COMPARATIVE PLANT VIROLOGY

SECOND EDITION

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CHAPTER

12

Plant-to-Plant Movement

Being obligate parasites, viruses depend for survival on being able to spread from one susceptible individual to another fairly frequently.

OUTLINE

I. INTRODUCTION

Because viruses cannot penetrate the intact plant cuticle and the cellulose cell wall (Figure 12.1B), plants have a barrier to infection. This problem is overcome either by avoiding the need to penetrate the intact outer surface (e.g., in seed transmission or by vegetative propagation) or by some method involving penetration through a wound in the surface layers, such as in mechanical inoculation and transmission by insects. There is considerable specificity in the mechanism by which any one virus is naturally transmitted.

II. TRANSMISSION VIA PLANT MATERIAL

A. Mechanical Transmission

Mechanical inoculation involves the introduction of infective virus or viral RNA into a wound on the plant's surface. When virus establishes itself successfully in the cell, infection occurs. This form of transmission occurs naturally with a few viruses such as *Tobacco mosaic virus* (TMV) and *Potato virus X* (PVX) that are very stable and reach high concentrations in the plant. TMV can readily contaminate hands,

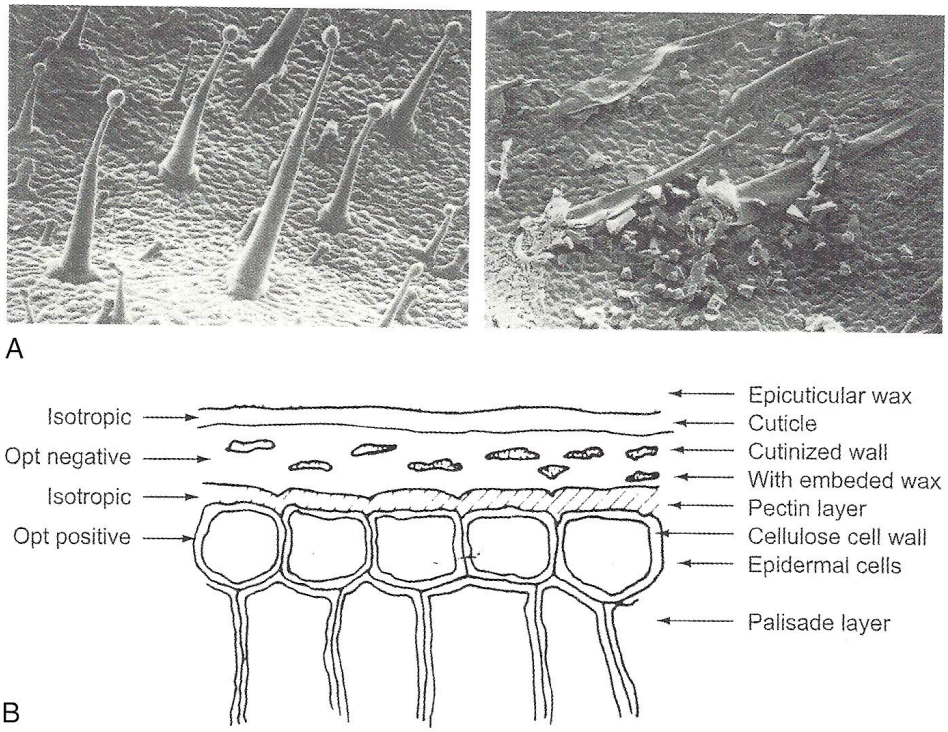

FIGURE 12.1 Leaf surface structure. A. Scanning electron micrographs of surface of *Nicotiana glutinosa* leaf before (left panel) and after (right panel) mechanical inoculation. Note leaf hairs in left panel that are broken in right panel. [From Hull (2002).] B. Diagrammatic representation of cross-section of the upper part of a leaf showing the barriers to virus infection. [From Eglinton and Hamilton (1967; *Science* **156**, 1322–1335), kindly used with permission of Dr. B.E. Juniper.]

clothing, and implements and can be spread by workers and, for instance, birds in tobacco and tomato crops. TMV may be spread mechanically by tobacco smokers because the virus is commonly present in processed tobacco leaf. For example, a survey showed that all 37 brands of cigarette and 60 out of 64 smoking tobaccos contained infectious TMV.

Mechanical transmission is of great importance for many aspects of experimental plant virology, particularly for the assay of viruses, often by local lesion production; in the propagation of viruses for purification; in host range studies; in diagnosis; and in the study of the early events in the interaction between a virus and susceptible cells. Mechanical inoculation is usually done by grinding up infected leaf tissue in a buffer—usually a phosphate buffer that contains additives that control nucleases and polyphenols—incorporating an abrasive such as celite or carborundum, and then rubbing the extract gently on the leaves of the recipient plant. The gentle application wounds the leaf surface without causing cell death (Figure 12.1A).

B. Seed Transmission

About one-seventh of the known plant viruses are transmitted through the seed of at least one of their infected host plants. Seed transmission provides a very effective means of introducing virus into a crop at an early stage, giving randomised foci of primary infection throughout the planting. Thus, when some other method of transmission can operate to

spread the virus within the growing crop, seed transmission may be of very considerable economic importance. Viruses may persist in seed for long periods so commercial distribution of a seed-borne virus over long distances may occur. Seed transmission rates vary from less than 1 to 100 percent, depending on virus and host.

Two general types of seed transmission can be distinguished. With TMV in tomato, seed transmission is largely due to contamination of the seedling by mechanical means. The external virus can be readily inactivated by certain treatments eliminating all, or almost all, seed-borne infection. In the second and more common type of seed transmission, the virus is found within the tissues of the embryo. The developing embryo can become infected either prior to fertilisation by infection of the gametes (indirect embryo invasion or gametic transmission) or by direct invasion after fertilisation. Generally speaking, for infection of the embryo from the mother plant, the earlier the plant is infected, the higher the percentage of seed that will transmit the virus. Obviously, for indirect embryo invasion by pollen, the infection takes place at pollination.

The direct route of seed infection from the mother plant poses problems in that symplastic connection is severed at meiosis. To infect the embryo, the virus has to reach either the floral meristems, which are beyond the limits of normal long-distance movement in the phloem (see Chapter 9), or the embryo itself. The route of direct embryo infection of peas by *Pea seed-borne mosaic virus* has been examined in detail (Box 12.1).

C. Pollen Transmission

Some viruses are transmitted from plant to plant via pollen. As with seed transmission, two mechanisms appear to operate in pollen transmission: gametic infection of the embryo and direct infection of the mother plant.

D. Vegetative Propagation

Vegetative propagation is an important horticultural practice, but it is also, unfortunately, a very effective method for perpetuating and spreading viruses. Economically important viruses spread systemically through most vegetative parts of the plant. A plant once systemically infected with a virus usually remains infected for its lifetime. Thus, any vegetative parts taken for propagation, such as tubers, bulbs, corms, runners, and cuttings, will normally be infected.

E. Grafting

Grafting is essentially a form of vegetative propagation in which part of one plant (the scion) grows on the roots (the stock) of another individual. Once organic union has been established, the stock and scion become effectively a single plant. Where either the rootstock or the individual from which the scion is taken is infected systemically with a virus, the grafted plant as a whole will become infected if both partners in the graft are susceptible. Grafting may succeed in transmitting a virus where other methods fail.

III. TRANSMISSION BY INVERTEBRATES

Many plant viruses are transmitted from plant to plant in nature by invertebrate vectors, members of the *Insecta* and *Arachnida* classes of the *Arthropoda*, and the *Dorylaimida* order of the *Nematoda*. Box 12.2 shows the orders of the *Insecta* that transmit plant viruses. Six of the orders contain insects that feed by chewing. The *Homoptera* feed by sucking sap from plants and are numerically the most important suborder containing plant virus vectors. Figure 12.2 shows three of the most common vectors of plant viruses: aphids, leafhoppers, and whitefly.

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BOX 12.1

DIRECT EMBRYO INFECTION OF PEAS BY *PEA SEED-BORNE MOSAIC VIRUS* (PSbMV)

The route by which PSbMV reached pea seeds has been studied in detail by comparing a variety (Vedette) in which the virus is seed transmitted with one (Progretta) in which it is not (Figure).

