Leafhopper Vectors and Plant Disease Agents

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Chapter 6

LEAFHOPPERS AND APHIDS AS BIOLOGICAL VECTORS: VECTOR-VIRUS RELATIONSHIPS

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6.1 INTRODUCTION

Insect transmissions of plants viruses may be categorized as *noncirculative* and *circulative* (Harris, 1977a, 1977b, 1978a, 1978c). In circulative ("biological" or persistent) transmission, virus is ingested via the maxillary food canal, absorbed, translocated and – following a latent period or incubation period – inoculated

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into plants in the form of virus-laden saliva ejected from the maxillary salivary canal: an *ingestion-salivation* transmission mechanism. Noncirculative is not synonymous with stylet-borne (mechanical); it is a more inclusive term, encompassing both nonpersistent and semipersistent transmissions.

Noncirculative transmissions are characterized by the absence of a detectable latent period, loss of vector inoculativity through ecdysis (nontransstadial), and the lack of evidence for transmissible virus entering the hemocoele and exiting via the vector's salivary system. Ideally, all viruses or their transmissions referred to in the literature as nonpersistent or semipersistent have been shown to meet at least the first two of the aforementioned criteria; but relatively few have been reported to have actually been tested on the basis of the third (Day and Venables, 1961; Harris, 1977a, 1978a; Pirone and Harris, 1977). Similarly, many persistent viruses have been classifed as circulative solely on the basis of transstadial passage, the presence of a latent period, and analogy with known circulative viruses. Thus far, at least for the vector groups treated here, this assumed synonymity of terminologies (i.e. nonpersistent and semipersistent = noncirculative, and persistent = circulative) appears to be a prescient conclusion. Nevertheless, possible exceptions to this assumption cannot be ruled out. The various observable phenomena that serve to separate noncirculative transmissions into nonpersistent, semipersistent, and "bimodal" types have been recently reviewed (Harris, 1977a, 1977b, 1978a).

This chapter deals primarily with plant virus transmission by aphids, leafhoppers, and delphacid planthoppers. Since the noncirculative/circulative system for categorizing transmissions seems applicable to each of these three major vector groups, it will be used here. Moreover, as implied in the title, most of what follows is an attempt to summarize our present knowledge of these insects as "biological" vectors, i.e. as transmitters of circulative viruses. Learning the fundamentals of vector-virus-plant interactions and how such interactions influence transmission is a first and necessary step towards creating a data bank for use in formulating ecologically sound approaches to disease control.

6.2 CIRCULATIVE VIRUSES: PROPAGATIVE VERSUS NONPROPAGATIVE

Some circulative viruses multiply in their vectors, whereas others do not. The former are referred to as *propagative* and the latter as *nonpropagative*. Many types of evidences have been presented in support of virus multiplication in vectors. Two techniques that unequivocally prove virus multiplication are those of serial passage of virus from insect to insect – either by injection technique or transovarial passage – until the dilution attained in the final inoculative insects exceeds with certainty the maximum dilution of the starting material that can be successfully inoculated (a dilution of 10^{-22} or greater; Black, 1950). Other techniques that indicate a high probability of virus multiplication include the following: methods of titrating for virus or viral antigen increase in virus-exposed insects after they are separated from a source of virus (e.g. infectivity bioassay, immunological procedures, and direct particle counting); demonstrable virus titer increases in inoculated vector cell cultures; demonstrable cross-protection between strains

of virus in the vector; demonstrable cytopathogenic effects of virus on the vector, and direct electron microscopic observations of virions and sites of viral synthesis in vector cells. The fate of virus in the vector might also be followed by infectivity bioassay tests and specific antibody staining techniques. Still other phenomena that suggest, but do not prove, multiplication include: the presence of a prolonged incubation period in the vector, the duration of which shows viral dosage and temperature-dependent responses typical of a biological system; prolonged persistence of individual vector inoculativity and transmission efficiency; transovarial passage; and pathological changes in the biology of virus-exposed insects, such as decreased fecundity, shortened life span, abnormal metabolism, and so on.

6.3 VECTORS OF PLANT VIRUSES AND PHYTOPATHOGENIC ORGANISMS

A compilation of the known insect and noninsect vectors of plant viruses and pathogens is presented in Table 1. In the table, emphasis is given to disease agents such as viruses, mycoplasmalike organisms (MLO's), rickettsialike organisms (RLO's), and spiroplasmas. Detailed coverages of the various types of insect involvements in the transmission of fungal and bacterial pathogens of plants will be presented elsewhere (Harris and Maramorosch, 1979).

TABLE I
List of Insect and Noninsect Vector Groups of Plant Disease Agents[a]

Vector Group	Disease Agents[b]
INSECTA	
HEMIPTERA	
Homoptera	
Sternorrhyncha	
Aphidoidea	
*Aphididae	virus; MLO (?) of Easter lily rosette, crimson clover rough vein, and sugarcane grassy shoot
Callaphididae	virus
Chaitophoridae	virus
Pemphigidae	virus
Thelaxidae	virus
Adelgidae	virus
Coccoidea	

Vector Group	Disease Agents[b]
*Pseudococcidae	virus (mainly of cocoa, but also sugarcane spike and pineapple latent)
Aleyrodoidea	
*Aleyrodidae	virus
Psylloidea	
*Psyllidae	MLO (citrus greening and pear decline), RLO (wheat chlorosis), proliferation disease agent of carrots (MLO/RLO?)
Auchenorrhyncha	
Cicadoidea	
*Cicadellidae	virus, MLO, RLO, spiroplasma
*Cercopidae	virus (sugarcane chlorotic streak), RLO (Pierce's disease), MLO (peach yellows)
Membracidae	virus (?) (pseudocurly top of tomato)
Fulgoroidea	
*Delphacidae	virus, MLO (rice grassy stunt)
Cixiidae	MLO (potato witches' broom, tomato big bud, *Phormium* yellow leaf)
Heteroptera	
Piesmidae	virus (beet leaf curl), RLO (sugarbeet witches' broom), MLO (beet savoy)
Miridae	virus (?), potato spindle tuber viroid
Lygaeidae	virus (*Centrosema* mosaic)
COLEOPTERA	
Chrysomeloidea	
*Chrysomelidae	virus, potato spindle tuber viroid
Bruchidae	virus
Curculionoidea	
Curculionidae	virus

Table I (cont.)

Vector Group	Disease Agents[b]
Apionidae	virus
Cucujoidea	
Coccinellidae	virus (experimental vectors)
Melooidea	
Meloidae	virus
THYSANOPTERA	
Thripidae	virus
ORTHOPTERA	
Acrididae	virus, potato spindle tuber viroid
Tettigoniidae	virus
LEPIDOPTERA	
Pieridae	virus (turnip crinkle, turnip yellow mosaic)
Sphingidae	virus (TMV)
Noctuidae	virus (TMV)
DIPTERA	
Agromyzidae	virus
DERMAPTERA	
Forficulidae	virus (turnip yellow mosaic)
NONINSECTA	
DORYLAIMIDA	
*Trichodoridae	virus (tobra-)
*Longidoridae	virus (nepo-)
ACARINA	
Eriophyidae	virus
Tetranychidae	virus (PVY; requires confirmation)

Table I (cont.)

Vector Group	Disease Agents[b]
CHYTRIDIALES	virus
PLASMODIOPHORALES	virus
GASTROPODA	virus (TMV)
Limacidae	
Endodontidae	
Zonitidae	

[a]The more important vector groups (in terms of numbers of vector species, and disease agents transmitted) are preceded by asterisks.
[b]Virus, mycoplasmalike organism (MLO), spiroplasma, and rickettsialike organism (RLO).

About 97% or 272 of the approximately 280 known arthropod-borne, plant viruses and pathogens are transmitted by insects. Most insect vectors, >80%, are in the suborder Homoptera. Of these homopterous vectors, ca. 57% occur in the Sternorrhyncha and ca. 43% in the Auchenorrhyncha. Auchenorrhynchous vectors include 130 species of leafhoppers (Cicadellidae), 20 species of planthoppers (Fulgoroidea: 17 in Delphacidae, and 3 in Cixiidae), and 10 species of spittle bugs (Cercopidae) (Carter, 1962, 1973; Ishihara, 1969; Nielson, 1962, 1978). Sternorrhynchous vectors include ca. 192 species of aphids (Aphidoidea: 173 in Aphididae, 10 in Callaphididae, 6 in Chaitophoridae, 2 in Pemphigidae, 1 in Adelgidae, and 1 in Thelaxidae), 19 species of mealybugs (pseudococcidae), 3 species of whiteflies (Aleyrodidae), and 4 species of psyllids (Psyllidae) (Carter, 1962, 1973; Kennedy *et al.*, 1962; Ossiannilsson, 1966; Roivainen, 1973, 1976, 1979).

Mealybug, psyllid, beetle, and nematode vectors were discussed in a recent international symposium on vectors of plant disease agents (Harris and Maramorosch, 1977b). Transmission by these and other vectors such as whiteflies, membracids, piesmids, mirids, thrips, leafminer flies, mites, and fungi will be covered in detail in a sequel to this book (Harris and Maramorosch, 1979).

6.4 APHID, LEAFHOPPER, AND DELPHACID PLANTHOPPER VECTORS

6.4.1 Aphids

The biology, feeding behavior and worldwide distribution of aphids make them ideally suited for transmitting plant viruses (Harris and Maramorosch, 1977a). Aphids (Aphidoidea), with ca. 192 vector species, transmit ca. 164 viruses and possibly 3 MLO's (Table I), and they account for more than 86% of all sternorrhynchous vectors. Indeed, these insects alone are responsible for the transmission of ca. 60% of all known, insect-borne, plant viruses and pathogens.

The total number of described aphid species is 3,742. Only ca. 300 species have been tested as vectors of any of ca. 300 different viruses in about the same

number of plant species. About 192 of the 300 species tested have been reported as vectors of at least one plant virus. More than half of the aphid species and most economically important virus vectors occur in the subfamily Aphidinae (Eastop, 1977). The majority of the 164 aphid-borne viruses are noncirculative (101 nonpersistent and 8 semipersistent), 38 are circulative (persistent), and the status of 17 is uncertain (Eastop, 1977). Most circulative aphid-borne viruses appear to be nonprogagative; those that are known with certainty to multiply in their vectors occur in the family Rhabdoviridae (section 6.8.2).

6.4.2 Leafhoppers

Leafhoppers (Cicadellidae), with 130 known vector species covering 10 subfamilies and 58 genera, transmit ca. 76 disease agents (ca. 38 viruses, 31 MLO's, 3 spiroplasmas, and 4 RLO's) and account for more than 80% of all auchenorrhynchous vectors (Nielson, 1962, 1978; Ishihara, 1969). Most leafhopper-borne viruses are transmitted in a circulative manner and multiply in their vectors, with the circulative, nonpropagative beet curly top virus and the noncirculative rice tungro virus group and maize chlorotic dwarf virus being notable exceptions.

6.4.3 Delphacid Planthoppers

As vectors, planthoppers (Delphacidae) have received far less attention from researchers than have aphids and leafhoppers. However, 17 vector species (11 genera) have been recorded; and, as a group, these vectors are responsible for the transmission of at least 12 disease agents, including 10 viruses and 2 MLO's (Table I; Kisimoto 1973). Transmission is circulative, and the viruses appear to multiply in their insect vectors.

6.5 NONCIRCULATIVE TRANSMISSION

The Plant Virus Subcommittee of the International Committee for Taxonomy of Viruses (ICTV) has endorsed eight groups of plant viruses that contain one or more noncirculative, aphid-transmitted members: potyviruses, carlaviruses, caulimoviruses, cucumoviruses, closteroviruses, and three monotypic groups, as yet unnamed, based on alfalfa mosaic, broad bean wilt, and parsnip yellow fleck viruses, respectively. The reader is referred to recent reviews by Shepherd (1977), Pirone and Harris (1977), and Harris (1978a) for more detailed information on the intrinsic properties of aphid-borne, noncirculative viruses.

Nonpersistent, noncirculative transmission. The stylet-borne hypothesis of virus transmission is based on experiments in which it was demonstrated that treating the stylets of viruliferous aphids with formalin or UV radiation renders the insects nonviruliferous. However, these experiments cannot provide conclusive proof that transmissible virus can be carried on the stylets, because such treatments might also inhibit the probing and feeding behavior responsible for transmission. Indeed, similar experiments could be used to argue against stylet-borne transmission. For example, exposing the stylets of viruliferous aphids to a number of other

antiviral agents, e.g. 8-azaguanine, milk, aphid saliva, and juice from crushed aphids, has no effect on vector inoculativity. Additionally, a flushing out of the salivary duct by saliva and the secretion of saliva over and around the stylets does not affect vector inoculativity. Therefore, if stylet-borne transmission, *sensu stricto*, can occur, it would not appear to be a *sine qua non* of noncirculative transmission (sections 7.2.3 and 7.2.4.4 in Harris, 1977a).

The importance of brief probing in nonpersistent transmission, especially in the acquisition phase, cannot be overemphasized. More pointedly, *what aphids do during brief probing* is of paramount importance in any attempt to understand the mechanisms of or explain the characteristics associated with both the acquisition and inoculation phases of transmission. Analyses of data obtained from membrane-probing and feeding experiments, from electronic monitoring of aphid probing and feeding, and from experiments involving sequential probing by aphids, first in isotopically labeled and then in nonlabeled leaf disks, indicate that aphid host-selection behavior, especially sap-sampling during superficial probes, plays an important, perhaps essential, role in the transmission process (Harris, 1977a).

