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Genomic RNA of an insect virus directs synthesis of infectious virions in plants

(viral host range/nodavirus/*Drosophila*)

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ABSTRACT **Newly synthesized virions of flock house virus (FHV), an insect nodavirus, were detected in plant cells inoculated with FHV RNA. FHV was found in whole plants of barley (*Hordeum vulgare*), cowpea (*Vigna sinensis*), chenopodium (*Chenopodium hybridum*), tobacco (*Nicotiana tabacum*), and *Nicotiana benthamiana* and in protoplasts derived from barley leaves. Virions produced in plants contained newly synthesized RNA as well as newly synthesized capsid protein. These results show that the intracellular environment in these plants is suitable for synthesis of a virus normally indigenous only to insects. Such synthesis involves, minimally, translation of viral RNA, RNA replication, and virion assembly. Inoculation of barley protoplasts with FHV virions resulted in synthesis of small amounts of progeny virions, suggesting that FHV virions are capable of releasing their RNA in plant cells. In *N. benthamiana*, virions resulting from inoculation with RNA were detected not only in inoculated leaves but also in other leaves of inoculated plants, suggesting that virions could move in this plant species. Such movement probably occurs by a passive transport through the vascular system rather than by an active transport involving mechanisms that have evolved for plant viruses.**

Viruses vary greatly in the range of cellular hosts that can support their synthesis. Some of the constraints, especially those involving large taxonomic distances, are attributable to characteristics of the whole organism such as cellular organization, immune systems, and vascular networks. The relative ability of similar cells to support virus synthesis is believed to depend primarily on the ability of the viral genome to enter into the cell interior—i.e., on such features as cell surface structure and existence of receptors.

The nature and extent of the constraints on virus synthesis are less clear once the genome has gained access to the cell interior. Virus synthesis is a complex phenomenon that requires satisfaction of an entire range of requirements interrelating translation, transcription, genome replication, and virion assembly, involving co-factors, membranes, and organelles and affected by environmental factors such as temperature, ionic conditions, and lytic agents.

It might be expected that cells of taxonomically diverse organisms would be unlikely to provide internal cellular conditions that are suitable for synthesis of a particular virus and, indeed, the host range of most viruses is confined narrowly within a taxonomic phylum. Nevertheless, there are a substantial number of examples of viruses whose host range extends beyond a single phylum and a small number of cases exist of viruses whose host range extends to two taxonomic kingdoms. Almost always these are viruses that multiply in insects that serve as vectors to vertebrate or to plant hosts. Especially in cases of insect vectored plant viruses the insect host range is quite narrow (1–3). Thus, particular viruses can accommodate to greatly different cellular environments but nevertheless can be fastidious in their requirements even among closely related hosts. Presumably, intimate contact between these vectors and the corresponding animals or plants has existed for many millenia, allowing viral adaptation to both vector and target hosts. We cannot be sure whether adaptation or the basic similarity of the interior of all cells is the primary factor governing host range of these viruses. It is thus an open question whether, in the absence of evolutionary adaptation, viruses can be synthesized in widely diverse cell types.

To address this question we introduced genomic RNA or intact virions of flock house virus (FHV), an insect virus, into plant cells and assayed for subsequent synthesis of virions. FHV, a small, spherical nodavirus, multiplies prodigiously in its usual host, the New Zealand grass grub (*Costelytra zealandica*) (4), and can be quantitated by plaque assay on cultured *Drosophila melanogaster* cells (5, 6). FHV has a simple genome consisting of two messenger-sense RNAs. RNA1 (3106 bases) encodes protein A (112 kDa), involved in RNA replication, and protein B (12 kDa), of unknown function. RNA2 (1400 bases) encodes virion capsid precursor protein α (44 kDa) (7–12).

We show herein that FHV RNA or virions, once inside plant cells, can initiate an infection cycle. This process culminates in synthesis of progeny virions containing newly synthesized RNA and coat protein. Our results indicate that in favorable cases the interior of plant cells fulfills all requirements for synthesis of an animal virus.

