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Authors: Irene Ontiveros[1,2], Juan Antonio Diaz-Pendón[2] and Juan José López-Moya[1]

[1]Centre for Research in Agricultural Genomics (CRAG, CSIC-IRTA-UAB-UB), Consejo Superior de Investigaciones Científicas (CSIC), Cerdanyola del Vallès, Barcelona, Spain.

[2]Institute for Mediterranean and Subtropical Horticulture "La Mayora" (IHSM-UMA-CSIC), Algarrobo-Costa, Málaga, Spain.

Corresponding Author: Juan José López-Moya

Address: Centre for Research in Agricultural Genomics (CRAG, CSIC-IRTA-UAB-UB), Consejo Superior de Investigaciones Científicas (CSIC), Edifici CRAG, Vall Moronta s/n, 08193-Cerdanyola del Vallès, Barcelona, Spain.

e-mail: juanjose.lopez@cragenomica.es

Chapter

Experimental transmission of plant viruses by aphids or whiteflies

Irene Ontiveros, Juan Antonio Diaz-Pendón and Juan José López-Moya

Abstract

Numerous species of plant viruses are naturally transmitted by insect vectors, mainly homopterans like aphids and whiteflies. Depending on the vector specificity and the mode of transmission, different durations of the periods for acquisition, retention, and inoculation are required for a successful transmission. Therefore, the experimental setup to perform controlled transmission experiments under laboratory conditions involves handling the vector organisms and managing the times for the different steps of the process to optimize and standardize the results. This chapter describes some basic procedures that can be applied to vector-mediated transmission experiments with selected viruses using aphids or whiteflies and different host plants.

Key words Non-persistent, semipersistent, circulative transmission, non-viruliferous, viruliferous vectors, Acquisition, Retention, Inoculation periods

1 Introduction

Plant viruses often rely on vectors as a means of transmission to survive in nature. By using vectors, they can reach new susceptible hosts where they can continue acting as obligate intracellular parasites, making the transmission process one of the most critical steps in the viral cycle [1]. Among vectors, phytophagous arthropods play an important role in the dissemination of most plant viruses, with insect vectors being the most common [2]. Many plant viruses are spread from plant to plant by species belonging taxonomically to the *Homoptera*, specifically the *Aphididae* (aphids) and *Aleyrodidae* (whiteflies) families [3-6]. Examples of viruses transmitted by aphids are potyviruses, cucumoviruses, caulimoviruses, luteoviruses, poleroviruses, alfamoviruses, closteroviruses, and nanoviruses, among others. In the case of whiteflies, examples are begomoviruses, criniviruses, ipomoviruses, carlaviruses, torradoviruses, and others. These two insects possess pierce-sucking mouthparts equipped with stylets that allow them to feed on plant tissues without causing significant damage, a characteristic that facilitates the establishment of new virus infections. The ability of these vectors to form large colonies and efficiently disperse themselves establishes them collectively as the most significant groups of vectors for plant viruses.

In addition to being an essential step in the cycle of numerous viruses, which certainly merits attention from the scientific community [7], there are other compelling reasons to establish protocols for conducting controlled vector transmission experiments. Currently, the rate of discovering new viruses [8] is steadily increasing, thanks to the application of powerful genomic tools that often yield complete genome sequence data from environmental samples. However, it is noteworthy that these sequences frequently lack relevant biological information [9]. For numerous newly discovered viruses, there is a lack of knowledge regarding their hosts or vector organisms. Consequently, identifying the vector of a new virus holds important implications for its precise taxonomical classification, as well as for effective

62 management if it will become a potential causal agent of new plant diseases threatening
63 crop production.

