Comparative Studies on Two Strains of Tobacco Rattle Virus

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SUMMARY

Differences in symptom expression and growth curve exist between two California strains, B and c of tobacco rattle virus, each of which possesses 3 components of different particle lengths and is serologically related to two English strains. With increasing age of infection from 3 to 35 days, the particle populations of strains B and c show a shift in the middle to bottom component ratio from about 2 to 0.9. An unstable 1600 to 1700 Å fraction recovered during periods of most rapid synthesis of the strain c may be implicated in the appearance of normally present 800 to 900 Å rods.

INTRODUCTION

Two closely related isolates of tobacco rattle virus (TRV) were separated by serial local lesion transfer from naturally infected pepper (Capsicum annum) (Semancik, 1966). No distinguishing properties were established between the purified isolates other than a difference in length between the noninfectious intermediate rods. Harrison & Woods (1966), in describing a number of English and foreign TRV strains, presented a strain (st'), the particle complement of which closely resembled the mixed culture first derived from naturally infected pepper.

This communication describes additional properties of the two California isolates of TRV, substantiating the existence of a strain relationship; and includes observations on the in vivo condition of the isolates in pure and mixed culture.

METHODS

Virus culture. Pure cultures of the two isolates, designated B and c (Semancik, 1966), were obtained after serial single lesion transfer from naturally infected pepper (Paulus, Thomason & Weathers, 1963). Local lesion assays were made on bean (Phaseolus vulgaris L. var. The Prince) in an incomplete block design with at least 8 half-leaves per treatment. Nicotiana clevelandii Gray served as the source for obtaining purified virus.

Purification and serology. Virus was purified by a previously reported method (Semancik, 1966), with the exclusion of the pH 5·0 precipitation step in experiments where the relative proportions of particles of different lengths were determined. Separation of components was achieved by centrifuging in the Spinco SW 25·1 rotor for 1½ to 2 hr at 24,000 rev./min. in a preformed density gradient column of 100 to 400 mg./ml. sucrose (Brakke, 1960) in 0·1 M-potassium phosphate buffer, pH 7·0. The gradients were scanned at 254 mμ and collected by means of an ISCO (Instrument-
Rabbits were bled for normal serum and then given 3 intravenous injections of the purified isolates (2 to 5 mg./injection) at 3-day intervals. A booster injection (4 to 5 mg.) was given 1 week later. The animals were bled 7 days later. Gel diffusion plates consisted of 0.5% Ionagar in physiological saline solution with 0.01% merthiolate.

Electron microscopy. Droplets of preparations from sucrose gradients were placed on Formvar+ carbon coated grids for about 2 min., then rinsed with repeated applications of droplets of deionized water. Excess liquid was removed by touching to filter paper. The preparations were shadowed with palladium. Negative staining of epidermal strips with 2% phosphotungstic acid, neutralized with NaOH, followed the method of Hitchborn & Hills (1965). Both shadowed and stained specimens were viewed on a modified RCA Model EMU-3 B electron microscope.

RESULTS

Properties of separated strains

Existence of an intimate relationship between the B and c isolates was suggested by their common source and the difficulty encountered in separating the mixed culture (Semancik, 1966). Symptom expression on a wide range of hosts was very similar; however, differences were observed on varieties of Nicotiana tabacum L. (Table 1). The most pronounced symptom differences were seen on varieties Xanthi and Samsun, in which the c isolates produced necrotic local lesions, while the B isolate caused a systemic reaction characterized by a necrotic etched line pattern accompanied by leaf distortion. Expressed sap of N. clevelandii infected with either strain produced no lesions after 10 min. at 55° or being diluted 10⁻³.

Table 1. Differential hosts of the B and c isolates of TRV

<table>
<thead>
<tr>
<th>Host</th>
<th>B isolate</th>
<th>C isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotiana sylvestris</td>
<td>Large necrotic lesions, systemic necrotic etching and leaf distortion</td>
<td>Similar symptoms; systemic symptoms less severe</td>
</tr>
<tr>
<td>Nicotiana tabacum L. var Samsun</td>
<td>Necrotic ringspots, systemic necrotic etching and leaf distortion</td>
<td>Necrotic ringspots</td>
</tr>
<tr>
<td>Nicotiana tabacum L. var. Xanthi-nc</td>
<td>Necrotic ringspots, systemic necrotic etching and leaf distortion</td>
<td>Necrotic ringspots</td>
</tr>
<tr>
<td>Nicotiana tabacum L. var. Turkish</td>
<td>Necrotic ringspots, systemic necrotic etching and leaf distortion</td>
<td>Necrotic ringspots; systemic symptoms less severe</td>
</tr>
<tr>
<td>Nicotiana clevelandii</td>
<td>Necrotic ringspots, systemic mottle, etching and leaf distortion</td>
<td>Similar reaction, but more severe</td>
</tr>
<tr>
<td>Amaranthaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gomphrena globosa L.</td>
<td>Large necrotic lesions, systemic leaf distortion</td>
<td>Large necrotic lesions</td>
</tr>
</tbody>
</table>
In order to facilitate fusion of precipitin bands in Ouchterlony gel diffusion, virus concentrations were equalized at E\textsubscript{280} and virus was added to wells 3 to 4 days before the antisera. Both isolates reacted with heterologous antiserum without the evidence of crossover spurs in the interwell region (Pl. 1, fig. 1). Antisera to both the $b$ and $c$ isolates also formed a continuous reaction line with an English strain of TRV and the PRN strain (kindly provided by Dr. R. M. Lister). The English strain possessed a larger proportion of short rods than any of the other strains, thus producing a precipitin line closer to the antisera wells because of more rapid diffusion of the short rods.

