**Insect vector-plant virus interactions associated with non-circulative, semi-persistent
transmission: current perspectives and future challenges**

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12 **ABSTRACT**

13 The non-circulative, semi-persistent (NCSP) mode of insect vector-mediated plant
14 virus transmission is shaped by biological, molecular and mechanical interactions that take
15 place across a continuum of processes involved in virion acquisition, retention and inoculation.
16 Our understanding of the interactive roles of virus, insect vector, and plant associated with
17 NCSP transmission is still evolving. Mechanisms exist that determine where and how virion
18 acquisition (from the plant) and retention (in the insect vector) are achieved, with both
19 processes being mediated by strategies involving viral capsid proteins, in some cases aided by
20 non-capsid proteins. In contrast, mechanisms underlying virion inoculation (to the plant)
21 remain poorly understood. Here, we review the established paradigms as well as fresh
22 perspectives on the mechanisms of NCSP transmission.

Introduction

“The journey of a thousand miles begins with a single step” - an adage by Lao Tzu, underscores a symbolic, but fitting characteristic of disease transmission. For plant viruses, the first step in transmission begins with overcoming the spatial distance that exists among plants. This is achieved, in most cases, by vectors, the most common of which are insects from the order Hemiptera. This large order of insects includes plant-feeding vectors in the families Aphididae (aphids), Aleyrodidae (whiteflies), Cicadellidae (leafhoppers), Fulgoroidea (planthoppers), Psyllidae (psyllids), Pseudococcidae (mealybugs), Coccidae (soft scales), as well as other vector and non-vector families. Phytophagous hemipterans use their stylets to feed (by sucking) on sap that flows within the phloem sieve elements in the vascular system located several cell layers beneath the plant surface. En route to the phloem, stylets may also probe and sample the contents of cells that are located outside the phloem [1,2]. Consequently, viruses in locations accessed by the stylets can be acquired and, if all goes well, transmitted by the vectors. Transmission can be seen as a continuum of three overlapping processes: 1) acquisition of virions when the vector samples/feeds on a suitable virus-infected plant, 2) passage of virions through (and their stable retention in) specific sites within the vector, and 3) inoculation of the retained virions into a suitable recipient plant during a subsequent sampling/feeding. Features observed in this continuum have helped frame our understanding of the different modes of virus transmission. Viruses that are transmitted in a non-circulative (NC) manner, unlike those with circulative, propagative (Whitfield et al, this volume) or circulative, non-propagative (Ghanim et al, this volume) modes of transmission, need not

circulate through, multiply, or invade the salivary glands of their insect vectors. NC transmission is contingent upon virion retention within the stylets or the foreguts of insect vectors [3,4]. Viruses exhibiting NC transmission and are not retained in their vectors beyond minutes to hours have a non-circulative, non-persistent (NCNP) mode of transmission; those that retain in and remain transmissible by their vectors for hours to days exhibit a non-circulative, semi-persistent (NCSP) mode of transmission [5] (and will hereafter be simplified as NCSP viruses). In both cases, virus transmissibility is lost when the vector molts. Here, we review the biology and mechanisms of NCSP transmission. The behaviors and mechanics of vector feeding, described briefly insofar as they are helpful in explaining NCSP transmission, have been examined comprehensively in other reviews [4,6,7].

Virus acquisition

NCSP viruses are transmitted by different but distinct insect vectors (Table 1). Transmission begins with virus acquisition. Optimal virus acquisition depends on whether or not virions are well positioned to capitalize on a vector's feeding behaviors (which encompass all known activities, including sampling and phloem-seeking/-feeding, identifiable by patterns of voltage fluctuation, known as electrical penetration graph [EPG] waveforms, generated during insect-plant contacts [7]). Microscopic examinations of the tracks created by stylet-insertion in plants, in correlation with EPG waveforms, have revealed nuances in the feeding behaviors of different hemipterans [8,9]. Aphids navigate their stylets in between cells located along the pathway en route to the phloem sieve elements and tend to issue frequent bouts of sampling

