

Variation of chrysanthemum virus B

F. A. HAKKAART¹ and D. Z. MAAT

Institute of Phytopathological Research (IPO), Wageningen

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Abstract

In an attempt to unravel the complexity of chrysanthemum virus B eight isolates were grafted onto the chrysanthemum indicator cultivars 'Fanfare', 'Good News', 'Mistletoe' and 'Pink Marble'. Six symptomatologically different entities could be distinguished. However, with antisera prepared against two of the isolates, no serological differences were detected. It is concluded that chrysanthemum virus B consists of many strains and that the number of strains that can be distinguished depends on the number of indicator cultivars used. The 'Good News', 'Yellow Doty', 'Nightingale', 'Improved Bronze Daisy' and 'September Morn' mosaic viruses described by Brierley and Smith (1958) as well as the vein mottle, dwarf mottle and necrotic mottle viruses named by Hollings and Stone (1967) should be considered as strains of chrysanthemum virus B.

Introduction

Of chrysanthemum many viruses are described corresponding to a great extent to Noordam's description of chrysanthemum virus B (cryptogram */*.*/*:E/E:S/Ap). They are identified not too well and are differentiated mainly on the basis of symptoms on indicator plants. The problem of the real identity of these viruses and their relation to chrysanthemum virus B became relevant again when the General Netherlands Inspection Service for Ornamental Plants (NAK-S), using serology as a diagnostic test for virus B, occasionally found this test unreliable. The question arose whether different viruses could be involved or whether a serological heterogeneity of virus B might exist in relation to symptomatological differences. The aim of the present paper is to answer this question.

Literature review

Since the introduction of the name chrysanthemum virus B by Noordam (1952) confusion exists about the identity of this virus. Noordam's virus B caused yellow local lesions in petunia about three weeks after inoculation, had antigenic properties and consisted of rod-shaped particles of 600×30 nm. Earlier, Keller (1951) had reported on a previously undescribed virus carried by the cultivar 'Blanche' which he called virus Q. Later, Brierley and Smith (1953) showed that this virus produced yellow local lesions in petunia and concluded that virus Q might be a mild strain of virus B

¹ Stationed at the Research Station for Floriculture, Aalsmeer.

or one of the components of a complex with B. Virus B, defined as giving local lesions in petunia, was found to be common in the United States.

Hollings (1957) first reported virus B in England. His isolates gave local lesions in petunia as well as in *Vicia faba*, except one giving no lesions in *V. faba*. This isolate he named chrysanthemum vein mottle virus. He suggested that virus B, vein mottle and perhaps Keller's virus Q constitute a group of viruses with features in common, but less closely related to one another than is usually implied by the term 'strain'. Unfortunately, Noordam (1952) did not mention *V. faba* as a test plant.

Brierley and Smith (1958) added to the confusion by describing eight mosaic viruses in chrysanthemum and naming them after the cultivar in which they had been found. Five of these (from the cultivars 'Good News', 'Yellow Doty', 'Nightingale', 'Improved Bronze Daisy' and 'September Morn') produced yellow local lesions in petunia and thus fit the description of virus B. By grafting onto 'Dynamo', 'Good News' and 'Mistletoe' symptomatological differences could be demonstrated between these viruses.

Hollings and Stone (1967) referred to a leaf mottling complex, the members of which (chrysanthemum vein mottle, dwarf mottle, necrotic mottle and virus B) were viruses with filamentous particles of about 700 nm, and infectious to petunia. Unfortunately the two new names dwarf mottle and necrotic mottle were introduced without further description of the entities. Later, Hollings et al. (1970) purified chrysanthemum virus B with particles of approximately 685 nm, prepared an antiserum and confirmed the serological relationship between the virus and carnation latent virus that had been reported by van Slogteren et al. in 1962.

Materials and methods

Virus isolates were kindly provided by the NAK-S, and designated according to the cultivar in which they occurred. All isolates ('Sirocco', 'Minstrel Rood', 'Paris', 'Souvenir de Jean Cot', 'Galaxie Sport', 'Roodkapje', 'Red Rolinda' and 'Lilian Hoek') caused yellow local lesions in petunia and had filamentous particles, and thus closely resembled the chrysanthemum virus B sensu Noordam (1952). They were transmitted to indicator cultivars ('Fanfare', 'Good News', 'Mistletoe' and 'Pink Marble') by grafting in twofold. Plants free of virus B served as controls. The isolate 'Lilian Hoek' proved to be contaminated with chrysanthemum stunt viroid, causing stunting in 'Fanfare', 'Good News' and 'Pink Marble', and yellow flecks in 'Mistletoe'. Because the stunt agent is not antigenic, its occurrence did not interfere with the serological experiments.

