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Arctium lappa L., a new host for Tobacco Ringspot Virus (TobRV) identified with immuno sorbent electron microscopy (ISEM)

Arctium lappa *L., en ny værtplante for Tobak ringplet virus (TobRV) identificeret med immuno sorbent elektronmikroskopi (ISEM)*

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Summary
Arctium lappa is a new host for Tobacco Ringspot Virus. Identification of Tobacco Ringspot Virus was made with the ISEM-method on a virus suspension stored for nearly ten years in PTA-stain.

Key words: *Arctium lappa* L., ISEM, diagnosis, Tobacco Ringspot Virus.

Resumé
Arctium lappa (glat burre) er en ny værtplante for Tobak ringplet virus (TobRV). Identifikation af Tobak ringplet virus er udført med ISEM på en virussuspension opbevaret ca. 10 år i PTA stain (kontrastfarve).

Nøgleord: *Arctium lappa* L., diagnose, ISEM, Tobak ringplet virus.

Introduction

In 1971 an *Arctium lappa* L. plant with yellow mosaic symptoms was collected from the roadside near Uppsala in Sweden. It was examined in a leaf dip sample in the electron microscope and also inoculated to a series of different test plants for identification of the virus.

The leaf dip examination revealed isometric virus particles app. 30 nm. Inoculation to different test plants failed, and a sample of the virus suspension in 2% PTA was stored away in a refrigerator (4°C). The introduction of reliable immuno sorbent electron microscopy (ISEM) opened up possibilities of identifying the virus nearly 10 years later. The virus suspension still stored in the refrigerator in 2% PTA was tested against 6 different antisera (NEPO) and also against antiserum of Tobacco Necrosis Virus (TNV).

Material and Methods

1971. *Inoculation to test plants.*

Plant sap from symptom bearing *A. lappa* was mechanically inoculated after collection to test plants rubbing of homogenate of *A. lappa* to *Chenopodium quinoa, Cucumis sativus, Phaseolus vulgaris* and *Nicotiana clevelandii.*

Leaf dip. Preparations were made with 2% PTA at pH 6.5 on formvar carbon coated grids with spray technique.

1981. *The ISEM test.*

The ISEM procedure following the method of *Roberts* and *Harrison* (1979) was repeated 3 times. The virus in the 2% PTA suspensions was tested against the following antisera:

Tomato Ringspot Virus
TomRV titer 1:256
Raspberry Ringspot Virus
RRSV 1:256
Tobacco Ringspot Virus
TobRV 1:256
Strawberry Latent Ringspot Virus
SLRV 1:512
Cherry Leafroll Virus
CLRV 1:1024
Tobacco Necrosis Virus
TN/A and TN/D 1:1280

Trapping

The grids were floated for 5 minutes on 10μl drops of antiserum diluted 1:1000 rinsed with 40 consecutive drops of 0.1 M phosphate buffer pH 7.0, and then placed for 20 minutes on 10μl drops of undiluted plant sap in PTA from the samples stored in the refrigerator for 10 years. The grids were finally rinsed with 40 drops of distilled water and stained with 6 drops of aqued uranyl acetate 2%. The antiserum was kindly supplied by Dr. *H. L. Paul* in Braunschweig.

Decoration

The decoration was performed according to *Milne* and *Luisoni* (1975) with antisera diluted 1:100 after the trapping of the virus particles.

Post inoculation

The same test plants as mentioned under »inoculation to test plants 1971«, were inoculated with the virus suspension in PTA after 10 years storage and carried out in the same manner as described.

Results

1974. *Inoculation to test plants*

The inoculation immediately after collection, for unknown reasons failed, and an identification of the virus could not be obtained.