Sap-sampling behavior, when occurring on a virus-infected plant, serves to contaminate the fore alimentary canal with virus-laden material ("cell sap" or protoplasm). The transmission cycle is completed when all or a portion of this virus-laden material is egested during subsequent sap-sampling probes in healthy plants. This unique, host-selection behavior of aphids – when coupled with their finely tipped stylets and their habit of inserting only the maxillary tips into cells from which they ingest sap and then closing these feeding sites with a salivary plug during stylet withdrawal – makes them ideally suited as vectors of noncirculative viruses. Virus is both acquired and released via the maxillary food canal: an *ingestion-egestion* transmission mechanism. Aphid vectors function more like flying syringes than "flying needles."

The aphid-plant interactions responsible for sap-sampling are not yet known. I (Harris, 1977a) hypothesized that the dendrites innervating the tips of the aphid's mandibles are contact chemoreceptors. Wensler (1977, personal communication) does not share this view, and she takes exception to the manner in which her research in this area was quoted and interpreted. She considers the structure of the mandibular innervation to be typical of chordotonal organs or scolopidia. Reportedly (Wensler, 1974), the two neurons with short dendrites extending into the mandibular base are not mechanoreceptors, whereas paired mechanoreceptive dendrites innervating the tip enable the aphid to monitor the movement and position of the stylet. Whatever is their mode of action, these receptors and others (e.g. the chemoreceptors of the pharyngeal gustatory organ and the mechanoreceptive pegs at the labial tip) are apparently part of the sensory transduction system that makes possible such behavioral manifestations as anticlinal groove localization, epidermal cell entry, sap sampling, deep probing, and feeding site selection.

Semipersistent, noncirculative transmission. Overall, semipersistent and bimodal transmission characteristics seem far more compatible with an ingestion-egestion transmission mechanism (Harris, 1977a, 1977b, 1978a, 1978b; Pirone and Harris, 1977). Lim and Hagedorn (1977) and Vanderveken (1977) favor van der Want's (1954) proposal that the surface-adherence properties of stylet and virion surfaces allow for differential adsorption of virus to and elution from the stylets. One might also consider the surface-adherence hypothesis in terms of an ingestion-egestion transmission mechanism. The varying degrees of mandibular contamination reported by Lim and Hagedorn (1977) may reflect the quantity and quality of available virus attachment sites within a particular vector. An aphid's fore alimentary canal, which is hypodermal in origin, is also lined with cuticle. Like the stylets, its cuticular lining or intima is shed during ecdysis as part of the exuvium. Semipersistence and increases in the probability of transmission with increases in the duration of the acquisition-access feeding period suggest that virus can accumulate in the vector's fore alimentary canal and resist being quickly flushed through by virus-free sap ingested during feeding on healthy plants. Virus that is acquired and retained in this way could subsequently be inoculated into plants by egestion (section 7.3.3, *semipersistent transmission*, in Harris, 1977a; Harris, 1978a). Ingested virus would have more numerous and more varied vector sites (both living and nonliving) with which to interact either directly or indirectly via intermediate helper agents. Murant and associates (1976) have proposed such a mechanism for the transmission of the semipersistent anthriscus yellows virus (AYV).

The aphid-borne, beet yellows virus (BYV) is phloem-restricted. Rather predictably, it is semipersistently transmitted. Using an electronic monitoring system, Chang (1968) demonstrated that BYV vectors require a minimum of ca. 5 min to reach the phloem. Once having reached a sieve element of a BYV-infected plant, aphids require an additional 5 min and 22 sec of fluid ingestion to become viruliferous. Similarly, the inoculation threshold for BYV approximates the time required for the vector to reach the phloem parenchyma. Acquisition-access feeding periods of more than 12 hr and inoculation feeds of at least 6 hr are necessary for optimum transmission (Watson, 1946; Sylvester, 1956a, 1956b; Russell, 1970). Feeding in the phloem would allow for maximum virus passage through and accumulation in the fore alimentary canal: the longer the feed, the greater the virus accumulation, and the longer the persistence of vector inoculativity. Beet yellows virus is retained by aphids for up to 3 days, with a half-life of ca. 8 hr (Watson, 1946; Sylvester, 1956a).

Among the Hemiptera, ingestion-egestion behavior does not seem to be confined to aphids. Storey (1939), for example, reported observing an occasional outward flow (egestion) of fluid from the stylets of leafhoppers, *Cicadulina mbila* Naude, that were feeding through wax membranes on sucrose solutions. He suggested that the outward flow ". . . may have been a voluntary action intended to clear the choked canal." I have observed similar egestion behavior on the part of the aphids (Harris and Bath, 1973; Harris, 1977a). Presumably egestion during and/or at the cessation of feeding in a sieve element would serve to clear blocking materials from the maxillary food canal and the salivary sheath canal. Occasional egestion of fluid might also help prevent the build up of P protein fibrils and callose at the

feeding site (Harris, 1977a). Crane (1970) reported egestion by the leafhopper *Hordnia circellata* (Bak.). Harris and associates (Harris, 1977a; Harris *et al.* 1979) developed techniques for critically studying the ingestion-egestion behavior of the leafhopper vector *Macrosteles fascifrons* (Stål) during membrane feeding. This leafhopper typically egests material following rather prolonged periods of ingestion. One insect was seen to intermittently egest previously ingested feeding solution over a 10-min period. Finally, Risk (1969) observed that the stinkbug *Euschistus conspersus* Uhler egests both during membrane feeding and when feeding in plants. In plants, egestion usually occurs after a "satiation" feed in the phloem, just prior to stylet withdrawal.

An ingestion-egestion mechanism, in combination with an ability of virus to accumulate and persist in the anterior portion of the vector's alimentary canal might also explain "semipersistent" transmission by leafhoppers (section 7.3.3, *semipersistent transmission*, in Harris, 1977a; Harris, 1978a). Rice tungro virus (RTV), rice tungro-like viruses (Shikata, 1978b; Ling and Tiongco, 1978), and maize chlorotic dwarf virus (MCDV) are prime suspects. There is no evidence to suggest that either RTV or MCDV is transmitted in a circulative manner. The vector-virus relationships of each of these viruses are characterized by the absence of a demonstrable latent period or incubation period, the absence of evidence for virus entering the hemocoele of the vector and exiting via the salivary system, a gradual decline in inoculativity once a viruliferous insect is separated from a source of virus, and nontransstadial passage. The prefix "semi" connotes a condition that is intermediate between two extremes. Rice tungro virus does not qualify as a persistent virus, and there are no known cases of nonpersistently transmitted, leafhopper-borne viruses. For these and other reasons, Ling and Tiongco (1978) have proposed the term "transitory" to describe the relationship of RTV with its leafhopper vector. Their definition of "transitory" would seem equally applicable to the vector-virus relationships of MCDV.

Ingestion-egestion behavior could also be involved in the transmission by leafhoppers of disease agents other than viruses. For example, although transstadial passage has not yet been tested, the characteristics of leafhopper transmission of Pierce's disease agent of grapevines suggest that the vector-pathogen relationship is a noncirculative one (Purcell, 1978). Retention of the pathogen in the alimentary canal and a simple inoculation mechanism, such as egestion, seem most compatible with the characteristics of a brief or nonexistent latent period, prolonged retention of inoculativity by vectors, and a broad vector range (low specificity). Pierce's disease agent is known to be transmitted by 24 species of leafhoppers (Frazier, 1965) and 4 species of spittle bugs (Severin, 1950).

A transmission mechanism similar to what I have proposed for noncirculative transmission by aphids and leafhoppers appears to be operative in the transmission of tobraviruses and nepoviruses by dorylaimid nematodes. In *Longidorus* species, virus is retained by specific association with the stylet or its cuticular guiding sheath. In *Xiphinema* species and trichodorid vectors, virus is retained at specific retention sites on the cuticle lining the esophagous (odontophore, esophagous

proper, and esophageal bulb). Inoculation of virus into punctured plant cells is thought to occur when the release of salivary fluids into and their passage through the esophageal lumen creates a backflow of materials (Wyss, 1977; Taylor, 1979).

The question of whether mealybugs (Pseudococcidae) transmit virus in a noncirculative or circulative manner is still unresolved (Roivainen, 1979). Ingestion-egestion behavior could conceivably play a role in this vector-virus association as well, even though transstadial passage has been recorded in some instances.

6.6 CIRCULATIVE LEAFHOPPER-BORNE VIRUSES

Most circulative, leafhopper-borne (Cicadellidae) viruses multiply (circulatve-propagative) in both their plant hosts and insect vectors, with the circulative, nonpropagative beet curly-top virus being a notable exception. Transovarial passage of wound tumor (WTV), rice dwarf (RDV) and potato yellow dwarf (PYDV) viruses has been reported (Fukushi, 1933; Black, 1953).

6.6.1 Reoviridae

The family Reoviridae (Fenner *et al.*, 1974; Fenner, 1975/76) includes viruses that multiply in vertebrates (genus *Reovirus*), invertebrates and insects (genus *Orbivirus*), and in plants and insects (genera not yet approved). In host cells, viral specific messenger RNA is synthesized via a virus-coded, RNA-dependent, RNA polymerase contained in the virus particles (Skehel and Joklik, 1969). Smith *et al.* (1969) found that virions contain at least seven different polypeptides in sizes corresponding to certain of the individual segments of viral RNA.

Hopper-borne (Cicadellidae and Delphacidae) phytoreoviruses have large isometric virions (70-80 nm in diameter), contain 10-22% double-stranded RNA by particle weight, multiply in both plant and vector hosts, are transmitted by vectors (leafhoppers or planthoppers) in a circulative (persistent) manner, are not sap-transmissible, and occur in the cytoplasm of their hosts' cells.

The taxonomy of the phytoreoviruses has not yet been clearly established. The leafhopper-borne WTV and RDV are probably members of the group, as are maize wallaby ear virus (MWEV) and leaf gall disease agent. A tentative listing of planthopper-borne members would include pangola stunt (PSV) (Kitajima and Costa, 1971), oat sterile dwarf (OSDV) (Lindsten and Gerhardson, 1971; Lindsten *et al.*, 1973), maize rough dwarf (MRDV) (Lovisolo, 1971), sugarcane Fiji disease (FDV) (Hutchinson and Francki, 1973), cereal tillering disease (CTDV) (Lindsten *et al.*, 1973), and rice black-streaked dwarf (RBSDV) (Shikata, 1974) viruses. Fiji disease virus, RDV, and MRDV do not appear to be serologically related (Ikegami and Francki, 1973), whereas MRDV shows serological relatedness to RBSDV (Luisoni *et al.*, 1973), PSV (Milne, personal communication in Shikata, 1977), MWEV, and leaf gall disease agent of maize and rice (Grylls, 1978). The genomes of the leafhopper-borne WTV and RDV are composed of 12 segments of dsRNA, whereas those of PSV, OSDV, FDV, MRDV, RBSDV (planthopper-borne), and reovirus type 2 are 10-segmented (Reddy and Black, 1973; Reddy *et al.*, 1974, 1975a, 1975b; Luisoni and Milne, 1978).

6.6.1.1 Wound Tumor Virus (WTV). Wound tumor virus is the name proposed by Black (1945) for the causative agent of big vein disease of clover (Black, 1944). Synonyms include clover wound tumor virus, clover big vein virus, *Aureogenus magnivena*, and *Trifoliumvirus nervicrassans* (Black, 1970a). The International Committee on Taxonomy of Viruses (ICTV) has placed WTV in the same family of double-stranded RNA viruses as reoviruses, the family Reoviridae (Maramorosch, 1966; Wildy *et al.*, 1967; Fenner, 1975/76). Similarities in size, shape, number and arrangement of capsomeres, dsRNA (Black and Markham, 1963), guanine-cytosine/adenine-uracil base ratios, RNA polymerase, and intracellular behavior are all factors relating WTV to reoviruses of man and a wide variety of lower animals (Gomatos and Tamm, 1963a, 1963b; Streissle and Maramorosch, 1963; Gamez *et al.*, 1967; Rosen, 1968; Maramorosch, 1970). These bases of similarity also suggest an affinity of WTV with other large polyhedral plant viruses such as RDV, MRDV, and FDV (Black, 1970). Whether these similarities reflect phylogenetic relationships or parallel evolution from different phylogenetic sources is not known (Maramorosch, 1966; Gamez *et al.*, 1967). Serological relatedness of WTV to any of the other probable members of the plant reovirus group has not been demonstrated. Also, sensitive passive hemagglutination tests failed to show a relatedness of WTV to any of three strains of reovirus (Gamez *et al.*, 1967).

Purified virus can be prepared from infected plants and leafhoppers. Crimson clover, *Trifolium incarnatum*, is the preferred plant for testing the inoculativity of vectors exposed to WTV *per os* by feeding on WTV-infected plants. Virus can also be assayed by abdominally inoculating leafhoppers with virus and then testing either for transmission to test plants or for the production of virus antigens in injected insects (Whitcomb, 1964; Reddy and Black, 1966). Inoculation of leafhopper cell monolayer cultures serves as an excellent assay system. Infected cells can be detected by electron microscopic examination or by staining with fluorescent antibody. Chiu and associates (Chiu *et al.*, 1966; Chiu and Black, 1967, 1969) demonstrated a linear relationship between the virus concentration in an inoculum and the number of infected cells in a culture, thus suggesting a theoretical cell-infecting unit (CIU) of a single particle. Kimura and Black (1972) came close to demonstrating this theoretical CIU in experiments utilizing cell monolayers of an AC20 cell line of *Agallia contricta* Van Duzee. Using Strohmaier's (1967) technique for sedimenting virus directly onto grids for subsequent quantitative analysis in the electron microscope, Streissle and associates (1968) determined that 1 g amounts of plant tumor or infected insect tissues contained an average of 5×10^{10} and 2×10^{10} virus particles, respectively.