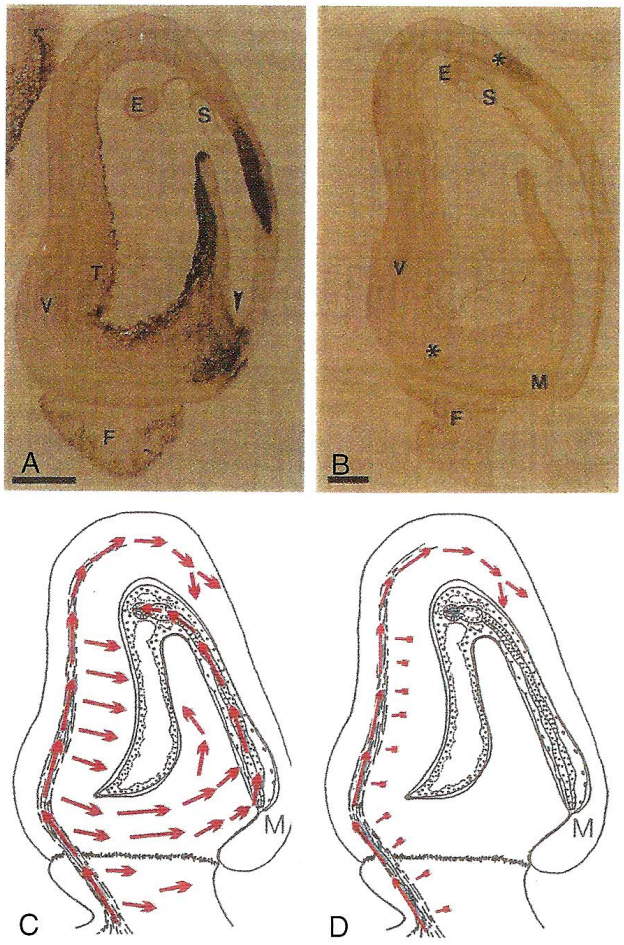

Fig. The pathway to seed transmission of *Pea seed-borne mosaic virus* (PSbMV) in pea. A and B. Analysis of the distribution of PSbMV in longitudinal sections through immature pea seed by immunochemistry using a monoclonal antibody to the virus coat protein shows that a cultivar-virus interaction, which is permissive for seed transmission (e.g., with *Pisum sativum* cv. Vedette in A) results in widespread accumulation of the virus in the testa tissues. In contrast, in the nonpermissive interaction (e.g., with cv. Progretta in B) virus enters the seed through the vascular bundle but is unable to invade the adjacent testa tissues extensively. In both cases there is a gradual reduction in the amount of accumulated virus after invasion such that in cv. Progretta only patches (asterisks) of infected tissue remain detectable. Systematic analyses of the immature seeds of different ages have identified the routes (red arrows) of virus invasion in the two cultivars (illustrated diagrammatically in C for cv. Vedetta and in D for cv. Progretta). The most consistent observation from all these studies is that the virus must reach the micropylar area of the testa for seed transmission to occur, a location providing the closest point of contact (arrowhead in A) between the testa tissues and the embryonic suspensor. In the nonpermissive interaction the virus appears to be blocked (denoted by red squares) in its ability to spread into and/or replicate in the nonvascular testa tissues. E, embryo proper; F, funiculus; M, micropylar region; S, suspensor; T, testa; V, vascular bundle. Bar marker = 500m. [This article was published in *Trends Microbiol.* **4,** A.J. Maule and D. Wang, Seed transmission of plant viruses: A lesson in biological complexity, pp. 153–158, Copyright Elsevier (1996).]

The virus moves through the testa of the immature seed after fertilisation and must reach the micropylar region of the seed for embryo infection to occur. The micropyle is in close contact with the base of the embryonic suspensor, which functions as a conduit for nutrient flow to support growth of the embryo. The suspensor is the route by which the virus invades the embryo itself, but it degrades as part of the seed development programme. This leaves a "window of opportunity" for embryo infection; this window of opportunity is taken by the virus in Vedette but not in Progretta. However, there is no symplastic connection between maternal and embryonic tissue, and it is still unknown how the virus crosses from the maternal testa cells to the embryonic suspensor.

BOX 12.2

VIRUS TRANSMISSION BY *INSECTA*

Seven of the 29 orders in the living *Insecta* feeding on living green land plants are vectors of plant viruses and are listed here with the approximate number of vector species in parentheses:

1. *Orthoptera*—chewing insects; some feed on green plants (27).
2. *Dermaptera*—chewing insects; a few feed on green plants (1).
3. *Coleoptera*—chewing insects; many feed on green plants; see table.
4. *Lepidoptera*—chewing insects; larvae of many feed on green plants (4).
5. *Diptera*—larvae of a few feed on green plants (2).
6. *Thysanoptera* (thrips)—some are rasping and sucking plant feeders (10).
7. *Hemiptera*—feed by sucking on green plants

Suborder *Heteroptera*, Families *Myridae*, and *Piesmatidae* (~ 4)
Suborder *"Homoptera"*,[a] see Table.

Distribution of plant virus vectors among selected *Homoptera* and *Coleoptera* families. [From Nault (1997; *Ann. Entomol. Soc. Am.* **90**, 521–541).]

Order, Suborder, Family	Common Name of Insect Group	No. Species Described	No. Vector Species	No. Viruses Transmitted
Homoptera				
Auchenorrhyncha				
Cicadidae	Cicada	3,200	0	0
Membracidae	Treehopper	4,500	1	1
Cercopidae	Spittlebug	3,600	0	0
Cicadellidae	Leafhopper	15,000	49	31
Fulgoroidea	Planthopper	19,000	28	24
Sternorrhyncha				
Psyllidae	Psyllid	2,000	0	0
Aleyroididae	Whitefly	1,200	3	43
Aphididae	Aphid	4,000	192	275
Pseudococcidae	Mealybug	6,000	19	10
Coleoptera				
Chrysomelidae	Leaf beetle	20,000	48	30
Coccinellidae	Ladybird beetle	3,500	2	7
Cucurlionidae	Weevil	36,000	10	4
Meloidae	Blister beetle	2,100	1	1

[a] *"Homoptera"* is a widely used generic term for several suborders of the *Hemiptera*.

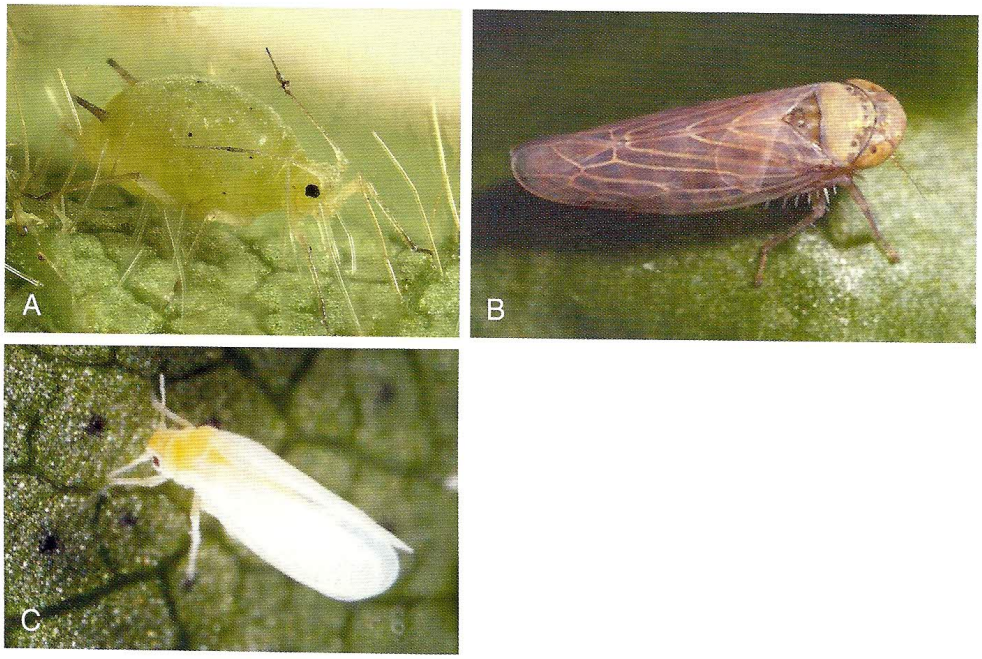

FIGURE 12.2 Major vectors of plant viruses. A. Aphid (*Aphis glycines*); note the stylet penetrating the leaf (from www.planthealth.info, Purdue University). B. Leafhopper (*Circulifer tenellus*). C. Whitefly (*Bemisia tabaci*).