64 There are excellent recent reviews that can be consulted for details about the relationship
65 between plant viruses and their insect vectors [10-13]. Also, in the case of whiteflies, basic
66 management procedures for transmission have been described recently [14].
67 Mechanistically, the successful transmission of viruses by insect vectors requires several
68 steps in the case of insect vectors: first, the acquisition of virions from an infected plant;
69 second, the acquired virions might need to be retained in the vector, either at specific sites
70 through binding to receptor-like elements in the digestive tract, or circulating from different
71 anatomical structures, mainly from the gut to the salivary glands; and finally, delivery of
72 virions is required, in many cases following salivation, allowing the virions to be deposited in
73 a susceptible host plant to start the infection. The duration of these steps (acquisition,
74 retention and inoculation) can serve to classify the modes of transmission according to the
75 length of the period when the vector keeps the virus infectivity. In non-persistent
76 transmission, the insect can transmit the virus almost immediately after acquiring it from an
77 infected source plant, and the capacity to transmit the virus is lost rapidly, often within a few
78 minutes. In contrast, persistent transmission requires longer periods of acquisition, often
79 followed by a period of retention before the vector finally becomes viruliferous and can
80 transmit the virus. Semipersistent transmission is an additional category reserved for viruses
81 with intermediate requirements for both acquisition and retention periods. A further
82 refinement of the classification also considers the route followed by the virus within the
83 insect vector, being either non-circulative or circulative transmission. In the non-circulative
84 case, there is only a temporary and reversible association of the virus with the anterior tract
85 of the digestive system (mouthparts or foregut). This type of transmission corresponds
86 mainly with non-persistent and semipersistent transmission, in which the virus is not retained
87 for long, and it can be immediately released and inoculated, completing the process only

during short periods of time. Both acquisition and inoculation are thought to occur during short feeding probes. Since the non-persistent transmission occurs within such a narrow timeframe, it is indeed difficult to control the spread of these viruses using insecticide treatments targeted to kill the vectors. On the other hand, circulative transmission requires the passage of virions through the insect's body. They first pass through the gut barriers to reach the haemolymph, and subsequently enter the salivary glands to be inoculated during salivation. In this case, the complete process might take days and often requires a period of latency since immediately after acquisition, the virus cannot be inoculated until the completion of its circulation within the vector. Additional categories can be established in this case when the virus replication only occurs in the plant hosts as non-propagative, being propagative if the virus is able to replicate as well in the vector. Interestingly, propagative viruses are parasites of both plants and insects, alternating between the two types of hosts to complete their cycles of dispersion, which could occasionally involve different development stages of the insect and even passage to the progenies.

In this chapter, our focus is on general procedures for testing the transmission of plant viruses using the two more common vector organisms: aphids and whiteflies. Alongside direct plant-to-plant transmission setups, we describe procedures based on artificial membrane feeding [15-16], which played a crucial role in identifying auxiliary factor(s) involved in the transmission of certain plant viruses [17-19].

2 Materials

2.1 Biological materials: viruses, host plants and insect colonies

Before starting the experimentation on vector transmission processes, it is essential to establish suitable conditions for obtaining viruses (*see* **Note 1**), host plants (*see* **Note 2**),

and insect colonies (*see* **Note 3**) following fundamental procedures in virology, plant science, and entomology (*see* **Note 4**).

2.2 Materials for containment and handling of vectors.

In addition to the biological materials, various devices are necessary to confine vectors while feeding during the acquisition and inoculation processes. These devices include:

1. Insect-proof cages (sized appropriately to accommodate plants during the acquisition and inoculation periods).
2. Containers and vials for capturing and immobilizing insects during fastening.
3. Paintbrush or aspiration device (*see* **Note 5**).
4. Clip cages (*see* **Note 6**).
5. Membrane feeding chambers prepared using stretched parafilm membranes and different feeding solutions (*see* **Note 7**)

Some examples are shown in Figure 1.

2.3 Systems for virus detection

To determine the presence of the virus in individual plants following transmission, an appropriate diagnostic protocol might be needed in advance. The procedure for virus detection can encompass various methods, ranging from simple observations of symptoms to more advanced molecular techniques such as serological methods (ELISA or western blot), hybridization using specific probes (dot-blot or tissue print), or methods based on PCR amplification (for DNA viruses) or RT-PCR (for RNA viruses).

3 Methods

All procedures will be conducted using plants and insects relocated to the designated area or room where the experiments will take place. In general, temperature and illumination should be maintained within ranges suitable for plant growth and insect rearing unless they need to be intentionally manipulated as variables within the experimental design.

The two primary methods for conducting transmission experiments are plant-to-plant transmission and artificial feeding. These approaches are described in the following subsections.

3.1. Plant-to-plant transmission by freely moving insect vectors.

This simplified method involves confining source and destination test plants together with potential vectors in a cage. The method can be used in studies to determine which insect species are capable of transmitting a specific virus, and it can be complemented by additional procedures to confirm unequivocally the identity of the vector and gather further information about the duration of the various stages of the transmission process.

1. Enclose infected source plants with test plants within a cage and introduce insect vectors at the desired density. The distance between the source and destination plants can be regarded as a parameter for the assay (*see* **Note 8**).
2. Allow unrestricted movement of vectors between plants, promoting mobility if required, and wait for the completion of the different periods for acquisition/inoculation.
3. Optionally, eliminate vectors through insecticide treatment (*see* **Note 9**).
4. Maintain the plants under suitable growing conditions, allowing sufficient time for virus infections to manifest.