The WTV particle is icosahedral and measures ca. 75 nm in diameter. An analysis of initial structural data suggested that the WTV capsid contained 92 capsomeres (Bils and Hall, 1962). However, later studies by Kimura and Black (unpublished data in Black, 1970a) suggest a particle structure similar or identical to rice dwarf virus (RDV). The RDV particle contains a total of 32 capsomeres, of which 12 consist of 5 substructural units and 20 of 6 substructural units (Fukushi *et al.* 1962; Kimura and Shikata, 1968). Each of the 180 substructural units is a hollow

tube measuring ca. 6 x 9.5 nm (Kimura and Shikata, 1968; Shikata, 1978a). Streissle and Granados (1968) reported that WTV, like reoviruses, has an inner structure or two protective coats and, by implication, more than one protein (Maramorosch, 1970). The inner and outer shells measure 4.5 and 5.0 nm, respectively.

Wound tumor virus has a sedimentation coefficient of 514 ± 10 S, a molecular weight of $68 \pm 2 \times 10^6$ daltons, an absorbance at 260 nm (1 cm light path) of 1.0 (8.8×10^{11} virions/ml), and thermal inactivation-point (10 min) of 50-60°C in vector extracts. Virions contain 22% dsRNA (ca. 38% G+C) of $15\text{-}17 \times 10^6$ daltons, and 78% protein by particle weight (Black and Markham, 1963; Gomatos and Tamm, 1963b; Kalmakoff *et al.*, 1969; Reddy and Black, 1973; Reddy *et al.*, 1974). The dsRNA of WTV has approximate molar percentages of nucleotides of G18.6, A31.1, C19.1, and U31.3 (Gomatos and Tamm, 1963b).

Wound tumor virus has not been observed causing disease in any plants in nature. It was first recovered, and only once, from a single species of leafhopper collected in the vicinity of Washington, D. C. Subsequently, it was found to experimentally infect two additional leafhopper species. Thus far, the leafhoppers (Agalliinae) *Agallia constricta* Van Duzee, *A. quadripunctata* Provancher, and *Agaliopsis novella* (Say) are the only species known to be susceptible to infection. No information is available on transmission through seed or by dodder. Only six instances of sap transmission of WTV to plants have been reported. WTV-inoculative leafhoppers can transmit the virus to a wide range of host plants. Experimentally susceptible species (forty-three) occur in at least 20 families of dicotyledonous plants(Black, 1945). The virus systemically infects many plant hosts, and disease symptoms include irregular vein enlargement, wart-like enations, and root as well as stem tumors. When stems of infected sweet clover, *Meliotus* sp., are wounded by scratching or pricking with a pin, a tumor develops at the site of wounding, hence. Black's (1945) reason for renaming the disease "wound tumour." *Trifolium incarnatum* (crimson clover), *Melilotus officinalis, M. alba* (sweet clovers), and *Rumex acetosa* (cultivated sorrel) serve as diagnostic species (Black, 1970a). Clone C10 of *M. officinalis* develops many root tumors containing high concentrations of virus (ca. 10^{12} virions/g) and is therefore an excellent propagative species (Black, 1951, 1970a).

Fate in plants. Virus antigen is mainly concentrated in spherical bodies in the cytoplasm of abnormal phloem (Nagaraj and Black, 1961). The relationship of these bodies to the spherules of Littau and Black (1952) or to the various viral inclusions, such as viroplasms, observed by electron microscopy is not known. The ultrastructure of WTV-induced plant neoplasia has been thoroughly studied by electron microscopy (Shikata *et al.*, 1964; Shikata and Maramorosch, 1966a, 1967a, 1969; Maramorosch, 1970). Ultrathin sections for electron microscopy were prepared from root and stem tumors of sweet clover, wart enations from sorrel, and enlarged veins from sweet clover leaflets. Wound tumor virus particles were observed in the cytoplasm of infected plant cells, but never in chloroplasts, mitochondria, or cell nuclei. Virions were observed scattered and in clusters in necrotic and nonnecrotic portions of tumor cell cytoplasm and in tracheidal cells

of root tumors, but not in epidermal cells of tumors or in the cortex of roots. Virus clusters enclosed in membranous structures and occasional strings of particles in open-ended tubular structures were observed in some stem and root tumor cells. In the initial stages of infection, it seems likely that WTV unleashes the neoplastic potentialities of plant cells; however, once this neoplastic tendency is set in motion, its sustenance is not dependent on the continued presence of detectable WTV. Wound tumor virus virions or antigens cannot be detected in plant tumors grown for several months in tissue culture (Streissle and Maramorosch, 1969; Maramorosch, 1970).

Fate in vectors. Leafhopper nymphs as well as adult males and females can transmit the virus (Maramorosch, 1950). The efficiency of WTV transmission by *Agallia constricta* depends on vector age at the time of acquisition-access feeding on diseased plants; nymphs are more efficient transmitters than adults. An analysis of data from experiments using abdominal puncture (Storey, 1933) and fluorescent antibody staining techniques suggests that both the susceptibility of gut epithelial cells to WTV infection and gut permeability to virus decrease with increasing vector age (Sinha, 1963, 1967).

Insects can be rendered inoculative by feeding on diseased plants or by syringe inoculation with infective plant extract, insect hemolymph, or partially purified virus suspensions (Maramorosch, 1956; Maramorosch and Jernberg, 1970). Once acquired by feeding on diseased plants, the virus undergoes a 13-15 day incubation period before the vector is able to transmit virus (Maramorosch *et al.*, 1949; Maramorosch, 1950).

Mutliplication of WTV in leafhoppers has been demonstrated by serial injection technique (Black and Brakke, 1952) and by electron microscopy of ultrathin sections of inoculative insects (Shikata *et al.*, 1964; Maramorosch and Shikata, 1965; Maramorosch *et al.*, 1965, 1969a, 1969b; Shikata and Maramorosch, 1965b, 1965c, 1965d, 1967a, 1967b, 1969; Granados *et al.*, 1967; Hirumi *et al.*, 1967; Granados *et al.*, 1968; Maramorosch, 1968, 1970). Electron microscopy of viruliferous leafhoppers revealed the presence of virions in the cytoplasm (never in cell nuclei) of fat body, malpighian tubules, hypodermis, tracheoblasts, muscle, mycetome, gut epithelium, all lobes of the salivary gland system (Shikata *et al.*, 1964; Maramorosch *et al.*, 1965; Shikata and Maramorosch, 1965d; Maramorosch, 1970), nervous system (Hirumi *et al.*, 1967), and certain types of hemocytes (Granados *et al.*, 1968). Figure 1 shows a microcrystalline inclusion of WTV in a salivary gland lobe of *A. constricta.*

After leafhoppers are fed on infected plants, virus is first observed in the lumen of the filter chamber, whence it infects gut epithelial cells (2-4 days postacquisition) and, having traversed the *tunica propria*, eventually invades and infects cells of most of the organs and tissues in the hemocoele. In the cytoplasm of infected cells, virions may occur free in the cytoplasm, in vacuoles, in defined, electron-dense, sometimes myelinated phagocytic structures (phagolysosomes?), in viroplasms, in microcrystalline inclusions, and, in a later stage of infection, in rows of particles in tubular structures.

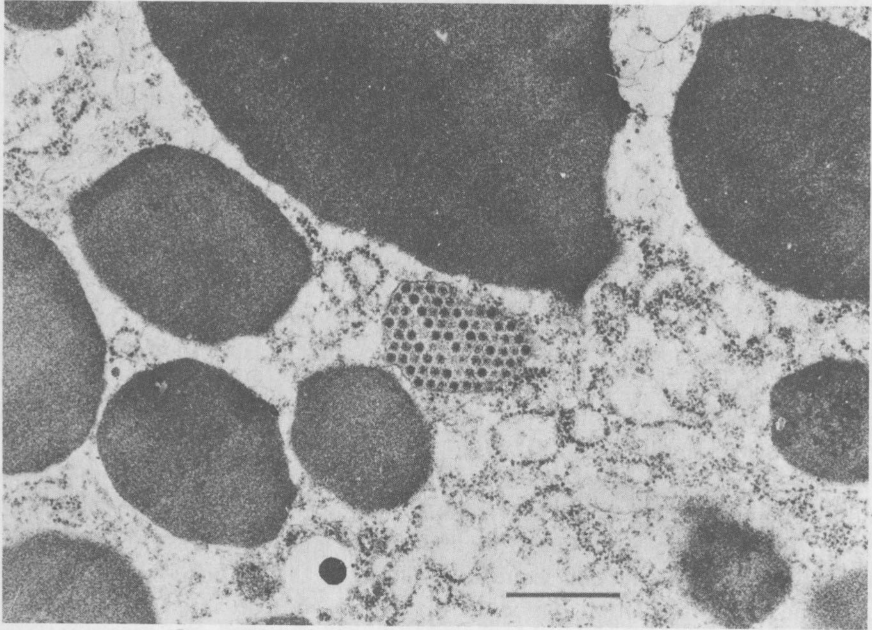


Fig. 1. Microcrystalline inclusion of wound tumor virus particles in a cell of a salivary-gland lobe of *Agallia constricta*. Bar, 500 nm. (Courtesy of E. Shikata.)

Serological studies indicate that WTV is also present in the ovaries (Sinha, 1968a). Virus is occasionally transovarially passed to progeny of infected females. The level of transovarial passage can be increased significantly by selection and breeding. The genetic mechanism controlling passage of WTV to progeny is independent of genetic variation in the ability of the leafhoppers to transmit potato yellow dwarf virus (Black, 1953, 1970a; Nagaraj and Black, 1961; Sinha and Shelley, 1965).

Fatbody tissues of viruliferous leafhoppers were found to contain the largest accumulations of WTV, often in the form of microcrystals (Shikata and Maramorosch, 1965b; Maramorosch *et al.*, 1969; Maramorosch, 1970, 1975). Crystalline arrangements were also seen in muscle and gut epithelial cells (Shikata and Maramorosch, 1965b, 1965c, 1965d), in hemocytes (Granados *et al.*., 1968), and rarely in the salivary glands (Maramorosch *et al.*., 1969a). These microcrystals probably represent stored virus: a possible "blind alley" for virions and thus a protective mechanism by which infected cells eliminate some virions from future participation in the infection process (Maramorosch, 1970, 1975). Electron microscopy of ultrathin sections of insects that acquire WTV by feeding or injection reveals numerous sites of WTV multiplication within the vector (Shikata and Maramorosch, 1967a, 1967b). Wound tumor virus assembly occurs in aggregates of finely textured, electron-dense materials in the cytoplasmic matrices of infected cells (Maramor-

osch, 1970). These electron-dense areas or viroplasms correspond in appearance with the "virus factories" described for several RNA viruses such as polio, mengo, reovirus, and others (Dales and Franklin, 1962; Dales *et al.*, 1965a, 1965b).

The appearance of assembly sites or viroplasms in vector cells or cells of infected plants is always followed by the formation of complete virus particles, first at the periphery and later within the entire viroplasmic matrix. Thus, the detection of viroplasms by electron microscopy provides a technique for the precise localization of virus assembly sites at the subcellular level. Viroplasms occur in gut cells, fat body, muscles, malpighian tubules, tracheoblasts, salivary gland, central nervous system, blood cells, and even in hypodermal cells. It appears, therefore, that numerous vector tissues are capable of supporting WTV multiplication. The presence of viroplasms and "empty shells" in hemocytes, and the late occurrence of microcrystals in these same cells, indicate that hemocytes act not only as carriers of virus to various sites in the vector but also as continuing sources of fresh virus (Granados *et al.*, 1968; Maramorosch, 1970, 1975).

The sequential infection of leafhoppers following WTV acquisition either *per os* or by abdominal injection has been studied by Shikata and Maramorosch (1965d, 1967b) and subsequently reviewed by Maramorosch *et al.* (1965, 1969a). In WTV-injected insects, the infection sequence is similar to that in plant-fed ones, except for a shortened incubation period in the vector and the absence of detectable virus in the gut lumen or epithelium (Maramorosch, 1975). The distribution of WTV antigens in leafhopper vectors has been studied, sometimes sequentially, using serological methods such as the precipitin ring test and fluorescent antibody techniques (Black and Brakke, 1952; Whitcomb and Black, 1959, 1961; Nagaraj *et al.*, 1961; Sinha and Black, 1962, 1963; Sinha and Reddy, 1964; Sinha *et al.*, 1964; Sinha, 1965a, 1967, 1969, 1974; Reddy and Black, 1966). Using fluorescent antibody technique, Sinha (1965a) was also able to detect WTV antigens in organs (brain, salivary glands, intestines, malpighian tubules, and ovaries) that had been excised from viruliferous *A. contricta* leafhoppers and maintained *in vitro* for 14 days. Whitcomb and Black (1961) measured the rate of WTV synthesis in insects by assaying the soluble antigen.

Pathological changes occur in the cells of the nervous system, fat body, blood, and gut of WTV-infected leafhoppers (Hirumi *et al.*, 1967; Shikata and Maramorosch, 1967b; Maramorosch *et al.*, 1969a; Maramorosch, 1970, 1975; Shikata, 1978a). Rather amazingly, however, despite numerous cytological lesions and extensive viremia, the life span and fecundity of infected insects appear normal (Maramorosch, 1975).

6.6.1.2 Rice Dwarf Virus (RDV). Selected synonyms for RDV include rice stunt, rice mosaic virus, *Oryze* virus 1, and *Marmor oryzae* (Iida *et al.*, 1972). The most commonly used English names, rice dwarf and rice stunt, are translations from the Japanese *ine isyuku-byo* (dwarf) and *ine ishuku-byo* (stunt). Although limited in geographical distribution, rice dwarf is perhaps the best known virus disease of plants in the world. Its notoriety undoubtedly stems from the fact that studies of the disease provided many historical landmarks in plant virology:

the first plant virus known to be insect-borne, transovarially passed to progeny of inoculative insects, propagative in its insect vector, and localized *in situ* by electron microscopy in cells of its plant and insect hosts.

