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Prunus necrotic ring spot virus (PNRV) identified with ISEM directly from the trees

Påvisning af Prunus *nekrotisk ringplet virus (PNRV) med ISEM direkte fra træerne*

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Summary

This report provides a guide to the use of immunosorbent electron microscopy (ISEM) in the detection of *Prunus* necrotic ring spot virus (PNRV) in trees throughout the year. The report describes in which part of the tree the highest concentrations of virus were found at different times of the year. The most suitable method for extracting the virus for ISEM preparations is also described. The methods are reliable and quick for single and limited amounts of samples tested with ISEM.

Key words: ISEM, *Prunus* necrotic ring spot virus, virus concentration, enzyme-linked immunosorbent assay (ELISA).

Resumé

Denne rapport giver retningslinjer for påvisning af *Prunus* nekrotisk ringplet virus (PNRV) med immunosorbent elektronmikroskopi (ISEM) direkte fra træerne året igennem. Der er oplysninger om anvendelse af forskellige dele af træerne, der indeholder den højeste koncentration af virus, som er tilgængelig for fremstilling af pålidelige præparater for ISEM-teknik. Der beskrives forskellige udtræksmetoder, som er særlig anvendelige på de forskellige årstider. Fra knopper i årets første fire og sidste fem måneder, hvor virus udtrækkes med diffusion i fosfatstødpude tilsat polyethylenglycol (PEG), og ved udmasning med karborundumpulver fra endnu ikke udfoldede eller meget unge blade i månederne maj–juni og til dels i juli måned. ISEM-metoden er pålidelig og hurtig for et begrænset antal prøver, som daglig kommer ind til afprøvning, og metoden er særdeles velegnet til samarbejde med Enzym-linked immunosorbent assay (ELISA) (5, 6), som er den foretrukne metode, når det gælder mange prøver (massetestninger).

Nøgleord: ISEM *Prunus* nekrotisk ringplet virus (PNRV), viruskoncentration, enzym-linked immunosorbent assay (ELISA).

Introduction

The ISEM method is a reliable method for electron microscopic diagnostic work with virus. The method is suitable for single or limited amounts of samples, but also corresponds well with ELISA testings (mass testings). Individual samples from cherry trees in some orchards were tested continuously for two years. Experience and results from these tests are given here as a guidance for ISEM testing of trees for *Prunus* necrotic ring spot virus (PNRV). As testified by ELISA reports, it is now possible to analyze samples direct from the trees throughout the year. Two different methods are described, where different parts of the trees are used in order to obtain the highest concentration of particles and to give the purest preparations suitable for »trapping« and »decoration«, which give the highest reliability of results with ISEM.

Materials and methods

Samples from sour cherries were regularly brought to the laboratory for testing from the beginning of January to the end of December. The following parts of the trees were tested for virus with the ISEM technique: leaf buds, folded and very young leaves, young leaves, mature leaves, flowers, flower buds and berries.

The material was prepared in two different ways: the ordinary »macerating« method with carborundum powder and phosphate buffer pH 7.0 and 0.1 M with 2 per cent poly ethylene glycol (PEG) and centrifuged at 10,000 rpm for 10 min. The »extraction method« was performed in the following way: Buds were cut into small pieces and left overnight also in 0.1 M phosphate buffer pH 7.0 with 2 per cent PEG and centrigfuged for 10 min. at 10,000 rpm at room temperature.

The ISEM technique (7) was used to decorate the particles, and identification took place in a 201 Philips electron microscope at 100,000 magnification. When scanning at least 5 squares (on a 400 mesh grid), 10–15 samples could be examined per hour.

Results

The method of extraction was most successful in the period from January to May and also from September to December. In the months where the trees were in leaf, the maceration method was most sucessful. The material tested during the first two periods were buds at different stages. Sometimes the material was very scarce, particularly in the months after the growing season until the end of the year; but even at low concentrations the decorated virus particles (Figs 1a and b) were easily detected in the electron microscope when using the extraction method. The concentration of virus particles was high in the months of April (Figs 1b and 2) and May, but then de-

