After 24 hours, guts from males treated with 100 μg of EFA were highly attractive to female beetles (Table 1). When bioassayed at a stimulus concentration of 2 male equivalents (two guts in approximately 0.05 ml of benzene), these guts were more attractive than guts from males that were allowed to produce pheromone naturally in logs. However, at a stimulus concentration of 0.02 male equivalents, extract from males which had produced pheromone in logs was significantly attractive (20.3 percent), whereas extract from males treated with EFA elicited only a trace response (Table 1). Evidently, males boring in logs produced greater amounts of the pheromone compounds than males treated with the hormone. There was a progressively weaker response as the EFA dose decreased, and after treatments with 10 μg no pheromone activity could be detected in the hindguts.

Only a trace response by females occurred to guts from control males treated with pure peanut oil (Table 1). In certain species of bumblebees and wasps, farnesol acts as an attractive pheromone (*9*). Therefore, we tested the attractiveness of 100 μg of EFA in 0.05 ml of benzene, but only 1 of 71 females responded positively to this stimulus.

Eighteen hours after treatment of male beetles with 100 μg of EFA, their gut extract elicited a significant response of 26.6 percent (25 of 94 females) when bioassayed at 2 male equivalents. However, similar treatments failed to induce pheromone production after 3, 6, and 12 hours in 13, 13, and 12 male beetles, respectively. Synthetic hormone applied topically apparently required more than 12 hours to elicit a response, whereas male beetles excised from host logs 3 hours after introduction possessed highly attractive hindguts. Evidently, boring into and feeding on the bark of host logs provide a stimulus which promotes rapid synthesis or release of the insect's juvenile hormone, or both, thereby inducing a more immediate physiological response. One suggestion is that topically applied EFA is slowly absorbed and converted to a hormonal terpene identical or closely related to "paper factor" (juvabione) (*10*) or cecropia juvenile hormone (*11*), while beetles in the bark receive a "paper factor" type stimulus directly from the host tree.

In nature, *I. confusus* males produce pheromone while feeding and release it by discharge of fecal pellets (*3*). Moreover, the terpene alcohol pheromone compounds are closely related to naturally occurring constituents in the host tree. Therefore, one hypothesis explaining the biogenesis of scolytid pheromones is that host tree precursor compounds are ingested and metabolized to produce pheromone in the hindgut (*12*). The fact that males treated with EFA were not allowed to ingest fresh host material supports the alternative hypothesis (*12*) that the pheromone compounds of *I. confusus* are true secretions. A further possibility is that absorbed EFA is metabolically converted by the beetle to form one or more of the known sex pheromone compounds (*2*).

Table 1. Response of *I. confusus* females to benzene extracts of hindguts from males 24 hours after topical application of 10,11-epoxyfarnesenic acid methyl ester (EFA) in peanut oil compared with response to hindgut extracts from males treated with peanut oil, or excised from host logs.

Stimulus (male equivalents)	Tested (No.)	Responders (No.)	Response (%)	Probability (χ^2)
1 μl of peanut oil				
2.0	103	8	7.8	Control
In log				
2.0	141	95	67.3	<.001
0.2	81	29	35.8	<.001
0.02	79	16	20.3	<.05
0.002	79	3	3.8	
100 μg of EFA in 1 μl of peanut oil				
2.0	101	82	81.2	<.001
0.2	119	57	47.9	<.001
0.02	102	10	9.8	<.70
0.002	102	1	1.0	
0.5 μl of peanut oil				
2.0	160	7	4.7	Control
50 μg of EFA in 0.5 μl of peanut oil				
2.0	80	41	51.3	<.001
0.2	78	22	28.2	<.001
0.02	80	4	5.0	<.90
25 μg of EFA in 0.5 μl of peanut oil				
2.0	80	12	15.0	<.01
10 μg of EFA in 0.5 μl of peanut oil				
2.0	83	2	2.4	

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Satellite-like Particle of Tobacco Ringspot Virus That Resembles Tobacco Ringspot Virus

Abstract. *A satellite-like nucleoprotein serologically indistinguishable from multicomponent tobacco ringspot virus, also resembles it in size, shape, and electrophoretic mobility. Although the protein shell of the nucleoprotein is similar to, if not identical to that of tobacco ringspot virus, each shell of the nucleoprotein contains many small strands of nucleic acid, which replicate only in mixed infections with the virus.*

The satellite virus (SV) of tobacco necrosis virus (TNV) was originally named "satellite" by Kassanis (*1*) because "it is always in association with, and dependent upon, the large virus particles" (that is, TNV). Similarly, the nucleoprotein, which is the subject of this report, appears to be dependent upon the multiplication of tobacco ringspot virus (TRSV), but I refer to it as "satellite-like" (SL) because its functional relation to TRSV has not yet been determined. The new particle, SL-TRSV, has the same shape and size as TRSV and is serologically related to TRSV, whereas SV-TNV is smaller than TNV and does not appear to be serologically related to TNV (*2*).

