THE RELIABILITY OF SEROASSAY FOR THE DETECTION OF CARNATION MOTTLE VIRUS IN CRUDE SAP[1]

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Abstract

Serological assay for carnation mottle virus in crude carnation sap by the double-diffusion technique was comparable to a bioassay method that used Chenopodium amaranticolor and Dianthus barbatus as indicator hosts. On only a few occasions were results for both methods divergent, Virus concentration, a major factor affecting the reliability of any seroassay method, fluctuated from month to month and differed among cultivars. The advantages and limitations of this serological method are discussed.

Introduction

Carnation mottle virus (C. Mot. V.) can be detected serologically in crude, undiluted sap by immunodiffusion tests in agar, even though the sap contains other constituents (2, 3, 6). Should such a test be as accurate and sensitive as an infectivity test that involves days of delay before the results are available, then its advantages would warrant its acceptance as a substitute or adjunct for the biological activity test.

To test its reliability, the seroassay method was compared monthly in the spring and summer of 1963 and 1964 with a conventional bioassay method. Serological estimates of the relative virus concentration in each sap sample were also determined at these times to gain some insight into seasonal changes in virus synthesis in various cultivars.

Materials and methods

Five plants were selected at random from each of the following carnation cultivars: 'Scania', 'Yellow Sim', 'Peace River', 'Petersen's Improved Sim', 'Peppermint Sim' and 'Flamingo'. All cultivars were grown in a commercial greenhouse at St. Catharines, Ontario. Each was tagged clearly to ensure that subsequent tests were made from the same source material. It was not known if the selected plants were infected with C. Mot. V.

A top lateral shoot weighing approximately 3-4 g was broken from each of the selected plants at monthly intervals between March and August in 1963 and 1964, collected in individual polyethylene bags, and brought to the laboratory on the day of testing. Enough sap was squeezed from each shoot to give a 1-ml sample.

The virus content of the crude sap from each of the 30 samples was assessed by the agar double-diffusion method with an antiserum that had been prepared in earlier experiments (3) against an isolate of C. Mot. V. from the cultivar 'Apollo'. The titer of the antiserum against its homologous antigen in crude undiluted sap of Dianthus barbatus L. was in excess of 1/256. The serum was unabsorbed but had given no reaction previously with sap from either healthy D. barbatus or D. caryophyllus L. However, control tests were conducted each month with undiluted sap prepared as above from a healthy seedling carnation clone.

Relative virus concentration was determined from serial twofold dilutions of each sap sample made in distilled water and tested against a constant serum dilution (1/16) in agar plates prepared as previously described (5). Approximately 0.1 ml antiserum was pipetted into the central well and 0.1 ml of each of the dilutions of every sap sample into the peripheral wells. All plates were incubated immediately at 27°C. After 24 and 48 hr, the highest dilution of each virus sample producing a visible precipitate was recorded.

A single reference isolate of C. Mot. V. was used with its antiserum to compare the homogeneity of the virus isolates, because variations in virus endpoint of any of the samples possibly might be due to major antigenic differences among the isolates. The reference isolate was placed in alternate peripheral wells and the other isolates were added to the adjacent ones. The precipitin patterns were noted after 48 hr and they were interpreted by the theories discussed by Crowle (1).

At the time of serological testing, bioassay tests were also made on each of the sap samples by the mechanical inoculation of both D. barbatus clone 26 and Chenopodium amaranticolor Coste & Reyn. These plants are also indicators of other unrelated sap-transmissible viruses of carnation, whose presence might affect the multiplication of C. Mot, V. in a mixed infection. The plants were maintained at

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70° F under a bank of fluorescent lights supplemented by incandescent light (14 hr/day) and observed for 21 days.

Results

Reliability of the seroassay method

Comparative serological and biological assays of crude saps extracted from the same 30 carnation plants between March and August were made in two successive years. The initial March test indicated that each of the selected plants was infected with C. Mot. V. before the start of the experiment (Table 1). It and the subsequent monthly tests established the fact that the results of both assay methods were highly correlated. In only two cases did infection occur on an assay plant when the serological reaction was negative. Crude sap from the leaves of a single plant of the cultivars 'Peace River' and 'Scania' did not react with C. Mot. V. antiserum in April 1963 and March 1964, respectively.