MATERIALS AND METHODS

Inoculation of Whole Plants. The following plants were tested for ability to support FHV synthesis: barley (*Hordeum vulgare*, cv. Morex), chenopodium (*Chenopodium hybridum*), cowpea (*Vigna sinensis*, cv. Queen Anne Black-eye), tobacco (*Nicotiana tabacum* cv. Havana 425), and *Nicotiana benthamiana*. Inoculation was by methods used routinely for plant viruses (13). For chenopodium, cowpea, tobacco, and *N. benthamiana*, leaves were dusted with carborundum and rubbed with 25 μl of a solution containing bentonite (5 μg/μl) and either FHV RNA (0.010 μg/μl) or virus (10^8 particles per μl). Barley inoculum was 10 μl and contained also 25 mM Tris·HCl, 25 mM Na_2HPO_4, 250 mM NaCl, and 5 mM EDTA (pH 7.7). Barley plants were inoculated at the single leaf stage; other plants were inoculated at the two- to six-leaf stages. One leaf was inoculated for each plant except cowpea, where both primary leaves were inoculated. Inoculated leaves were rinsed with distilled water prior to incubation at 24°C under a 12-hr photoperiod. At harvest, leaves were weighed and then homogenized with mortar and pestle in an equal mass of isotonic buffer (IB; 100 mM NaCl/35 mM

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Abbreviations: FHV, flock house virus; pfu, plaque-forming unit(s).
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Pipes/10 mM KCl/1 mM $MgCl_2$/1 mM $CaCl_2$, pH 6.8). Storage was at −80°C.

Inoculation of Protoplasts. Barley protoplasts were prepared and inoculated as described (14). Typically, 5–10 × 10^6 protoplasts were obtained from 4 g of leaves. Briefly, leaves from six-day-old barley plants were minced and then digested with cellulase (Calbiochem) and pectinase (Macerozyme; Yukult Honsha, Tokyo). Residual fibrous material was removed by filtration through a nylon screen, and protoplasts were collected by centrifugation onto a 20% sucrose cushion. Protoplast samples were pelleted and resuspended in 10% mannitol to which was added a suspension of FHV RNA or virions and then a solution containing polyethylene glycol (6000) and $CaCl_2$. In most experiments 10^5 protoplasts were inoculated with 2 μg of RNA (the amount contained within 8 × 10^{11} virions) or with gradient-purified virions (amounts ranging from 1 × 10^{10} to 1.6 × 10^{13}). After inoculation, protoplasts were rinsed three times with 10% mannitol, resuspended at 2 × 10^5 per ml in protoplast medium, and incubated at 30°C, with constant illumination.

Transfection of *Drosophila* Cells. *Drosophila* cells were propagated at 26°C (5) in Schneider's insect medium (15) containing 15% fetal bovine serum (GIBCO). For transfection, cells were rinsed three times with IB (see above), resuspended in IBDD (IB adjusted to pH 6.0 and containing 320 μg of DEAE-dextran per ml), and then inoculated by addition of a small amount of RNA, as described (8). Cells were then rinsed with Schneider's medium lacking serum and suspended at 1 × 10^6 per ml in medium containing serum.

Plaque Assays. Plaque assays on *Drosophila* cells were as described (6), with minor modifications. Briefly, *Drosophila* cells were mixed with plant homogenates or protoplast lysates for 1 hr. Cells were then poured into tissue culture dishes, and, after cells had attached to the dishes, plating buffer was replaced with 1% agarose overlay. Plaques were visualized after 44–50 hr by staining 2 hr to overnight at 26°C with 0.5 ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (3 mg/ml in IB) (16). For plant homogenates and lysates, infectivity was calculated per unit volume of homogenate or lysate and then was converted into plaque-forming units (pfu)/mg of plant tissue (each μl of homogenate contained 0.5 mg of plant tissue and 0.5 mg of buffer). The resulting numbers were multiplied by the mass of the leaf to give pfu per leaf.

Extraction and Electrophoresis of Virion RNA. Protoplasts and *Drosophila* cells were lysed with Nonidet P-40 (0.5%, final concentration), and virus was purified by differential centrifugation, including a final sucrose gradient step (5). To recover virion RNA, gradient fractions containing virus were made 0.2 M in NaCl and 1% in SDS and then were extracted twice with an equal volume of phenol (equilibrated in 10 mM Tris·HCl/100 mM NaCl/1 mM EDTA, pH 8.0) and once with an equal volume of $CHCl_3$. After ethanol precipitation, RNA was dissolved in distilled water and subjected to electrophoresis on a 10-cm 1% agarose gel (buffer was 89 mM Tris/89 mM borate/1 mM EDTA, pH 8.3). En^{3}Hance (New England Nuclear) was used in fluorography.