The literature contains many historical accounts of rice dwarf in Japan (Hino, 1927; Ishikawa, 1928; Murata, 1931; Fukushi, 1935a, 1969; Katsura, 1936). The disease was recorded in Shiga Prefecture as early as 1883. Insect involvement in the disease cycle was discovered in 1894 by H. Hashimoto, a rice grower, who noted that healthy rice seedlings developed disease symptoms when they were enclosed in cheesecloth cages and infested with leafhoppers (Ishikawa, 1928). The species of leafhopper used in the tests is not known since he did not publish his experiments. Involvement of the leafhoppers *mon-yokobai, Recilia (=Inazuma) dorsalis* (Motschulsky) (Takata, 1895, 1896; Shiga Agricultural Experiment Station, 1898), and *tsumagura-yokobai, Nephotettix cincticeps* (Uhler) (Shiga Agricultural Experiment Station, 1900; Takami, 1901), was subsequently reported. *N. apicalis* (Motsch.) is also a vector of RDV (Nasu, 1963). All three vectors belong to the subfamily Deltocephalinae. It is obvious from Takata's publications and those of the Shiga Agricultural Experiment Station (1898-1908) that, initially, rice dwarf etiology was attributed to the leafhoppers themselves. According to Murata (1931), the role of leafhoppers (*N. cincticeps*) as vectors of an unknown causative agent of rice dwarf was first demonstrated in 1902 by N. Onuki of the Imperial Agricultural Experiment Station. And Onuki's conclusions were subsequently confirmed by T. Nishizawa of the Shiga Agricultural Experiment Station (1908, reports 7 and 8) and by H. Ando (1910). The disease is present in ca. 9 districts of Japan (Iida, 1965, 1969) and in Korea (Park, 1966).

The host range of RDV includes about a dozen species of plants in the Gramineae: *Alopecurus aequalis, A. japonicus, Avena sativa, Echinochloa crusgalli* var. *frumentosa, E. crusgalli* var. *oryzicola, Glyceria acutiflora, Hordeum sativum* var. *hexastichon, H. sativum* var.*vulgare, Oryza sativa, O. cabensis, Panicum miliaceum, Paspalum thunbergii, Phleum pratensis, Poa annua, Secale cereale, Triticum aestivum* (Shinkai, 1962; Iida, 1969). Rice, *Oryza sativa*, and *E. crusgalli* var. *oryzicola* (cockspur or barnyard grass) are diagnostic species; rice also serves as a propagative and assay species. Diagnostic symptoms include fine chlorotic specks and general stunting (Fukushi, 1934). Young test seedlings are best for assaying transmission by vectors; virus preparations may be bioassayed by injecting them into non-virus-exposed vector nymphs and then, following an appropriate incubation period, testing the inoculativity of the injected insects (Fukushi and Kimura, 1959). Transmission by dodder has not been reported, and seed transmission in rice does not occur (Fukushi, 1934). Field and laboratory tests have revealed that several rice varieties possess resistance to dwarf disease (Kimura *et al.*, 1969; Sakurai, 1969; Ishii *et al.*, 1969; Ling, 1972) and, in addition, some varieties are resistant to the vector, *N. cincticeps* (Ishii *et al.*, 1969).

Physical and biological properties of RDV. Virus can be partially purified from infected rice leaves by a simple extraction procedure followed by one cycle of differential centrifugation (Toyoda *et al.*, 1965; Shikata, 1966; Iida *et al.*, 1972;

Ling, 1972). The resuspended pellet obtained in this manner contains a high concentration of virus particles as evidenced by electron microscopy (Toyoda *et al.*, 1965). Fukushi *et al.* (1962) used a similar scheme to purify virus from inoculative leafhoppers. Virions isolated by differential centrifugation often appear surrounded by an outer envelope (Fukushi *et al.*, 1962). And accumulations of isolated particles are accompanied by thin membranous structures, presumably host-cell derived, cytoplasmic in origin, and lipoprotein in composition (Shikata, 1966). The particle envelopes are clearly seen in virus preparations that are only lightly shadowed or shadowed from two directions; the width of the shadows from enveloped particles are always wider than the actual diameter of the particles themselves (Shikata, 1966). Further purification can be accomplished either by treating the virus preparation with phospholipase of snake venum or pancreation to remove enveloping materials and subsequently eluting the virus from a DEAE-cellulose column with 0.2-0.25M NaCl (Toyoda *et al.*, 1965), or by rate zonal and quasi-equilibrium zonal density-gradient centrifugation (Kimura *et al.*, 1968).

Intramuscular injection of rabbits with an emulsion of purified virus in Freund's adjuvant provides a 1/2000 to 1/8000-titer antiserum that reacts in precipitin or agar gel-diffusion tests with viral antigen in both plant and insect extracts (Kimura, 1962b). Virus titers in various parts of diseased plants can be measured via precipitin ring and ring-time tests. Virus antigen can be detected in diseased plant tissue or in smears of individual viruliferous insects using fluorescein-conjugated antibody (Kimura and Miyashima, unpublished data, in Iida *et al.*, 1972). Rice dwarf and WTV have not been found to be serologically related; and RDV and rice black-streaked dwarf virus do not exhibit mutual protection in rice plants (Shinkai, 1961).

The dilution end-points of RDV in various media are as follows: $10^{-3} - 10^{-5}$ in extracts of eggs from infective female leafhoppers (Fukushi and Kimura, 1959; Fukushi, 1969); 10^{-4} in extracts of viruliferous *N. cincticeps*; 10^{-3} or 10^{-2} in sap of leaves and stems or roots, respectively, of diseased rice plants (40 days post-inoculation) (Kimura, 1962a); and 10^{-4} in sap from chlorotic portions of diseased leaves. Virus *in vitro* is infective after 48 hr but not after 72 hr. The thermal inactivation point (10 min) of RDV is 40-45°C in plant sap and 45-50°C in extracts of viruliferous insects. Virus remains infective for as long as a year in inoculative insects or diseased leaves that are stored at -30° to -35°C (Fukushi and Kimura, 1959; Kimura and Fukushi, 1960).

Fukushi *et al.* (1960) published the first electron micrographs of RDV. The virions (510 S) are icosahedral in shape and ca. 70 nm in diameter. The RDV capsid contains 32 capsomeres, 12 consisting of 5 substructural units and 20 of 6 units to give a total of 180 substructural units on the particle surface (Fukushi *et al.*, 1962; Kimura and Shikata, 1968). Each substructural unit is a hollow tube measuring ca. 6 x 9.5 nm (Kimura and Shikata, 1968). The virus consists of 11% double-stranded RNA (16-17 x 10^6 daltons) with a base composition of 27.8% adenine, 20.8% uracil, 22.8% guanine, and 21.4% cytosine (Miura *et al.*, 1966; Reddy *et al.*, 1974). Nucleic acid extracted from purified virus consists of 12 segments of different lengths and is not infective (Fujii-Kawata and Miura, 1970). Optical rotatory dispersion and circular dichroism data confirm the double-helix structure of RDV ribonucleic acid (Samejima *et al.*, 1968). Sato *et al.* (1966) reported a helix-to-helix distance of 1.3 nm along the common axis of the helices.

Transmission by vectors. Of the 3 known vector species of leafhoppers, *R. dorsalis (=Inazuma dorsalis), N. apicalis,* and *N. cincticeps*, the latter is the principal vector in the field. Shinkai (1962) was unable to get transmission using *N. impicticeps* Ishihara (=*N. bipunctatus*). Not all members of a given group of insects are capable of transmitting virus, and the proportion of potential transmitters varies depending on the vector species and the locality in which it was collected. The proportions of active transmitters for unselected *N. apicalis* (Nasu, 1963), *N. cincticeps* (Shinkai, 1962) and *R. dorsalis* (Hashioka, 1964) are 23%, 0-69%, and 2-43%, respectively. Proportions of transmitters for selected *N. cincticeps* and *I. dorsalis* leafhoppers are 90% and 43%, respectively. The majority of potential *N. cincticeps* transmitters acquire virus when given a 1-day acquisition-access feeding period on diseased rice plants; but with 1st and 2nd instar nymphs, the acquisition threshold is rarely as brief as 1 min for *N. cincticeps* and 30 min for *R. dorsalis* (Shinkai, 1962). Shinkai (1962) reported inoculation thresholds of 10 min for *R. dorsalis* and 3 min for *N. cincticeps*. Approximately half of the transmitting *N. cincticeps* can inoculate virus into healthy rice seedlings during a 30-min feeding period.

The incubation period in *N. cincticeps* varies from 4-58 days, with most transmitters transmitting after 12-35 days (Iida and Shinkai, 1969). Shinkai (1962) reported incubation periods of 9-42 days in *R. dorsalis*, with the majority of transmitters beginning to transmit after 10-15 days. Viruliferous nymphs retain inoculativity after ecdysis. And once having acquired virus, potential transmitters do not necessarily inoculate plants consistently on a daily basis, but most do retain their inoculativity for life. Shinkai (1962) demonstrated retention periods as long as 64 days for *N. cincticeps* and 93 days for *R. dorsalis*.

Virus-plant interactions. Rice dwarf disease symptoms generally appear in rice plants in late June after the plants have been transplanted to paddy fields; however, occurrence of the disease in seedlings in seedbeds is not unusual. As the name rice dwarf implies, diseased plants become noticeably stunted due to shortening of the internodes. Development of numerous diminutive tillers produces a rosette appearance. Inhibition of root growth is manifest in small roots that extend out horizontally from infected plants. The severity of stunting depends on the age at which plants are inoculated. Shinkai (1962) reported that height reduction decreases from 70% to nil as the time of inoculation increases from the 3-leaf to the 14-leaf stage. Similarly, the duration of the incubation period in the plant is dependent on the age of the plant at the time of inoculation: 8-10 days up until the 10-leaf stage and progressively longer for later inoculations. Plants remain susceptible to infection up to the 13-leaf stage, the 16th-leaf stage being the last (Ling, 1972), and inoculation at this time results in an incubation period of 27 days (Shinkai,

1962). Early inoculated plants may live until harvest, but they produce no panicles or a few worthless ones.

Cytological studies reveal that chlorotic cells occur in the mesophyll tissue adjacent to vascular bundles in infected plants. These cells contain disintegrated chloroplasts and numerous vacuoles of variable sizes. Large, irregularly shaped, inclusion bodies measuring 3-10 x 2.5-8.5 μm often occur in the cytoplasm adjacent to the nuclei (2.5-3.5 μm) of infected cells. Although mainly limited to the mesophyll, the inclusion bodies are sometimes seen in the epidermis and chloroplast-deficient parenchyma cells as well (Fukushi, 1931, 1934; Hirai *et al.*, 1964). Spherule bodies containing WTV-antigen have been observed in the cytoplasm of phloem-derived tumor cells in WTV-infected plants (Nagaraj and Black, 1961; Littau and Black, 1952). Starch accumulates in the leaves of RDV-infected plants, presumably due to inhibition of starch translocation (Daikubara, 1904).

Electron microscopy revealed the presence of RDV virions in mesophyll cells adjacent to vascular bundles and in cells of chlorotic portions of leaf tissue from diseased rice plants (Fukushi *et al.*, 1962; Shikata, 1966). Infected mesophyll cells adjacent to vascular bundles contained no chloroplasts or only disintegrated ones. Compared to healthy rice leaves, the palisade cells of infected leaves were shortened, whereas the parenchyma cells were somewhat larger and filled with cytoplasmic structures (Shikata, 1966). Virions occur loosely arranged, arranged in rows in the endoplasmic reticulum, or scattered throughout the cytoplasm of infected cells in young leaves at 10-20 days postinoculation (Fukushi *et al.*, 1962; Shikata, 1966). Infected cells of older diseased leaves (30 days postinoculation) sometimes contained large accumulations of compactly or regularly arranged (crystalline) virions in their cytoplasm. No organelles in a size range of host cell nuclei and resembling X-bodies or inclusion bodies were observed (Shikata, 1966). But since masses of virus particles sometimes occurred beside or in close contact with the nuclei of infected cells, and since these masses appear to be surrounded by thin membranes (Fukushi *et al.*, 1962), either they or viroplasms could represent the inclusion bodies described by researchers using light microscopy (Fukushi, 1931, 1934; Hirai *et al.*, 1964). Strings of RDV particles enclosed in tubular structures, sometimes arranged in several layers and measuring 60-80 nm in diameter, also occurred in the cytoplasm of some infected leaf cells (Shikata, 1966). Virions were never observed in the nuclei, mitochondria, or chloroplasts of host cells. Membrane-enveloped virions were seen in chromium-shadowed, diseased-leaf sections that were previously treated with xylene to dissolve the methacrylate embedding resin (Shikata, 1966).

Virus-vector interactions. That RDV is indeed propagative in its leafhopper vectors has been demonstrated by transovarial passage (Fukushi, 1933, 1934, 1935b, 1939, 1940, 1969; Shinkai, 1958, 1960, 1962, 1965), by passage of virus through a series of insects using injection technique (Fukushi and Kimura, 1959; Kimura, 1962a; Fukushi, 1969), by localization of virions (Fig. 2) and viroplasms in cells of vectors by electron microscopy (Fukushi *et al.*, 1960, 1962; Fukushi and Shikata, 1963a, 1963b; Nasu, 1965, 1969; Shikata, 1966, 1978a; Shikata and

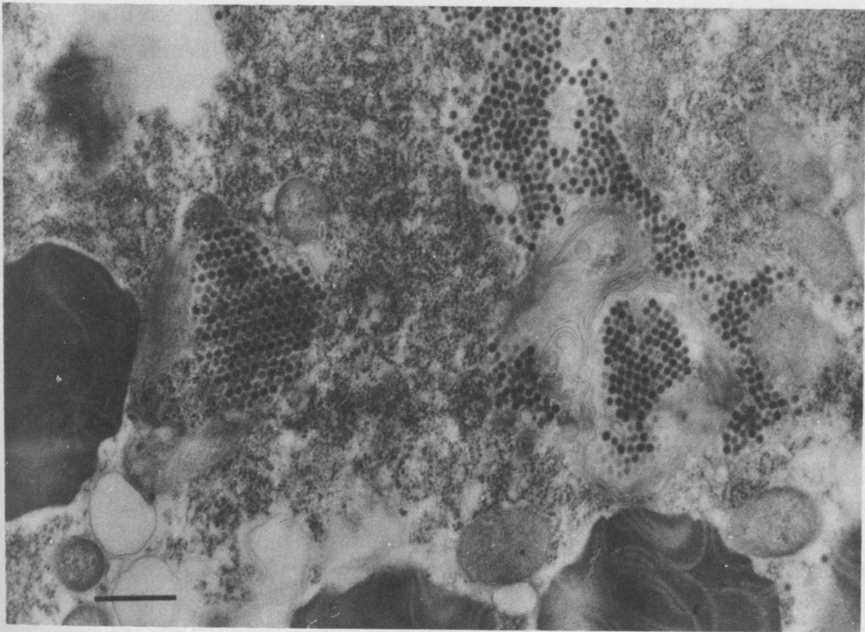


Fig. 2. Dense aggregates and microcrystalline formations of rice dwarf virus particles in the cytoplasm of a salivary gland cell of *Nephotettix cincticeps*. Bar, 500 nm. (Courtesy of E. Shikata.)

