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Silene armeria, a test plant for carnation etched ring virus

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Abstract

A test plant grown from seed was discovered which may be useful for the detection of carnation etched ring virus (CERV) in carnations. In *Silence armeria* plants, infected with CERV, necrotic line patterns and blotches may develop about 2–3 weeks after inoculation, but this reaction is not very reliable. When *S. armeria* is infected with carnation mottle virus (CaMV) the plants show hardly perceptible symptoms consisting of a faint mosaic of the leaves and slight stunting of the plants. However, when *S. armeria* plants, infected with CaMV, are inoculated with CERV, necrotic lines, rings and blotches develop quite clearly. Thus infection with CaMV makes *S. armeria* a useful indicator for CERV. Also in the carnation cultivar 'Joker' a synergism exists between the two viruses, severe symptoms resulting from addition of CaMV to CERV-infected plants that otherwise show few if any symptoms.

Introduction

The name "carnation etched ring" virus was first mentioned in 1961 (Hollings and Stone, 1961). No test plant grown from seed has been available for diagnostic purposes and therefore, grafting on the sensitive carnation cultivar 'Joker' has been practised as a method of detection. Progress in the study of CERV has been slow, the main reason being the unsatisfactory method of diagnosis available. The freedom from virus of a vegetatively propagated plant of a carnation cultivar such as 'Joker', even from the virus which it is expected to indicate, can always be questioned. A test plant grown from seed was therefore badly needed.
In this paper some experiments are described with *Silene armeria*, which was found to be a suitable test plant for CERV.
The data obtained give reason to re-evaluate the original description of the virus symptoms in carnations. To avoid confusion it therefore seems appropriate to give first a detailed review of the literature.

Review of literature

Hollings and Stone (1961) gave the following description of symptoms: "Conspicuous chlorotic spots, flecks and rings, similar to those caused by carnation ringspot virus,

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were seen on the younger leaves of carnation plants of the variety 'Cocomo Sim' but, although mottle virus was present, ringspot virus could not be detected. Grafting affected plants to other Sim varieties and to 'Joker', already infected with mottle virus, induced the additional leaf symptoms after three months. After a further four to six weeks, whitish etched necrotic flecks and rings appeared on the middle leaves of grafted plants. These necrotic symptoms also developed in the original 'Cocomo Sim' plants and have been seen in several other varieties. Because these necrotic symtpoms appeared to be characteristic for the disease, and sufficiently distinctive for diagnosis, the name "etched ring" seemed appropriate. Small, irregular rings and streaks also developed on the stems of several infected varieties, though less frequently than on the leaves. Of the varieties tested so far 'Joker' has shown the most severe and conspicuous symptoms, and is currently being used as an indicator".
In 1962 transmission by sap and by *Myzus persicae* (Sulzer) f. *dianthi* was reported (Hollings et al., 1962) and in 1963 the same authors purified the virus, prepared an antiserum and saw in electron micrographs spherical particles with a diameter of 28 mμ. Some confusion arose, however, when in 1964 the same authors reported that the virus described in their 1963 report was not concerned with the symptoms of etched ring.
In 1965 purified preparations were made from the carnation cultivars 'Dusty' and 'Joker', showing symtoms of CERV, but in which no other carnation virus could be detected (Hollings and Stone, 1965). Differentially centrifuged leaf extracts contained polyhedral particles ca 29 mμ in diameter, produced a single specific zone in density-gradient centrifugation, and were used to immunize rabbits. The antiserum reacted specifically with purified preparations of the virus, whether or not CaMV was also present. CERV did not infect six species of test plants which react to one or more of the other carnation viruses.

Materials and Methods

Inoculations were carried out by crushing leaf samples in a mortar with phosphate buffer (pH 7) and rubbing the sap with the finger on the leaves of the test plants, previously dusted with carborundum, after which they were rinsed with tap water.
A seed lot of *S. armeria* was obtained by courtesy of Professor S. J. Wellensiek, Laboratory of Horticulture, Wageningen. *S. armeria* was tested during the months with short days when this plant remained in the vegetative stage (Wellensiek, 1966).

