Virus Transmission by Leafhoppers, Planthoppers and Treehoppers (Auchenorrhyncha, Homoptera)

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I. INTRODUCTION

Conti (1985) summarized the first report of transmission of a plant virus by an insect, a leafhopper. In 1883, a Japanese rice farmer suspected that a dwarfing disease of rice was associated with leafhoppers, and a year later, he demonstrated experimentally the causal relationship of leafhoppers to the disease. The leafhopper species used in the study was not identified nor were the results published. Investigations 20 years later at the Imperial and Shiga Agricultural Experiment Station in Japan led to the detection of both vector and non-vector

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leafhopper species, and showed that feeding the former species on infected rice plants made them vectors of the disease agent. In subsequent studies, the disease agent was identified as the *Rice dwarf virus* and the vectors as *Recilia dorsalis* and *Nephotettix cincticeps*.

That the first discovered hopper-borne plant virus infects a monocotyledonous host is no surprise. Of the nearly 60 plant viruses discovered to date that have a hopper (Auchenorrhyncha) vector, fewer than 10% infect dicotyledonous plants (Table I). Many of the hopper vectors are delphacids, all of which are grass feeders, as are most of the known cicadellid leafhopper vectors. Bias towards research on these species would be expected with the large number of economically important viruses that infect three of the world's most important grain crops: rice, wheat, and maize (corn). This bias towards hopper vectors of monocotyledonous plant-infecting viruses may not reflect the true host plant distribution of hopper-transmitted viruses (Nault, 1997).

Researchers may have barely tapped the number and diversity of hopper-borne viruses. For example, in the *Fulgoroidea*, the superfamily that includes 20 planthopper families, the delphacids are unlikely to be the only important vector group, e.g. a cixiid is reported as a vector of an unclassified coconut virus (Table I) as well as several plant-infecting phytoplasmas (O'Brien and Wilson, 1985). With the exception of the delphacids, which account for approximately 16% of the more than 7000 described *Fulgoroidea*, most planthoppers have a tropical distribution and feed on perennial dicotyledonous hosts. Tropical viruses have not been as well studied as viruses of temperate crops, or viruses of cultivated or wild perennials. It is quite likely that, as more attention is given to viruses of tropical perennials, planthopper vectors from the other 19 Fulgoroid families will be discovered. Similarly, many of the more than 15 000 cicadellid leafhopper species described, which may represent only one-third of extant species (Nielson, 1985), also have tropical distributions and feed on perennials. Leafhoppers are potential vectors of a wide array of plant viruses of tropical perennials. Although a membracid treehopper has been identified as a vector of a single virus of subtropical distribution, additional vectors are likely to be discovered.

The Fulgoroidea, Cicadellidae and Membracidae are the most likely families in which vectors will be found, not only because of the many known species and perhaps many more undiscovered species, but because they feed primarily on the phloem. The cicadas and cercopids, which feed in the xylem, in which viruses rarely occur, are unlikely to be virus vectors. Thus, much of what is known concerns viruses and vector species from temperate North America, Europe, Australia and Japan and annual graminaceous crops, mostly rice, wheat and maize (corn).

In this chapter we also use examples from hopper-borne mollicutes (phytoplasmas and mycoplasmas), which are transmitted in a similar way to persistently transmitted, propagative plant viruses. Earlier reviews on transmission of hopper-borne viruses and mollicutes are by Corbett and Sisler (1964), Maramorosch and Harris (1979), Nault and Rodriguez (1985), Denno and Perfect (1994) and in Nault and Ammar (1989), Ammar (1994a) and Nault (1997).

TABLE I

Plant viruses transmitted by leafhoppers, planthoppers and treehoppers arranged according to their mode of transmission, virus group and vector family

Transmission mode (and virus family and genus)	Virus	Vector family	Vector genus
Semipersistent (Foregut-borne)			
Caulimoviridae			
Badnavirus	Rice tungro bacilliform	Cicadellidae	*Nephotettix*
Sequiviridae			
Waikavirus	Maize chlorotic dwarf	Cicadellidae	*Graminella* and six others
	Rice tungro spherical		*Nephotettix, Recilia*
Persistent:circulative			
Geminiviridae			
Mastrevirus	Maize streak	Cicadellidae	*Cicadulina*
	Chloris striate mosaic		*Nesoclutha*
	Panicum streak virus		*Cicadulina* (Briddon *et al.*, 1992)
	Paspalum striate mosaic		*Nesoclutha*
	Pearl millet streak		*Cicadulina* (Briddon *et al.*, 1996b; Ikegami *et al.*, 1992)
	Miscanthus streak		
	Sugarcane streak		*Cicadulina* (Ammar, 1994b)
	Wheat dwarf		*Psammotettix* (Bendahmane *et al.*, 1995)
Curtovirus	Beet curly top	Cicadellidae	*Circulifer*
	Chickpea chlorotic dwarf		*Orosius* (Horn *et al.*, 1994)
	Tobacco yellow dwarf		*Orosius*
	Tomato pseudo-curly top	Membracidae	*Micrutalis* (Simons, 1962)
Nanovirus	Coconut foliar decay	Cixiidae	*Myndus* (Julia, 1982)
Persistent:propagative			
Marafivirus	Bermuda grass-etched line	Cicadellidae	*Aconurella*
	Maize rayado fino		*Dalbulus* and three others
	Oat blue dwarf		*Macrosteles*
Rhabdoviridae			
Nucleorhabdovirus	Cereal chlorotic mottle	Cicadellidae	*Nesoclutha, Cicadulina*
	Oat striate mosaic		*Graminella*
	Potato yellow dwarf*		*Aceratagallia, Agallia, Agalliopsis*
	Rice transitory yellowing		*Nephotettix*
	Sorghum stunt mosaic		*Graminella*
	Wheat striate mosaic*		*Endria, Elymana*
	Winter wheat mosaic		*Psammotettix*
	Barley yellow striate mosaic*	Delphacidae	*Laodelphax*
	Colocasia bobone disease		*Tarophagus*
	Cynodon chlorotic streak		*Toya*
	Digitaria striate		*Sogatella*
	Finger millet mosaic		*Sogatella, Peregrinus*
	Iranian maize mosaic		*Ribautodelphax*
	Maize mosaic		*Peregrinus*
	Maize sterile stunt		*Sogatella, Peregrinus*
	Northern cereal mosaic		*Laodelphax, Muellerianella, Ribautodelphax, Unkanodes*
	Wheat chlorotic streak*		*Laodelphax*
	Wheat rosette stunt		*Laodelphax*

continued

TABLE I (*continued*)
Plant viruses transmitted by leafhoppers, planthoppers and treehoppers arranged according to their mode of transmission, virus group and vector family