5. Quantify the transmission (rate number of infected plants/total number of test plants) by directly observing symptoms or employing a detection system (*see* **Note 10**).

3.2. Plant-to-plant transmission under restricted mobility conditions of the insect vectors

1. Collect insects (*see* **Note 11**) in adequate containers and fasten them during a period determined experimentally (*see* **Note 12**).
2. Allow insects to access the infected plant (either complete or detached leaves) for the desired acquisition period (*see* **Note 13**). Alternatively, insects could be reared directly on virus-infected plants, therefore being already viruliferous at the time of collection, and consequently, this second step could be skipped.
3. Transfer insects to the test plants (*see* **Note 14**) and allow them to feed during the required inoculation period. For small plants, they can be individually covered with a containment cage such as an inverted beaker, while for larger plants, the restriction of mobility can be achieved using clip cages or by bagging the leaf or branch where the inoculation feeding will take place.
4. Uncover plants (or remove clip cages or bags) and eliminate vectors with insecticide treatment (optional).
5. Maintain the plants under suitable growing conditions, allowing sufficient time for virus infections to manifest.
6. Quantify the transmission (rate number of infected plants/total number of test plants) by directly observing symptoms or employing a detection system.

3.3. Transmission after virus acquisition through artificial feeding

1. Collect non-viruliferous insects in adequate containers and fasten them during a period determined experimentally.
2. Prepare artificial membranes with stretched parafilm (*see* **Note 15**) and the solutions to be tested (*see* **Note 16**).
3. Transfer the insects to the feeding chambers and allow them to feed through the parafilm on the solution containing the tested components. This can be done either in a single feeding, using individual components or mixes of different elements to be tested, or in sequential steps to provide each component in the desired order (see section 3.4). The insects should have access to the virus preparation during the acquisition period as determined for the assay.
4. Transfer insects to the test plants and allow them to feed during the required inoculation period.
5. Uncover plants (or remove clip cages or bags, in case these were used) and eliminate vectors with insecticide treatment (optional)
6. Maintain the plants under suitable growing conditions, allowing sufficient time for virus infections to manifest.
7. Quantify the transmission (rate number of infected plants/total number of test plants) by directly observing symptoms or employing a detection system.

3.4. Sequential feeding experiments

The processes of acquisition and inoculation can be divided into distinct sub-periods to carry out sequential feeding on various plant tissues and/or artificial membranes. This approach allows for refinement in the design of targeted experiments (*see* **Note 17**).

4 Notes

1. Before most types of transmission experiments, the virus infection might need to be initiated in hosts that will be used as a transmission source. This usually involves mechanical inoculation for certain viruses, rubbing manually an extract on leaves dusted with an abrasive (such as carborundum). Buffers to prepare crude extracts should be consulted in the relevant literature for each specific virus, but also examples for many viruses can be found in the dedicated database of "Description of plant viruses" available online at https://www.dpvweb.net/ [20]. For other viruses, live inocula might be needed and preserved through vegetative propagation or even through vector transmission. Finally, purified virions could be used in artificial membrane feeding for virus acquisition, either alone or assisted by auxiliary factors.
2. Virus-free seed stocks or other uninfected sources of propagules are a good system to initiate the growth of experimental plants. To exclude undesired visits of potential vectors, confinement in growth chambers or greenhouses will be desirable, or alternatively, the use of insect-proof cages (see Fig. 1) might be required during the whole cultivation cycle. Updated and valuable resources about handling plants for experiments with pathogens and pests can be found in the relevant literature and dedicated collections and databases, for instance at the European and Mediterranean Plant Pathology Organization (EPPO) website https://gd.eppo.int/ , (Guidelines for the production of biological reference material. EPPO Bull, 51: 499-506. https://doi.org/10.1111/epp.12781).
3. Laboratory-established colonies (such as clonal parthenogenetic aphids and progenies populations of individually selected male and female whiteflies) are generally better than heterogeneous colonies or wild-captured individuals, which can carry other pathogens and might require to perform quarantine/acclimation periods before their use as vectors. Depending on the virus under study and the host range,