probes into these cells, including epidermal cells, early in the process [9,10] (Figure 1a). In the case of whiteflies, although the stylet pathway to the phloem is also intercellular, they tend to engage in intracellular probes only much later, when their stylet bundles have reached deep into the plant tissue [11] (Figure 1a). Mealybugs also maneuver their stylets intercellularly, although they tend to make fewer (but longer) intracellular probes than aphids [12,13]. Leafhoppers are less nimble in intercellular stylet movement; because their stylets are much stouter, they tend to penetrate plant tissues in a direct route to the phloem, destroying cells along the way [14,15]. These disparate feeding behaviors in effect have intriguing biological implications for virus transmission. For example, because many viruses with an NCNP mode of transmission generally invade all plant cell types (i.e. they are tissue generalists), they stand a good chance of being acquired by aphids during early intracellular probing [16^{*}]. In contrast, phloem tropism is more common among NCSP viruses; as such, virion acquisition is favored during phloem-feeding, and this is well represented by a number of whitefly-, leafhopper- and mealybug-transmitted viruses (Table 1). However, exceptions do exist, as exemplified by the aphid-transmitted *Cauliflower mosaic virus* (CaMV) and the whitefly-transmitted viruses in the genera *Ipomovirus* and *Torradovirus*, and *Cowpea mild mottle virus*, none of which are phloem-restricted (Table 1).

What about the molecular mechanisms of virus acquisition? While much of it remains unknown, recent investigations of CaMV have yielded novel findings showing that virion acquisition is associated with transmission activation (TA), a phenomenon in which a complex termed the transmission body (TB), containing CaMV encoded proteins (P2 and P3) and virions, disintegrates within an infected plant cell when triggered by mechanical or

chemical stresses during stylet probing. This facilitates the re-localization of P2-virions on the cortical microtubules as mixed-networks, the function of which is to promote the distribution of P2 (see section on virus retention) and virions throughout the cell periphery in order to enhance their acquisition by aphid vectors [17^{••},18[•]] (Drucker, this volume).

Virus retention

Upon ingestion, virion-filled fluid enters the opening at the apex of the maxillary stylets and moves up the maxillary food canal until it reaches the precibarium and cibarium. From here, aided by the compression of the cibarium and closing of the precibarial valve, fluid enters the pharynx and esophagus (Figure 1b). Along this ingestion route, opportunities arise for virions of NCSP viruses to be retained at precise locations within their insect vectors. Two virion retention sites have been identified: the maxillary stylets and a region comprising the cibarium, anterior pharynx and posterior esophagus (generically referred to as the foregut) [19-23^{••}].

Retention in the maxillary stylets

Virions of CaMV are retained at the apex of the aphid's maxillary stylets in a common duct (termed the acrostyle) formed by the merger of the food and the salivary canal (Figure 1b and Table 1) [23^{••},24]. The interaction is specific as it is seen only in the acrostyles of aphid species that transmit CaMV. The virus-driven mechanism underlying CaMV transmission is unequivocal and has been referred to as the "helper strategy" [25] (Table 1). The "helper"

aspect of this model refers to the interaction between viral encoded non-capsid proteins P2 and P3, aptly named aphid transmission factors (ATFs); their concurrent association with the interactions between P3 and the coat protein (P4) in the assembled virion, and the physical link that these combined interactions provide to “help” attach the virion to the acrostyle [26]. Retention of CaMV in the acrostyle was determined by several approaches, among which binding assays using dissected maxillary stylets to determine the retention specificity of *in vitro* expressed P2-GFP (Figure 1b), and of *in vitro* expressed P2 and purified P3-virion complexes, were insightful [23]. The “helper strategy” first emerged from studies of non-persistent transmission in members of the genus *Potyvirus* (family *Potyviridae*). Unlike in CaMV, a solitary viral non-capsid protein, the helper component (HC-Pro), serves as the bridge between the potyvirus virion and the retention site in the vector’s maxillary stylets [27]. There is clear evidence that the loss of transmission of CaMV and potyviruses with specific mutations in their ATFs/HC-Pros and coat proteins, as reported in many studies, is due to defective virion retention [23^{**},28-31]. In the case of *Tomato torrado virus* (ToTV; genus *Torradovirus*), which is not known to encode any viral non-capsid proteins similar to HC-Pro or the CaMV ATFs, reverse transcriptase (RT)-PCR detected ToTV sequences in the probosces (Table 1), but not in the heads, of viruliferous whiteflies, suggesting that ToTV may also be retained in the maxillary stylets [32].

Retention in the foregut

Studies of *Lettuce infectious yellows virus* (LIYV; genus *Crinivirus*) have shown that virions are retained in the foregut of its specific whitefly vector (*Bemisia tabaci* New World

