make a cleft tangentially in the main stem near one of the leaf nodes. Insert the virus-infected scion into the slit as described above.

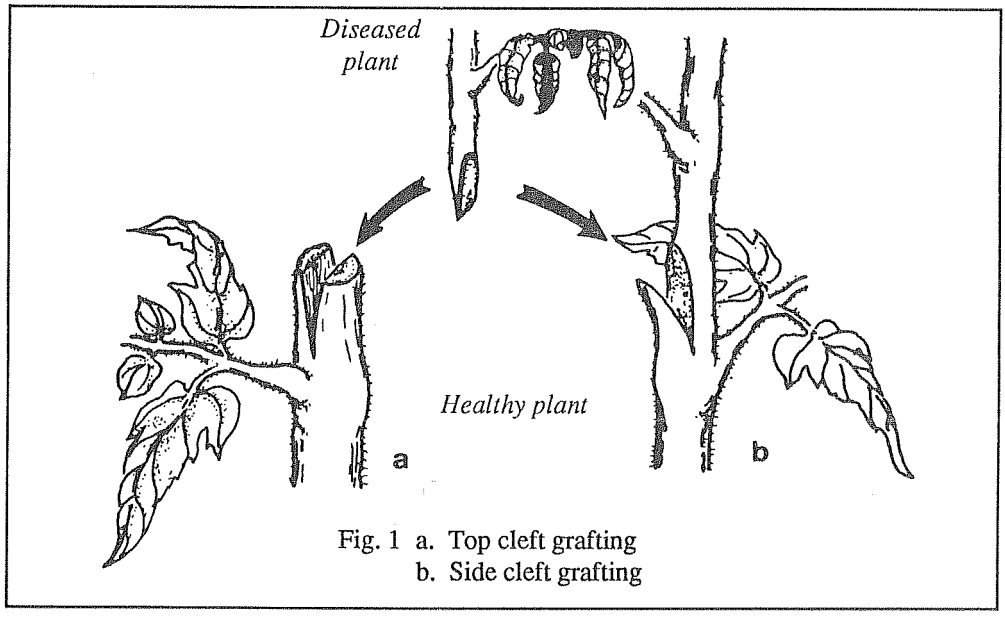

Fig. 1 a. Top cleft grafting
b. Side cleft grafting

b. Approach grafting (Fig. 2)

Cut the stems of a virus-infected and a virus-free plant lengthwise so that the cambium is exposed. Choose stems of similar thickness. Join the cut portions and then wrap the union with plastic tape. Cut back the growing tip of the healthy plant to promote the development of lateral buds. If the infection is systemic, virus symptoms will be observed on the previously healthy plant.

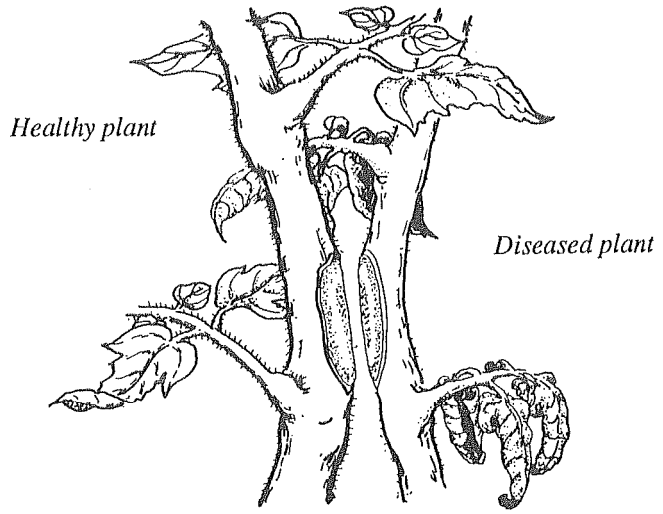

Fig. 2. Approach grafting

c. Other grafting methods — consult the literature.

C. Transmission by Dodder

Dodder (*Cuscuta* spp.) is a parasitic plant which attaches itself to other plants and draws nutrients from them by means of root-like haustoria. Several species of *Cuscuta* are known to transmit viruses. The most common ones are *C. campestris* and *C. subinclusa*. Dodder plants used for transmission work must be grown from seed so that they will be virus-free. Germination of *Cuscuta* is difficult to obtain; a 30-minute treatment of the seeds with concentrated H_2SO_4 is needed before germination occurs.

Place the virus-free dodder plant in close contact with the virus-infected plant. The dodder will wrap itself around the stems and leaves of the virus-infected plant and send out haustoria to form a union with the virus-infected plant. Sap is then passed from the virus-infected plant to the dodder. After the dodder has become well established on the diseased plant, train its stems towards the healthy plant. Virus symptoms will eventually appear on the healthy plant.

D. Transmission by Insects, Mites and Nematodes

Transmission experiments are used to:

- determine the vector of a plant virus,
- assay viruses which are not mechanically transmitted,
- obtain information about the mode of transmission in nature.

1. Materials Needed

a. Cages

- Wooden plant cage (Fig. 3a)

  The cage size should be approximately 35 × 35 × 50 cm. Cover the sides either with fine wire or plastic netting (15 mesh/cm) or a saran screen. The top and front door of the cage may be covered

with a glass plate. For whiteflies, use a cage with two wooden side walls. Each wall should have a round access hole approximately 18 cm in diameter, just large enough for a hand to pass through. The whiteflies are prevented from escaping during handling by black cloth tubes attached to the holes at one end and held closed by rubber bands at the other.

- Plastic cylinder plant cage (Fig. 3b)

  Cover the top of a 20 cm diameter plastic cylinder with cheesecloth and press the bottom into the soil of the pot. If you are not using a potted plant, place fresh leaves in a test tube filled with water inside a plastic cylinder. Use cellulose nitrate plastic or butyrate plastic as some other materials such as cellulose acetate with diethyl phthalate are toxic to plants and insects.

- Plastic cylinder leaf cage (Fig. 3c)

  Use this kind of cage for transmission tests which utilize small numbers of insects. Make the cage using sections of plastic tubes approximately 3 cm in diameter and 1.5 cm long and cover one side with a screen made from a nylon stocking or any other fine meshed material. Transfer the insects through a small hole in the wall of the tube and then close it with a cork. Attach the cages to the leaves with the aid of hairclips. Attach the hairclips by heating them and pushing them through the wall of the plastic tubes.