Transmission mode (and virus family and genus)	Virus	Vector family	Vector genus
Persistent:propagative (cont.)			
Reoviridae			
Phytoreovirus	Rice dwarf*	Cicadellidae	*Nephotettix, Recelia*
	Rice gall dwarf*		*Nephotettix, Recelia*
	Wound tumor*		*Agallia, Agalliopsis, Aceratagallia*
Fijivirus	*Arthraterum* blue dwarf	Delphacidae	*Javesella, Dicranotropis,*
	Cereal tillering disease		*Laodelphax, Dicranotropis*
	Fiji disease*		*Perkinsiella*
	Oryzae ciation disease		*Javesella*
	Maize rough dwarf*		*Laodelphax, Delphacodes, Javesella, Sogatella*
	Oat sterile dwarf*		*Javesella, Dicranotropis Ribautodelphax*
	Pangola stunt		*Sogatella*
	Rice black streaked dwarf		*Laodelphax, Unkanodes*
	Rice ragged stunt		*Nilaparvata*
	Echinochloa ragged stunt		*Sogatella*
Tenuivirus	European wheat striate mosaic*	Delphacidae	*Javesella*
	Maize stripe*		*Peregrinus*
	Rice grassy stunt		*Nilaparvata*
	Rice hoja blanca*		*Sogatodes*
	Rice stripe*		*Laodelphax, Terthron, Unkanodes*
	Rice wilted stunt		*Nilaparvata* (Chen and Chiu, 1989)
	Maize yellow stripe	Cicadellidae	*Cicadulina* (Ammar *et al.*, 1989)

* indicates that transovarial transmission of the virus has been reported with some vectors.
For further references see Nault and Ammar (1989) and Ammar (1994a).

II. SEMIPERSISTENT TRANSMISSION OF FOREGUT-BORNE VIRUSES

Three plant viruses are known to be transmitted in a semipersistent manner by leafhoppers (Table I). Two are waikaviruses, *Maize chlorotic dwarf virus* (MCDV) and *Rice tungro spherical virus* (RTSV), and one a badnavirus, *Rice tungro bacilliform virus* (RTBV). These three viruses are located mainly in phloem tissues of their host plants, although RTBV has also been found in xylem (Hibino, 1989; Nault and Ammar, 1989; Cruz *et al.*, 1993).

MCDV is mainly transmitted by *Graminella nigrifrons*, which can acquire MCDV from infected plants or inoculate healthy ones in 15 min (Choudhury and Rosenkranz, 1983), whereas *Nephotettix virescens* requires 30 min to

acquire or inoculate RTSV (Inoue and Hirao, 1981). However, longer acquisition and inoculation access periods increase transmission efficiency for both viruses (Nault *et al.*, 1973; Ling and Tiongco, 1979). The minimum acquisition and inoculation periods required are assumed to be the time required for the leafhopper vector to reach the phloem. With MCDV or RTSV, the average persistence of inoculativity by leafhopper vectors at 25°C or warmer is less than 24 h, but when starved or at cooler temperatures virus inoculation is retained for a few days. Both sexes of the adult leafhoppers as well as nymphs can transmit these viruses, but inoculativity is lost upon moulting (Ling and Tiongco, 1979; Choudhury and Rosenkranz, 1983).

The *Rice tungro bacilliform virus* (RTBV) can be transmitted by its leafhopper vector *N. virescens* only from plants co-infected with RTSV, or from plants singly infected with RTBV if leafhopper vectors had previous access to RTSV-infected plants (Hibino, 1989). However, when the agrobacterium-mediated transfer technique was used to transfer RTBV DNA to maize plants, tungro disease symptoms were induced (Dasgupta *et al.*, 1991). Furthermore, RTSV-inoculative leafhoppers held for 2–3 days on healthy plants, so that their ability to transmit RTSV is lost, can acquire and transmit RTBV for another 3–4 days (Cabautan and Hibino, 1985). Also, when leafhoppers carrying RTSV were fed RTSV antiserum, they could not transmit RTSV, but could acquire and transmit RTBV (Hibino and Cabautan, 1987). These results suggest that a helper factor or component, other than the RTSV virions, is required by leafhopper vectors to acquire and transmit RTBV. However, it is not known whether acquisition and transmission of RTSV by its leafhopper vector similarly requires a helper component.

A helper component from infected plants is apparently required for transmission of purified MCDV by *G. nigrifrons* (Hunt *et al.*, 1988; Creamer *et al.*, 1993). Purified MCDV (WS strain), acquired by membrane feeding, was transmitted by leafhoppers that had been fed first on maize plants infected with another strain (M1) of MCDV. Conversely, feeding on MCDV (WS)-infected plants allowed transmission of purified MCDV-M1, indicating that the putative helper component is not strain specific. Viruliferous *G. nigrifrons* lost the ability to transmit MCDV-M1 after feeding for 24 h on healthy plants, but retained the ability to acquire and transmit purified MCDV-WS for up to 36 h. Another vector species of MCDV (*Amblysellus grex*) also transmitted purified MCDV-WS after an initial acquisition feeding on MCDV-M1-infected plants, indicating that the MCDV helper component may not be species specific (Creamer *et al.*, 1993). However, unlike the helper component of the aphid-borne, non-persistently transmitted potyviruses, which has been isolated and fairly well characterized (Pirone and Perry, this volume), efforts to isolate and characterize the putative helper component of MCDV have so far been unsuccessful.

Transmission electron microscopy was used to investigate possible retention sites for MCDV in some vector and non-vector species that had fed on MCDV-infected plants (Ammar and Nault, 1991). Virus-like particles (VLP) were found embedded in a semi-opaque matrix attached to the cuticular lining of the

oesophagus, cibarium, precibarium and occasionally to the inner surface of the maxillary food canal in *G. nigrifrons*, two other vector species and the inefficient vector *Dalbulus maidis*. However, these VLP were not found in the non-vector delphacid planthopper, *Peregrinus maidis*. The matrix, but not the VLP, was seen in *G. nigrifrons* after a 4-day feeding period on healthy plants following MCDV acquisition. It was concluded that the matrix-embedded VLP were MCDV virions attached to putative retention sites on the cuticular lining of the foregut in vector leafhoppers. Ammar and Nault (1991) further suggested that the matrix in which the VLP were embedded might be the helper component required for leafhopper transmission of MCDV.

With MCDV, the occurrence of similar retention sites in an efficient vector (*G. nigrifrons*) and in an inefficient vector (*D. maidis*), but not in a non-vector (*P. maidis*) suggests that, in addition to the attachment (or binding) of virions to the foregut cuticle, other factors may play a role in vector specificity of this virus (Ammar and Nault, 1991). Wayadande and Nault (1993) found that MCDV is inoculated by *G. nigrifrons* when producing X-waveforms, the electronically monitored signal associated with phloem probing. The X-waveforms of *G. nigrifrons* were qualitatively similar to those of four other MCDV-vector species, but were distinct from those of the inefficient vector *D. maidis* and non-vector species. Thus, it was suggested that MCDV inoculation by *G. nigrifrons* is associated with extravasation (expulsion of fluid from the leafhopper's food canal formed by the maxillary stylets and the precibarium), which may be absent or rare in non-vector or poor vector leafhopper species.