A. Relationships Between Plant Viruses and Insects

The transmission of viruses from plant to plant by invertebrate animals is of considerable interest from two points of view. First, such vectors provide the main method of spread in the field for many viruses that cause severe economic loss. Second, there is much biological and molecular interest in the relationships between vectors and viruses, especially as some viruses have been shown to multiply in the vector. Such viruses can be regarded as both plant and animal viruses. Even for those that do not multiply in the animal vector, the relationship is usually more than just a simple one involving passive transport of virus on some external surface of the vector ("the flying pin"). Transmission by invertebrate vectors is usually a complex phenomenon involving specific interactions between the virus, the vector, and the host plant, coupled with the effects of environmental conditions. Most of the detailed studies on virus transmission and virus-vector relationships have been made using aphids. Many of the features described following for aphid transmission are applicable to transmission by vectors from other insect orders.

As a general rule, viruses that are transmitted by one type of vector are not transmitted by any of the others. This specificity is not only at the level of vector type, family, genus, or species but can be even at the level of biotype. There are two basic interactions between viruses and their biological vector. They may be taken up internally within the vector, termed *persistent, internally borne* or *circulative*, or they may not pass to the vector's interior, in which case they are termed *nonpersistent, externally borne,* or *noncirculative* (Box 12.3).

BOX 12.3

RELATIONSHIPS BETWEEN PLANT VIRUSES AND THEIR VECTORS

There are two major types of interaction between a virus and its vector: nonpersistent and persistent. Features of the interactions are outlined in the table here, and the pathways that the viruses have with their vector are shown in the figure.

Virus Transmission Group			Transmission Characteristics						
Site in vector	Type of transmission	Virus product interacting with vector	Acquisition time (max. dose)	Retention time (half-life)	Transtadial passage	Virus in vector haemolymph	Latent period	Virus multiplies in vector	Transovarial transmission
Externally – borne	Nonpersistently transmitted stylet-borne	Capsid +/– helper factor	Seconds to minutes	Minutes	No	No	No	No	No
	Nonpersistently transmitted foregut-borne (semipersistent)	Capsid +/– helper factor	Minutes to hours	Hours	No	No	No	No	No
Internally-borne	Persistent circulative		Hours to days	Days to weeks	Yes	Yes	Hours to days	No	No
	Persistent propagative		Hours to days	Weeks to months	Yes	Yes	Weeks	Yes	Often

(continued)

BOX 12.3 *(continued)*

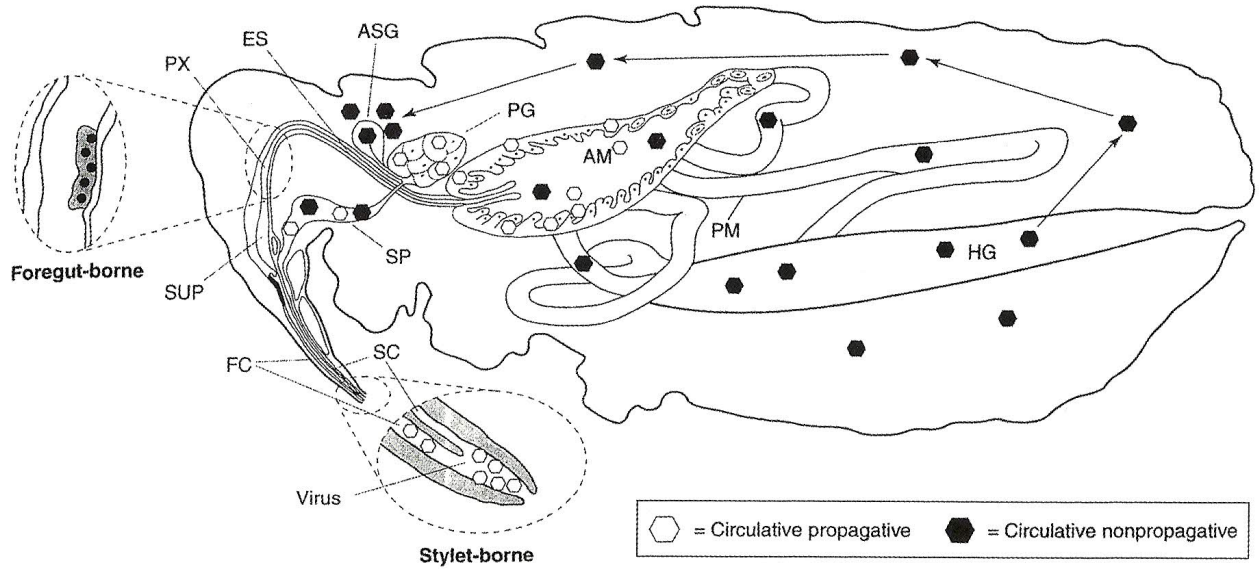

Figure. Mechanism of transmission of plant viruses by arthropods with piercing-sucking mouthparts. The general anatomy of the alimentary system and the salivary system is shown; the areas relevant to virus transmission are labeled. One inset shows a detailed view of the distal end of the mouthparts where the food canal (FC) and the salivary canal (SC) empty into a common space. One current model of transmission of stylet-borne (nonpersistent, noncirculative) viruses suggests that the transmissible virus is retained at the distal tip of the stylets and then released by salivary secretions as the insect salivates during feeding. A second inset shows a detailed view of the foregut-borne (semipersistent, noncirculative) with virus particles attached to the cuticle lining of the foregut, a region that would include the sucking pump (SUP), pharynx (PX), and esophagus (ES). Notice that the virus is embedded in a matrix material attached to the cuticle. The origin or composition of the matrix material is unknown. The circulative nonpropagative viruses pass through the foregut into the anterior midgut (AM), posterior midgut (PM), and then into the hindgut (HG). They do not infect the gut cells but are transported through the posterior midgut and hindgut cells and released into the haemocoel (body cavity). Current information indicates that these viruses specifically associate with the accessory salivary glands (ASG) and are transported across the ASG cells and then released into the salivary canal (SC). The circulative propagative viruses will infect midgut cells and subsequently infect other tissues. These viruses ultimately associate with the principal salivary glands (PG) and possible ASG prior to their release into the SC. SP, salivary pump. [This article was published in *Encyclopedia of virology*, Vol. 1. S.M. Gray and D.M. Rochon (A. Granoff and R. Webster, Eds.), Vectors of plant viruses, pp. 1899–1910, Copyright Elsevier (1999).]

Essentially, there are three stages in the transmission cycle:

1. The *acquisition phase,* in which the vector feeds on the infected plant and acquires sufficient virus for transmission.
2. The *latent period,* in which the vector has acquired sufficient virus but is not able to transmit it. For externally borne viruses, there is little or no latent period.
3. The *retention period* is the length of time during which the vector can transmit the virus to a healthy host.

B. Nonpersistent Transmission by Insects

1. *Features of Nonpersistent Transmission*

Of the over 300 known aphid-borne viruses, most are nonpersistent. The following virus genera have definite members transmitted in a nonpersistent manner: *Alfamovirus, Caulimovirus* by *Myzus persicae, Closterovirus, Cucumovirus, Fabavirus, Macluravirus,* and *Potyvirus.* These genera include viruses with helical and isometric particles and with DNA and RNA mono-, bi-, and tripartite genomes. There are no known nonpersistent viruses transmitted by leafhoppers.

Nonpersistently transmitted viruses are acquired rapidly from plants, usually in a matter of seconds. During this time, aphid's stylet does not usually penetrate beyond the epidermal cells, and when it penetrates beyond the epidermis into the mesophyll and vascular tissue, the transmission rate declines rapidly. The initial host-finding behaviour of aphids is short probing, thought to sample the epidermal cells' sap, and fits very well with this mechanism. Since the sampling is especially brief on nonhosts for the aphid, the vectors of nonpersistent viruses are often noncolonisers of that species.

With a nonpersistent virus there is little or no latent period, and aphids begin to lose the ability to infect immediately after the acquisition feed. The rate at which infectivity is lost depends on many factors, including temperature and whether they are held on plants or under some artificial condition.