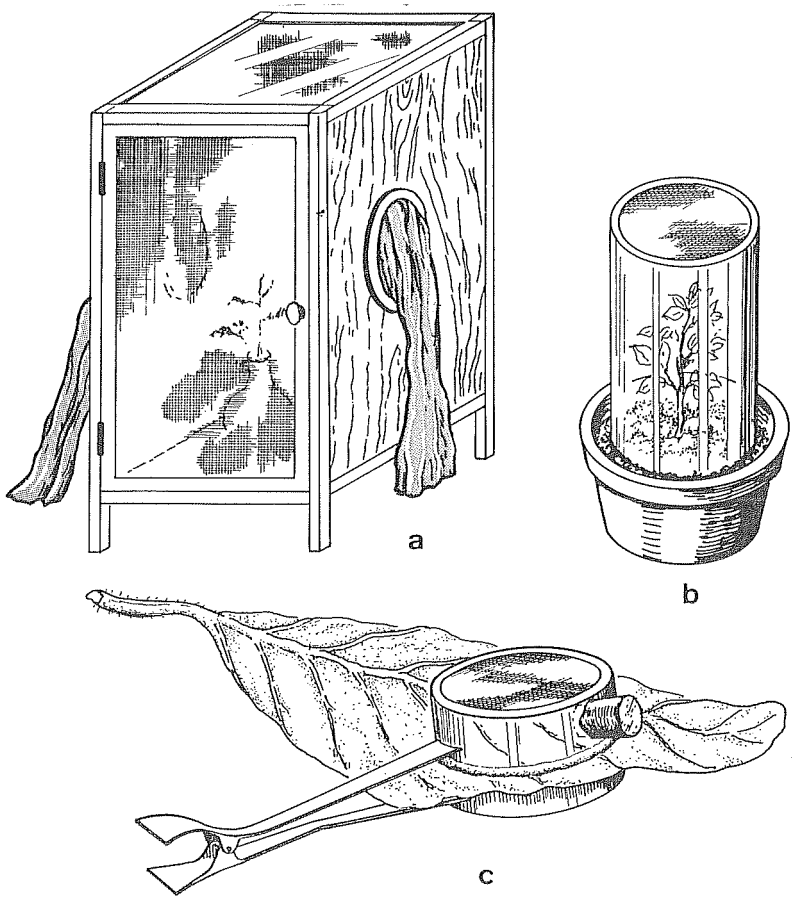

Fig. 3 a. Wooden plant cage
b. Plastic cylinder whole plant cage
c. Plastic cylinder leaf cage

- Plastic or glass containers

  Use these containers to transport insects collected in the field. They should have a screen cover and be large enough to allow for ample space and ventilation.

b. Tools for handling

- Artist's brush

  A pointed artist's brush is generally used for aphids. Moisten the tip to make the insect adhere to the brush.

- Aspirator (Fig. 4)

  Use an aspirator for handling very active insects (e.g., leafhoppers and whiteflies). The aspirator consists of a small glass bottle closed with a two-hole rubber stopper. Insert a small straight glass tube through one of the holes. Its outer end is connected to a piece of rubber tubing which serves as a mouthpiece, and the inner end is covered with a small piece of screen. Then insert a slightly longer glass tube which has been bent to the desired shape into the other hole. Insects are sucked into the bottle through this tube.

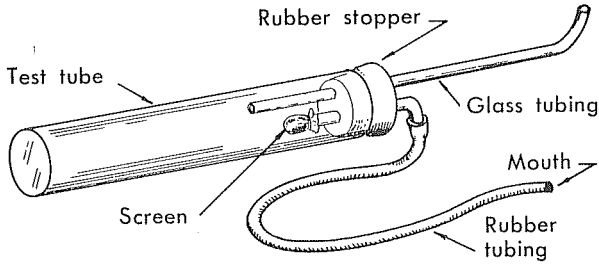

Fig. 4. Aspirator

- A single hair

  Use hairs for very small insects (e.g. thrips) and mites.

  Fasten the hair to a toothpick or a thin wooden stick.

  For easier handling of the insects, a small amount of carbon dioxide may be used to immobiliz them.

c. Test plants

Use the same plant species from which the insects are collected in the field as test plants.

2. Collection of Vectors in the Field

a. Collect insects in the field by:

- sweeping and brushing over the vegetation with a net
- beating the plant and collecting the fallen insects on a dark sheet spread below
- collecting individual insects with an artist's brush
- collecting plant material on which the insect is present
- trapping the insects using the following:

Color traps	Aphids and whiteflies are attracted by yellow color and can be caught in yellow pans filled with water. Thrips are attracted by the color blue.
Light traps	Most insects are attracted to blue ultraviolet light.
Suction traps	Insects are sucked in by a stream of air.
Sticky traps	Insects are caught on surfaces covered with a sticky substance. The surfaces are often painted with colors attractive to insects.

b. Collect mites in the field by:

- Collecting plant material on which mites are present.

c. Collect nematodes in the field by:

- Collecting soil from the rhizosphere and roots.

3. Maintenance of Vectors

- Generally, vectors are maintained on host plants which are not susceptible to the virus being studied.
- Conditions which favor host plant growth also favor the development of vectors. Most vectors can be reared on their host plants or on detached parts, such as leaves of these plants. Certain vectors can also be maintained on artificial diets.
- Keep the plants on which the vectors are reared absolutely free of pesticides.
- Transfer vectors collected in the field first to healthy virus indicator plants to determine whether the vectors are virus-free. If the virus indicator plants develop symptoms, the field-collected vectors were carrying virus.
- If the virus is not carried in the vector's eggs (transovarially) the eggs can be used to start a virus-free insect culture. They may be placed on wet blotting paper until they hatch. The nymphs can then be transferred to healthy plants.

4. Inoculation of Plants

- Virus-free insects are placed on a virus-infected test plant to feed (acquisition-feeding). Depending on the virus, it may take from a few seconds to a few days for the insects to become infected. The acquisition period varies with the insect, the virus and the host plant.
- After the vectors have acquired the virus, transfer them immediately to a virus-free test plant for transmission feeding (inoculation feeding). Some vectors can transmit the virus immediately, but others can only do so after a latent period which may vary from a few hours to several weeks. This latent period, i.e., the time between acquisition and transmission, can be determined by successive transfers of the vectors to virus-free test plants at hourly intervals after the acquisition feeding.

  Some insects, such as certain aphids which carry the virus on their stylet, retain it for as little as 30 minutes. Most leafhoppers and certain aphids which carry the virus in their gut are able to transmit the virus throughout their lifetime. Aphids which carry the virus in their hemolymph can also transmit the virus throughout their lifetime, even after molting.
- After the inoculation feeding kill the insects with insecticides or fumigants.
- Observe the inoculated plants for the development of typical virus symptoms for one to three months.

5. Control Plants and Vectors

- To check the possibility of the vector culture being infected with virus and to detect virus-like symptoms caused by vector feeding only, transfer some vectors from the culture plants directly to the test plants without letting them feed on a virus source.
- Transfer vectors collected from the field to test plants to ensure that they are not already viruliferous.
- Place vector-free plants in a greenhouse to detect accidental virus spread and to ensure that the test plants are not infected before inoculation.

6. Use of Pesticides

Vectors die or develop poorly when placed on plants sprayed with pesticides. In cases where pesticides must be applied, use compounds that are only toxic for a few days. Afterwards, place only a few vectors on these plants to determine whether they can thrive.

V. IDENTIFICATION OF VIRUSES

A. Determination of Size and Shape

The size and shape of a virus is determined by electron microscopy. Crude plant extracts are normally used, because the particle dimensions are best preserved that way. Purified preparations of elongated viruses often contain high amounts of broken or aggregated particles.

1. Materials Needed

a. Support grids

- Grids are needed to carry the support film to which the virus is attached.
- Grids of 3 mm diameter and 150-400 mesh number are mostly used. (The mesh number refers to the number of apertures per linear inch.)
- Copper or nickel grids are generally used.
- The grids must be coated with an electron-transparent film so that they can support the virus particles. Coating of grids requires skill and practice and it is often easier to obtain precoated grids from a virologist at a cooperating institute.
- The following materials can be used for coating:
  - Collodion (0.2% in amylacetate)
    This support film is easy to prepare.
  - Formvar (0.2% to 0.5% in chloroform or ethylenedichloride)
    Although slightly more difficult to prepare, this support film has the advantage of being more stable.

