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Symptom expression and variation of rose mosaic

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Abstract

Three isolates of *Prunus* necrotic ringspot virus (PNRV) causing line pattern in rose and one causing yellow mosaic were compared on four greenhouse and on five outdoor rose cultivars with an isolate of the virus causing Stecklenberger disease in morello and with an isolate of apple mosaic virus (AMV) from apple.

All PNRV isolates were readily transmissible through budding in rose (up to 100%). AMV was hard to transmit from apple to rose, but as easily from rose to rose as the PNRV isolates. In the greenhouse, first symptoms were produced 28 days after budding. Budding of outdoor roses in early September usually gave first symptoms next spring, but sometimes not until late during the second year after budding. In the course of time symptom production was erratic and often on a few leaves only.

Nature and severity of symptoms greatly depended on cultivar and to a lesser extent on the isolate used. Symptomatologically all isolates, including AMV, behaved like members of a continuum, with AMV in certain cultivars causing characteristic rose (yellow) mosaic but usually being more virulent on rose.

Introduction

So far, virus infections have not created serious problems in the important rose industry in the Netherlands. This may be due in part to the general use of rose seedlings as rootstocks in this country. Strawberry latent ringspot virus was isolated once from affected plants occurring spotwise in a greenhouse rose crop (Bos, 1973). This virus and *Arabis* mosaic virus were also found to occur occasionally in vegetatively propagated *Rosa rugosa* rootstocks (Van Hoof and Caron, 1974). The cause of a peculiar bud proliferation of newly budded outdoor roses still is uncertain. Graft transmissibility could not be proved and no known virus seems to be involved (Bos and Perquin, 1975).

Irregular line patterns and chlorotic or yellow bands (rose mosaic) can sometimes be observed in rose plants grown in greenhouses and in the open. These occur especially in cultivars of American origin, presumably mainly because of having originally been raised on vegetatively propagated rootstocks (R. 'Manetti'). Since ca 1965 inconspicuous line pattern symptoms were occasionally observed in 'Baccara' roses (Fig. 1). The infection was tentatively associated with the replacement of seedling rootstocks by imported vegetatively propagated rootstocks of *R. indica* 'Major'. Mechanical transmission to herbaceous hosts, purification, electron microscopy and serology readily demonstrated the presence of *Prunus* necrotic ringspot virus (PNRV) (Bos, 1968). Since roses grown for certification in the Netherlands are visually in-

Fig. 1. Rose 'Baccara' on *R. indica* 'Major' rootstock with line pattern and spotting due to natural infection by *Prunus* necrotic ringspot virus.

Fig. 1. Roos 'Baccara' op onderstam van R. indica 'Major' met figuurbont en vlekking ten gevolge van natuurlijke infectie met het necrotische-kringvlekkenvirus van Prunus.

spected, experiments were made on expression and variation of symptoms of 'rose mocaic' under greenhouse and field conditions. Such information is essential to evaluate visual indexing techniques.

Some literature on rose mosaic. Thomas and Massey (1939) were the first to report on three apparently distinct mosaic diseases of rose in California. These were designated rose mosaic 1, 2 and 3. Brierley and Smith (1940) distinguished rose mosaic and yellow mosaics, from which K.M. Smith (cf. Smith, 1957) inferred the existence of rose mosaic virus (*Rosa* virus 1) and rose yellow mosaic virus (*Rosa* virus 2). Mosaic could also be induced in rose with apple mosaic virus (e.g. Thomas, 1937; Thomas and Massey, 1939) and the virus of peach yellow bud mosaic (Thomas and Rawlins, 1939). Subsequently, some of the rose mosaic diseases were associated with specific viruses. Cochran (1950), Gilmer (1956), Kirkpatrick et al. (1962) and others reported the isolation of *Prunus* necrotic ringspot virus (PNRV) and Halliwell and Milbrath (1962) of tomato ringspot virus from mosaic-diseased roses. By 1966 the evidence suggested that PNRV was the principle virus involved in rose mosaic (Traylor et al.,

1966). However, a special rose mosaic virus (RMV) was also described (Fulton, 1967). This was found to be only distantly related serologically to PNRV, but closely related to apple mosaic virus (Fulton, 1968, a, b). More recently Casper (1973) compared six virus isolates from hybrid tea roses with three isolates of AMV and two of PNRV. He found a wide range of serological relationships; two rose isolates clearly belonging to the AMV serotype and three to the PNRV serotype. One siolate, however, reacted to a high degree with antisera of both serotypes. These tests demonstrated the existence of a continuous serological spectrum of isolates from rose extending from the PNRV to the AMV serotype.