RESULTS

Detection of Infectious Virus in Inoculated Leaves of Plants. In initial experiments to test whether FHV (which is normally propagated in *Drosophila* cells) could be produced in plants, we inoculated single leaves of a number of plant species with FHV RNA. After incubation at 24°C for periods of 1–18 days, homogenates of individual inoculated leaves were tested for FHV infectivity by applying aliquots to *Drosophila* cells in a standard FHV plaque assay.

FHV infectivity was detected in most homogenates derived from leaves of barley (6 of 7 homogenates tested), chenopodium (6 of 6), cowpea (7 of 7), and *N. benthamiana* (30 of 30). Only one of eight homogenates from tobacco contained detectable levels of virus. Some homogenates contained sufficient virus to clear *Drosophila* cell lawns and were therefore reassayed after dilution with buffer. There was great variation from plant to plant within each species, preventing reliable quantitation of increased virus titer over time. However, no FHV was detected in homogenates from plants that had been incubated 5 hr or less. Generally, barley gave lower plaque titer than chenopodium, cowpea, and *N. benthamiana*.

Results from one set of tests are shown in Table 1. It may be seen that two of the three barley test plants produced virus. Yields were 4 and 170 pfu/mg of leaf tissue, respectively. On a per leaf basis, these yields for barley were 180 and 3800 pfu. All chenopodium, cowpea, and *N. benthamiana* plants produced virus, with median average yields of 1.8 × 10^5, 1.6 × 10^6, and 1.2 × 10^5 pfu per leaf, respectively. One cowpea plant gave a titer of 34,000 pfu/mg (4.2 × 10^7 per leaf), the highest we have observed. The only plant species failing to produce virus in this experiment was tobacco.

Detection of Infectious Virus in Protoplasts Derived from Barley Leaves. Barley protoplasts were inoculated with FHV RNA in a manner typically used for their inoculation with brome mosaic virus RNA (13). Protoplasts were lysed after 2 days and aliquots of the lysate were assayed on *Drosophila* cells, as above. In the experiment summarized in Table 1, an aliquot derived from 0.00016 mg of leaves produced 51 plaques. This corresponds to 3 × 10^5 pfu/mg of tissue. This yield is higher than that obtained from inoculation of intact barley leaves, reflecting higher efficiency of transfection of barley cells in the form of protoplasts. In this experiment ≈10^7 protoplasts were obtained from 4 g of leaves; thus the yield of virus per protoplast was 130 pfu.

To estimate the proportion of protoplasts infected by FHV RNA, transfected protoplasts were plated together with untreated *Drosophila* cells. After 2 days, an average of 156 plaques was counted in two plates, each receiving 5000 protoplasts and 4 × 10^6 *Drosophila* cells. Thus, ≈3% of the inoculated protoplasts released enough virus to produce plaques during the period of the experiment. This probably represents a minimum estimate of the proportion of protoplasts that synthesized virus.

Nature of the Infectious Agent Produced in Plant Cells. The plaque assay in *Drosophila* is sensitive primarily to virions, but viral RNA is capable of producing a low level of infection (unpublished observations). To test whether the infectious agents produced in plant cells were virions, plant homogenates that displayed infectivity on *Drosophila* were treated with RNase or with antiserum against FHV. As a control, purified FHV virions were treated similarly. As shown in Table 2, homogenate infectivity and also that of the FHV virion control were destroyed by antiserum but were not reduced by RNase. Thus, the infectious material consisted of virions. Production of such virions must have required translation of FHV RNA to yield capsid protein, followed by encapsidation of FHV RNA by such protein.

