Quantity of Virus Required for Aphid Transmission of a Potyvirus

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ABSTRACT

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Aphids (*Myzus persicae*) were allowed a 10-min acquisition access, through a membrane, to solutions that contained helper component and 1 ng/μl of tobacco etch virus or tobacco vein mottling virus in the presence of ^{125}I. Individual aphids were then placed in a gamma counter to determine ^{125}I content and transferred to a tobacco seedling to test for transmission. Volumes of feeding solution acquired by aphids, calculated from their ^{125}I content, typically ranged from approximately 300 pl to approximately 1 pl. Similar volumes were calculated when ^{125}I-labeled virus was used to determine volumes acquired by aphids, indicating that there was not selective uptake of either the isotope or the virus. The number of virus particles in these volumes was calculated to be from approximately 4,000 to approximately 10. From 5 to 17% of the aphids transmitted virus, and there was no correlation between the number of particles acquired and the ability to transmit. The amount of virus required for transmission in these experiments was in the femtogram range and thus well below the limits of detection for currently available systems; this is of significance for attempts to detect viruliferous aphids for epidemiological studies or for predictive schemes.

The question of the minimum number of virus particles required for transmission of nonpersistent viruses by their natural vectors, aphids, is of practical as well as theoretical importance. There has been considerable recent interest in the application of such techniques as enzyme-linked immunosorbent assay (ELISA) for detecting viruliferous aphids trapped in or around crops, so that predictive control strategies can be developed (19). Information on the minimum dose of inoculum required for successful infection would thus be useful in establishing the level of sensitivity required for detection.

An estimate of a minimum infectious dose of 10^2–10^3 particles of the nonpersistently transmitted cucumber mosaic virus (CMV) was made by Walker and Pirone (22). This was based on measurement of the volume of tritiated water contained within the stylet of an aphid and calculation, with data from experiments in which aphids transmitted CMV from solutions of known virus concentration, of the number of CMV particles in this volume. Using a similar approach, the amount of ^{32}P taken up by probing aphids was used to calculate the volume of feeding solution acquired by aphids in a 10-min acquisition access. Data from experiments in which aphids transmitted from solutions that contained known concentrations of potato virus Y were then used to estimate that transmission could occur with the acquisition of a few hundred particles (Pirone, *unpublished*). The evidence used to make the estimations in both of these studies was merely correlative, however, in that different groups of aphids were used to determine the volumes and the ability to transmit.

To approach the question directly, we allowed aphids to acquire, through a membrane, solutions that contained known concentrations of either unlabeled virus in the presence of ^{125}I or of virus labeled with ^{125}I. The amount of virus acquired by individual aphids was determined and each aphid was then tested for its ability to transmit. An abstract of portions of this research has been published (17).

MATERIALS AND METHODS

Virus purification and iodination. The isolates of tobacco vein mottling virus (TVMV) and the HAT isolate of tobacco etch virus (TEV) have been described elsewhere (14,16). The viruses were purified by method 1 of Mohgal and Francki (12) from systemically infected tobacco (*Nicotiana tabacum* L. "Burley 21") that had been mechanically inoculated 2–4 wk previously. Virus concentrations were determined spectrophotometrically (extinction coefficient = 2.4 cm^2 mg^{-1} at 261 nm) (18). Virus was radioiodinated using Iodogen (Pierce Chem. Co., Rockford, IL). A glass scintillation vial was coated with 10 μl of a 100-mg/ml solution of Iodogen in chloroform and dried in a stream of dry nitrogen. One milligram of virus was reacted on ice for 20 min with 1 mCi $Na^{125}I$. All reactions were made at 4 C. Labeled virus was separated from unreacted $Na^{125}I$ by gel filtration on Sephadex G-25 equilibrated with 50 mM sodium borate buffer, pH 8.0. Specific radioactivity was generally about 1,000 cpm/ng virus for TEV preparations, but we were able to achieve this level of activity with only one TVMV preparation. Specific activities were determined by counting serial dilutions of known concentrations of labeled virus in a single-well gamma counter (Mini Instruments Ltd., Essex, England).

Preparation of feeding solutions. For experiments in which ^{125}I was used as a volumetric marker, the 25-μl feeding solution contained TVMV at a concentration of 1 ng/μl or 10 ng/μl, 1.25 mCi ^{125}I (as NaI), and an amount of helper component predetermined to assure efficient transmission (13). The presence of these amounts of $Na^{125}I$ was found not to have an effect on virus transmissibility, in preliminary experiments. The suspending buffer (TSMS) was 100 mM Tris-SO_4, pH 7.2, 20 mM $MgSO_4$, which contained 20% sucrose. The feeding solution for experiments in which labeled virus was used contained 350–700 ng/μl of ^{125}I-labeled virus and helper component in TSMS.