Results

Preliminary experiment with 'Joker'
One hundred shoots of each of the carnation cultivars 'Bella Sim', 'Tangerine Sim' and 'Orange Triumph' were grafted on 'Joker' plants. The developing lateral shoots of the 'Joker' stocks showed two syndromes:
(1) few necrotic flecks and rings on the leaves, sometimes enlarging to blotches, but no conspicuous chlorosis in the youngest leaves;
(2) more severe symptoms consisting of chlorosis in the youngest leaves, conspicuous necrotic lines and rings on the other leaves, sometimes enlarging to blotches, sometimes with streaks and flecks on the stems (Fig. 1).

The second type of syndrome corresponds completely with the original description by Hollings and Stone (1961), but the first type is different. From the 'Joker' plants showing the first type of symptoms, no CaMV could be isolated, whereas from the plants with the severe type it could. The following hypothesis seems appropriate: CERV alone causes in 'Joker' only a disease with necrotic leaf symptoms without obvious chlorosis. When, however, CaMV is also present a more severe disease results with the symptoms described above under 2 and illustrated in Fig. 1. According to this hypothesis the symptoms described by Hollings and Stone (1961) apply to the complex of CaMV and CERV but not to CERV alone. In fact the 'Cocomo Sim' plants from which the CERV symptoms were originally described, also contained CaMV.

Experiments with Silene armeria

During a search for a suitable test plant for CERV it was found that when *S. armeria* was inoculated with sap from 'Joker' plants containing CaMV and showing the symp-

Fig. 1. Symptoms of a mixed infection with carnation etched ring and carnation mottle virus in the carnation cultivar 'Joker'. Four leaves on the left with chlorotic symptoms, next four leaves with necrotic symptoms. Healthy leaf on the extreme right.

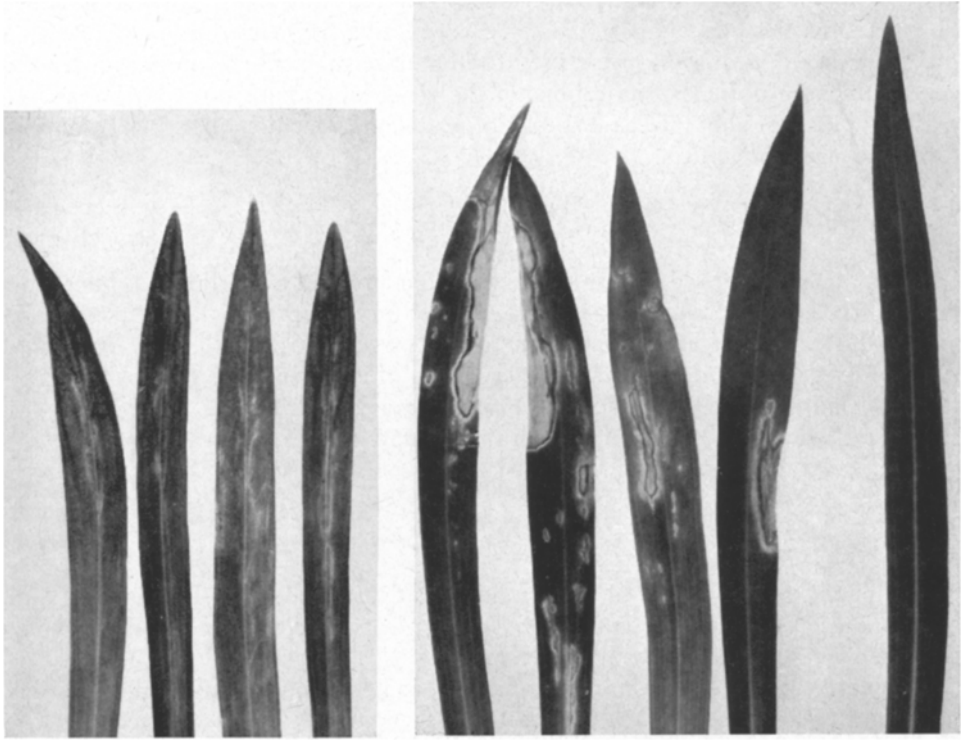

Fig. 1. Symptomen van een gemengde infectie met het "carnation etched ring" – en het "carnation mottle"-virus in de anjercultivar 'Joker'. Links vier bladeren met chlorotische symptomen, daarnaast vier bladeren met necrotische symptomen. Geheel rechts gezond blad.

