

Properties and Behaviour of a Virus Depending for its Multiplication on Another

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SUMMARY

The Rothamsted culture of tobacco necrosis virus contains two serologically unrelated viruses one of which, called the 'satellite virus' (SV), causes no lesions and multiplies detectably only when the other (TNV) is present. It decreases the size of necrotic local lesions formed by TNV. Inocula containing both viruses gave the same, fewer or more lesions than inocula containing only TNV; the results depended on the ratio of the two viruses in the mixed inoculum, on the species and age of the test plants and, particularly, on the temperature at which the plants were kept after the inoculation. The concentration of TNV decreased when SV was also present; the extent of the decrease depended on the ratio of the two viruses in the inoculum and other factors. Inoculation with SV 5 days before inoculation with TNV still decreased lesion size and led to the multiplication of SV. Phenol-disrupted SV affected lesion size only when inoculation with TNV followed within a few hours, but when inoculation with TNV was delayed by up to 2 days, SV sometimes multiplied detectably. SV has the smallest particle reported for any plant virus; diameter *c.* 17 m μ and weight equivalent to a molecular weight of about 1.9×10^6 , of which 20% is nucleic acid. It is exceptionally stable, retaining infectivity after 17 years at 3° and after heating for 10 min. at 90°. TNV was much more susceptible to inactivation by heat and by ultraviolet light than SV. The two differed greatly in electrophoretic mobility and were readily separated from mixed preparations by electrophoresis.

INTRODUCTION

The Rothamsted culture of tobacco necrosis virus was long known to contain spherical particles of two sizes (Bawden & Pirie, 1942, 1950) but the significance of this was not understood until they were found to be serologically unrelated viruses, the smaller of which multiplies detectably only in the presence of the larger. The simultaneous multiplication of the two produces smaller necrotic local lesions in French bean than when the large particles are multiplying alone; and the ratio of large to small lesions falls as the proportion of small particles in the inoculum increases; a great excess of small particles can decrease the number of lesions (Kassanis & Nixon, 1960, 1961). The experiments now to be described show other effects of interaction between the two viruses and how their interactions depend on the species of host plant and changes in the environment. Also, by using the

proportion of large to small lesions formed by a mixed inoculum as a method of assaying small particle infectivity some of the properties of the small virus particles have been determined. For need of a better name, the virus with small particles was called 'satellite virus' (SV) because it is always in association with and dependent on the large virus particles.

METHODS

The virus inocula were prepared as described by Kassanis & Nixon (1961). The satellite virus (SV) was obtained from the top zone after centrifugation in sucrose gradients of purified preparations of an isolate of the Rothamsted culture. The ratio of small to large particles varied in different preparations, depending on the season and the age of the tobacco plants, from 10:1 to 250:1. The total yield of virus was smaller in summer than winter. The particles of two sizes separated into zones best when the preparations contained no more than 2 mg./ml. of virus. Plants inoculated with purified preparations of SV produced no lesions and the virus did not multiply detectably in them. SV can be activated by two tobacco necrosis viruses, TNVa or TNVb (Kassanis & Nixon, 1961), but in the present work TNVb was mainly used. All virus dilutions were in water. Purification, infectivity tests and particle counts with the electron microscope were made as before (Kassanis & Nixon, 1961).

The proportion of SV surviving after various treatments was measured by comparing its effect on the lesion formed by TNVb with untreated SV of known concentration. The treated SV was inoculated at one dilution and the untreated at 4 dilutions, after mixing with constant amounts of TNVb (enough to give about 20-60 lesions/half bean leaf). The numbers of large lesions were plotted against the concentration of untreated SV, and the concentration of SV remaining active in the treated preparation was estimated by interpolation. This method assumes that loss of the ability of SV to affect the size of lesions produced by TNVb coincides with loss of infectivity; there is evidence to show that the assumption is correct.

