Neth. J. Pl. Path. 77 (1971) 127–133

Saponaria vaccaria 'Pink Beauty', a new test plant for carnation mottle virus

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Accepted 3 March 1971

Abstract

Saponaria vaccaria 'Pink Beauty' was found to be a test plant for carnation mottle virus. Its value was compared with that of *Chenopodium amaranticolor* for indexing for the virus, and this bioassay was compared with a serological diagnosis. Serological double-diffusion tests gave the quickest reactions, but proved to be the least sensitive; addition of 1 *M* urea did not increase sensitivity. Local lesions resulting from infectivity tests on detached leaves of *C. amaranticolor* in a climate room could be counted after 1 week and these tests were about equally sensitive as those on leaves on intact plants of this species in the glasshouse, which were read after about 10 days. Infectivity tests on *Saponaria vaccaria* 'Pink Beauty' resulted in systemic symptoms 10 to 14 days after inoculation; this method appeared to be the most sensitive.

Introduction and literature

In several countries, like the Netherlands, Great Britain, Denmark and France, considerable efforts are being made to control carnation mottle virus (CaMV) (Cryptogram:x/x : x/x : S/S : S/O) in carnation mother plants. For diagnosis of CaMV both bio-assay and serological methods are advocated. In the Netherlands at this moment *Chenopodium amaranticolor* is almost the only indicator plant used.

The oldest test plant for CaMV mentioned in the literature is *Dianthus barbatus* and it was used by Kassanis (1955) in his pioneer work on carnation viruses. Nowadays, this test plant has fallen into disuse because it has given inconsistent results and was found unreliable in detecting CaMV (Hollings and Stone 1964; Hakkaart, unpublished).

Hollings (1956) was the first to mention *C. amaranticolor* as an indicator plant for CaMV. Hollings and Stone (1964) considered this species as the best indicator host. There are 2 modifications of the test in use at this moment: one with detached leaves and another with leaves on intact plants. Serology is another approach to diagnose CaMV and the opinions on its merits diverge. By comparing precipitin-tube tests and gel-diffusion tests with infectivity tests on *C. amaranticolor*, Hollings and Stone (1964) concluded that the infectivity test was more sensitive and reliable and should be used for indexing carnation mother plants. Kemp (1967), however, working with crude

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carnation sap, found that a serological assay by the double-diffusion technique was comparable to a bioassay with *C. amaranticolor* and *D. barbatus* as indicator hosts. An improvement in the sensitivity of the double-diffusion technique was reported by Devergne and Cardin (1967) who added 1 *M* urea to the medium.

During a search for test plants for carnation etched ring virus, *Saponaria vaccaria* 'Pink Beauty' appeared to be sensitive to CaMV and was therefore supposed to have value as indicator (Hakkaart, 1970). In the literature *S. vaccaria* has already been mentioned as a systemic host of CaMV by Kemp (1964), but no details on symptoms, incubation period or sensitivity were recorded; Kemp presumably used the wild form of the plant.

In the present paper the sensitivity to CaMV of the test plant method with *S. vaccaria* 'Pink Beauty' is compared with that of other diagnostic methods for this virus.

Materials and methods

A. Diagnostic methods

For the diagnostic methods compared see the legends to Table 1.

B. Inoculum

Three inoculum sources were used: a. leaves from plants of the carnation cultivar 'Joker' with a longstanding infection, b. leaves of *C. amaranticolor* with local lesions 2 to 3 weeks after inoculation, and c. leaves of *S. vaccaria* 'Pink Beauty' systemically infected about 3 weeks after inoculation. The inoculum source b was obtained from a and c from b. Leaf material was wrapped in cotton wool and squeezed out. A series of dilutions up to ten-fold was made of the crude sap with 1/30 M NaK-phosphate buffer (pH 6.8). Each dilution was inoculated to 10 *C. amaranticolor* leaves and 10 *S. vaccaria* plants, and ten wells were filled for the double-diffusion tests.

C. Serology

The medium used in the double-diffusion tests consisted of 1% agar (Noble), 0.8% NaCl (w/v) and 0.01% merthiolate (v/v). In the modification also used, 1 *M* urea was added to the medium. The diameter of the wells, which were 6 mm apart, was 3 mm. All tests were also done with normal serum, with which no reactions were obtained. The antiserum was kindly provided by Ir D. H. M. van Slogteren, Flowerbulb Research Institute, Lisse. The dishes were incubated at room temperature and were evaluated one day after preparation.