For antiserum production virus was purified from chrysanthemum (isolates 'Red Rolinda' and 'Lilian Hoek') mainly according to Maat (1973), but to homogenize 100 g of leaves, 250 ml tris-citric acid buffer (0.5 M tris) pH 9, containing 0.4% Na₂SO₃·7H₂O, 0.2% sodium diethyldithiocarbamate and 0.1% sodium thioglycollate, 50 ml diethyl ether and 50 ml carbon tetrachloride were used. For resuspension 0.03 M phosphate-citric acid buffer pH 7 was used with or without additions. Rabbits were injected intramuscularly with an emulsion of virus and Freund's incomplete adjuvant as well as intravenously without adjuvant.

Serological tests were performed according to the micro-precipitin test under paraffin oil and (for reactions with normal plant antigens) to the Ouchterlony double

diffusion test. As test antigens purified viruses ('Red Rolinda' and 'Lilian Hoek') or clarified extracts (all isolates mentioned) were used. Clarification was performed by homogenizing leaves (chrysanthemum) in buffer, diethyl ether and carbon tetrachloride and centrifuging at low speed. Antisera and purified virus were diluted with saline. Clarified extracts were diluted with trisbuffer pH 9 containing 0.5% Na₂SO₃.

Experiments and results

Graftings were performed in 1972 and 1973. In 1972 the isolate 'Red Rolinda' and the indicator 'Fanfare' were not tested. Because the results of 1973 confirm those of 1972, only those of 1973 will be mentioned.

Graftings were made on 2 and 3 May 1973 and the symptoms were regularly recorded until the end of the experiment in the fall. The symptoms consisting of veinbanding, mosaic and leaf deformation started a fortnight after grafting (Table 1).

Table 1. Symptoms caused by isolates of chrysanthemum virus B in chrysanthemum indicator cultivars.

| Isolate | Indicator cultivar | | | |
|----------------------|--|--------------------------------------|---|-------------------------------|
| | Fanfare | Good News | Mistletoe | Pink Marble |
| Sirocco | veinbanding, mosaic, severe necrosis and crinkle | veinbanding, mosaic | veinbanding, mosaic, slight necrosis | mosaic |
| Minstrel Rood | veinbanding, mosaic and slight necrosis | as above | as above | mosaic |
| Paris | as in Sirocco | as above | as above | no symptoms |
| Souvenir de Jean Cot | mild mottling | as above | as above | no symptoms |
| Galaxie Sport | as in Sirocco | as above | as above | no symptoms |
| Roodkapje | as in Minstrel Rood | as above | as above | no symptoms |
| Red Rolinda | stunting, severe crinkle, even death | veinbanding, mosaic, malformation | veinbanding, mosaic, malformation | no symptoms |
| Lilian Hoek | as in Sirocco, stunting ¹ | as in Sirocco, stunting ¹ | as in Sirocco, yellow flecks ¹ | mosaic, stunting ¹ |

¹ Caused by chrysanthemum stunt viroid.

Tabel 1. Symptomen veroorzaakt door chrysantevirus B in chrysante-indicatorrassen.

Based on the presence or absence of symptoms in 'Pink Marble' the isolates could be divided into two groups (Table 1). When using 'Pink Marble' only, one could easily conclude the occurrence of two different entities of the virus.

However, in 'Good News' all isolates produced veinbanding and mosaic, except the isolate 'Red Rolinda', which in addition showed severe leaf deformation (Table 1 and Fig. 1). The results with 'Mistletoe' (Table 1 and Fig. 2) supported those in 'Good News', but in these two cultivars the results did not parallel those in 'Pink Marble'.

Fig. 1. 'Good News' with chrysanthemum virus B isolates 'Sirocco' (A), showing veinbanding and mosaic, and 'Red Rolinda' (B), showing veinbanding, mosaic and malformation.