[NW] species; formerly *B. tabaci* biotype A) (Figure 1b and Table 1) and that the retained virions function in virus transmission. This was demonstrated by transmission assays conducted in parallel with membrane feeding and immunofluorescent localization (a typical example of the visualization of virion retention is presented in Figure 1b). The presence of specific signals in the foreguts of a significant number of virion-fed vectors corresponded with LIYV transmission. In contrast, insignificantly few diet-fed vectors harbored signals in their foreguts, and no corresponding LIYV transmission was observed. The number of virion-fed or diet-fed non-vectors (*B. tabaci* Middle East-Asia Minor 1 [MEAM 1] species; formerly *B. tabaci* biotype B) that harbored signals in their foreguts was also insignificant, and, collectively, none of them transmitted the virus [20^{••},33]. Using similar approaches, the specific retention and transmission of the crinivirus *Lettuce chlorosis virus* (LCV) by its whitefly vectors (both the *B. tabaci* MEAM 1 and NW species) have also been demonstrated recently (Chen and Ng, Unpublished data). LIYV transmission is mediated by the proverbial “capsid strategy” (Table 1), wherein transmission determinants are present on the virion capsid and no helper proteins are involved [5]. The LIYV virion is composed of four capsid components [34]. Of these, the minor coat protein (CPm) plays a pivotal role in mediating virion retention to the vector’s foregut and this was determined using a combination of biochemical and molecular approaches and by transmission assays [20^{••}]. The CPm determinants of LIYV transmission are unknown. However, there is information implicating the involvement of the CPm C-terminus in transmission, and that of the N terminal (approx. 71 amino acids) portion in interactions required for virion encapsidation (Tom Smith, personal communication). First, a natural mutant of LIYV (1-5b), with a 211-amino acid long CPm (the result of a >50% truncation of the

wild type [WT] C-terminus), is defective in transmission and foregut retention (compared to the WT) [20**], and the truncated CPm does not appear to be incorporated in the virion (Figure 2) [35]. Second, a mutant (R6M6) engineered to produce an even shorter CPm (containing 71 amino acids from the WT N-terminus) is also transmission defective, but the truncated CPm appears to be incorporated in the virion (Figure 2). Thus, the first 71 residues may represent a domain that is essential for the interactions possibly needed for encapsidation. The fact that this truncated protein is insufficient for vector transmission suggests that the vector recognition domain probably lies towards the carboxyl terminus. Because the 1-5b CPm is not incorporated into the virion, this suggests that additional residues beyond the first 71 are not necessary for assembly but may in fact have a deleterious effect on protein folding. Ultimately, a high-resolution structure of the CPm will help identify the domains or amino acids involved in virion retention and transmission.

Evidence of virion retention in the foregut has also been obtained from the transmission electron microscopy examinations of viruliferous aphid and leafhopper vectors of the *Anthriscus yellows virus* (AYV)/*Parsnip yellow fleck virus* (PYFV) complex and *Maize chlorotic dwarf virus* (MCDV), respectively (Figure 1c-e and Table 1) [19,21,22]. The “helper strategy” has been implicated in the transmission these viruses [36,37]. For the AYV/PYFV complex, the helper function is supplied by AYV, the transmissible member in the complex. As yet, neither the identities of the helper proteins nor concrete evidence of their modes of action in mediating virion retention/transmission has been determined for AYV and MCDV.

Retention in yet to be identified locations

Interestingly, some members of the whitefly-transmitted *Ipomovirus* (a genus of *Potyviridae*) (Table 1) encode an HC-Pro while others do not [38]. It will be worthwhile to determine if ipomoviruses with an HC-Pro, or with other viral encoded proteins that substitute for the HC-Pro, are retained in their vectors' maxillary stylets; and whether those without an HC-Pro are retained in locations other than the maxillary stylets (such as the foregut). The leafhopper-transmitted *Rice tungro spherical virus* (RTSV)/*Rice tungro bacilliform virus* (RTBV) complex [39] as well as several other aphid-, whitefly-, leafhopper-, mealybug-, and soft scale-transmitted viruses are other examples of NCSP viruses for which information on virion retention sites and mechanisms are largely unavailable (Table 1). For RTSV/RTBV, a helper dependent transmission strategy has been shown, with the helper function being provided by RTSV, the transmissible member of the complex. However, the identity of the helper protein(s) and the mechanisms underlying its/their function are unknown.

Virus inoculation

The mechanics of NCSP virus inoculation have been inferred from EPG waveforms and/or other features associated with vector (mostly aphid and whitefly) feeding, including the transmission of stylet- and foregut-borne viruses [16*,20,40-43]. Together, the information has helped strengthen two hypotheses (ingestion-salivation and ingestion-egestion) explaining the mechanical actions that mediate virion inoculation. Because salivation and ingestion (occurring in that order) take place during both sampling and phloem-feeding [42,44,45], the ingestion-salivation hypothesis provides a logical explanation for how previously ingested virions, now

retained at the tip of the maxillary stylets (at the point of convergence with the salivary canal; Figure 1b), are released and inoculated when contacted by the flow of watery saliva from the salivary canal. In aphids, watery saliva secreted during phloem-feeding is purportedly swept up the food canal with the ingested sap [4]. Thus, it is conceivable that the mixture of saliva and sap may also contact virions that are retained past the point of maxillary stylets/salivary canal convergence and, possibly, as far up as in the foregut. Therefore, ingestion-salivation appears relevant to the inoculation of both stylet- and foregut-borne viruses. The contents of aphid saliva have been studied and found to contain proteins implicated in modulating plant defense responses [46-50]. However, whether or not and how aphid saliva is involved in triggering virion inoculation is unknown. The role, if any, of saliva in mediating virion inoculation by other hemipteran vectors has also not been demonstrated.