Fig. 2. Symptoms of a mixed infection with carnation etched ring and carnation mottle virus in *Silene armeria*

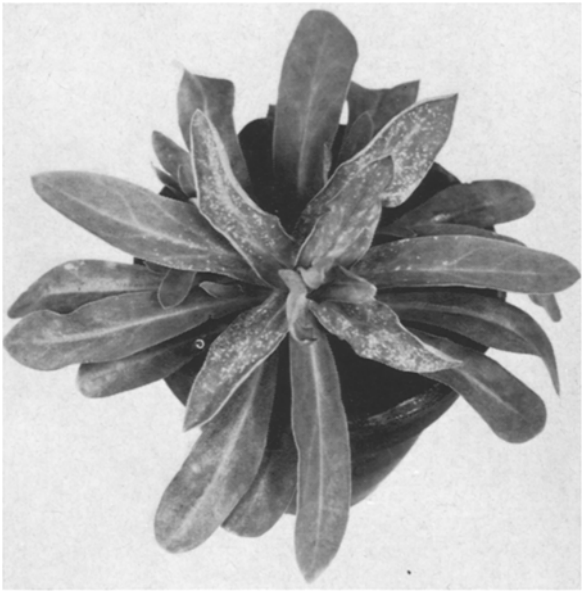

Fig. 2. Symptomen van een mengsel van het "carnation etched ring"- en het "carnation mottle"-virus op Silene armeria

toms described above under 2, the plants reacted with systemic symptoms consisting of conspicious flecks and rings (Fig. 2). When *S. armeria* plants were infected with CaMV from *Chenopodium amaranticolor* infected with this virus, the plants remained nearly symptomless except for a sometimes slightly paler colour of the leaves, a faint mosaic, and slight stunting of growth (Fig. 3). In these plants CaMV could be demonstrated about one week after sap inoculation on *C. amaranticolor*. Sap from 'Joker' plants without CaMV, but showing the slight necrotic symptoms, inoculated on *S. armeria* plants, caused flecks and rings on the leaves, though in some instances the reaction remained absent. This led to the idea of using CaMV-infected *Silene* plants as test plants for CERV.

Experiment 1. S. armeria plants to be used as test plants were first inoculated with CaMV from *C. amaranticolor* leaves. After 18 days a sample of eight of these plants was checked for the presence of CaMV to confirm that the inoculation had been successful. All proved to be infected.
The CaMV-infected *S. armeria* plants were divided into groups of eight, which were inoculated with sap from:
(1) CaMV–free 'Joker' leaves without any symptoms.
(2) CaMV-free 'Joker' leaves with only a few necrotic lines and rings (syndrome 1).
(3) CaMV-infected 'Joker' leaves showing conspicuous chlorotic and necrotic leaf symptoms (syndrome 2).

Fig. 3. Symptoms in *S. armeria*. Left, two healthy leaves; centre, two leaves infected with carnation mottle virus; right, two leaves infected with a mixture of carnation etched ring and carnation mottle viruses.

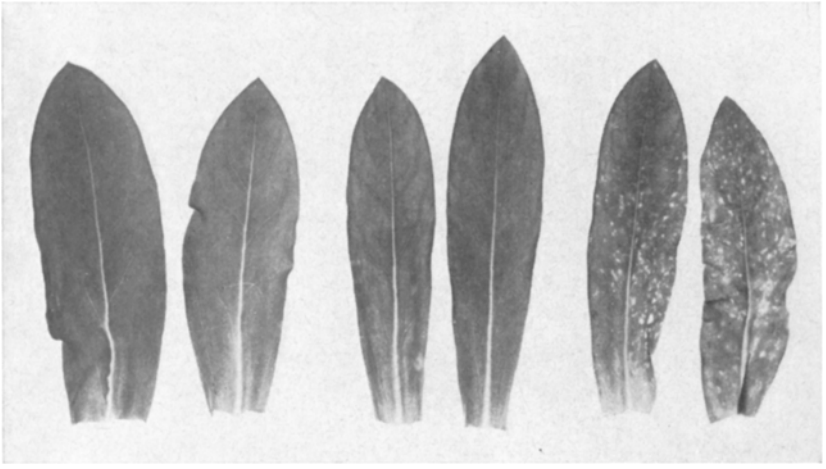

Fig. 3. Symptomen op S. armeria. Links twee gezonde bladeren; midden twee bladeren besmet met het "carnation mottle"-virus; rechts twee bladeren besmet met een mengsel van het "carnation etched ring"- en "carnation mottle"-virus.