Feeding behaviour was also monitored in adult females of the leafhopper *N. virescens*, which had been starved for 2 h following a 4-day acquisition access period on rice plants infected with both RTSV and RTBV (Dahl *et al.*, 1990). Four waveform patterns were observed that indicated probing, salivation, phloem feeding and xylem feeding. Of 16 females tested, 11 transmitted the viruses in overnight feeding, while the other five transmitted them only once in ten serial transfers. The average duration of phloem feeding was longer in transmitting females than in those that did not transmit. It was suggested that transmission of these viruses was associated with phloem feeding; the minimum phloem-feeding period for virus transmission was 30 s. In addition to the effects of feeding behaviour on virus transmission, the possible inhibitory effects of the vector's salivary secretions on plant viruses (Nishi, 1969) during the acquisition, retention or inoculation phases of transmission may be important.

III. PERSISTENT TRANSMISSION OF CIRCULATIVE VIRUSES

Eleven geminiviruses are persistently transmitted in a circulative (non-propagative) manner by cicadellid leafhoppers, whereas one geminivirus, *Tomato pseudo-curly top virus* (TPCTV) is transmitted in this manner by a membracid treehopper (Table I). Mastrevirus, of which *Maize streak virus* (MSV) is the type

member, contains several viruses that are transmitted by leafhoppers. Curtovirus, which has only one well-characterized virus, *Beet curly top virus* (BCTV), is also transmitted by leafhoppers.

The minimum acquisition access periods (AAP) for MSV, particles of which have been observed in mesophyll and phloem tissues of infected maize leaves (Ammar, 1994b), was 15 s by *Cicadulina mbila*, 15 min by *C. ghaurii*, and 1 h by *C. arachidis* (Asanzi *et al.*, 1995). The minimum inoculation access period (IAP) for MSV, however, was 5 min by *C. mbila*, and 1 h by *C. ghaurii* and *C. arachidis*. Transmission efficiency by these two species was closely associated with log AAP and log IAP. MSV transmission by these two species ranged between 15% and 45%, with *C. arachidis* being the less efficient vector. Similarly, with *C. mbila*, transmission efficiency of MSV was significantly greater after a 50-h AAP compared with a 3-h AAP (Reynaud and Peterschmitt, 1992). Also, using enzyme-linked immunosorbent assay (ELISA), there were more ELISA-positive insects after 50-h than after 3-h AAP. However, a decrease in the proportion of ELISA-positive insects occurred from day 17 post-AAP. Using a calibration curve obtained with purified MSV, which detected as little as 0.15 ng of virus/insect, a mean value of 0.36 ng of MSV/leafhopper was found 3 days after the AAP, whereas 14 days later there was only 0.20 ng of virus/insect. These results suggested that MSV does not multiply in *C. mbila*.

The latent period (LP) of geminiviruses in their leafhopper vectors ranges from 4 to 19 h (Harrison, 1985). MSV transmission is trans-stadial, i.e. nymphs do not lose inoculativity upon moulting, but apparently is not transovarial or vertical, i.e. inoculativity is not passed from adult females to their progeny. When the abdomens of leafhoppers from an inactive/inefficient vector race of *C. mbila* were punctured with a fine needle, either before or just after acquisition of MSV from diseased plants, they became more efficient MSV vectors than unpunctured hoppers (Storey, 1933). This classic experiment demonstrated that the gut wall in leafhoppers is a possible barrier to MSV transmission. However, non-vectors and inefficient *Cicadulina* spp. vectors of MSV acquire and retain smaller amounts of virus than the efficient vector *C. mbila* (Boulton and Markham, 1986). Markham (1992) reported that MSV passes from the gut of its vector, *C. mbila*, to the haemocoel via the filter chamber and anterior cells of the ventriculus (midgut) by receptor-mediated endocytosis. Some species within the genus *Cicadulina* apparently lack these gut receptors but maintain them in the salivary glands; thus they can transmit needle-injected but not orally acquired MSV. This suggests that the transmission mechanism of MSV is different from that of luteoviruses that also are persistently transmitted (by aphids) in a circulative manner (Reavy and Mayo, this volume).

For *Chickpea chlorotic dwarf geminivirus* (CCDV), transmitted by the leafhopper *Orosius orientalis*, transmission characteristics were similar to those of MSV (Horn *et al.*, 1994). Also, using ELISA, CCDV did not appear to multiply in its vector and its concentration decreased when the leafhopper fed on a non-host of the virus. It is possible that other geminiviruses may multiply in

their vectors at a very low rate that may be detectable using molecular methods, e.g. DNA hybridization or polymerase chain reaction (PCR).

The role of the viral coat protein in vector specificity of geminiviruses has been shown by gene replacement experiments (Briddon *et al.*, 1990). Chimaeric clones were constructed in which the coat protein encoded by the whitefly-transmitted *African cassava mosaic virus* (ACMV) was replaced by that of the leafhopper-transmitted BCTV. Tobacco plants were infected with the chimaeric DNA by agroinoculation and the resultant virus, which induced typical ACMV symptoms, was injected into *Circulifer tenellus*, the leafhopper vector of BCTV. This leafhopper transmitted the chimaeric ACMV, indicating that the specificity of leafhopper transmission of BCTV is determined mainly by the virus coat protein

The treehopper-transmitted TPCTV has acquisition and inoculation thresholds in its membracid vector, *Micrutalis malleifera*, of less than 1 h, and a latent period of 24–48 h; retention of inoculativity was positively correlated with the length of AAP (Simons, 1962). TPCTV transmission can also be achieved by injecting the treehopper vector with crude sap or partially purified preparations of the virus. Both adults and nymphs are efficient vectors of TPCTV, and the nymphs retain virus inoculativity after moulting. The LP 50 of TPCTV in *M. malleifera* was estimated to be 15 h after a 6 h AAP. Briddon *et al.* (1996a), indicated that the genome of TPCTV had features typical of both Mastreviruses and Curtoviruses. The coat protein, although distinct from all previously characterized geminiviruses, was more similar to the leafhopper-transmitted, than those of the whitefly-transmitted, geminiviruses. This provides support for the view that vector specificity of geminiviruses is determined mainly by the coat protein.

IV. PERSISTENT TRANSMISSION OF PROPAGATIVE VIRUSES

At least 41 plant viruses, belonging to five groups (or genera), are transmitted either by leafhoppers or planthoppers in a persistent propagative manner (Table I). Propagative viruses are those that have been proven to multiply in their vectors. Such multiplication can be demonstrated by transmission (bioassay) studies in which virus is passed serially, by injection from one insect to another, until the dilution injected attained into the final inoculative insects exceeds the dilution endpoint of the initial inoculum (Banttari and Zeyen, 1976; Sinha and Chiykowski, 1969; Harris, 1979; Nault, 1997). For example, *Rice stripe virus* (RSV), was serially injected into its planthopper vector *Laodelphax striatellus*, until its dilution (presuming non-multiplication in the vector) reached 1.25×10^6, which exceeds the dilution endpoint of this virus (Okuyama *et al.*, 1968). Transmission electron microscopy of thin sections of viruliferous insects, combined with immunolabelling of virus particles or virus-encoded proteins, can also give evidence of virus multiplication in the vector, by demonstrating the presence of aggregated virus particles, protein products or sites of virus assembly in vector cells and

tissues (Ammar and Nault, 1985; Shikata, 1979). However, more direct evidence of virus multiplication in the vector is obtained by quantitative serology using ELISA, dot blot, tissue blot or other assays that show an increase in virus titre in the vector after a relatively short AAP on a virus source (Gingery *et al.*, 1982; Falk *et al.*, 1987; Nault and Gordon, 1988).

