PN-ABD-864

RESEARCH GUIDE
GUIA DE INVESTIGACION
GUIDE DE RECHERCHE

63794

CIP

CIP Research Guide 21

USE OF INDICATOR PLANTS FOR DETECTION OF POTATO VIRUSES

1989

Upali Jayasinghe, Carlos Chuquillanqui

INTERNATIONAL POTATO CENTER (CIP)
CENTRO INTERNACIONAL DE LA PAPA (CIP)
CENTRE INTERNATIONAL DE LA POMME DE TERRE (CIP)

CIP Research Guide 21

USE OF INDICATOR PLANTS FOR DETECTION OF POTATO VIRUSES

1989

Upali Jayasinghe, Carlos Chuquillanqui

CIP
Apartado 5969
Lima, Peru

Location:
Av. La Universidad s/n
La Molina - Lima

Tel. 366920
Telex 25672 PE
Cable CIPAPA, Lima

CIP Research Guides (CRGs)

CIP Research Guides (CRGs) describe technologies that have been developed and used by CIP and National Potato Programs. The CRGs have been produced to promote exchange of information among scientists and are regularly updated to ensure that they describe the most recent advances.

Jayasinghe, U.; Chuquillanqui, C. 1989. Use of indicator plants for detection of potato viruses. CIP Research Guide 21. International Potato Potato Center, Lima, Peru. 29 pp.

USE OF INDICATOR PLANTS FOR DETECTION OF POTATO VIRUSES

1 Production of indicator plants
2 Glasshouse management
3 Mechanical inoculation
4 Grafting
5 Vector transmission
6 Development of symptoms
7 Additional study

Viruses cause most serious potato diseases, that cannot be controlled by chemical means. Erradication of potato viruses requires complicated procedures that depend on relyable detection methods. One of these methods consists in the use of indicator plants.

Potato viruses may also cause disease in plants other than potato (crops, ornamentals and wild species); even plants of other families. Symptoms may be completely different from those produced in potatoes. The property of potato viruses to infect other plants and to produce clear symptoms is used in their detection. This document describes how to grow indicator plants under optimum conditions, free from insects, diseases, and nutrient deficiencies, and how to inoculate, and evaluate them.

1 PRODUCTION OF INDICATOR PLANTS

Seed bed preparation. Prepare a mixture of sand (preferably quartz) and peat moss (1:1 v/v) according to the following procedure:

- Sieve sand and peat moss separately through a wire mesh (100 mesh).
- Wash the sand several times to eliminate salts.
- Mix sand and peat moss.

  If these materials are not available, use any other substrate that has a similar structure.

- Sterilize the mixture in one of the following ways.

  a Steam sterilization at 100 ºC for 30 minutes is the best way but facilities may not always be available.

  b In warm regions, use solar heat:

    - Spread the substrate evenly over a metal surface and expose it to the sun for 2 weeks. Turn the substrate periodically and moisten it with water.
    - You may also seal the moist substrate in a plastic bag and expose it to sunlight for 2 weeks. Turn the bag perodically.

- When the mixture is cool (two to three hours later) add 40 g of ammonium nitrate, 200 g monophosphate and 50 g sulphomac per 100 kg mixture.

Planting of seeds. All indicator plants are grown from seed (no cuttings).

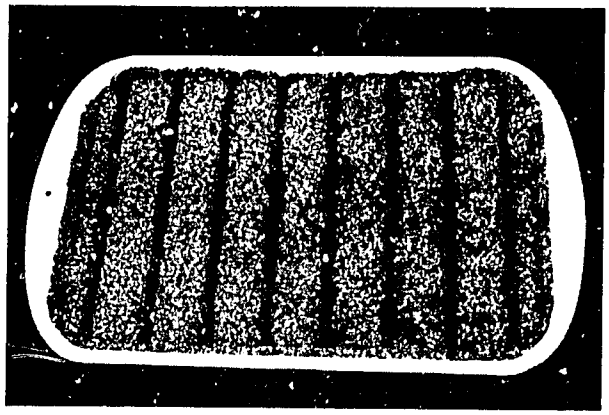
- To improve uniformity of germination you may treat seeds for 24 hours with 1500 ppm gibberelic acid. Dry the seeds for at least 24 hours.
- Fill seed trays of about 40 x 30 x 5 cm or any other suitable container with the substrate.
- Prepare furrows of 0.5 cm depth at a distance of 3-4 cm. (Fig. 1)
- Place seeds of the same indicator plant in one furrow. (Fig. 2) Seeds of different host plants can be planted in one tray. (Fig. 3)
- Cover with a thin layer of soil and irrigate gently.