Materials and methods

Graft transmission tests were made by budding two to three buds each into three young stems of four cultivars of greenhouse roses and five cultivars of outdoor roses. Buds were taken from greenhouse rose plants of 'Baccara', 'White Satin' and 'Orange Garnet' with mild line pattern symptoms. From these plants or from plants with comparable symptoms, I had isolated earlier PNRV. 'Orange Garnet', heat treated by F. A. Hakkaart (IPO, stationed at Research Station for Floriculture, Aalsmeer), served as a check. Similarly, buds were taken from outdoor 'Queen Elizabeth' with severe yellow line and band patterns (Fig. 2) and known to contain PNRV. Furthermore, buds were used from a Stecklenberger-diseased morello cherry tree (*Prunus cerasus* var. *austera*) (provided by Th.G.E. Clevers, Plant Protection Service) and from apple with apple mosaic virus (AMV) (provided by F.A. van der Meer, IPO; Casper (1973) later produced his AMV antiserum to my virus isolate from this material). Non-budded plants were included as controls.

Fig. 2. Rose 'Queen Elizabeth' with 'yellow mosaic' due to natural infection by *Prunus* necrotic ringspot virus.

Fig. 2. Roos 'Queen Elizabeth' met 'geelmozaïek' ten gevolge van natuurlijke infectie met het necrotische-kringvlekkenvirus van Prunus.

In the outdoor experiment some plants were budded two or three times, the second or third time from plants showing symptoms after the first budding (indicated in Table 2 with an asterisk). In this trial, bud inoculations were also made from 'Baccara' plants showing symptoms after having been budded from 'White Satin' and from 'Verhage' plants previously budded with 'Queen Elizabeth', both in the greenhouse.

The experiment with greenhouse roses was performed at the Research Station for Floriculture at Aalsmeer. Visually virus-free roses were provided by F. A. Hakkaart. The trial with outdoor roses was made at the IPO, Wageningen. Certified roses were provided through P. J. Taconis, Plant Protection Service.

Results

The greenhouse roses were budded during May 1969 and kept under observation for 17 months. The results are summarized in Table 1. In all instances, the non-budded controls remained free of symptoms. No symptoms were produced after budding from heat-treated 'Orange Garnet' roses and from apple containing AMV. With the three virus isolates originating from greenhouse roses (1–3) first symptoms were produced after 28 days. With 'Queen Elizabeth' in 'Verhage' the first symptoms appeared in the second season. In all cases of sensitivity, transmission rates were high, up to 100%. PNRV from morello produced no symptoms in 'Carol'. There was no clear difference in susceptibility between cultivars as appears from the percentage of total symptom-bearing plants.

There was no basic difference among the virus isolates in type of symptoms produced. Symptoms tended to be most severe with the 'Queen Elizabeth' isolate but this also depended greatly on cultivar. With this isolate line pattern symptoms were rather yellow in 'Baccara' and 'Zorina' but mild in 'Verhage'. The 'Baccara' isolate sometimes produced yellow spots and rings on 'Carol'.

Table 1. Summary of rose varietal test in the greenhouse at Aalsmeer 1969–1970.

Virus isolates from	Rose cultivars					
	Verhage	Carol	Baccara	Zorina	total	per cent
1. Baccara	3/3[1]	3/3	3/3	2/4	11/13	85
2. White Satin	3/4	1/4	4/4[2]	4/4	12/16	75
3. Orange Garnet	2/3	4/4	4/4	0/4	14/15	93
4. Orange Garnet (heat treated)	0/4	0/4	0/4	0/4	0/16	0
5. Queen Elizabeth	4/4[2]	4/4	4/4	4/4	16/16	100
6. morello with Stecklenberger	4/4	0/4	1/4	4/4	9/16	56
7. apple with apple mosaic	0/4	0/4	0/4	0/4	0/16	0
8. non-budded control	0/4	0/4	0/4	0/4	0/16	0
total	16/30	12/31	16/31	18/32	62/124	
per cent	53	39	51	56	50	

[1] Numbers of plants showing symptoms out of total number of plants budded.
[2] Plants used for budwood numbers 8 and 9 of the outdoor experiment of Table 2.

