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A comparison of electron microscopy and serology with infectivity tests for the detection of chrysanthemum virus B

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Abstract

Two methods for the detection of chrysanthemum virus B in chrysanthemums were compared with the conventional infectivity test on *Petunia* plants. The reliability of electron microscopy tests was not inferior to that of infectivity tests provided that plants not yielding visible particles in the first test were tested a second time. A newly developed micro-agglutination test was least sensitive and is not recommended.

Introduction

When indexing chrysanthemum mother plants for the presence of chrysanthemum virus B (CVB) infectivity tests on *Petunia* plants (Noordam, 1952) may be used. These tests require much time and glasshouse space. A quicker method is the micro-precipitin test adapted for chrysanthemums by Hakkaart et al. (1962). The sensitivity of this serological method has proved satisfactory, but the pretreatment of plant sap is time-consuming, making the method not as rapid as desired. An attempt was therefore made to simplify serological diagnosis by developing a micro-agglutination method to make utilisation of crude sap possible.

The recent development of quick and simple preparation techniques, such as negative staining of crude sap specimens (Hitchborn and Hills, 1965), makes the electron microscope another potential tool for routine diagnosis. In working on filamentous chrysanthemum viruses Hollings and Stone (1967) found electron microscopy of quick leaf-dip preparations to be more sensitive, reliable and rapid than inoculation to *Petunia* and grafting to 'Good News' and 'Mistletoe' chrysanthemums. However, the method did not indicate which virus of the leaf mottling complex (chrysanthemum vein mottle, dwarf mottle, necrotic mottle and virus B) was present.

In this paper results are reported of experiments comparing three indexing methods for CVB. It was thought best to apply each method in its optimal form and to compare the results obtained in testing a number of plants of unknown state of health.

Materials and methods

The tests were carried out with 240 glasshouse-grown chrysanthemum plants, cv. 'Chatsworth', previously used in an experiment on the spread of chrysanthemum

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viruses. A number of these plants had been infected by aphids using some other plants in the glasshouse as virus sources. Each plant was tested in three ways. For each test three leaves of about the same age were taken, one leaf from each of three stems.

Infectivity tests. The leaves were crushed in a mortar with 1–2 ml of a solution of 1/30 M Na_2HPO_4-KH_2PO_4 (Sörensen buffer, pH 7) to which Na_2SO_3 was added to make a 0.6% solution. The extract was rubbed with the forefinger on the leaves of two plants of *Petunia hybrida* cv. 'Celestial' previously dusted with carborundum and rinsed with water. When yellow local lesions developed after about 2 weeks the plant was considered infected with a virus of the leaf-mottling complex. When no local lesions had appeared after 5 weeks the test was considered negative.

Micro-agglutination test. A subsample of 0.5 g was taken from the three leaves, wrapped in cotton wool and placed in a hand press. Then 1.5 ml of the same buffer solution used in the infectivity test was added to the cotton wool. Sap was pressed out and mixed immediately with the buffer. The sample was turned over and after a second pressing extract was used for serology by dipping a glass rod first into the extract and then into a drop of antiserum placed in a petri dish. "Dilutions" were made by dipping the glass rod with extract once or twice into the drop of antiserum. After each dipping the glass rod was cleaned with cotton wool. Drops of extract. with normal serum and also with saline were used as controls. Paraffin oil was poured over the drops and the dishes were incubated at 37 °C for 2–3 h. When a flocculation occurred the plant was considered infected with CVB. The antiserum was kindly provided by Ir D. H. M. van Slogteren of the Laboratory of Flowerbulb Research at Lisse.

Electron microscopy. Strips of tissue were peeled from the undersides of the leaves and placed in a drop of 2% potassium phosphotungstate (pH 6.5). Copper grids, covered with a thin film of Formvar, backed with carbon, were used. The grid was held for a few seconds against the drop, allowed to dry and used for examination. The tools were flamed after the preparation of each specimen. One grid was prepared per leaf sample, but when no virus particles were observed, a second grid was prepared in a similar way from strips of tissue peeled from three other leaves of the same plant. When elongated particles of about 680 mμ were observed (Fig. 1), the plant was considered infected. In some cases only one virus-like particle was observed after scanning the grid. To avoid possible confusion with crystals, the observation of at least two particles was considered necessary for a sample to score positive. This number of two particles was arbitrarily chosen. Scanning was done with a Philips EM 300 at ×18,000.

The three tests were carried out in 1968 nearly simultaneously. Electron microscopy was done from 13 September until 1 November, testing for infectivity from 23 September until 26 November and serology from 20 September until 5 November.

Results

Evaluation of the infectivity tests was complicated by contamination of a number of the plants with tomato aspermy virus (TAV). *Petunia* is also susceptible to this virus, producing grey local lesions and systemic symptoms. When the reaction to

Fig. 1. Two electron micrographs of plant sap with particles of chrysanthemum virus B. Magnification × 44,280 (Photographs T.F.D.L., Wageningen).

