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DESCRIPTION OF SYMPTOMS AND ASSESSMENT OF LOSS CAUSED BY SOME VIRUSES IN THE CARNATION CULTIVAR 'WILLIAM SIM'[1]

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A study was made of the influence of carnation mottle, ring spot and vein mottle viruses on carnations of the 'William Sim' variety by artificial infection of virus-free plants of one single clone. No clear leaf symptoms were obtained by artificial infection with the mottle virus, but leaf symptoms appeared on plants infected with the ring spot virus and the growth of these plants was markedly reduced. The carnation vein mottle virus caused a definite leaf flecking. A depressing effect on yield was demonstrated with all three viruses. Vein mottle was serious, particularly because it caused flower colour breaking. The ring spot virus, also, had a depressing effect on the quality of the flowers, causing a high percentage of split calices. The carnation mottle virus also caused a reduction in the quality of the produce, though not so intense as that caused by the ring spot virus. A combination of the two last-mentioned viruses proved slightly more serious than the ring spot virus alone.

INTRODUCTION

Little is known about the influence of the various carnation viruses on the growth and flower production of carnation plants, since for an accurate assessment of these effects in named varieties virus-free plants are essential. It is almost certain that all carnations in commercial culture and especially those of the 'Sim' varieties were formerly infected with the carnation mottle virus at least. However, QUAK (1957) succeeded in obtaining mottle-free plants of the varieties 'Pink Sim' and 'Harvest Moon' by a combination of heat treatment and meristem culture. As testing for other viruses gave negative results these plants were considered virus free.

In the Netherlands this method has been put into practice on a large scale and virus-free plants of a considerable number of important varieties have been produced in this country (VAN OS, 1964). The availability of this healthy material has enabled this study to be made of the symptoms caused by the various carnation viruses singly and an evaluation of their effects on the growth and flower production of carnation plants.

There is some confusion about the identity and nomenclature of the carnation viruses. In the present paper we shall follow KASSANIS's (1955) nomenclature.

EXPERIMENTAL DESIGN

A clone of virus-free cuttings of the variety 'William Sim', obtained by meristem culture, was checked plant by plant for freedom from viruses by sap inoculation on *Chenopodium amaranticolor* Coste & Reyn. The plants were then divided into six lots which were inoculated with carnation mottle virus, carnation ring spot virus, carnation vein mottle virus, carnation latent virus

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and a combination of carnation mottle and ring spot viruses, one lot being left uninoculated. The plants were grown in plots in greenhouse beds, as described below, the plots being so spaced that the plants in different plots could not touch each other. Hardboard shields prevented root contact in the soil between plants in the different plots.

MATERIALS AND METHODS

Virus isolates

Four virus isolates were kindly supplied by Ir. D. H. M. VAN SLOGTEREN at Lisse, viz.

1. Carnation mottle virus in the carnation variety 'William Sim'. Sap inoculation on *C. amaranticolor* gave local lesions after 7–9 days, but no reaction was obtained on *Gomphrena globosa* L. No serological reaction occurred with carnation latent antiserum whereas a single precipitation line appeared in gel diffusion tests with bivalent antiserum, prepared against mottle and ring spot viruses.
2. Carnation ring spot virus in *Nicotiana tabacum* L. var. 'White Burley'. Sap inoculation on *C. amaranticolor* gave local lesions after about three days and a systemic reaction in *G. globosa*. The virus was transferred to *Phaseolus vulgaris* L., from which plant the inoculations on carnations were made.
3. Carnation vein mottle virus in *Dianthus barbatus* L. Sap inoculation on *G. amaranticolor* gave local lesions after about twelve days, but no reaction on *G. globosa*. A negative serological reaction was obtained with antiserum prepared against carnation latent virus and bivalent antiserum prepared against carnation mottle and ring spot viruses.
4. Carnation latent virus in *D. barbatus*. Sap inoculation on *C. amaranticolor* and *G. globosa* gave no symptoms. A positive serological reaction was observed with antiserum prepared against carnation latent virus. However, the sap did not react with bivalent antiserum prepared against carnation mottle and ring spot viruses.

Inoculations

1. Carnation mottle virus

Fresh leaves (from carnation plants infected with this virus) were crushed in a mortar. The sap was squeezed through cheese cloth and after dilution (1:20) rubbed with the forefinger on the plants, previously dusted with carborundum powder. After inoculation the plants were rinsed with water. All plants were inoculated three times, viz. on 17 January, 24 January and 8 March 1962.

