

Heat treatment experiments with carnations for the elimination of carnation mottle and etched ring viruses¹

F. A. Hakkaart² and J. Jordanova

Institute of Phytopathological Research (IPO), Wageningen, The Netherlands
Bulgartzvet, State Economic Enterprise, Sofia, Bulgaria

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Abstract

Plants of the carnation cultivar 'Joker', infected with carnation mottle and etched ring viruses, were subjected to a heat treatment at 38°C for 132 days. At intervals lateral shoots were taken and rooted. In some of these shoots carnation mottle virus could no longer be detected, even after repeated indexing on *Chenopodium amaranticolor*. Extension of the duration of the heat treatment resulted in an increase in the percentage of mottle-free plants, but the absolute number decreased owing to deterioration of the plants due to the prolonged heat treatment. A heat treatment of 132 days did not inactivate carnation etched ring virus in the plants.

Introduction

During recent years there has been some discussion about the possibility of eliminating carnation viruses, especially carnation mottle virus, by heat. Hollings and Stone (1964) could not inactivate carnation mottle virus (CaMV) by growing plants at 38°C, although its concentration in the apical tissues was diminished. Unfortunately, the duration of their experiment is not mentioned. Paludan (1964) succeeded in inactivating CaMV by keeping carnation plants (cultivar names not mentioned) at 37°C. After 56 days 10% of tips ranging from 3-5 cm proved to be CaMV-free, and after a heat treatment of 84 days this percentage had risen to 54. The virus testing was carried out 240 days after the heat treatment. Not all cultivars were equally easy to cure. Nevertheless, by prolonged heat treatment Paludan (1965) was able to free from CaMV the carnation cultivars 'Laddie Sim' and 'Petersons New Pink Sim', in which it apparently has been more difficult to inactivate this virus than in other cultivars. After 240 days, CaMV had been inactivated in 35% of the treated 'Laddie Sim' and in 32% of 'Petersons New Pink Sim'.

Brierley (1964) reported that carnation ringspot, mosaic, streak, etched ring (CERV), mottle and latent viruses can be eliminated by dry heat (38°C) in 2 months. Short stem tips were taken, rooted in 1 week and indexed after another week. It was not mentioned

¹ Part of this work was carried out during a stay of Mrs. J. Jordanova at the IPO, Wageningen.

² Stationed at the Experimental Station for Floriculture, Aalsmeer, The Netherlands.

how long the plants were maintained for visual observation of symptoms.

For the present work carnation plants of the cultivar 'Joker' were used, known to be infected with CaMV and showing symptoms of CERV. The cultivar 'Joker' was chosen because it shows conspicuous CERV-symptoms.

Materials and methods

Rooted cuttings of the carnation cultivar 'Joker' were transplanted into four wooden boxes, there being 35 plants in a box. Before application of the heat treatment, the tops of these plants were taken, rooted and kept untreated. The 79 resulting plants were each tested serologically for the presence of CaMV; all were infected. Serological testing for the presence of carnation ringspot virus gave negative results with all plants. Four months after rooting, all 79 plants showed severe symptoms of CERV. It was therefore assumed that all plants used for the heat treatment were infected with CaMV and CERV, but not with carnation ringspot virus.

The experiment was started 2 days after topping, on 2 November 1966. The boxes were placed in a heat chamber maintained at 38°C, illuminated by six fluorescent tubes per m² for 18h a day. Twenty-eight days after the beginning of the heat treatment, all side shoots of the plants in one box were removed, rooted and kept in an aphid-proof glasshouse. The same was done with shoots of the other three boxes after 70, 112 and 132 days, respectively. When taken, the side shoots measured 2–5 cm in length. These plants were tested for the presence of CaMV by sap inoculation on *Chenopodium amaranticolor*. The first tests were carried out about 2 months after the shoots were removed from the heat chamber. The plants were considered to be CaMV-free if they passed five tests on this host without causing the typical local lesions. The last test was carried out in November 1967. Details of testing dates appear in Table 1.

Because during this experiment no test plant for CERV that could be grown from seed was available and the symptoms even on the sensitive carnation cultivar 'Joker' were not considered as reliable, a return to the original situation was effected by re-inoculating the CaMV-free plants with this virus. The inoculum originated from *C. amaranticolor* leaves after a passage through *Silene armeria* plants to ensure hundred percent transmission. Re-infection with CaMV was done because of the synergism between CaMV and CERV (Hakkaart, 1968). In this way it was possible to check the elimination of CERV.