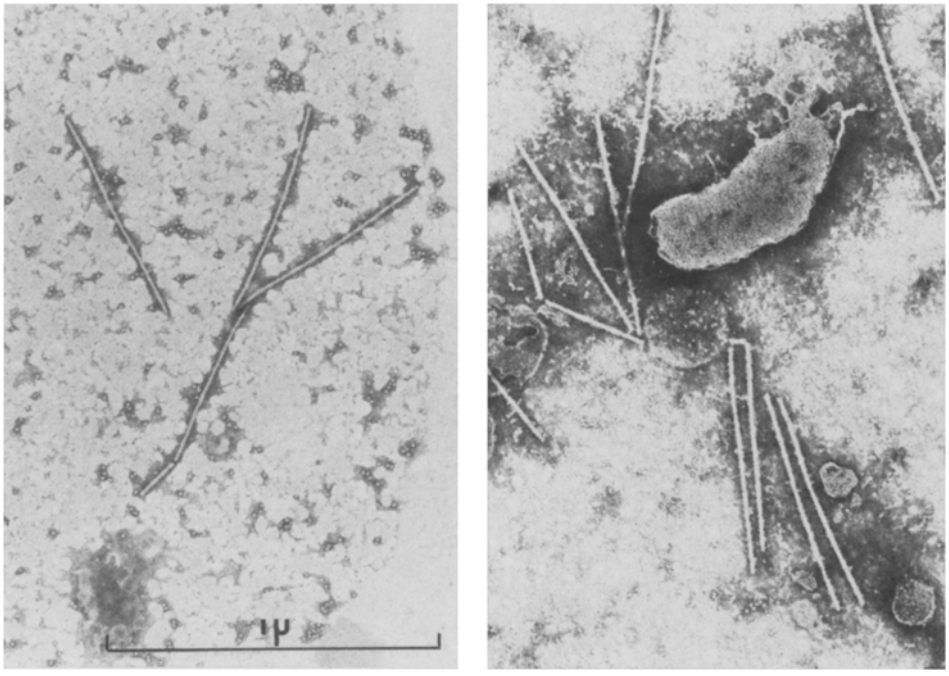

Fig. 1. Twee elektronenmicroscopische opnamen van plantesap met deeltjes van het chrysante-B-virus. Vergr. 44.280 × (Foto's T.F.D.L., Wageningen).

TAV is rapid and severe, CVB cannot be reliably diagnosed. Therefore, in the absence of TAV yellow local lesions were considered diagnostic for CVB; similarly when TAV was present, but yellow local lesions developed clearly. However, when the reaction to TAV was very severe and no yellow local lesions developed, the test was considered inconclusive for CVB.

Results obtained have been combined and are presented in Table 1. More than half of the plants reacted positively and about one fifth negatively in all three types of test. The divergent cases are of special interest for evaluating the three methods. Twenty plants giving no reaction in the agglutination test were undoubtedly infected because they reacted in both the other tests. The infectivity test was not always reliable either (four failures when both other tests were positive), nor was the electron microscopy test (three failures when both other tests were positive). From these figures it is concluded that the agglutination method is the least sensitive and is not recommended for indexing work.

The data in the electron microscopy test were obtained using a second grid if no particles were seen in the first one. To study the effect of a second grid the results of infectivity and electron microscopy tests were further elaborated and compared with those obtained when using only one grid. With one grid 148 infected plants were

Table 1. Comparison of three diagnostic methods for the detection of chrysanthemum virus B in chrysanthemums.

Number of plants (out of 240 tested)	Results obtained with the following tests		
	infectivity	serology	electron microscopy (2 grids used)
127	+	+	+
42	−	−	−
20	+	−	+
3	+	+	−
4	−	+	+
4	+	−	−
6	−	−	+
2	−	+	−
	154	136	157
5	inconclusive	+	+
21	,,	−	−
5	,,	−	+
1	,,	+	−
		142	167

Table 1. Vergelijking van drie diagnostische methoden voor het aantonen van chrysante-B-virus in chrysanten.

detected against 157 with two grids. For these 9 alterations in the recording 60 repetitions were necessary. Of course, the reliability of the *Petunia* test, also, may be improved by repeating the test, but this is laborious.

Discussion

The reliability of the electron microscopy test is not inferior to that of the *Petunia* test. An advantage of electron microscopy is the rapidity with which the diagnosis can be made. The whole procedure was a matter of minutes. An important aspect is the time required for examination before a preparation may be safely considered as free from CVB. In many cases particles were detected immediately. In my experience the first 2 min of examination were decisive and I found it of little use to search a preparation for more than 3 min.

Although many samples were also infected with TAV this virus could not be readily detected with the electron microscope. The routine detection of this isometric virus is at this moment better done by infectivity or serological tests. Furthermore, the electron microscope does not differentiate between viruses of the leaf mottling complex of chrysanthemum (vein mottle, dwarf mottle and necrotic mottle viruses and CVB). The identity of these viruses is still obscure and only CVB has been described in sufficient detail. However, the three first mentioned viruses also seem to have elongated particles and for routine indexing of mother plants the identity of the virus rods may be of minor importance. A more important drawback of electron microscopy for large-scale screening work is the cost of using the instrument. Only when these expenses can be reduced to nearly those of the conventional methods, may electron microscopy have a future for indexing work.

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Samenvatting

Een vergelijking van de elektronenmicroscopie en serologie met infectietoetsing voor het aantonen van het chrysante-B-virus

Twee methoden voor het aantonen van het chrysante-B-virus in chrysanten werden vergeleken met de gebruikelijke infectietoetsing op *Petunia*. De betrouwbaarheid van de elektronenmicroscopie deed niet onder voor die van de infectietoetsing wanneer van de planten, waarin geen virusdeeltjes werden waargenomen een tweede preparaat onderzocht werd. Een gewijzigde micro-agglutinatiemethode bleek het minst gevoelig en wordt niet aangeraden.

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