The mixed culture as originally isolated from pepper survived through at least six serial single lesion passages in cucumber (*Cucumis sativus* var. National Pickling) (Semancik, 1966) and retained the whole range of particle lengths. Also, during these early studies, a mixed culture of $b$ and $c$ displaying four discrete zones in sucrose gradient centrifugation would produce at times, upon successive passages in *Nicotiana clevelandii*, virus giving three zones, always of the $c$ type; suggesting that the $c$ isolate
might possess a competitive advantage over the B isolate in the infection of *N. clevelandii*. Controlled experiments designed to establish this competitive phenomenon consisted of separating a fraction containing predominantly long (1750 to 1900 Å) rods of B and C, standardizing at $E_{260}$ and applying to *N. clevelandii* in various ratios of B:C. Fig. 1 presents the sedimentation profile of virus purified from plants 6 days after inoculation with B:C of 1:5, 1:1 and 5:1. The C strain comprised the bulk of the virus when inoculum included a fivefold excess or an equal part of C. Even when applied with a fivefold excess of B, the C strain produced a disproportionate amount of its characteristic particle present in the middle component. These results tend to confirm the previous observations that the C strain has a competitive advantage in mixed infections.

![Graph showing virus infectivity](image)

**Fig. 2.** The virus infectivity of sap (—■—■), and purified virus (●—●) as number of lesions per half bean leaf; and the specific infectivity (○—○) of the B strain of TRV obtained from leaves of *Nicotiana clevelandii* after various periods of infection.

**Age of infection and the virus condition**

Harrison & Nixon (1959) reported an unexplained variation in the long to short particle ratio of 1:2 to 1:18 for the PRN strain of TRV. These observations prompted a more extensive investigation of the condition of the particle population at various stages in the infection process. *Nicotiana clevelandii* plants, infected with pure B and C and kept at 23 to 27°C, were harvested at intervals from 3 to 35 days after inoculation and stored at −20°C. Both strains produced necrotic ringspots on inoculated leaves and a systemic mottle with areas of etched necrosis. The reactions to the C isolate appeared slightly more severe than those to the B strain. Infectivity tests were made using sap and the concentration of virus in purified preparations was estimated spectrophotometrically (Figs. 2, 3). Infectivity and amount of purified virus increased rapidly with both isolates, reaching a maximum about 1 to 2 weeks after inoculation and decreasing by about 80% at the 35-day harvest. Growth of the host plant could
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not account for this decrease. The c strain increased more rapidly and attained a greater concentration.

The specific infectivity of purified virus was determined by standardizing virus preparations at $E_{260}$ with a Beckman DU-2 spectrophotometer before testing for infectivity. Considerable differences in specific infectivity were observed with the two isolates. The c strain maintained about 75% of the maximum specific infectivity by the end of the 35-day period, whereas the b strain possessed only about 30%. The maintenance of high specific infectivity by strain c is similar to the growth behaviour of tobacco mosaic virus (Goodchild, Cohn & Wildman, 1958); while the reduction shown by strain b was similar to the growth behaviour of alfalfa mosaic virus (Kuhn & Bancroft, 1961) and a number of spherical viruses.

![Graph](image-url)

Fig. 3. The virus infectivity of sap (---) and purified virus (---), as number of lesions per half bean leaf; and the specific infectivity (- - -) of the c strain of TRV from leaves of Nicotiana clevelandii after various periods of infection.