Tabel 1. Samenvatting van de rozerassenproef in kas te Aalsmeer 1969–1970.

Table 2. Summary of rose varietal test in the open at Wageningen 1968–1971.

Virus isolates	Rose cultivars						
	Peace	Étoile de Hollande	Masquerade	New Yorker	Betty Prior	total	per cent
1. Baccara 9-9-68 (27-8-70)[1]	3/4[2] (3/4)[3]	2/3[5]	3/3	1/3(1/3)	2/4(2/4)	11/17(6/11)	65(55)[4]
2. White Satin 28-8-68	2/4	3/4	2/4	2/4	3/4	12/20	60
3. Orange Garnet 28-8-68 (4-9-69)	1/4	3/3	2/4	0/2(0/2)	3/4[5]	9/17(0/2)	53(0)
4. Orange Garnet heat treated 9-9-68	0/4	0/3	0/3	0/4	0/4	0/18	0
5. Queen Elizabeth 28-8-68 (4-9-69)	1/3	4/4	3/3	2/2(2/2)	3/4[5]	13/16(2/2)	81(100)
6. morello with Stecklenberger 9-9-68 (4-6-69, 27-8-70)	3/4(3/3)	2/4(2/2)	2/4(2/2)	1/4(1/2)	3/4[5]	11/20(8/9)	55(89)
7a. apple mosaic PD3 30-8-68 (27-8-70)	0/4	3/3[5](2/2)	3/4(3/3)	3/4(3/3)	3/4(3/4)	12/19(11/12)	63(92)
7b. apple mosaic Golden Del. PD3 8-9-69	0/3	0/4	0/4	0/4	0/4	0/19	0
8. Baccara with Wh.S. exp. Aalsmeer 4-9-69	2/3	3/3	3/3	1/3	3/3	12/15	80
9. Verhage with Q.El. exp. Aalsmeer 4-9-69		2/8	2/8			4/16	25
total	12/33(6/7)	20/39(4/4)	20/40(5/5)	10/30(7/12)	20/35(5/8)	84/177(27/36)	45(75)
per cent	36(86)	51(100)	50(100)	33(58)	57(63)	47(75)	

[1] Dates of budding and, in parentheses, of rebudding.
[2] Total number of plants reacting out of total number of plants budded.
[3] In parentheses number of plants reacting out of number of plants rebudded.
[4] In parentheses percentage of plants reacting out of total number of plants rebudded.
[5] Plants used for budwood for later buddings.

Tabel 2. Samenvatting van veldproef met rozerassen te Wageningen, 1968–1971.

The outdoor roses were first budded in early September 1968 and kept under observation through 1971. The results are summarized in Table 2. Budding in September usually gave the first symptoms the following spring. Often symptoms did not appear until late that year or some time during the second season after budding (Table 3). Percentages of successful transmission after the first budding, not recorded separately in Tabel 2, were slightly less than in the greenhouse, but further buddings, made from plants infected as a result of the first budding, usually gave higher results (up to 100% for 'Queen Elizabeth'). Although not recorded in Table 2, unbudded spare plants of the experiment never produced symptoms. Also, no symptoms were produced with buds from heat-treated 'Orange Garnet', as in the greenhouse test. Similarly,

Table 3. Course of symptom expression in part of the plants budded during September 1968 in outdoor trial at Wageningen.