Propagative viruses usually require longer latent periods, and are retained longer, in their vectors than circulative (non-propagative) viruses (Sinha, 1973; Conti, 1985). Nault (1994) indicated that the mean latent period ± SE of 13 propagative viruses, from four virus groups, was 368 ± 41 h, compared to 23 ± 4.1 h for 10 circulative (non-propagative) viruses from three groups. For non-propagative viruses, the latent period is presumed to be the time necessary for the virus to circulate in the vector, i.e. from ingestion of virus from a virus source to inoculation of virus. However, with propagative viruses, the longer latent period may also be necessary for virus multiplication in various tissues of the vector before moving into the salivary secretions of the vector. Another important difference between propagative and non-propagative viruses, is that transovarial transmission has been reported and confirmed only with propagative viruses (Sinha, 1981; Nault and Ammar, 1989).

A. *MARAFIVIRUS* GROUP

Three serologically related marafiviruses are transmitted by leafhoppers (Table I). Acquisition and inoculation thresholds for these viruses range from several minutes to several hours; longer feeding periods result in more efficient transmission. A latent period of 7 days or longer post acquisition of virus from infected plants, is required before the leafhoppers become inoculative. However, injection of *Maize rayado fino virus* (MRFV) into the haemocoel of vector insects decreased this period to 1–3 days and increased the transmission rate by leafhoppers (Gamez and Leon, 1988; Nault *et al.*, 1980). Marafiviruses were proven to multiply in their vectors but transovarial transmission of these viruses has not been reported (Banttari and Zeyen, 1976; Gingery *et al.*, 1982; Gamez and Leon, 1988).

B. RHABDOVIRIDAE

Seven plant rhabdoviruses are transmitted by cicadellid leafhoppers and 11 are transmitted by delphacid planthoppers (Table I). Other plant rhabdoviruses are transmitted by aphids, lacebugs (piesmids) or mite vectors (Jackson *et al.*, 1987). Acquisition thresholds of <1 min for *(American) wheat striate mosaic virus* (AWSMV) and 5–15 min for *Rice transitory yellowing virus* (RTYV) are probably due to the more general distribution of AWSMV in mesophyll as well phloem plant cells (Lee, 1967), compared to the restricted distribution of RTYV in the phloem and occasionally in bundle sheath cells (Chen and Shikata, 1971). Inoculation thresholds for both of these viruses were <15 min.

The latent period in the vector for RTYV was 3–66 days. With AWSMV, the latent period was shorter in efficient vectors compared with inefficient ones (Slykhuis, 1963). Some plant rhabdoviruses are transmitted transovarially, usually at a very low rate (1–4%) in their vector leafhoppers, planthoppers or aphids (Sinha, 1981).

C. REOVIRIDAE

Three plant reoviruses of the genus *Phytoreovirus* are transmitted by cicadellid leafhoppers and ten viruses of the genus *Fijivirus* are transmitted by delphacid planthoppers (Table I). The acquisition and inoculation thresholds of plant reoviruses range from a few minutes for *Rice dwarf virus* (RDV), which has been found in the mesophyll of infected leaves, to several hours for most others that have been found mainly in phloem (Conti, 1984; Francki and Boccardo, 1983). Latent periods range from 2 days for *Rice ragged stunt virus* (RRSV) in *Nilaparvata lugens* to 2 months for *Pangola stunt virus* (PaSV) in *Sogatella furcifera*; most latent periods are between 7 and 14 days. The vector usually retains virus inoculativity for life, frequently with intermittent transmission (Conti, 1984). The three leafhopper transmitted phytoreoviruses are transovarially passed to the progeny of their vectors, with efficiencies ranging from 1.8% to 100%. Only a few of the studied fijiviruses, however, are transovarially transmitted (0.2–17%) in their planthopper vectors (Sinha, 1981; Conti, 1984).

D. *TENUIVIRUS* GROUP

Six tenuiviruses are transmitted by delphacid planthoppers, and a tenui-like virus is transmitted by a cicadellid leafhopper (Table I). Acquisition thresholds range from 10 min to 4 h, whereas inoculation thresholds range from 30 s to nearly an hour. Latent periods in the vector range from 3 to 36 days, but most are between 7 and 21 days. Virus retention periods in the vector are up to 84 days post acquisition, but inoculativity usually declines with vector age. Transovarial transmission to a large proportion of the progeny of the vector (21–100%) has been reported for most tenuiviruses (Falk and Tsai, 1998; Nault and Ammar, 1989). However, no transovarial transmission has been reported for *Rice grassy stunt virus* or *Rice wilted stunt virus* in their planthopper vector *N. lugens* (Chen and Chiu, 1989), or for *Maize yellow stripe virus* (MYSV) in its leafhopper vector *Cicadulina chinai* (E.D. Ammar and E.A. Khalifa, unpublished observations).

For MYSV, acquisition and inoculation thresholds were 30 min each, the latent period ranged from 4.5 to 8 days, and the retention period was up to 27 days (Ammar *et al.*, 1989). ELISA tests on viruliferous leafhoppers indicated that MYSV multiplies in its vector (Mahmoud *et al.*, 1996; E.D. Ammar and E.A. Khalifa, unpublished observations). MYSV may represent a new group of

propagative leafhopper-borne viruses or a subgroup of tenuiviruses transmitted by leaf hoppers. *Maize fine stripe virus,* recently reported from Peru and apparently related to MYSV, is transmitted by the leafhopper *Dalbulus maidis* (Injante-Silva *et al.*, 1997).

V. TRANSMISSION BARRIERS AND VECTOR COMPETENCE

With vector-borne viruses, the terms 'vector specificity', 'vector efficiency' and 'vector competence' are often used to describe the comparative ability of certain species, biotypes or lines of vector insects to transmit a certain virus or a virus strain (Hardy, 1988). Also, differences in transmission correlated with the age, sex or wing-form of vector insects have been reported with several viruses (Harris, 1979; Nault and Ammar, 1989).

Before an insect can transmit any circulative/propagative virus they must have: (1) ingested a virus; (2) the virus must have entered the cells of the insect midgut; (3) the virus must then be released from these cells into the haemocoel; (4) virus must enter the salivary glands; (5) virus must be released into the saliva; and (6) the insect must feed on a susceptible host.