The nucleoprotein SL-TRSV was discovered in bean plants previously inoculated with a laboratory source of TRSV. The new component appears to be noninfectious in both cowpea *Vigna sinensis* (Torner) Savi, cv. Blackeye and bean *Phaseolus vulgaris* L. cv. Black Valentine plants, which are hosts

of TRSV. No lesions were induced when SL-TRSV, at a concentration equivalent to 0.26 optical density units per milliliter at 260 nm, was rubbed onto cowpea leaves. The TRSV induced lesions under the same conditions when its concentration was 1/20 that of SL-TRSV (in optical density units per milliliter). Yet a 2000-fold dilution of the apparently noninfectious SL-TRSV preparation, when mixed with TRSV, induced, in addition to typical TRSV lesions, some lesions which were distinct from those induced by TRSV (strain ST) alone (*3*). The atypical lesions induced in cowpeas by the SL-TRSV–TRSV mixture are dark, usually surrounded by a clear zone, and very small (Fig. 1; 1 mm or less, including the clear zone). Lesions induced by TRSV (strain ST) alone are brown, necrotic, and much larger (Fig. 1) (4 to 6 mm). Triturations of 10 to 15 of the small lesions in 3 ml of phosphate buffer (0.02 *M*, *p*H 7), with or without bentonite, induced no lesions in cowpeas. An extraction from one large lesion in the same volume of buffer induced 10 to 50 large lesions in the opposite primary leaves of the same plants. A mixture (1 : 1 by volume) of the same two extracts (from small plus large lesions) induced mainly small lesions, and no infectivity was detected in extracts from 10 to 15 of these small lesions. When 40 to 45 of these small lesions were triturated in 3 ml of buffer, the extract induced an average of 12 small lesions per leaf. The nucleic acid extracted from SL-TRSV is likewise biologically active only when mixed with TRSV or with the infectious nucleic acid from TRSV. In doses of more than 20 μg/ml the nucleic acid from SL-TRSV induced no lesions in cowpeas, but in doses of 0.008 μg/ml the same nucleic acid preparation, when mixed with TRSV, induced some tiny atypical lesions.

No multiplication of SL-TRSV could be detected in purified (*3*, *4*) products from symptomless bean or cowpea plants which had previously been inoculated with SL-TRSV or its nucleic acid. Typical TRSV, without detectable SL, was obtained by purification from other plants which had been inoculated at the same time with TRSV alone. A third group of plants, which had been inoculated with mixtures of SL-TRSV and TRSV, yielded a mixture of SL-TRSV and TRSV.

The relative amount of SL-TRSV quickly increased in the mixture of TRSV + SL-TRSV in inoculated (as well as in the systemically invaded) leaves of bean and cowpea plants. For example, when mixed inoculum of TRSV and SL-TRSV contained 5 percent or less SL-TRSV, the yield of SL increased in systemically invaded leaves, while that of TRSV did not (Table 1). The yield of TRSV per gram of tissue in systemically invaded tissue, 7 days after the plants had been inoculated, was 20 percent that of TRSV in inoculated tissue harvested only 4 days after inoculation. In contrast, the yield of SL-TRSV from systemically invaded tissue in the same plants at 7 days was 20 times as large as it was in the inoculated tissue at 4 days (Table 1).

Table 1. Yield of SL-TRSV and TRSV in the same tissue with increase in age of infection. Optical density, O.D.

Route of infection	Elapsed time (days)	Yield per gram of leaf		
		TRSV (O.D. units)	SL-TRSV	
			O.D. units	(%)
Inoculation	4	0.62	0.15	20
Inoculation	6	.62	.28	31
Systemic invasion	7	.13	3.06	96

When a high percentage (95 percent) of the inoculum was SL-TRSV, only minute amounts of SL-TRSV and no infectious product could be detected in the purified product from 300 g of tissue. The SL-TRSV was detected by biological assay only; the preparation induced no lesions alone, but induced small lesions characteristic of SL-TRSV if pure TRSV (2.5 μg/ml) was added.