2. Carnation ring spot virus

The same method was used here, with the exception that the infective sap was obtained from French bean plants and was diluted 1:10 prior to inoculation. The inoculation dates were 19 January, 25 January and 6 March 1962.

3. Carnation vein mottle virus

At first sap inoculation was tried. Sap of infected *D. barbatus* plants was diluted 1:10 and inoculated in the same way as carnation ring spot and mottle viruses on 15 January, 26 January and 6 March 1962. However, no symptoms

appeared in the carnation plants. Since tests on *C. amaranticolor* also gave negative results, it was concluded that the inoculation method had failed. Therefore, inoculation by means of the aphid *Myzus persicae* was attempted. Aphids were allowed to feed for a short period (1–5 minutes) on *D. barbatus* plants infected with vein mottle virus, after which they were transferred to the virus-free carnation plants. The next day the aphids were killed. Ten aphids were used for each plant. Each plant was inoculated once. The infection experiments with aphids were carried out from 18 to 20 April and from 1 to 8 May 1962.

4. Carnation latent virus

At first sap inoculation was tried with *D. barbatus* sap (1:10) from plants infected with the carnation latent virus. Each plant was inoculated on 18 January, 25 January and 7 March 1962. After about a month, serological tests for the presence of the latent virus proved negative. Therefore, aphid inoculation by means of *M. persicae* was performed in the same way as with the vein mottle virus. Inoculation dates were 30 May and 20–27 June 1962.

Tests for presence of the viruses

To detect the presence of carnation ring spot, mottle and vein mottle viruses in the inoculated carnation plants dry inoculation was performed on the leaves of *C. amaranticolor* previously dusted with carborundum. The appearance of local lesions after about 3–5 days, 7–9 days and 12 or more days was considered to be of diagnostic value for carnation ring spot, mottle and vein mottle viruses, respectively.

To demonstrate the presence of both carnation mottle and ring spot viruses in the same plant, leaf samples were serologically tested against a bivalent antiserum prepared against these two viruses. Fresh leaves were crushed in a mortar and the sap obtained was squeezed through cheese cloth. Thereafter the sap was put into holes in agar-gel plates and tested according to the gel-diffusion method as described by VAN SLOGTEREN (1959). By means of standard samples of known virus content it was possible, by the appearance of two precipitation lines, to demonstrate the presence of these two viruses in the same plant.

To detect the presence of carnation latent virus fresh leaves were crushed in a mortar. The sap thus obtained was squeezed through cheese cloth, then mixed with an equal volume of saline and the mixture centrifuged at 3000 r.p.m. for 10 minutes. The supernatant was serologically tested according to the microprecipitin method under paraffin oil as developed by VAN SLOGTEREN (1955). Results were read after 2–3 hours at 37° C.

Cultural details

The cuttings were struck on 9 December 1961 and the rooted cuttings transplanted on 4 January 1962. The young plants were planted on 9 March 1962 in steamed soil in benches in an aphid-proof glasshouse with forced aeration. The first flowers were picked on 11 July 1962. The experiment was finished on 31 August 1963.

Results

Effectiveness of inoculations

To see if the inoculation with the carnation mottle virus had been successful, the plants were checked for the presence of this virus on 16 March 1962. Of the 144 plants inoculated, 136 gave a positive reaction on *C. amaranticolor*. The eight remaining plants were tested again on 9 April and then reacted positively. It was concluded that all plants of the mottle group contained the virus.

The presence of the ring spot virus was checked on 20 March 1962, when 113 plants reacted positively. The remaining 31 plants, although giving no reaction on 20 March, later showed clear ring spot symptoms.

Testing the plants inoculated with both mottle and ring spot viruses gave 133 double infections on 2 May 1962. The remaining eleven plants were tested again on 23 July and then gave also a double precipitation line in a gel-diffusion test. It was therefore concluded that all plants of this group contained both viruses.

The inoculation with vein mottle virus was not so successful. Only 71 plants reacted positively on 1 June.

The inoculation with carnation latent virus was almost a total failure. Serological testing on 16 April gave negative reactions for all plants. Testing on 18 September resulted in only one positive reaction. It was concluded that the experiment could give no information on the influence of this virus on yield and quality of carnation plants.