An antiserum to SV was prepared by injecting a rabbit with SV purified by two successive centrifugations in sucrose gradients. A total of 0.7 mg. of SV was injected into a rabbit in three injections; the first was intravenous and a month later two intramuscular injections were given on successive days. The virus injected intramuscularly was emulsified in Bacto Adjuvant, Complete (Difco Laboratories). The rabbit was bled a month after the last injection and when titrated against SV at 20 mg./l. the serum precipitated when diluted 1/400. This antiserum did not precipitate TNVa or TNVb; nor did antisera to these viruses precipitate SV, thus confirming earlier evidence that the large and small virus particles are serologically unrelated (Kassanis & Nixon, 1961).

The concentration of purified virus preparations was estimated from measuring their optical density at 260 $m\mu$, and the concentration of the virus in sap by determining the highest dilution at which a specific precipitate was produced with the homologous antiserum. The sap used in serological tests was frozen, thawed, left for a day at 20°, centrifuged and heated at 45° for 10 min. before again being centrifuged at 8000 g. When the increase of virus in bean leaves was investigated, the water lost from the necrotic leaves was estimated by comparing their water content with that of uninoculated leaves and the loss was compensated for by

adding an appropriate amount to the leaves when they were ground before extracting the sap. Most of the experiments were made between autumn and spring when the glasshouse temperature fluctuated between 16° and 20°. The temperature in summer fluctuated between 20 and 30°, which sometimes was too high for virus multiplication.

RESULTS

Interference during lesion formation

The necrotic local lesions formed by inocula containing only TNVa or TNVb were all large, but inocula that also contained SV gave some small lesions, the number of which depended on the amount of SV in the inoculum (Fig. 1). The total number of lesions (large and small) formed by mixed inocula (constant TNVa or TNVb and varying amounts of SV) was sometimes the same over a wide range of concentrations of SV (Table 3 in Kassanis & Nixon, 1961), sometimes more and sometimes fewer (Fig. 1), depending on the age and species of the inoculated plants and their growing conditions before and after inoculation. A decrease was usual only with high concentrations of SV and when all the lesions were small, but in

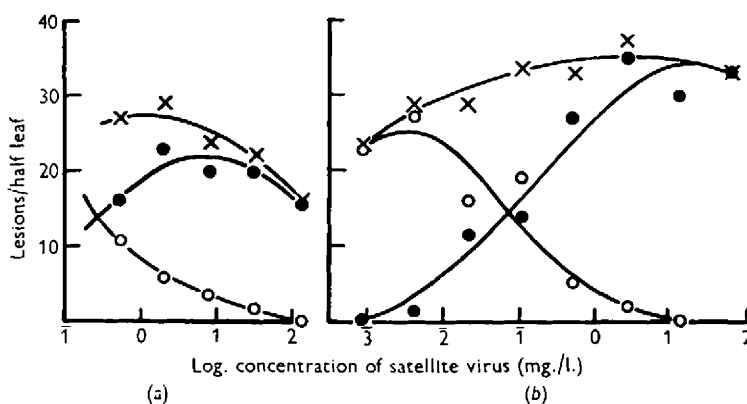


Fig. 1. Lesions/half leaf of French bean inoculated with mixtures of constant TNVb (0.3 mg./l.) and different amounts of SV. \times — \times , total number of lesions; \circ — \circ , large lesions; \bullet — \bullet , small lesions. (a) and (b), experiments made at different times of the year.

summer it happened with lower concentrations of SV than in winter. Increases occurred mostly in winter and only when the ratios of the two viruses were such that not all lesions were small.

The number of lesions produced by a given inoculum of tobacco necrosis virus depends on the temperature at which the plants are kept (Kassanis, 1952). An experiment was therefore made to find whether the temperature also affected the extent to which TNVb and SV interacted. Bean plants were inoculated with constant amounts of TNVb mixed with varying amounts of SV and placed in glass chambers kept at 17°, 22° and 26°. The lesions appeared 1 day sooner at 26° than at the lower temperatures, but were fewer and all large. The highest concentration of SV decreased lesion numbers at all temperatures, and did so more at higher than at lower temperatures (Table 1). It is interesting that, with 1 mg./l. of SV, the total number of lesions decreased at 26°, but increased at 22° and even more at 17°.