Most seeds germinate best at 22-24 ºC; in cold regions, artificial heating may be necessary. Sow more seeds than the number of plants required, because emergence may be erratic. Utilize the plants emerging first, and discard the rest.

CIP has a limited quantity of seeds available. If desired, send your request to CIP. Enclose an import permit, if necessary for your country.

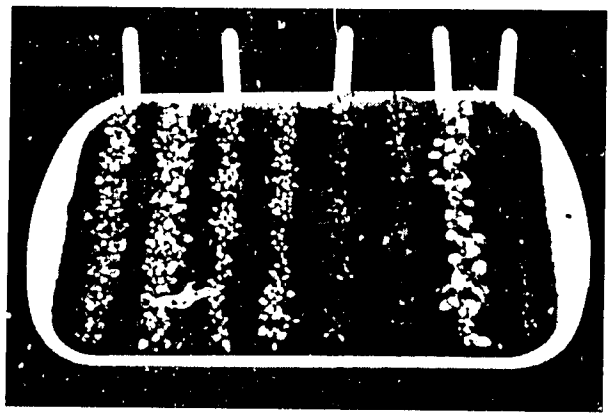
Transplanting. At a certain age (table 1.1) transplant the seedlings individually into pots filled with a sterilized planting substrate (sand : soil : moss = 1:1:1). Prepare the substrate as previously described, but use 36 mesh for sieving. Use pots of 5 cm diameter. Clay pots are better than plastic pots.

- Clean and sterilize the pots before use.
- Loosen the soil in the seed trays (Fig. 6) and lift the seedlings carefully. (Fig. 5)
- Plant the seedlings in 2-3 cm deep holes. (Fig. 6)
- Slightly press the soil to hold seedlings in place.
- Irrigate immediately.

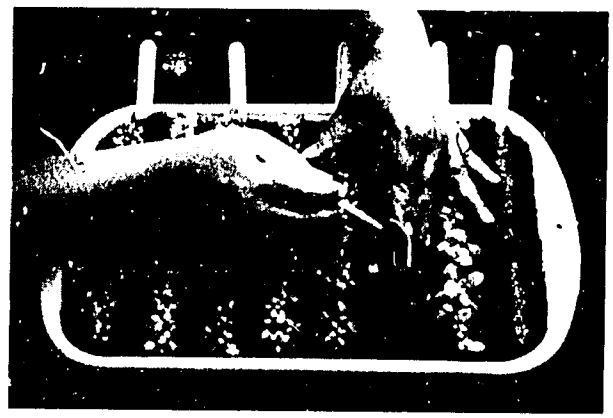


1

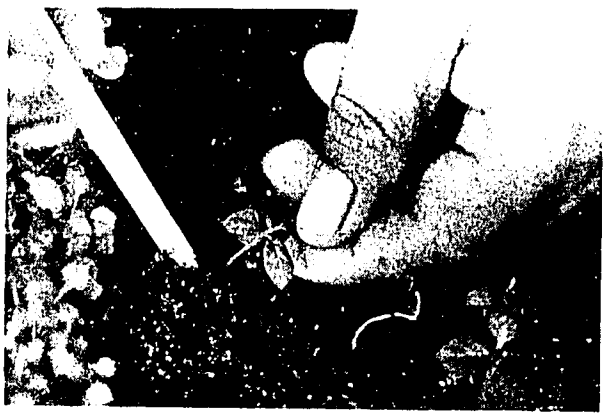
2



3



4



5

6

Table 1.1. Number of days from sowing to transplanting and inoculating (for the conditions of CIP, Lima)

		transplanting	inoculating
Gomphrena globosa	*	28	46
Nicotiana rustica		28	46
Nicotiana glutinosa	*	28	46
Nicotiana tabacum "White Burley"	*	28	46
Nicotiana clevelandii		28	46
Nicotiana debneyii	*	28	46
Nicotiana benthamiana		17	49
Nicotiana occidentalis		23	49
Nicotiana tabacum "Samsun"		28	46
Nicandra physaloides	*	28	46
Physalis floridana	*	17	31
Datura metel	*	17	31
Datura stramonium	*	17	31
Chenopodium quinoa	*	17	31
Chenopodium murale	*	17	31
Chenopodium amaranticolor	*	28	46
Lycopersicum esculentum "Rutgers"	*	17	31

* used at CIP for routine work

2 GLASSHOUSE MANAGEMENT

The glasshouse should provide optimum conditions for vigorous plant growth.