The route of some propagative plant viruses has been studied in their vector leafhoppers and planthoppers, e.g. for WTV in its leafhopper vector *Agallia constricta* using immunofluorescence microscopy (Sinha, 1965), and for AWSMV in its vector *Endria inimica* using infectivity bioassays (Sinha and Chiykowski, 1969). AWSMV was recovered from the alimentary canal of its vector 2 days, and from the salivary glands 4 days after a 1-day AAP on diseased plants.

The multiplication of *Maize stripe virus* (MStV) in its planthopper vector *P. maidis* has been followed by quantitative serology (Nault and Gordon, 1988). ELISA values for MStV increased significantly from 2 to 23 days postacquisition. On day 7, more midguts than ovaries were infected, whereas no virus was detected in the salivary glands 7–9 days postacquisition. On days 16 and 23, however, these three organs were infected in most tested specimens.

Four groups of barriers to transmission of propagative viruses in their vectors have been identified (Hardy, 1988; Ammar, 1994a): (1) midgut infection barrier; (2) dissemination (including midgut-escape and salivary gland infection) barriers; (3) salivary gland escape barrier; and (4) transovarial transmission barriers.

A. MIDGUT INFECTION BARRIER

The insect midgut consists mainly of a single layer of epithelial cells, with extensive microvilli on the lumen side, and a porous basal lamina on the haemocoel side. The morphology of the midgut, however, differs markedly between leafhoppers, planthoppers and other homopteran vectors, e.g. aphids and whiteflies (Ammar, 1985; Cheung and Purcell, 1993). Midgut infection thresholds of virus concentration have been demonstrated for several arboviruses in

mosquitoes (Hardy, 1988). However, with plant viruses the term 'acquisition threshold' usually refers to the minimum time required for exposure or feeding on diseased plants, after which an insect vector can become viruliferous (virus-carrying or virus-infected). This 'acquisition threshold' possibly involves both the time necessary for the insect stylets to reach the plant tissue from which the virus can be acquired, e.g. mesophyll or phloem, as well as a 'threshold titre' of virus that must be ingested before infection of the vector can occur (Ammar, 1994a). This is indicated by several studies that show a positive correlation between longer AAP on diseased plants and greater efficiency of transmission for many propagative plant viruses by their vectors. For example, the percentage of *Nephotettix nigropictus* transmitting *Rice gall dwarf virus* (RGDV) increased from 12% to 96% when the AAP on diseased plants was increased from 4 h to 12 days, although the latent period of RGDV in the vector did not differ significantly (13.6–14.6 days) in either case (Inoue and Omura, 1982). Also, with RGSV, the percentage of inoculative planthoppers increased from 10% to 61.5% as the AAP increased from 30 min to 3 days (Mathew and Basu, 1986).

A gut barrier has been demonstrated in leafhopper transmission of several propagative viruses including *Wound tumor virus* (WTV), *Maize mosaic virus* (MMV) and MRFV. Efficiency of WTV transmission usually decreases with the age of the vector following acquisition of virus from infected plants. Results from abdominal puncture and fluorescent antibody techniques on WTV in its leafhopper suggest that both the susceptibility of the midgut epithelial cells to infection and gut permeability to this virus decrease with increasing vector age (Sinha, 1963). This might explain why most propagative viruses tested are transmitted more efficiently by vectors when the virus is acquired from diseased plants by nymphs rather than by adults (Nault, 1994). For example, with *Sorghum stripe virus* (an isolate of MStV) transmitted by *P. maidis*, first-instar nymphs were more efficient in transmission (64%) compared with the second to fourth instars (50%) or with adults (33%) (Narayana and Muniyappa, 1996).

For MMV, a much larger proportion of planthoppers (*P. maidis*) tested were positive using ELISA following injection (85%), than when virus was acquired from plants (42%) (Falk and Tsai, 1985). Similarly, the *Iranian maize mosaic virus* (IMMV), which is transmitted naturally by *Ribautodelphax notabilis* (Izadpanah, 1989), was experimentally transmitted by *P. maidis* with very low efficiency (0.4% to 1.6%) when acquired from plants, but with much greater efficiency (64%) when acquired by injection into the haemocoel (E. D. Ammar, R. Gomez-Louengo and D. T. Gordon, unpublished observations). Two delphacid planthoppers, *Toya propinqua* and *Sogatella vibix* could not transmit *Maize rough dwarf virus* (MRDV) orally, but were able to transmit it if their gut was needle-punctured following virus acquisition from diseased plants (Harpaz and Klein, 1969). Furthermore, the latent period in the vectors of several propagative plant viruses is much shorter when the virus is acquired by injection than when from plants, e.g. for MRFV (Nault *et al.*, 1980). Also, with MMV in its planthopper vector *P. maidis*, the average time between virus injection and its

detection by ELISA was only 4 days, compared to 12.3 days after acquisition from diseased plants (Falk and Tsai, 1985). The above results suggest the involvement of a midgut (infection or escape) barrier for these viruses in their vectors but the mechanism(s) by which it functions is unknown.

B. DISSEMINATION BARRIERS

For *Fiji disease virus* (FDV) the first, second and possibly third nymphal instars, but apparently not the adults, of the planthopper vector *Perkinsiella saccharicida* can acquire the virus from infected plants (Egan *et al.*, 1989). Only about 15% of the planthoppers contained detectable FDV in ELISA, even though they were reared on infected sugar cane for three generations and fewer than half of these infected insects (6%) transmitted FDV. Francki *et al.* (1986) suggested that the feeding behaviour of the vector might be incompatible with the distribution of FDV in plant tissue (for acquisition) and/or introduction of virus to susceptible plant tissue (for inoculation). Equally plausible, however, is the occurrence of a midgut (infection or escape), or other, dissemination barrier, to FDV in its vector.

When the MMV concentration of inocula injected into the haemocoel of the vector, *P. maidis*, ranged between 0.25 and 25 μg/ml, the percentage of ELISA-positive planthoppers, the antigen concentration for these ELISA-positive individuals, and the minimum time between injection and the first serological detection of MMV, were found to be dose dependent (Falk and Tsai, 1985). Also with MStV, when acquired from plants by its vector *P. maidis*, virus concentration, as determined by ELISA, was substantially greater following a 7-day compared to that of a 1-day AAP (Ammar *et al.*, 1995).