All sap samples gave a positive reaction on C. amaranticolor but on 11 occasions no response occurred on D. barbatus 26. In 1963, six failures to infect the latter host were associated with the cultivar 'Yellow Sim', two with 'Petersen's Improved Sim', and one each with 'Peace River' and 'Scania'. They occurred more often between May and July than in March, April or August. In 1964, D. barbatus failed to become infected after inoculation on only one occasion. Only twice did a negative response occur on D. barbatus 26 with sap extracted from the same plant in different months,

Precipitin zones appeared in the agar plates within 24 hr and they were usually sharp and distinct. On occasion, these sharp zones later became diffuse towards the antigen wells, which indicated excess virus antigen in the crude sap. Infectious sap induced local lesions on the inoculated leaves of C. amaranticolor in four days and on those of D. barbatus in 12 days.

In March 1963 there was no evidence that any other sap-transmissible carnation virus was present in the selected plants, Later, however, carnation ringspot virus (CRSV) was detected in three plants. Crude sap from two plants of 'Peace River' and from one of 'Flamingo' induced systemic symptoms on the uninoculated leaves of D. barbatus 26 in April and August 1963, respectively. These symptoms were identical with those Kemp and Heald (4) showed to be characteristic of CRSV infection in this clone. CRSV was not associated with either of the plants in which C. Mot. V. was not detected serologically. Incidentally, the sap from healthy seedling carnations produced no precipitin reaction when reacted with C. Mot. V. antiserum nor did it produce visible symptoms on either of the assay plants.

Frequency distribution of the relative concentration of C. Mot. V. in the crude sap samples

In the 360 sap samples taken in the spring and summer of 1963 and 1964, virus concentrations ranged from 1/1 to 1/16. On two occasions, although the virus was detected by bioassay, it could not be measured serologically. The frequency distributions of the concentrations in 1963 and1964 had the same range but differed in their prevalence at the high and low values (Table 2). The mode of the concentration distribution in a 11 samples and that calculated for the scatter in both 1963 and 1964 was 1/4.

Factors influencing the C. Mot. V. concentration

Month—Considerable fluctuation in the virus concentration in individual plants occurred from month to month in both 1963 and 1964 (Table 3). In general, the lower concentrations were more prevalent in June, wherras thr higher ones occurred most often in March and August.

Cultivar—Concentration differencrs also existed between the diffrrcnt cultivars. The mode of the concentration distribution for individual cultivars varird noticeably (Table 4). In both years, the modal concentration of the virus in 'Scania' was low, whereas it was high in 'Petersen's Improved Sim' and 'Flamingo'.

Strains and unrelated viruses—Only a single plant of 'Peace River' contained an isolate of C. Mot. V. that was antigenically distinct from the reference virus. The concentration of this distinct strain was never below the modal concentration of 'Peace River' at any sampling date in either year. Neither was it associated with sap samples that failed to react serologically. No evidence was found to suggest that the isolates from 'Scania', in which the concentration was consistently low, were serologically or biologically different from the reference virus.

Although CRSV was found to be associated with C. Mot. V. in three plants, this unrelated virus had no apparent effect on the C. Mot. V. concentration in the sap. It was never detected in samples that failed to react with C. Mot. V. antiserum. Neither was the estimated concentration of C. Mot. V. in these doubly infected plants below the most frequent concentration in the same cultivar infected only with C. Mot. V.

Discussion and Conclusions

The agar double-diffusion technique appears to be a reliable, rapid and practical method for the detection of C. Mot, V. in crude sap. Although the results of the serological tests were closely correlated with those of the bioassays, it might be argued

Table 1. Number of positive serological and infectivity reactions of crude sap extracts from 30 carnation plants belonging to six cultivars sampled during six-month periods in 1963 and 1964

Cultivar	Year tested	Serological reaction						Infectivity: C. amaranticolor						Infectivity: D. barbatus 26					
		Mar.	Apr.	May	June	July	Aug.	Mar.	Apr.	May	June	July	Aug.	Mar.	Apr.	May	June	July	Aug.
Petersen's Imp. Sim	1963	5*	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	1964	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Yellow Sim	1963	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	4	2	2
	1964	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Peace River	1963	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5	5	4	5
	1964	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Flamingo	1963	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5		5	5
	1964	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Scania	1963	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	1964	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Peppermint Sim	1963	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	1964	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	5

* Number of sap samples giving a positive serological or infectivity reaction of five samples tested.

that the former appeared to be more consistently reliable under our conditions. In no case did virus infection go undetected in two successive sero-assays of the same plant. On the other hand, consistent results with the bioassay evidently depend on the assay host. C. amaranticolor was considerably more sensitive to C. Mot. V. than D. barbatus 26. The latter plant failed to respond to the virus in sap samples on 11 occasions when both bioassays on C. amaranticolor and seroassays were positive.