Ventilation. During hot seasons, aphid proof nets can cause excessive heat. Forced ventilation may be needed. Use an electric exhaust fan. Inlets of the fan must be insect proof.

Shading. To avoid extreme summer heat, shade the glasshouse during the hottest hours of the day.

Lighting. During winter, supplementary lighting (to extend illumination to 16 hours) may be necessary. Daylight florescence tubes and a few incandescent bulbs (25 W each) are sufficient to maintain proper plant growth.

Hygiene. Apply the following hygiene measures to prevent contamination.

- Remove all plant debris.
- Remove weeds growing inside and outside the glasshouse.
- Do not take plants from field into the glasshouse, unless they have been treated with chemicals to control pathogens and pests. Keep plants separately from indicator plants.
- Do not enter the glasshouse immediately after visiting a field.
- Use a clean laboratory coat when entering the glasshouse.
- Wash hands before entering the glasshouse.
- Do not touch indicator plants. When working with indicator plants use paper towels to hold the leaves.

Inoculate indicator plants by mechanical or sap inoculation, grafting, or vectors. See the following sections

3 MECHANICAL INOCULATION

With exception of a few viruses, such as potato leafroll virus (PLRV) and Solanum apical leafcurl virus (SALCV), most of the potato viruses can be transmitted by mechanical or sap inoculation.

Materials:

- Sterilized pestle and mortar.
- Indicator plants.
- Carborundum powder (600 mesh).
- Sterile cotton swabs.
- Plant material to be tested (young leaves, petals, tubers, roots), approximately 2-3 g.
- 0.01M phosphate buffer, pH 8.0. Prepare the following solutions with distilled water:

  Solution A: $NaH_2PO_4.H_2O$ - 27.6 g/l

  Solution B: $Na_2HPO_4.7H_2O$ - 53.65 g/l or $Na_2HPO_4.12H_2O$ - 71.64 g/l.

  Mix 2.65 cm^3 of solution A and 47.35 cm^3 of solution B and make up to 100 cm^3 with distilled water.

Procedure:

- Wash hands with soap.
- Label indicator plants with date, origin of sample, etc.
- Dust carborundum powder onto leaves of indicator plants (Figs. 7 + 8).

- Add about 2 cm^3 buffer into the mortar and grind the plant material to be tested (Fig. 9).
- Soap a cotton swab or your fingertip in the sap and wet all leaves of an indicator plant (Figs. 10 + 11). Start each stroke from the petiole, and end at the leaf tip. Support the leaf with a paper towel. Avoid excess pressure.
- Inoculate a "control set" of indicator plants with pure buffer.
- Immediately after inoculation wash the sap from the inoculated leaves with a gentle water spray (Fig. 12).
- Place the inoculated plants into the glasshouse.
- Remove all used material, clean the table, and wash hands before proceeding with the next test.

7

8

9

10

11

12

4 GRAFTING

All potato viruses, including viruses that cannot be transmitted by mechanical or sap inoculation, can easily be inoculated to indicator plants by grafting. However,

- Donor and recipient host must be compatible,
- the virus should be systemic in the indicator plant.

Materials:

- Sharp, thin blade knife, new razor blade, or scalpel.
- Bast (raffia, which has been made supple with water) or strips of parafilm (1 x 3 cm).
- Sticks for supporting the plants.
- Plastic bags (slightly bigger than the plants to be grafted).
- Indicator plants (recipient host, "stock"). Indicator plants used for grafting should be more mature than plants used for mechanical or sap inoculation. The stem of the stock should be strong and thick enough to support the graft.
- Plant material to be tested (donor host, "scion").

Several procedures for grafting exist. For detection of potato viruses, most commonly used are:

- side grafting,
- approach grafting.

Side grafting. Side grafting requires that donor and recipient host are well compatible.

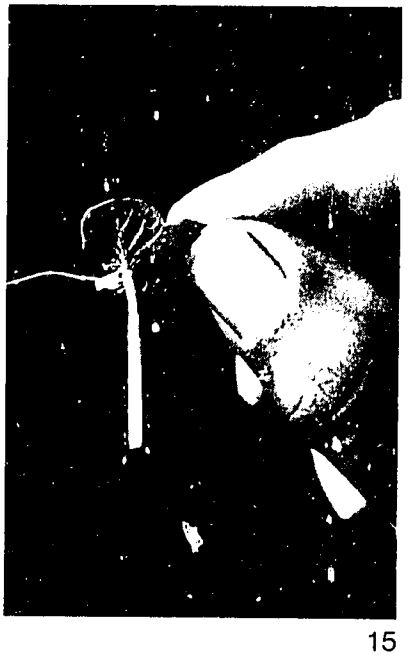
Procedure:

- From the indicator plant (stock) remove most of the larger leaves. If necessary support the plant with a stick.
- Cut an oblique, 0.5 - 1 cm deep incision downwards into the stem (Fig. 13 + 14).
- From the donor host (scion) select a young growing tip with one or two leaves.
- Trim the stem of the scion to a 0.5 - 1 cm long wedge (Fig. 15).
- Insert the scion into the incision and fix the graft with raffia or parafilm (Figs. 16 + 17).
- Cover the grafted plant with a moist plastic bag (Fig. 18).
- Place the plant on a cool, shaded place.
- Check the plant daily and make sure that the plastic bag is moist.
- After 3-4 days, remove the bag and transfer the plant to the glasshouse.

For side grafting, apical stem tips, single leaves, or tuber pieces can be used as scion.

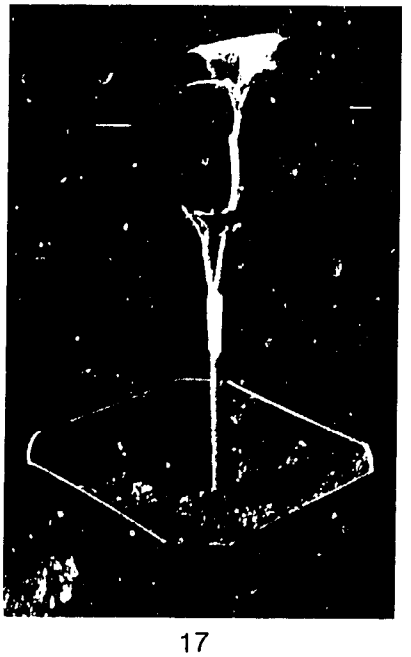
13

14



15

16



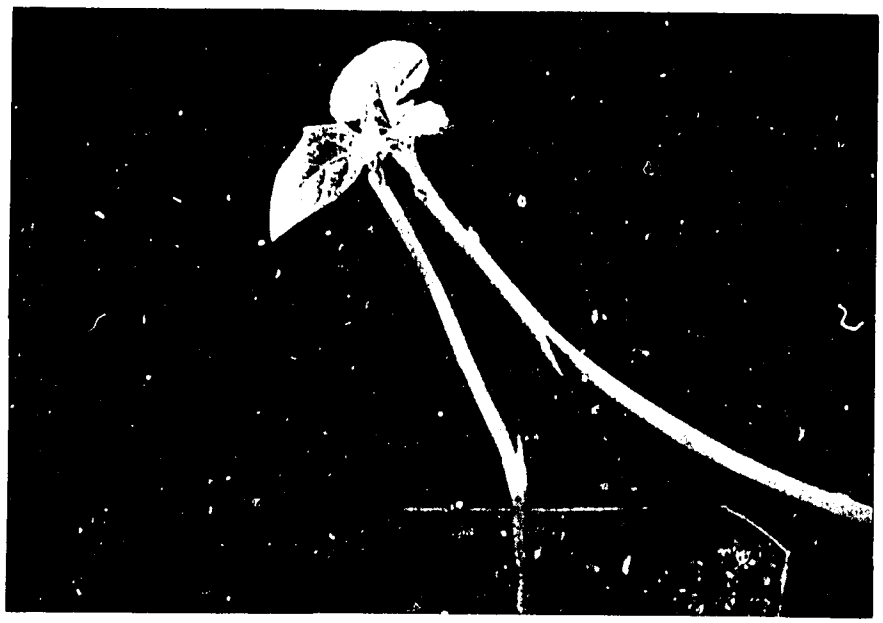
17

18

Approach (or inarch) grafting. Both the stock and scion are maintained on their own root system. Aproach grafting is useful when plants are not compatible for side grafting.

Procedure:

- On the donor plant, cut an oblique 1 cm deep insicion downward into the stem, about 4-5 cm above the soil level.
- At the same height, cut an upward insicion into the stem of the indicator plant (scion). The stem should have similar thickness as the donor plant (Fig. 19).
- Insert the scion into the stock and fix the graft with raffia or parafilm (Figs. 20 + 21).
- Support the plants with a stick.



19

20

21

5 VECTOR TRANSMISSION

Aphids are the most important vectors of potato viruses. Aphid-transmitted potato viruses can be divided into two groups:

- non-persistent,
- persistent.