231 a cautionary principle to avoid contaminations will be to rear insects in a non-host
232 plant for the virus, although such a possibility might not always be available. As a
233 general principle, different rooms or chamber facilities would serve to keep the
234 insects separated from the experimental plants until the setting up of the
235 transmission experiments. As in the case of biological materials, information on
236 insect vectors can be found at EPPO, for instance, about whiteflies (*Bemisia tabaci*.
237 EPPO Bulletin, 34: 281-288. https://doi.org/10.1111/j.1365-2338.2004.00729.x), and
238 at a dedicated website of the French Institute of Agricultural Research INRAE
239 (https://www6.inrae.fr/encyclopedie-pucerons_eng/).
240 4. Proper management of plant host and insect vectors is essential for successful
241 transmission experiments. Both plants and insects are living organisms, and their
242 susceptibility and performance can be influenced by environmental conditions and
243 even circadian rhythms. Also, the influence of insect behavior needs to be
244 considered [21]. As a rule, repetitions of the experiments must be performed at
245 similar times of the day and in a comparable environment. If possible, controlled light
246 and temperature conditions could be preferred to standardize the repetitions. In
247 practical terms, a record of the conditions during each repetition should be kept for
248 future reference and statistical treatment of the data.
249 5. To preserve their behavioral capacities, insect individuals must be handled carefully,
250 keeping tools clean and minimizing the force required to remove them from the
251 rearing plant. This involves gently collecting them using a fine paintbrush or
252 aspiration devices. An example of a home-made design for a collection device is
253 shown in Fig. 1c, which uses a commercial sample tube (30 ml, cylindrical with
254 conical bottom and screw cap) prepared using two silicon flexible thin hoses that
255 pass through perforated holes in the cap of a tube, and, therefore, can be screwed
256 into different collecting tubes. One of the hoses is open and can be attached to a

pipette tip to concentrate the aspiration, while the second hose should be connected to the vacuum source outside and covered with a fine mesh inside the device to avoid losing insects when the vacuum is applied. Holes for passing the two hoses through the container cap must be sealed to avoid leaks and consequently create a negative pressure in the tube for the suction of the insects.

6. Clip cages should be constructed using an openable hatch that could be clamped onto a leaf while maintaining moderate pressure to prevent insects from escaping, for example, using soft ring pads to avoid pressure damage on the leaf surface. To facilitate the transfer of insects, tubes of the same model as the ones used for the collection of vectors can be transformed. The construction involves first cutting the base of the tube to allow gluing and securing the hatch clamp, leaving the original screw-on cap on the other side of the cage to confine the insects, allowing ventilation through a mesh-covered opening. Additionally, this design can serve to transfer insects from the collection tube using a connector formed by two opposite caps, glued and perforated, to communicate two compatible tubes (Fig. 1f).
7. Under certain circumstances, purified virus particles and auxiliary factors can be supplied to the vectors using artificial feeding (see Fig. 3). For pierce-sucking insects, membranes can be prepared using stretched parafilm membranes placed above a darkened cylinder where the insects have been previously placed. The solution for feeding consists of an artificial diet placed either in a sandwich of two membranes forming a sachet (Fig. 3a to 3f), or above a single membrane with a spacer and a cover.
8. For the most straightforward transmission experiment confining together infected and test plants with the vector, negative control of a similar cage without including insects must be considered to rule out the possibility of other vector organisms acting regardless of the presence of the presumed vector. This is particularly important

when testing viruses collected directly from field samples that might carry unidentified pests. Quarantine periods followed by detailed inspection and a few passages (if possible) under controlled laboratory conditions should be considered. While caging together source and test plants, it is advisable to avoid direct contact to preclude other types of contact-driven transmission. The distances between plants must be adjusted previously to account for the flying capacity/other mobility conditions of the insects.

9. Insecticide treatment(s) are performed once the period of inoculation is finalized, directly spraying on the plants (Fig 2q) with a commercial product previously tested as effective on the same colony of insects. The treatment must be repeated, if necessary, especially in the case of whiteflies, to avoid the future risk of a new generation of vectors coming from eggs laid by females during the inoculation period. When the inoculation has been restricted to confined areas of leaves, one possibility to avoid infestation by next-generation whiteflies is to remove the leaves used for inoculation once the virus has been able to move to other parts of the plant through cell-to-cell and systemic movement.
10. Sampling at different intervals might be required to properly detect the presence of the virus after transmission. In case a mechanical inoculated control was included during the experiment (Fig. 2l to 2n), a good indication of the timing of appraisal for transmission rates can be deduced
11. The stylets of sucking insects, when inserted into plant tissues, can be fragile and easily torn inadvertently during collection. In the case of actively feeding aphids, gently touching their abdomen with the paintbrush (Fig, 2a) activates a reflex response that rapidly retracts the stylet. This reflex can be observed through changes in the relative position of the antennae, which move from being parallel to the body to going forward and touching the surroundings in preparation for walking away. Once

