Two Properties of Raspberry Ringspot Virus Determined by its Smaller RNA

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Raspberry ringspot virus (R/1: 2.4/43 + 1.4/30 (or 2 x 1.4/46): S/S:S/Ne, nepovirus group) is a multicomponent plant virus with two sizes of RNA having mol. wt. of 2.4 x 10^6 (RNA-1) and 1.4 x 10^6 (RNA-2) (Murant et al. 1972). When obtained by centrifuging in sucrose density gradients or by electrophoresis in polyacrylamide gels, preparations of RNA-2 mostly did not infect the local lesion assay host, Chenopodium amaranticolor Coste & Reyn., and those of RNA-1 were only moderately infective, whereas mixtures of the two kinds of preparation were very infective (Harrison, Murant & Mayo, 1972). No such increase in infectivity occurred when one RNA species from raspberry ringspot virus was mixed with the other from tobacco ringspot virus (another nepovirus), or when either of the RNA species from raspberry ringspot virus was u.v. irradiated before use. A possible explanation of these results is that each RNA species carries genetic information not carried by the other. To test this hypothesis, we inoculated plants with mixtures of RNA-1 and RNA-2 taken from two distinctive strains of raspberry ringspot virus, to see whether virus would be produced with properties combining those of the two strains. The results are described in this paper.

The two isolates of raspberry ringspot virus used were the type culture of the ENGLISH strain (strain E), originally obtained from Rubus procerus P. J. Muell. (Cadman, 1960) and characterized by Murant et al. (1972), and the type culture of the SCOTTISH strain (strain S) (Harrison, 1958). These two isolates are serologically related but not identical, and whereas strain S produces systemic ringspot patterns followed by recovery in Petunia hybrida Vilm., strain E produces ringspot symptoms followed by a generalized yellowing of the young leaves.

The viruses were purified from systemically infected leaves of Nicotiana clevelandii Gray; RNA was obtained from the virus particles by treatment with pronase + SDS, then heated in electrophoresis buffer containing 8 M-urea and fractionated by electrophoresis in polyacrylamide gels (Murant et al. 1972). RNA-1 and RNA-2 were extracted from the two u.v.-absorbing bands in the gels using tissue grinders, and the preparations diluted with 0.06 M-phosphate buffer, pH 8.0, for use as inoculum (Harrison et al. 1972). RNA species from strains E and S are designated by the suffices (E) and (S) respectively.

The infectivities of mixtures of RNA-1 and RNA-2 were greater than the sum of the infectivities of each component alone, irrespective of whether the two RNA species came from the same or different virus strains (Table 1). In the experiment quoted, a large increase in infectivity was obtained when each RNA-2 was mixed with either dilution of RNA-1(E), but only when mixed with the greater dilution of RNA-1(S), which was relatively more infective than RNA-1(E) when inoculated alone. The infectivity of RNA-1 decreased more rapidly with dilution when inoculated alone than when in the presence of a constant amount of RNA-2. Thus nearly all the lesions produced by mixtures containing RNA-2 and the lesser concentration of RNA-1 seemed to result from an interaction between the two RNA species.

Several of these lesions were excised, ground singly in two drops of 0.03 M-phosphate buffer (pH 7.4), and each extract inoculated to a separate Chenopodium quinoa Willd.
Short communications

Table 1. Hybridization of two strains of raspberry ringspot virus

<table>
<thead>
<tr>
<th>RNA species in inoculum*</th>
<th>Infectivity†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RNA-1 at 1/2</td>
</tr>
<tr>
<td>RNA-1 (e)</td>
<td>5</td>
</tr>
<tr>
<td>RNA-2 (s)</td>
<td></td>
</tr>
<tr>
<td>RNA-1 (e) + RNA-2 (s)</td>
<td>248</td>
</tr>
<tr>
<td>RNA-1 (s) + RNA-2 (s)</td>
<td>210</td>
</tr>
<tr>
<td>RNA-1 (s)</td>
<td>250</td>
</tr>
<tr>
<td>RNA-2 (s)</td>
<td>1</td>
</tr>
<tr>
<td>RNA-1 (s) + RNA-2 (s)</td>
<td>352</td>
</tr>
<tr>
<td>RNA-1 (s) + RNA-2 (e)</td>
<td>555</td>
</tr>
</tbody>
</table>

* RNA-1 preparations were used at final dilutions of 1/2 or 1/10; RNA-2 preparations were used at a final dilution of 1/2 throughout.
† Figures were total numbers of lesions in eight half-leaves of Chenopodium amaranticolor.

Fig. 1. Gel-diffusion serological tests with hybrid isolates of raspberry ringspot virus derived from single lesions produced by: 1, RNA-1 (s)+RNA-2 (s); 2, RNA-1 (e) + RNA-2 (e); 3, RNA-1 (e) + RNA-2 (s); 4, RNA-1 (s) + RNA-2 (e). S, antiserum to strain s; B, antiserum to strain s absorbed with strain e. The antigen preparations were saps from infected Chenopodium quinoa leaves.