The ingestion-egestion hypothesis proposes that retained virions are released and inoculated into the plant during egestion (a mechanical function aided by reversing fluid flow in the cibarium [Figure 1b]) [40]. Based on this model, virions of stylet- and foregut-borne viruses located in the path of the reversed fluid flow can both be egested. If virion inoculation is a purely mechanical event mediated only by egestion, then it is imperative that the force generated by fluid flow must be sufficient to dislodge and expel the retained virions. Unfortunately, technology has yet to develop the capability to determine the dynamics of fluid flow inside the maxillary stylets or foregut. Based on existing evidence, it is more than likely that the inoculation of both foregut- and stylet-borne viruses may be mediated by a combination of salivation and egestion. In one probable scenario, a mixture of saliva and sap, along with prevailing dietary conditions (Ng, unpublished data), may work cooperatively to

facilitate the release of virions, while egestion takes place at the same time or afterwards to complete the inoculation process.

Clearly, the interactions involved in virion release during the inoculation phase are unknown for NCSP viruses. Future investigations are needed to address the fundamental mechanisms underlying this process; the question of what factors (sap and saliva component as well as dietary conditions) are involved in virion inoculation will be particularly pertinent.

Conclusions

An unhindered continuum of processes (virion acquisition, retention and inoculation) contributing to successful NCSP transmission requires the concerted actions of all three participants – the virus, the vector, and the plant. Transmission can be affected by sub-optimal virion acquisition due to a virion distribution scheme (in the plant) that is not in-sync with vector feeding behavior. The discovery of a specialized mechanism, TA, seen in the transmission of CaMV, a tissue generalist, indicates how changes in virion distribution within an infected cell play a role in optimizing virion acquisition. In this regard, it will be interesting for future studies to determine if the transmission of phloem-restricted NCSP viruses also involves TA or mechanisms that are similar to it.

Loss of virus transmission may arise from defective virion retention. So far, CaMV and LIYV are the only NCSP viruses for which definitive retention sites and mechanisms have been revealed through the use of transmission defective mutants. Similar studies will be needed to determine the retention sites and mechanisms of other NCSP viruses. For LIYV and

other criniviruses, a structure-based approach to studying the CPm's function in virion retention will further contribute to a mechanistic view of the molecular bases involved in this process.

Another consideration in understanding virion retention mechanisms is the identities of the receptors in the maxillary stylets and foregut to which stylet- and foregut-borne viruses, respectively, are attached. Because the surfaces of the foregut and maxillary stylets are covered with cuticle consisting of a chitinous intima embedded in a protein matrix [24,51], it is conceivable that cuticular proteins may function as receptors. This hypothesis is, in part, supported by data from Uzest et al [23**], demonstrating that treatment of dissected maxillary stylets of aphid vectors with specific proteases (with or without chitinase treatment) abolished CaMV P2 binding (Figure 1b). The identity of this and other receptors of NCSP viruses and proof of their role in virion retention await future discovery. In the case of LIYV and LCV, because both viruses are retained and transmitted specifically by *B. tabaci* NW, this suggests that the same receptors in the foregut of *B. tabaci* NW may accommodate the retention of LIYV and LCV. Alternatively, there may be different receptors in the foregut of *B. tabaci* NW that interact independently with both viruses. Co-localization studies of LIYV and LCV within *B. tabaci* NW will help resolve this in the near future.

Understanding the behavioral, cellular and molecular bases underlying the tripartite relationship among NCSP virus, vector and plant holds the potential for new opportunities aimed at breaking the transmission continuum, such as disrupting virion acquisition and interfering with virion retention or release.

