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Virus Detection in Potato Seed Production

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Virus detection by indicator plant inoculation

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INTERNATIONAL POTATO CENTER (CIP) Lima – Peru

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Objectives. Study of this bulletin should enable you to:

- explain nature and effects of potato viruses.
- describe principles and objectives of virus detection.
- explain principles and discuss application of the most common methods of virus detection (see sections 4, 5 and 6).

Study materials.

- Potato plants showing different virus symptoms.
- Indicator plants with different virus symptoms.
- Illustrations or actual samples of serological methods.

Practicum.

- Practice virus detection in field and storage.
- Demonstrate those serological methods that are easily feasible under your local situation.

Questionnaire.

1. Which plant parts are invaded by most viruses?
2. How big can yield losses be?
3. How can virus diseases be controlled?
4. Why should viruses be detected as soon as possible after infection?
5. On which criteria would you base choice of detection methods?
6. Under wich considerations can production of completely virus free seed be undesirable?
7. Which pre-conditions are required for symptom observation in the field?
8. What are principal virus symptoms on potatoes?
9. What are the advantages of symptom observation in the field?
10. When is symptom observation in the field the method indicated?
11. What are the pre-conditions for application of the indicator plant test?
12. Describe the principle of serological tests.
13. Why is the latex test more sensitive than microprecipitation?

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Virus Detection in Potato Seed Production

1 Introduction
2 Nature and effect of potato viruses
3 Principles of virus detection
4 Symptom observation in the field
5 Indicator plant test
6 Serological tests
7 Additional reading

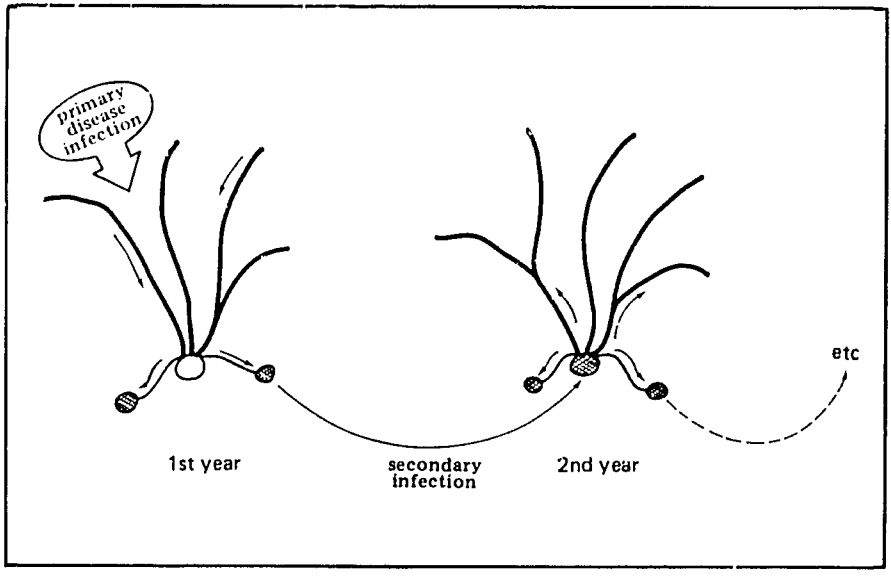
1 INTRODUCTION.

Viruses, extremely small disease-causing agents, can reduce potato yields significantly. Once in a potato plant, most viruses are transmitted to following generations through infected seed tubers. In most practical cases, viruses can only be eliminated by eradication of infected plants in a seed production program. Virus infections are not always visible on the potato plant. Several detection methods are available, some more dependable than others. Choice of method depends on the practically desired degree of virus reduction in a field and on the facilities available.

2 NATURE AND EFFECT OF POTATO VIRUSES.

Viruses are very small; medium size particles for example 500 nm = 0,0005 mm. They can only be seen with the aid of an electron microscope. They multiply in living cells and are transmitted to healthy plants by insects, nematodes, fungi, or mechanical contact. Successful primary transmission results in so called **primary infection**. Whenever the plant is susceptible, the virus multiplies and further invades the plant tissue.

As the infected plant grows and matures, most viruses may invade all vegetative plant parts, including tubers. Most potato viruses pass from crop to crop through infected tubers. Infections originating from diseased seed tubers are termed **secondary infection.**



Following initial virus infection, diseased seed tubers transmit most viruses. Disease severity increases from crop to crop. The disease cycle is interrupted by eliminating virus diseased plants from seed multiplication.

Some viruses infect pollen grains or ovules and are transmitted through botanical seed.

Losses caused by potato viruses are both quantitative (yield reduction) and qualitative (reduced market value). Type and severity of losses depend on plant and environmental conditions and the virus involved. Some 20 viruses cause disease in potato. Potato virus X, S or M (PVX, PVS, PVM) can reduce yields by 5% to 40%, others, such as potato leafroll virus (PLRV) or combined infections of potato viruses X and Y (PVX + PVY), cause yield losses up to 90%.