Different strains of the same virus may differ in the efficiency with which they are transmitted by a particular aphid species. Some strains may not be transmitted by aphids at all. Different strains of the same nonpersistent virus do not usually interfere with each other's transmission, as is sometimes found with propagative viruses.

Aphid species vary widely in the number of different viruses they can transmit. At one extreme, *M. persicae* is known to be able to transmit a large number of nonpersistent viruses, whereas other aphids transmit only one virus. These differences in part reflect the extent to which different aphid species have been tested, but there is no doubt that real differences in versatility occur. Among species that transmit a given virus, one species may be very much more efficient than another. For instance, marked differences were found in the efficiency with which *Potato virus Y* was transmitted by different species even when acquisition feed and test feed times were standardised (Figure 12.3). This can reflect the initial feeding behaviour of different aphid species on the test plant species.

2. *Virus-Vector Relationships*

As just noted, when they alight on a leaf, aphids may make brief probes into the leaf—often less than 30 seconds. Thus, the initial behaviour of such aphids on reaching a leaf is ideally suited to rapid acquisition of a nonpersistent virus. Sap sampling on a virus-infected plant will contaminate the stylet tips, the food canal, and the foregut. These sites have been favoured for the retention site of virus that will be injected subsequently into a healthy plant following another exploratory probing feed.

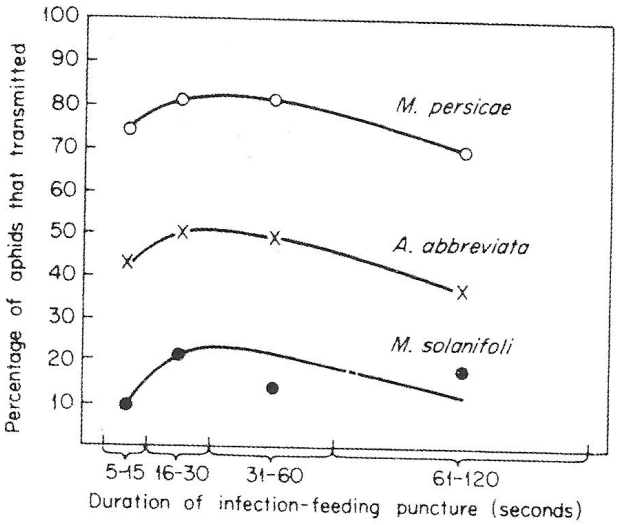

FIGURE 12.3 Relative efficiencies of three aphis species in transmitting *Potato virus Y* after defined acquisition feeding times. [Reprinted with permission from Bradley and Rideout (1953; *Can. J. Zool.* **31**, 333–341).]

The weight of evidence favours the food canal in the maxillae as the site where infective virus is retained during nonpersistent transmission, but it must be remembered that this evidence identifies sites of accumulation but does not give any indication as to whether that virus is transmitted.

There are two phases to the interaction involved in nonpersistent virus transmission: retention of the virus at a specific site and release of the virus. All nonpersistently transmitted viruses have a simple structure of nucleic acid encapsidated in simple icosahedral or rod-shaped particles by one or more coat protein species. Thus, it is the capsid protein that is available for any interactions. Two forms of interaction have been identified in the retention phase: one in which there appears to be direct interaction between the virus capsid and the site of retention in the aphid and the other in which a nonstructural virus-encoded protein is involved. This nonstructural protein is termed a *helper component*, *helper factor*, or *aphid transmission factor*.

a. Direct Capsid Interaction. It is thought that *Alfalfa mosaic virus* and *Cucumber mosaic virus* (CMV) transmission involves direct links between the capsid and the binding site within the aphid vector. The prime evidence is that purified virus can be transmitted from artificial feeding systems without the addition of other proteins or factors. Most of the detailed evidence is for CMV. The efficiency of aphid transmission of heterologously assembled particles between the genomes and capsid proteins of a highly aphid-transmitted (HAT) and a poorly aphid-transmitted (PAT) strain of CMV segregated with the source of coat protein. The amino acid differences between the coat proteins of HAT and PAT strains of CMV are associated with both vector transmissibility and virion stability.

The minor coat protein of *Closteroviruses*, which encapsidates only a teminal part of the viral genome to give "rattlesnake" particles (see Figure 5.1), is thought to be involved in aphid transmission.

b. Indirect Interaction Involving Helper Components. The interactions involving helper components of two groups of viruses, the potyviruses and caulimoviruses, have been studied in detail. The helper components of potyviruses are encoded by the virus and have the following properties in relation to virus transmission:

- The helper factor of one potyvirus may or may not permit the aphid transmission of another potyvirus when tested in an *in vitro* acquisition system. Thus, there is some specificity in the phenomenon.
- The helper component must be acquired by the aphid either during or before virus acquisition. If it is provided after the virus, there is no transmission.
- Potyvirus helper components have MWs in the range of 53 kDa and 58 kDa. They are cleaved from the polyprotein encoded by the virus as a product that, as well as helper

component activity, has various other activities including being a protease. Thus, it is termed HC-Pro.

- Purified helper component can be used to facilitate the transmission of potyviruses by feeding through artificial membranes.
- The biologically active form of helper component appears to be a dimer.

By studying the effects of mutations in the coat protein and helper component on aphid transmission of various potyviruses, a picture appears of some of the molecular interactions involved. The current hypothesis is that the helper component forms a bridge between the virus capsid and the aphid stylet (Figure 12.4A). Close to the N-terminus of the coat protein is the amino acid