Controls were also groups of eight:
(4) *S. armeria* plants inoculated with CaMV in phosphate buffer, but not inoculated with carnation sap.
(5) *S. armeria* plants rubbed with phosphate buffer only.
(6) *S. armeria* plants without any inoculation or rubbing with phosphate buffer.
About 16 days after the inoculation with carnation sap (groups 1, 2 and 3) a systemic reaction consisting of necrotic lines and rings began to appear on the *S. armeria* plants. Two weeks later, thus about a month after inoculation, the reactions reached a maximum of severity and then began somewhat to decline. The groups 1, 2 and 3 behaved identically and all 24 plants became obviously diseased. The *S. armeria* plants with CaMV (group 4) showed hardly perceptible symptoms consisting of a slightly paler colour of the leaves, a faint mosaic and some stunting of growth. The buffer-inoculated plants (group 5) showed only the light damage caused by the rubbing, whereas the non-inoculated, non-rubbed plants (group 6) showed no symptoms at all.
Apparently the symptomless (group 1), the slightly necrotic (group 2) and the severely chlorotic and necrotic 'Joker' plants (group 3) all contained a virus which could be reliably detected by sap inoculation on CaMV-infected *S. armeria* plants.
In group 3 CaMV was present in the inoculum, while in groups 1 and 2 the CERV component met the CaMV in the *S. armeria* plants. For the reactions of the test plants this apparently made no difference.
Another important point is that CaMV-free 'Joker' plants without any necrotic symptoms gave reactions on CaMV-infected *S. armeria* plants. This raises doubts about the value of 'Joker' as an indicator, because apparently this cultivar may at

times be a symptomless carrier of CERV. The assay method with CaMV-infected *S. armeria* plants is more sensitive than the grafting method using 'Joker' as an indicator.

A further conclusion is that the virus from 'Joker' can easily be transmitted mechanically, at least from 'Joker' to CaMV-infected *S. armeria* plants.

Neither in the 'Joker', nor in the *S. armeria* plants could carnation ringspot virus be detected. It is concluded that carnation ringspot virus is not involved in the syndromes described in this paper.

Experiment 2. This experiment concerned the reliability of *S. armeria* as a test plant with or without CaMV. Sap from a number of carnation plants was inoculated, separately, on two CaMV-infected and two CaMV-free *S. armeria* plants. The CaMV-infected test plants had been inoculated with CaMV 14 days prior to inoculation with carnation sap. The inocula consisted of sap from each of six CaMV-free 'Joker' carnations and four CaMV-free 'William Sim' carnations, each group coming from a single clone. Controls were similar to those in experiment 1.

Results are given in Table 1. The first reactions began to appear 12 days after inoculation; reached a peak in severity about 7 weeks after inoculation, after which the severity of symptoms in the new growth began gradually to decline. After 56 days from the date of inoculation the number of reacting plants remained constant. The control plants showed no necrotic symptoms.

Several conclusions may be drawn. Firstly, the sensitivity of the CaMV-infected *S. armeria* plants in detecting CERV in the samples was higher than that of the CaMV-free *S. armeria* plants. Furthermore, the severity of the reactions was greater on the CaMV-infected than on the CaMV-free *S. armeria* plants. The CaMV-free plants were

Table 1. Results of testing for the presence of etched ring virus in ten carnation plants using two *S. armeria* plants with and two without carnation mottle virus

Carnation plant		*Number of reacting S. armeria plants at various intervals after inoculation*											
		12 days		*14 days*		*16 days*		*19–23 days*		*28 days*		*37–56 days*	
		+	−	+	−	+	−	+	−	+	−	+	−
'Joker'	1	1	0	2	0	2	0	2	0	2	0	2	1
	2	0	0	0	1	2	1	2	1	2	1	2	1
	3	1	0	1	1	1	1	2	2	2	2	2	2
	4	1	0	2	1	2	1	2	1	2	2	2	2
	5	1	0	2	1	2	1	2	1	2	1	2	1
	6	0	0	2	0	2	0	2	1	2	1	2	2
'William Sim'	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	2	0	2	0	2	1
	4	1	0	1	0	2	2	2	2	2	2	2	2
Total		5	0	10	4	13	6	16	8	16	9	16	12

