Serological Properties of Prunus Necrotic Ringspot and Apple Mosaic Virus Isolates from Rose

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ABSTRACT

Antisera against three isolates of apple mosaic virus (ApMV) and two isolates of Prunus necrotic ringspot virus (PNRV) were used in comparative serological tests against six virus isolates from hybrid tea roses. The isolates showed a wide range of serological reactions, but two isolates belonged clearly to the ApMV serotype, whereas three belonged to the PNRV serotype. One isolate reacted to a high degree with all antisera of both serotypes. These tests show the existence of a continuous spectrum of virus isolates from rose extending from the PNRV serotype to the ApMV serotype.

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Prunus necrotic ringspot virus (PNRV) was first reported by Cochran (4) to occur naturally in roses. His findings were based on production of typical PNRV symptoms when rose was indexed to peach. There are several other reports of the occurrence of PNRV in roses based also on host reactions for virus identification (8, 10) and serological tests (11). Serological tests with antisera against PNRV and with an antiserum prepared against rose mosaic virus (RMV) were conducted by Fulton (6). These tests showed that RMV is not identical with, but is related to, PNRV. According to serological reactions, RMV and apple mosaic virus (ApMV) are identical, and both names can be used as synonyms (7). De Sequeira (5) and Fulton (7), both using the definition of Kassanis (9), considered ApMV and PNRV to be serotypes of the same virus.

MATERIALS AND METHODS.—The present work was undertaken to examine the serological reactions of six virus isolates from roses (*Rosa dilecta* Rehd.) collected in Oregon and California with five antisera prepared against several isolates from the ApMV and the PNRV serotypes. For comparison, two PNRV isolates from peach and the type culture of ApMV (ATCC PV 32) were included in the investigation. The origin and description of each virus isolate are given in Table 1.

Virus transfers were made from rose to cucumber (*Cucumis sativus* L. 'National Pickling') as follows: To 1 g of young rose leaves showing symptoms, we added 1 ml of 4% polyethyleneglycol 6000 in 0.02 M HEPES buffer (N-2-Hydroxyethylpiperazine-N-2-ethanesulfonic acid) (Calbiochem Co., Los Angeles, Calif.), pH 8. The stabilizing effect of HEPES on PNRV was reported by Casper et al. (3). The material, ground in a mortar, was used to inoculate cotyledons (previously dusted with 600-mesh Carborundum) of 12 cucumber plants for each virus isolate. Some minutes later, the plants were rinsed with water and shaded for 18-24 hr.

In the first passage on cucumber, usually only one to a few lesions showed on any of the inoculated cotyledons. In the second passage on cucumber, three to fifteen lesions appeared on each infected cotyledon within 3 to 5 days.

A modified Ouchterlony agar gel test (3) was used for serological identification of the viruses. In cases where only a few lesions occurred, single lesions were punched out of inoculated cucumber leaves to insure adequate virus concentration for the serological test. The leaf material was wrapped in cheesecloth and squeezed in a hand press together with the same amount (w/v) of 0.02 M phosphate buffer, pH 8. The resulting crude sap was used immediately to fill holes in the agar gel. Each hole filled with crude sap was surrounded by three holes with three antiserum dilutions in physiological saline, ranging from 1:4 to 1:256. First readings could be taken several hours later; the last reading was taken after 10 days.

Five antisera were used for the serological tests. Three antisera were prepared against different isolates of the ApMV serotype: Antiserum 1 by R. W. Fulton, Madison, Wisc., against a RMV isolate; Antiserum 2 by our laboratory in Braunschweig, Germany, against an ApMV isolate from Holland; Antiserum 3 by R. W. Fulton, against an ApMV isolate from Paradise Valley, Calif. (ATCC PV 32). Two antisera were made against two isolates of the PNRV serotype: Antiserum 4 by R. W. Fulton, against PNRV, strain G; and Antiserum 5 by our laboratory in Braunschweig against a PNRV isolate causing Stecklenberg disease in sour cherries in Germany. All antisera were adjusted to a final dilution endpoint of 1:256 for comparative purposes.