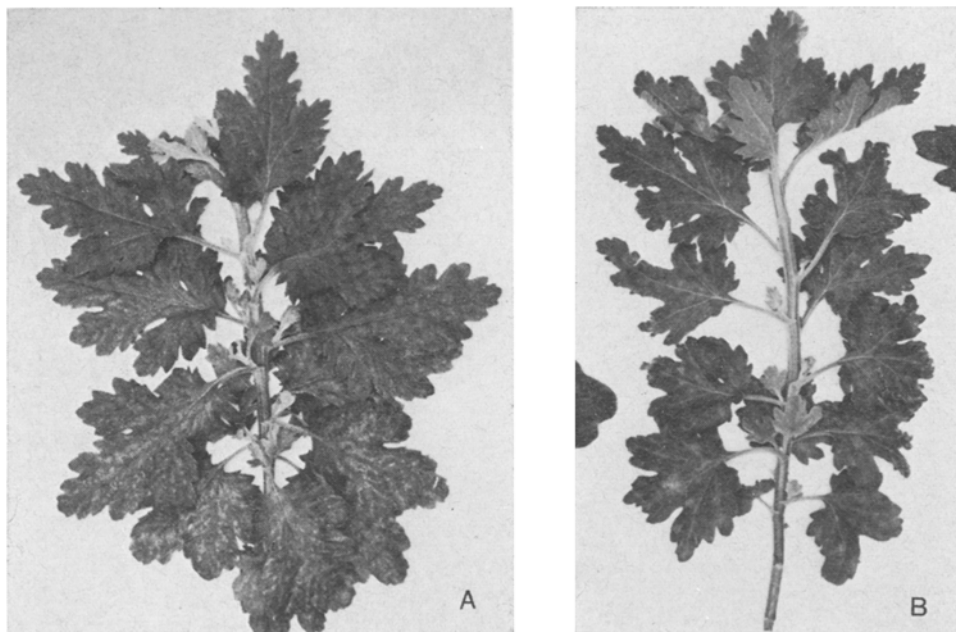


Fig. 1. 'Good News' met de chrysantevirus-B-isolaten 'Sirocco' (A) en 'Red Rolinda' (B).

'Fanfare' further complicated the picture. A series of syndromes could be distinguished (Table 1 and Fig. 3).

Thus, with the four indicators the eight isolates could be grouped into six symptomatologically different entities. With the cultivars used only the isolates 'Paris' and 'Galaxie Sport' and also 'Sirocco' and 'Lilian Hoek' could not be distinguished.

The antisera reacted to the same endpoint (1024) with purified preparations of 'Red Rolinda' and 'Lilian Hoek'. When tested against clarified extracts of the eight isolates mentioned in *'Materials and methods'*, the titres of both sera to all isolates were 64, whereas the titres against samples from healthy plants were 1 or 4. Thus, in these experiments the isolates investigated could not be distinguished serologically.

Discussion

With the two antisera prepared and the test method used, no serological differences were demonstrated, indicating a close relationship between the isolates. They therefore may be considered as strains of one virus. It is very unlikely, that the incidental unreliability of the serological test used by the NAK-S is caused by antigenic heterogeneity of the virus.

By increasing the number of chrysanthemum indicator cultivars, the number of symptomatologically different entities corresponding to the description of Noordam's

Fig. 2. 'Mistletoe' with chrysanthemum virus B isolates 'Sirocco' (A), showing veinbanding, mosaic and slight necrosis, and 'Red Rolinda' (B), showing veinbanding, mosaic and malformation.



Fig. 2. 'Mistletoe' met de chrysantivirus-B-isolaten 'Sirocco' (A) en 'Red Rolinda' (B).

virus B increases. Since the isolates were taken arbitrarily, many more different forms may exist.

Apart from serology the experiments described above resemble those of Brierley and Smith (1958). However, the interpretation is different. These authors considered each entity as a separate virus. We have not been able to detect serological differences between the isolates studied. The only remaining distinction on the basis of host varietal reaction does not justify their description as different viruses. In conclusion we consider our six entities together with the five 'viruses' of Brierley and Smith (1958) and the members of the leaf mottling complex of Hollings and Stone (1967) as strains of one virus, viz. virus B. Unfortunately it is impossible to relate any one of the six strains found here with one of the 'viruses' on basis of descriptions and photographs.

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Fig. 3. 'Fanfare' with chrysanthemum virus B isolates 'Souvenir de Jean Cot' (A), showing mild mottling, 'Minstrel Rood' (B), showing veinbanding, mosaic and slight necrosis, 'Paris' (C), showing veinbanding, mosaic, necrosis and crinkle, and 'Red Rolinda' (D), showing stunting, crinkle and necrosis.

