Hitching a ride
Vector feeding and virus transmission

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The majority of plant viruses rely on insect vectors for transmission. Insects with piercing-sucking mouthparts are the most common and efficient vectors because they are able to inject viruses into specific plant tissues. Acquisition and inoculation of viruses occurs during specific vector feeding behaviors, and feeding behavior varies greatly among insects with piercing-sucking mouthparts. In this review we provide an overview of the feeding behavior of the major insect vectors with piercing sucking mouthparts: aphids, whiteflies, mealybugs, hoppers and thrips. We briefly review the different mechanisms of plant virus transmission by these insects, and discuss how each mechanism requires a vector that engages in specific feeding behaviors, and how differences in feeding behavior among these insects can determine which viruses they are capable of transmitting. We also discuss recent findings indicating that plant viruses can directly modify their vector’s behavior in a way that enhances transmission to a host plant.

Feeding strategies used by piercing-sucking insects fall into two basic categories: sheath feeders, which make up the vast majority of vectors and cell rupture feeders (also known as lacerate and flush feeders).2,3 Sheath feeders produce gelling saliva which solidifies and forms a sheath around the stylet bundle. This gelling saliva is different from the watery saliva which is produced immediately before ingestion from various tissues. Hereafter salivation will be used for all references to watery salivation. Most of these insects preferentially feed on vascular tissue. Cell rupture feeders usually do not produce salivary sheaths and preferentially feed from non-vascular tissue. Sheath feeders include: aphids, whiteflies, mealybugs and some hopper species and cell rupture feeders include thrips and some hopper species. Sheath feeders are usually characterized as phloem feeders or xylem feeders based on which tissue the insect predominantly ingests from. However it is important to note that almost all piercing-sucking insects ingest some amount of xylem sap during feeding.4 All piercing-sucking insects initiate feeding by inserting their stylets either, intracellularly (into cells), intercellularly (between cells) or, rarely, through stomata. Although most species within a given insect family generally use just one of these stylet insertion strategies, all three may be used by different species within a family or individuals within a species. Due to the diversity of feeding behaviors among individuals, it is difficult to describe a general scheme that applies to all insects within a genus or even species. Therefore, the feeding behaviors reported herein are those most representative of important vector species on preferred host plants.

Feeding behavior of piercing-sucking insects occurs inside plant tissue and is not directly observable. The most useful and widely used technique to study these feeding behaviors is the electrical penetration graph (EPG).5,6 EPGs allow real time detection, monitoring, quantification and analysis of different feeding behaviors of insects with piercing sucking mouthparts. The technique was first invented by McLean and Kinsey in 1964 and over the past 40 years the system has evolved with new technologies and strategies employed.7-10 Users of EPG systems are currently able to examine feeding behavior in fine detail and detect variations in feeding behavior that can change in a fraction of a second. EPGs work on the basis of a simple circuit, incorporating a feeding insect and plant into this circuit. As the insect engages in different feeding behaviors, EPGs measure fluctuations in voltage level which occur in distinct patterns termed waveforms. Each waveform is produced by a specific feeding behavior.

When studying a new insect/plant system, EPG waveforms must be characterized by correlating them with their underlying biological activities. This characterization requires the use of techniques that identify the feeding behavior occurring during a specific waveform. These techniques include, but are not limited to: severing of stylets during a specific waveform followed by histology to examine stylet placement inside plant tissue, histological examination of salivary sheaths left inside plant tissue.
after recording an EPG, observation and chemical analysis of honeydew produced during a specific waveform to determine the feeding source, simultaneous EPG recording and observation and videography of the insect as it feeds on leaves, or simultaneous EPG recording and observation and videography of the stylets in artificial diet. Once waveforms are correlated with biological activities, EPGs can be used to observe the behaviors in real time. Researchers generally assign letter and number codes to EPG waveforms. For ease of understanding, we will generally refer to the behaviors instead of the letter and number codes designating specific waveforms. Specific attention will be paid to those feeding behaviors most important in virus transmission.