  Both support films can be further stabilized by depositing onto them a layer of carbon. However, this can only be done using a vacuum evaporation apparatus found in electron microscopy departments of universities and research institutes.

b. Fine pointed stainless steel forceps (for manipulating the grids)

c. Grid box (for storage and transport of the grids)

d. Double-distilled water

e. Chemicals

- Stains:
  - Uranyl acetate (UAC)
  - (Phosphotungstic acid (PTA) was formerly widely used but yielded inferior results)
- Grid coating chemicals:
  - Formvar (polyvinyl formaldehyde)
  - Collodion (parlodion = nitrocellulose)

f. Electron microscope

If an electron microscope is not available, send the specimen grids to a virologist at a cooperating institute for examination.

Materials required for sample preparation are available from many companies, e.g. from:

Balzers Union AG
Postfach 75
FL-9496 Balzers
Furstentum Lichtenstein

TAAB Laboratories
52 Kidmore End Road
Emmer Green
Reading, UK

Ask for nearest representatives.

2. Sample Preparation

Samples for electron microscopic examination can be prepared in various ways (leaf squash, leaf dip and epidermal strip methods). The leaf squash method is universally applicable and is, therefore, described here in detail.

It allows relatively large pieces of leaf tissue to be used and thus to obtain preparates representative of a large number of infected cells.

Preparation of the virus-infected sample:

- Place a 1/4 cm^2 leaf sample (punch out a piece from the leaf with the large opening of a pasteur pipet) on a clean microscope glass slide.
- Place 1 to 2 drops of phosphate buffer on the leaf sample and crush it with a glass rod (flattened at one end) until cellular material is homogenized.
- Dilute with more phosphate buffer until a faint green color is obtained.
- Push the crushed leaf remnants to the side and remove the clear sap with a pasteur pipet.
- Place one drop of the clear liquid on a clean parafilm membrane.
- Place the grid, coated surface downward, on top of the drop. Leave for five minutes.
- Remove grid with forceps and rinse the coated surface with 40 consecutive drops of double-distilled water.
- Stain with 5 drops of 1% uranyl acetate in double-distilled water.
- Remove excess stain by very lightly touching the edge of the stain drop with a pointed strip of filter paper.

3. Electron Microscopic Examination

- Examine the specimen at magnifications of 30,000 to 50,000×.
- Take photographs at the same magnifications for demonstrating particle structures. For particle length measurements take photographs at approximately 5,000× to obtain more particles to be measured per picture.
- For determination of size, a minimum of at least 100 particles should be measured.

- Contaminating plant debris such as chloroplast fragments and ribosomes may make it difficult to recognize virus particles, especially the isometric ones. Use more dilute plant extracts (faintly green color) to avoid such problems.

B. Determination of the Physical Properties of the Virus

1. Thermal Inactivation Point

The thermal inactivation point (TIP) is the temperature required to completely inactivate the virus in crude sap during a 10-minute exposure.

Method

- Homogenize the infected leaf tissue with a small amount of buffer. Pass the crude sap through cheesecloth. With a pipet, add 2 ml of the sap to each of eight screw-capped test tubes, being careful not to let the sap drip along the walls of the test tube. Heat each tube in a water bath for 10 minutes. Preliminary testing should be at 10°C intervals (30° to 100°C).
- After heating, cool the tubes immediately in ice cold water. Use the samples to inoculate test plants, preferably those which will react to the virus with local lesion formation.
- Observe the test plants for symptom development for four days to three weeks, and record the temperature range in which virus activity ceases (e.g., 60°-70°C). For determination of the exact TIP, divide this temperature range into five smaller intervals (e.g., 59°, 62°, 65°, 68° and 71°C). Heat five test tubes with sap prepared in the same manner described earlier and inoculate the test plants. The lowest temperature at which no symptoms appear on the inoculated test plants is the TIP.

2. Longevity In Vitro

Longevity in vitro (LIV) is the length of time the virus is infective in crude sap kept at room temperature (approximately 20°–22°C).

Method

- Use a clarified extract similar to that used for testing the TIP, but with 0.01% antibiotic, such as Streptomycin or Aureomycin, added. These antibiotics prevent bacterial contamination.
- Fill 10 screw-capped test tubes each with 2 ml of the sap.
- Inoculate the test plants (preferably local lesion hosts) with sap from the individual test tubes at 10 time intervals (e.g., 1, 3, 6, 9, 12, 15, 30, 60, 90, 150 days) and observe them for symptom development.
- If symptoms appear in plants inoculated after 60 days of storing the inoculum at room temperature but not after 90 days, the LIV is between 60 and 90 days. For an exact LIV determination, test at two-day intervals within the 60-90 day range.

3. Dilution End Point

The dilution end point (DEP) is the highest dilution of plant sap in which a virus is still infectious.

Method

- Homogenize virus-infected leaf tissue in a small amount of buffer.
- Several dilutions are then made from this original sap: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}.
  - 10^{-1} dilution: 1 ml undiluted sap + 9 ml buffer (shake well)

- 10^{-2} dilution: 1 ml of 10^{-1} dilution + 9 ml buffer (shake well)
- 10^{-3} dilution: 1 ml of 10^{-2} dilution + 9 ml buffer (shake well)

- Use a similar procedure to make additional dilutions.
- Inoculate host plants, preferably local lesions hosts, with undiluted sap and with each dilution, record the highest dilution which still produces symptoms on the inoculated plants.

C. Determination of Host Range

Grind one part infected plant tissue with five parts buffer using a mortar and pestle (see also section IV.A.2 and A.3). Inoculate various test plants directly with the sap or after passing it through two layers of cheesecloth.

D. Determination of Insect Vectors

Place insects on infected plants for acquisition feeding. After feeding, place the insects on healthy plants for transmission feeding and observe the plants for symptom development (see also section IV.D.4).

E. Determination of Serological Relationship with Other Viruses

Most serological methods are based on the precipitation produced when antibodies (the antiserum) and antigens (the virus) combine. This reaction is highly specific. Antisera must be prepared from purified or semipurified virus preparations. Many antisera can also be ordered from:

ATCC (American Type Culture Collection)
12301 Parklawn Drive
Rockville, Maryland 20852
USA

or:

DSM
Messeweg 11/12
D-3300 Braunschweig
Germany

The most commonly used serological tests are the drop precipitin (microprecipitin) test, the Ouchterlony agar gel double diffusion test, immunoelectron microscopy (IEM) and the enzyme-linked immunosorbent assay (ELISA).

These tests are applicable to virus identification from crude sap, clarified sap and purified preparations. They require special training, and, in the case of IEM and ELISA, special equipment which is not commonly available.

1. Microprecipitin Test

Single drops of antiserum and antigen are placed close to each other on a glass slide or on the bottom part of a plastic petri dish. The drops are carefully mixed with a toothpick, and incubated at room temperature for 1-6 hours, taking care to prevent the drops from drying out. The formation of the precipitate is observed through a microscope. A dark field microscope is usually best to visualize the precipitate.