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279

280 **Figure legends**

281 **Figure 1.** Aphid and whitefly feeding behaviors associated with the transmission of NCSP
282 viruses, and sites in the anterior alimentary canal of specific hemipteran vectors where the
283 NCSP viruses that they transmit are retained. (a) Comparison of the phloem-seeking and -
284 feeding behaviors between aphid and whitefly. In this diagrammatic illustration, both insects
285 are on the abaxial (under)side of the leaf. (Left) Oblique frontal view of an adult apterous aphid
286 and, (right) lateral view of an adult whitefly, revealing the stylet tracks (st) created during the
287 intercellular movement of the maxillary stylets (ms) and the intracellular probing/sampling of
288 cells in the epidermal and mesophyll layers (indicated by yellow stars) and/or the vascular
289 bundle (indicated by dark blue stars) where the phloem (represented by purple colored cells) is
290 located. Note the difference in the probing/sampling locations of the two insects. (b)
291 Diagrammatic sagittal section through the head of a generic hemipteran insect vector (art work
292 courtesy of Greg Walker), illustrating the ingestion (green arrows), salivation (blue arrows) and
293 egestion (red arrows) pathways as well as the retention sites of known stylet- (blue box) and
294 foregut-borne (translucent, blue-shaded region) viruses. As noted in the text, the ingested fluid
295 is a mixture of sap and, presumably, saliva. Insect anatomical features relevant to the
296 transmission of NCSP viruses are indicated. Insets b1 and b2: widefield fluorescence
297 microscopy images of the whole-mounted maxillary stylets of aphid vector *Acyrtosiphon*
298 *pisum*. The maxillary stylets were treated (b1) or not treated (b2) with proteinase K before
299 being subjected to *in vitro* binding using the green fluorescent protein (GFP)-marked P2 protein

(P2-GFP) of CaMV; only the stylets that did not receive proteinase K treatment (b2) contained GFP fluorescence in the acrostyle (as) located at the tip of the stylet bundle (reproduced from reference [23^{**}], copyright [2007] National Academy of Sciences, U.S.A). Inset b3: confocal laser scanning microscopy image of the whole-mounted head (posterior view) of a whitefly vector (*Bemisia tabaci* New World species) that fed on LIYV and then sequentially on biotin-labeled anti-LIYV antibody and quantum dot (QD)₆₀₅-labeled streptavidin, showing the presence of specific QD signals (see enlargement in inset b4) in the foregut (reproduced from reference [33]). The positions of the eye, cibarium (cb) and maxillary stylets (ms) are indicated. (c) Transmission electron micrograph of an ultra-thin section through the head of a leafhopper (*Graminella nigrifrons*) that fed on MCDV, showing the presence of spherical virion-like particles (VLPs; V) associating with the cuticular intima (CI) of the pharynx, as well as in the lumen (L) of the pharynx (reproduced from reference [21] with permission). (d - e) Transmission electron micrographs of ultra-thin sections through the head of an aphid vector (*Cavariella aegopodii*) that fed on AYV (d) or the AYV/PYFV complex (e), showing the presence of spherical VLPs (reproduced from reference [22] with permission).

Figure 2. Encapsidation, transmission and virion retention of wild type (WT) and minor coat protein (CPm) mutants of LIYV. (Left panel) Genetic map of the WT full-length LIYV CPm (WT; amino acid position [aa] 1 – 452), mutants with truncated CPms, 1-5b (aa 1 – 211) and R6M6 (aa 1 – 71), and the 1-5b mutant engineered with a restored full-length CPm (1-5bM1). The putative domains corresponding to aa 1 – 71 (domain I) and aa 72 – 452 (domain II) are indicated by the double-ended arrows. The numerical scale above the genetic maps indicates the relative sizes of the CPms. (Right panel) Immunogold-labeling TEM (IGL-TEM), transmission and virion retention analyses. IGL-TEM analyses: the filamentous virions/virion-like particles of WT, 1-5b, 1-5bM1 and R6M6 were tested by using antibodies produced against the LIYV CPm, followed by anti-rabbit IgG labeled with 5-nm gold particles. Micrographs of 1-5b and 1-5bM1 are reproduced from Stewart et al [35] and Chen et al [20], respectively. Each bar represents 200nm. Arrows indicate gold labeling sites. Transmission analyses: (+) and (-) indicate transmission success and absence of transmission, respectively, by *Bemisia tabaci* New World (NW) species as reported in Stewart et al [35] and Chen et al [20]. Data for transmission of R6M6 is unpublished. Virion retention analyses: (+) indicates presence of virion retention in the foreguts of most of the *B. tabaci* NW (averaging 39% for WT and 34% for 1-5bM1) as reported in Chen et al [20], and (-) indicates defective virion retention in the foreguts of most of the *B. tabaci* NW (averaging 3% for 1-5b) as reported in Chen et al [20]. NT denotes not tested.