Plant viruses transmitted by arthropods are classified into 4 transmission types based on the length of time the vector retains the ability to transmit the virus after the virus has been acquired, the movement/retention site of the virus in the insect (whether the virus circulates through the body of the insect or is retained on the cuticle), and whether or not the virus is able to replicate inside of the vector. Nault (1997) gives an excellent detailed description of the biological properties that characterize these categories which we will briefly explain here. For extensive reviews on virus-vector interactions please see references 1 and 11–19.

The insect taxa that utilize these various transmission modes are well summarized in Table 3 of Hogenhout et al. (2008). Almost all (99%) noncirculative, nonpersistent viruses are transmitted by aphids. For an extensive review on this transmission mode, see Ng and Falk (2006). These viruses are retained at the tips of the maxillary stylets in the area where the food and salivary canals come together to form the common duct and vectors lose the ability to transmit them in minutes to hours after initially acquiring them. These viruses are inoculated during the initial phase of brief intracellular punctures by the stylet tips when the aphid salivates into epidermal and mesophyll cells. Noncirculative, semi-persistent viruses are retained either on the lining of the foregut lumen or at the tips of the maxillary stylets in the common duct area (present in aphids and whiteflies) and vectors usually lose the ability to transmit them within several hours after acquisition. These viruses are inoculated during either extravasation or egestion of previously ingested material or salivation. Transmission characteristics of these viruses are poorly understood and highly variable; see Ng and Falk (2006). The final destination of circulative viruses, so named because they circulate through the body of the insect, is the salivary glands. After invasion of the salivary glands, the virus is secreted into the plant when the insect salivates, but for transmission to occur, the insect must inject its saliva into the appropriate plant cells. For reviews on this mode of transmission see Hogenhout et al. (2008), Gray and Gildow (2003), and Whitfield et al. 2005. Vectors of circulative, persistent, nonpropagative viruses retain the ability to transmit these viruses essentially for life, but because the virus does not replicate in the insect, transmission rates decrease over time. Circulative, persistent propagative viruses differ from the nonpropagative viruses in that the virus replicates in the vector and therefore after acquisition, these viruses are generally transmitted at high rates for the entire life of the vector. The circulative viruses are almost all inoculated directly into phloem sieve elements during salivation, with a few notable exceptions such as *Pea enation mosaic virus* and the *Tospoviruses*.

### Aphids

Aphids are by far the most important vector group in terms of number of viruses transmitted, and the damage these viruses inflict to crops; consequently, aphids are the best studied vectors. Aphid transmitted viruses include all four transmission types. Aphids are polymorphic, some individuals develop wings as adults (alates) and some never develop wings (apterates). Both alates and apterates are capable of transmitting viruses; however, alates are thought to be most important in virus epidemiology due to their propensity to disperse great distances. Several studies have shown that virus infected plants are often highly attractive to aphids due to physiological changes resulting in virus symptoms (i.e., yellowing) and alteration of volatile composition. Furthermore, plant infection may affect aphid feeding behavior in a way that is likely to result in increased virus acquisition and subsequent inoculation, and the effect on feeding behavior may differ according to the mode of transmission of the particular virus. For example, Mauck et al. (2009), reported that aphids were attracted to plants infected with the nonpersistent *Cucumber mosaic virus* but once on these plants; they did not feed for prolonged periods of time. This behavior enhanced transmission because nonpersistently transmitted viruses are most effectively acquired and inoculated during brief stylent penetrations rather than long phloem sap ingestion penetrations. In the case of the persistent-circulative *Potato leafroll virus*, aphids were highly attracted to and preferred to settle and engage in phloem sap ingestion on infected plants vs. healthy ones; again enhancing transmission since these persistent-circulative viruses are acquired and inoculated during feeding on the phloem. Usually after landing on a plant they begin to tap the plant surface with their rostrum, hypothetically to locate intercellular grooves where they preferentially insert their mouthparts. They will indiscriminately probe any smooth surface they land on, including glass slides. During the initial brief stylent insertions, often referred to as ‘exploratory probes’, the aphid makes brief intracellular punctures (or potential drops in the EPG literature). Aphids have very characteristic patterns of behavior during these extremely short intracellular punctures which generally last for 5–10 sec. They all begin with salivation, followed by an unknown behavior, and end with ingestion of a minute amount of cell sap, presumably for chemosensory evaluation. This brief ingestion is believed to carry cell sap to the gustatory receptors located in the precibarium and cibarium to assess host plant quality. Surprisingly, these brief intracellular punctures usually do little or no detectable damage to the cells. These intracellular punctures are critical to the transmission of certain viruses and they are thought to lay at the foundation of the coevolutionary relationship between nonpersistent, noncirculative viruses and their aphid vectors.