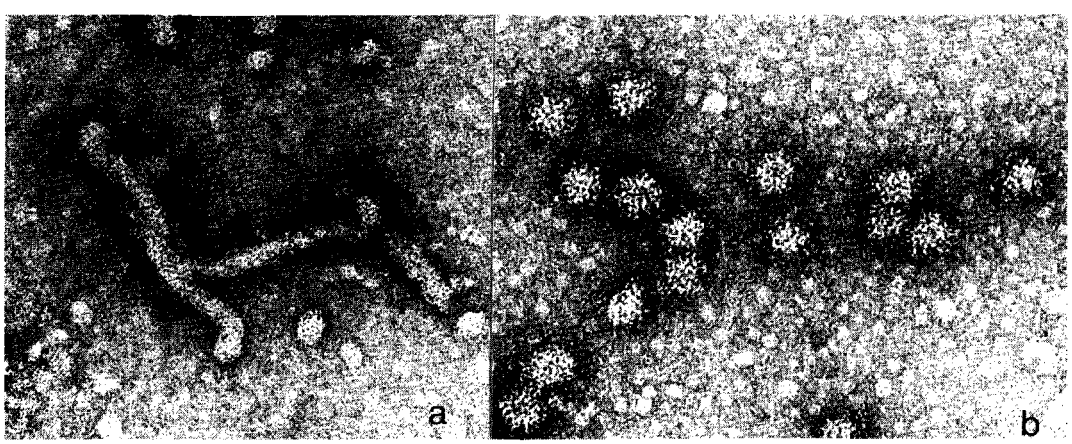

Fig. 1. Decorated PNRV particles with a. irregular and b. regular shape × 140,000

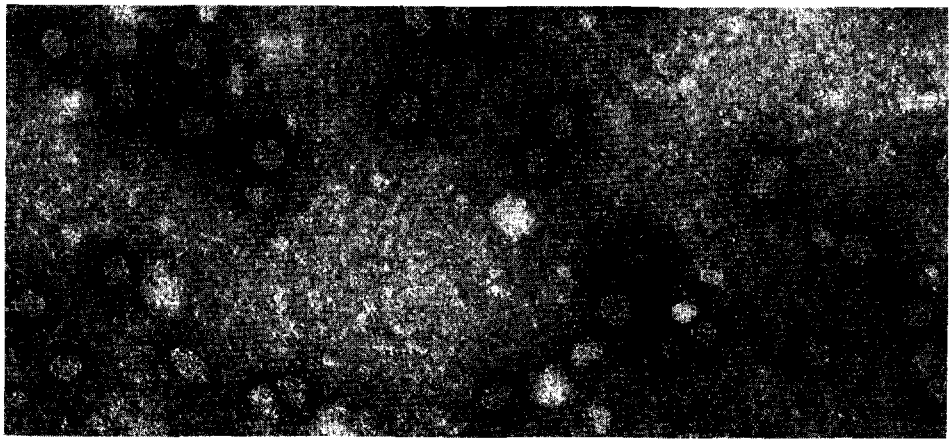

Fig. 2. Undecorated, trapped PNRV particles × 140,000

Table 1. *Prunus* necrotic ring spot virus (PNRV) throughout the year materials and methods

Months	Part of the tree	Method	Symbols
January	buds	extraction	
February	buds	extraction	
March	buds	extraction	
April	buds	extraction	
May	flowers, new leaves	maceration	
June	folded/new leaves	maceration	
July	folded/new leaves	maceration	
August	new leaves, buds	maceration	
September	buds, new leaves	maceration & extraction	
October	buds	extraction	
November	buds	extraction	
December	buds	extraction	

creased slowly until about August. August was the most difficult month for testing and definitely the month where the lowest virus concentration was found both in leaves and in buds. The procedure throughout the year and the methods described appears in Table 1.

It is found to be an advantage for the ISEM technique to clear the sap by centrifugation especially when the virus concentration is low. Glow discharge cleaning of the support film (formvar) was also found to be of great use for detection of the virus particles in low concentrations, as the

background stays completely neutral and staining becomes more uniform.

Conclusion and discussion

Testing for PNRV with ELISA technique (1, 3, 4, 6) at different times of the year led to the same results as reported here. No reports have been given until now on the identification af PNRV with ISEM, although ISEM had the advantage of enabling a small number of samples to be conveniently handled. The extraction methods have solved the problem of extremely low virus concentrations.

The short new branches which were tested in this work mainly carried flower buds. Apical buds were found to have a high virus content.

Samples are usually prepared in 2 per cent PTA, which means that they can be stored and the serological testing performed later when convenient (2). Whether this can also be done with samples tested with ELISA has not been included in our investigations. It must be taken into consideration that extremely low temperatures are reported to decrease the virus concentration in the trees(8).

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