References and recommended reading

Papers of particular interest, published within the period of review have been highlighted as:

- of special interest
- of outstanding interest

1. Dietrich CH: **Auchenorrhyncha (cicadas, spittlebugs, leafhoppers, treehoppers, and planthoppers)**. In *Encyclopedia of insects*, edn 2nd. Edited by Resh VH, Cardé RT. Burlington, MA, USA: Elsevier; 2009:56-64.
2. Gullan PJ, Martin JH: **Sternorrhyncha (jumping plant-lice, whiteflies, aphids, and scale insects)**. In *Encyclopedia of insects*, edn 2nd. Edited by Resh VH, Cardé RT. Burlington, MA, USA: Elsevier; 2009:957-966.
3. Blanc S, Drucker M, Uzzest M: **Localizing Viruses in Their Insect Vectors**. *Ann Rev Phytopathol* 2014, **52**:403-425.
4. Ng JCK, Walker GP: **Whitefly feeding behavior and its relationship with non-circulatively transmitted viruses**. In *Vector-Mediated Transmission of Plant Pathogens*. Edited by Brown JK: APS Press, St. Paul, MN; In press.
5. Ng JCK, Falk BW: **Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses**. *Annu Rev Phytopathol* 2006, **44**:183-212.
6. Moreno A, Tjallingii WF, Fernandez-Mata G, Fereres A: **Differences in the mechanism of inoculation between a semi-persistent and a non-persistent aphid-transmitted plant virus**. *J Gen Virol* 2012, **93**:662-667.

- 363 7. Walker GP: **A beginner's guide to electrical monitoring of homopteran probing behavior.** In
364 *Principles and applications of electronic monitoring and other techniques in the study of*
365 *homopteran feeding behavior.* Edited by Walker GP, Backus EA: Thomas Say publications
366 in entomology, Entomological Society of America; 2000:14-40.
- 367 8. Backus EA, Bennett WH: **The AC-DC Correlation Monitor: New EPG design with flexible**
368 **input resistors to detect both R and emf components for any piercing-sucking**
369 **hemipteran.** *J Insect Physiol* 2009, **55**:869-884.
- 370 9. Tjallingii WF, Esch TH: **Fine-Structure of Aphid Stylet Routes in Plant-Tissues in Correlation**
371 **with Epg Signals.** *Physiol Entomol* 1993, **18**:317-328.
- 372 10. Fereres A: **Aphid behavior and the transmission of noncirculative viruses.** In *Vector-*
373 *Mediated Transmission of Plant Pathogens.* Edited by Brown JK: APS Press, St. Paul, MN;
374 In press.
- 375 11. Johnson DD, Walker GP: **Intracellular punctures by the adult whitefly Bemisia argentifolii**
376 **on DC and AC electronic feeding monitors.** *Entomol Exp Et Appl* 1999, **92**:257-270.
- 377 12. Calatayud PA, Rahbe Y, Tjallingii WF, Tertuliano M, Leru B: **Electrically Recorded Feeding-**
378 **Behavior of Cassava Mealybug on Host and Nonhost Plants.** *Entomol Exp Et Appl* 1994,
379 **72**:219-232.
- 380 13. Cid M, Fereres A: **Characterization of the Probing and Feeding Behavior of Planococcus**
381 **citri (Hemiptera: Pseudococcidae) on Grapevine.** *Ann Entomol Soc Am* 2010, **103**:404-
382 417.

- 383 14. Stafford CA, Walker GP: **Characterization and correlation of DC electrical penetration**
384 **graph waveforms with feeding behavior of beet leafhopper, *Circulifer tenellus*.**
385 *Entomol Exp Et Appl* 2009, **130**:113-129.
- 386 15. Backus EA, Serrano MS, Ranger CM: **Mechanisms of hopperburn: An overview of insect**
387 **taxonomy, behavior, and physiology.** *Ann Rev Entomol* 2005, **50**:125-151.
- 388 16. Martin B, Collar JL, Tjallingii WF, Fereres A: **Intracellular ingestion and salivation by aphids**
389 **may cause the acquisition and inoculation of non-persistently transmitted plant**
390 **viruses.** *J Gen Virol* 1997, **78**:2701-2705.
- 391 • The authors show that acquisition and inoculation of non-persistently transmitted
392 viruses are identifiable by specific EPG waveforms associated with brief, distinct
393 intracellular punctures made by aphids.
- 394 17. Martiniere A, Bak A, Macia JL, Lautredou N, Gargani D, Doumayrou J, Garzo E, Moreno A,
395 Fereres A, Blanc S, et al.: **A virus responds instantly to the presence of the vector on**
396 **the host and forms transmission morphs.** *Elife* 2013, **2**.
- 397 •• The authors show that transmission activation of CaMV involves the interaction
398 among virions, viral encoded proteins (P2 and P3) that form a specialized transmission
399 body within infected plant cells, the plant cell microtubule network, and the aphid
400 vector.
- 401 18. Martiniere A, Gargani D, Uzest M, Lautredou N, Blanc S, Drucker M: **A role for plant**
402 **microtubules in the formation of transmission-specific inclusion bodies of Cauliflower**
403 **mosaic virus.** *Plant J* 2009, **58**:135-146.