the antennae move and the aphid begins to ambulate, it becomes a safe moment to collect them without causing anatomical damage. Similarly, a convenient way to collect whiteflies is by attracting them to light sources, which can be achieved by shading a portion of the rearing boxes and leaving only the front part illuminated, or using yellow cards near the rearing plant. Both systems can stimulate their flight and facilitate their aspiration away from feeding areas on the plant. Once collected, whiteflies can be transferred between the different devices, connecting containers, and even the feeding clip cages or artificial membranes using gentle shaking, tapping, and swift movements of the sample tubes by a trained experimenter. Alternatively, connecting the different components prepared with compatible tube caps can be adopted using glued double connectors made with two perforated caps. Small clip cages with different designs that can be secured to leaves are good systems to allow the feeding of insects only in the selected part of the plant where they are confined. In the cases of whiteflies feeding for sufficiently long periods, eggs can be laid by females on the leaves, and labeling of the temporarily infested leaves might be required to facilitate their later removal before the hatching without affecting the results.

12. Keeping the insects isolated from plants for a fasting period (around one hour is often sufficient) might increase their focus on feeding when released, and might have other positive effects like clearance of blocking stuff from hypothetical virus-specific receptors to favor acquisition. The containers used for fasting must be dry and with an adequate volume for the number of individuals or alternatively sufficiently ventilated to avoid suffocation in case of too crowded groups; for instance, glass vials of about 2-4 ml with tight caps can be used for groups of up to 50-100 aphids, which can be collected individually using a fine paintbrush (Fig. 2b-2c). In the case of whiteflies, since they are attracted to light sources, covering the cage with an opaque

fabric material would help move them to a section of the cage and facilitate their collection using a device with gentle aspiration. Once collected inside the tubes, the insects can be moved to the bottom by gently tapping the vials on the bench or table surface. For whiteflies, placing the tubes in cold conditions (such as on ice) for a few seconds before opening them helps to avoid the risk of escape.

13. Rearing viruliferous vectors (on virus-infected plants) could simplify the experimental procedures to proceed directly to the inoculation step. In other cases, the acquisition requires placing the insect on the virus source. This can be done by providing access of insects to the infected plant tissue: in the case of wingless aphids, this can be done on detached leaves on a petri dish over a wet filter paper. The petri dish can be placed inside a larger plate or tray surrounded with soapy water to better confine them and avoid escapes (see Fig 2h). Aphids can also be placed directly on whole infected plants and recovered manually later. Since whiteflies fly actively, the acquisition can be forced on small, clipped cages on infected leaves or released inside cages containing the acquisition source. Another system adequate for whiteflies could be to connect the clip-cage used for acquisition with a second clip-cage for inoculation, creating a tunneled bridge that the whiteflies can use to move from infected to test plants and trimming the leaf section can make it easier to dry out and favor the migration of insects. In either case, it is important to allow individuals to feed on the infected plants for the duration of the acquisition access period. In the case of non-persistent viruses and aphid vectors, a stopwatch can be used to measure the time (Fig 1j). Direct observation with a magnifying glass or a binocular microscope could reveal their behavior during feeding (see comments on note 10). To further investigate feeding behavior, entomological equipment such as Y-tube olfactometers and colored sticky cards can be used to monitor vectors [22]. Additionally, the implementation of techniques such as wiring individual insects and

recording their feeding patterns using an electrical penetration graph (EPG) system could provide valuable insights [21].

14. Control of temperature can be used to reduce the mobility of insects for a while, but with certain time limits to avoid undesired mortality. The collection tubes containing insects can be placed momentarily on an ice bucket or left in a cold room for a few minutes, right before transferring them to other tubes, clip cages or the membrane feeding chambers.
15. Artificial diets containing sugar are more attractive to insects. The percentage of sucrose (usually from 10 to 20%) can be experimentally optimized to maximize transmission rates in preliminary experiments. The diet can be contained in between two stretched layers of parafilm forming a sachet and placed at one end of a cylindrical chamber made with a short tube section (Fig. 3a to 3f), or for short feeding periods, the solution on the parafilm membrane can be covered with a glass coverslip, using a plastic flat washer as a spacer. In the case of collected whiteflies, they can be released through the other end of the tube, which would be covered by a ventilated lid (such as a perforated cap covered with a fine mesh). In all cases, zenithal illumination helps the movement of the insects toward the membrane.
16. The purification of virions and auxiliary factors allows combining them into artificial diets. The concentrations of the different components would need to be established experimentally, with expected linearity for a range of the concentrations chosen. Under certain conditions, the transmission percentages of serial dilutions can be used to titer the activity of a component better than using a concentration measurement.
17. Sequential feeding serves to identify the requirements of auxiliary factors for transmission [18]. The versatile system could even combine steps for the acquisition

of components transiently expressed in plant tissues and later supplemented with membrane feeding on purified virions [23].