RESULTS.—All isolates, except RV-1, are known to cause localized necrotic reaction on *Prunus serrulata* Lindl. 'Shiro-fugen'. In general, symptoms caused by most of the rose isolates in cucumber were similar, but could vary depending on the age of the cucumber seedlings and on environmental conditions

TABLE 1. Symptomology of standard apple mosaic virus and Prunus necrotic ringspot virus isolates and 6 virus isolates from hybrid tea roses on original hosts, on Shiro-fugen, and when sap-transmitted to cucumber

Virus isolate	Origin	Symptoms on rose	Test on *Prunus serrulata* 'Shiro-fugen'	Symptoms on National Pickling cucumber	
				Cotyledons	True leaves
RV-1	Unidentified rose cultivar from California	Yellow mosaic	Negative	Very faint, light green lesions	Heavy mottle, retarded growth, no plants died
RV-2	Red Delight rose from Oregon	Yellow mosaic	Positive	Large, chlorotic lesions turning yellow	Heavy mottle, retarded growth, all plants died
RV-3	Unidentified rose cultivar from Oregon	Mosaic, leaf-pinching	Positive	Large, irregular chlorotic lesions, often with a darker ring in the center	Heavy mottle, retarded growth, some plants died
RV-4	Unidentified rose cultivar from California	Severe mosaic, leaf distortion	Positive	Chlorotic lesions	Heavy mottle with necrotic areas, sometimes retarded growth, no plants died
RV-5	Greenhouse Lovita rose from Oregon	Mosaic	Positive	Chlorotic, and some necrotic lesions	Heavy mottle, retarded growth, many plants died
RV-6	Unidentified rose cultivar from Oregon	Leaf distortion	Positive	Necrotic, very small lesions expanding into large necrotic areas	Only a few showed systemic infection; mild, light-green mottle over the whole leaf
ApMV-1	ATCC PV 32	Not tested	Positive	Chlorotic lesions turning yellow	Heavy mottle, retarded growth, some plants died
PNRV-1	Peach from Oregon	Not tested	Positive	Chlorotic lesions	Heavy mottle, very retarded growth, a few plants died
PNRV-2	Recurrent strain from peach from Oregon	Not tested	Positive	Chlorotic lesions	Very heavy mottle, many plants died

like temperature of the greenhouse and light intensity. These conditions also greatly influenced the killing of cucumber seedlings by some isolates. The best symptom production on cucumber occurred in late winter when greenhouse temperatures were held at ca. 25-28 C day and night, and the low intensity sunlight was supplemented by low intensity incandescent lights during 3-hr light interruptions nightly. Symptoms (Table 1) were observed when cucumber seedlings, inoculated after cotyledon leaves had fully opened, were kept in the greenhouse between 25 and 28 C.

Two isolates, RV-1 and RV-6, differed remarkably in symptom expression from all other isolates. In the first passage from rose to cucumber, RV-1 showed very faint symptoms on only a few cotyledons and no systemic infection. In the second passage, almost all inoculated cotyledons became infected, but symptoms on cotyledons remained faint despite strong systemic infection. At the other extreme, RV-6 initially caused pinpoint necrotic lesions which soon expanded until half of the cotyledon became necrotic. Systemic infection rarely occurred with this isolate. Isolates RV-4 and RV-5 produced lesions mostly of the chlorotic type like those typical for PNRV or ApMV, but RV-5 also formed some of the atypical, necrotic lesions characterizing RV-6.

Serological tests (Fig. 1) showed that the five rose isolates either belonged to the ApMV serotype, to the PNRV serotype, or reacted to a high degree with all antisera against both serotypes. RV-1 and RV-2 belong to the ApMV serotype. RV-1 did not react with PNRV antisera adjusted to a dilution endpoint of 1:256, but if we used antisera against PNRV with a higher titer, e.g., 1:512 or 1:1,024, we obtained reactions up to serum dilutions of 1:4 and 1:8, respectively. RV-3 reacted well with all antisera of both serotypes. RV-4 and RV-5 are similar in serological behavior to PNRV 1 and 2. Isolate RV-6 was unique among the rose isolates tested in producing necrotic but no chlorotic lesions on cucumber cotyledons. Nevertheless, RV-6 belonged definitely to the PNRV serotype.