- The authors show that microtubules are required for the formation of electron-lucent inclusion bodies (also called transmission bodies in subsequent work), consisting of the CaMV encoded proteins P2 and P3.

19. Ammar ED, Nault LR: ***Maize chlorotic dwarf virus-like particles associated with the foregut in vector and non-vector leafhopper species.*** *Phytopathol* 1991, **81**:444-448.

20. Chen AY, Walker GP, Carter D, Ng JC: **A virus capsid component mediates virion retention and transmission by its insect vector.** *Proc Natl Acad Sci U S A* 2011, **108**:16777-16782.

- The first study demonstrating that whitefly transmission of LIYV is mediated by a capsid component (the minor coat protein)-mediated foregut retention mechanism.

21. Childress SA, Harris K: **Localization of virus-like particles in the foreguts of viruliferous *Graminella nigrifrons* leafhoppers carrying the semi-persistent *Maize chlorotic dwarf virus*.** *J Gen Virol* 1989, **70**:247-251.

22. Murrant A, Roberts I, Elnagar S: **Association of Virus-Like Particles with Foregut of Aphid *Cavariella-Aegopodii* Transmitting Semi-Persistent Viruses Anthriscus Yellows and Parsnip Yellow Fleck.** *J Gen Virol* 1976, **31**:47-57.

23. Uzest M, Gargani D, Drucker M, Hebrard E, Garzo E, Candresse T, Fereres A, Blanc S: **A protein key to plant virus transmission at the tip of the insect vector stylet.** *Proc Natl Acad Sci U S A* 2007, **104**:17959-17964.

- The authors show that aphid transmission of CaMV is mediated by a CaMV P2-mediated virion retention mechanism in the tip of the aphid's stylet bundle.

24. Uzest M, Gargani D, Dombrovsky A, Cazevieille C, Cot D, Blanc S: **The "acrostyle": A newly described anatomical structure in aphid stylets.** *Arthropod Str Dev* 2010, **39**:221-229.

- 426 25. Pirone TP, Blanc S: **Helper-dependent vector transmission of plant viruses.** *Annu Rev*
427 *Phytopathol* 1996, **34**:227-247.
- 428 26. Uzest M, Blanc S: **Molecular mechanisms involved in the non-circulative virus-vector**
429 **interaction.** In *Vector-Mediated Transmission of Plant Pathogens*. Edited by Brown JK; In
430 press.
- 431 27. Pirone TP, Perry KL: **Aphids: Non-persistent transmission.** *Adv Bot Res* 2002, **36**:1-19.
- 432 28. Fernandez-Calvino L, Goytia E, Lopez-Abella D, Giner A, Urizarna M, Vilaplana L, Lopez-Moya
433 JJ: **The helper-component protease transmission factor of tobacco etch potyvirus**
434 **binds specifically to an aphid ribosomal protein homologous to the laminin receptor**
435 **precursor.** *J Gen Virol* 2010, **91**:2862-2873.
- 436 29. Blanc S, LopezMoya JJ, Wang RY, GarciaLampasona S, Thornbury DW, Pirone TP: **A specific**
437 **interaction between coat protein and helper component correlates with aphid**
438 **transmission of a potyvirus.** *Virology* 1997, **231**:141-147.
- 439 30. Wang RY, Powell G, Hardie J, Pirone TP: **Role of the helper component in vector-specific**
440 **transmission of potyviruses.** *J Gen Virol* 1998, **79**:1519-1524.
- 441 31. Leh V, Jacquot E, Geldreich A, Hermann T, Leclerc D, Cerutti M, Yot P, Keller M, Blanc S:
442 **Aphid transmission of cauliflower mosaic virus requires the viral PIII protein.** *EMBO J*
443 1999, **18**:7077-7085.
- 444 32. Verbeek M, van Bekkum PJ, Dulleman AM, van der Vlugt RAA: **Torradoviruses are**
445 **transmitted in a semi-persistent and stylet-borne manner by three whitefly vectors.**
446 *Virus Res* 2014, **186**:55-60.