In practice, virus-infected plants cannot be "cured." Generally, disease severity and dissemination increases from one growing season to the next, unless the disease cycle is interrupted. An effective way to interrupt the disease cycle is to exclude virus-diseased plants from seed multiplication. This requires detection of virus infections.

3 PRINCIPLES OF VIRUS DETECTION.

Virus detection techniques seek to identify the cause of virus infections

- as soon as possible after infection, and
- before the infected plant becomes a reservoir for further infection.

Several detection techniques vary in sensitivity and complexity. None of the techniques is completely accurate. Efficiency depends also on skill and training of a technician.

An optimum technique should combine high sensitivity with simplicity of application and low cost. Since none of the techniques is ideal for all situations, base choice of methods on the following criteria considering given possibilities of your specific situation:

- desired degree of virus reduction
- stage of seed multiplication,
- viruses involved,
- facilities available.

Desired degree of virus reduction. The limits of health tolerance depend on need of a seed production program. Some viruses cause only insignificant losses or are not prevalent in the region. Indiscriminate production of **virus free seed** can be **undesirable** and costly. A seed production program should be restricted to the most prevalent and severe viruses.

Stage of seed multiplication. Seed multiplication is conducted in various steps. If it is only final multiplication, simple and cheap virus detection methods are suitable. If individual mother plants for basic seed production must be tested, more sensitive detection techniques are needed as those plants are the basis of the entire program.

Viruses involved. One detection method may be effective for a specific virus but not for another. Thus, choice of methods depends also on the virus involved.

Facilities available. Some detection methods require a minimum of pre-conditions such as symptom observation in the field that needs only experienced inspectors. Use of other techniques depends on availabity of greenhouses, laboratories, equipment and materials. An indicator plant test can only be conducted effectively when insect-proof greenhouses are available. Serological methods depend on availability of antisera.

The most common techniques to be discussed in detail here are:

- symptom observation in the field,
- indicator plants test,
- serological tests.

Additional techniques are restricted to specific viruses or applications, such as the callose test for potato leafroll virus (before ELISA was developed), electrophoresis for potato spindle tuber viroid, and electron microscopy for virus particles in plant sap extracts.

4 SYMPTOM OBSERVATION IN THE FIELD.

Most potato viruses induce symptoms in infected potato plants and occasionally in tubers. Symptom observation in the field is inexpensive and does not require specific facilities. Only the observer needs to be trained.

On growing plants, reduced growth, yellowing, mosaic, necrosis and leaf deformation may be observed. Tuber symptoms include spindly shape, cracks, deformation and necrosis. Environmental factors, such as temperature, light intensity and soil fertility (mainly excess of nitrogen) influence symptom expression. In some circumstances, infected plants show only mild symptoms or no symptoms at all (symptomless or latent infections). Some viruses may produce similar symptoms on the same potato variety. In other cases different varieties may react differently to the same virus. Combined infections by various viruses change symptom expression. Virus symptoms can be confused with non-parasitic causes or with symptoms caused by other pathogens.

Difficulty in detecting mild or latent infections is another disadvantage of observing symptoms in the field. Nevertheless, symptom observation detects the most important viruses.

Symptom observation in the field is useful when large potato populations must be examined in later stages of seed multiplication. It is the method indicated for programs seeking infections which reduce yields significantly.

CIP's publication, "The Potato: major diseases and nematodes," may be a useful aid in observing virus symptoms in the field. See also Cortbaoui, 1980 (section 7).

Symptom observation in the field is well able to detect most important viruses. Plant symptoms include leaf yellowings (left) and leaf deformations (right).

5 INDICATOR PLANT TEST.

Many potato viruses also infect other plants, including those from other genera and families. Some host plants react with conspicuous and characteristic symptoms, such as different types of systemic mosaics or local lesions. These characteristic symptoms on so called **indicator plants** help detect and identify potato viruses, even when they are latent.

Characteristic symptoms on indicator plants include systemic mosaics (left; *Nicotiana debneyi*) and local lesions (right; *Solanum demissum* A6).

Some common indicator plants are: A6 clone (*S. demissum* x *S. tuberosum* "Aquila"), *Chenopodium amaranticolor*, *Gomphrena globosa*, and *Physalis floridana*.

Indicator plants are generally cultivated from seed in greenhouses. At a certain growth stage they are inoculated mechanically or by insect vectors (principally aphids). The first typical symptoms can be observed about a week after inoculation.

The indicator plant test is sensitive to many viruses and relatively simple to conduct. It is recommended for testing mother plants or basic seed material. Indicator plant cultivation must be synchronized so that plants of correct growth stage are available at the precise time. The method requires growing facilities (greenhouses) and materials (pots, soil, simple laboratory materials).