Maramorosch, 1969), and by propagation and visualization of virions in vector cell cultures (Mitsuhashi, 1965; Mitsuhashi and Nasu, 1967). Multiplication of virus in the vector is also confirmed by data indicating that RDV effects changes, sometimes pathological, in the cytology, histology and biology of its vectors (Shinkai, 1962; Jensen, D., 1963, 1969; Maramorosch and Jensen, 1963; Fukushi, 1969; Nakasuji and Kiritani, 1970).

Transovarial passage. One technique for proving multiplication of a virus in a vector is to demonstrate transovarial passage of virus from a single viruliferous female to a sufficient number of progeny in a sufficient number of generations so that, in the absence of virus multiplication, the dilution end-point of the virus would surely be exceeded in the final-generation offspring. Black (1950) estimated that a dilution factor of 10^{-22} ought to be exceeded to prove multiplication. Fukushi (1933, 1935b) found that insects hatched from eggs deposited by viruliferous females, *N. apicalis* var. *cincticeps*, were often viruliferous. In an experiment conducted over a 374-day period (Fukushi, 1939, 1940, 1969), RDV was transovarially passed through 6 generations; only the initial viruliferous mother was allowed access to RDV-infected rice plants. Since there was no evidence of a decline in either the number of insects that became viruliferous per generation or the percentage of plants inoculated by the congenitally viruliferous progeny, the experi-

ment provided strong evidence for multiplication of RDV in the vector. Moreover, since RDV appears to be far less deleterious to congenitally viruliferous *N. cincticeps* than it is to other vector species (Shinkai, 1960), the experiment further suggests that RDV could survive in vector populations for long periods, possibly indefinitely, in the absence of RDV-infected source plants.

In tests where inoculative mothers acquire virus as nymphs and complete the incubation period at the time of egg laying, the resulting congenitally inoculative progeny begin transmitting virus to plants 3-4 days after hatching (Fukushi, 1933; Shinkai, 1962). An average of 15 days (range 1-38 days) elapses before most progeny from infective *N. cincticeps* females begin to transmit. Most progeny remain inoculative as nymphs, and as adults they may continue to transmit for life, or as long as 88 days, without renewed access to a virus source. Transovarial passage of RDV also occurs in *I. dorsalis* (Shinkai, 1958) and *N. apicalis* (Nasu, 1963), but at a lower frequency than in *N. cincticeps*. Thirty-two to 100% of the offspring of viruliferous *N. cincticeps* females are rendered congenitally viruliferous, compared to 0-64% for progeny from inoculative *R. dorsalis* (Shinkai, 1965). Congenitally viruliferous *R. dorsalis* nymphs begin to transmit RDV 3-14 days after hatching (Shinkai, 1962, 1965). The number of viruliferous progeny decreases markedly in successive generations; virus was passed through eggs to progeny of the 3rd but not the 4th generation. Offspring of congenitally viruliferous females seemed to die prematurely; and this premature death is the probable cause for the disappearance of virus from *I. dorsalis* colonies that rely entirely on transovarial passage of RDV for the maintenance of vector inoculativity (Shinkai, 1958, 1962; Fukushi, 1969).

To date, there are no known cases of transovarially transmissible viruses being transmitted through the sperm of infective males. Thus progeny from crosses between noninfective females and infective males are entirely free from virus. However, progeny from such crosses have a greater ability to acquire and transmit RDV than do ones derived from crosses between nonviruliferous parents. Fukushi (1969) pointed out that this phenomenon is intelligible if one assumes that virus is capable of multiplying in and being transmitted by potential transmitters only and that vector susceptibility to virus is an hereditary characteristic controlled by a dominant factor(s). If this is so, then crosses between nonviruliferous females and infective males (where at least one and possible both parents are potential transmitters) are more likely to yield larger numbers of susceptible progeny or potential transmitters than are crosses between nonviruliferous parents. In the latter crosses, both parents may be non-RDV-susceptible, nontransmitters; and the likelihood of either one or both parents being potential transmitters is diminished. Rather predictably, on the basis of Fukushi's hypothesis, when the progeny from crosses between infective parents, between infective females and nonviruliferous males, and between nonviruliferous parents are reared on RDV-infected rice plants, 92, 68, and 12% of the progeny, respectively, were subsequently shown to be transmitters (Fukushi, 1969).

Electron microscopic studies by Nasu (1965, 1969) have elucidated the mechanism by which virus is passed from viruliferous females to their progeny. Virus invasion of mycetocytes in the ovarioles of viruliferous females appears to be linked with the selective migration of free-state L and H symbiotes from the hemolymph into mycetocytes. Virions in the mycetocytes have an affinity for the surface membranes of L symbiotes. Progeny are inoculated with RDV when virus-containing parent mycetocytes enter neighboring oocytes at the yolk-forming stage. During embryogenesis, virus multiplies first in the mycetome and then in various germinal cells in the cytoplasm of developing progeny.

Fate of RDV in vector cells. Electron microscopic observations on the localization of RDV in viruliferous vectors are essentially the same as those for WTV-infected leafhoppers (Shikata, 1978). Virions have been localized in the cytoplasm only of cells of practically every vector organ and tissue, including the fat body, gut, malpighian tubules, tracheoblasts, gut epithelium and integumental hypodermis, muscle, mycetome, salivary glands, blood, and ovarian tubules. In the cytoplasm, virions occur free or loosely scattered, or in aggregates in any of the following types of situations: in vacuoles (endocytic?); in rows inside tubular structures; in and around the periphery of nonmembrane-delineated, viral assembly sites known as viroplasms; in defined, electron dense, often myelinated structures (heterophagosomes or phagolysosomes?: Harris *et al.*, 1975), and in crystalline arrays derived from dense aggregates of virions in either defined structures or mature viroplasms.

In preliminary experiments, Mitsuhashi (1965, 1969) and Mitsuhashi and Nasu (1967) demonstrated that RDV could multiply in vector cell cultures derived from embryos of viruliferous *N. cincticeps*, as well as in ones that are derived from nonviruliferous insects and subsequently artificially inoculated with virus. Later studies using autoradiography and immunoelectron microscopy with ferritin-labeled antibody confirmed that the viroplasmlike areas observed in RDV-infected cells of monolayer cultures were the sites of RDV nucleic acid and protein accumulation, and virus assembly (Nasu and Mitsuhashi, 1968).

Rice dwarf virus and several other hopper-borne viruses can be detected in infected plant tissues and in viruliferous vectors using highly sensitive, hemagglutination tests (Saito, 1969).

6.6.1.3 Maize Wallaby Ear Virus (MWEV). Maize wallaby ear virus occurs in Australia and was first reported by Tryon (1910). The leafhoppers *Cicadulina bimaculata* (Evans), *C. bipunctella* and *Nesoclutha pallida* (Evans) are known to serve as vectors (Schindler, 1942; Grylls, 1978). Transmission by vectors is obligatory, but transmission by grafting is possible (Grylls, 1978). The virus presumably multiplies in its vectors, and a high incidence of transovarial passage in *C. bimaculata* has been recorded (Grylls, 1978).

The viral eitology of the disease has been confirmed by electron microscopy and by infectivity bioassay of purified virus preparations from diseased plants and viruliferous leafhoppers (Grylls, 1975, 1978). Membrane-surrounded particles

measuring 70-80 nm in diameter, including the membranes, and resembling maize rough dwarf virus (MRDV) (Conti and Lovisolo, 1971) can be seen in the salivary glands of viruliferous leafhoppers. Membraneless particles similar in size (45-50 nm) to the nucleocapsids of the membrane-bounded particles observed in salivary glands occur in partially purified fractions prepared either from MWEV-infected plants or viruliferous insects. Virus particles have not yet been observed in thin sections of galls, leaf veins or roots of diseased maize. The virus shows serological affinity to MRDV; and its 10-segmented, double-stranded RNA resembles but is different from those of MRDV and sugarcane Fiji disease virus (FDV) (Reddy *et al.*, 1976). These latter characteristics seemingly make MWEV a potential candidate for the family Reoviridae (Fenner *et al.*, 1974; Fenner, 1975/76).

As with rugose leaf-curl virus (section 6.6.2.3), rickettsialike bodies (RLB's) have also been observed in the salivary glands of viruliferous and nonviruliferous insects, and in some MWEV-infected maize. These RLB's were sometimes seen together with virus particles in viruliferous *C. bimaculata*. The relationship of these bodies to maize wallaby ear disease is not known; however, since noninoculative leafhoppers can be rendered viruliferous by injection with partially purified preparations of MWEV, the bodies do not seem essential to transmission (Grylls, 1978).

6.6.1.4 Leaf Gall Disease of Maize and Rice. The agent(s) responsible for leaf gall disease of maize and rice in Australia is transmitted by the leafhoppers (Deltocephalinae) *Cicadulina bimaculata* and *C. bipunctella*. Partially purified fractions prepared from either diseased plants or inoculative insects contain 45 to 50-nm, viruslike particles (inner nucleocapsid core?) that are indistinguishable from those of MWEV. Partially purified virus reacts with MWEV and MRDV antisera (Grylls, 1978). Gel electrophoresis of RNA extracted from partially purified virus prepared from inoculative leafhoppers resulted in an electrophoretic profile closely paralleling that of MWEV processed at the same time (Grylls, 1978).

6.6.2 Small Spherical or Polyhedral Viruses

6.6.2.1 Beet Curly-Top Virus (CTV).

Physical and biological properties. Until recently, very little was known about the etiology of curly-top disease except that the causative agent, presumably a virus, passed through ordinary filters such as Berkefeld V, N, and W, the Mandler medium and fine grades, and the Chamberland filter candles, L1, 3, 5, 7, 9, and 13. In 1973, Duffus and Gold subjected dialyzed phloem exudate from CTV-infected shepherd's purse. *Capsella bursa-pastoris* (L), to density gradient electrophoresis. Electron microscopic examination of shadowed preparations taken from infective zones of the electrophoretic columns revealed small, "spherical" particles measuring 19-20 nm in diameter and occurring mostly in clumps. The particles are similar in size to those found in phloem exudate passed through gladocol membranes with estimated pore sizes of ca. 25 nm (Bennett, 1971). The zone of highest infectivity, 14-17 cm from origin, appeared to be completely separated

from all particulate materials other than the characteristic 19-20 nm particles. The latter were absent from noninfectious zones.

That the particles observed by Duffus and Gold (1973) are indeed CTV virions is further confirmed by Mumford (1974a, 1974b) who purified CTV from infected 'Turkish' tobacco. Extracts from diseased plants were clarified with chloroform and butanol. Virus was concentrated by precipitation with polyethylene glycol and NaCl. Clarified virus concentrates were partially purified on sucrose-density gradients and then further purified by gel chromatography on agarose. The resulting purified virus preparations had maximum and minimum UV-light absorptions at 260 and 240 nm, respectively, and contained isometric particles (not seen in fractions from healthy control preparations) measuring 18-22 nm in diameter. Mumford (1974b) also followed the virus through his purification regime using a plant-infectivity bioassay in which non-CTV-exposed leafhoppers were fed virus preparations through membranes and then placed on healthy sugar beet seedlings to test for inoculativity.

Curly-top virus is immunogenic. In further studies (Duffus and Gold, 1973), CTV antisera were prepared using either clarified phloem exudate or the most infectious zones of several density-gradient electrophoresis runs as antigen for intramuscular injections of rabbits. Serological neutralization of CTV infectivity was demonstrated by feeding nonviruliferous leafhoppers through membranes either directly on virus-antigen reactants or on the zones obtained by first subjecting the virus-antigen reactants to density-gradient centrifugation. Leafhoppers were then caged individually on healthy sugar beet seedlings to test their inoculativity. The infectivity of three CTV isolates was almost completely neutralized with antiserum against curly-top phloem exudate and partially so with the lower titer antiserum produced against curly-top electrophoresis zones. Duffus and Gold pointed out that neutralization of infectivity by immune sera could be a valuable tool in clarifying the geographic distribution, origins, and interrelationships of curly-top viruses. Additional improvements in CTV purification technique permitted the production of antiserum suitable for developing latex flocculation serological assay for detecting and estimating concentrations of CTV in extracts of diseased plant tissue (Mumford, 1974a, 1977).

In plant sap, curly-top virus has the following properties: a thermal inactivation point (10 min) of 75-80°C; a dilution end-point of 1:1000 or 1:20,000 when membrane-fed leafhoppers are tested for inoculativity by placing them on plants individually or in groups of ten, respectively (Bennett, 1935); and a longevity *in vitro* of 7 days in filtered and unfiltered extracts of diseased beet leaves, and 14 days in unfiltered or 28 days in filtered water washings of alcohol precipitates of leaf juice. The dilution end-point of virus in juice from crushed viruliferous leafhoppers is 1:24,000 (Severin and Freitag, 1933). The virus can remain infective for 10, 6, 5, 4 and 2 months in dried phloem exudate, in dried viruliferous leafhoppers, in alcoholic precipitate of phloem exudate, in dried beet tissue, and in alcoholic precipitate of beet leaf and root juice, respectively.