Results

From Table 1 it appears that CaMV can be eliminated by heat treatment. The low number of plants obtained after 70 days of heat treatment was due to a rooting failure. After a stay of about 4 months in the heat chamber the condition of the carnation plants had deteriorated so much that the experiment had to be finished; thus it was not possible to investigate the effect of a longer duration of the heat treatment.

All 'Joker' plants in which CaMV was not inactivated showed symptoms of CERV. So in these plants CERV was not eliminated. The plants in which CaMV had been inactivated were re-inoculated with CaMV on 15 November 1967, when indexing for the absence of CaMV had been finished. All of them became infected with this virus. Some 6 weeks after inoculation with CaMV, symptoms of CERV began to appear,

Table 1. Elimination of carnation mottle virus from carnation plants of the cultivar 'Joker' by heat treatment

Duration of heat treatment in days	Date of shoot harvest	Number of shoots harvested from a box	Number of shoots rooted	Dates of indexing for carnation mottle virus ¹	Plants free from car- nation mottle virus	
					Number	%
0	—	—	—	—	0	0
28	30 Nov. 66	33	—	—	0	0
70	11 Jan. 67	40	27	—	0	0
112	22 Febr. 67	33	3	8 May 4 Aug. 18 Aug. 13 Sept. 9 Nov.	1	33
132	14 March 67	11	31	8 May 20 June 4 Aug. 13 Sept. 9 Nov.	17	55
			11	24 May 4 Aug. 18 Aug. 13 Sept. 9 Nov.	9	82

¹ The dates of indexing of the apparently cured shoots only are given; the dates of indexing of the plants that proved to be still infected have been omitted

Tabel 1. Eliminering van het "carnation mottle"-virus uit anjerplanten van de cultivar 'Joker' door warmtebehandeling

and after about 8 weeks all 'Joker' plants showed etched ring symptoms. It was concluded that CERV had not been eliminated from any of the plants.

Discussion

The results obtained confirm the findings of Paludan (1964, 1965) that CaMV can be eliminated by heat treatment. Evidently, the method of meristem culture is not the only way in which this virus can be eliminated. In our experiments, too, the efficiency of heat treatment increased with the duration of the treatment. At a certain stage, however, the plants deteriorated so much that although a higher percentage of CaMV-free plants was obtained, the absolute number decreased. It was not possible to prolong the treatment until 240 days, as Paludan (1965) was able to do, because our plants did not withstand such a long exposure.

In many cases CaMV could be detected in the not-cured shoots about 2 months after return from the heat chamber, but sometimes even a longer period was required for detection of this virus. It is, therefore, clear that a heat treatment experiment such as reported by Brierley (1964), in which the plants were tested 2 weeks after return from the heat chamber, cannot afford valuable results.

CERV was not eliminated in our experiment. Perhaps an exposure to heat for a period longer than 132 days is necessary, or meristem culture may be the only way in which plants free from this virus may be obtained. However, nothing is known about the occurrence of CERV in commercial stocks. When this virus occurs only incidentally in a cultivar its elimination can be effected by a process of testing and discarding. Curative measures would only have to be considered if a cultivar were found to be fully infected.

Samenvatting

Warmtebehandelingsproeven met anjers voor de eliminering van het "Carnation mottle" - en het "Carnation etched ring" - virus

Planten van de anjercultivar 'Joker', die besmet waren met het "carnation mottle" - en "carnation etched ring" - virus, werden gedurende 132 dagen bij een temperatuur van 38°C gehouden. Met tussenpozen werden er scheutjes van de planten afgehaald en beworteld. In een aantal van deze scheutjes bleek het "carnation mottle"-virus, zelfs na vijfmalige toetsing op *Chenopodium amaranticolor*, niet meer aantoonbaar. Verlenging van de warmtebehandeling gaf een toename van het percentage genezen planten, hoewel het absolute aantal afnam als gevolg van beschadiging door de warmtebehandeling. Een warmtebehandeling van 132 dagen leverde evenwel geen planten op die vrij waren van het "carnation etched ring"-virus.

References

- Brierley, P., 1964. Heat cure of carnation viruses. Pl. Dis. Repr 48: 143.
Hakkaart, F. A., 1968. *Silene armeria*, a test plant for carnation etched ring virus. Neth. J. Pl. Path. 74:150-158.
Hollings, M. and Stone, O. M., 1964. Investigation of carnation viruses. I. Carnation mottle. Ann. appl. Biol. 53: 103-118.
Paludan, N., 1964. Inaktiveringsforsøg med virus-inficeret nellikemateriale. Maanedoversigt PlSygd. 412:83-88.
Paludan, N., 1965. Inaktiveringsforsøg med nellike - spactning-virus. Maanedoversigt PlSygd. 418: 65-68.