6 SEROLOGICAL TESTS.

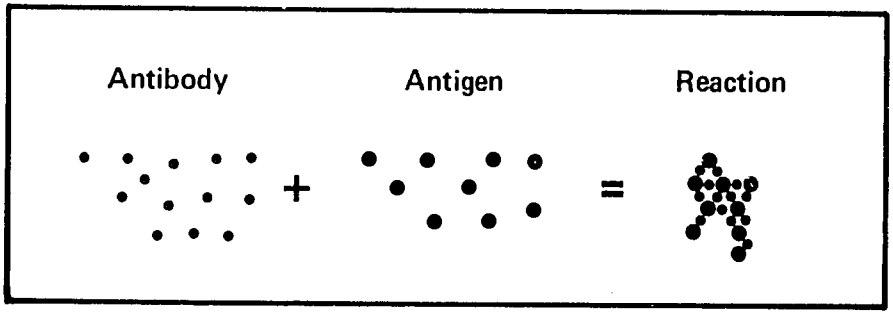
All serological tests are based on the same principle.

When warm-blooded animals (rabbits, for example) are injected with **antigens** – disease-causing agents or some organic molecules – other proteins, the so called **antibodies**, are formed in their blood serum. Many potato viruses are good antigens.

The serum can be separated and is called **antiserum**. When properly prepared, an antiserum is so specific that it reacts only with the virus (antigen) which induced the production of antibodies.

When antibodies and antigen are combined **in vitro** a reaction takes place. This reaction can be observed in different ways, depending on the method employed. A positive reaction indicates the presence of the virus under consideration.

Most common serological tests are microprecipitation, latex test and ELISA.

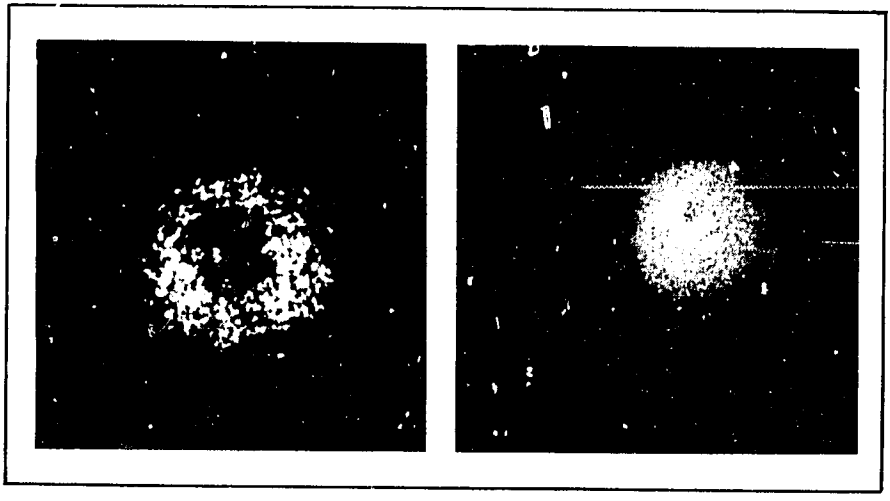


When antibodies and antigen are combined in vitro a reaction takes place.

Microprecipitation. A drop of antiserum is mixed with a drop of clarified plant sap. After a certain time, a flocculation can be observed, preferably with the aid of dark-field microscopy. Flocculation occurs with infected sap samples but not with healthy samples.

Latex test. The latex test is similar to microprecipitation. Sensitivity of the test is increased by joining the small antibody molecules to relatively large (0.85 μm) latex spheres before the test is carried out. (Latex is an organic macromolecule of polystyrene).

A drop of antibody-sensitized latex is mixed with a drop of plant sap. If sap contains virus, this will cause latex particles to flocculate. The reaction is visible without a microscope. The latex test is 100 to 1000 times more sensitive than microprecipitation, does not require clarification of the plant sap, shows a quicker reaction, and needs less antiserum. It does require some chemicals and laboratory facilities.



In the latex test, flocculation (left) is visible without using a microscope. (Right no - negative - reaction).

ELISA. The term ELISA is an acronym formed from EnzymeLinked Immuno sorbent Assay. It requires more preparative steps than the techniques mentioned previously. It is the most sensitive serological method in use and allows quantitative estimation of virus concentration in sap samples.

Generally, serological tests are preferred for virus diagnosis because of their suitability for testing a large number of samples. Antisera are available for most potato viruses. Serology is usually less sensitive than the indicator plant test.

ELISA is the most sensitive of the three serological techniques described, followed by the latex test. Microprecipitation, although least sensitive, is useful when laboratory facilities are not available to conduct the other tests.

Both microprecipitation and latex test may show non-specific flocculation to sap components other than the target virus. The reaction may fail when the quantity of antibodies considerably exceeds the quantity of virus particles, or vice-versa. This is the reason samples are tested at two different dilutions.

7 ADDITIONAL READING.

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