D. Infectivity tests

For the infectivity test with *C. amaranticolor* the full-grown leaves just beneath the top of the plants were used for inoculation on the intact plant; the detached leaves to be inoculated were taken from the same positions. The *S. vaccaria* plants were about 3 weeks old when inoculated and had about 4 developed leaves at this age. Both cotyledons and all leaves were inoculated. The leaves were dusted with carborundum (500 mesh) and inoculated by rubbing with a small disposable plastic sponge saturated with sap (see under inoculum). The inoculated plants were rinsed with water. The detached leaves were placed in plastic trays, as used in routine tests by the Netherlands

Table 1. Comparison of the sensitivities of serological and bioassay methods for the detection of carnation mottle virus.

Dilution of sap	Source of inoculum														
	Carnation 'Joker'					*Chenopodium amaranticolor*					*Saponaria vaccaria*				
	S.d.d.	C^1	C^2	C^3	S.v.	S.d.d.	C^1	C^2	C^3	S.v.	S.d.d.	C^1	C^2	C^3	S.v.
Undiluted	++	10/m	10/m	10/m	10	++	10/m	10/m	10/m	10	++	10/m	10/m	10/m	10
10^{-1}	+	10/m	10/m	10/m	10	+	10/m	10/m	10/m	10	+	10/m	10/m	10/m	10
10^{-2}	–	10/m	10/m	10/m	10	–	10/m	10/m	10/m	10	–	10/m	10/m	10/m	10
10^{-3}	–	10/m	10/m	10/m	10	–	10/m	10/m	10/m	10	–	10/m	10/m	10/m	10
10^{-4}	–	10/m	10/34	10/25	10	–	10/13	10/22	10/47	10	–	10/m	10/m	10/m	10
10^{-5}	–	10/19	10/14	9/19	10	–	9/4	6/2	10/10	10	–	10/m	10/m	10/m	10
10^{-6}	–	8/9	3/2	9/12	10	–	7/1	5/0.5	10/4	10	–	10/m	10/m	10/m	10
10^{-7}	–	8/4	7/1	8/3	10	–	2/0.4	3/0.6	5/1	10	–	10/m	10/m	10/m	10
10^{-8}	–	8/4	5/2	5/2	9	–	4/0.6	2/0.4	3/0.4	8	–	10/43	10/8	10/9	10
10^{-9}	–	7/2	6/1	6/4	8	–	5/1.0	4/0.6	6/1.7	6	–	10/16	5/1.2	19/7	10
10^{-10}	–	4/1.0	3/0.5	2/0.4	5	–	0/0	0/0	0/0	0	–	10/11	4/0.4	9/3	10
Total number of infected leaves or plants	–	95	84	89	102	–	77	70	84	94	–	110	99	108	110

S.d.d.: serological test by double-diffusion, both without and with 1 *M* urea
C^1: infectivity test on 10 leaves of *Chenopodium amaranticolor* on an intact plant
C^2: infectivity test on 10 detached leaves of *C. amaranticolor* of the same batch as C^1
C^3: infectivity test on 10 detached leaves of *C. amaranticolor* of a batch which is routinely used for diagnostic purposes by the Netherlands General Inspection Service for Ornamental Crops at Roelofarendsveen
C^1, C^2 and C^3 nominator denotes number of infected leaves out of 10 leaves inoculated; denominator denotes average number of local lesions per leaf
m: many local lesions.

Tabel 1. Vergelijking tussen de gevoeligheid van serologische en bioassaymethoden om het vlekkerigheidsvirus van anjer op te sporen.

General Inspection Service, and exposed to 19 °C and 16 h of fluorescent light a day. The inoculated plants of *Chenopodium* and *Saponaria* were placed in a glasshouse maintained at about 22 °C in daylight. The experiments were performed from July until the middle of October 1970. Lesions on detached *Chenopodium* leaves were counted 1 week after inoculation; those on intact plants 3 days later. The reaction on *Saponaria* plants was read about 2 weeks after inoculation.

Results

The serological double-diffusion tests yielded the normal precipitation lines, but with urea these lines were slightly more diffuse. In all tests clear lines were formed with undiluted sap and weaker lines with a dilution of 1:10. In no case a line was obtained with a (sap) dilution of 1:100. The dilution end-points were the same whether or not urea was present, and therefore in Table 1 all results with both media are given in one column.

Inoculation of the *Chenopodium* leaves resulted in the wellknown local lesions, which need no further description.

S. vaccaria 'Pink Beauty' reacted 8 to 10 days after inoculation with vein chlorosis, followed by systemic mottling and deformation of the new leaves (Fig. 1 and 2). Sometimes local chlorotic spots developed on the leaves as a first sign of infection, but these local symptoms were not always present. In a further stage the mottling became more distinct and sometimes darkgreen patches of tissue appeared among the mottled areas. Growth of the plant was stunted.

Six experiments were made which all showed the same tendency. For brevity only three experiments are summarized in Table 1.