These results strongly suggest that the interactions between TNVb and SV differ at different seasons mainly because of difference in temperature.

From these results it can be argued that, for a lesion to form, at least one of the susceptible sites (situated in one or more neighbouring cells) has to be occupied by TNVb. But when the concentration of SV is high or when the susceptible sites are few (by keeping the plants at a high temperature after inoculation or by other treatments), then the chances are that all the sites will be occupied by SV and the lesion will not be formed. The fact that with susceptible plants kept at relatively low temperatures considerably higher concentrations of SV were needed to decrease the total number of lesions suggests that each infectable centre may contain several susceptible sites.

Table 1. *Effect of temperature on number of large and small lesions formed by tobacco necrosis virus (TNVb) simultaneously inoculated with satellite virus (SV) at different concentrations*

| Con- centra- tion of SV* (mg./l.) | Small lesions | | | Large lesions | | | Total of small + large lesions | | |
|---|---|-----|-----|---------------|-----|-----|-----------------------------------|-----|-----|
| | 17° | 22° | 26° | 17° | 22° | 26° | 17° | 22° | 26° |
| | Number of lesions from 8 bean-half leaves | | | | | | | | |
| 25 | 227 | 98 | 0 | 0 | 0 | 4 | 227 | 93 | 4 |
| 5 | 915 | 614 | 0 | 94 | 4 | 22 | 1009 | 618 | 22 |
| 1 | 1097 | 522 | 0 | 113 | 33 | 36 | 1210 | 555 | 36 |
| 0.2 | 333 | 299 | 0 | 259 | 189 | 74 | 502 | 488 | 74 |
| 0.04 | 139 | 116 | 0 | 558 | 384 | 180 | 697 | 500 | 180 |
| 0.008 | 38 | 13 | 0 | 513 | 341 | 137 | 551 | 354 | 137 |

* Mixed with TNVb at 0.3 mg./l.

The interaction between TNVb and SV is also influenced by the age of the plants and the conditions in which they are kept before inoculation. When bean plants of two ages were inoculated with the same inocula (1.2 mg./l. of TNVb mixed with different amounts of SV), the large lesions expressed as a percentage of the small were 5, 18, 59 in young plants and 9, 38 and 138 in old plants, respectively, for 2.4, 0.6 and 0.15 mg./l. of SV, suggesting that, as plants age, their resistance to infection by SV increases more than to TNVb.

The susceptibility of the plants to virus infection can be increased by keeping them at 36° for a day or two before inoculation (Kassanis, 1952). To see whether their preinoculation treatment affects the interaction between the two viruses, bean plants were kept for one day at 36° or 18° and then inoculated on opposite half leaves with TNVb alone and TNVb mixed with SV (0.25 mg./l. for each virus). The plants pretreated at 36° produced about twice as many lesions as plants kept at 18° before inoculation. The presence of SV increased the total number of lesions on both lots of plants, by 42% in plants pretreated at 36° but by only 17% in plants kept at 18°, suggesting that the pretreatment increases susceptibility to infection by SV more than by TNVb. The interaction between SV and TNVb also depends on the species of plant. In *Nicotiana glutinosa* L. lesions look the same whether inoculated with TNVb alone or mixed with SV. When bean and *N. glutinosa* plants were inoculated on opposite half leaves with TNVb alone (0.6 and 6 mg./l., respectively, for the two species) or together with SV (10 mg./l.), the two inocula produced the

same number of lesions in beans, whereas in *N. glutinosa* the mixed inoculum produced on average only 14% of the number of lesions produced by TNVb alone. Hence concentrations of SV that do not affect the number of lesions produced by TNVb in beans have a large effect in *N. glutinosa*.