Symptoms

As virus-free plants had been infected artificially with separate virus isolates it became possible to study the symptoms caused by the various single carnation viruses. There is no doubt that many descriptions of symptoms of carnation virus diseases in the literature relate to mixtures of viruses.

1. Carnation mottle virus

Plants infected with this virus showed no visible symptoms at any time during the season although tests on *C. amaranticolor* proved the presence of the virus in the plants.

2. Carnation ring spot virus

Symptoms consisting of irregular yellow spots appeared on the leaves. Sometimes they had a necrotic centre. With the ageing of the leaves the yellow spots often became necrotic. The leaves were occasionally malformed (Fig. 1). The leaf symptoms remained visible throughout summer and winter. In summer the colour of the flowers was somewhat paler than that of the virus-free flowers, but this difference tended to disappear in winter. The vigour of the plants was clearly reduced.

3. Carnation vein mottle virus

The young leaves exhibited vein clearing and flecks of a darker green colour than the normal tissues (Fig. 1). On old leaves symptoms tended to disappear. The green spots were most clear in summer, but less distinct in winter. The flowers showed a colour breaking (Fig. 2) which was especially distinct in summer, but less easily detectable in winter.

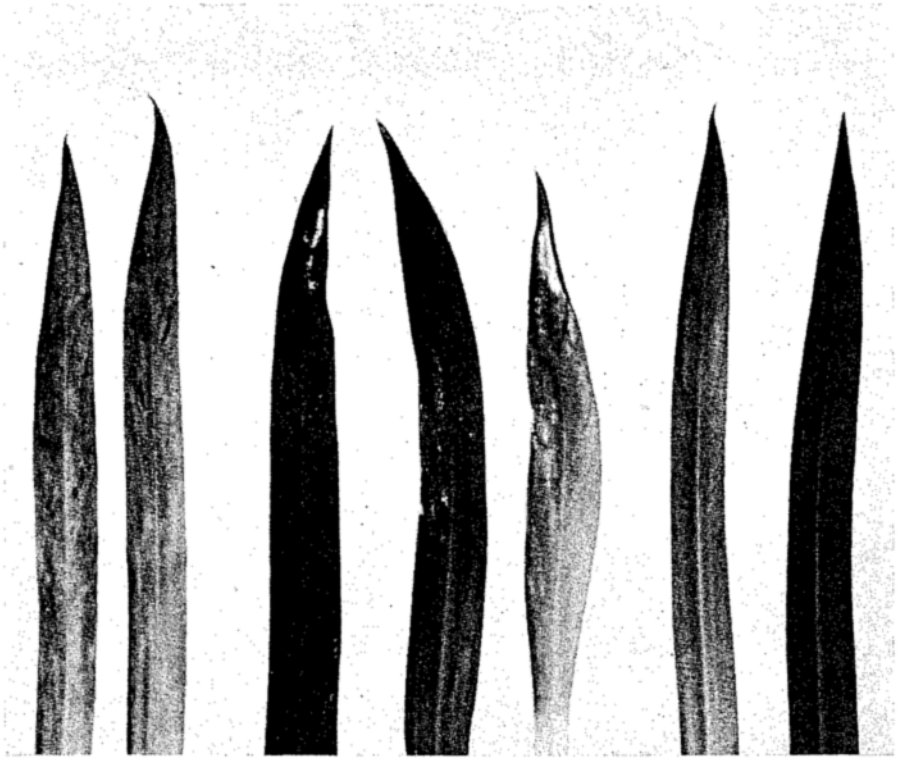

FIG. 1. Leaves of carnation cultivar 'William Sim', showing symptoms after infection with carnation viruses.

Right: two leaves of virus-free plants.

Middle: three leaves with ring spot symptoms.

Left: two leaves with vein mottle symptoms.