The ability of the planthopper *Sogatodes orizicola* to support multiplication of *Rice hoja blanca virus* (RHBV) appears to be genetically controlled, apparently segregating in the progeny as a single recessive gene. There was no evidence of sex linkage but a strong maternal influence on progeny transmission ability was detected (Zeigler and Morales, 1990). For WTV, ultrastructural and immunological studies indicated that virus accumulations were high in various organs of an efficient vector, *Agallia constricta*, but low (with no virus detected in the salivary glands) in an inefficient vector, *Agalliopsis novella* (Granados *et al.*, 1967). The shorter latent periods reported in efficient compared with inefficient vectors for several plant viruses, probably indicate faster replication and/or transport of virus in the tissues of efficient vectors. Direct evidence of the correlation between lower virus titre and longer latent period has been obtained with MStV in the planthopper vector *P. maidis* (Ammar *et al.*, 1995). An example of the specificity of replication of a propagative plant virus in potential vectors is provided by two strains of *Potato yellow dwarf virus* (PYDV) in leafhoppers. One strain is transmitted by *Aceratagallia sanguinolenta* and by other *Aceratagallia* spp. but not by *Agallia constricta*, whereas a second strain is transmitted by *A. constricta* but not by *A. sanguinolenta* (Black, 1970). Hsu *et al.*

(1977) found no differences in the susceptibility of cell lines from vector and some non vector species to PYDV strains. However, in another non vector of PYDV, *Dalbulus elimatus*, the *A. constricta*-transmitted strain did not multiply, whereas the *A. sanguinolenta* transmitted strain did, but much less efficiently than in cell lines of vector species. This indicates a dissemination (multiplication) barrier to PYDV in the cells of some non vector species but not others. Adam and Hsu (1984) suggested that differences in the G (glycosylated) protein might be related to the selective transmission of PYDV strains by their leafhopper vectors. The G protein, which protrudes from the virion envelope, functions in the attachment of rhabdoviruses to host recognition sites on the plasma membrane during the early stages of infection (Jackson *et al.*, 1987); this was apparently the case also with PYDV in insect cell cultures (Gaedigk *et al.*, 1986).

The ability of WTV to replicate in vector cell monolayers has also been used to study the specificity of its transmission. This virus readily infected cell lines from two leafhopper vector species, *A. constricta* and *A. novella*, but only with difficulty infected cell lines from one non vector, *A. sanguinolenta*, and failed to replicate in the cell lines from another non vector, *D. elimatus* (Hsu *et al.*, 1977). Leafhopper-transmitted WTV isolates were converted to vector non-transmissible ones by long-term maintenance in host plants by vegetative propagation. Loss of vector transmissibility of WTV, as well as its replication in vector cell monolayers, was found to be associated with the deletion of segments 2 and 5 of the 12 dsRNA segments present in the leafhopper-transmissible isolate (Nuss, 1984). These results suggest that the gene products of segments 2 and 5 are required for replication of WTV in the insect vector, but not in the host plant. The products of these segments comprise the outer capsid (shell) of the virus, which indicates that these proteins may be involved in the recognition of vector cells, virus penetration into them, or both. Since removal of the outer protein coat by protease treatment apparently caused no loss of infectivity to vector cell monolayers, Nuss (1984) suggested that the products of segments 2 and 5 might perform multiple functions in the replication. More recently, Tomaru *et al.* (1997) obtained a non transmissible isolate of RDV that lacked the ability to infect cells from the leafhopper vector *Nephotettix cincticeps*. Analysis of purified virus by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) showed that of six structural proteins, the P2 outer capsid protein, encoded by genome segment 2, was absent from the non transmissible isolate. The authors concluded that the P2 protein is essential for infection of vector cells by the virus, and thus influences RDV transmissibility by vector insects.

C. SALIVARY GLAND ESCAPE BARRIER

The morphology and ultrastructure of the salivary glands of leafhoppers, planthoppers and other homopterans differ markedly (Ammar, 1985, 1986; Wayadande *et al.*, 1997). With several propagative plant viruses, transmission by

inoculative insects is usually intermittent (Nault and Ammar, 1989; Nault and Gordon, 1988). More failures in daily transmission by *N. cincticeps* of RTYV occurred at >15–20°C than at 25–30°C and also later in the retention period (Chen and Chiu, 1980). Infectivity assays of RGDV indicated that virus titre in whole insect vectors remained high up to 40 days postacquisition, although transmission efficiency decreased with advancing age of vector leafhoppers (Omura *et al.*, 1988). Several plant viruses have been detected serologically in individuals that are unable to transmit the virus to host plants, e.g. only 10–24% of *Dalbulus* spp. leafhoppers exposed to MRFV-diseased plants transmitted virus although about 80% were positive for this virus by ELISA (Gamez and Leon, 1988). Similar results were obtained with MRDV, FDV and MStV in their planthopper vectors (Conti, 1985; Francki *et al.*, 1986; Nault and Gordon, 1988). More direct evidence of a salivary gland escape barrier is provided by serological studies on MStV in *P. maidis*. Of 31 planthoppers with salivary glands that were positive for MStV by ELISA, 24 insects failed to transmit the virus to host plants (Nault and Gordon, 1988).

WTV apparently does not multiply equally well in all lobes of the salivary glands in its leafhopper vector as antigens were found mainly at the anterior lobes (Sinha, 1973). An ultrastructural study of MMV in various organs of *P. maidis* showed differences that might explain how this virus is produced and transported within the salivary glands of the vector (Fig. 1) (Ammar and Nault, 1985). In maize leaf cells and in most of the vector's tissues examined, including midgut epithelium, epidermis, fat tissue, nerve cells and the 'accessory' salivary glands, MMV particles were found to bud mainly through the inner nuclear membrane, and to accumulate in perinuclear spaces (Fig. 2). However, in secretory cells of the principal salivary glands, MMV particles bud mainly through the plasma membrane and accumulate in intercellular and extracellular spaces (Fig. 1). These spaces are apparently connected with the extracellular vacuoles and canaliculi that lead to the salivary ductules and ducts (Ammar, 1986). Thus, Ammar and Nault (1985) suggested that this intercellular–extracellular route allows efficient discharge of MMV into the vector's saliva during feeding. This may be one mechanism by which some viruses overcome a salivary gland escape barrier in their vectors.

D. BARRIERS TO TRANSOVARIAL TRANSMISSION

The morphology of the female reproductive system in leafhoppers and planthoppers has been described by Ammar (1985) and Cheung (1995). For a virus to be transmitted transovarially, a virus must cross additional barriers in the vector. Following successful invasion of the haemolymph, the virus must cross the ovarian and ovariole sheaths and the follicular epithelium, before it can have access to the developing oocytes. If infection of the oocytes does not occur early in oogenesis, presumably the developing chorion would provide another substantial barrier (Turell, 1988).

Fig. 1. Rhabdovirus particles of maize mosaic virus (arrowheads) budding through plasma membrane (arrows) in a salivary gland cell of the planthopper vector *P. maidis*. Particles accumulate in intercellular spaces (IS) and in secretory vesicles (SV). Inset shows higher magnification of boxed area (top right). Scale bar: 200 nm (inset).