Virus-plant relationships. Curly-top virus appears to be confined to North America, especially in sugar beet areas west of the Rocky Mountains, and possibly to Argentina, Brazil, and Turkey (Bennett *et al.*, 1946; Bennett and Costa, 1949; Bennett and Tanrisever, 1958). These curly-top viruses are strikingly similar, but slight differences among them in general behavior, host range, and symptomatology have been noted. The host range of the North American virus is very broad, with host species occurring in the Cruciferae, Violacea, Chenopodiacea, Geraniaceae, Tropaeolaceae, Cucurbitaceae, Polygonaceae, Malvaceae, Caryophyllaceae, Leguminosae, Umbelliferae, Dipsaceae, Amaranthaceae, Nyctaginaceae, Compositae, Solanaceae, Linaceae, Boraginaceae, and Valerianaceae (Severin, 1929; Smith, 1972). With such a wide host range, it is difficult to pick out particular species as indicator plants; however, *Datura stramonium* and *Nicotiana tabacum* are most commonly used for this purpose, probably because they are easy to grow and have served well in this capacity for so many other viruses.

In infected beets, degeneration of primary sieve tubes occurs, followed by primary hypertrophy and necrosis of pericycle or phloem-parenchyma cells adjacent to the sieve tubes. Primary hyperplasia of cells in areas farther removed from the affected sieve tubes occurs, and a large proportion of these hyperplastic cells undergoes changes characteristic of differentiating sieve tubes. This hyperplastic tissue, in which sieve-tubelike cells predominate, is striking and allows one to identify the disease in its early stages. The sieve plates of phloem elements in hyperplastic areas seem not to complete their development, for callus fails to develop. In later stages of infection, the sieve-tubelike cells and, if present, their companion cells die and collapse. This secondary necrosis is followed by secondary hypertrophy and hyperplasia of nearby parenchyma cells, thus giving rise to proliferations resembling callus. As noted by Esau (1935), these sequential histopathological changes strongly suggest that virus moves in the phloem through the mature sieve tubes.

The virus is not normally sap transmissible, but beets can be mechanically inoculated by making repeated punctures with insect pins into the crown through drops of expressed juice or phloem exudate from CTV-infected beets (Severin, 1924; Severin and Freitag, 1933; Bennett, 1934, 1935). Seed transmission is not known to occur (Severin, 1921), but transmission by grafting and dodder have been recorded (Smith and Boncquet, 1915; Johnson, 1941; Bennett, 1944).

Transmission characteristics. Viruliferous vectors retain their inoculativity after ecdysis (Severin, 1924; Freitag, 1936; Bennett and Wallace, 1938). Transovarial passage of virus to progeny of viruliferous females is not known to occur. Curly-top virus appears to be confined to phloem tissues, and its specific vector, *Circulifer tenellus* (Baker), is primarily a phloem feeder. The Argentine, Brazilian and Turkish curly-top viruses are transmitted by *Agalliana ensigera* Oman, *Agallia albidula* Uhl., and *Circulifer opacipennis* (Lth.), respectively.

Curly-top virus must undergo a latent period in the vector before it can be transmitted (Smith and Boncquet, 1915; Carsner and Stahl, 1924; Severin, 1921, 1931; Bennett and Wallace, 1938). Bennett and Wallace (1938) allowed leafhoppers a 1-hr acquisition-access feeding period and then transferred them individually and at 1-hr intervals to series of healthy test plants. Using this technique, they demonstrated that the minimum latent period is more than 3 but not more than 4 hr. They also demonstrated that insects could acquire (acquisition threshold) virus from or inoculate (inoculation threshold) it into plants during feeding periods of not more than 1 min duration. Using groups of leafhoppers, Severin (1931) reported an occasional transmission threshold as brief as 20 min. If confirmed, such a brief transmission threshold could be interpreted as suggesting that, in rare instances and under proper conditions, leafhoppers may transmit CTV in a noncirculative fashion as well (via a stylet-borne and/or ingestion-egestion mechanism?).

Reports on CTV retention by viruliferous leafhoppers once they are separated from a continuing source of virus fall in a range of 1-161 days (Boncquet and Stahl, 1917; Carsner, 1919; Severin, 1924; Freitag, 1936; Bennett and Wallace, 1938; Wallace and Murphy, 1938). It is not unusual for insects to retain their inoculativity for life; and most researchers have reported some retention periods in excess of 100 days. Severin (1934) found that overwintering females lost their inoculativity in an average time of 83.9 days.

Virus-vector relationships. Available evidence suggests that CTV circulates but does not multiply in its beet leafhopper vector. Transmission is transstadial but not transovarial; and multiplication in the vector has not been demonstrated using serial injection technique. Negative data that might be interpreted as favoring nonpropagation in the vector include: 1, the latent period in the vector is brief, ca. 4 hr (Bennett and Wallace, 1938); 2, there is a gradual decline in the inoculative capacity of viruliferous leafhoppers once they are separated from a virus source (Severin, 1924; Freitag, 1936; Bennett and Wallace, 1938); 3, the virus concentration in the vector decreases steadily with time once the vector is separated from a virus source (Bennett and Wallace, 1938; Bennett, 1962); 4, an insect's inoculative capacity is positively correlated with the amount of virus taken up during an acquisition-access feeding period (Freitag, 1936; Bennett and Wallace, 1938; Bennett, 1962); 5, viruliferous insects that eventually lose their ability to transmit become inoculative again when allowed an additional feeding on a diseased plant (Freitag, 1936; Bennett and Wallace, 1938; Giddings, 1950); and 6, the beet leafhopper can acquire, carry and transmit two or three distinct curly-top virus strains simultaneously (absence of cross protection) (Giddings, 1940, 1950).

Using a feeding technique, Severin (1931) showed that virus was present in the mouthparts of viruliferous leafhoppers; he was unable to detect virus in the blood and salivary glands. Later, Bennett and Wallace (1938) demonstrated the presence of CTV in the blood, salivary glands, alimentary tract, and feces of viruliferous insects. The blood appeared to serve as the chief virus reservoir and as an especially favorable medium for the preservation of virus infectivity. They also detected virus in several nonvector insects which were previously fed on CTV-infected beets: including the aphids *Myzus persicae* (Sulz.), *Aphis rumicis* L., and *Pempigus betae* Doane, the leafhoppers *Aceratagallia californica* (Baker), *Phlepsius strobi*

(Fitch), and *Empoasca solana* (DeLong), and the thrips *Hercopthrips femoralis* (Reuter). Although these nonvector insects were unable to transmit the virus they acquired, some of them acquired large quantities of virus which, when separated from a virus source, they retained for periods ranging from less that a day (*H. femoralis*) to 21 days (*A. californica*). Smith (1941) demonstrated the presence of virus in vector saliva.

Maramorosch (1955) succeeded in mechanically transmitting CTV to non-CTV-exposed leafhoppers via the injection technique. The duration of the latent period of the virus in the recipient insects depended on the dose of virus they received. Injection with CTV-containing juices from viruliferous insects at a 1:30 dilution resulted in latent periods in recipient insects ranging from 1 to 9 days, whereas inocula diluted 1:300 resulted in ones ranging from 5-20 days.

6.6.2.2 Oat Blue Dwarf Virus (OBDV).

Physical and biological properties. Oat blue dwarf virus has been purified from OBDV-infected oats, *Avena sativa* L. 'Rodney,' using a cellulose column chromatography system combined with further purification by sucrose density-gradient centrifugation (Banttari and Zeyen, 1969). The virus is relatively stable, compared with other phytarboviruses. Sucrose density-gradient centrifugation of eluates from columns containing either healthy or OBDV material produced two bands (top and bottom or top and middle, respectively) in density-gradient tubes; whereas, a third, bottom band occurred only in tubes containing OBDV fractions. Virus was followed through the purification regime by a plant-infectivity bioassay in which non-OBDV-exposed leafhoppers were injected with various purification fractions and then individually tested for inoculativity on healthy oat seedlings. Fractions assayed in this manner included nondiluted, 1:100-diluted and 1:1000-diluted clarified sap, centrifuged column eluates, and nondiluted, 1:100-diluted and 1:1000-diluted bottom component of OBDV from density gradient tubes. All the aforementioned fractions proved to be infective.

Electron microscopic examination of OBDV fractions revealed the presence of small, spherical or polyhedral particles measuring 28-30 nm in diameter. The particles were mainly concentrated in the bottom band from OBDV density-gradient tubes, but they were occasionally seen in the top and middle bands as well. Similarly, infectivity was primarily associated with the bottom band component, but some infectivity was also associated with the top and middle bands. The virus has maximum and minimum UV-light absorptions at 260 nm and 240 nm, respectively, which are typical for nucleoproteins. The value for its E260/280 is 1.63. These data in combination with the association of infectivity and viruslike particles only with fractions from OBDV-infected plant material appear to prove that the 28-30 nm particles are indeed virions of OBDV (Banttari and Zeyen, 1969).

Further analysis of purified OBDV preparations on linear-log sucrose density gradients yielded a single virus zone with a sedimentation coefficient of 119 S. The viral genome consists of single-stranded RNA with a sedimentation coefficient of 31.9 S and a molecular weight of ca. 2.13×10^6 daltons. The viral RNA is

alkali-labile, susceptible to ribonuclease (0.1 μg/ml) degradation, and resistant to deoxyribonuclease (50 μg/ml) (Pring *et al.*, 1973).

Virus-plant relationships. The host range of OBDV is known to include 18 species of plants representing 7 families (Westdal, 1968). Symptomatology in infected oats includes deep blue-green coloration, stunting, shortened flag leaves, blasted heads, and abnormally increased tillering (Goto and Moore, 1952). Westdal (1968) reported that the most characteristic symptom was the occurrence of enations in veins on the abaxial surfaces of leaves. Banttari and Moore (1962) demonstrated that the agents of "flax crinkle" (Banttari and Frederiksen, 1959; Frederiksen and Goth, 1959) and "blue dwarf of oats" were the same. Blue dwarf of oats has been reported in Kansas (Sill *et al.*, 1954), Canada (Creelman, 1965) and North Dakota (Timian, personal communication in Zeyen and Banttari, 1972). Raatikainen (1970) has reported a leafhopper-spread disease called European oat blue dwarf.

The virus occurs in low titer in extract from infected plants (Westdal, 1968; Banttari and Zeyen, 1971), presumably because of its being phloem restricted. The histology and ultrastructure of OBDV-infected oats have been studied by light and electron microscopy (Zeyen and Banttari, 1972). The earliest stages of infection were detected by examining serial cross sections of leaves still in the leaf whorl. The virus has pathological effects on the differentiation of vascular and adjacent tissues: primarily procambium, phloem and adjacent parenchyma, and parenchyma-derived tissue. After a sieve-tube element within a procambial strand has fully differentiated, pathological changes are first noted in the form of a multiplaned derangement of the normal pattern of division among procambial cells. Hyperplasia and limited hypertrophy of phloic procambium are most common; xylary procambium is much less affected. The majority of phloem elements in hyperplastic areas are parenchymatous, lack sieve plates, and are rarely accompanied by companion cells. Hyperplasia of parenchyma cells adjacent to hyperplastic phloem may also occur. In a later stage of infection, portions of the hyperplastic phloem areas often necrose and collapse; and this collapse sometimes produces lacunae in the affected leaf tissue. Zeyen and Bantarri (1972) also observed the occasional development of nonfunctional xylem elements which likely arise from parenchymatous cells on the margin of hyperplastic phloem.

Electron microscopic observations of virions only in phloem elements of infected plant tissue confirm the hypothesis that the virus is phloem-restricted. The greatest accumulations of virions were seen in the region between immature and fully vacuolated phloem elements, thus implicating virus synthesis in immature elements (Zeyen and Banttari, 1972). Virus crystals were often large enough to be seen by light microscopy.

Virus-vector relationships. Oat blue dwarf virus is not sap transmissible, so infectivity bioassay is accomplished by checking leafhoppers for inoculativity after they have been injected with test inocula. The obligatory vector of OBDV is the aster leafhopper (Deltocephalinae), *Macrosteles fascifrons* (Stål) (Banttari and

Moore, 1962; Banttari and Zeyen, 1970; Long and Timian, 1971). About 30% of insects taken from wild populations of *M. fascifrons* are transmitters; however, higher transmission levels can be obtained using insects derived from selective breedings of the vector (Hsu, 1973; Timian and Alm, 1973). Transovarial passage of virus to progeny of viruliferous females does not occur (Banttari and Moore, 1962; Timian and Alm, 1973).

In 1976, Banttari and Zeyen presented convincing evidence that OBDV is not only circulative but also propagative in its vector. Thus, conclusive evidence for multiplication of plant viruses in their vectors is no longer confined to viruses having 50-nm or larger diameters (Black, 1969). Data in support of OBDV multiplication in *M. fascifrons* include the following: 1, the virus requires a minimal incubation period of ca. 6 days (20-25°C) in the vector; 2, following the 6-day incubation period, the level of transmission gradually increases to a maximum at about 28 days before gradually declining; 3, virus has been serially passed through eight populations of previously virus-free leafhoppers, a number sufficient to effect a dilution (1×10^{-18}) far exceeding the dilution end-point of the inoculum (1×10^{-5}) injected into the initial "donor" insect; and 4, membrane-bounded aggregates, and nonmembrane-bounded crystalline and paracrystalline aggregates of OBDV have been observed in the neural lamella of the supraesophageal ganglia and in fat body cells of viruliferous leafhoppers (Fig. 3) (Banttari and Zeyen, 1976).