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Figure Captions

Fig. 1. Devices used for handling insect vectors in virus transmission experiments. The image shows a commercial portable insect-proof cage (BugDorm, measuring 47.5 x 47.5 x 47.5 cm) with aphid-infested tobacco plants inside (a). The cages are stackable and can be placed in growth chambers (b) for rearing different colonies of insects or conducting experiments that require vector confinement. To create small membrane feeding chambers, 30 ml tubes (polystyrene containers 25 x 90 mm with polypropylene caps) can be employed (c, left) by cutting the bottom and applying black adhesive tape for darkening (c, right). A closer look from above shows a cylinder (d) ready to be covered with a stretched parafilm membrane. The same tubes can be used to prepare various components for handling whiteflies. These include an aspiration device (e, left), clip cages that can be attached to tubes (e, center), or other clip cages (e, right, and disassembled in f) using two perforated caps glued together in opposing orientations to connect them.

Fig. 2. Procedure for conducting plant-to-plant aphid transmission experiments. Apterous adult aphids reared on infested plants (a), are carefully collected using a

paintbrush (b) and placed in glass vials for a fasting period (c). The experimental arena for acquisition consists of a plate covered with wet filter paper and positioned inside a larger plate filled with soapy water (c). Small susceptible plants (d) are prepared in a tray to receive the virus, acquired from a leaf collected from an infected source plant (e). The aphids after fasting are grouped by tapping the vials, deposited on the detached infected leaf, and evenly distributed (f), with the acquisition time measured using a stopwatch (h). To prevent unwanted aphid visits, control plants (negative and positive: mock and mechanically inoculated, respectively) are covered with inverted sample tubes (h). Once the acquisition period is completed, the aphids are individually transferred to each test plant until the desired number of vectors per plant is reached and then covered (i). This process is repeated for all the test plants (j). After distributing the aphids, the controls are uncovered, dusted with carborundum (l), and either mock-inoculated or manually inoculated with a fresh extract of infected tissue (m-n), to be later rinsed and covered (o). Upon completion of the inoculation period, the plants are uncovered (p) and sprayed with insecticide (q) to eliminate the vectors. The percentage of transmission is calculated based on the number of infected plants, which are diagnosed once the positive control exhibits symptoms or tests positive.

Fig. 3. Procedure for conducting membrane feeding transmission experiments. Apterous adult aphids are collected and placed for a fasting period, as shown in Fig. 2. In this case, chambers with artificial membranes are prepared using cylindrical section of rigid plastic tubes (polystyrene, diameter 2 cm, height 2.5 cm), and the exterior is darkened using black tape. Parafilm is then stretched in two dimensions and tightly wrapped around the top of the cylinder to form a membrane (a-c). A 15% sucrose solution is carefully placed on top of the membrane (d) and covered with an additional layer of stretched parafilm to create a

sachet (e, f). The fasting aphids are introduced into the cylinder (g) and allowed to crawl until they reach the membrane attracted by the light. To cover the bottom of the chamber, a dark slide is used (h). The aphids can pierce the membrane with their stylets to access the sucrose solution, and the acquisition time is measured using a stopwatch (i). Once the acquisition period is completed, the cylinder is lifted, and the aphids are individually collected using a paintbrush (j). For the inoculation, the aphids are individually transferred to the test plants, as shown in Fig. 2.