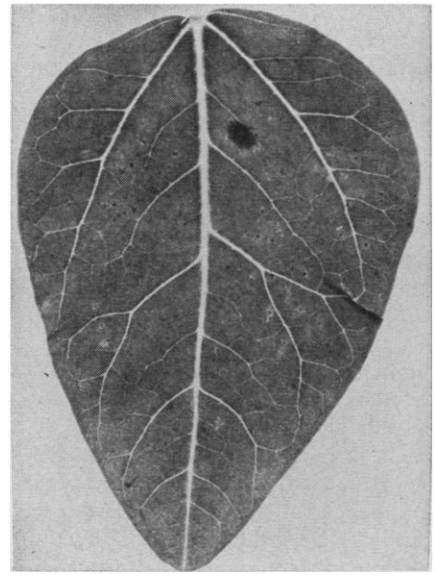

Fig. 1. Lesions induced by a mixture of SL-TRSV and TRSV. All lesions except the largest (upper to the right of the midrib) contain a high percentage of SL-TRSV. Only the largest lesion contains a detectable amount of TRSV.

The similarity of SL-TRSV to TRSV is indicated by sucrose density-gradient analysis. The ultraviolet absorption profile (after 3 hours of centrifugation at 24,000 rev/min; SW-25.1 rotor; Beckman model L ultracentrifuge) of the components from purified TRSV, containing as much as 30 percent SL-TRSV, was indistinguishable from the ultraviolet absorption profile of purified TRSV that contained no SL-TRSV (*3*, *4*). When more SL-TRSV was present, the SL-TRSV, although almost coinciding in position with that of bottom component of TRSV, appeared more heterogeneous than bottom component usually does.

Comparison of the sedimentation rate of bottom component of TRSV with that of the biologically active SL-TRSV was also carried out in the analytical untracentrifuge (Beckman model E, with ultraviolet optics). Prior to this comparison, the SL preparation (which contained 5 to 10 percent TRSV) and TRSV (bottom component only) were purified further by centrifugation to equilibrium in a CsCl gradient (in an SW-65L rotor in the model L2 ultracentrifuge). After removal of CsCl from bottom component and from a major portion of the SL fraction by dialysis, the samples were centrifuged simultaneously in separate cells. Both samples appeared homogeneous. The sedimentation coefficient ($s_{20,w}$) of the SL-TRSV was 122, and that of TRSV (bottom component) was 126. The latter figure agrees with published figures (*3*). There was no significant difference in size and shape (based on the average from measurements of 50 particles from each sample), and exclusion of stain (negatively stained) when SL-TRSV and TRSV were compared by electron microscopy.

The nucleic acid content of SL-TRSV appears somewhat higher than the nucleic acid content of the middle component of TRSV, if their protein shells are identical (maximum/minimum at 258/240 nm for SL averaged 1.43, and for middle component 1.35), but it was less than the nucleic acid content of bottom component based on the position of SL-TRSV in a CsCl gradient. The sedimentation coefficient ($s_{20,w}$) of the satellite nucleic acid was only 7, when centrifuged in 0.02*M* phosphate buffer (*p*H 7), and that of the infectious nucleic acid of TRSV was 32 (*5*). The

thermal denaturation curves of both nucleic acids indicated that they are single stranded; thus, the nucleic acid of SL-TRSV is much smaller than that of TRSV and much smaller than one would have predicted from either the sedimentation coefficient or the apparent nucleic acid content of SL-TRSV (nucleoprotein). Furthermore, if the relation between sedimentation coefficient and molecular weight suggested by Gierer (*6*) holds, a molecular weight of only 86,000 is indicated for the nucleic acid of SL-TRSV compared with a molecular weight of 1.2 million for the nucleic acid from middle component of TRSV. If one considers that the nucleic acid content of SL-TRSV is greater than that of the middle component of TRSV but less than that of bottom component [which is about 2.2 million (*5*)], we must then conclude that each particle of SL contains 14 or more strands of nucleic acid of about the same size, and that some strands, perhaps all, are biologically active in conjunction with TRSV infections.