Virus isolates and rose cultivars	Serial plant number	Year and month														
		1969					1970					1971				
		5	6	7	8	9	5	6	7	8	9[1]	5	6	7	8[1]	9
Baccara in																
Étoile de Hollande	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	2	−	−	+	+	+	−	−	−	+		−	−	−		+
	3	−	−	+	+	−	−	−	−	+		+	−	−		+
Masquerade	1	−	−	+	−	−	−	−	−	+		−	−	−		−
	2	−	−	−	−	−	−	+	−	+		−	−	−		−
	3	−	−	−	−	−	−	−	−	+		−	−	−		−
White Satin in																
Étoile de Hollande	1	−	−	−	−	−	−	−	−	−		?	−	−		−
	2	−	−	−	+	+	−	−	−	+		−	−	?		+
	3	−	−	−	−	−	−	−	−	+		−	+	?		+
	4	−	−	−	+	+	−	+	−	+		?	+	+		+
New Yorker	1	−	−	+	−	−	−	−	−	−		−	−	−		−
	2	−	−	−	−	−	−	−	−	−		−	?	−		−
	3	−	−	+	−	−	−	−	−	−		?	+	+		+
Betty Prior	1	−	−	−	−	−	−	−	−	−		−	−	−		−
	2	−	−	−	+	+	−	−	−	−		−	−	−		−
	3	−	−	−	+	+	−	−	−	?		−	?	−		−
	4	−	−	−	+	−	−	+	−	?		−	−	−		−
Queen Elizabeth in																
Étoile de Hollande	1	−	−	+	+	−	+	+	+	+		+	+	+		+
	2	−	+	−	+	−	−	−	−	?		−	−	−		−
	3	+	+	+	−	−	−	+	−	+		+	+	+		+
	4	+	+	+	+	+	+	+	−	+		+	+	+		+
morello Stecklenberger	in															
Betty Prior	1	−	−	−	−	−	−	−	−	?		−	−	−		−
	2	−	−	+	+	+	−	−	−	−		?	?	+		?
	3	−	−	+	+	+	−	−	−	−		?	?	−		+
	4	−	+	+	+	+	−	−	−	+		−	−	−		+

[1] No observations made.

Tabel 3. Verloop van de symptoomexpressie in deel der planten geoculeerd in september 1968 in de veldproef te Wageningen.

Fig. 3. Rose 'Masquerade' with 'yellow mosaic' two years after budding from 'Baccara' of Fig. 1 with line pattern symptoms.

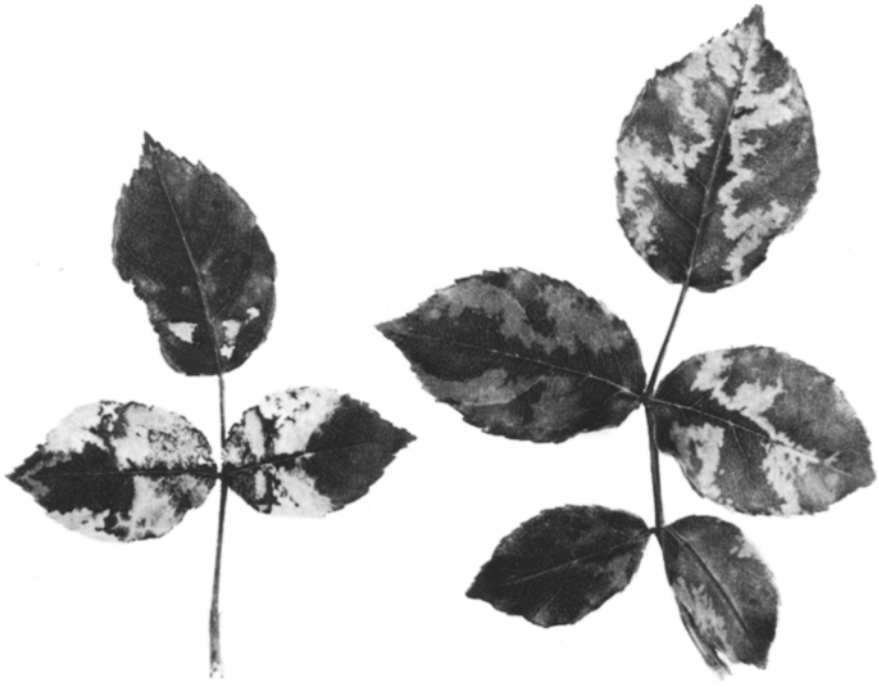

Fig. 3. Roos 'Masquerade' met 'geelmozaïek' twee jaar na oculatie vanuit 'Baccara' van Fig. 1 met figuurbont.

the plants inoculated with the AMV material used in 1969 (same inoculum as used in the greenhouse test) yielded negative results. However, after transmission by direct budding from apple in 1968 (Table 2) infection succeeded in one plant of 'Étoile de Hollande' only. Later budding from this rose plant led to infection in 92% of the budded plants. As in the greenhouse test, there was no apparent difference in susceptibility among the cultivars used.