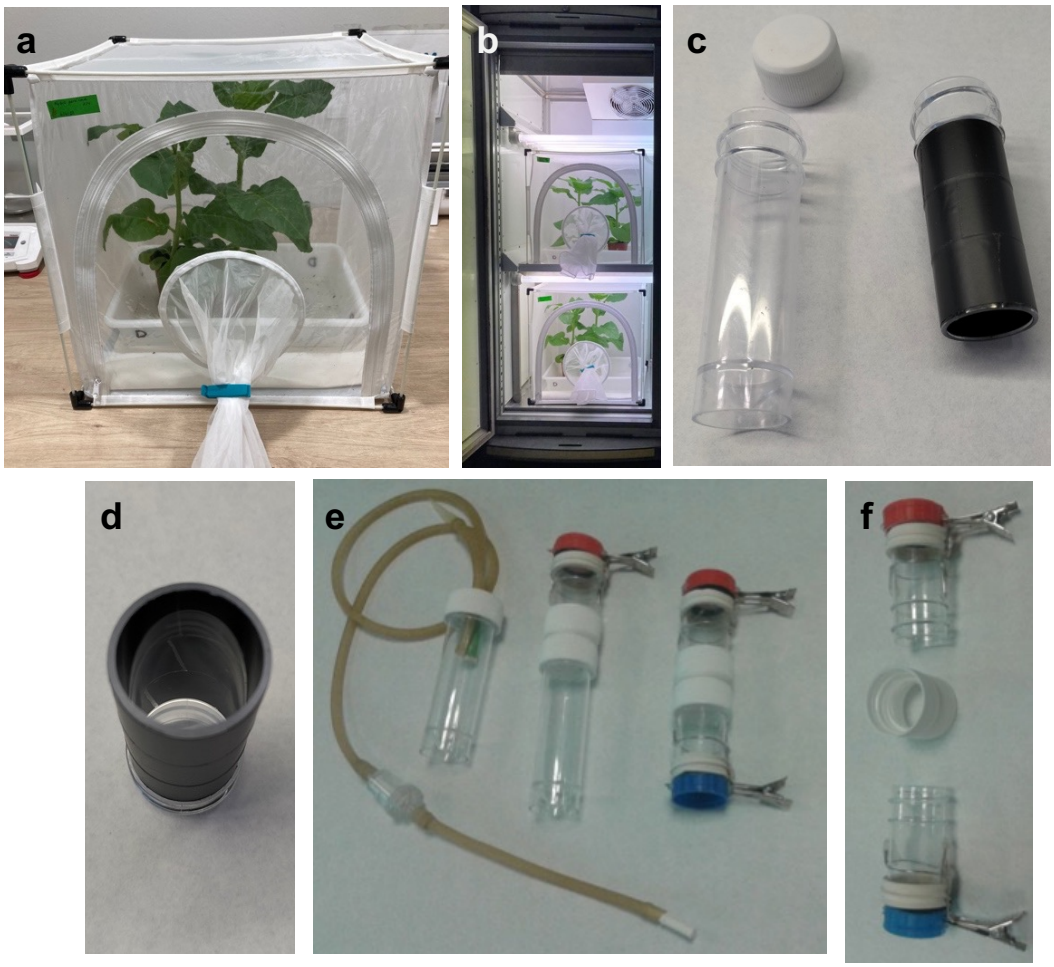

Fig. 1. Devices used for handling insect vectors in virus transmission experiments. The image shows a commercial portable insect-proof cage (bugdorm, measuring 35x35x35 cm) with aphid-infested tobacco plants inside (a). The cages are stackable and can be p`laced in growth chambers (b) for rearing different colonies of insects or conducting experiments that require vector confinement. To create small membrane feeding chambers, 20 ml tubes can be employed (c, left), by cutting the bottom and applying black adhesive tape for darkening (c, right), A closer look from above shows the cylinder (d), ready to be covered with a strectched parafilm membrane. The same tubes can be used to prepare various componets for handling whiteflies. These include an aspiration device (e, left), clip cages that can be attached to tubes (e, center) or to other clip cages (e, right, and disassembled in f) using two perforated lids glued together in opposing orientation to connect them.

Fig. 2. Procedure for conducting plant-to-plant aphid transmission experiments. Apterous adult aphids reared on infested plants (a), are carefully collected using a paintbrush (b) and placed in glass vials for a fasting period (c). The experimental arena for acquisition consists of a plate covered with wet filter paper and positioned inside a larger plate filled with soapy water (c). Small susceptible plants (d) are prepared in a tray to receive the virus, acquired from a leaf collected from an infected source plant (e). The aphids after fasting are grouped by tapping the vials, deposited on the detached infected leaf, and evenly distributed (f), with the acquisition time measured using a stopwatch (h). To prevent unwanted aphid visits, control plants (negative and positive: mock and mechanically inoculated, respectively) are covered with inverted sample tubes (h). Once the acquisition period is completed, the aphids are individually transferred to each test plant until the desired number of vectors per plant is reached, and then covered (i). This process is repeated for all the test plants (j). After distributing the aphids, the controls are uncovered, dusted with carborundum (l), and either mock-inoculated or manually inoculated with a fresh extract of infected tissue (m-n), to be later rinsed and covered (o). Upon completion of the inoculation period, the plants are uncovered (p) and sprayed with insecticide (q) to eliminate the vectors. The percentage of transmission is calculated based on the number of infected plants, which are diagnosed once the positive control exhibits symptoms or tests positive.