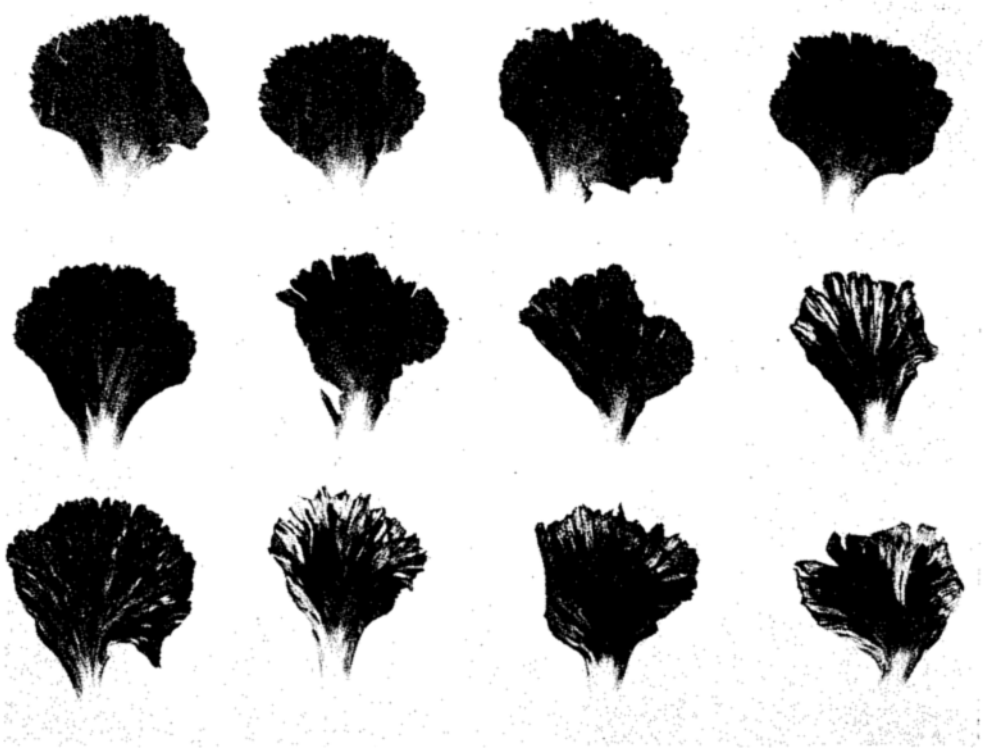

FIG. 2. Colour breaking in carnation flowers, cultivar 'William Sim', caused by vein mottle virus.

Top row: petals from virus-free plants.

Second and third row: petals from infected plants, showing various grades of colour breaking.

4. Carnation latent virus

The infected plant showed no symptoms.

Effect on yield

Records were taken from each plant individually. The flowers were picked, graded, measured and weighed. Two complications occurred. Firstly, a number of plants died in the course of the experiment as a result of infection with a foot rot fungus. Secondly, although the utmost care was taken to avoid spreading of the viruses, in the case of the mottle virus this could not be prevented. Spread of other viruses was not detected.

At the end of the experiment, therefore, the plants were individually checked for the presence of mottle virus. The situation as it was on 31 August 1963 is given in Table 1. Only plants without any virus and those with none but the intended virus were taken into consideration. The yield of flowers graded for quality is given in Table 2 and shown graphically in Fig. 3. The viruses also exerted an influence on the average flower weight, as is shown in Fig. 4.

TABLE 1. Situation on 31 August 1963 with respect to number and health of remaining carnation plants.

	Unsuccessful inoculations	Plants killed by foot rot	Unintended mottle infections	Plants with intended virus only
Virus-free	–	13	71	60
Mottle	0	18	–	126
Ring spot	0	19	106	19
Mottle and ring spot	0	14	–	130
Vein mottle	2	8	97	37

TABLE 2. Average number of flowers produced per plant in the course of the experiment.

	Number of first quality flowers	Statistical meaning	Number of second quality flowers	Statistical meaning	Number of third quality flowers	Statistical meaning	Number of flowers with split calices	Statistical meaning	Total number of flowers	Statistical meaning
Virus free	13.1		6.8		1.2		4.5		25.6	
Mottle	11.0	++	6.1	N.S.	1.4	not	5.0	N.S.	23.6	(+)
Ring spot	2.2	++	1.9	++	0.2	calcu-	17.3	++	21.6	+
Mottle and ring spot	1.7	++	1.4	++	0.3	lated	18.4	++	21.7	+
Vein mottle	9.2	++	5.6	(+)	1.2		5.9	N.S.	21.8	+

Statistical significance of the differences has been calculated in comparison with the virus-free object.
++ highly significant ($P < 0.01$), + significant ($P < 0.05$), (+) nearly significant ($P < 0.10$), N.S. not significant