Origin of RNA in Plant-Derived Virions. Virions produced in plant cells could, in principle, have been synthesized by translation of coat protein, followed by encapsidation of inoculum RNA. To show that FHV RNA is synthesized in plant cells, we proceeded as follows. Protoplasts were inoculated with FHV RNA, and [^{32}P]orthophosphate was added after 1 hr. After 48 hr, FHV particles were purified, and virion RNA was extracted and subjected to electrophoresis on an agarose gel. The fluorogram shown in Fig. 1 indicates that radioactive—i.e., progeny—RNA was recovered from particles synthesized in protoplasts (lane c). We thus conclude that barley protoplasts, and, by inference, cells of whole barley plants, provide an environment suitable for FHV RNA

Table 1. FHV yield in plants and plant protoplasts

Plant	Tissue analyzed, mg	No. of plaques	pfu per mg	pfu per leaf
Barley				
Leaf 1	10	0		
	1	0		
Leaf 2	5	20	4	1.8×10^2
	0.5	0		
Leaf 3	2.5	TMTC		
	1.0	168	168	3.8×10^3
	0.12	29		
Chenopodium				
Leaf 1	0.5	TMTC		
	0.25	200	800	3.0×10^5
Leaf 2	0.5	TMTC		
	0.25	233	932	1.8×10^5
Leaf 3	0.5	TMTC		
	0.25	251	1,004	3.4×10^5
Cowpea				
Leaf 1	1.0	96	96	1.6×10^5
	0.5	58		
Leaf 2	0.5	TMTC		
	0.025	114	4,600	1.6×10^6
	0.005	19		
Leaf 3	0.025	TMTC		
	0.005	168	34,000	4.2×10^7
N. benthamiana				
Leaf 1	1.0	16	1.6	2.7×10^2
	0.17	0		
	0.005	0		
Leaf 2	5	TMTC		
	0.17	178	1,047	1.6×10^5
	0.005	9		
Leaf 3	5	TMTC		
	0.17	196	1,153	1.2×10^5
	0.005	8		
Tobacco				
Leaf 1	22	0	0	0
Leaf 2	22	0	0	0
Barley protoplasts	0.0016	TMTC		
	0.00016	51	3.2×10^5	3×10^7

The results of a set of assays of extracts derived from a number of plant sources are shown. Protoplast lysates and *N. benthamiana* homogenates were prepared 2 days after inoculation with FHV RNA; other homogenates were prepared after 14 days. Lysates and homogenates (diluted where necessary) were subsequently titered by plaque assay on *Drosophila*. For protoplasts, ≈40 leaves, weighing an average of 100 mg, were used to prepare 10×10^6 protoplasts. The "pfu per leaf" figure for protoplasts is calculated for a typical leaf—i.e., one weighing about 100 mg. Homogenates prepared 1–5 hr after inoculation, and lysates prepared 1 hr after inoculation, had no infectivity. The limits of detection were 4 pfu/mg of starting tissue for the protoplast lysate and 0.6 pfu/mg for all homogenates except those from chenopodium, where it was 3 pfu/mg. pfu per mg and pfu per leaf are shown only for the statistically most significant result for each sample. TMTC, too many plaques to be counted with precision.

synthesis and contain any host factors required by the FHV replicase for synthesizing the minus-strand intermediate and the genomic, messenger-sense RNA.

Inoculation of Whole Plants with FHV Virions. The above results indicated that free FHV RNA could induce virion synthesis in plant cells. We tested next whether intact virions could initiate the process. However, detection of newly synthesized virions following inoculation of plants with intact virions was technically difficult. Leaves of barley, chenopodium, cowpea, *N. benthamiana*, and tobacco were inoculated with FHV virions by methods routinely used with plant viruses. Virions could be detected (by plaque assay) in homogenates prepared immediately after inoculation, even after thorough rinsing of the leaves and after application of a virion inoculum containing 80 times less RNA per unit volume than had been used with free FHV RNA. Virion concentration decreased in all species during 2- or 14-day incubation (Table 3). We were thus unable to detect the presence of progeny virions in whole plants inoculated with virions. The decrease in infectivity occurring with time could be due to instability of virions in plants and/or spread of virions away from the site of inoculation.

Table 2. Plant-derived infectivity: Resistance to RNase and sensitivity to antibody

Virus source	Infectivity, pfu/5 μl			
	No treatment	Preimmune	Anti-FHV	RNase A
Barley	55	53	0	45
Chenopodium	124	128	0	114
Cowpea	75	88	0	66
N. benthamiana	81	76	0	77
FHV	69	63	0	56

Plant homogenates and sucrose gradient-purified FHV (5, 6) were diluted in Schneider's insect medium (15) such that ≈80 plaques would be expected per 5 μl in a *Drosophila* plaque assay. Aliquots (100 μl) were then treated with 1 μl of either water, undiluted rabbit serum, or RNase A (stock concentration = 10 mg/ml). After 1 hr at 37°C, aliquots (5 μl) were titered by plaque assay. Data presented are average plaque counts from duplicate plates. No plaques were produced upon assay of similarly diluted homogenates from control plants.