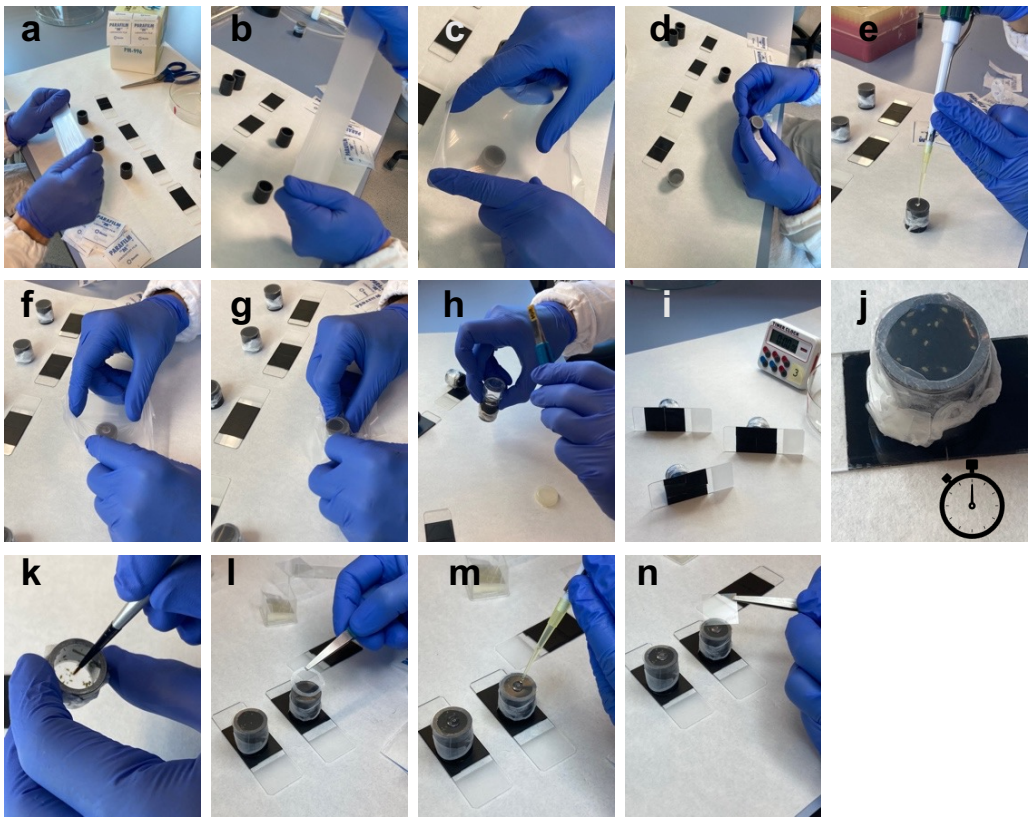

Fig. 3. Procedure for conducting membrane feeding transmission experiments. Apterous adult aphids are collected and placed for a fasting period as shown in figure 2. Chambers with artificial membranes are prepared in this case using cylindrical section of rigid plastic tubes (diameter 2 cm, height 2.5 cm), and the exterior is darkened using black tape. Parafilm is then stretched in two dimensions and tightly wrapped around the top of the cylinder to form a membrane (a-d). A 15% sucrose solution is carefully placed on top of the membrane (e) and covered with an additional layer of stretched parafilm to create a sachet (f, g). The fasting aphids are introduced into the cylinder and allowed to crawl until they reach the membrane attracted by the light. To cover the bottom of the chamber, a dark slide is used (i). The aphids can pierce the membrana with their stylets to access the sucrose solution, and the acquisition time is measured using a stopwatch (j). Once the acquisition period is completed, the cylinder is lifted, and the aphids are individually collected using a paintbrush (k). For the inoculation, the aphids are individually transferred to the test plants, as shown in figure 2. For very short acquisition periods, an alternative procedure to the sachet method involves using a plastic washer as spacer (l), followed by the addition of the feeding solution (m), and placing a microscopy coverslip on top (n).