The SL-TRSV reacted with antiserum to TRSV when SL was diluted to the same concentration as TRSV (in optical density units per milliliter) that was barely detected in microprecipitin tests, or that was barely detected in Ouchterlony double-diffusion tests. In the latter test at higher concentrations of antigen, when SL-TRSV and TRSV were in adjacent wells and opposite the well containing TRSV antiserum, the reaction bands were continuous with no spur formation. Furthermore, when SL-TRSV and TRSV were subjected to electrophoresis for 22 hours in sucrose gradients (0.002*M* phosphate buffer, 0.05*M* NaCl, *p*H 7) (*7*), both apparently migrated to the same position. The SL-TRSV appears to be a nucleoprotein containing many small strands of nucleic acid which are biologically active in the presence of TRSV, but the protein shell which encloses these strands is similar to the protein shell of TRSV.

The SL-TRSV is similar to some plant viruses with functionally incomplete particles in that it is unable to replicate alone. These dependent plant-virus particles relate biologically in their respective systems as symbionts or as parasites. Components of alfalfa mosaic virus, cowpea mosaic virus, or tobacco rattle virus relate symbiotically (*8*). With the exception of the long particle of tobacco rattle virus, no particle of this group of viruses can replicate alone. But the long particle of tobacco rattle virus does not code for its protein shell and depends on the short particle (that cannot replicate alone) for this function.

The parasitic dependency of SL-TRSV on TRSV more closely resembles the parasitic dependency of SV-TNV for TNV, its activator. The SL-TRSV, like SV-TNV becomes the larger part of the virus population in mixed infections with its specific activator. The TRSV and TNV replicate and form complete particles in the absence of their respective satellites. The TRSV, like TNV, codes for its own coat protein.

Although the SL-TRSV–TRSV system resembles the SV-TNV–TNV system, many biological as well as structural differences are evident. The activator (TRSV) is a multicomponent virus; TNV is a single-component virus. The TRSV and SL-TRSV commonly infect hosts systemically; TNV and its satellite are commonly localized in primary infection sites. The SL-TRSV has the same size and shape as TRSV and is serologically indistinguishable from TRSV, and each protein shell of SL-TRSV encloses many small nucleic acid strands. The SV-TNV is smaller than, and serologically unrelated to, TNV, and each shell of SV-TNV contains a single nucleic acid strand. Since the small nucleic acid strands of SL-TRSV can be activated by TRSV or its infectious nucleic acid after extraction, these strands were small before extraction and may occur as subunits within the shell. However, different strands cannot be ruled out at present.

The origin of plant viruses, which are unable to replicate alone, is uncertain. The SV-TNV and the functionally incomplete particles of tobacco rattle virus, alfalfa mosaic virus, and cowpea mosaic virus have been known in virus preparations for many years (*8*). The strain of TRSV used in this study to activate SL-TRSV has been increased and purified routinely in this laboratory since 1963. The ability of SL-TRSV to rapidly become the larger part of the population in mixed infections with TRSV indicates that SL-TRSV probably has not been continuously carried along as an active contaminant with TRSV. Thus it may be that the origin of SL-TRSV as a "contaminant" in our population of TRSV was a recent event.

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Cytosine to Thymine Transitions from Decay of Cytosine-5-^{3}H in Bacteriophage S13

Abstract. *Decay of cytosine-5-^{3}H incorporated into bacteriophage S13 DNA causes a molecular rearrangement of the cytosine molecule undergoing the decay. The molecular rearrangement produces a cytosine to thymine coding change with an efficiency approaching one. Decay of either thymidine-(methyl)-^{3}H or cytosine-6-^{3}H is less than 1 percent as effective in causing either cytosine to thymine or thymine to cytosine transitions.*

Radioactive decay of uracil-5-^{3}H incorporated into *Escherichia coli* (*1*) is more mutagenic than that of other tritiated precursors (*2*, *3*). *Escherichia coli* efficiently interconverts uracil and cytosine (*4*) so that cells grown in the presence of uracil-^{3}H will have uridine-^{3}H and cytidine-^{3}H incorporated into their RNA and cytidine-^{3}H incorporated into their DNA (*5*). For each disintegration, the mutation frequency is threefold greater when bacteria are labeled with uracil-5-^{3}H than when labeled with thymidine-(methyl)-^{3}H (*2*).