\+ = *S. armeria* with CaMV
− = *S. armeria* without CaMV

Tabel 1. Resultaten van de toetsing op aanwezigheid van "etched ring"-virus in tien anjerplanten op twee S. armeria planten met of zonder "carnation mottle"-virus

all checked for the absence of CaMV about 1 month after inoculation with the carnation saps. All proved to be free from this virus. It is therefore clear that CERV can cause symptoms on CaMV-free *S. armeria* plants, but both the number of infected test plants and the severity of the reactions increase when these test plants also contain CaMV.
It was also found that all six 'Joker' plants were infected with CERV, whereas two out of four 'William Sim' plants reacted negatively. It may therefore prove relatively easy to find CERV-free plants by testing e.g. of an important cultivar such as 'William Sim'. With 'Joker' this may be more difficult.
It is interesting to note that the same phenomenon of outgrowing of the symptoms that is seen generally in carnation plants was also observed in the *S. armeria* plants.

Experiment 3. After it was established that it was advantageous for the sensitivity of the testing method to use CaMV-infected *S. armeria* plants, the question arose if it was necessary to add CaMV at a definite time before inoculation with the carnation sample or whether it was possible to inoculate both viruses simultaneously.
Three treatments were compared:
(1) Inoculation of *S. armeria* plants with CaMV 7 days before the inoculation with the carnation sap.
(2) Inoculation of *S. armeria* plants with CaMV, followed by rinsing with water; then, after an immediate second dusting with carborundum, inoculation with the carnation sap and rinsing again with tap water.
(3) Inoculation of *S. armeria* plants with a mixture consisting of sap from a CaMV-infected *S. armeria* plant and sap of the carnation plant.

Table 2. The effect of differences in time of inoculation of *S. armeria* plants with carnation mottle virus on the reaction to carnation etched ring virus.
A = mottle infection 7 days before etched ring infection
B = etched ring infection immediately after mottle infection
C = simultaneous mottle and etched ring infection

		Numbers of reacting test plants on different dates after inoculation (Groups of three plants inoculated per sample)																	
		15 days			*18 days*			*22 days*			*28 days*			*34 days*			*39 days*		
		A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
'Joker'	1	0	1	0	1	1	2	1	1	2	1	3	3	1	3	3	3	3	3
	2	1	0	0	2	1	1	3	1	3	3	3	3	3	3	3	3	3	3
	3	0	1	3	2	1	3	3	2	3	3	3	3	3	3	3	3	3	3
	4	2	0	1	2	2	2	2	2	2	2	3	2	3	3	3	3	3	3
	5	1	0	1	2	1	2	2	2	2	3	2	2	3	2	2	3	3	3
	6	2	0	1	3	1	1	3	1	1	3	2	2	3	3	2	3	3	3
Total		6	2	6	12	7	11	14	9	13	15	16	15	16	17	16	18	18	18

Tabel 2. De invloed van het inoculeren op verschillende tijdstippen van S. armeria planten met "carnation mottle"-virus op de reactie op het "carnation etched ring"-virus.
A = "mottle"-infectie 7 dagen voor de "etched ring"-infectie
B = "etched ring"-infectie onmiddellijk na "mottle"-infectie
C = gelijktijdige "mottle"- en "etched ring"-infectie

Each treatment was applied to three *S. armeria* plants. Carnation inocula were taken from the same six 'Joker' plants as used in experiment 2. Results are given in Table 2. It may be concluded that about 4 weeks after inoculation the three methods all showed the same efficiency.