Inoculation of Barley Protoplasts with FHV Virions. As with whole plants, no time-dependent increase in virions was detected in protoplasts (10^5) inoculated with virions (10^{10}) (Table 3).

However, we were able to detect radioactive progeny virions after barley protoplasts were inoculated with nonradioactive virions and then incubated in medium containing [^{32}P]orthophosphate. Such progeny virions contained radioactive FHV RNA (Fig. 1, lanes d and e), albeit in lesser amounts than could be obtained following inoculation with free FHV RNA (lane c).

We conclude that FHV virions are able to release RNA in infectious form within barley protoplasts. Thus any host factors or environmental circumstances required for FHV disassembly exist within barley protoplasts and probably also within cells of whole barley plants.

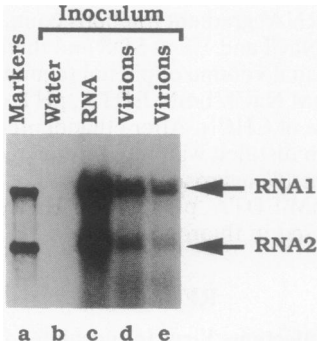

FIG. 1. Electrophoretic analysis of RNA from virions synthesized in barley protoplasts. Transfected protoplasts (10^5, in 0.5 ml of medium) were incubated in the presence of [^{32}P]orthophosphate [150 μCi (1 Ci = 37 GBq), added after 1 hr]. After a total of 48 hr at 30°C, virions were purified and RNA was extracted and electrophoresed on a 1% agarose gel. Inoculum was water (lane b), FHV RNA (2 μg, containing genetic material from 8×10^{11} virions; lane c), or FHV virions (8×10^{11} or 1.6×10^{13}, lanes d and e, respectively). Lane a contains [^{3}H]RNA from *Drosophila*-derived virions.

Table 3. FHV titer in leaves and protoplasts inoculated with virions

Plant	Median infectivity titer, pfu/mg	
	Early harvest	Late harvest
Barley	9,000	60
Chenopodium	300	100
Cowpea	7,000	200
N. benthamiana	1,000	900
Tobacco	500	<0.6
Barley protoplasts	14,000	13,000

Six plants of each species (four for tobacco) were inoculated with FHV virions, rinsed with distilled water, and then incubated at 24°C. Half of the plants were harvested early (after 2 hr for barley, 3 hr for cowpea, 4 hr for chenopodium and *N. benthamiana*, 5 hr for tobacco), and half were harvested late (after 2 days for *N. benthamiana*, 14 days for all other plants). For barley protoplasts, only one preparation was used, and early and late harvests were after 1 hr and 2 days, respectively. FHV infectivity was subsequently measured by plaque assay.

Time Course of Virus Synthesis in Barley Protoplasts. Although we were unable to quantitate a time-dependent increase of FHV titer in whole plants, we did find it possible in plant protoplasts. Fig. 2*A* shows a time course of virion accumulation (as measured by plaque assay) after inoculation of barley protoplasts with FHV RNA. Virions were first detected 8 hr after infection, increased exponentially from about 8–12 hr after infection, and then leveled off, reaching ≈60 pfu per protoplast, somewhat less than the 130 pfu per protoplast found in the experiment of Table 1.