This test is economical because it uses small amounts of both antiserum and antigen. The test is also very sensitive.

The microprecipitin test can also be conducted in small tubes. However, this variant of the test requires more antiserum and virus than the drop test.

2. Ouchterlony Agar Gel Double Diffusion Test

This test is usually performed in a petri dish filled with agar to a depth of 5 mm (0.8% Noble agar, agarose or Ionagar in distilled water). Cut round holes into the agar using a corkborer or hollow steel tube in a pattern such that a center well is surrounded by six to eight peripheral wells.

Add antiserum to the center well. Fill the surrounding wells with the viruses to be tested either in purified form or in crude sap. Include healthy plant sap as the control. Both the viruses and the antibodies diffuse out of their respective wells into the agar. Where the viruses and antibodies meet in optimal proportions, a visible precipitin band forms between compatible antigens (virus) and the antiserum well.

This test works best with spherical viruses; elongated viruses longer than TMV do not diffuse readily through the agar medium. Sodiumdodecylsulfate (SDS) which breaks the elongated particles into smaller sub-units which can diffuse more readily may have to be added to the agar or to the virus-containing sap.

3. Immunoelectron Microscopy (IEM)

In IEM, serology is combined with electron microscopy. Two methods are used:

The *decoration technique* allows the coating of the virus particles with specific antibodies to be seen in the electron microscope.

The *immunosorbent electron microscopy method* is used to specifically trap virus particles from plant extracts on antiserum-coated support films, and thus yields a high sensitivity of virus detection.

4. Enzyme-linked Immunosorbent Assay (ELISA)

ELISA is applicable to virus identification from crude sap, clarified sap and purified preparations. It is particularly useful for testing large numbers of samples such as in virus surveys or in screening of large populations for resistance to virus.

a. Materials needed

- Micropipets
- Polystyrene plates (usually 96 well plates are used)
- Chemicals for the preparation of buffers
- Virus-specific gamma globulins (usually prepared from antiserum produced in rabbits)
- Enzyme conjugated virus-specific gamma globulins (for direct ELISA) (alkaline phosphatase is the enzyme most frequently used)
- Enzyme conjugated anti-rabbit antibodies (for indirect ELISA)
- Enzyme substrate (*p*-nitrophenylphosphate is most frequently used)

b. Methods

Direct double antibody sandwich ELISA (DAS-ELISA) (Fig. 5)

- Coating of wells with virus specific gamma globulins — this step is not compulsory
- Addition of test samples containing virus
- Addition of enzyme conjugated virus-specific gamma globulins
- Addition of substrate
- A color change indicates the presence of the specific virus

Indirect ELISA

- Coating of wells with virus-specific gamma globulins (this step is not compulsory)
- Addition of test samples containing virus
- Addition of virus-specific gamma globulins (usually from rabbit)
- Addition of enzyme-conjugated second antibody (usually anti-rabbit antibodies)
- Addition of substrate
- Color change indicates presence of the specific virus

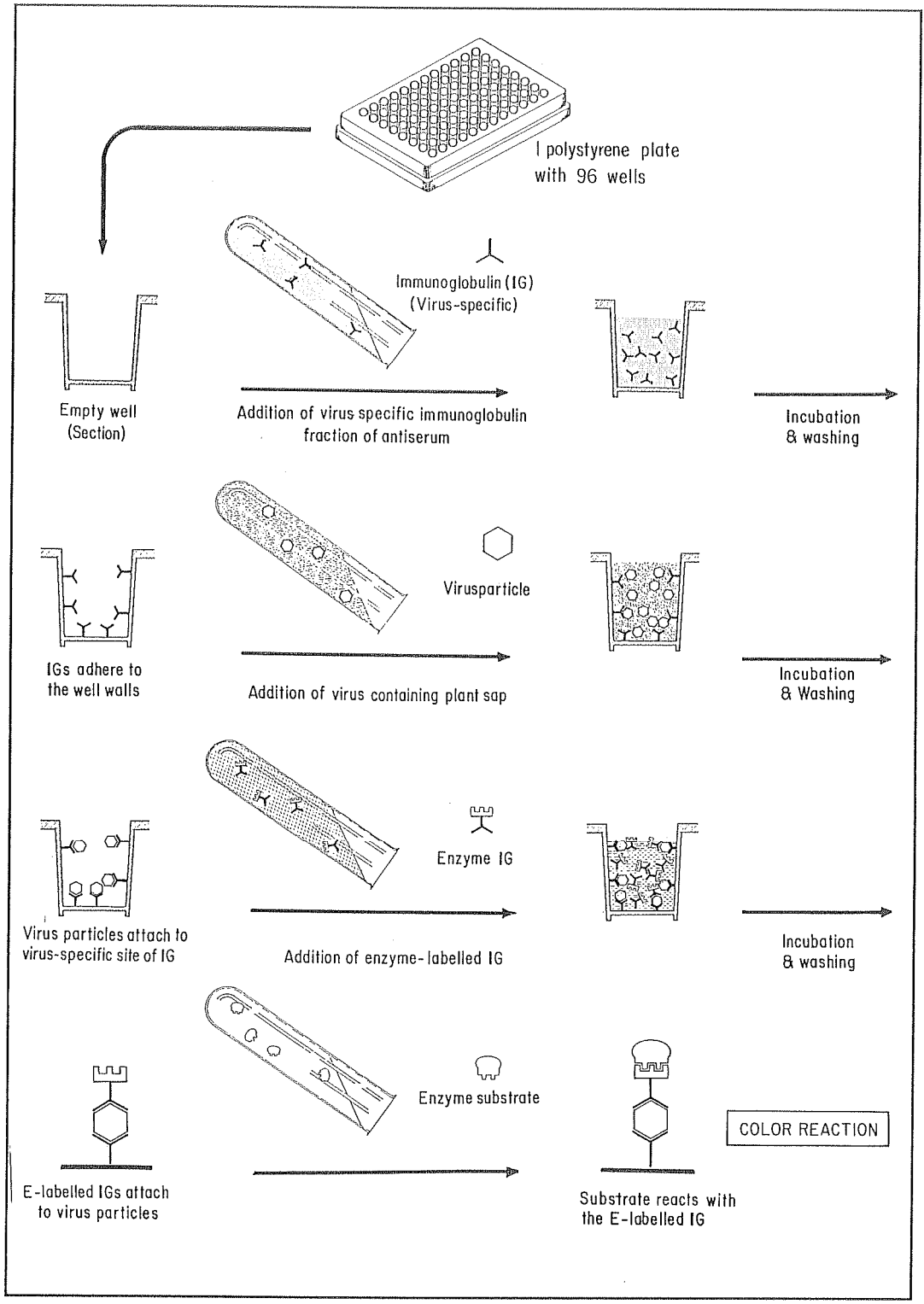

Fig. 5. Enzyme-linked immunosorbent assay
Direct method [Double antibody sandwich (DAS)]

VI. STORAGE OF VIRUSES

Various methods can be used to store viruses in infected plant material.

- The most widely practiced method of maintaining viruses is to keep them in suitable, actively growing storage hosts.
- Another way is to preserve the virus in frozen infected tissue (freshly harvested virus-infected plant material sealed in plastic bags and stored in a freezer at -20°C). However, infectivity may be reduced or lost during prolonged storage and through repeated freezing and thawing.
- Leaf material can also be rapidly dried and stored over calcium chloride ($CaCl_2$) at 0-4°C. Infectivity is generally maintained longer by this method than by freezing virus-infected plant materials.