Under field conditions symptom variation among the virus isolates from rose and morello was similar to that observed in the greenhouse experiment. Differences among isolates in one cultivar varied as much as among cultivars budded with a single isolate. 'Masquerade' usually reacted with rather severe and yellow symptoms when inoculated with the mild isolates from 'Baccara' (Fig. 3), 'White Satin' and morello. The isolate from 'Queen Elizabeth' usually produced the most severe symptoms on all cultivars (Fig. 4). AMV often produced yellow bands and line patterns indistinguishable from those of 'rose mosaic' However, this isolate generally was severe and tended to produce a true mosaic rather than line pattern (Fig. 5). 'Betty Prior' plants suffered badly from infection, affected plants only attaining half of their normal size with most leaves expressing symptoms.

In the outdoor experiment observed during three consecutive years, there was consi-

Fig. 4. Rose 'Étoile de Hollande' with intense 'yellow mosaic' one year after budding from 'Queen Elizabeth' of Fig. 2 with yellow mosaic.

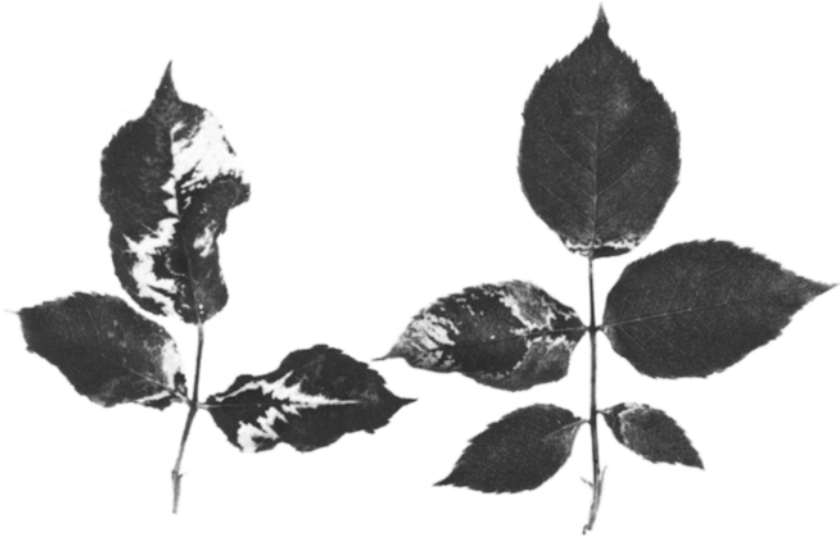

Fig. 4. Roos 'Étoile de Hollande' met intens 'geelmozaïek' een jaar na oculatie vanuit 'Queen Elizabeth' van Fig. 2 met geelmozaïek.

Fig. 5. Rose 'Betty Prior' with oak-leaf patterns and severe mosaic after budding from 'Etoile de Hollande' with apple mosaic virus.

Fig. 5. Roos 'Betty Prior' met eikebladpatronen en ernstig mozaïek na oculatie vanuit 'Étoile de Hollande' met appelmozaïekvirus.

derable fluctuation in the occurrence of symptom expression and to some extent in severity. Symptoms often were not observed for part of a season or sometimes for a whole season. Some of this variability is illustrated in Table 3. Often only one or a few leaves showed symptoms.

Discussion

The results obtained have demonstrated that the isolates used are readily transmissible by budding. It must be admitted, however, that usually budwood was taken from selected branches showing clear leaf symptoms and on each plant at least six buds were inserted. When testing individual rose buds for rose mosaic on 'Shirofugen' flowering cherry, Fleisher et al. (1971) found low transmission rates and ascribed this to uneven distribution of the virus in the infected plants.

There is a great variation in symptom severity, depending on virus isolate and cultivar infected. In symptoms, no clear-cut distinction exists between rose mosaic (weakly chlorotic, often narrow line patterns) and rose yellow mosaic (yellow and often broad bands and oak-leaf patterns), since both types can be produced by a single isolate in different cultivars. However, in general the yellow 'Queen Elizabeth' isolate tended to produce rather severe and yellow symptoms, comparable to those of rose yellow mosaic as often described in the literature. The virus isolated from this cultivar was serologically similar to the isolate obtained from 'Baccara' and no spurs were found in agar-gel diffusion tests using an antiserum to the first and a PNRV antiserum provided by R. W. Fulton, Madison, Wisc. USA (D. Z. Maat, IPO, Wageningen, personal communication). Thus, they were all true PNRV serotype isolates.