Most tenuiviruses are transovarially transmitted to a large proportion of the progeny in their planthopper vectors. For example, *Rice stripe virus* (RStV) was reported to pass through the eggs of a single infective female of *Laodelphax striatellus* for 40 generations, with 90% of the progeny insects of the 40th gen-

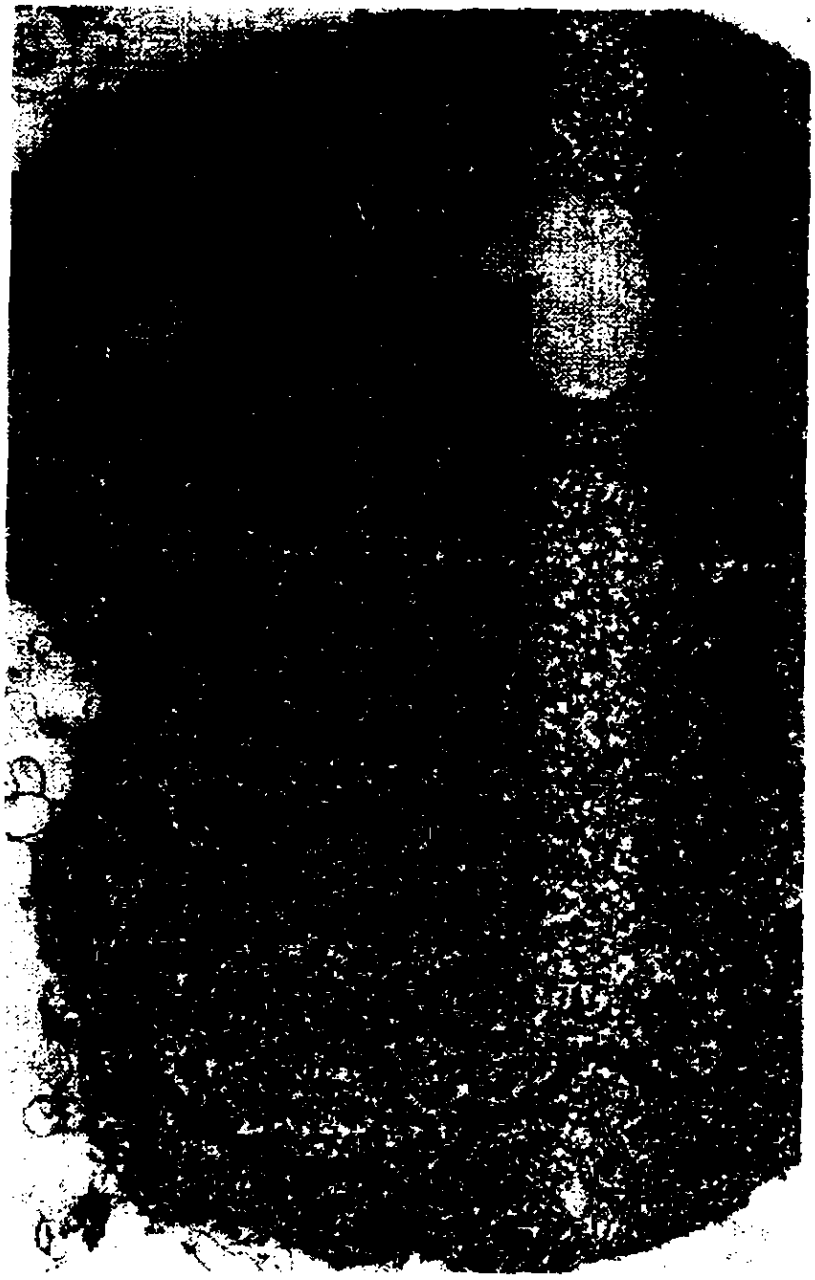

Fig. 2. Rhabdovirus particles of *Iranian maize mosaic virus* budding through nuclear membrane (arrows) and accumulating in massive arrays in perinuclear space, finally replacing most of the cell cytoplasm. N, nucleus; V, virions.

eration being inoculative with RStV (Shinkai, 1962). On the other hand, only a few rhabdoviruses and no marafiviruses are transovarially transmitted (Table I). However, within each virus group different rates of transovarial transmission have been reported for different viruses and for the same virus in different vectors, e.g. only 2–10% for WTV in *A. novella*, and up to 80% for the same virus in *A. constricta* (Sinha, 1981). Furthermore, the efficiency of transovarial transmission may be different between vector races of the same species, as well as between virus isolates. Races of leafhoppers with high rates of transovarial transmission of WTV were also efficient in transmitting WTV to host plants (Sinha, 1981).

Efficiency of transovarial transmission of three geographically distinct isolates of MStV was positively correlated with that of oral transmission of these isolates by the planthopper vector *P. maidis* (Ammar *et al.*, 1995). Efficient female planthopper vectors of another *Tenuivirus*, RHBV, transmitted virus transovarially to their progeny, regardless of the male parent and progeny genotype. In progeny receiving RHBV maternally, however, virus titre was lower and more variable in insects with an ELISA-negative male parent than in insects with two ELISA-positive parents (Zeigler and Morales, 1990). With *European wheat striate mosaic virus* (EWSMV), if females of the planthopper vector *Javesella pellucida* acquired the virus orally as adults, little or no transovarial transmission occurred; but if the virus was acquired at the nymphal stage, most of the progeny inherited the virus and were able to transmit immediately after hatching (Sinha, 1973). With another tenuivirus, RStV, and a phytoreovirus, RGDV, the rate of transovarial transmission decreased with the age of females, and at high temperatures in the case of RStV (Chen and Chiu, 1980; Raga *et al.*, 1988).

The presence of virus particles in the ovaries of female vectors does not necessarily mean that the virus is transovarially transmitted. Particles of MMV, which is not transmitted transovarially or venereally (during mating), by its planthopper vector *P. maidis*, were found in follicular cells of female ovaries, and in the epithelium of the ejaculatory ducts in males, as well as in most other organs examined from this vector (Ammar and Nault, 1985). However, MMV particles were detected in only one out of five ovaries examined. With MStV, which is transmitted transovarially in *P. maidis* to a large proportion of the progeny, all tested ovaries, oviducts and bursae copulatrix contained MStV antigens; virus was also detected in single eggs (Nault and Gordon, 1988). These studies suggest the involvement of some transovarial transmission barriers.

Nasu (1965) obtained results suggesting that RDV particles attach selectively to the surface of L-symbiotes, present in the cytoplasm of the mycetocytes, which migrate to the adjacent oocytes. However, other mechanisms for introduction of virus into the oocytes in leafhopper and planthopper vectors are feasible but remain uninvestigated.

VI. OTHER FACTORS AFFECTING VECTOR COMPETENCE

A. ENVIRONMENT

Temperature seems to be the main environmental factor affecting the rate of virus transmission, the length of the latent period and the retention of virus inoculativity in vectors (Matthews, 1991). For example, 100% transmission rate of *Sorghum stunt mosaic virus* (SSMV) was obtained, with a latent period of 11 days, when the leafhopper vector *Graminella sonora* was reared at 26–30°C, whereas at 16°C a latent period of 28 days was necessary to obtain high levels of transmission (Creamer and He, 1997). On the other hand, temperatures from

27° to 36°C prevented the spread of WTV from the midgut to the haemolymph and salivary glands of its leafhopper vector *A. constricta* (Sinha, 1967). Transovarial transmission also may be affected by temperature. For RStV, in its planthopper vector *L. striatellus*, the percentage of viruliferous females that passed virus to a high proportion of their progeny was 82.6% at 17.5°C, and only 12.5% at 32.5°C (Raga *et al.*, 1988).