Noncirculative, nonpersistent viruses are transmitted almost exclusively by aphids probably because of the frequency with which...
which they make these brief intracellular punctures and the behaviors in which they engage during these punctures. Aphids make more brief intracellular punctures on route to the phloem than any other vector group, sometimes probing into nearly every cell along the path, or 1 puncture every 2 min. Additionally, they usually make brief intracellular punctures during short probes, which are the most effective probes for transmission of nonpersistent viruses. Finally, only aphids are known to engage in salivation and ingestion during the brief intracellular punctures, which as indicated previously, are responsible for inoculation and acquisition on nonpersistent viruses.

The stylet pathway to the phloem is tortuous and largely intracellular; except for the brief intracellular punctures along the stylet pathway. As the styles approach the phloem, the stylet pathway may become highly branched, indicating that phloem location is often a trial and error process, and that aphids use cues obtained from intracellular sampling to locate the phloem. Once the aphid reaches the phloem, it will often continue to make intracellular punctures, each with a salivation and ingestion phase and puncture several sieve elements multiple times before settling into sustained phloem sap ingestion (defined as ingestion from a sieve element for longer than 10 min) which may last several hours or days. Sieve element penetrations that eventually attain phloem sap ingestion always begin with about 40–60 sec of salivation into the sieve element before the onset of ingestion. This large number of phloem salivation events into multiple sieve elements probably increases the likelihood of inoculating phloem limited viruses into a viable sieve element. Salivation into phloem sieve elements may counteract phloem sealing responses that would interfere with ingestion of phloem sap and many phloem limited viruses have taken advantage of this sieve element salivation behavior in order to facilitate inoculation.

From the beginning of contact with the plant surface it typically takes aphids several hours to reach a phloem sieve element and engage in sustained ingestion, however, aphids have been reported to reach the phloem in as little as 5 min. This timing may depend on several factors including but not limited to: aphid species, starvation of the aphid prior to plant exposure and plant species and cultivar.

Whiteflies

Whiteflies preferentially feed on the abaxial side of leaves in minor veins and all life stages are obligate phloem feeders. Newborn first instar nymphs are referred to as “crawlers” because they are mobile for a brief time while they search for a suitable settling site. They have only a few hours to tap into a phloem sieve element before they starve to death. All subsequent instars are sessile and therefore during the nymphal stage they do not play a role in virus dissemination. Each new instar forms new styles each after each molt and therefore must once again locate the phloem. Once tapped into the phloem, nymphs can feed from a single sieve element throughout their entire instar, alternating between phloem sap ingestion and a non-ingestion behavior that is presumed to be salivation. There is conflicting evidence regarding whether initial styllet penetration through the epidermis is intra- or intercellular, but once past the epidermis there is consensus that the penetration route is primarily intercellular.