  Materials Needed

  - glass jar or petri dish
  - granular anhydrous calcium chloride
  - cotton, tissue paper or gauze
  - razor blades
  - cellophane tape or parafilm

  Method

  - Place granules of anhydrous calcium chloride in the bottom of a glass jar or petri dish.
  - Cover them with a thin layer of cotton, tissue paper or gauze.
  - On top of this layer place a 5-10 g sample of virus-infected plant material which has been finely chopped with a clean razor blade.
  - Cover the container and seal it with tape or parafilm. Store it in a refrigerator. To dry the leaf sample completely, it may be necessary to open the container several times and replace the moist calcium chloride granules.
  - For final, permanent storage, transfer to a tightly sealed vial containing a small amount of $CaCl_2$ in the bottom.

- Freeze Drying

With the exception of the use of fresh hosts, this method is considered the safest. Special equipment and additives are usually needed for virus stabilization.

VII. CONTROL OF VIRUS DISEASES

Unlike fungi and bacteria, viruses, so far, cannot be controlled by using chemicals. Some antiviral compounds are known but they are still in the developmental stages. High costs, phytotoxicity and regulatory considerations have prevented their use on a large scale. Indirect control measures and plant and vector resistance thus remain the only practical methods for controlling viruses.

To effectively control virus attacks, the virus has to be correctly identified and its epidemiology and ecology understood. Some of the methods most commonly used to control viruses are described below.

A. Control of the Vector

1. Chemical

a. Pesticides

- Insecticides provide an effective control for persistent insect-transmitted viruses where the vector requires several hours or days to acquire and transmit the virus.
- Viruses transmitted in a nonpersistent manner are not controlled because they can be transmitted by the insect before it is killed by insecticides.
- Insecticides should be used not only on the crop but also on weeds surrounding it because these weeds can be alternate hosts or sources of the virus.
- In the case of nematode-transmitted viruses, the use of nematicides and soil fumigants can be effective for virus control (caution: these chemicals are highly toxic and should be applied with special care).

b. Oils

- Various types of oils, such as vegetable, mineral, synthetic and essential oils have been tried to control virus transmission.
- Best efficiency has been obtained with mineral oils. Some of the oils which have been commercially used on vegetables are: Sunspray 6E(R), Sunspray 7E(R) and JMS Stylet-Oil(R).

  These oils are usually used at concentrations of approximataly 0.75% and have to be applied at high spray pressure (400 psi) using special nozzles (TX-4(R) and TXVS-5(R) have been used extensively). The oil droplets should have a diameter of 0.2 mm to give the best control.
- The appropriate dilution of the oil has to be determined for each crop species because of its reported phytotoxicity.
- The mechanism by which oil acts in preventing virus transmission by insects is not exactly understood. In the case of aphid-borne nonpersistent viruses, oil suppresses both acquisition and inoculation mechanisms.

- Oils are shown to be effective in reducing aphid-transmitted nonpersistent, semipersistent and persistent viruses, and whitefly-transmitted viruses.
- Oils have been successfully used in commercial production of peppers, squash and tomatoes in the USA and some other countries.

2. Non-chemical

a. Barrier crops

- Barrier crops are useful for controlling aphid-borne viruses.

  Example:

  Papaya ringspot virus incidence could be reduced in Taiwan by planting corn around young papaya seedlings. Aphids land first on the taller, more attractive corn on which they probe briefly, causing them to lose any of the nonpersistently transmitted papaya ringspot virus they might have carried.

- Barrier crops which are additionally treated with insecticides will increase their effectiveness.

b. Insect traps

- Color, light (aphids are strongly attracted to the color yellow and reflected light of 500-700 nm), suction traps and hormone (pheromone) traps are most commonly used.

  Example:

  Yellow sticky polythene sheets erected on the windward side of pepper fields have been used in Israel to reduce incidence of potato virus Y (PVY) and cucumber mosaic virus (CMV) in this crop.

c. Reflective mulches

- Aluminum-coated or white polyethylene plastic mulches are commonly used.
- These mulches are thought to act as a repellent by reflecting ultraviolet light and thus confusing the aphids in their landing attempt.

  Example:

  Reflective mulches are used to control CMV and PVY in pepper fields, and watermelon mosaic virus in squash plantings in the USA.

d. Insect parasites

- Controlling virus vectors such as whiteflies and thrips through predators is widely practiced on important crops such as tomatoes, peppers and cucurbits grown in greenhouses, screenhouses and under protective cover.
- The effectiveness of this practice on field-grown crops has not yet been established.

e. Avoidance of the vectors

- Adjust the sowing time of the crop so that it does not coincide with high vector population, particularly at the seedling stage, when the plants are particularly susceptible to virus infection.
- The safest way to avoid virus vectors is to grow the crop in an insect-free nethouse. However, this is expensive and practiced only in very valuable crops. Growing of seedlings which are most susceptible to virus infection in a screenhouse up to the time of transplanting in the field may help delay the onset of virus incidence in a crop.
- Recently, "floating nets" which loosely cover crops have been tried to protect vegetable seedlings and young plants from virus vectors.

B. Elimination of the Source of Inoculum

1. Removal of Infected Plants

- To effectively limit the spread of a virus within a crop, remove virus-infected plants while still young so that they will no longer serve as a source of infection for secondary virus spread.
- Remove volunteer plants emerging at the edges of a planting or within a field since they may be potential virus carriers.

2. Eradication of Weeds and Alternative Hosts

- Eradicate weeds from within and around the crop to remove potential reservoirs of viruses.

  Example:

  CMV and PVY have a very wide host range, infecting many weed species commonly found growing near the crop.

- The removal of alternate hosts is difficult to practice in the tropics where mixed cropping and intercropping are widely used. For example, when hot or sweet peppers are planted in the vicinity of tomato plants, chances are high that aphids will introduce the viruses present in the tomatoes to the pepper plants. These two crops have many viruses in common.

3. Modification of Cultural Techniques

- Crop-free period

  Continuous cropping may lead to virus and/or vector build up. A crop-free period or the planting of a non-susceptible crop can break this cycle.

  Example:

  Celery mosaic virus became so severe because of the overlapping of growing seasons of celery crops in southern California, that celery free periods of 3-5 months were established and have subsequently contributed to the suppression of this virus.

  AVRDC studies have shown that tobacco mosaic virus (TMV) was found in soils up to 8 months after the harvest of the tomato crop, regardless of what crop was grown afterwards. The virus was found in the soil even when irrigated rice had followed the tomato crop. The continuous planting of tomato or any other TMV-susceptible crops could thus lead to a high incidence of the virus.

- Growing a crop away from the inoculum source

  Growing crops away from virus sources is another useful technique to aid the production of a virus-free crop. This is frequently used in the production of virus-free brassica seed plants and 'seed' potato tubers.

4. Use of Clean Planting Material

a. Seed-propagated planting material

- Select seeds from healthy looking plants.
- In cases where virus infection results in seed discoloration or abnormalities, select only healthy looking seeds.
- Use certified seeds.