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Quantitation of radioactivity in feeding solutions. Samples (5μl) from each feeding solution were serially diluted, and three 5-μl aliquots of each dilution were absorbed to a glass fiber filter in the bottom of a 500-μl Eppendorff tube. The samples were counted in the gamma counter and the counts were used to calculate the disintegrations per minute per unit volume. This value and the virus concentration in the feeding solution were used to calculate the volume and amount of virus imbibed by each aphid, based on the uptake of ^{125}I.

Aphid handling and testing. Aphids (*Myzus persicae* Sulz.) were reared as previously described (9). After several hours of preacquisition fasting, 5–10 aphids were placed in feeding chambers which differed from those previously described (9) only in that the feeding solution above the Parafilm membrane was covered with stretched Parafilm, rather than with a coverslip, to prevent evaporation. The feeding chambers were placed under a dissecting microscope, and aphids that appeared to be probing the membrane at the end of the 10-min acquisition access period were selected for further processing.

Individual aphids were placed in 500-μl Eppendorf tubes which were contained in an ice bath. Chilling the aphids reduced their mobility considerably and minimized postacquisition probing of the surface of the tube, which might have led to egestion of virus. A 3-mm glass ball was placed in the tube to prevent the aphid from walking up the sides of the tube during counting and thus affecting the counting geometry. The tube was then placed in the single-well gamma counter and counted for 50 sec, after which the aphid was removed and placed on a test plant for several hours or overnight. After the aphid was removed, the test plant was retained for 7–10 days to determine whether it became infected. Each plant that became infected could thus be correlated with the amount of radioactivity contained in the aphid that inoculated that plant.

RESULTS

Determination of quantity of virus acquired. In these experiments, the ^{125}I content of each aphid was used to calculate the volume of feeding solution acquired by that aphid. The amount of virus taken up by the aphid was then calculated on the basis of this volume. The results of these experiments are summarized in Table 1.

When the concentration of virus in the feeding solution was 1 ng/μl, 5–17% of the aphids transmitted (Table 1, top). The number of particles acquired ranged from fifteen to several thousand, with about 80% of the transmission occurring with aphids that acquired fewer than 500 particles, as shown in the expanded presentation of data for transmitters in Table 2. The range of particle numbers acquired by aphids that did not transmit was similar to that acquired by the transmitters (Table 1, top).

When the concentration of virus in the feeding solution was increased to 10 ng/μl, the range of volumes acquired was similar to that acquired from 1 ng/μl solutions. As would be expected, the number of particles in these volumes was calculated to be about 10 times that in the 1 ng/μl solution. The frequency of transmission increased to about 40% (Table 1, bottom).

Correlation of volume acquired with virus particle content. Determination of the number of virus particles acquired on the basis of the volume of solution acquired assumes that there is not selective uptake of either the isotope used to determine the volume or of the virus. To determine whether the volumes acquired were representative of the number of particles acquired, we conducted experiments with ^{125}I-labeled virus. The amounts of virus in the feeding solutions for these experiments had to be several orders of magnitude higher than those used to determine minimum particle numbers, to be able to detect radioactivity with the gamma counter. Furthermore, radioiodination of the virus resulted in reduced virus infectivity. Thus, these experiments were

TABLE 2. Frequency distribution of volumes and corresponding numbers of virus particles acquired by transmitting aphids from solutions containing 1 ng/μl of virus in the presence of ^{125}I

Range of volumes (pl)	Range of particles[a] (no.)	Number of transmitters (total:39)[b]	Total transmitters (%)
<5	15–46	8	21
5–<10	84–116	8	21
10–<20	163–219	9	23
20–<40	252–456	6	15
40–<80	...	0	0
80–<160	966–1,505	4	10
160–<320	2,000–3,202	3	8
320–640	4,380	1	3

[a]Number of particles calculated to be in the actual, experimentally determined volumes within the range indicated in column 1.
[b]Expanded presentation of the data for the 39 transmitters in Table 1, top.

TABLE 1. Determination of numbers of potyvirus particles acquired by aphids using ^{125}I as a volume marker

			Range of acquired[a]		
			Disintegrations per minute	Volumes (pl)	Particles
1 ng/μl					
Experiment	Virus	Transmitters			
1	TEV	5	3,138–63,977	18–362	216–4,380
2	TVMV	5	2,560–37,943	17–265	204–3,180
3	TVMV	12	921–7,118	2.8–23	34–252
4	TVMV	17	213–46,771	1.2–267	15–3,202
		Nontransmitters			
1	TEV	95	491–147,180	2.8–833	33–9,996
2	TVMV	95	118–22,046	0.8–154	10–1,848
3	TVMV	88	145–39,311	0.4–112	5–1,344
4	TVMV	83	186–53,092	1.1–309	13–3,708
10 ng/μl					
Experiment	Virus	Transmitters			
3	TVMV	10	1,100–12,100	3–330	360–3,960
4	TVMV	7	568–30,341	28–1,730	336–20,760
		Nontransmitters			
3	TVMV	10	255–4,076	0.7–11	84–1,320
4	TVMV	13	275–24,278	1.5–139	180–16,680

[a]Individual aphids were counted in a gamma counter to determine ^{125}I content. The efficiency of the counter was determined, experimentally, to be 60%. The volume of feeding solution acquired was calculated from the ^{125}I content, and the number of particles in this volume was then calculated. Only aphids containing counts exceeding background are included, because 210 aphids that did not exceed background levels did not transmit virus in these and in several preliminary experiments.

useful only in establishing the virus particle-volume ratio. The results, summarized in Table 3, show that the volumes calculated from the amount of ^{125}I-labeled virus acquired by the aphids coincided with the range of the volumes calculated on the basis of $Na^{125}I$ in solution (Table 2).