Similar to aphids, the stylet pathway to the phloem is tortuous and intercellular; however, whiteflies make far fewer intracellular punctures than aphids. The great majority (90–100%) of intracellular punctures last for less than 10 sec, however occasionally, longer ones lasting up to 22.5 sec are made. Jiang et al. (1999) reported that whiteflies make an average of 6 intracellular punctures before reaching the phloem. Furthermore, whiteflies never make these brief punctures during short probes (<1 min), and rarely make them during the early stages of feeding. This extremely low number of intracellular punctures may explain why whiteflies transmit only 2–3 nonpersistently transmitted plant viruses; most viruses transmitted by whiteflies are persistent or semi-persistent phloem limited viruses. Even whitfly transmitted Ipomoviruses which are in the same family as the nonpersistent aphid transmitted Potyviruses have a semipersistent relationship with their whitfly vectors. The few intracellular punctures produced by whiteflies occur once the styles are close to the phloem, likely to aid in locating sieve elements and some of these may be penetrations into phloem sieve elements. The feeding behaviors that occur during these intracellular punctures do not follow the stereotypical pattern that aphid punctures do.

Adult whiteflies are primarily phloem sap feeders, but occasionally ingest xylem sap. Phloem salivation always precedes phloem sap ingestion. Jiang et al. (1999, 2000), reported relatively long periods of phloem salivation preceding phloem sap ingestion for Bemisia tabaci (biotypes A and B) feeding on tomato (most last <5 min in Jiang et al. 1999 and an average of 6.98 min in Jiang et al. 2000), but in our experience, the duration of phloem salivation prior to phloem sap ingestion for B. tabaci (biotype B) feeding on a number of plant species is similar to that in aphids: usually 20–60 sec (Walker GP, unpublished data). After salivating into the sieve element, whiteflies engage in phloem sap ingestion which can last from minutes to hours. Whiteflies are capable of reaching sustained phloem sap ingestion within 16 min, although most individuals require more than an hour to reach phloem after being placed on a plant. Whiteflies appear to require long bouts of phloem sap ingestion in order to acquire enough virions to enable subsequent inoculation irrespective of the type of virus.

Mealybugs

Mealybug nymphs and adult females are wingless, and while the nymphs are mobile and disperse on wind currents, adult females are relatively sedentary, and their feeding behaviors appear to be somewhere between whiteflies and aphids. The winged adult males do not feed. The stylet pathway to the phloem is intercellular and contains several intracellular punctures. Mealybugs appear to have less control over fine stylet movements than aphids and produce fewer (8–20/h) and longer intracellular punctures (20 sec) along the entire route to the phloem. Mealybugs rarely produce short probes (<1 min) and often reach the phloem after a single probe. However, it takes mealybugs a relatively long
time to reach the phloem. Some mealybugs were unable to tap into phloem sieve elements even after a period of 20 h, but most are able to reach the phloem in 1–6 h.77,78 Mealybug stylets are exceedingly long and are coiled within their body when they are not feeding.65 Stylet withdrawal from the plant is a slow and arduous task and involves a special mechanism to recoil the stylets. This unique mouthpart morphology may explain the propensity of mealybugs to make a single stylet insertion and the inability to reach the phloem quickly as is seen with other hemipterans. Phloem salivation is likely to occur before phloem sap ingestion, but this has not been experimentally proven. Once in the phloem, mealybugs may continue to feed from the same sieve tube for several days.77,78 Xylem ingestion is also a predominant feeding behavior for some mealybug species.79 Mealybug vectored viruses often exist as a complex of viruses, such as the mealybug wilt of pineapple complex which is made of three pineapple mealybug wilt associated viruses.79-81 All mealybug transmitted viruses appear to have a semipersistent mode of transmission based on retention times; however *Grapevine leafroll-associated virus* 3 was found in the salivary glands of its mealybug vector, suggesting a circulative mode of transmission.82 Mealybug transmitted viruses appear to have a high rate of acquisition and low rate of inoculation.78