- Treat the seeds by chemicals or heat.

  Example:

  Tomato mosaic virus (ToMV) and tobacco mosaic virus (TMV) can be eliminated from tomato seed coats by a 30-minute treatment with a solution of 12.5% trisodiumphosphate. Internally carried ToMV or TMV can be eliminated from tomato seeds by heating them at 78°C dry heat for 2 to 3 days. It is important however, to bring the seed moisture content to approximately 4–6% before the treatment; otherwise germination will be affected.

b. Vegetatively propagated plant material

- Clean plant material or virus-free plants can be obtained by heat treatment, meristem tip culture and a combination of heat treatment followed by meristem tip culture. Absence of virus is usually confirmed by virus indexing.

C. Cross Protection

This control method is based on the theory that a plant infected with one strain of a virus is often protected from infection by other related strains.

Examples:

When artificially inoculated at the seedling stage with experimentally produced mild strains (attenuated strains) tomato plants have been shown to be less severely damaged when subsequently infected with the naturally occurring strains of ToMV. This method, however, is no longer used because resistant varieties have become available.

Mild or attenuated strains of CMV have been used in Japan and China for controlling the virus in tomato plantings.

Cross protection is also used in the case of citrus tristeza virus and papaya ringspot virus.

D. Resistance

- The above virus control methods are usually only partially effective. Crop resistance to the virus or the vector is the ultimate solution for virus control.
- The principal aim is to produce cultivars that are able to withstand losses from serious virus diseases and at the same time have acceptable horticultural traits. Ideally, resistance should prevent entry, multiplication and movement of the virus in the host and should be effective against all strains of the virus. In reality, there are several types of resistance to infection (Table 1).
- There are three major problems to resolve in producing virus or vector-resistant cultivars:
  - finding good sources of resistance,
  - combining resistance with other desirable horticultural qualities,
  - anticipating the durability of the resistance factors.
- Sources of resistance can be found in:
  - existing cultivars
  - primitive cultivars or wild types (land races)
  - closely related species
  - other genera of the same botanical family.

Table 1. Types of plant reactions to virus infection.

RESISTANCE TYPE	REACTION OF VIRUS IN THE HOST				
	Virus replication	**Virus spread in plant**	**Virus titer**	Symptom	Yield reduction
Extreme resistance (immunity)	-	-	-	-	-
Resistance to systemic spread	+ or +/-	- or +/-	low or high	+ or -	- or +
Hypersensitivity (quick cell death)					
local	+ or +/-	-	low	necrosis	- or +
systemic	+	+	low	necrosis	+ or ++
Latency	+	+ or -	low or high	-	- or +/-
Tolerance	+	+	high	- or +	- or +/-
Full susceptibility	+	+	high	+/++	+/++

+ = present; - = absent; +/- = limited; ++ = extensive.

The first two sources of resistance are most desired by breeders and pathologists because they can be readily found and used immediately in a resistance breeding program. Other species as sources of resistance may be difficult to use because of genetic incompatibility and/or close linkages with other undesirable characters.

- For resistance screening the following points need to be considered:
  - Plant populations must be uniformly infected by artificial inoculation so that resistant plants can be easily distinguished from susceptible ones.
  - Virus-inoculated plants should be protected from infection with other viruses or more important, different strains of the same virus which may not be distinguished serologically.
  - Screening for resistance to sap-transmissible viruses is usually done by mechanical inoculation (see Section IV.A, Sap Transmission). Inoculum can be applied with fingers, q-tips, or with spray guns if large numbers of plants are to be inoculated.
  - Screening for resistance to viruses that are not mechanically transmitted has to be done by other means such as by viruliferous insects or by grafting (see Sections III and IV).
  - Screening for resistance can be done in the lab or greenhouse by artificial inoculation or under field conditions, using infection of the viruses by their natural vectors. To ensure high virus incidence however, release of virus-infected vectors and/or the interplanting of susceptible individuals is recommended. These plants will serve as source of inoculum.
  - The assessment of the degree of infection and the disease incidence must be reliable. Visual symptom observation alone is not considered very reliable, particularly when symptoms are very mild, when latent infection is present or when screening is done in the field under natural conditions where more than one virus may prevail. Confirmatory tests (such as by serology or inoculation to susceptible hosts) should be made to supplement visual observations to determine the presence or absence of the virus in the plants.
- Recently, genetically engineered, transgenic plants with virus resistance have been developed. Various technologies have been used to achieve resistance such as for example, the introduction of the gene for the virus coat protein or of the satellite RNA into the plant genome via a bacterial vector *(Agrobacterium tumefaciens)*, electroporation or a "gene gun".
- The use of antiviral plant-produced proteins may also receive attention in the future.

E. Exclusion

Most countries have import and quarantine regulations aimed at preventing the entry and introduction of specific virus diseases, especially those not yet known to be widely established, and controlling the spread of virus diseases on a worldwide basis.

APPENDICES

Appendix Table 1. Common viruses of peppers.

Virus	Particle size (nm)	Vector[1]	Host range	Geographic distribution
Isometric (spherical) Viruses				
Beet western yellows (BWYV)	26	A	wide, mainly Dicotyledonae	Europe, USA Japan
Belladonna mottle (BMV)	27	B	Solanaceae	Europe, USA
Broad bean wilt (BBWV)	25	A	wide, mainly Dicotyledonae	Argentina, Egypt, Europe, Japan, Morocco
Cucumber mosaic (CMV)	28	A	wide	Worldwide
Nasturtium ringspot	28-32	A	Solanaceae	Italy
Petunia asteroid mosaic (PeAMV)	30	5	*Capsicum* spp., *Humulus* sp., *Vitis* sp.	Europe, North Africa
Tobacco necrosis (TNV)	26 & 17	F	wide	Worldwide
Tobacco ringspot (TobRV)	28	N	wide	North America, Europe, Australia
Tobacco streak (TSV)	27-35	T	wide	USA, New Zealand, Argentina, Europe, Japan
Tomato aspermy (TAV)	30	A	wide	USA, Europe
Tomato black ring (TBRV)	30	N	wide	Europe
Tomato bushy stunt (TBSV)	30	5	wide	USA, Europe, North Africa
Tomato ringspot (TomRV)	28	N	wide	Japan, Europe, Chile, USA
Tomato spotted wilt (TSWV)	70-90	T	wide	Worldwide (temperate/ subtropical regions)

(Continued)

Appendix Table 1. Common viruses of peppers.