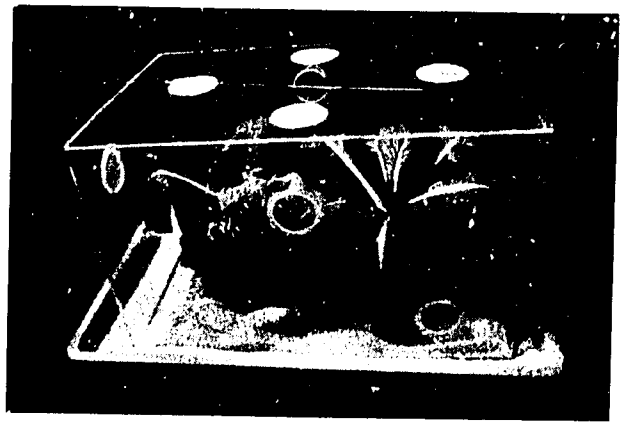
Non-persistant viruses are acquired by the aphid after a few seconds of feeding on an infected plant. They can be transmitted to a healthy plant again within a few seconds. An example is PVY.

Persistant viruses are acquired by the aphid after 20 to 30 minutes of feeding. Usually, they have a latent period of several hours to days in the aphid before they can be transmitted to a healthy plant. For transmission, again a longer feeding period is needed. An example PLRV.

If aphid vectors are needed regularly, pure colonies should be maintained. For maintenance of aphids, cages of about 40 x 40 x 60 cm may consist of wooden frames covered with an aphid proof mesh (<0.3 mm; Fig. 22).

Cages can also be made with transparent perspex (40 x 25 x 25 cm; Fig. 23). Open large holes on sides and top, and cover them with nylon mesh. Perspex cages usually do not have a base. Placed them in plastic or aluminium trays filled with sand. Water the plants in the cages by moistening the sand.

22



23

The most important aphid vector of potato viruses is Myzus persiace. Use a magnifying lens (10x) to identify aphids.

- Myzus persiace usually colonizes the lower mature leaves of the potato plant.
- Aphids are green to peach in color; sometimes translucent.
- The abdomen is oval (egg-shaped). The width of the abdomen is almost same as the distance from thorax to the cornicle basis.
- The head has prominent inward-pointing antenna tubercles.
- Winged forms have a distinct black patch on the abdomen.

Rearing virus free aphids. Because viruliferous adults do not transmit potato viruses to nymphs, virus free colonies can easily be established.

- Take a small leaf from a young, healthy chinese cabbage plant (Brassica pekinensis) and cover the petiole end with wet cotton wool. Fix the cotton wool with parafilm.
- Place the leaf in a plastic petri dish (14 cm diameter). Preferably open a hole in the lid, and cover the hole with an aphid proof mesh. Check the leaf daily, and moisten the cotton wool with distilled water using an injection syringe.
- In the field, collect a potato leaf containing winged aphids. If winged aphids are not available, use wingless adults.
- Disturb the aphids by touching the abdomen with a moist camel hair brush (No. 0).
- When aphids withdraw their stylets and begin to move, transfer them onto the cabbage leaf. Close the petri dish.
- With a magnifying glass, observe the leaf daily for the appearance of new-born aphids.
- Transfer nymphs onto a healthy cabbage plant, and place the plant in a cage.

After 3-4 weeks, depending on temperature, the chinese cabbage plant will be full of aviruliferous aphids.

Continuous illumination prevents formation of winged aphids, but also good plant growth. Illumination of 16 hours allows good plant growth and reduces the appearance of winged aphids.

Trouble shooting. In rearing virus-free aphids, several problems may occur.

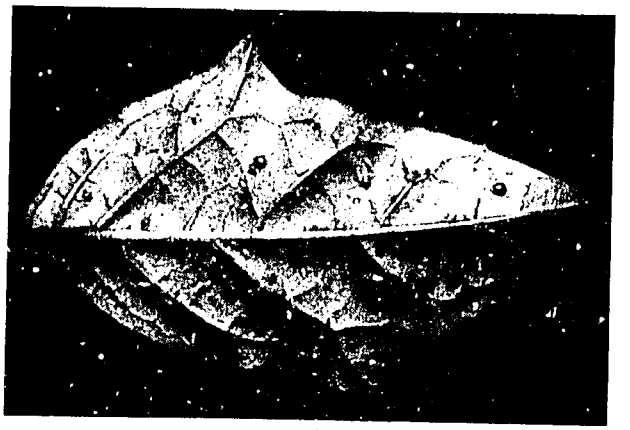
Insects parasitizing on aphids may be controlled by specific insecticides. Insecticides, however, affect vigor of aphids. Prevention is best. Check colonies regularly for parasite appearance. If possible use a double layered net in the cages and rear two colonies at one time. If one gets parasitized the other can be saved. (Fig. 24)

Fungal parasites may become a problem when humidity within the cage is high. Water the plants carefully. (Fig. 25).