AMV was difficult to transmit from apple to rose, but once in rose the virus could be readily transferred from rose to rose. In certain cultivars even this virus produced symptoms indistinguishable from those of PNRV, but in general the isolate was much more virulent.

Thus, symptomatologically on rose our rose virus isolates characteristically behaved like true PNRV. AMV from apple is also able to incite rose mosaic which is sometimes indistinguishable from that of PNRV but usually much more severe, at least with the one isolate studied. Fulton (1968b) and Casper (1973) isolated viruses from rose that were serologically indistinguishable from AMV. In fact, Casper's AMV antiserum 2 had been prepared to my AMV isolate. Our results with rose cultivars corroborate the results of Casper's (1973) serological studies. We seem to be dealing with a continuum of closely related entities. Borderlines between them may be more arbitrary still than suggested by Fulton (1968a) when discussing the relationships among the ringspot viruses of *Prunus*. Opinions may differ as to the existence of a special rose mosaic virus as concluded by Fulton (1967, 1968b), and will greatly depend on whether the person concerned is a 'splitter' or a 'lumper'. This problem seems identical to that encountered within other morphological groups, e.g. the cluster of viruses more or less related to bean yellow mosaic virus (Bos, 1970).

The immense variation within such groups and even of individual viruses hampers diagnosis and indexing as well.

Although of limited reliability for roses (Fleisher et al., 1971), *Prunus serrulata* 'Shirofugen' is often used for indexing PNRV and related viruses. Casper (1973)

found one of his rose yellow mosaic isolates not to react on this indicator, whereas all other isolates, including AMV, did. Fleisher et al. (1971) claim that certified planting stocks of roses should also be inspected visually several times before releasing. However, as demonstrated in this publication, this technique is not reliable either. Serological techniques of indexing cherry trees for PNRV (Schade and Schimanski, 1974) have not yet been worked out for roses. Here again, variation of the viruses possibly involved should be taken into consideration. Therefore, studies of practical virus problems should never be based on single virus isolates, but observations on natural variation be included.

Samenvatting

Symptoomexpressie en -variatie van rozemozaïek

In een kasproef (Tabel 1) en een buitenproef (Tabel 2) werden op vier kasrassen en vijf buitenrassen van roos door oculatie drie isolaten van het necrotische-kringvlekkenvirus van *Prunus* (PNRV) uit roos met figuurbont (Fig. 1) en één uit roos met geelmozaïek (Fig. 2) vergeleken met een isolaat van het virus uit morel met Stecklenbergerziekte en met een isolaat van het appelmozaïekvirus (AMV) uit appel.

Alle PNRV-isolaten gingen bij roos gemakkelijk over door oculatie (soms zelfs tot 100%) (Tabel 1 en 2). AMV was moeilijk over te brengen van appel op roos maar gemakkelijk van roos op roos. In de kas verschenen de eerste symptomen van PNRV al 28 dagen na oculatie. Oculatie van buitenrassen vroeg in september gaf meestal het volgende voorjaar maar soms ook pas laat in het tweede jaar na oculatie de eerste afwijkingen. In de loop van de tijd was de vorming van symptomen zeer wisselvallig. Ze kwamen vaak slechts op enkele bladeren of blaadjes voor maar waren niet zelden tijdelijk geheel afwezig (Tabel 3).

De aard en hevigheid van de symptomen was sterk afhankelijk van het geöculeerde rozeras en in mindere mate van het virusisolaat (Fig. 3–5). Symptomatologisch gedroegen alle isolaten, met inbegrip van AMV, zich als leden van een aaneensluitende reeks. Daarbij veroorzaakt het AMV in bepaalde rozerassen karakteristiek roze-(geel)mozaïek, maar was het in het algemeen virulenter op roos (Fig. 5) dan de PNRV-isolaten. Deze variatie in symptoomexpressie en het vaak tijdelijk afwezig zijn van verschijnselen heeft consequenties voor de visuele beoordeling bij keuring.

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