Table 2. Numbers and ratios of large and small lesions when mixed inocula (TNVb and SV) are inoculated at different dilutions

| Inocula | Size and number of lesions on 6 half-leaves of French bean | | Large/small |
|--|--|-------|-------------|
| | Large | Small | |
| Purified preparations (TNVb 0.8 mg./l. and SV 0.6 mg./l.) | | | |
| Undiluted | 107 | 50 | 2.1 |
| Diluted 1/2 | 82 | 16 | 5.1 |
| Diluted 1/4 | 35 | 1 | 35.0 |
| Diluted 1/8 | 20 | 0 | — |
| Sap from tobacco plants inoculated with the Rothamsted culture (TNVb + SV) | | | |
| Diluted 1/5 | 32 | 403 | 0.08 |
| Diluted 1/25 | 19 | 85 | 0.22 |
| Diluted 1/125 | 13 | 17 | 0.76 |
| Diluted 1/625 | 3 | 1 | 3.0 |
| Diluted 1/3125 | 1 | 0 | — |

Table 3. Numbers and ratios of large to small lesions with mixed inocula containing various concentrations of tobacco necrosis virus (TNVb) and constant amounts of satellite virus (SV) at 0.6 mg./l.

| Concentration of large virus (mg./l.) | Size and number of lesions on 8 half-leaves of French bean | | Large/small |
|---------------------------------------|--|-------|-------------|
| | Large | Small | |
| 8.8 | 53 | 427 | 0.12 |
| 4.4 | 47 | 273 | 0.17 |
| 2.2 | 22 | 170 | 0.13 |
| 1.1 | 7 | 46 | 0.15 |

When a constant amount of TNVa or TNVb was mixed with decreasing amounts of SV, the ratio of large to small lesions in beans increased (Fig. 1). The ratio of large to small lesions also increased when a mixture of TNVb and SV was inoculated at different dilutions (Table 2). Hence the effect on lesion size depends not only on the ratio of the two viruses in the inoculum but also on the absolute concentration of SV; dilution presumably lessens the chances of TNVb and SV particles meeting in the same cell of infection (multiple infection) and interfering with each other. When beans were inoculated with TNVb at different concentrations in a constant concentration of SV, the ratio of large to small lesions remained about the same (Table 3). Fewer lesions were produced by the diluted TNVb; but at a constant concentration of SV the chances that TNVb and SV particles will meet in the same cell are also unchanged.

In all the above experiments TNVb and SV were inoculated simultaneously as

mixtures, but lesion size can be affected when the two are inoculated at different times. When TNVb was inoculated first, however, SV had to be inoculated soon afterwards to affect lesion size. About half the lesions were small when the interval between the two inoculations was 30 min., about one-quarter when the interval was 5 hr. and all lesions were large when the interval was 1 day. By contrast, bean plants inoculated with 40 mg./l. of SV produced only small lesions when re-inoculated with TNVb 5 days later.

Interference in virus multiplication

TNVb multiplies less extensively in leaves inoculated with a mixture of the two viruses than when inoculated alone; the extent to which its multiplication is depressed depends on the ratio of the two viruses in the inoculum. With inocula containing much more SV than TNVb, the amount of TNVb was too small to be detected serologically (Table 4). Other factors than the ratio of the two viruses in the inoculum can influence the virus concentration. SV reached higher concentrations in bean than in tobacco leaves, whereas TNVb inoculated alone reached higher concentrations in tobacco than in bean (Table 5). As SV seems completely dependent on TNVb it is interesting that it multiplies better in the species of plant less well suited for the multiplication of TNVb. In both bean and tobacco, TNVb was more effective than TNVa in stimulating the multiplication of SV.

Table 4. Concentration of tobacco necrosis virus (TNVb) and satellite virus (SV) in single and mixed infections in tobacco plants, 6 days after inoculation

| Inocula* (mg./l.) | Reciprocals of precipitation end point | |
|-------------------|--|----|
| | TNVb | SV |
| TNVb 9 | 16 | 0 |
| TNVb 9+SV 3 | 8 | 32 |
| TNVb 9+SV 9 | 2 | 16 |
| TNVb 9+SV 27 | 0 | 16 |

* 'Celite' was added in the inocula.