The serological test, even in the modified version with urea, was appreciably less sensitive than the bioassay methods.

Fig. 1. *Saponaria vaccaria* 'Pink Beauty', showing mottling and leaf deformation caused by carnation mottle virus.

Fig. 1. Saponaria vaccaria 'Pink Beauty', met vlekken en bladmisvorming, na inoculatie met anjer-vlekkerigheidsvirus.

Fig. 2. *Saponaria vaccaria* 'Pink Beauty'. On the left 2 healthy leaves; the other leaves show mottling caused by carnation mottle virus.

Fig. 2. Saponaria vaccaria 'Pink Beauty'. Links 2 gezonde bladeren; de overige bladeren met vlekken na inoculatie met anjervlekkerigheidsvirus.

C. amaranticolor proved a very sensitive indicator plant. The numbers of infected leaves obtained for C^1, C^2 and C^3 are not much divergent, although there is a tendency of C^1 (leaves on intact plants) to be more sensitive than C^2 (detached leaves of the same batch). The highest numbers of infections were obtained in the *Saponaria* test. The difference between this test and the *Chenopodium* test (C^1) becomes even greater when one considers that in routine testing minute numbers of local lesions, e.g. 1 lesion per leaf, may easily be overlooked. The systemic symptoms of *Saponaria*, however, do not give rise to such inaccuracies.

Discussion

We can confirm the results of Hollings and Stone (1964) who claimed that the infectivity test on *C. amaranticolor* is more sensitive than sero-assay with the double-diffusion technique in detecting CaMV. Consequently, we must disagree with Kemp (1967) who stated that both methods are equally sensitive. The improvement obtained by Devergne and Cardin (1967) by adding 1*M* urea to the medium could not be confirmed in our tests. The reason is not known. In our opinion sero-assay with the double diffusion technique has value when quick information is wanted about the health condition of large numbers of plants, but has no merits when building up CaMV-free stocks.

From the experiments *S. vaccaria* 'Pink Beauty' emerges as an extremely sensitive CaMV indicator. A disadvantage is that it has a longer incubation period than *Chenopodium* and that it is not possible to perform more than 1 test on a single plant because the reaction is systemic.

There are, however, other properties making this plant very attractive as a carnation virus indicator. *S. vaccaria* 'Pink Beauty' is a test plant for a 50 nm isometric virus, which we consider as an incitant of the etched ring disease for which *C. amaranticolor* is no indicator (Hakkaart, 1970). Furthermore. the plant is an indicator of a 29 nm isometric virus, isolated from the carnation cultivar 'Orange Triumph', non-infectious to *C. amaranticolor*, still under study (Hakkaart, in press). It is also a host of a rodshaped virus, presumably carnation latent virus, to which again *C. amaranticolor* is not susceptible (Hakkaart, 1970). Carnation ringspot virus is infectious to *C. amaranticolor*, but also to *S. vaccaria* 'Pink Beauty' (Hakkaart, 1970), which is of little importance because the virus has been eradicated from the Netherlands. Details of these properties will be published in a following paper.

We now advocate the use of *S. vaccaria* 'Pink Beauty' in conditioned glasshouses for testing stocks of carnation mother plants with a very low incidence of CaMV to detect the last traces of this virus and to discover several other viruses, to which *C. amaranticolor* is not susceptible.

Acknowledgments

The authors wish to thank the Netherlands General Inspection Service for Ornamental Crops (NAK-S) for providing the facilities for the experiments with detached leaves.

Samenvatting

Saponaria vaccaria 'Pink Beauty', een nieuwe toetsplant voor het vlekkerigheidsvirus van anjer.

Na de ontdekking, dat *Saponaria vaccaria* 'Pink Beauty' gevoelig is voor het vlekkerigheidsvirus van anjer, werd de waarde van de toetsplantenmethoden met deze en andere indicatorplanten onderling vergeleken en met de gebruikelijke serologische toets voor dit virus. De serologische dubbel-diffusiemethode leverde het snelst resultaten op, maar was het minst gevoelig. Ook toevoeging van 1 *M* ureum aan het medium vergrootte deze gevoeligheid niet. Inoculatie van losse bladeren van *Chenopodium amaranticolor* in een klimaatkamer leverde lokale lesies op, welke na een week konden worden geteld. Deze methode was vrijwel even betrouwbaar als toetsing op bladeren aan intacte planten van dezelfde soort in de kas, welke reacties na 10 dagen afgelezen konden worden. De traagste, maar gevoeligste methode was de infectietoets met *S. vaccaria* 'Pink Beauty' die ook waarde heeft voor het aantonen van enkele virussen, waarvoor *C. amaranticolor* niet vatbaar is.

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