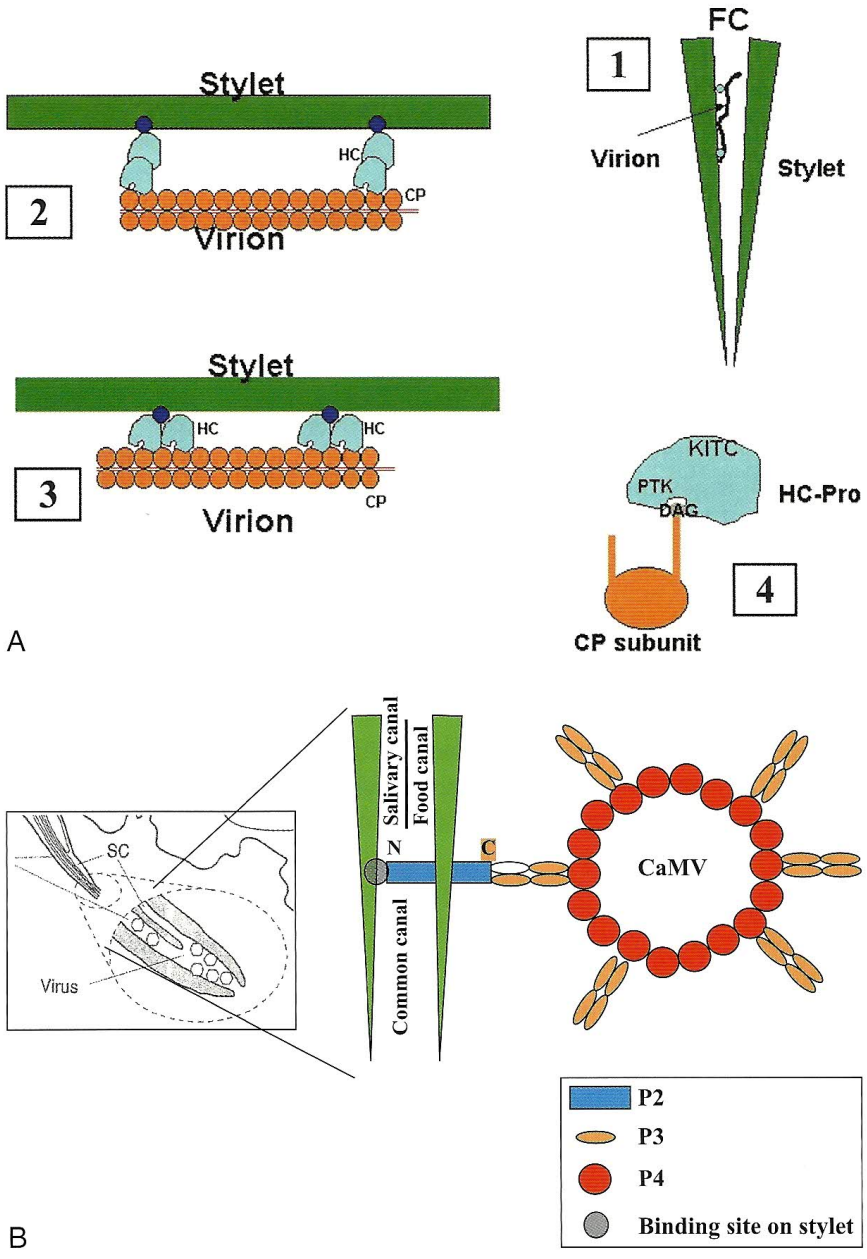

FIGURE 12.4 Models for the interactions between viruses, transmission helper factors, and vector. A. Possible interactions between the HC-Pro, the aphid's stylets, and the potyviral coat protein. (1). Position of the virion particles close to the apical section of the food canal. (2). A model assuming an association between two molecules of HC-Pro. Note that one molecule of the HC-Pro is bound to a "receptor" on the stylet, while the second HC-Pro molecule is bound to the coat protein subunit. (3) A model assuming that a dimer is needed to bind to the "receptor" on the stylet. Both HC-Pro molecules are linked to coat protein subunits. (4) A proposed structural binding between the PTK motif of HC-Pro and the DAG motif found on the N-terminus of the coat protein subunit. [This article was published in *Virus-insect-plant interactions*, B. Raccah, H. Huet, and S. Blanc (K.F. Harris, O.P. Smith, and J.E. Duffus, Eds.), Potyviruses, pp. 181–206, Copyright Elsevier (2001).] B. Interaction involved in the aphid transmission of *Cauliflower mosaic virus* (CaMV). The panel on the left is from the figure in Box 12.3. The rest of the figure shows diagrammatically the interactions between a CaMV particle and the aphid's stylet (see text). N and C are the N-terminal and C-terminal regions of P2, respectively.

triplet DAG (aspartic acid-alanine-glycine) that is important for transmissibility. Mutagenesis shows that both the DAG sequence itself and the context of the surrounding amino acids affects transmissibility. Biochemical and immunological analyses indicate that this N-terminal region is located on the external surface of the virus particle. Two important regions have been identified in HC-Pro. One, characterised by the amino acid sequence PKT (proline-lysine-threonine), appears to be involved in the interaction with the capsid protein. The other, termed the KITC (lysine-isoleucine-threonine-cysteine) region, appears to be involved with the HC-Pro retention on the aphid's stylets.

Little is known about the mechanisms of release of nonpersistent viruses from the site of binding in the aphid's stylet, but three theories have been proposed:

1. The mechanical transmission theory suggests that the virus is simply inoculated by the stylet.
2. In the ingestion-egestion theory, release is effected by regurgitation and salivation.
3. Since the food and salivary canals of the stylets fuse near the tip of the maxillary stylet, nonpersistently stylet-borne viruses could be released by saliva alone.

Cauliflower mosaic virus (CaMV), and presumably other caulimoviruses, requires a helper component (or aphid transmission factor) when being transmitted by *M. persicae*. The CaMV helper component system has the following properties:

- As with potyviruses, the helper component must be acquired by the aphid either during or before virus acquisition.
- Helper components of other caulimoviruses can complement defects in CaMV helper component.
- The CaMV helper component system involves two noncapsid proteins: the 18 kDa product of ORFII (P2) and the 15 kDa product of ORFIII (P3); (for CaMV gene map, see Profile 4).
- In infected cells, P2 is found in crystalline electrolucent inclusion bodies (see Figure 2.6) and P3 in association with virus particles.
- P2 interacts very strongly with microtubules with binding domains in two regions: one near the N-terminus and the other near the C-terminus.

Thus, the CaMV helper component system is more complex that that of potyviruses. The virus can be transmitted from an *in vitro* acquisition system containing baculovirus-expressed P2 and sap from a plant infected with a P2-defective isolate but not from P2 + purified virus; however, the virus can be transmitted when P3 is added to the purified virus. Secondary structure predictions of P2 suggest two domains: the N-terminus being predominantly β-sheet and the C-terminus predominantly α-helix; the two domains are separated by a predicted random structure. The 61 amino acid C-terminal domain interacts with partially purified virus and with the 30 N-terminal amino acids of P3. Mutations of the N-terminal domain abolish its ability to facilitate transmission but do not affect its ability to bind to semipurified virus. This leads to a model for how the CaMV helper system operates (Figure 12.4B). P3, which forms a tetramer, binds to the virus capsid composed mainly of P4, with the C-terminus of P2 binding to P3. The bridge is completed by the N-terminus of P2 binding to a nonglycosylated protein embedded in the chitin matrix of the common food/salivary duct of the aphid stylet (Figure 12.4B). The role of the microtubule binding activity of P2 is unknown, but it is noted that the microtubule binding domains overlap the P3 and aphid binding domains.

As with nonpersistent viruses, nothing is known about the molecular details of virus release from the vector, but as the virus is retained in the common food/salivary duct at the tip of the aphid's stylet, it is thought that release could be effected by the aphid's saliva.

C. Persistent Transmission by Insects

The main features of persistent transmission are summarised in Box 12.3. Viruses transmitted in this manner are usually transmitted by one or a few species of aphid. Yellowing and leafrolling symptoms are commonly produced by infection with persistently transmitted viruses. Viruses that are internally borne in their aphid vectors may replicate in the vector (propagative) or may not (circulative). For an aphid to become a transmitter by either type of relationship, the virus has to be ingested from the infected plant and reach the salivary glands, usually via the hemolymph, to be egested into the healthy plant. Thus, it has to pass at least two barriers: the gut wall and the wall of the salivary glands.

1. *Circulative Viruses*

a. Features of Circulative Virus: Vector Interaction. Circulative viruses are usually phloem-limited, and thus the vector must feed for a longer time to acquire the virus (see Box 12.3). Luteoviruses are the most studied of the circulative (persistent) viruses for which there is no demonstration of replication in the vector.

The minimum acquisition time can be as little as 5 minutes but is usually several hours. This is followed by a latent period of at least 12 hours, after which the virus can be transmitted with an inoculation access time of 10 to 30 minutes. The aphids then remain capable of transmitting for at least several days.

As just noted, persistently transmitted viruses have to cross at least the gut and salivary gland barriers. Particles of *Cereal yellow dwarf virus-RPV* (CYDV-RPV) associate only with the cell membranes of the hindgut of the aphid vector *Rhopalosiphon padi*. It is suggested that the particles entered the hindgut cells by endocytosis into coated pits and coated vesicles and accumulated in tubular vesicles and lysosomes (Figure 12.5A).

Particles are then released into the haemocoel by fusion of the tubular vesicles with the basal plasmalemma. Aphid salivary glands comprise two principal glands and two accessory glands. *Potato leafroll virus* particles have been seen in the basal lamina and plasmalemma invaginations of accessory salivary cells (Figure 12.5B). Particles were also found in tubular vesicles in the cytoplasm near salivary canals and in coated pits connected to the canal membrane. The basal lamina and the basal plasmalemma function as independent barriers to transmission of different luteovirus-aphid combinations. From these studies the route that luteoviruses take across the two barrier tissues in their aphid vector would appear to be by incorporation into coated vesicles and transport across the cell(s). Thus, the main sites of interaction for the virus particles is with the plasma membrane on the gut side of the gut wall cells and with two plasma membranes on the haemocoel side of the salivary gland accessory cells, which suggests a receptor-mediated interaction.

Because purified luteoviruses can be aphid transmitted from *in vitro* acquisition, it is likely that no noncapsid proteins are involved. The capsid comprises the major capsid protein and a minor amount of a larger protein translated via a read-through of the coat protein stop codon (see Chapter 7). Particles containing just the major coat protein without any read-through protein are not transmissible, which led to the widespread assumption that the read-through portion was required for aphid transmissibility. However, there is no clear picture of the luteovirus component of the receptor-mediated recognition.

Several aphid proteins of Mr ranging from 31 to 85 kDa have been shown to interact with purified luteoviruses *in vitro*. Antisera raised against two of these proteins, P31 and P44, react specifically with extracts of accessory salivary glands from vector aphids, suggesting that these proteins might be involved in luteovirus-specific recognition at this site.