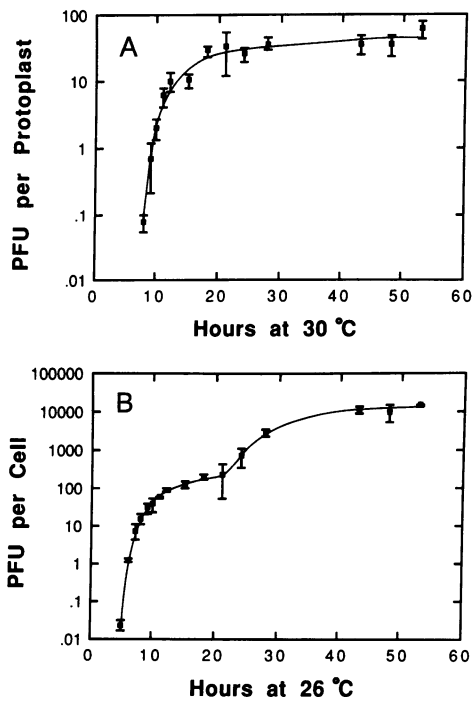

FIG. 2. FHV growth curves in transfected barley protoplasts (*A*) and *Drosophila* cells (*B*). Protoplasts (1×10^6) were transfected with 20 μg of FHV RNA, and aliquots (2×10^4 protoplasts in 100 μl) were seeded into wells of a microtiter plate. Incubation was at 30°C, with illumination. For *Drosophila* transfections, 5×10^6 cells were transfected with 1.5 μg of RNA in the presence of DEAE-dextran (8). Aliquots (10^5 cells in 100 μl of growth medium, with serum) were seeded in microtiter wells and incubated at 26°C. At intervals, three wells containing protoplasts and one containing *Drosophila* cells were treated with 5 μl of 10% Nonidet P-40 to halt the infection. For infectivity determination, the resulting lysates were diluted with Schneider's insect medium, and three 5-μl aliquots were plaque titered on *Drosophila* cell lawns. For protoplasts, data points are averaged from three wells, and error bars represent one standard deviation about the mean. For *Drosophila* cells, error bars represent one standard deviation about the mean plaque count from the three plaque assay plates.

For comparison, we measured the time course of FHV synthesis in *Drosophila* cells inoculated with FHV RNA (Fig. 2*B*). Virions (as measured by plaque assay) were first detected 5 hr after infection. Yield increased exponentially from about 5 to 10 hr after infection, leveled off, and then increased again. The second increase started reproducibly at about 24 hr after infection. In Fig. 2*B*, the initial plateau occurred at ≈240 pfu per cell, whereas the second burst resulted in a yield of 16,000 pfu. We interpret the first phase of virion synthesis as arising from infection of a small percentage of the cells with RNA and the second phase as arising from infection of the remaining cells by progeny virions released from cells infected earlier. From Fig. 2*B* and the assumption that the number of cells doubled during the course of the experiment, it is possible to calculate that ≈3% of the *Drosophila* cells were transfected by FHV RNA. This is typical of the level of FHV RNA transfection of *Drosophila* cells (12) and the same as the level of transfection obtained for transfection of barley protoplasts in the experiment of Table 1 (see above).

We interpret the absence of a second wave of virion production in barley protoplasts as indicating inability of progeny virions to initiate further infection. Overall, we consider the kinetics of FHV synthesis in protoplasts to be consistent with the hypothesis that, once its genomic RNA is inside a plant cell, FHV carries out a normal infection cycle. This cycle includes a lag period during which the input RNA is translated and is followed by RNA replication, capsid protein synthesis, and virion assembly.

The results of the experiments of Fig. 2 *A* and *B* show that the ratio of virions produced by barley protoplast transfection to that produced by *Drosophila* cell transfection is 60/240 = 0.25.

The plaque assay for FHV yields about 1 plaque per 300 FHV virions (6). We can thus calculate that each successfully transfected barley protoplast yields $\approx 60 \times 300/0.03 = 6 \times 10^5$ virions, whereas each successfully transfected *Drosophila* cell yields $\approx 240 \times 300/0.03 = 2.4 \times 10^6$ virions. We judge that the synthesis of FHV virions in barley protoplasts is thus quite comparable to that found in its natural host under comparable conditions of transfection.

Symptoms and Distribution of Progeny Virus in Plants. No overt symptoms were detected in any inoculated plants. However, we did obtain evidence for spread of virus in *N. benthamiana*. Two days after inoculation of *N. benthamiana* with RNA, we detected virions not only in all of the inoculated leaves but also in other leaves, particularly leaves immediately above the inoculated leaf. In one experiment, seven leaves were titered from each of 18 RNA-inoculated plants. These were the inoculated leaf, the next three leaves above, and the next three below. All 18 inoculated leaves produced virus (Table 4), in amounts ranging from 10 to 3600 pfu/mg; the median value was 410. When leaves immediately above the one inoculated were assayed, 14 of the 18 contained detectable amounts of virus. The highest titer observed in such homogenates was 4.5 pfu/mg. Thirty-three of the remaining 90 leaves (i.e., leaves two or three above the inoculated one, or one, two, or three below it) also produced virus, although yields were very low in most instances. However, one leaf, three below the site of inoculation, had a titer of 900 pfu/mg, higher than many of the inoculated leaves.