Honeydew produced by aphids promotes growth of saprophitic fungi. Black growth on the surface of leaves is an indication that the colony should be renewed.

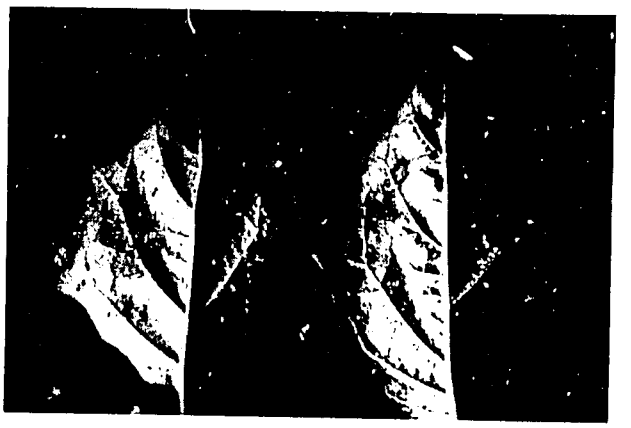
Mites are most difficult to control. Although mites do not attack aphids, the aphid population tends to decline. Start a new colony after disinfecting cages and tables with a suitable miticide. (Fig. 26).

Inoculation of indicator plants. The same materials and procedures - with small variations - can be used to transmit non-persistant and persistant viruses.



24

25



26

Materials:

- Aviruliferous apterous aphids (one leaf from the aphid colony).
- Petri dish (14 cm diameter).
- Indicator plants (young plants).
- Camel hair brush (No. 0) or any other soft hair brush.
- Beaker with distilled water.
- Small pieces of filter paper.
- Leaf of the host to be tested.
- Plastic beakers (250 - 500 cm^3) with a hole in the bottom that is covered with an aphid proof mesh.

Procedure:

- Disturb the aphids on the cabbage leaf with the moist camel hair brush or by breathing on them.
- Transfer aphids to a moist filter paper in a petri dish.
- Close the petri dish and keep it for 1 hour at a cool shaded place to starve the aphids.
- Place a leaf from the plant to be tested in another petri dish and transfer the aphids. Make sure that the aphids feed on the leaf.
- Non-persistant viruses: Let aphids feed for 2-10 minutes.
  Persistant viruses: Let aphids feed for 24-48 hours. The leaf will be dry in 24 hours, use moist cotton wool to prevent drying.
- Transfer at least five aphids to each indicator plant.
- Cover the indicator plant with an inverted plastic beaker.
- Non-persistant viruses: Let aphids feed for 15 minutes.
  Persistant viruses: Let aphids feed for 24-48 hours.
- Kill the aphids with an insecticide.
- Place the inoculated indicator plants in the glasshouse for symptom development and observation.

In all aphid transmission experiments, use the appropriate control to distinguish virus symptoms from symptoms caused by other factors. Feed a group of aphids on healthy plants and transfer them to indicator plants.