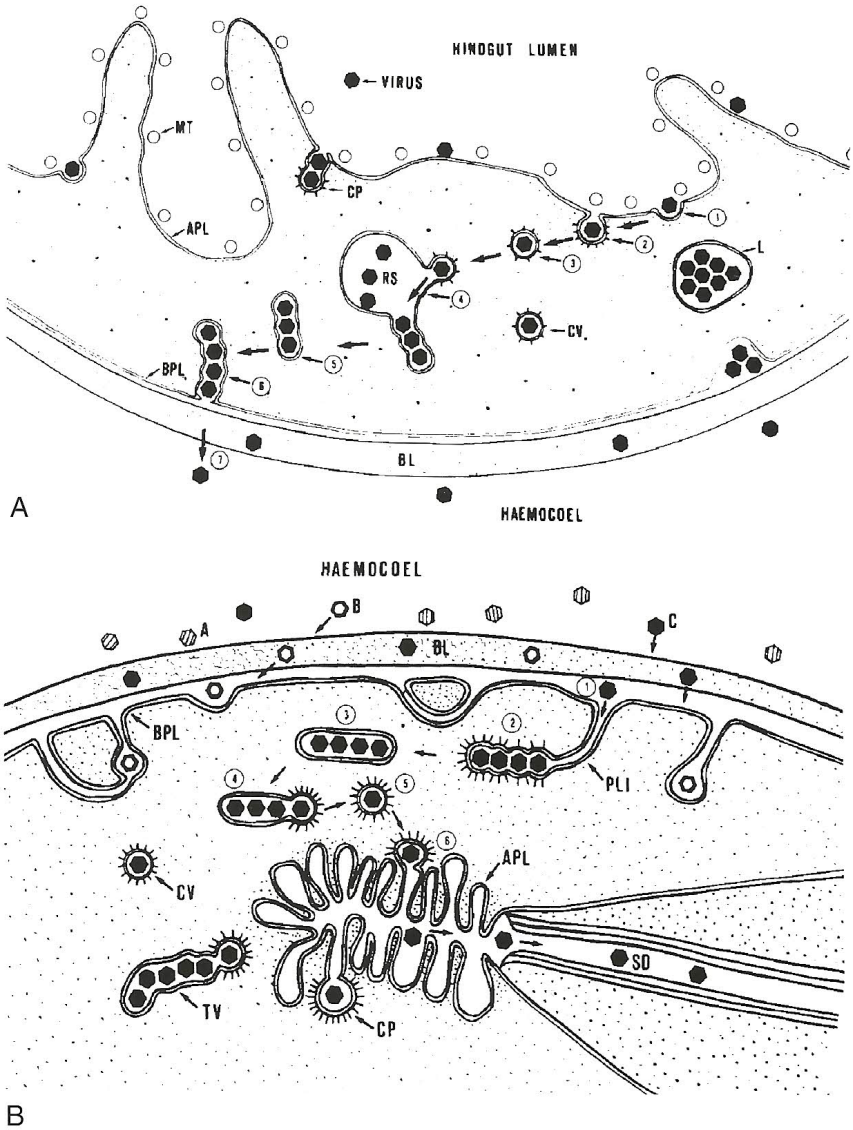

FIGURE 12.5 Models for interactions and transcellular transport of luteoviruses in aphid vectors. A. Transcellular transport through aphid gut epithelium. Visualisation of endocytotic- and exocytotic-associated ultrastructure supports receptor-mediated endocytosis as a mechanism regulating vector-specific luteovirus acquisition. Based on this model, luteoviruses recognised at the gut-cell apical plasmalemma (APL) bind to the membrane (1) initiating virus invagination (2) into coated pits (CP). Coated pits bud off the APL as virus-containing coated vesicles (CV), which transport the virus (3) to larger uncoated vesicles, called receptosomes (RS), which act to concentrate the virus (4). Tubular vesicles containing linear aggregates of virus form on the receptosomes (5) transport the virus to the basal plasmalemma (BPL) and fuse with the BPL, allowing release of the virus from the cell (6). Luteoviruses can then diffuse through the gut basal lamina (BL) and into the haemocoel (7). Eventually, receptosomes (endosomes) mature into lysosomes (L), and any virus particles remaining in the lysosome are probably degraded. MT, microtubules. B. Luteovirus interactions with accessory salivary glands (ASG) determining vector-specific transmission. Luteoviruses in the haemocoel first encounter the extracellular basal lamina (BL) surrounding the ASG. The BL acts as a selective barrier to luteovirus transmission. Depending on the aphid biotype and the specific luteovirus, the virus particles may be prevented from penetrating the BL (A) or may diffuse through (B, C) to the basal plasmalemma (BPL). A second selective barrier occurs at the BPL. Luteovirus particles not recognised at the BPL remain outside the cell in the pericellular space (B). Luteoviruses recognised by putative virus receptors (C) on the BPL (1) are encytosed by coated pits (2) and accumulate into tubular vesicles (TV) in the cytoplasm (3). The TV adjacent to the microvilli-lined canals formed by the apical plasmalemma (APL) bud off coated vesicles (CVs) (4) containing individual virions. The CVs transport the virus (5) to the canals, fuse with the APL (6), forming coated pits (CP), and release the virus into the canal lumen allowing transport of luteovirus out of the aphid along with salivary secretions. PLI, plasmalemma invagination; SD, salivary duct. [From Gildow (1999; in *The Luteoviridae*, H.H. Smith and H. Barker, Eds., pp. 88–112, CAB International, Wallingford, UK).]

Another binding aphid protein, which interacts with many luteoviruses and other viruses, is the 60 kDa symbionin or GroEL from the endosymbiotic bacterium *Buchneria* spp. This protein, found readily in aphid hemolymph, is a member of the molecular chaperone family that is responsible for stabilising the structure of proteins. The interaction of luteoviruses with symbionin is determined by the read-through domain of the minor capsid protein. The luteovirus-symbionin interaction is essential for the retention of the virus in the hemolymph.

b. Dependent Transmission. As with certain nonpersistent viruses, some persistent viruses require a helper factor—in this case, a virus—to be present in the plant before aphid transmission can occur. For persistent viruses dependent on another virus, it is the presence of the virus itself in a mixed infection that provides the assistance. This type of dependent transmission is due to phenotypic mixing together during replication of the two viruses in the plant, resulting in the encapsidation of the genome of one virus in coat protein subunits of the other virus.

Umbraviruses do not encode a coat protein. For their aphid transmission they associate with a helper luteovirus, which is presumed to supply the coat protein and thus aphid transmission properties. Each definitive Umbravirus species is associated with a specific luteovirus. These systems have the following characteristics:

- Both viruses are transmitted in a circulative nonpropagative manner.
- The dependent virus (umbravirus) is sap transmissible, but the helper is not.
- The dependent virus is only transmitted by aphids from source plants that contain both viruses; in other words, aphids already carrying helper virus cannot transmit the dependent virus from plants infected only with this virus.
- Evidence from a variety of experiments indicates that the dependent virus is transmitted by the aphid vector only when its RNA is packaged in a protein shell made of the helper virus protein.

This phenotypic mixing must take place in doubly-infected plants. *Groundnut rosette virus* depends on its satellite RNA as well as on *Groundnut rosette assistor virus* for transmission by *Aphis craccivora* (see Box 2.1).

2. Propagative Viruses

Propagative viruses can be considered to be viruses of the insect that have become adapted to plants. Two plant virus families, *Reoviridae* and *Rhabdoviridae*, and the *Tenuivirus* and *Marafivirus* genera contain members that replicate in their leafhopper vectors. Such replication usually has little effect on the hoppers. However, from the virus point of view, replication in the vector has two important consequences: Once they acquire virus, the vectors normally remain infective for the rest of their lives, and replication in the vector is often associated with transovarial passage of the virus, thus giving it a means of survival over winter that is quite independent of the host plant. With viruses that replicate in their vectors, there is usually a high degree of specificity between vector and virus or even strains of a virus.

Many of the virus:host interactions resemble those found in animal viruses, with some genes that adapt the virus to either animals or plants. In the plant reoviruses, particular genome segments code for gene products required for replication in the insect but not in the plant. The rhabdo- and tospoviruses need glycoprotein spikes for infecting insects but not for plants. On the other hand, the sc4 gene product in plant rhabdoviruses (see Profile 13 for genome map) that facilitates cell-to-cell spread in plants is not found in animal rhabdoviruses.