Virus	Particle size (nm)	Vector[1]	Host range	Geographic distribution
Gemini Viruses				
Curly top (CTV)	18-20	L	wide, Dicotyledonae	North America, Europe, Turkey
Tobacco leaf curl (TLCV)	15-20 × 25-30	W	Solanaceae (Compositae)	India, Japan, Sri Lanka
Bacilliform Viruses				
Alfalfa mosaic (AMV)	5 components: 18 × 18 18 × 29 18 × 38 18 × 49 18 × 58	A	wide, Dicotyledonae	Worldwide
Filamentous Viruses				
Chili veinal mottle (CVMV)	750	A	narrow, Solanaceae	Southeast Asia
Pepper mild mosaic	714	A	Solanaceae	Venezuela
Pepper mottle (PeMV)	737	A	Solanaceae	El Salvador, USA, India, Thailand (?)
Pepper severe mosaic	761	A	Solanaceae	Argentina
Pepper veinal mottle (PVMV)	770 850	A	Solanaceae	West Africa, India (?)
Peru tomato virus (PTV)	775	A	Solanaceae	Peru
Potato virus Y (PVY)	730	A	wide, mainly Solanaceae	Worldwide
Tobacco etch (TEV)	730	A	wide, Dicotyledonae	North and South America, Sudan, Nigeria

(Continued)

Appendix Table 1. Common viruses of peppers.*(Concluded)*

Virus	Particle size (nm)	Vector[1]	Host range	Geographic distribution
Rod-Shaped Viruses				
Bell pepper mottle (BePMV)	300	C	Solanaceae	Argentina
Pepper mild mottle (PMMV)	312	C	*Capsicum* spp.	Europe, North America, Japan, Australia, Taiwan
Tobacco mosaic (TMV)	300	C	wide	Worldwide
Tobacco mild green mosaic (TMGMV)	310	C	Solanaceae Umbelliferae Commelinaceae Gesneriaceae	Worldwide
Tomato mosaic (ToMV)	300	C	wide	Worldwide
Potato aucuba mosaic	580	C	mainly Solanaceae	Worldwide
Potato virus X (PVX)	515	C	mainly Solanaceae	Worldwide
Tobacco rattle (TRV)	2 components: 21-23 x 46-117 21-23 x 185-197	N	wide	USA, Europe, Brazil, Japan

[1] A = aphid; C = contact; F = fungus; L = leafhopper; N = nematode; S = soil; T = thrips; W = whitefly.

Appendix Table 2. Major viruses of tomato.

Virus	Particle size (nm)	Transmission Vector[1]	Mech[2]	Seed[3]	Host range	Geographic distribution
Isometric (spherical) Viruses						
Cucumber mosaic (CMV)	35	A, np	+	-	wide	Worldwide
Tobacco ringspot (TobRV)	28	N	+	+	Solanaceae	Canada
Tobacco spotted wilt (TSWV)	70-90	T	+	+	wide	Worldwide
Tobacco streak (TSV)	27-35	T	+	?	wide	USA, Japan, Argentina, Europe, New Zealand
Tomato aspermy (TAV)	30	A, np	+	-	wide	Europe, USA
Tomato bushy stunt (TBSV)	30	?[4]	+	?	Solanaceae	England, Argentina, Morocco, Mexico
Geminiviruses						
Curly top (CTV)	18-20	L, p	-	?	wide	USA, Europe, Turkey
Tobacco leaf curl (TLCV)	15-20 × 25-30	W, p	-	-	intermediate	Tropics and subtropics
Tomato yellow leaf curl (TYLCV)	18-20	W, p	-	-	Solanaceae	Middle East, North Africa
Bacilliform Viruses						
Alfalfa mosaic (AMV)	5 components: 18 × 18, 18 × 29, 18 × 38, 18 × 49, 18 × 58	A, np	+	?	wide	Worldwide
Filamentous Viruses						
Peru tomato	775	A, np	+	-	Solanaceae	Peru
Potato virus Y (PVY)	730	A, np	+	-	wide	Worldwide
Tobacco etch (TEV)	730	A, np	+	-	wide	North & South America

(Continued)

Appendix Table 2. Major viruses of tomato. ***(Concluded)***

Virus	Particle size (nm)	Transmission Vector[1]	Mech[2]	Seed[3]	Host range	Geographic distribution
Rod-Shaped Viruses						
Potato virus X (PVX)	515	-[5]	+	-	narrow	Worldwide
Tobacco mosaic (TMV)	300	-[5]	+	+	wide	Worldwide
Tobacco rattle (TRV)	2 components 21-23 × 46-117 21-23 × 185-197	N	+	?	wide	Europe, USA, Brazil, Japan
Tomato mosaic (ToMV)	300	-[5]	+	+	wide	Worldwide

[1]A = aphid, N = nematode, T = thrips, W = whitefly, np = nonpersistent, p = persistent.
[2]+ = mechanical transmission by sap is possible; - = mechanical transmission by sap is not possible.
[3]+ = the virus is transmitted through seed in tomato; - = the virus is not transmitted through seed in tomato; ? = not known.
[4]The virus is soilborne.
[5]The virus is transmitted by contact.

Appendix Table 3. Common viruses of Chinese cabbage.

Virus	Particle size (nm)	Transmission			Host range	Geographic distribution
		Insect[1]	Sap[2]	Seed[3]		
Isometric (spherical) Viruses						
Beet western yellows (BWYV)	25	A, p	-	-	wide	Worldwide
Cauliflower mosaic (CaMV)	50	A, np or sp	+	-	Cruciferae	Worldwide
Cucumber mosaic (CMV)	28	A, np	+	?	wide	Worldwide
Radish mosaic (RaMV)	25-30	B	+	?	Cruciferae	Europe, USA, Japan
Turnip crinkle (TCV)	30	FB	+	?	wide	Europe
Turnip rosette	28	?	+	?	Cruciferae Compositae Resedaceae Solanaceae	Scotland
Turnip yellow mosaic (TYMV)	28	FB	+	-	Cruciferae	Western Europe
Filamentous Viruses						
Turnip mosaic (TuMV)	720	A, np	+	-	wide	Worldwide

[1]A = aphid; B = beetle; FB = flea beetle; np = nonpersistent; p = persistent, sp = semipersistent; not exactly known, probably biting insects.

[2]+ = can be transmitted mechanically; - = can not be transmitted mechanically.

[3]- = the virus is generally not seed-transmitted in Chinese cabbage; ? = not known.

Appendix Table 4. Common viruses of sweet potato.

Virus	Particle size (nm)	Transmission Insect[1]	Transmission Sap[2]	Host range	Geographic distribution
Isometric (spherical) Viruses					
Caulimovirus	28	?	?	?	Puerto Rico, Pacific Islands
Cucumber mosaic (CMV)	28	A, np	+	wide	Ghana, Israel, USA
Sweetpotato ringspot	28	?	?	wide	Papua New Guinea
Geminiviruses					
Sweet potato leaf curl (SPLCV)		W	-	*Ipomoea* spp.	Taiwan
Filamentous Viruses					
Sweet potato feathery mottle (SPFMV)	850	A, np	+	*Ipomoea* spp. *Nicotiana benthamiana*, *Chenopodium amaranticolor*, *C. quinoa*	Worldwide
Sweet potato latent (SPLV)	700-750	?	+	Convolvulaceae, *Chenopodium* sp., *N. benthamiana*	Taiwan
Sweet potato mild mottle (SPMMV)	800-950	W	+	wide, (45 species 14 plant families)	East Africa
Sweet potato vein mosaic (SPVMV)	761	A, np	?	Convolvulaceae	Argentina
Sweet potato yellow dwarf (SPYDV)	750	W	+	Convolvulaceae, *Chenopodium* spp., *Gomphrena globosa*, *Sesamum orientale*, *Datura stramonium*, *Cassia occidentalis*	Taiwan
Others					
Sweet potato virus disease[3] (SPVD)				*Ipomoea* spp.	Nigeria

[1]A = aphid, W = whitefly, np = nonpersistent; ? = not known.

[2]+ = mechanical transmission is possible; ? = not known.