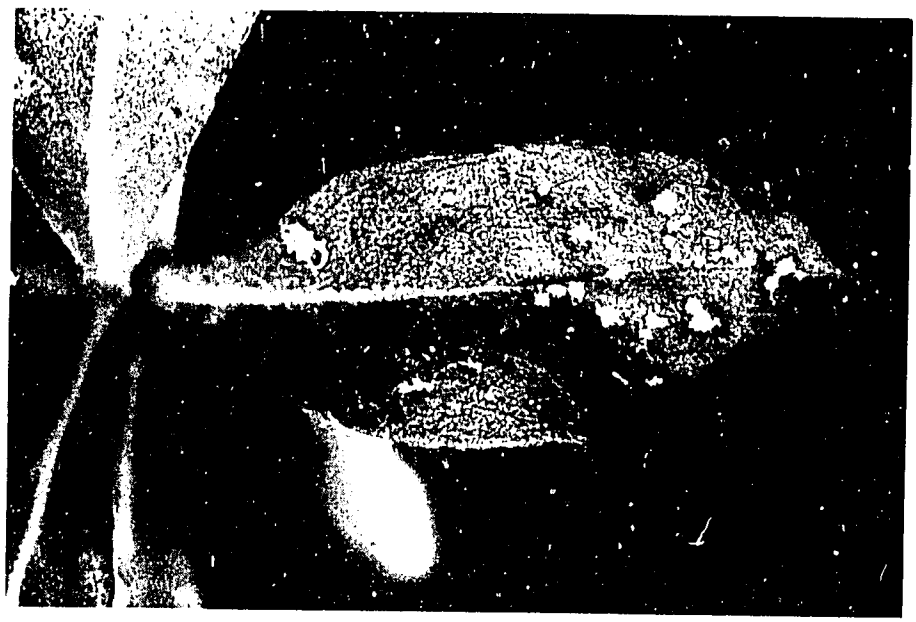
6 DEVELOPMENT OF SYMPTOMS

Inoculated plants should be observed daily for development of symptoms. The majority of potato viruses produce clear symptoms under cool conditions. Speed of symptom appearance depends on temperature, light, and virus involved. Symptoms can be divided into two groups:

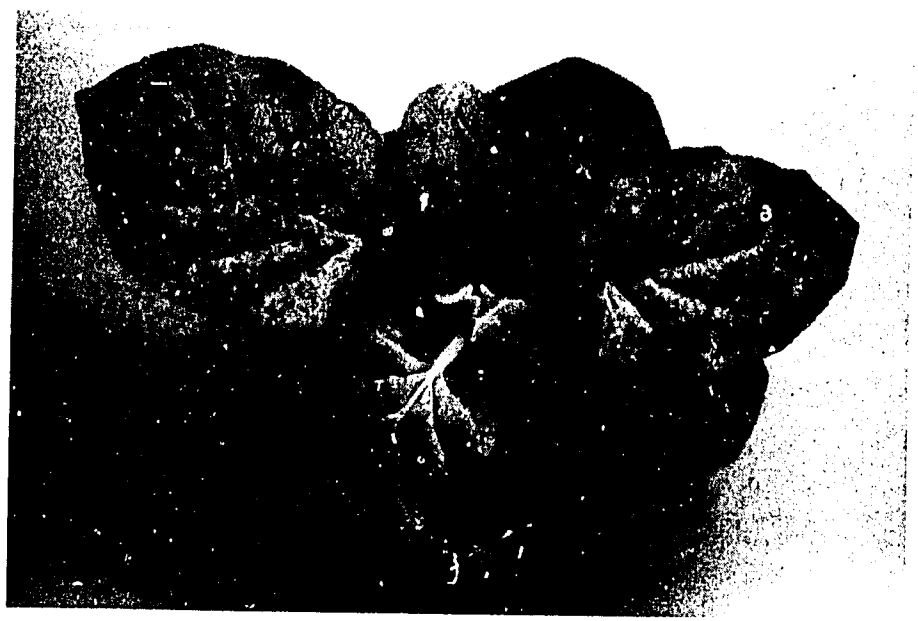
- Local symptoms remain restricted to the site of inoculation (Fig. 27).
- Systemic symptoms appear on non-inoculated leaves (Fig. 28).

Certain potato viruses produce only local symptoms in certain indicator hosts while others produce both local and systemic symptoms. (See table)

We recommend to keep a pure local isolate of a virus for further studies and for serology as a positive control. Field grown plants, are normally infected with more than one virus. Follow the indications given in Figs. 29 + 31 to separate the viruses and produce pure cultures.



27



28

Fig. 29 Separation of PVX and PLRV from a plant with mixed infection.

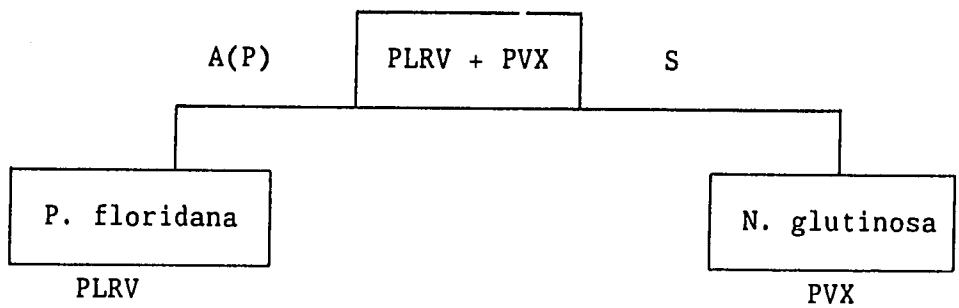


Fig. 30 Separation of PVY and PLRV from a plant mixed infected with PVY and PLRV.