Table 5. Comparison of the concentration of the two tobacco necrosis viruses (TNVa and TNVb) when inoculated alone or together with SV in bean and tobacco plants

| Inocula | Reciprocals of precipitation end-point | | | |
|---------|--|-----|-----------|----|
| | Bean | | Tobacco | |
| | TNVa or b | SV | TNVa or b | SV |
| TNVb* | 4 | 0 | 16 | 0 |
| TNVb+SV | 0 | 128 | 1 | 16 |
| TNVa | 2 | 0 | 8 | 0 |
| TNVa+SV | 0 | 32 | 0 | 4 |

* TNVb and TNVa were at 12 mg./l. and SV at 31 mg./l. The tobacco plants were inoculated with the aid of 'Celite'.

Concentrations of TNVb and SV were affected differently by changing temperature. Although TNVb alone and SV (in plants with both viruses) reached maximum concentration in tobacco plants kept at about 20°, the concentration of SV de-

creased relatively more than that of TNVb when the temperature was raised to 26°. The multiplication of TNVb was inhibited by SV more at lower than at higher temperatures, which agreed with the effects of temperature on the type of lesion. At 26° all the lesions formed by mixed inocula were large (Table 1).

The multiplication of the two viruses depended on whether they were inoculated simultaneously or not. When SV was inoculated together with, or 1 day before, TNVb, SV multiplied more than when inoculated after TNVb. The multiplication of TNVb was decreased more by SV when the two viruses were inoculated together than when TNVb was inoculated 1 day before SV. When TNVb was inoculated one day before SV all the lesions were large, but SV nevertheless multiplied under these conditions (Table 6).

Table 6. *Concentration of tobacco necrosis virus (TNVb) and satellite virus (SV) inoculated at different times to tobacco plants*

| First inoculation | Second inoculation | Reciprocal of precipitation end-point | |
|-------------------|--------------------|---------------------------------------|----|
| | | TNVb | SV |
| 3 Jan. TNVb* | 4 Jan. SV | 16 | 8 |
| 3 Jan. TNVb | 4 Jan. Water | 32 | 0 |
| 2 Jan. SV | 3 Jan. TNVb | 8 | 32 |
| 2 Jan. Water | 3 Jan. TNVb | 8 | 0 |
| 3 Jan. TNVb + SV | — | 2 | 32 |

* TNVb and SV were used at 10 mg./l.; 'Celite' was added in all the inocula.

Properties of SV

Purification. Most of the TNVb present in purified preparations of the mixed Rothamsted culture could be precipitated by ammonium sulphate at 10 to 15% saturation leaving about 75% of SV in the supernatant fluid, as shown by serological and infectivity tests. The average numbers of lesions per leaf were 61, 89 and 5, respectively, with the mixture, with the precipitate (containing most of the TNVb), and with the supernatant fluid (containing most of the SV). To remove the remaining TNVb the preparations of SV were centrifuged in sucrose gradients. Purified SV inhibited the lesions formed by TNVb at concentrations as low as 1 µg./l. Concentrations less than 3 mg./l. were not precipitated by homologous antiserum.

Purified preparations of SV scattered light much less than preparations of the same concentration of TNVa or TNVb, and when centrifuged at 75,000 g for 3 hr. they formed crystalline pellets. Pellets freshly suspended in water were turbid and contained many crystals of different sizes, most of which dissolved in 24 hr. at 4°. Under the microscope the crystals appeared as rhombic plates, and electron micrograph measurements showed them to consist of particles the size of SV (Pl. 1, fig. 1). The rhombic plates resembled the small crystals photographed by Bawden & Pirie (1945, Fig. 3) from concentrated purified preparations of the Rothamsted culture left for some weeks at 0°.