B. CO-INFECTION OF THE VECTOR WITH OTHER PLANT PATHOGENS

Some leafhoppers and planthoppers transmit more than one virus, sometimes simultaneously. However, very few combinations of propagative plant viruses have been studied for possible interaction in their common vectors. Simultaneous transmission by the leafhopper *A. constricta* of WTV and PYDV apparently can occur without any evidence of interaction between them (Nagaraji and Black, 1962). Access of planthoppers to MMV-infected plants within 0–14 days before or after access to MStV-infected plants significantly decreased transmission of MStV by *P. maidis*. In contrast, access to MStV-infected plants usually had no effect on the acquisition and transmission of MMV. Plants previously infected with either virus were partially protected from infection by the other. In both plants and vectors, however, MMV apparently interfered with multiplication of MStV (Ammar *et al.*, 1987). Mutual interference was reported between the *Oat blue dwarf virus* (OBDV) and aster yellows mycoplasma-like organism (*Phytoplasma*) in their common leafhopper vector *Macrosteles quadralineatus* (= *furcifera*) (Atcham and Banttari, 1986). Since both viruses and mollicutes apparently have a similar route in their vectors (Markham, 1983), it is possible that interference between propagative viruses, or between viruses and mollicutes in the same vector, can be explained as a result of competition for replication sites or substrates.

C. DIFFERENCES IN GENDER

Male and female leafhoppers behave differently on plants (Hunt and Nault, 1991; Lopes *et al.*, 1995) and this affects transmission patterns. However, no work has been done to show that the mechanism of virus transmission differs between males and females.

VII. EFFECTS OF PROPAGATIVE VIRUSES ON THEIR VECTORS

Most of the early reports of pathogenic effects of some plant viruses on their leafhopper or planthopper vectors have now either been refuted or shown to involve mollicutes previously assumed to be viruses, e.g. Western X-disease mycoplasma-like organism (MLO) and corn stunt *Spiroplasma* (Whitcomb,

1972; Purcell and Nault, 1991). For corn stunt *Spiroplasma*, transmitted by *Dalbulus* leafhoppers, it was found that in well-adapted coevolved vector species, the plant pathogen was not only non-pathogenic, but may benefit the vector. In contrast, poorly adapted *Dalbulus* species are frequently inefficient vectors and in those that do transmit their longevity and fecundity are greatly reduced, compared to non-infected leafhoppers (Purcell and Nault, 1991).

Reports that RStV decreases the longevity and fecundity of its planthopper vector *L. striatellus* (Nasu, 1963) were later refuted by work that indicated that RStV had no deleterious effects on this efficient vector (Raga *et al.*, 1988). EWSMV was also reported to decrease the fecundity and increase embryonic abnormalities in its vector *J. pellucida* (Watson and Sinha, 1959) but Kisimoto and Watson (1965) later suggested that these abnormalities might have been due to inbreeding of vector colonies in the laboratory. Further studies on the effects of EWSMV on its vector, were made by Ammar (1975a,b). When EWSMV was acquired from diseased plants by *J. pellucida*, no deleterious effects were observed on adult longevity, fecundity, or embryonic or nymphal development, but when EWSMV was acquired transovarially for one or two generations, nymphal mortality was increased by 13–17% and adult longevity was reduced by 14%. However, the deleterious effects on *J. pellucida* of inbreeding for two generations were much greater than those of EWSMV. No pathogenic effects of MStV on the longevity or fecundity of *P. maidis* have been observed (Tsai and Zitter, 1982; E. D. Ammar and L. R. Nault, unpublished observations). The capsid protein of MStV was detected at a smaller concentration in infected, inoculative, planthoppers compared to that in infected maize plants, whereas the major non capsid protein, also encoded by MStV, was detected only in infected plants but not in planthopper vectors (Falk *et al.*, 1987). If MStV replicates differently in plant and vector tissues, this might explain its lack of adverse effects on the vector compared to its effects on maize plants. These studies indicate that, in well-adapted vectors, genotypes naturally have been selected that minimize or eliminate pathogenicity of plant pathogens to their vectors.

Why, or how, propagative plant viruses usually have deleterious affects on their plant hosts, while having little or no adverse affects on their insect vectors has been investigated in a few cases. Ultrastructural studies with MMV revealed small aggregates of virus particles in tissues from the planthopper vector *P. maidis* (Fig. 1), whereas massive and often crystalline aggregates of particles of MMV or its relative IMMV occurred in most cell types of infected maize leaves (Fig. 2). In addition, a larger proportion of budding MMV particles with intermediate lengths was observed in insect compared to plant tissues (Ammar and Nault, 1985; McDaniel *et al.*, 1985). These results suggest a slower rate of MMV replication and/or assembly in insect compared to plant tissues (E. D. Ammar and L. R. Nault, unpublished). Quantitative studies on RDV showed that the accumulation of virus-encoded proteins in rice leaves is much greater than in the leafhopper vector *N. cincticeps* (Suzuki *et al.*, 1994). With RGDV phloem parenchyma cells were severely affected and the cells appeared fully occupied

by virus particles and viroplasm, in diseased rice plants, whereas in viruliferous *N. lugens*, virus infection never occupied large areas of the infected cells. Thus, insect vectors might have some regulatory mechanism(s) that decreases or slows down virus multiplication in the vector compared to its multiplication in plant cells; phagocytic-like vesicles were found only in RGDV-infected leafhoppers, and they might provide such a mechanism in the vector (Kimura and Omura, 1988).

For PYDV, which induces strong symptoms in infected plants, no cytopathic effects were observed in an infected vector cell line. However, when the inoculum contained a large concentration of PYDV, some cytopathic effects, leading to cell death were observed (Hsu, 1978). With WTV, despite the cytological changes found in infected leafhopper vectors, these vectors apparently showed no decrease in fecundity or longevity (Maramorosch, 1975). Persistent productive WTV infections of vector cell cultures caused no apparent changes in cell growth rate or protein synthesis capability (Nuss and Dall, 1990). Peterson and Nuss (1986) reported that WTV-specific polypeptide synthesis and viral genome RNA accumulation increased to a maximum level during the first 5 days following vector cell culture inoculation, and then decreased as infected cells were passaged. In contrast, viral-specific mRNAs were present at approximately the same level in the acute phase and in the early stage of the persistent phase of infection. Transcripts isolated from persistently infected cells were inefficiently translated *in vitro*. These results indicate that the level of viral polypeptide synthesis associated with the persistent phase of WTV infection in vector cells is related to a change in the translational activity of viral transcripts.

VIII. EPILOGUE

Research on hopper transmission of plant viruses has lagged behind that of hopper transmission of plant spiroplasmas and phytoplasmas, but as knowledge of the molecular and genetic bases for the transmission of hopper-borne viruses increases new strategies for controlling plant virus diseases by manipulating the plant genome are likely to emerge. Many more hopper-borne viruses will be reported, especially those that infect tropical perennials where potential hopper vector species are found in abundance, and molecular methods will substitute for the slow and laborious methods used today in studying virus transmission.

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