Both assay methods have at least one similar disadvantage. Virus concentration, a major factor affecting the precipitin reaction and infectivity, fluctuates considerably from month to month and among different cultivars. Extremely low concentrations or possible uneven distribution of the virus in carnation shoots, or both, at any time could influence the successful application of either test. Failure to detect the virus conceivably could occur in June, when the environmental conditions in Ontario apparently encourage lower concentrations. The presence of the virus might remain undetected in a cultivar such as 'Scania', in which the concentration is consistently lower than in other cultivars.

In spite of this potential weakness, seroassay was very reliable under Ontario conditions in 1963 and 1964. This method has the advantage over bioassay in that it not only reduces the time for testing but eliminates the need for extensive greenhouse facilities. Such a test could serve, at least, in a primary screening program for the establishment of C. Mot. V.-free mother plants with the purpose

Table 2. Freauencv distribution of relative concentrations of carnation mottle virus in 36Q crude sap samples

Relative concentration*	1963 (No.)	1963 (%)	1964 (No.)	1964 (%)	Total (No.)	Total (%)
Below detectable leve1	1	0.5**	1	0.5**	2	0.5***
1/1	8	4.4	3	1.6	11	3.2
1/2	47	26.1	42	23.3	89	24.7
1/4	55	30.6	54	30.0	109	30.2
1/8	54	30.0	49	27.2	103	28.6
1/16	15	8.3	31	17.2	46	12.7

* Highest dilution of a sample to produce a precipitin reaction.

** Frequency of each concentration calculated as a percentage of the samples extracted each year.

*** Frequency of each concentration calculated as a percentage of all samples.

Table 3. Frequency distribution of relative concentrations of carnation mottle virus in crude sap extracted at monthly intervals in 1963 and 1964

Relative concentration*	Year	Sampling date: March	April	May	June	July	August
Below detectable level	1963	0	1	0	0	0	0
	1964	1	0	0	0	0	0
1/1	1963	0	1	0	5	2	0
	1964	0	1	0	1	1	0
1/2	1963	2	2	9	18	10	6
	1964	5	6	4	12	11	4
1/4	1963	8	12	13	6	10	6
	1964	6	15	5	10	10	8
1/8	1963	18	11	8	1	5	11
	1964	10	8	13	4	8	6
1/16	1963	2	3	0	0	3	7
	1964	8	0	8	3	0	12

* Highest dilution of a sample to produce a precipitin reaction.

Table 4. Frequency distribution of the relative concentrations of carnation mottle virus in crude sap samples extracted from six commercial cultivars in 1963 and 1964

Relative concentration*	Year	Cultivar sampled** PIS	YS	PR	FL	SC	PS
Below detectable level	1963	0	0	1	0	0	0
	1964	0	0	0	0	1	0
1/1	1963	1	0	1	0	3	3
	1964	0	0	1	0	1	1
1/2	1963	5	7	7	5	11	12
	1964	0	6	5	2	1	7 1 2
1/4	1963	6	15	7	6	1	3 8
	1964	8	9	8	5	1	1 1 3
1/8	1963	13	8	12	12	3	6
	1964	9	1	4	8	1	4 0 4
1/16	1963	5	0	2	7	0	1
	1964	13	1	8	9	0	0

* Highest dilution of a sample to produce a precipitin reaction.

** PIS = Petersen's Improved Sim; YS = Yellow Sim: PR = Peace River: FL = Flamingo; SC = Scania; PS = Peppermint Sim.

of dividing an apparently healthy carnation population into two groups. The one would be exempt from further examinations because the test detected the virus, and the other group would be examined further by the most sensitive bioassay method available if the serological test failed to detect the virus.

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