A preparation of SV twice separated in sucrose gradient columns contained 2.0% phosphorus, in agreement with the results of Bawden & Pirie (1942) who found the phosphorus content of all tobacco necrosis virus isolates to be between 1.7

and 2.0%. The particle of SV is one-third to one-half that of TNVb and therefore contains correspondingly less nucleic acid; this has been suggested as a possible reason for its apparent inability to multiply unaided (Kassanis & Nixon, 1961). Since then, brome grass mosaic virus has been reported to contain the smallest infective nucleic acid, with a molecular weight of 1×10^6 (Bockstahler & Kacsberg, 1961). The equivalent molecular weight of SV calculated from the sedimentation constant (50 S) is 1.85×10^6 (Bawden & Pirie, 1945), and of its nucleic acid 0.37×10^6 , which is much smaller than the brome grass mosaic virus.

Particle size. Measurements of the diameter of SV particles were from 14.3 to 21.2 $m\mu$ according to the technique used for mounting the preparations (Kassanis & Nixon, 1961). Because of the uncertainty about the true size of SV, rows of particles were measured on several electron micrographs of the carbon copies of shadowed crystals of SV. The result depended on which direction in the crystal the measurement was made; one way gave the diameter as 14.4 and the other 16.7 $m\mu$. With a diameter of 16.7 $m\mu$ and 1.35 density (the density of bushy stunt virus) the particles would have a weight equivalent to a molecular weight of 1.9×10^6 , which is close to that calculated from sedimentation constant (Bawden & Pirie, 1945). Hence 16.7 $m\mu$ is possibly the actual diameter of SV.

Table 7. *Inactivation of satellite virus by heat*

20 mg./l. of virus in 0.5 ml. lots were heated for 10 min. at different temperatures and mixed with equal volumes of tobacco necrosis virus (TNVb) at 1.2 mg./l.

| Treatment | Size and number of lesions on 8 French bean half-leaves | | Survival (%) |
|---------------|--|---------------|-----------------|
| | Large lesions | Small lesions | |
| Unheated | 0 | 170 | — |
| Heated at 70° | 21 | 128 | 8 |
| 80° | 47 | 114 | 4 |
| 85° | 95 | 169 | 2 |
| 90° | 195 | 25 | 1 |
| 95° | 199 | 0 | 0 |

Inactivation by heat. SV was heated for 10 min. at different temperatures. After heating it was mixed with constant amounts of TNVb, inoculated to beans, and the large and small lesions counted separately. A few small lesions were formed with SV heated at 90° but none after heating at 95° (Table 7). Serological tests on the sap from inoculated leaves confirmed that SV heated at 95° did not multiply. Heating for 10 min. at 70° destroyed over 90% of the infectivity, whereas heating at 90° did not affect the ability to precipitate with antiserum. Loss of infectivity without obvious change of the precipitation titre is a feature of many spherical viruses. TNVb lost infectivity in 10 min. between 75° and 80°, whether heated alone or with SV.

Stability. The activity of purified preparations of SV stored at 3° remained unaltered for several months. That some activity remained after 17 years was shown by tests on several purified preparations of the Rothamsted culture of TNV given to me by Mr N. W. Pirie. These had been left at 3° since 1943, and produced no lesions on bean but when mixed (at 50 mg./l.) with active TNVb they decreased the size of

25% of the lesions. A 17-year-old preparation of the 'potato' isolate of TNV contaminated with the Rothamsted culture of TNV (Bawden & Pirie, 1942) was also inoculated to beans; it produced a few lesions and virus from these was multiplied in tobacco. Partially purified virus prepared from the tobacco contained mostly large particles but also some small ones. The presence of SV suggests that the 'potato' isolate, a stable tobacco necrosis virus (Bawden & Pirie, 1942), is another that can activate SV.

Ultraviolet inactivation. TNVb is inactivated $2\frac{1}{2}$ times faster than SV by ultraviolet irradiation at a wavelength of $254\text{ m}\mu$ (Fig. 2).