Whereas aphids are vectors of many of the persistent circulative viruses, most of the persistent propagative viruses are leafhopper- or

planthopper-transmitted. However, several members of the *Rhabdoviridae* replicate in their aphid vector, including *Sowthistle yellow vein virus (SYVV).*

The latent period of SYVV in the vector is long and depends strongly on temperature. Characteristic bacilliform particles have been observed in the nucleus and cytoplasm of cells in the brain, subesophageal ganglion, salivary glands, ovaries, fat body, mycetome, and muscle. Virus particles appear to be assembled in the nucleus. The virus can be serially transmitted from aphid to aphid by injection of hemolymph, and infection is associated with increased mortality of the aphids. Decreased life span varies with different virus isolates. However, since infected aphids live through the period of maximum larviposition, the intrinsic rate of population growth was hardly affected. The virus is transmitted through the egg of *Hyperomyzus lactucae*, about 1 percent of larvae produced being able to infect plants. Continuous passage of SYVV in the aphid by mechanical inoculation gives rise to isolates that have lost the ability to infect the plant host.

3. *Thrip Transmission of Tospoviruses*

Transmission of tospoviruses by thrips has several unusual features. Only the first and early second larvae stages can acquire the virus and the competence to acquire decreases with age of the larvae. *Tomato spotted wilt virus* (TSWV) can be acquired or transmitted by first instar nymphs of *Frankliniella occidentalis* in feeding periods of as short as 5 minutes, but the median acquisition access period on infected *Impatiens* plants is more than 100 minutes. The median latent period varies with temperature being 84 hours at 27°C and 171 hours at 20°C. Individuals may retain infectivity for life, but their ability to transmit may be erratic. The virus is not passed through the egg.

As with other internally borne persistently transmitted viruses, tospoviruses have to pass several barriers in the vector, which suggests that there is/are a receptor-mediated mechanism(s). TSWV is enveloped with spikes made up from two virus-coded glycoproteins, extending from the envelope (see Profile 17). Passage of TSWV through plants can result in envelope-deficient isolates. Feeding *F. occidentalis* on plants infected with wild-type and an envelope-deficient isolate showed that the thrips only became infected when they acquired intact virus particles. These and other experiments suggest that the viral glycoproteins bind to a receptor in the vector's midgut. Two proteins from *F. occidentalis* have been shown to bind to TSWV glycoproteins, one of which is localised to the larval thrip midgut and the other present throughout the thrip's body.

A detailed study of the route that TSWV takes through *F. occidentalis* showed that the first infections were found in the midgut (Mg1) region about 24 hours postacquisition (hpa). These infections increased in intensity but remained restricted to the Mg1 epithelium for some time. In late larval stage, it spread to the circular and longitudinal midgut muscle tissues. By the adult stage, the visceral muscle tissues of the midgut and foregut were infected. Infection of the salivary glands was first observed 72 hpa, and at the same time, the ligaments connecting the midgut with the salivary glands became infected. There was no evidence for TSWV in either the haemocoel or the midgut basal lamina. It appeared that the virus reached the salivary glands through the ligaments connecting Mg1 to the salivary glands. This is a different route to that conventionally proposed for persistent viruses, which is movement through the haemocoel from the gut cells to the salivary glands.

D. Virus Transmission by Beetles

Leaf-feeding beetles have chewing mouthparts and do not possess salivary glands. They regurgitate during feeding, which bathes the mouthparts in sap. This regurgitant will contain virus if the beetle has fed on an infected plant. Beetles can acquire virus after a single bite

and can infect a healthy plant with one bite. However, beetle transmission is not a purely mechanical process. There is a high degree of specificity between beetle vector and virus, and some very stable viruses, such as TMV, are not transmitted by beetles. The viruses that are transmitted belong to the *Tymovirus*, *Comovirus*, *Bromovirus*, and *Sobemovirus* genera.

Sometimes one beetle species will transmit a particular virus with high efficiency, while a related species does so inefficiently. It is suggested that the regurgitant fluid of the beetles contains an inhibitor that prevents the transmission of non-beetle-transmitted viruses but does not affect those that are transmitted. There is good evidence that the inhibitor is an RNase.

E. Nematode Transmission of Viruses

1. *Features of Nematode Transmission*

Two genera of plant viruses are transmitted by nematodes. Nepoviruses are transmitted by species in the genera *Xiphinema* and *Longidorus*, and tobraviruses are transmitted by species of *Trichodorus* and *Paratrichodorus*. All three tobraviruses are nematode transmitted, but only about one-third of the nepoviruses are transmitted by these vectors. With the exception of *Tobacco ring spot virus* (TRSV), which is reported to also have aphid vectors, none of the viruses in these two genera is known to have invertebrate vectors other than nematodes; some nepoviruses are pollen transmitted.

Nematodes are difficult vectors to deal with experimentally because of their small size and their rather critical requirements with respect to soil moisture content, type of soil, and, to a lesser extent, temperature. To overcome these problems, five criteria have been proposed for establishing the nematode vectoring of viruses.

- Infection of a bait plant must be demonstrated.
- Experiments should be done with hand-picked nematodes.
- Appropriate controls should be included to show unequivocally that the nematode is the vector.
- The nematode should be fully identified.
- The virus should be fully characterised.

A common method for detecting nematode transmission has been to set out suitable "bait" plants (such as cucumber) in a sample of the test soil. These plants are grown for a time to allow any viruliferous nematodes to feed on the roots and transmit the virus and for any transmitted virus to replicate. Extracts from the roots and leaves of the bait plants are then inoculated mechanically to a suitable range of indicator species (see Chapter 13).

2. *Virus-Nematode Relationships*

The nematode transmission of a virus has been divided into seven discrete but interrelated processes: ingestion, acquisition, adsorption, retention, release, transfer, and establishment. Ingestion is the intake of virus particles from the infected plant, and although it does not require a specific interaction between nematode and virus, it needs a specific interaction between the nematode and plant. In the acquisition phase, the ingested virus particles are retained in an intact state, and specific features on the surface of the particle are recognised by receptor sites in the nematode feeding apparatus leading to adsorption. Once adsorbed, infectious particles can be retained in the nematode for months or even years but not after moulting. Release of the virus particles is thought to occur by a change in pH caused by saliva flow when the nematode commences feeding on a new plant. In the transfer and establishment phases, the virus particles are placed in the healthy plant cell and start replicating and causing infection.

There is specificity in the relationships between nematodes and the viruses they transmit with often an apparent unique association between the virus isolate and the vector species. There are some cases of different virus isolates

sharing the same vector species or, conversely, one particular virus isolate being transmitted by several nematode species. There are 13 trichodorid species known to be tobravirus vectors, but only one or two of these transmits each tobravirus. There is a substantial degree of specificity between the nematode vector and the tobravirus serotype. Several nepoviruses are transmitted by more than one vector species, but there can be differences in the observations under laboratory and field conditions.

Once acquired, viruses may persist in transmissible form in starved *Longidorus* for up to 12 weeks, in *Xiphinema* for about a year, and much more than a year in *Trichodorus*. Transmission does not appear to involve replication of the virus in the vector. Plant virus particles have never been observed within nematode cells. Consistent with this is the fact that no evidence has been obtained for virus transmission through eggs of nematode vectors.

Specificity of transmission does not appear to involve the ability to ingest active virus, since both transmitted and nontransmitted viruses have been detected within individuals of the same nematode species. Sites of retention of virus particles within nematodes have been identified by electron microscopy of thin sections. Nepovirus particles are associated with the inner surface of the odontostyle of various *Longidorus* species and with the cuticular lining of the odontophore and esophagus of *Xiphenema* species. Tobravirus particles have been observed absorbing to the cuticular lining of the esophageal lumen.

The genetic determinants for the transmissibility of the nepoviruses *Raspberry ringspot virus* and *Tomato black ring virus* are encoded by RNA2, which expresses, among other proteins, the viral coat protein (see Profile 6 for nepovirus genome organisation).