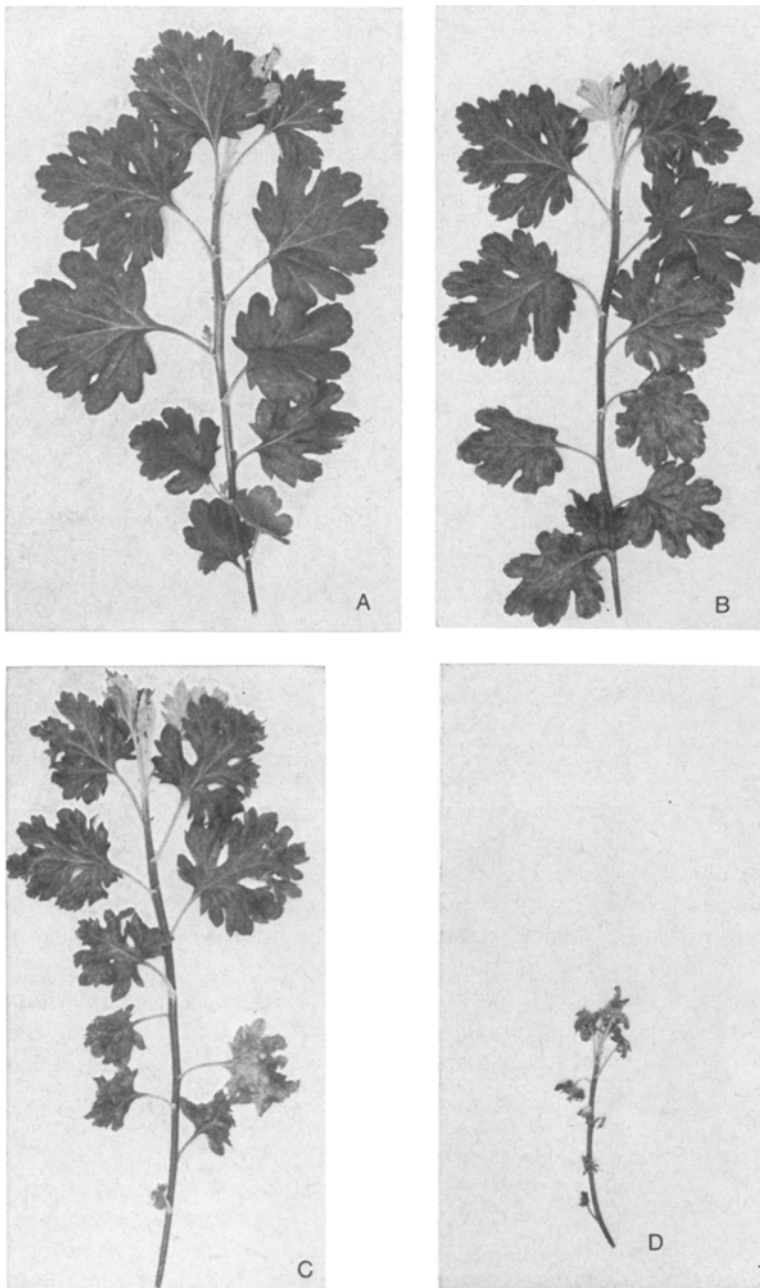


Fig. 3. 'Fanfare' met de chrysantemivirus-B-isolaten 'Souvenir de Jean Cot' (A), 'Minstrel Rood' (B), 'Paris' (C) en 'Red Rolinda' (D).

Samenvatting

Variatie van het chrysantevirus B

In een poging de complexiteit van het chrysantevirus B te ontrafelen werden acht isolaten geënt op de chrysante-indicatorrassen 'Fanfare', 'Good News', 'Mistletoe' en 'Pink Marble'. Zes symptomatologisch verschillende eenheden konden worden onderscheiden (Tabel 1; Fig. 1, 2 en 3), die echter serologisch niet verschilden. De conclusie wordt getrokken dat van het virus vele stammen voorkomen en dat het aantal te onderscheiden stammen afhangt van het aantal indicatorrassen dat men gebruikt. Ook de vijf mozaiekvirussen van Brierley en Smith (1958) en de leden van het 'leaf mottling complex' van Hollings en Stone (1967) dienen als stammen van het chrysantevirus B te worden beschouwd.

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Address

Instituut voor Plantenziektenkundig Onderzoek (IPO), Binnenhaven 12, Wageningen the Netherlands