The resulting single-lesion isolates were tested serologically by the gel-diffusion method against (1) strain s antiserum, and (2) strain s antiserum absorbed with strain e. Sap from infected C. quinoa plants was used as the antigen. In these tests the absorbed antiserum reacted only with strain s and the unabsorbed antiserum reacted with both strains, producing a spur with strain s when the two strains were in adjacent wells. All but one of the thirty-seven isolates tested had the serological specificity of the strain contributing RNA-2 to the inoculum (Table 2; Fig. 1). The exceptional isolate, which was obtained from the inoculum RNA-1 (s) + RNA-2 (e), behaved serologically like strain s. It probably resulted from contamination of RNA-1 (s) with RNA-2 (s). Thus, the serological specificity of raspberry ringspot virus was determined by RNA-2. Tests with several of the single-lesion isolates showed that RNA-2 also determined the ability of virus isolates to cause yellowing in P. hybrida (Table 3; Fig. 2). A few isolates were obtained from lesions produced by RNA-1 preparations alone. These isolates behaved serologically and symptomatically like the strain that was the source of the inoculum. Thus the infectivity of preparations of RNA-1 was most plausibly explained by their contamination with RNA-2, both types of molecule being needed for lesions to be produced.
Table 2. Serological specificity of hybrid isolates of raspberry ringspot virus

<table>
<thead>
<tr>
<th>Inoculum used to produce lesions from which isolates were derived</th>
<th>Number of single lesion isolates</th>
<th>Serological specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed*</td>
<td>Tested</td>
</tr>
<tr>
<td>RNA-1 (E) + RNA-2 (E)</td>
<td>10/12</td>
<td>8</td>
</tr>
<tr>
<td>RNA-1 (E) + RNA-2 (S)</td>
<td>12/16</td>
<td>12</td>
</tr>
<tr>
<td>RNA-1 (S) + RNA-2 (S)</td>
<td>5/8</td>
<td>5</td>
</tr>
<tr>
<td>RNA-1 (S) + RNA-2 (E)</td>
<td>14/16</td>
<td>12</td>
</tr>
</tbody>
</table>

* Numerator is the number of single lesion isolates obtained, denominator is the number of attempts made. The source lesions were taken from the set of plants inoculated with RNA-1 at 1/10 (Table 1).

Table 3. Symptoms produced in Petunia hybrida by hybrid isolates of raspberry ringspot virus

<table>
<thead>
<tr>
<th>Inoculum used to produce lesions from which isolates were derived</th>
<th>Systemic symptoms produced in P. hybrida (number of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Severe yellowing</td>
</tr>
<tr>
<td>RNA-1 (E) + RNA-2 (E)</td>
<td>4</td>
</tr>
<tr>
<td>RNA-1 (E) + RNA-2 (S)</td>
<td>0</td>
</tr>
<tr>
<td>RNA-1 (S) + RNA-2 (S)</td>
<td>0</td>
</tr>
<tr>
<td>RNA-1 (S) + RNA-2 (E)</td>
<td>11</td>
</tr>
<tr>
<td>Strain E†</td>
<td>10</td>
</tr>
<tr>
<td>Strain S†</td>
<td>0</td>
</tr>
</tbody>
</table>

* This isolate had the serological specificity of strain S (see Table 2).
† Stock cultures were used as inoculum.

We therefore conclude that raspberry ringspot virus has a divided genome; we have identified two properties that are determined by RNA-2, and RNA-1 seems essential for infection. This confirms our previously expressed view (Harrison et al. 1972) that a split genome may prove to be characteristic of nepoviruses. It now seems improbable that the larger RNA is formed by the joining of two of the smaller RNA molecules, as suggested by Diener & Schneider (1966).

Our results indicate similarities in behaviour between raspberry ringspot virus and members of the comovirus group. Thus both the larger and the smaller RNA of cowpea mosaic virus are needed for infection to occur (Bruening & Agrawal, 1967; van Kammen, 1968) and, with bean pod mottle virus, there is evidence that the nucleoprotein particle that contains only the smaller RNA species determines serological specificity (Moore & Scott, 1971). Raspberry ringspot virus resembles tobacco rattle virus in the distribution of functions among RNA species. Thus with tobacco rattle virus the shorter nucleoprotein particle, and hence the smaller RNA, determines both serological specificity and the ability to cause yellow symptoms in P. hybrida (Sänger, 1968; Lister & Bracker, 1969). Indeed it is conceivable that these two properties are different expressions of the same cistron. With tobacco mosaic virus, too, there is evidence of a correlation between the properties of the coat protein of some mutants and the production of yellow symptoms (Jockusch & Jockusch, 1968). However, particles of raspberry ringspot virus contain only one type of protein, of mol. wt. 54000 (Mayo, Murant & Harrison, 1971), and the cistron
Fig. 2. Petunia hybrida plants systemically infected with hybrid isolates of raspberry ringspot virus derived from single lesions produced by: top left, RNA-1 (i) + RNA-2 (e); top right, RNA-1 (s) + RNA-2 (s); bottom left, RNA-1 (s) + RNA-2 (e); bottom right, RNA-1 (e) + RNA-2 (s). Note the yellowing shown by the plants on the left.

Coding for this would occupy less than half of RNA-2. Similarly, the smaller RNA species of tobacco rattle virus is larger than would be needed to code merely for the protein in the virus particles. What causes the leaf yellowing produced by these virus strains is not known, although Hirai & Wildman (1969) suggested that the yellowing of leaves infected with tobacco mosaic virus results from a repression of transcription of chloroplast DNA, possibly by a virus-coded protein, and the yellowing produced by other viruses may be similarly explained. Such a repressor may or may not be virus coat protein.

Whether or not the serological specificity and ability to cause yellow symptoms prove to be expressions of the same cistron in raspberry ringspot virus, the importance of the
results we describe is that they indicate an approach to studying the genetic control of its biological properties and, presumably, those of other nepoviruses. This should lead to a better understanding of the mechanisms underlying the biological behaviour of these viruses.

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REFERENCES

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