**Hoppers**

Leafhoppers are able to transmit semipersistent and persistent viruses and, along with the planthoppers, transmit the largest number of persistent-propagative viruses of any vector group.12,16 Hoppers exhibit the most diverse feeding behaviors of all the different vector groups with different hopper species categorized as preferentially feeding from phloem, xylem or mesophyll. In general, most hopper vectors are those that feed preferentially from phloem,12 and therefore we will focus on hoppers with this feeding type. For a thorough description of the different types of cell rupture feeding utilized by leafhoppers, see Backus et al. (2005). Although many leafhoppers are classified as phloem feeders, they still spend a significant amount of time feeding from other tissues.83-85 Leafhoppers have been reported to feed actively on non-phloem tissue (xylem and mesophyll) for up to 75% of the time observed.83,84-85 The hoppers have much larger mouthparts than the other vector groups and because of this they do not feed as delicately. Likely due to the size of their mouthparts, most leafhopper use an intracellular route to reach the phloem,80 but some, such as *Cicadulina storeyi*, use a largely intercellular pathway.91 Planthoppers and leafhoppers are able to reach the phloem in as little as 30 sec.12,92 They are also able to inoculate phloem limited viruses in only a few minutes.12,92-95

Hoppers vary greatly in the behaviors they engage in upon reaching a phloem sieve element. Some hoppers always engage in phloem salivation before phloem sap ingestion.85,91 Some only occasionally engage in phloem salivation before phloem sap ingestion,84,92 some alternate between phloem salivation and phloem sap ingestion86 and some engage in long bouts of extravasation before phloem sap ingestion.97,98 It is during this extravasation behavior that leafhopper foregut bound semipersistent viruses, such as *Maize chlorotic dwarf virus* are transmitted.25,26 Interestingly Wayadande and Nault (1993) showed that the EPG waveform associated with leafhopper extravasation (X wave) has a different pattern depending on whether or not the leafhopper is a vector or non-vector species. Leafhoppers are also highly variable in the time spent in phloem sap ingestion. Leafhoppers usually engage in phloem sap ingestion for only 5–30 min at a time but will occasionally ingest phloem sap for several hours.84,96,99 The strategies that hoppers use during phloem sap ingestion are not entirely clear. The fact that they predominantly engage in sustained phloem sap ingestion for short periods of time, and that the stylets of some species are larger than the diameter of phloem sieve elements indicate that they may destroy these cells during their feeding process. However, they are able to efficiently transmit a large number of persistent-propagative viruses and spiroplasmas that are completely limited to the phloem which indicates that at least some phloem cells in the immediate feeding area remain intact. Stafford and Walker (2009) (Fig. 10) provided evidence that salivary secretions of the beet leafhopper extend into cells beyond those penetrated by the stylet tips, which would enable the movement of phloem limited pathogens into viable phloem cells. Nymphal stages are more efficient vectors than the adults for many hopper transmitted viruses.12,95,100,101 This may be because the stylets of the nymphal stages are smaller than those of the adults and therefore nymphal feeding behaviors may be less destructive.

**Thrips**

Thrips mouthparts are unique within the Hemipteroid superfamily. The stylets are asymmetrical and housed within a flexible structure called the mouthcone. Thrips have a single left mandibular stylet, often referred to as a peg, which is occasionally used to puncture plant tissue before insertion of the maxillary feeding canal and therefore it is not understood exactly how thrips secrete saliva during feeding, especially when they ingest and salivate concurrently. Thrips are cell rupture feeders and do not feed from phloem. Due to the type of feeding damage that they inflict, thrips have been mistakenly referred to as raspers. They exhibit three main types of probing behaviors: noningestion probes, short- and long-ingestion probes.103 Noningestion probes are very short, (around 1 sec) and are initiated by insertion of the mandibular peg, which occurs when the thrips thrusts its head downward and backward.103-106 Thrips may produce multiple noningestion probes in rapid succession thrusting their heads up and down many times; this is referred to as head nodding.104 Thrips salivate before and during noningestion probes, but do not actively ingest plant sap.103 Noningestion probes likely leave cells intact and available for virus replication; therefore these probes are believed to be most important for virus inoculation, similar to the very short exploratory probes produces by aphids.103 Several authors have reported that insertion of the mandibular peg precedes all probing behaviors,102,104 however we found that short- and long-ingestion probes usually begin with an insertion of the maxillary stylets only.103 During short ingestion
probes thrips first salivate into, and then empty out individual epidermal and mesophyll cells. During long ingestion probes, thrips engage in sustained bouts of ingestion, (which may last over 1 h) potentially from multiple previously damaged cells or the xylem. Due to the destructive nature of these probe types, they likely play a very minor role in virus inoculation; but depending on the food source they likely play a major role in virus acquisition.