DISCUSSION.—The results of the serological tests

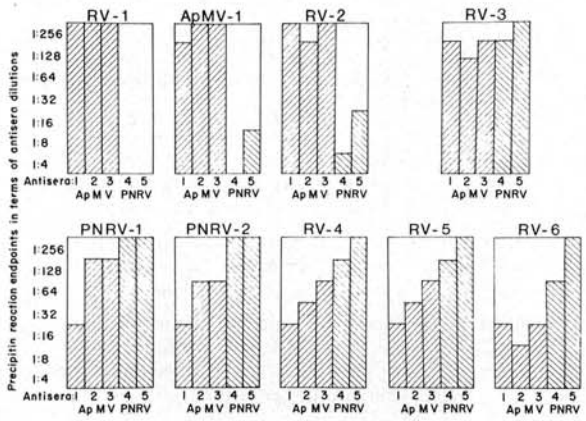

Fig. 1. Serological reactions in agar gel of nine apple mosaic virus and Prunus necrotic ringspot virus isolates from rose (RV-1 through RV-6), peach (PNRV 1 and 2), or apple (ApMV-1) against five different antisera. The antisera are: 1 = rose mosaic virus antiserum prepared by R. W. Fulton; 2 = apple mosaic virus antiserum prepared in Braunschweig; 3 = apple mosaic virus antiserum prepared by R. W. Fulton; 4 = Prunus necrotic ringspot virus antiserum prepared by R. W. Fulton; and 5 = Prunus necrotic ringspot virus antiserum prepared in Braunschweig. All antisera were adjusted to a titer of 1:256. The serological endpoints which we obtained are indicated by the heights of the bars.

are, in general, in agreement with the findings of Fulton (7), who placed ApMV and RMV into one group according to serological behavior, and considered PNRV and Danish plum line pattern virus to be in a separate but related group. However, the present quantitative study with five antisera forces us to acknowledge the existence of a rather wider range of serological reactions from rose virus sources than has been previously reported.

The ApMV-type isolates, RV-1, RV-2, and also the standard ApMV isolate all showed clear but distant heterologous reactions to PNRV antisera. On the other hand, the PNRV-type isolates, RV-4, RV-5, RV-6, and the standard PNRV isolates all had fairly high heterologous reactions to ApMV antisera.

Isolate RV-3 could logically be placed in either serological group because it reacts with PNRV and ApMV antisera nearly to the same high degree. So far, no isolate with such serological behavior has been reported. In its serological properties, this isolate seems to be a link between ApMV and PNRV. But these results could mean that RV-3 is a mixture of strains of both serotypes. The lesions produced by this isolate appeared uniform in shape and color. However, since ApMV and PNRV strains generally produce similar lesions on cucumber, homology of lesion morphology is no assurance that isolate RV-3 is a pure strain. Further investigation may answer this question.

RV-5, clearly belonging to the PNRV serotype, showed not only lesions of the chlorotic type common for PNRV but also some necrotic lesions similar to those of RV-6. Since RV-5 reacts serologically similar to RV-6, this isolate may be a mixture of a strain like RV-6 and a common PNRV strain.

The broad and continuous spectrum of serological reactions obtained from these relatively few rose virus isolates may reflect the existence of many different individual virus strains in the vegetatively propagated rose cultivars with which we have worked. Alternatively, mixtures of virus strains in these cultivars may account for this variation. In any case, the demonstrated serological variability in virus isolates from hybrid tea roses provides an explanation for the great variation in virus symptoms which have been noted in roses (6, L. C. Cochran, *personal communication*). Investigation of more isolates from other rose sources will probably expand the range of serological reactions even more.

Roses and hops (1, 2) are the only natural hosts from which both ApMV and PNRV have been isolated. It seems possible that the hop plant is another host for isolates with a wide range of serological properties. In cherry and peach only PNRV has been found, whereas in apple ApMV but not PNRV has been identified.

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