Oat blue dwarf virus is the smallest, single-stranded RNA virus for which evidence of multiplication in both plants and insects has been demonstrated.

6.6.2.3 (Datura) Rugose Leaf-curl Virus (RLCV). This virus was originally recovered from its leafhopper vector, *Austroagallia torrida* Evans, before it was recognized in the field (Grylls, 1954; Grylls and Day, 1966). The disease was then recognized in *Datura tatula*, one of the first hosts on which viruliferous leafhoppers were colonized (Grylls, 1954). The experimental host range includes more than a dozen plant species, most of which are legumes (Grylls, 1954; Smith, 1972). Red and white clover appear to be the most important field hosts. Recovery and resistance to reinfection have been noted in plants established from recovered stolons of several stoloniferous clover species (Grylls and Day, 1966; Jones, 1973).

The suspected viral etiology of the disease has been confirmed by electron microscopic localization of the viruslike particles in the salivary glands of viruliferous leafhoppers and in partially purified preparations from infected plants (Grylls *et al.*, 1974). Viruslike particles in the salivary glands of vector are membrane-bounded and, together with their surrounding membranes, measure 80-85 nm in diameter. The surrounding membranes are apparently lost during partial purification of virus from either viruliferous insects or RLCV-infected plants; and the membraneless, spherical or polyhedral particles measure 45-50 nm in diameter (Grylls *et al.*, 1974; Grylls, 1978). To date, virus particles have not been seen *in situ* in thin sections of diseased plant tissue. Additional data are needed before the taxonomic positioning (Reoviridae?) of RLCV and its serological relatedness to other viruses can be determined.

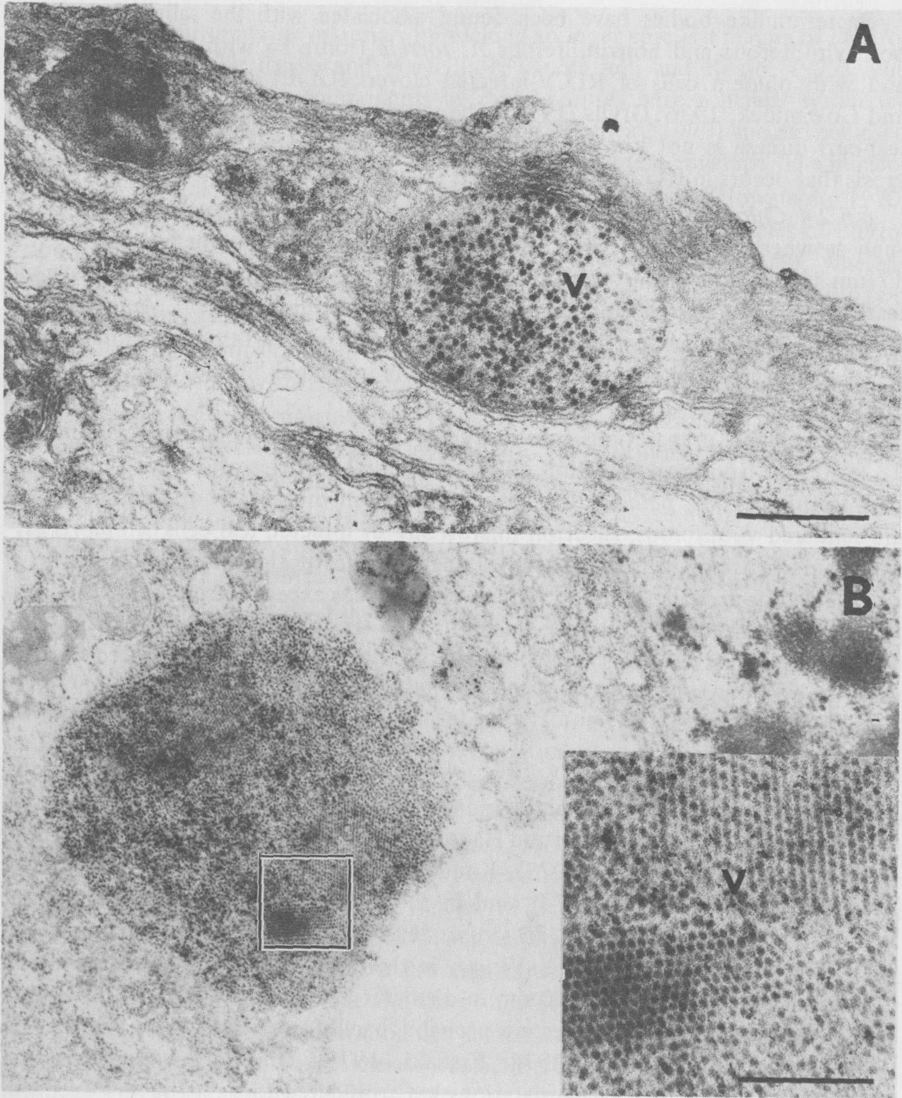


Fig. 3. Virions of oat blue dwarf virus in the cytoplasm of *Macrosteles fascifrons*. *A*, membrane-bounded aggregate of particles in the cytoplasm of cells of the neural lamella surrounding the supraesophageal ganglion of an adult aster leafhopper. *B*, crystalline and paracrystalline aggregate, not membrane-bounded, of virions in the cytoplasm of a cell from a fat body of an adult insect. Inset, an enlargement of the area enclosed by the square. *V*'s = virions. Bars, 1μm. (From Banttari and Zeyen, 1976.)

Bacteriumlike bodies have been found associated with the salivary glands of both viruliferous and nonviruliferous *A. torrida* (more so with viruliferous ones) and with phloem cells of RLCV-infected clovers (Grylls *et al.*, 1974; Behncken and Gowanlock, 1976; Grylls, 1978). The relationship of these organisms to rugose leaf-curl disease is not known; however, they could be related to the phloem necrosis that occurs in RLCV-infected plants (Grylls, 1954, 1978).

6.6.2.4 Chloris Striate Virus (CSV). Chloris striate is a disease of small grains such as wheat, oats, and barley, and grasses in Australia. Polyhedral particles ca. 17 nm in diameter have been observed in some extracts from diseases plants and leafhoppers, *Nesoclutha pallida* (Evans), carrying the disease agent. And when extracts containing such particles were injected into or fed through membranes to nontransmitting leafhoppers, the insects were rendered inoculative (Grylls and Waterford, 1976; Grylls, 1978).

Purified virus preparations contain single and paired (doublets or structures ca. 18 x 30 nm; Goodman and Bird, 1978) polyhedral particles ca. 18 nm in diameter. And leafhoppers, *N. pallida*, that are fed through membranes on these preparations are rendered viruliferous. Electron microscopy of infected leaf cells of *Chloris gayana* plants – using a tilting goniometer stage – indicates that doublets also occur *in situ*. The virus appears to invade all host plant tissues except the epidermis, and particles are usually confined to the nuclei of cells (Hatta *et al.*, 1978; Francki *et al.*, 1978). *Chloris* striate virus likely belongs to the ICTV's newly proposed Geminivirus group of plant viruses that have circular ssDNA genomes (Goodman, 1977; Harrison *et al.*, 1977).

6.6.3 Rhabdoviridae

Members of the family Rhabdoviridae (from the Greek *rhabdos* meaning rod) have been reviewed by Simpson and Hauser (1966), Howatson (1970), Hull (1970), Hummeler (1971), Francki (1973), Knudson (1973), Peters and Schultz (1975), Wagner (1975), Shepherd (1977), and in the C.M.I./A.A.B. Descriptions of Plant Viruses. The group includes ca. 75 viruses that multiply in plants, vertebrates, and invertebrates (Fenner, 1975/76). Viruses in the group are bullet-shaped or bacilliform and range from 45 to 100 nm in diameter and from 130 to 430-500 nm in length. Most phytorhabdoviruses are probably bacilliform *in situ* (Howatson, 1970; Hummeler, 1971; Knudson, 1973; Francki, 1973). Bullet-shaped particles observed in negatively stained preparations are considered to be artifacts (MacLeod, 1968; Ahmed *et al.*, 1970; Peters and Kitajima, 1970; Francki, 1973; Russo and Martelli, 1973; Peters and Schultz, 1975). To what extent this might be true of the assumedly bullet-shaped particles of animal rhabdoviruses is not known (David-West and Labsoffsky, 1968; Bergold and Munz, 1967; Yang *et al.*, 1969; Peters and Schultz, 1975). It is interesting to note that recent data suggest that vesicular stomatitis virus (VSV) has true bacilliform particles (Orenstein *et al.*, 1976).

The generally accepted schematic or model for both plant and animal rhabdoviruses consists of a helically wound nucleoprotein filament enveloped by a lipoprotein membrane studded with an array of short spikes protruding from its surface (Martelli and Russo, 1977). Whether or not the area interior to the nucleocapsid

helix is hollow or contains materials mimicking an inner core is a matter of controversy (Francki, 1973; Russo and Martelli, 1973; Martelli and Russo, 1977). The nucleocapsids or internal components of rhabdoviruses have a helical symmetry with a basic pitch of 4.5-5.5 nm (Howatson, 1970; Francki, 1973) and are enclosed in an outer, bilayered, 10-nm thick, host-derived lipoprotein envelope. The nucleocapsids of animal rhabdoviruses are evidently bullet-shaped (Howatson, 1970; Wagner, 1975). Some believe that nucleocapsids of bacilliform particles of phytorhabdoviruses may also be bullet-shaped (Peters and Kitajima, 1970), whereas others have suggested that the bacilliform particles result from the fusion of two bullet-shaped nucleocapsids at their planar ends (Francki, 1973; Wagner, 1975). The genome of the nucleocapsid is a single molecule of single-stranded, complementary or negative RNA with a molecular weight of 3.5-4.0 x 10^6 daltons or greater. Transcription of the complementary RNA genome is presumably accomplished via a nucleocapsid-associated, RNA-dependent, RNA polymerase (transcriptase) (Baltimore, 1971; Francki and Randles, 1972, 1973, 1975; Wagner, 1975; Shepherd, 1977).

The RNA is closely associated with the N protein moeity of the nucleocapsid; N protein subunits are generally believed to be helically arranged along the cylindrical portion of the nucleocapsid. The N protein subunits are probably interrelated with the glycoproteins (G protein) of the surface projections or spikes that protrude 6-10 nm from the surface of the viral envelope (Brown *et al.*, 1974), but the nature of this interrelationship is not yet understood (Hull, 1976). In both plant and animal rhabdoviruses the protein G is the primary source - in some viruses possibly the only source - of glycopolypeptides (Wagner, 1975; Ziemiecki and Peters, 1976b). The G protein in VSV appears to function in enabling particles to attach to the plasma membrane of host cells (Wagner, 1975). Similarly, glycopolypeptides in the spikes of plant rhabdoviruses may be required for particle attachment to the plasma membranes of insect cells (Francki, 1973; Knudson, 1973). In all, virions contain five major proteins: L, G, N, NS, and M (M_1 and M_2).

Insect-borne phytorhabdoviruses are transmitted in a circulative manner, and they multiply in both their plant and insect hosts (circulative-propagative). In host cells, virion synthesis and maturation are associated with nuclear and/or cytoplasmic membranes. For some of these viruses, there is evidence that nucleocapsid synthesis may also occur in the nucleoplasm or in the cytoplasm in association with viroplasmlike areas (Richardson and Sylvester, 1968; Wolanski and Chambers, 1971; Shikata, 1978a). Russo *et al.* (1975) suggest that even assumedly cytoplasmic rhabdoviruses – when maturing in close proximiity to the nucleus – may acquire their envelope from the outer lamella of the nuclear membrane. In their insect hosts, plant rhabdoviruses may appear in the form of bullet-shaped, bacilliform, or "long bacilliform" particles. Such forms are thought to represent immature particles, mature particles, and dimeric particles (i.e. two bullet-shaped nucleocapsids fused together at their planar ends and encased in the same membrane), respectively. Most phytorhabdoviruses are not sap-transmissible; a few, about eight, are sap-transmissible, but often are so only with difficulty.

The reader is referred to the recent treatise of Martelli and Russo (1977) for an up-to-date review of the morphological and physicochemical properties of rhabdoviruses, especially plant rhabdoviruses, and their morphogeneses.

Francki (1973) listed sixteeen plant viruses belonging to the Rhabdoviridae. Shikata (1978a) has organized eleven of these for which vectors are known into aphid, leafhopper, and planthopper-borne groups. Martelli and Russo (1977) list 29 plant rhabdoviruses. Some of these have no known vectors; twelve are transmitted by "leafhoppers" (six by planthoppers and six by leafhoppers); nine are transmitted by aphids; and one, sugarbeet leaf curl virus (SLCV), is transmitted by a piesmid vector.

The leafhopper-borne rhabdovirus group includes potato yellow dwarf (PYDV), rice transitory yellowing (RTYV), Russian winter wheat mosaic (WWMV) (vectors = *Psamotettix striatus* [L.] and *P. alienus* [Dhlb.]), oat striate mosaic (*Graminella nigrifrons* [Forbes]), cereal chlorotic mottle (CCMV), and American wheat striate mosaic (WSMV) viruses. Pigeon pea proliferation virus is suspected of being leafhopper-borne, but a vector has not yet been recorded (Maramorosch *et al.*, 1974).

6.6.3.1 Potato Yellow Dwarf Virus (PYDV). There are two serologically related but distinct forms of PYDV (Liu and Black, 1978): the *sanguinolenta* yellow dwarf virus (SYDV) that is transmitted by *Aceratagallia sanguinolenta* (Provancher) but not by *Agallia constricta* Van Duzee, and the *contricta* form (CYDV) that is transmitted by *A. constricta* but not by *A. sanguinolenta. Aceratagallia lyrata* (Baker), *A. obscura* Oman, *A. curvata* Oman, *Agallia quadripunctata* (Provancher), and *Agalliopsis novella* (Say) are also recorded vectors of PYDV.