Thus, when $Na^{125}I$ was in the feeding solution, the acquired volumes ranged from <5 to 640 pl, whereas with ^{125}I-labeled virus, volumes acquired ranged from <20 to 600 pl. Despite the high concentrations of virus fed to the aphids, only about 20% of the transmitters contained counts that exceeded the background level of 35–60 counts per minute (cpm) ($\bar{x}$ = 46 cpm). The other 80% had thus acquired < 20 pl of feeding solution, because a volume of less than 20 pl would not contain counts that exceeded background.

DISCUSSION

It is obvious from the data that there can be a several hundred-fold difference in the amount of solution acquired by individual aphids. For this reason, we have not attempted to subject the data to statistical analysis, but rather to present the data in such a form that the variability will be evident. Furthermore, the fact that aphids rapidly lose the ability to transmit nonpersistent viruses precluded the possibility of the repeated counting of each aphid, which would be needed to more accurately determine the volume contained in each. Repeated counting, in experiments not monitored for transmission, showed that there could be up to a two-fold difference in the number of counts from a particular aphid if the number of counts was near the background level, although the variability was about 10% with aphids that contained higher levels of radioactivity (data not shown). Given even a two-fold level of imprecision, the data show that the process of transmission of potyviruses by aphids requires relatively few virus particles.

From the data in Table 2, it may appear that aphids that acquired more than 500 particles were less likely to transmit, but this distribution occurs because few aphids acquired volumes corresponding to more than 40 pl under our experimental conditions. The combined data (not shown) for both transmitters and nontransmitters tabulated in Table 1, top, revealed that 82% of all aphids acquired less than 40 pl, corresponding to fewer than 500 particles. The fact that the probability of transmission does not depend on the number of particles acquired is compatible with the often-described fact that aphids that make brief acquisition probes into potyvirus-infected plants are as likely, or even more likely, to transmit virus than aphids that make longer probes or those that feed during acquistion access (15). It is also compatible with the hypothesis that only those virus particles that are retained, in the presence of helper component, within the maxillary stylets or in the foregut are involved in transmission (1).

Mechanical (manual) inoculation of a local lesion host with tobacco mosaic virus (TMV) using standard inoculation techniques resulted in the occurrence of one detectable infection for every 5×10^4 to 1×10^5 particles in the inoculum (20,22). Similar experiments with TEV resulted in one detectable infection for approximately every 10^7 particles in the inoculum (23). Microinjection of picoliter volumes of a TMV suspension resulted in the infection of single cultured tobacco cells with doses of 72–620 particles per cell (10). The latter values are similar to those obtained in the present study with aphid-inoculated (presumably cell-injected) potyviruses. Comparison of leaf rubbing and cell injection methods of inoculation could thus lead to the conclusion that the latter is a far more efficient method of inoculation. Although this is doubtless true for these comparisons, when microliter suspensions of virus are applied mechanically, plants can be infected with doses of tens to hundreds of particles of TMV (23) or even fewer particles of turnip mosaic virus (7).

The numbers of potyvirus particles acquired by aphids that were subsequently able to transmit, in our study, correspond to amounts of virus in the femtogram (10^{-15} g) range. The lower limits of virus detection in aphids with ELISA tests are generally in the nanogram range (4,6,21), although for the nonpersistent cucumber mosaic virus, detection at the picogram level has been reported (8). We have been able to detect picogram quantities of potyviruses using a dot blot immunobinding assay (2). Even these picogram levels of detection are several orders of magnitude above those that would be required to detect aphids carrying transmissible quantities of potyviruses. Hence, the reliability of immunological systems for epidemiological studies or for predictive purposes is likely to considerably underestimate, or even possibly to fail to detect, virus-carrying aphids. This situation is the reverse of that for vectors of propagative viruses, in which ELISA tests have been reported to overestimate the number of potential vectors (3,5,11).

TABLE 3. Volume of feeding solution acquired by aphids calculated on the basis of labeled virus acquired[a]

Concentration of virus in feeding solution	350–700 ng/μl
No. of aphids counted	290
Range of volumes acquired (all aphids)	<20–600 pl
No. of aphids transmitting virus	53
Range of volumes acquired by transmitters	<20–300 pl
No. of transmitters acquiring <20 pl	42

[a]Totals of four experiments using ^{125}I-labeled TEV or TVMV.

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