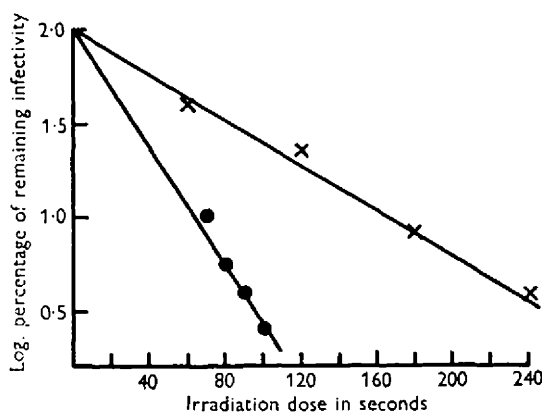


Fig. 2. The inactivation of SV and TNVb (both 20 mg./l.) by ultraviolet light. 20 cm. from source in films 0.07 cm deep. \times — \times , SV; \bullet — \bullet , TNVb.

Ultraviolet absorption. The ultraviolet absorption spectrum of SV was similar to that of TNVb, with a maximum at $260\text{ m}\mu$ and a minimum at $241\text{ m}\mu$. The extinction coefficient for SV was 6.5 (for 1 mg./ml.).

Electrophoretic mobility. In 0.066M-phosphate buffer (pH 7.0) SV was stationary even after 3 hr. at a potential gradient of 9.5 v./cm., whereas TNVb had a mobility of $-7.4 \times 10^{-5}\text{ cm.}^2\text{ sec.}^{-1}\text{ v.}^{-1}$. The difference in mobility between the two viruses was found useful in separating them from purified preparations of the Rothamsted culture. SV remained in the descending arm and after $2\frac{1}{2}$ hr. at a potential gradient of 9.5 v./cm., TNVb moved away and the arm was disconnected. Preparations of the Rothamsted culture usually have much less TNVb than SV and electrophoresis gave clean preparations of SV. The two viruses were also separated from a preparation of the Rothamsted culture enriched by adding TNVb so that the mixture contained per weight more of TNVb than SV. SV prepared from this run was almost free from TNVb particles (Table 8). Preparations of SV obtained by electrophoresis from ordinary preparations of the Rothamsted culture were as free from TNVb as those obtained by centrifugation in sucrose gradient columns. Separation by electrophoresis was preferred because it was less wasteful, and its efficiency was not impaired by high virus concentration or by aggregated SV in the mixture. Purified preparations of the Rothamsted culture containing aggregated SV gave three zones when centrifuged in sucrose gradients; the lowest zone was difficult to remove from the tube through the middle zone containing TNVb (Kassanis & Nixon, 1961).

Nucleic acid of SV. When preparations of SV were disrupted with phenol (Geirer & Schramm, 1956) they were still infective and still gave many of the phenomena shown by intact virus. Such disrupted preparations were inactivated in less than a day at 20°. They presumably consisted largely of nucleic acid, for they were rapidly inactivated by dilute pancreatic ribonuclease, which does not inactivate intact virus. Fresh preparations of nucleic acid did not produce lesions when inoculated alone to bean or tobacco and no virus was later recoverable from such inoculated plants. When inoculated together with TNVb, however, these nucleic

Table 8. *Separation of satellite virus (SV) from a mixture with TNVb by electrophoresis*

| Inoculum | Average particle count | | TNVb/SV | Infectivity test Amount of virus (mg./l.) | |
|---------------------------------------|------------------------|-----|---------|---|----|
| | TNVb | SV | | 300 | 30 |
| | | | | Average number of lesions per leaf | |
| From descending arm | 1 | 435 | 0.002 | 2 | 0 |
| From ascending arm | 134 | 33 | 4.0 | 65 | 17 |
| The mixture before electrophoresis | 64 | 111 | 0.57 | 37 | 14 |