Experiment with 'Joker'

The question arose whether in carnations an interaction of the two component viruses as demonstrated in experiment 2 also occurs. Therefore the two CaMV-free 'Joker' plants of experiment 1 were infected with CaMV, one showing no symptoms, the other showing only slight necrotic symptoms, but both giving a severe reaction on CaMV-infected *S. armeria* plants. Both plants started showing chlorotic symptoms on the young leaves 7 weeks after the inoculation. Severe necrotic symptoms began to develop several weeks later and 70 days after inoculation both plants showed the complete syndrome consisting of chlorotic and necrotic leaf symptoms. The presence of CaMV was proved by sap inoculation on *C. amaranticolor*. Because CaMV alone cannot cause these serious necrotic symptoms, the conclusion is that there was a synergism between CERV, whose presence in the plants was demonstrated by the tests on *S. armeria* plants, and CaMV added later on.

Discussion

It is a matter for discussion how best to deal with the situation revealed by the experiments which have been described. In our opinion the situation is as follows.

In the carnation cultivar 'Joker' CERV alone causes slight necrotic symtoms, sometimes on only a few leaves of the plant; occassionally it may be completely latent, and in no case does it cause chlorotic symptoms. When CaMV is added a severe syndrome results with conspicuous chlorotic and necrotic leaf symptoms and even sometimes symptoms on the stems.

CERV alone can infect *S. armeria* plants and may cause clear necrotic symptoms. However, when such *S. armeria* plants are or become infected with CaMV, the sensitivity of this test plant, as well as the severity of reaction increase.

A re-appraisal of the original description of CERV symptoms is therefore needed. The carnation plants from which the symptoms were described by Hollings and Stone (1961) also contained CaMV but apparently this virus was considered as irrelevant. In our view the description given by these authors applies to the complex CERV plus CaMV.

'Joker' is considered to be the carnation cultivar most sensitive to CERV. However, in our experiments it was shown that in CaMV-free 'Joker' plants the symptoms were slight or absent. The value of the testing method with 'Joker', therefore, becomes doubtful.

The testing method on *S. armeria* has some limitations. This long-day plant has a rather short juvenile phase and rapidly forms flowering stems during the summer months. We found the results in the months with short days, when the plant is in the rosette stage, more satisfactory than in the months with long days and therefore do not advocate its use during the summer months. Another limitation is that the plant is a systemic host and is not suitable for testing work with detached leaves.

It is not known if CERV occurs naturally in *S. armeria*, neither is it known if the virus

is transmitted by seed. If this proved to be so, extra care would be needed in harvesting the seed by taking it from healthy plants
S. armeria is susceptible to other carnation viruses, but has no value as an indicator of CaMV. So it cannot replace *C. amaranticolor* in the routine tests for this virus. When it is considered important to remove both CaMV and CERV from commercial stocks, two different test procedures are necessary.
One of the properties of CERV revealed by our experiments is that the virus can easily be transmitted by sap from carnation to *S. armeria*. No work was done on aphid transmission, purification, serology or morphology, nor is much known about the occurrence of the virus in commercial stocks.
The severity of symptoms of the complex of CaMV and CERV, at least at certain stages of growth and under certain conditions, makes elimination of them from commercial stocks desirable.

Samenvatting

Silene armeria, een toetsplant voor het "carnation etched ring"-virus

Een uit zaad gekweekte tosplant werd ontdekt, die van belang kan zijn voor het aantonen van het "carnation etched ring"-virus in anjers. Wanneer *Silene armeria* besmet wordt met dit virus treedt er ongeveer 3 weken na de inoculatie een systemische reactie op bestaande uit necrotische lijnen, kringen en soms vlekken op de bladeren. Deze reactie is evenwel niet erg betrouwbaar. Wordt echter het "etched ring"-virus geïnoculeerd op planten van *S. armeria* die reeds met het "carnation mottle"-virus besmet zijn – welk virus zwakke symptomen zoals een lichte bontheid in het blad en wat groeivertraging veroorzaakt – dan ontstaan weer de necrotische lijnen, kringen en vlekken. Hierdoor kan de plant als een betrouwbare indicator voor het "etched ring" – virus worden beschouwd. In de anjercultivar 'Joker' kan ook een dergelijk synergisme aangetoond worden tussen de twee genoemde virussen. Wanneer 'Joker'-planten zonder "mottle"-virus, maar met "etched ring"-virus, besmet worden met "mottle-virus, verschijnt een heftig ziektebeeld bestaande uit bontheid van het jonge blad en hevige necrotische verschijnselen op de oudere bladeren.

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