- 447 33. Ng JCK: **A quantum dot-immunofluorescent labeling method to investigate the**
448 **interactions between a crinivirus and its whitefly vector.** *Frontiers Microbiol* 2013, **4**.
- 449 34. Tian T, Rubio L, Yeh HH, Crawford B, Falk BW: **Lettuce infectious yellows virus: in vitro**
450 **acquisition analysis using partially purified virions and the whitefly Bemisia tabaci.** *J*
451 *Gen Virol* 1999, **80**:1111-1117.
- 452 35. Stewart LR, Medina V, Tian T, Turina M, Falk BW, Ng JCK: **A mutation in the *Lettuce***
453 ***infectious yellows virus* minor coat protein disrupts whitefly transmission but not in**
454 ***planta* movement.** *J Virol* 2010, **84**:12165-12173.
- 455 36. Elnagar S, Murrant AF: **The role of the helper virus, anthriscus yellows, in the transmission**
456 **of parsnip yellow fleck virus by the aphid *Cavariella aegopodii*.** *Ann Appl Biol* 1976,
457 **84**:169-181.
- 458 37. Hunt RE, Nault LR, Gingery RE: **Evidence for infectivity of maize chlorotic dwarf virus and**
459 **for a helper component in its leafhopper transmission.** *Phytopathol* 1988, **78**:499-504.
- 460 38. Dombrovsky A, Reingold V, Antignus Y: **Ipomovirus - an atypical genus in the family**
461 **Potyviridae transmitted by whiteflies.** *Pest Manag Sci* 2014, **70**:1553-1567.
- 462 39. Hull R: **Molecular biology of rice tungro viruses.** *Ann Rev Phytopathol* 1996, **34**:275-297.
- 463 40. Harris KF: *An ingestion-egestion hypothesis of noncirculative virus transmission.* Edited by
464 Harris KF, Maramorosch K. New York: Academic Press; 1977.
- 465 41. Moreno A, Palacios I, Blanc S, Fereres A: **Intracellular salivation is the mechanism involved**
466 **in the inoculation of cauliflower mosaic virus by its major vectors *Brevicoryne***
467 ***brassicae* and *Myzus persicae*.** *Ann Entomol Soc Am* 2005, **98**:763-769.

- 468 42. Powell G: **Intracellular salivation is the aphid activity associated with inoculation of non-**
469 **persistently transmitted viruses.** *J Gen Virol* 2005, **86**:469-472.
- 470 43. Johnson DD, Walker GP, Creamer R: **Stylet penetration behavior resulting in inoculation of**
471 **a semipersistently transmitted closterovirus by the whitefly Bemisia argentifolii.**
472 *Entomol Exp Et Appl* 2002, **102**:115-123.
- 473 44. Moreno A, Garzo E, Fernandez-Mata G, Kassem M, Aranda MA, Fereres A: **Aphids secrete**
474 **watery saliva into plant tissues from the onset of stylet penetration.** *Entomol Exp Et*
475 *Appl* 2011, **139**:145-153.
- 476 45. Jiang YX, De Blas C, Barrios L, Fereres A: **Correlation between whitefly (Homoptera :**
477 **Aleyrodidae) feeding behavior and transmission of tomato yellow leaf curl virus.** *Ann*
478 *Entomol Soc Am* 2000, **93**:573-579.
- 479 46. Harmel N, Letocart E, Cherqui A, Giordanengo P, Mazzucchelli G, Guillonneau F, De Pauw E,
480 Haubruge E, Francis F: **Identification of aphid salivary proteins: a proteomic**
481 **investigation of Myzus persicae.** *Insect Mol Biol* 2008, **17**:165-174.
- 482 47. Vandermoten S, Harmel N, Mazzucchelli G, De Pauw E, Haubruge E, Francis F: **Comparative**
483 **analyses of salivary proteins from three aphid species.** *Insect Mol Biol* 2014, **23**:67-77.
- 484 48. Rao SAK, Carolan JC, Wilkinson TL: **Proteomic Profiling of Cereal Aphid Saliva Reveals Both**
485 **Ubiquitous and Adaptive Secreted Proteins.** *Plos One* 2013, **8**.
- 486 49. Chaudhary R, Atamian HS, Shen Z, Briggs SP, Kaloshian I: **Potato Aphid Salivary Proteome:**
487 **Enhanced Salivation Using Resorcinol and Identification of Aphid Phosphoproteins.** *J*
488 *Prot Res* 2015, **14**:1762-1778.

- 489 50. Sharma A, Khan AN, Subrahmanyam S, Raman A, Taylor GS, Fletcher MJ: **Salivary proteins**
490 **of plant-feeding hemipteroids - implication in phytophagy.** *Bull Entomol Res* 2014,
491 **104**:117-136.
- 492 51. Andersen SO: **Cuticle.** In *Encyclopedia of insects*. Edited by Resh VH, Cardé RT. Burlington,
493 MA, USA: Elsevier; 2009:245-246.

Figure 1

Figure 2