Leaf dip preparation

Preparates from *A. lappa* revealed a very high concentration of isometric virus particles (Fig. 1) by the test performed shortly after the collection

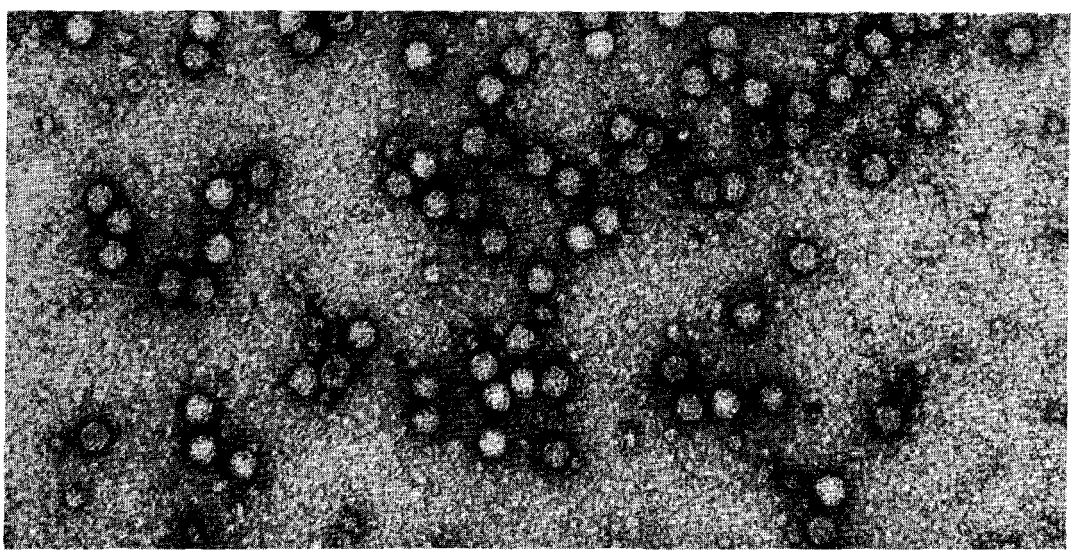

Fig. 1. Leaf dip preparation from *Arctium lappa*.
Leaf dip præparation fra A. Lappa. × 111.200.

of the plant, but no identification was possible with the leaf dip after the inoculation to test plants failed. The virus suspension was stored away in 2% PTA and sealed with a rubber stopper.

1981. *The ISEM test*
The examination of the virus suspension in PTA 10 years later with the ISEM trapping and decoration revealed many more virus particles than with the ordinary leaf dip method (Fig. 2 and 3). The decoration of the virus particles with TobRV antiserum was quite clear (Fig. 3). A measurement of app. 100 virus particles gave the result of 29 nm with 2% U:A:

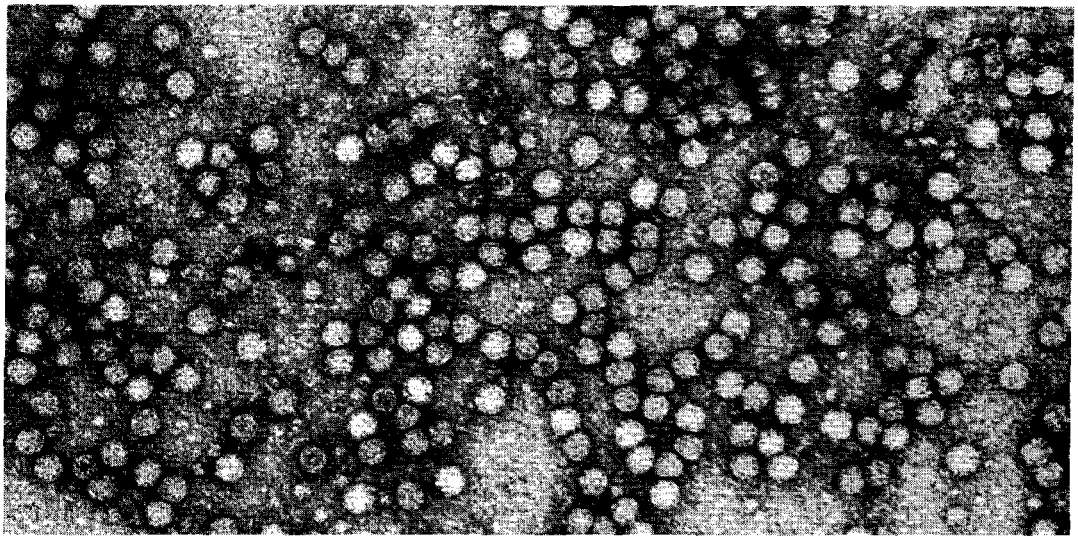

Fig. 2. ISEM test with trapped particles from *Arctium lappa* stored in 2% PTA for 10 years.
ISEM analyse med »fangede« partikler fra A. lappa *opbevaret 10 år i 2% PTA.* × *111.200.*

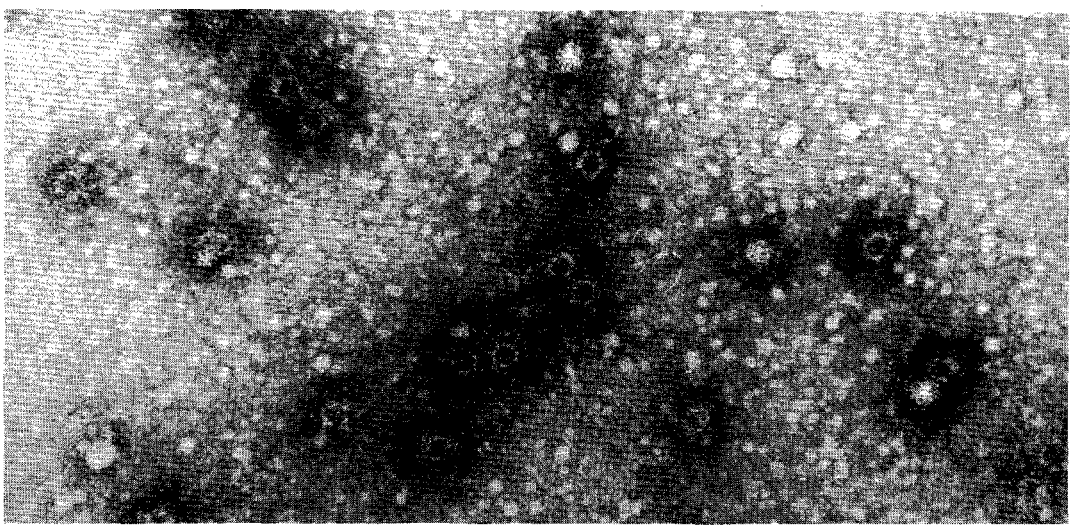

Fig. 3. ISEM test with decorated particles, identifying the virus as Tobacco Ringspot Virus.
ISEM analyse med dekorerede partikler, som identificerer viruset som Tobak ringplet virus. × *111.200.*

Discussion

Why the inoculation to test plants *Chenopodium quinoa, Cucumis sativus, Phaseolus vulgaris* and *Nicotiana clevelandii* failed in 1974 cannot be explained. The test plants are recommended for TobRV (*Gibbs et al.*, 1970) and also by *Tu* (1980). Nevertheless it is surprising that the virus reacted with its antigen 10 years later after storage in PTA. Even though the serological reaction in the ISEM test is based on a reaction with the coat protein and not related to infectivity of the particle.

The inoculation test in 1981 with the virus from the PTA suspension did not give any results as the PTA undoubtly had damaged the singel stranded infectious RNA.

The illustrations of the ISEM test with the decorated virus particles (Fig. 3) and also the increased amount of virus particles in the trapping preparation (Fig. 2) compared to the ordinary leaf dip preparation (Fig. 1) of the same virus suspension with 10 years interval is convincing, and there is no doubt in reporting the virus from *A. lappa* as being TobRV and thereby adding a new plant to the host range of this virus.

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