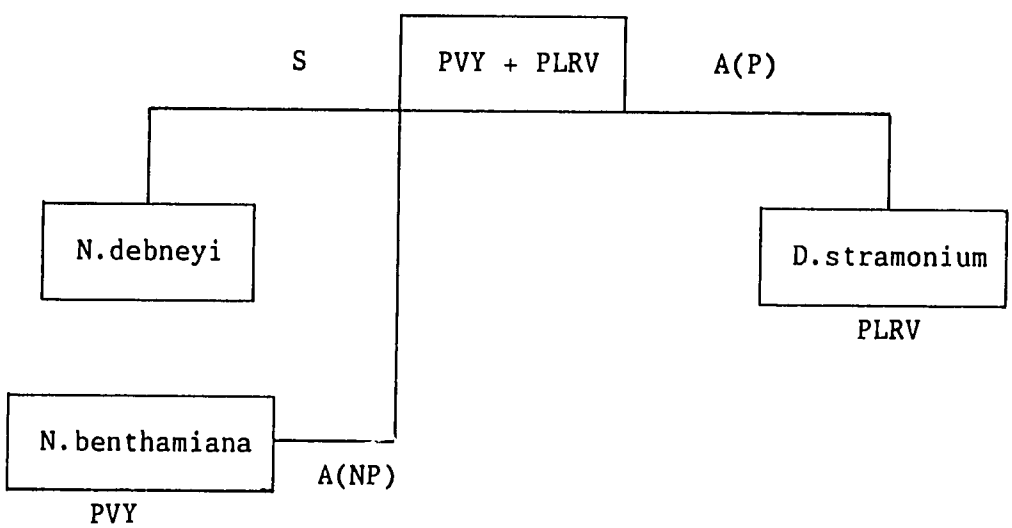
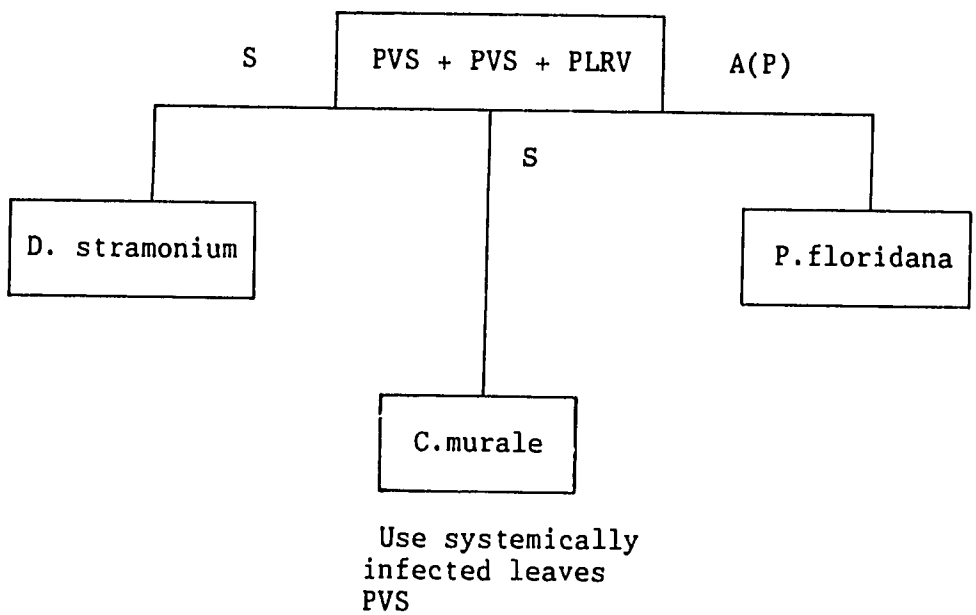


Fig. 31 Separation of PVX, PVS and PLRV from a plant mixed infected with PVX, PVS and PLRV.



A(P) = Aphids M.persiace persistant
(A(NP) = Aphids non-persistant
S = Sap transmission

7 ADDITIONAL STUDY

Hooker, W.J. 1982. Virus diseases of potato. Technical Information Bulletin 19. International Potato Center, Lima, Peru. 17 pp.

Raman, K.V. 1985. Transmission of potato viruses by aphids. Technical Information Bulletin 2. International Potato Center, Lima, Peru. 23 pp.

Salazar, L.F. 1982. Virus detection in potato seed production. Technical Information Bulletin 18. International Potato Center, Lima, Peru. 14 pp.

Nordham, D. 1973. Identification of plant viruses. Methods and experiments. Pudoc, Wageningen, The Netherlands. 204 p.

XVII-TR-E-10-0-300

Processed and printed by CIP's Training and Communications Department
August 1989
Copies printed: 300