acid preparations decreased the size of the lesions and such inoculated plants later contained normal SV. To influence the size of lesions produced by TNVb, the nucleic acid and TNVb had to be inoculated simultaneously or the TNVb within an hour or so of the nucleic acid. This is a striking difference from the intact virus which produces its effects when inoculated several days before the TNVb. The difference between the behaviour of the nucleic acid and intact virus presumably largely reflects the greater instability of the nucleic acid. There was, however, some result from an early inoculation with nucleic acid, for although the lesions were all large some SV could later be extracted from such plants. In two experiments out of five, when TNVb was inoculated as long as 2 days after nucleic acid, sap extracted 6 days later contained some SV, although only about 6% of the amount from leaves inoculated with TNVb immediately after nucleic acid. In the other three experiments of this kind, SV was not detected when the inoculation with TNVb was delayed for 2 days. Different glasshouse temperatures may have caused the inconsistency. Since the nucleic acid preparations lost their infectivity within a day at 20°, it seemed that *in vivo* some nucleic acid was either protected from inactivation by cellular components, or had produced an undetectable amount of new virus at some sites, where multiplication reached detectable amounts after infection with TNVb. TNVb when inoculated with the nucleic acid of SV, as with whole SV, sometimes produced twice as many lesions as when inoculated on its own.

DISCUSSION

The interactions between TNVb and SV seem to differ from the various types already described. There is nothing novel in the multiplication of TNVb being decreased by SV, except that such interference is usually between related strains, whereas TNVb and SV are serologically unrelated. However, tobacco severe etch

virus can suppress the multiplication of potato virus Y, which is not related to it (Bawden & Kassanis, 1945). Nor is there anything new in the idea that one virus may increase the amount to which another multiplies; but the previous examples have all been with viruses clearly able to multiply on their own (Rochow & Ross, 1955; Kassanis, 1961). The striking feature of the interaction between TNVb and SV is that SV apparently depends completely on TNVb for its ability to multiply. However, it is necessary to say apparently, for although when inoculated alone to plants it is not pathogenic and it does not multiply detectably, it is necessary to consider the limitations of the techniques used in detection. The most sensitive technique is the ability to affect the size of lesions produced by TNVb and this can be done by preparations containing as little as 1 $\mu\text{g./l.}$ Serological tests are very much less sensitive than infectivity tests: the minimum amount detectable is 3 mg./l. Obviously, the SV could multiply many times before it became detectable serologically and even by infectivity tests. Attempts were made to demonstrate multiplication in leaves inoculated with SV alone by extracting the sap 10 days later and mixing it with TNVb and inoculating to beans, but all the lesions were large.

There are three reasons for suspecting that SV induces changes in the plant cells and so withstands inactivation, or perhaps multiplies to a limited extent. First, plants inoculated many days earlier with SV still contain considerable amounts of SV when later re-inoculated with TNVb. This could be explained simply by particles from the initial inoculum surviving intact for days in the inoculated leaves until their replication is set going by TNVb. However, the idea conflicts with the behaviour of other viruses as stable as SV, which are inactivated within a few days of being inoculated in hosts where they do not multiply. Secondly, under certain conditions, the infectivity of TNVb is greater when inoculated with SV or its nucleic acid, than when inoculated on its own; some particles of TNVb which otherwise could not have infected can do so in the presence of SV. This suggests that SV changes cells so they become more susceptible to TNVb. Thirdly, and perhaps more important, is the fact that some SV can be recovered from plants inoculated with SV nucleic acid 2 days before they are inoculated with TNVb, for it seems highly improbable that the fragile nucleic acid could survive unharmed for 2 days while waiting to be activated by TNVb. It may be protected from inactivation by association with some host component, perhaps by entering a state comparable to that of bacteriophage in lysogenic bacteria, or it may have initiated virus multiplication which has proceeded only to an undetectable degree.

These are possibilities, but the main picture from the results is one of SV being completely dependent on an attendant virus to create conditions in which it can multiply. The attendant virus seems to lose from the association, because it multiplies less in doubly-infected leaves than on its own. The one suggestion of gain is that occasionally more infection occurs with inocula of TNVb + SV than with inocula of TNVb alone. However, even when this happens most of the lesions are smaller than they would be with TNVb alone and at each infection site its multiplication is restricted.

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EXPLANATION OF PLATE

Crystal of staellite virus (platinum shadowed carbon replica). $\times 54,000$.