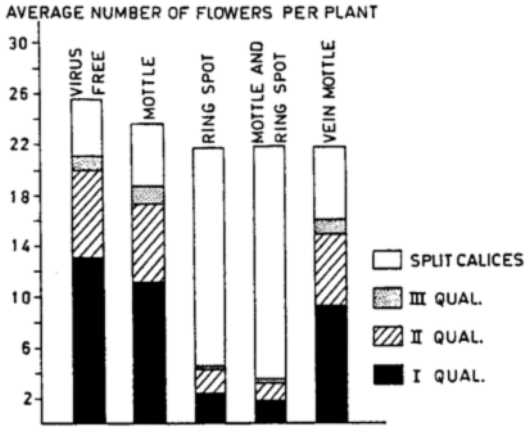

FIG. 3. Influence of carnation viruses on number and quality of flowers of the cultivar 'William Sim'.

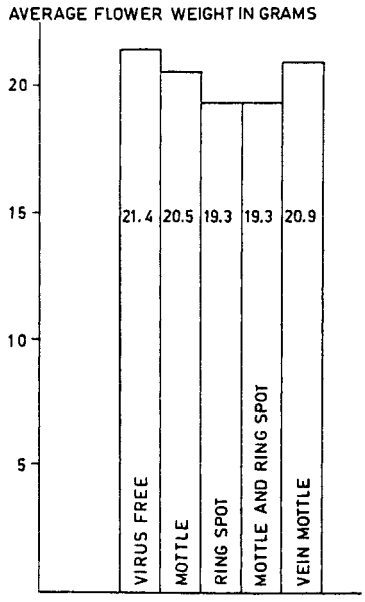

FIG. 4. Influence of carnation viruses on flower weight of the cultivar 'William Sim'.

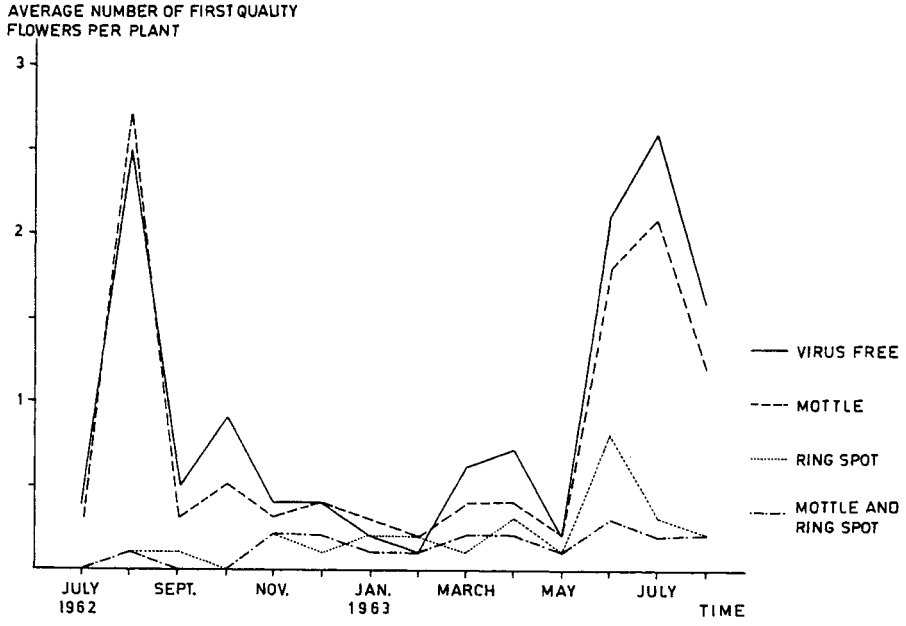

FIG. 5. Influence of carnation viruses on the number of first quality flowers in the course of the experiment.

The influence of the viruses on yield during the course of the cultural period was also studied. The effect on the number of first quality flowers during the course of the experiment may be seen in Fig. 5 and on the number of split flowers in Fig. 6.

