

Testing for Plant Diseases in Plant Material

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EXCLUSION OF PESTS

Controlled propagation of quality plants and plant products requires more than horticultural excellence. It also requires tests or indexing procedures to ensure freedom from specific pests and pathogens.

Plant diseases can be controlled by chemicals, altering cultural conditions, breeding or engineering resistant cultivars, and by various administrative methods. One aspect of the administrative approach aims to exclude specific pests and pathogens. This exclusion of pests and pathogens can be of interest from:

- 1) An international point of view and be done for example by prohibiting the movement of plants from an infested country into one which is free of the particular pest or disease.
- 2) A national point of view and be done by preventing the spreading of a disease from one nursery to another.
- 3) From the point of view of the single grower and be done for example by preventing the spreading of the disease from the production area to the propagation area. One should look at specific diseases and find out if there are only one or several host plants.

In most situations absence of the pathogen or pest must be demonstrated in order to get a certificate that guarantees freedom for specific diseases, but in some cases a tolerance may be allowed. A low percentage of infection in a seed crop may be allowed because at that level it is not considered damaging. Above a given level the risks of damage are considered too high and the crop is rejected.

INDEXING

When dealing with exclusion, indexing is essential for this purpose and implies that some assessment for the presence of a specific pathogen or pest on a given crop or consignment has been made. Where a pathogen is established the ultimate objective of indexing is usually eradication. But it must of course be cost effective to achieve this goal.

There are different factors which influence the success of indexing:

- The plant material.
- The sample size.
- The specificity and sensitivity of the diagnostic methods.
- The biology of the plant and pathogen.
- The possibility of re-introduction of the pathogen.

Reducing the incidence of a pest or pathogen is likely to reduce the intensity of disease but because all indexing methods have a threshold of detection below which they give a negative reaction, they are unlikely to guarantee the absence of a specific pathogen in a single indexing exercise.

Ten years ago Danish potato growers had a major problem with potato ring rot caused by *Clavibacter michiganensis* subsp. *sepedonicus*. Through the use of meristem culture for production of the prebasic potatoes and indexing the seed

potatoes no cases of potato ring rot in Danish seed production are present and only a few incidences per year are found in the final production (Table 1).

The situation is similar for the production of *Pelargonium*. A number of countries have severe problems with respect to *Xanthomonas campestris* pv. *pelargonii*, however, in Denmark an effective system of indexing has been introduced and the inspectors never see the disease.

METHODS OF DIAGNOSIS

The different methods used in indexing for diagnosis should be cheap, rapid, specific, and/or sensitive.

Cheap and Rapid. Cheap and rapid are the two issues covering the costs of actual staff input and the length of time it takes to obtain the results of indexing procedures. Some virus testing methods include grafting and take many months before results are obtained. Many diagnostic methods are labour intensive because bacterial isolation, purification, and identification by traditional bacteriological methods are required.

Specificity. Specificity means that the actual pathogen has been found by a certain method and the taxonomic parameters are acceptable.

Sensitivity. All the diagnostic methods have a threshold of detection. Two major factors affect this threshold — the efficiency of sampling and the test methods which are used to detect the presence of the pathogen in a selected sample. A negative result in an indexing test means only that the target organism was not found in that sample.

Sampling, Extraction, and Diagnostic Methods.

Bacteria. As the density of pathogenic bacteria is generally higher in older than in younger plant parts, the samples to be tested should be taken from the base of the stem. The optimal time of year for sampling depends on the actual pathogen to be detected. Bacteria are extracted by shaking the plant material in water. After shaking one of the following diagnostic methods is used: immunofluorescens, ELISA, DNA-based methods, bioassays, isolation, or protein profiles.

Viruses. Factors that influence the success of indexing tests for viruses include the type of plant tissue collected, distribution of virus in the plant, time of the year, stage of plant maturity, time and temperature at which plant samples are stored, and presence of different virus strains. Samples to be tested usually are prepared by squeezing the leaves in an extraction buffer with a press or a power driven crusher (Pollähne roller press). After squeezing the sample is tested by one of the following diagnostic methods: ELISA, indicator plants, electron microscopy, DNA-based methods, or protein profiles.

Fungi. Testing for fungi is generally limited to specific instances where symptomatic plant parts are sampled and tested for the presence of fungi. The diagnosis of fungi is done by isolation, bioassay, and light microscopy.

Table 1. Testing for potato ring rot in seed potatoes.

Year	Samples (no.)	Area of control (ha)	Infected samples (no.)
1992	2813	10,357	30
1993	3643	7647	9
1994	1388	5505	8
1995	1476	5660	0