Tospoviruses are circulative- persistent-propagative viruses exclusively transmitted by thrips and they must be acquired during the nymphal stages. Persistent-propagative viruses have the most intimate association with their vectors because the virus replicates inside of the vector. Tospoviruses likely evolved from an animal virus originally infecting their thrips vector. Tospoviruses provide an excellent example of how vector feeding behavior directly influences transmission of the virus. Because these viruses are introduced into plants with thrips saliva, they are initially able to enter only into cells that thrips feed on. Therefore, they are unique among the circulative viruses because transmission does not involve the phloem tissue.

Conclusions

We recently showed that vector infection with Tomato spotted wilt virus alters thrips feeding behavior in a way that increases the probability of virus transmission. Taken together with the fact that closely related animal viruses within the Bunyaviridae also cause increased biting rates in infected vectors, we proposed that the ability of the virus to modify its vector's feeding behavior evolved as a mechanism to enhance virus transmission. We also proposed that the evolutionary advantage conferred by this trait has caused it to be conserved among plant- and animal-infecting members of the Bunyaviridae. Our data further underscores the importance of vector feeding behavior in virus transmission and we feel that future studies will identify many more virus-vector interactions that result in direct changes in vector feeding behavior. As with the indirect change in vector behavior caused by virus infected plants, we hypothesized that the direct alterations to feeding behaviors caused by vector infection by viruses may be dependent on the mode of transmission. Cuticle borne viruses may block the food canal and forestall triggering salivation or egestion to clear the stylets, in much the same way as Yersinia pestis clogs the foregut of fleas causing them to regurgitate into their host. Circulative viruses which must invade the salivary glands to be secreted into the plant likely alter vector salivary secretions and propagative viruses that infect multiple tissues in the vector, especially neural tissues, are likely to greatly impact overall vector behaviors. Furthermore, interaction of virions with receptors in the gut or simply the ingestion of virions may affect feeding behavior.

The continuation of vector feeding behavior studies is essential to further our understanding of complex plant-virus-vector interactions. The study of vector feeding behavior has always required the use of a great number of cutting edge techniques. As we move into the “omics” age, the utilization of genomics, transcriptomics and proteomics offers additional tools to further our understanding of why insects feed the way they do and how viruses exploit this. Studies on the composition of salivary proteins are allowing us to answer questions about why aphids must engage in extensive salivation into the phloem before they are able to engage in sustained phloem sap ingestion. These types of studies may also be able to explain why leafhoppers salivate into the phloem only sometimes and why thrips behavior is modified when their salivary glands are infected with tospoviruses.

In conclusion, our understanding of plant virus-vector interactions has come a long way since the early hypothesis of vectors acting essentially as flying “dirty needles,” where viruses spread by simple contamination of the insects’ mouthparts. It is now clear that both acquisition and inoculation depend on the insects engaging in very specific behaviors and that behavioral differences among piercing-sucking insects, all of which may superficially appear to be potential vectors, may play a significant role in determining which species actually are vectors and which are not. The recent work on thrips and tospoviruses also demonstrates that in addition to viruses “selecting” vectors based on the insects’ innate species-specific feeding behaviors, viruses can also directly modify the insects’ feeding behavior to their own advantage. We believe that future studies on feeding behavior of plant virus vectors will continue to make new discoveries and shed further light on the intimate relationship between plant viruses and their vectors.

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