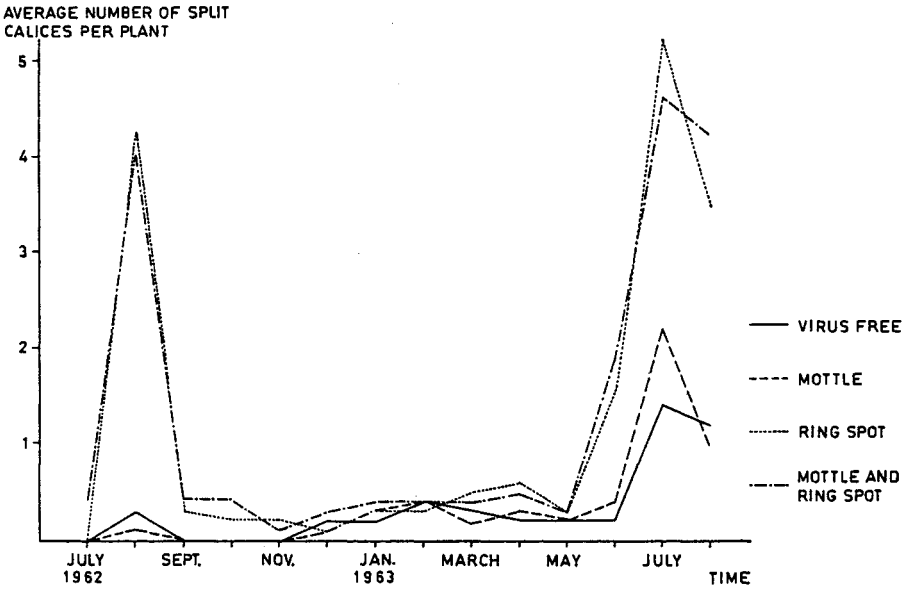

FIG. 6. Influence of carnation viruses on the number of split calices in the course of the experiment.

DISCUSSION

The symptoms described occurred after artificial infection of originally virus-free plants. Of course, the question may be raised whether a virus other than the three viruses studied might have been present in the plants used. For instance, the carnation etched ring virus, recently discovered (HOLLINGS & STONE, 1960), might be mentioned in this respect. Conspicuous pale green rings and oval markings on the older leaves and stems have been described as characteristic for this virus. Unfortunately, no non-carnation host plant of the virus is yet known and proof for absence of this virus cannot easily be given. However, no symptoms of the kind described for etched ring were seen and no symptoms whatsoever developed in the uninoculated plants during the course of the experiment. Moreover, the sap of these plants reacted negatively on known test plants and with known antisera. It was therefore concluded that the plants were virusfree and could legitimately be used in an experiment of the kind undertaken.

The carnation ring spot virus proved to be a serious virus and exerted a clear influence both on quality and quantity of the flowers. In particular, it caused a marked increase in the number of flowers with split calyx. Of course, the question of calyx splitting is a complex one, but plants infected with ring spot are more prone to it than virus-free plants.

The effect of the mottle virus was not so severe, but there was a definite depressing effect on quantity and quality of the yield. In recent years attention has been focused on large-scale application of the technique of meristem culture (VAN OS, 1964) and it may be concluded that the resulting elimination of the carnation mottle virus, among others, is of practical importance.

The carnation vein mottle virus caused colour breaking in the flowers of the variety used and the deleterious effect of the virus was obvious. The flowers were graded irrespective of the colour breaking, so that the influence of the virus was even greater than may be concluded from Table 2 and Fig. 3.

Because of the failure of the infection with the carnation latent virus no information on the influence of this virus could be obtained.

SAMENVATTING

Om de invloed van „carnation mottle-", „carnation ring spot-" en „carnation vein mottle"-virus op anjers te kunnen bestuderen werden virusvrije planten van een bepaalde kloon van het ras 'William Sim' kunstmatig met genoemde virussen geïnfecteerd.

Er verschenen geen ziekteverschijnselen op bladeren van planten, besmet met het „carnation mottle"-virus, maar duidelijke bladsymptomen konden worden toegeschreven aan het „carnation ring spot-virus, welk virus de groei van het gewas nadelig beïnvloedde. Het „carnation vein mottle"-virus veroorzaakte niet alleen vlekken in het blad, maar ook breking van de bloemkleur.

Elk van de drie bestudeerde virussen had een nadelige invloed op de oogst. Het „vein mottle"-virus was vooral van belang vanwege de breking in de bloemkleur. De schade, veroorzaakt door het „ring spot"-virus, uitte zich in de grootte en vooral in de kwaliteit van de oogst, waarbij een hoog percentage bloemen met gescheurde kelken optrad. Het „carnation mottle"-virus veroorzaakte eveneens een reductie van de grootte, maar vooral van de kwaliteit van de oogst, doch in mindere mate dan het „ring spot"-virus. Een combinatie van de twee laatstgenoemde virussen bleek iets schadelijker te zijn dan „ring spot"-virus alleen.

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