These results indicate that, in *N. benthamiana*, FHV can accumulate not only in inoculated leaves but also in other leaves. Such accumulation occurs most frequently in leaves immediately above the one that was inoculated. Judging from the relatively low titers in noninoculated leaves, it is likely

Table 4. Distribution of FHV in *N. benthamiana*

Leaf	Proportion containing FHV
Three above inoculated	8/18 (MT = 2.4 pfu/mg)
Two above inoculated	7/18 (MT = 1.5 pfu/mg)
One above inoculated	14/18 (MT = 4.5 pfu/mg)
Inoculated	18/18 (MT = 3600 pfu/mg)
One below inoculated	6/18 (MT = 1.1 pfu/mg)
Two below inoculated	9/18 (MT = 1.0 pfu/mg)
Three below inoculated	3/18 (MT = 900 pfu/mg)

Infectivity was scored positive when at least two plaques were produced by 5 mg of homogenate. No plaques were observed upon assay of 40 mg of control homogenate. MT, maximum titer.

that they contain primarily virions transported after synthesis in inoculated leaves. However, we cannot rule out the possibility that inoculum RNA or progeny RNA or progeny virions were transported from the inoculated leaf and subsequently induced virion synthesis in noninoculated leaves, especially in the exceptional cases in which large accumulation of virions was detected.

In all other plant species tested, virions were detectable only in the inoculated leaf. We conclude that, in these species, neither FHV virions nor unencapsidated RNA induce systemic infection. Localization of infection may be due to the inability of FHV RNA (or virions) to escape from infected cells, to move within these plant species, or to initiate infection after transport to other leaves.

DISCUSSION

The foregoing results show that the FHV genome, once inside plant cells, can complete the FHV infection cycle and thus that these plant cells can substitute for animal cells in supporting such essential viral processes as translation, RNA replication, and particle assembly. Nevertheless, FHV should not be regarded as a plant virus. It does not have a natural route of infection of plants, it shows no obvious symptoms, and probably it does not induce virus synthesis at secondary sites.

In most plants inoculated with RNA, the resulting virions were found only in the inoculated leaf, even after extended periods. In *N. benthamiana*, FHV virions did accumulate in noninoculated leaves, but virus amounts were generally low in such leaves. Thus in all species tested, FHV virion synthesis was initiated efficiently only in the inoculated leaves. This suggests that initiation of infection requires physical breeching of cell walls—i.e., by our artificial inoculation methods—but that, thereafter, FHV synthesis proceeds normally in those cells.

The fact that FHV did accumulate in noninoculated leaves of *N. benthamiana* indicates that FHV can move through such plants. Accumulation of particles in noninoculated leaves is most readily explained by synthesis of particles in the inoculated leaf, followed by release and transport to other leaves. Movement of plant virus particles through resistant plants, without multiplication, has been reported. For example, potato virus X particles can accumulate in leaves of resistant strains of potato after sections of such plants are grafted to potato plants infected with this virus (17, 18). In the exceptional cases where large amounts of FHV were detected in noninoculated leaves of *N. benthamiana*, it is possible that inoculum RNA, progeny RNA, or progeny virions moved to distant leaves and initiated infection therein. In this respect it should be noted that plaque assay on *Drosophila* lawns is an extraordinarily sensitive method for detecting virions, far more sensitive, for example, than detection by means of radioactivity. Thus experiments to measure the course of infection in uninoculated leaves would be very difficult.

Plant viruses are believed to encode a protein involved in the movement of virions from initially infected cells (19–21). It seems unlikely that FHV, an insect virus, would encode such a protein. *N. benthamiana* supports the systemic movement of an extraordinary number of plant viruses. It is conceivable that *N. benthamiana* cells are structurally more permissive to virion release than the cells of other plants tested or that *N. benthamiana* encodes a protein that facilitates cell movement. Virion release, by whatever method, coupled with passive transport through the vascular system could account for the presence of FHV in uninoculated leaves. Their presence thus nowise contradicts the accepted tenets of plant virus movement.

Our results show that FHV, a virus normally proliferating in invertebrates, can be synthesized in plants, once the viral genome has been introduced. This suggests that the internal conditions of diverse organisms may be sufficiently similar to accommodate the synthesis of simple viruses.

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