By making reciprocal pseudo-recombinants between a nematode transmissible and a nontransmissible isolate of the tobravirus *Tobacco rattle virus* (TRV), it was shown that transmissibility segregated with RNA2. As noted in Profile 15, tobravirus RNA2 is variable in size, and, besides encoding the viral coat protein, encodes one to three nonstructural proteins. A recombinant virus, in which the coat protein gene of a nematode nontransmissible isolate of *Pea early browning virus* (PEBV) was replaced with that of a highly nematode transmissible isolate of TRV, was not transmitted by nematodes, which indicated that more than one of the RNA2 genes was involved. Mutations in the 29 kDa and the 23 kDa nonstructural genes of PEBV both abolished nematode transmission without affecting particle formation, as did removal of the C-terminal mobile region of the coat protein. It is suggested that the nonstructural proteins may be transmission helper components analogous to those in some aphid and leafhopper virus transmission systems.

IV. FUNGAL TRANSMISSION OF VIRUSES

Several viruses have been shown to be transmitted by soil-inhabiting fungi or protists. The known vectors are members of the class *Plasmodiophoromycetes* in the division *Myxomycota* or in the class *Chytridiomycetes* in the division *Eumycota*. Both classes include endoparasites of higher plants. Species in the chytrid genus *Olpidium* transmit viruses with isometric particles, while species in two plasmodiophorus genera, *Polymyxa* and *Spongospora*, transmit rod-shaped or filamentous viruses.

The two chytrid vectors, *Olpidium brassicae* and *O. bornavirus*, are characterised by having posteriorly uniflagellate zoospores, while those of the three plasmodiophoral vectors, *Polymyxa graminis*, *P. betae*, and *Spongospora* subterranean, are biflagellate. All five species are obligate parasites of plant roots and have similar development stages (Figure 12.6). They survive

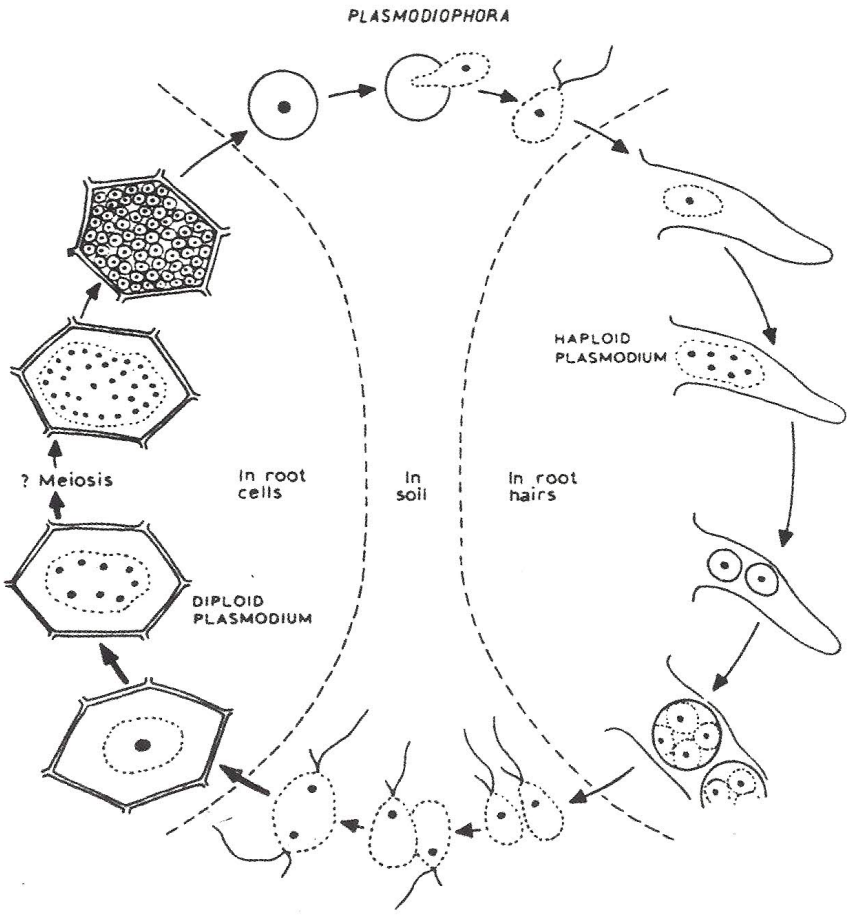

FIGURE 12.6 Life cycle of a plasmodiophoral fungus. On the left-hand side is the diploid stage in root cells; on the right-hand side is the haploid stage in root hairs. Between are the phases in the soil where plant-to-plant virus transmission can occur. [Reprinted from *Matthews' plant virology*, 4th ed., R. Hull, Transmission 1: by invertebrates, nematodes and fungi, pp. 485–532, Copyright (2002), with permission from Elsevier.]

between crops by resting spores that produce zoospores that infect the host. The zoospores form thalli in the host cytoplasm. In the early stages of infection, the cytoplasm of thalli is separated from the host cytoplasm by a membrane, but later the thallus forms a cell wall. The entire thallus is converted into vegetative sporangia or resting spores.

Various degrees of host specificity exist in both the chytrid and plasmodiophoral vectors, with some isolates having a wide host range and others a narrow host range. The wide host range isolates tend to be better virus vectors than are the narrow ones.

Two types of virus-fungal vector relationships have been recognised, termed *in vitro* and *in vivo*. The *in vitro* virus-vector relationship is found between the isometric viruses of the *Tombusviridae* and two *Olpidium* species. Virions from the soil water adsorb onto the surface of the zoospore membrane and are thought to enter the zoospore cytoplasm when the flagellum is "reeled in." It is unknown how the virus passes from the zoospore cytoplasm to the host cytoplasm, but it is thought that this occurs early in fungal infection of the root. Reciprocal exchange of the coat proteins of *Tomato bushy stunt virus* (not transmitted by *O. bornavirus*) and *Cucumber necrosis virus* (CNV; transmitted by *O. bornavirus*) showed that the coat protein is involved in the uptake of the virus by the zoospore. One amino acid in the coat protein of CNV is important for

transmissibility, and binding studies showed that this is associated with recognition of the virus by *O. bornavirus* zoospores.

The model for the *in vivo* virus-vector relationship is demonstrated by the interactions between *Beet necrotic yellow vein virus* (BNYVV) and *Polymyxa betae*. The virus is within the zoospores when they are released from the vegetative sporangia or resting spores and infects the new host when these zoospores establish their own infection of the root. The processes of virus acquisition and release by the zoospores are unknown. The read-through domain from the coat protein (see Profile 2 for genome organisation of BNYVV) is implicated in the fungal transmission. BNYVV RNAs 3 and 4 also have an indirect effect on the transmission, most likely through controlling factors such as spread and accumulation of the virus in the root system.

V. VIRUSES OF OTHER KINGDOMS

In moving to a new host, viruses of vertebrates and invertebrates do not have to cross barriers such as the cuticle and cell wall that face plant viruses. As the respiratory and digestive tracts comprise very large areas of living cells surrounded just by the plasmamembrane, most viruses of vertebrates are transmitted by the respiratory and faecal-oral route. Some, loosely termed arboviruses (e.g., rhabdoviruses, bunyaviruses, and flaviviruses), are transmitted by arthropods in a manner similar to that of propagative persistent plant viruses, the usual vectors being blood-sucking insects such as mosquitoes. From the insects' point of view, the vectors of arboviruses and the analogous plant viruses are the alternate host, the vertebrate, and plant. There is mother-to-child vertical transmission (analogous to seed transmission) of some viruses of vertebrates and invertebrates. Bacterial viruses spread either through cell division or through the surrounding liquid medium.

VI. SUMMARY

- Plant viruses must cross two barriers—the cuticle and the cell wall—before they can infect a plant; this is done by mechanical damage.
- The plant virus can be introduced either from plant material or by a biological vector.
- Introduction from plant material can be by mechanical damage (e.g., breaking leaf hairs), through seed or pollen, or by grafting or vegetative propagation.
- Biological vectors are invertebrates (arthropods or nematodes) and fungi and protests.
- Each plant virus is usually transmitted in nature by just one of the preceding methods.
- Plant viruses have very specific and intricate interactions with their biological vectors.
- There are two basic interactions with insect vectors: nonpersistent or stylet borne, in which the virus interacts with the insect's mouth parts, and persistent or circulative, in which the virus passes though the insect's gut wall into the haemocoel and then into the salivary glands, from where it is injected into the plant.
- The interactions involved the virus coat and in some cases additional virus gene products.
- Interactions with nematode and fungal vectors are also detailed and involve the virus coat protein and sometimes additional viral factors.

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