Changes in particle length distribution after various periods of infection were investigated by rate zonal sedimentation of purified virus in sucrose density gradients followed by scanning in an ISCO fractionator. A pronounced decrease in the non-infectious, intermediate-length rod with progressive age of infection is apparent in both strains (Figs. 4, 5). Estimation of relative concentration of the infectious component (bottom) and the noninfectious component (middle) by compensating polar planimeter confirmed the decrease in the intermediate-length rod (middle) (Table 2). This intermediate-length rod is 1050 to 1150 Å in the b strain and 800 to 900 Å in the c strain. In both isolates the infectious length is 1750 to 1900 Å and the minor, slowest sedimenting component is about 450 to 500 Å (Semancik, 1966). In addition to these components another sedimenting species, designated B', was detected in preparations from the 3- and 7-day harvests of the c growth curve (Fig. 5). This fraction,
sedimenting very near the bottom component, was noninfectious and included a large number of particles that appeared to be either in a stage of end-to-end aggregation or fragmentation (Pl. 1, fig. 2). A histogram of a typical B' population (Fig. 6) confirmed...
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the presence of 1600 to 1700 Å and 1900 to 2000 Å fractions as well as a large proportion of the typical 800 to 900 Å rods. The presence of the small amount of 1900 to 2000 Å rods was attributable to the proximity of sedimentation, and to mixing when sampling; however, the large amount of 800 to 900 Å particles could not be explained the same way.

Table 2. Changes in the relative proportion of short rods (middle) to long rods (bottom) with age of infection

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Relative area (middle/bottom)</th>
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<tbody>
<tr>
<td></td>
<td>TRV-a</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>2.1</td>
</tr>
<tr>
<td>7</td>
<td>2.0</td>
</tr>
<tr>
<td>14</td>
<td>1.6</td>
</tr>
<tr>
<td>21</td>
<td>1.2</td>
</tr>
<tr>
<td>28</td>
<td>0.9</td>
</tr>
<tr>
<td>35</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Fig. 6. Particle length distribution of purified TRV-c (upper) and the B' fraction (lower) derived from Nicotiana clevelandii 3 days after inoculation. Sedimentation position of B' fraction is indicated in Fig. 5.

Since the 1600 to 1700 Å particle was present only during the early stages of infection when virus synthesis was most rapid, it may have been an unstable breakdown product of the infectious long rods which were subsequently split into two stable equal lengths.
Aggregation of two of the 800 to 900 Å particles as demonstrated with prolonged treatment at pH 6.5 with the PRN strain (Harrison & Nixon, 1959) must certainly be considered; but the inability to recover the 1600 to 1700 Å form after about 14 days, at which time large quantities of the 800 to 900 Å rods still exist, does not support this idea.

The whole range of short noninfectious particles was seen in epidermal strip specimens.

**DISCUSSION**

Tobacco rattle virus, which has a variety of particle lengths, exists in a number of variant forms both in Europe and America. Although identical in many properties, the isolates derived from pepper in California stimulated distinct host reactions and differed in particle length of the middle component. Antisera to both strains showed good serological activity with two English strains of TRV. Using isolates of TRV from Oregon and California, which resembled the c strain from pepper in particle profile, Harrison & Woods (1966) could not detect any serological relation with an antiserum to another English strain (sp). This antiserum (sp) reacted with the PRN strain which was found to react with antisera to both the b and c isolates in the present studies. Thus, the absence of a serological relationship between European and American strains of TRV is not consistent with these data.

With successive propagations, the short particle population of the sp isolate was reported to change from a single component of 1140 Å to two components with a bimodal distribution about 950 and 1100 Å (Harrison & Woods, 1966). The latter distribution strikingly resembles that obtained with the mixed b and c cultures (Semancik, 1966). Pertinent to this is the rate of increase of the c isolate which ensures its eventual predominance in a mixed infection with b. Once they were completely separated the stability of the b and c strains through successive propagations would favour the presence of a mixture in the case of the sp isolate.

Although the variation in the ratio of short to long (middle component:bottom component; $M:B$) particles for the b and c strains did not approach the level reported for the PRN strain (Harrison & Nixon, 1959), age of infection did result in a progressive decrease in $M:B$. Since the major change occurred while virus yield was rapidly decreasing (14 to 35 days), the disproportionate decrease in the middle component might have been due to greater lability of the incomplete rod. The recovery of the B' (1600 to 1700 Å) unstable component suggests that the complete infectious particle may have been degraded to the B' form which was then rapidly converted to two 800 to 900 Å fragments. This possibility removes the often suggested requirement that some multiple of the fragment must equal the length of the infectious rod. *In vitro* degradation studies in progress may be helpful in this interpretation.

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REFERENCES


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EXPLANATION OF PLATE

Fig. 1. Ouchterlony gel-diffusion plate 2 weeks after addition of antiserum; (b) antiserum to B isolate; (c) antiserum to C isolate; 1, tobacco rattle virus B isolate; 2, C isolate; 3, PRN strain; 4, English strain.

Fig. 2. Electron micrograph of B' particle population from rate zonal sedimentation in sucrose density gradient showing presence of 1600 to 1700 Å and 800 to 900 Å particles. (×45,000.)
Plate 1

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(Facing p. 162)