[3]A disease due to synergistic action of 2 viruses, a strain of SPFMV and an unidentified whitefly-transmitted agent. Symptoms caused by either agent alone are relatively mild or non-existent in sweet potato.

Appendix Table 5. Major viruses of soybean.

Virus	Particle size (nm)	Transmission			Host range[4]
		Insect[1]	Sap[2]	Seed[3]	
Isometric (spherical) Viruses					
Bean pod mottle (BPMV)	30	B	+	+ (very low)	narrow (mainly Leguminosae)
Blackgram mottle (BGMV)	28	B	+	?	narrow
Broad bean wilt (BBWV)	25	A, np	+	-	wide
Cowpea chlorotic mottle (CCMV)	26	B	+	-	intermediate
Cowpea mosaic (CpMV)	20-24	B	+	+ (low)	intermediate (mainly Leguminosae)
Cowpea mottle	30	B	+	+	intermediate (mainly Leguminosae)
Cowpea severe mosaic (CSMV)	25	B	+	-	intermediate (mainly Leguminosae)
Cucumber mosaic (CMV)	29	A, np	+	?	wide
Indonesian soybean dwarf (ISDV)	25-30	A, p	-	-	narrow
Japanese soybean dwarf (SDV)	26	A, p	-	-	narrow
Peanut stunt (PSV)	30	A, np	+	+ (low)	wide
Southern bean mosaic (SBMV)	30	B	+	-	narrow (Leguminosae)
Soybean chlorotic mottle	50	?	+	-	narrow
Soybean mild mosaic	26	A, np	+	+	intermediate
Soybean stunt (SSV)	25-28	A, np	+	+	intermediate
Subterranean clover red leaf	27, 30	A, p	-	-	intermediate
Tobacco ringspot (TRSV)	28-30	T, N	+	+	wide
Tobacco streak (TSV)	25-35	T	+	+	wide
Tomato spotted wilt virus (TSWV)	85	T	+	-	wide

(Continued)

Appendix Table 5. Major viruses of soybean.

Virus	Particle size (nm)	Transmission			Host range[4]
		Insect[1]	Sap[2]	Seed[3]	
Filamentous Viruses					
Adzuki bean mosaic (AZMV)	750	A, np	+	+	narrow (Leguminosae)
Bean common mosaic (BCMV)	750	A, np	+	+	intermediate
Bean yellow mosaic (BYMV)	750	A, np	+	-	wide
Blackeye cowpea mosaic (BlCMV)	750	A, np	+	+	(Leguminosae)
Cowpea aphid-borne mosaic	750	A, np	+	+	intermediate
Cowpea mild mottle (CMMV)	650-700	W, sp.	+	+	intermediate (Leguminosae, Solanaceae)
Peanut mottle (PMV)	750	A, np	+	-	narrow (Leguminosae)
Peanut stripe (PStV)	750	A, np	+	+ (low)	intermediate
Soybean mosaic (SMV)	750	A, np	+	+	narrow
Rod-Shaped Viruses					
Peanut clump	245,160,190	F	+	+	intermediate
Soybean yellow vein	500-550	?	+	?	narrow (soybean)
Tobacco rattle (TRV)	2 components: 46-117 185-197	N	+	?	wide
Bacilliform Viruses					
Alfalfa mosaic (AMV)	5 components: 18 × 18 18 × 29 18 × 38 18 × 49 18 × 58	A, np	+	+	wide

(Continued)

Appendix Table 5. Major viruses of soybean. ***(Concluded)***

Virus	Particle size (nm)	Transmission			Host range[4]
		Insect[1]	Sap[2]	Seed[3]	
Geminiviruses					
Bean golden mosaic (BGMV)	19	W	+/-	-	narrow
Mungbean yellow mosaic (MYMV)	18	W	-	-	narrow (Leguminosae)
Soybean crinkle leaf	18	W	-	-	wide
Others					
Groundnut rosette	?	A[5]	+	-	narrow

[1]A = aphid; B = beetle; F = fungus; N = nematode; np = nonpersistent; p = persistent; T = thrips; W = whitefly.
[2]+ = can be transmitted mechanically; - = cannot be transmitted mechanically; +/- = can be transmitted with difficulty.
[3]+ = seed-transmitted in soybean; - = not seed-transmitted in soybean.
[4]Narrow host range = 1-3 families; intermediate host range = 3-10 families; wide = more than 10 families.
[5]Helper virus required.

Appendix Table 6. Major viruses of mungbean.

Virus	Particle size (nm)	Transmission			Host range[4]
		Insect[1]	Sap[2]	Seed[3]	
Isometric (spherical) Viruses					
Blackgram mottle	28	B	+	+	narrow
Cowpea chlorotic mottle (CCMV)	26	B	+	-	intermediate
Cucumber mosaic (CMV)	28	A, np	+	+	wide
Tobacco ringspot (TRSV)	28-30	T, N	+	+	wide
Tomato spotted wilt (TSWV)	85	T	+	-	wide
Bacilliform Viruses					
Alfalfa mosaic (AMV)	5 components: 18 × 18, 18 × 29, 18 × 38, 18 × 49, 18 × 58	A, np	+	+	wide
Geminiviruses					
Mungbean yellow mosaic (MYMV)	18 × 30	W	-	-	narrow (Leguminosae) Compositae? Graminaceae?
Filamentous Viruses					
Adzuki bean mosaic (AZMV)	750	A, np	+	+	narrow (Leguminosae)
Bean common mosaic (BCMV)[5]	750	A, np	+	+	intermediate
Bean yellow mosaic	750	A, np	+	-	wide
Blackeye cowpea mosaic (BlCMV)	750	A, np	+	+	intermediate (Leguminosae)
Cowpea aphid-borne mosaic (CAMV)	750	A, np	+	+	intermediate (Leguminosae)
Cowpea mild mottle (CMMV)	650	W, sp	+	+	intermediate
Peanut mottle (PMV)	750	A, np	+	-	narrow (Leguminosae)

(Continued)

Appendix Table 6. Major viruses of mungbean. ***(Concluded)***

Virus	Particle size (nm)	Transmission			Host range[4]
		Insect[1]	Sap[2]	Seed[3]	
Others					
Mungbean mosaic	700-750	A, np	+	+	narrow (Leguminosae)
Mungbean leaf crinkle	?	A, WF, B	+	+	narrow (Leguminosae)

[1]A = aphid; W = whitefly; T = thrips; N = nematode; B = beetle; np = nonpersistent; p = persistent.
[2]+ = can be transmitted mechanically; - = cannot be transmitted mechanically;
[3]+ = the virus is generally seed-transmitted but not necessarily in mungbean; - = the virus is generally not seed-transmitted.
[4]Narrow host range = 1-3 families; intermediate host range = 3-10 families; wide = more than 10 families.
[5]Sometimes referred to as the mungbean strain of BCMV (M-BCMV).

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The Journal of General Virology (triannual; covers all virus diseases. Concentrates on biochemical, biophysical and cytological aspects.)

Virology (monthly; includes all virus diseases, not only those of plants; concentrates on biochemical, biophysical aspects, serology, strain differentiation, protein and nucleic acid analyses.)

Journal of Phytopathology (Phytopathologische Zeitschritt) (monthly; covers all aspects of plant pathology including viruses.)