Virions of PYDV measure 380 x 75 nm in sections of diseased plants (MacLeod *et al.*, 1966), 290 x 75 nm in vectors (Chiu *et al.*, 1970), and 290 x 75 nm in vector cell monolayers (Chiu *et al.*, 1970). Virus in plant sap has a thermal inactivation point (10 min) of ca. 50°C, a longevity *in vitro* (23-27°C) of 2.5-12 hr in sap of *Nicotiana rustica*, and a dilution end-point between 10^{-3} and 10^{-5} in phosphate buffer. Purification methods have been reported by Brakke (1951, 1953, 1955, 1956) and Whitcomb (1965). Particles appear to be of one type, with a sedimentation coefficient (S20, w) of 810-950 S, a buoyant density in sucrose of 1.17, and a molecular weight of ca. 1100 x 10^6 daltons (Brakke *et al.*, 1951; Brakke, 1958). The cell infecting unit appears to be a single particle (Chiu *et al.*, 1970). It has been shown that the PYDV particle contains more than 20% lipid (Ahmed *et al.*, 1964) at least three different proteins, an a single-stranded RNA with a molecular weight of ca. 4.3 x 10^6 daltons.

Virus-plant and virus-vector relationships. The experimental host range of PYDV includes twelve solanaceous species, the legumes *Vicia faba* and *Trifolium incarnatum*, and *Callistephus chinenesis* in the Compositae (Black, 1938; Hougas, 1951). The virus is found sporadically in North America and can be spread in infected seed potatoes. Diagnostic species are potato (*Solanum tuberosum), Trifolium incarnatum* and *Nicotiana rustica.* The latter species also serves as a propagative and assay host. Unlike most phytorhabdoviruses, PYDV is mechanically transmissible. It is easily transmissible to the local lesion host *N. rustica* using leaf-abrasion inoculation technique; with difficulty, virus can also be transmitted to *T. incarnatum* by pricking inoculum into the crown of plant. Whitcomb and Sinha (1964) demonstrated that,

under proper conditions, the numbers of lesions produced on *N. rustica* can be increased several fold by the presence of healthy, host-plant extracts or high concentrations of sucrose. Tests for seed transmission in *N. rustica* proved negative; no information on transmission by dodder is available (Black, 1970b).

The virus undergoes a minimum incubation period of 6 days in the vector. Occasional transovarial passage of the *constricta* form of PYDV has been demonstrated (Black, 1943, 1953). Particle assembly appears to be restricted to the nuclei of infected cells (MacLeod *et al.*, 1966). Electron microscopic studies of PYDV-infected plants and leafhoppers indicate that the principal sites of virus assembly and accumulation are the nuclear membrane and perinuclear space, respectively; however, intranuclear assembly also occurs (MacLeod *et al.*, 1966; MacLeod, 1968; Chiu *et al.*, 1970). In later stages of infection, virus is found in the cytoplasm of infected cells. Sinha (1965b) found that PYDV (New Jersey strain) is capable of systemically invading *A. constricta.* Non-PYDV-exposed insects were rendered viruliferous by injection with inocula prepared from extracts of various organs from viruliferous insects. In this way, virus was recovered from all organs and tissues tested, including blood, intestine, brain, fat body, malpighian tubules, salivary glands, mycetomes, ovaries, and testes. Potato yellow dwarf virus (SYDV form) also multiplies in monolayers of *A. sanguinolenta* cells (Chiu *et al.*, 1970; Hsu and Black, 1974). It is possible to infect 100% of vector cells in a monolayer. After an eclipse period of 9 hr, there is an exponential increase from the 9th to the 29th hr during which the virus population doubles every 80 min. Virus antigens and particles can be demonstrated in inoculated cells by fluorescent antibody and electron microscopic techniques, respectively. Specific fluorescence is observed only in cell nuclei in the early infection stage; but, later, antigen can be detected in the cytoplasm too.

6.6.3.2 Rice Transitory Yellowing Virus (RTYV). Particles of RTYV seem to differ from those of other phytorhabdoviruses in being bullet-shaped in ultrathin sections of diseased hosts as well as in dip preparations. Thus, in particle morphology, RTYV more closely resembles the seemingly bullet-shaped animal rhabdoviruses (Wagner, 1975). Particles measure 96 x 120-140 nm in preparations of crude leaf extracts or leaf-dip preparations from infected rice plants, 94 x 180-210 nm in ultrathin sections of infected rice plants, and 92 x 216 nm in the cytoplasm of salivary gland cells in viruliferous *Nephotettix cincticeps* Uhler (Chen and Shikata, 1971; Chen and Shikata, 1972). The center-to-center distance of cross striations on the surface of the inner core (45 nm wide) or nucleocapsid is ca. 3 nm. The viral envelope is 3-layered and ca. 21 nm thick (Chen and Shikata, 1971).

Purification methods have not yet been reported, but partial purification is possible using low speed centrifugation (Hsieh and Roan, 1967). Moderately high titered antiserum can be obtained by intravenous injection with partially purified virus (Su, 1969). Transmission by its leafhopper vectors is obligatory; therefore, the properties of the virus have been determined by an infectivity bioassay in which non-RTYV-exposed leafhoppers are tested for inoculativity following injection with sap from RTYV-infected rice. Virus has a longevity *in vitro*

of 36 hr at 28-33°C and 11 days at 0-2°C, a thermal inactivation point between 55.5 and 57.5°C, and a dilution end-point between 10^{-5} and 10^{-6} (Hsieh, 1967; Shikata, 1972).

Virus-plant and virus-vector relationships. In Taiwan, RTYV causes severe yellowing and stunting of rice, its only known plant host. Infected plants gradually recover under greenhouse conditions and produce symptomless leaves, thus the name "transitory" yellowing. Rice plants infected with RTYV are symptomatologically indistinguishable from ones infected with rice tungro virus (Shikata, 1972). But, of course, the latter virus is transmitted in a nonciruclative (semipersistent) manner by its vector *N. impicticeps* Ishihara, and its particles are isometric and 30 nm in diameter (Gálvez, 1971).

Pathological changes in the histology of RTYV-infected rice has been described by Su (1969). Parenchymatous cells around vascular bundles contain spherical or cylindrical inclusion bodies. Similar inclusions also occur in smaller parenchyma cells in the leaf and root. Disintegrated chloroplasts with a decreased affinity for hematoxylin and Giemsa stains occur in mesophyll cells. Electron microscopic examinations of infected rice leaves reveals virus-particle accumulations in the perinuclear spaces of phloic and parenchymatous cells bordering vascular bundles. The nuclear membrane appears to be the site of both nucleocapsid synthesis and viral maturation (envelopment) by budding, with the outermost layer of the viral envelope originating from the inner lamella of the nuclear membrane. Particles are sometimes seen aligned with their longitudinal axes perpendicular to the nuclear membrane and their blunt ends attached to its inner lamella, thus forming a continouous connection between viral envelope, nucleocapsid, and inner lamella (Chen and Shikata, 1971; Shikata, 1978a). Enveloped particles were often enclosed in a membranous structure either in groups or individually. Since ribosomes are frequently attached to the outer surface of these structures, the enclosing membrane presumedly arises from the outer lamella of the nuclear membrane. Except for occasional, intranuclear, cytoplasmic invaginations of membrane-bounded clusters of particles, virus in the nucleus was always associated with the nuclear membrane. No visible degenerative changes were noted in the nucleoli of infected cells, but the surrounding nucleoplasm showed a marked decrease in the amount of chromatin material.

The leafhoppers *Nephotettix apicalis, N. cincticeps* and *N. impicticeps* are known vectors of RTYV (Chiu *et al.*, 1968; Chiu, personal communication in Shikata, 1972). Different strains of the vectors vary in their transmission efficiency (Chiu *et al.*, 1968). Acquisition thresholds for *N. apicalis* and *N. cincticeps* are 5 min and 15 min, respectively; and, once having acquired virus and after an incubation period of 3-29 days (*N. apicalis*) or 21-30 days (*N. cincticeps*), vectors remain inoculative for life. Congenital transmission has not been observed (Chiu *et al.*, 1968). Bullet-shaped particles (92 x 215 nm) have been observed associated with cytoplasmic membranes in vacuolated areas of the salivary glands of viruliferous *N. cincticeps* (Fig. 4). Additionally, viruslike (RTYV) tubular structures, 5-10 times longer than typical RTYV particles, were localized in the nuclei and cyto-

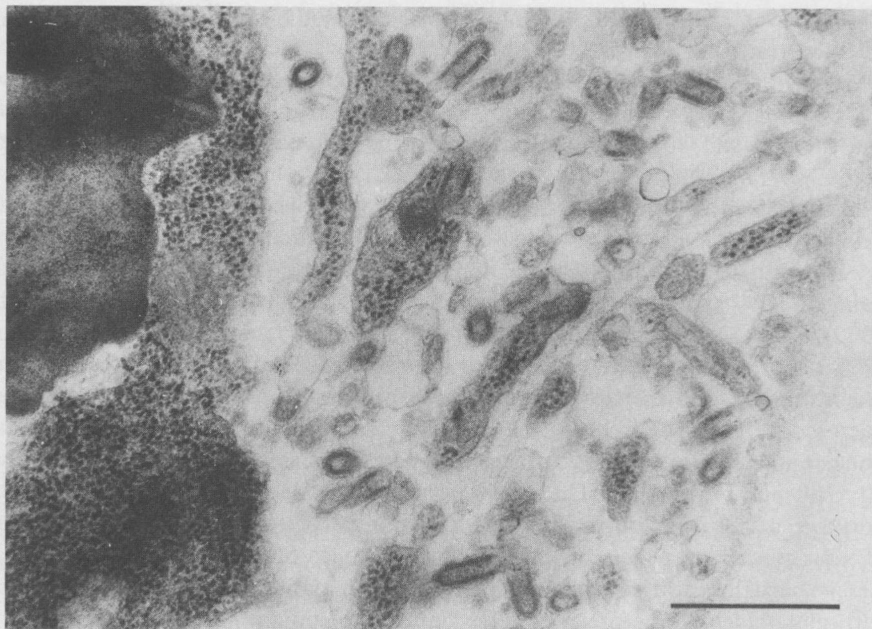


Fig. 4. Bullet-shaped particles of rice transitory yellowing virus associated with cytoplasmic membranes in a vaculoated area of a salivary gland cell of *Nephotettix cincticeps*. Bar, 500 nm. (Courtesty of E. Shikata.)

plasm of gut epithelial cells (Chen and Shikata, 1972; Shikata, 1978a). Infectivity bioassay experiments showed a high recovery of virus from gut tissues of viruliferous insects, but virus was not recovered from malpighian tubules or fat body (Chen and Shikata, 1972).

6.6.3.3 American Wheat Striate Mosaic Virus (WSMV). Virus can be purified by differential and density-gradient centrifugation (Lee, 1968; Ahmed *et al.*, 1970). Based on particle weight, the virus contains the following: ca. 5% single-stranded RNA with approximate molar percentages of nucleotides of G27, A20, C24, and U29; ca. 68% protein; ca. 24% lipid, and a suspected carbohydrate content of 3%. The virus sediments as three components, one major and two minor ones. The main component has a sedimentation coefficient (S20,w) of ca. 900 S; one minor component has a higher and the other a lower "S" value than the main component (Sinha and Behki, 1972). The virus has an absorbance at 260 nm (1mg/ml, 1 cm light path) of 3.1 and an A 260/280 of 1.25 (Sinha and Behki, 1972) and contains 25,000, 59,000 (N protein), 92,000 (G protein), and 145,000-dalton structural proteins (Trefzger-Stevens and Lee, 1977).

The virus is weakly immunogenic. Antiserum prepared by intramuscular injection of rabbits with partially purified preparations had titers of 1/160, 1/40, and 1/5 in ring-precipitin, tube-precipitin and gel-diffusion tests, respectively (Sinha and Behki, 1972). Sinha and Thottappilly (1974) compared the sensitivity of three serological tests for detecting WSMV purified from infected plants. A virus

concentration as low as 15.6 μg/ml could be detected by precipitin ring or precipitin tube tests, but at least 1000 μg/ml of virus was needed to obtain a positive reaction in agar-gel, double-diffusion tests. As determined by ring tests, the titer of WSMV antigen in extracts of infected wheat was 160 in leaves or culms, 80 in awns and sheaths, and 40 in roots; the antigen titer in extracts of viruliferous leafhoppers, *Endria inimica* (Say), was 80 (Sinha, 1968b).

Since the virus is not sap-transmissible, its stability has been studied by injecting virus extracts into non-WSMV-exposed insects which are later tested for inoculativity. The virus has a thermal inactivation point (10 min) of ca. 55°C, a dilution end-point of 10^{-3}, and a longevity *in vitro* of ca. 3 days at +4 or -10°C (Lee and Bell, 1962).

The morphology of the WSMV particle varies depending on its *milieu (in situ* versus *in vitro)* and the treatment it receives prior to being observed in the electron microscope. Lee (1967, 1968) reported that negatively stained, purified preparations contained two types of bullet-shaped particles measuring ca. 170 x 80 nm and 90 x 80 nm, respectively. These particles were considered artifacts resulting from breakage of the bacilliform, 250-270 x 80 nm particles observed in thin sections of virus-containing pellets. Ahmed *et al.* (1970) also observed bullet-shaped particles in partially purified preparations; but, when similar preparations were fixed with glutaraldehyde before negative staining with sodium phosphotungstate, they observed mainly intact, bacilliform particles measuring ca. 260 x 80 nm. Lee (1967) found that particles in thin sections of infected plant tissue were mostly bacilliform and ca. 250-300 x 60-80 nm. The question of particle morphology is perhaps best summarized by Sinha (1971) who observed bacilliform (250 x 75 nm), bullet-shaped (200 x 75 nm), and long bacilliform (415 x 75 nm) particles both *in situ* in infected plants and in extracts of infected leaves that had been first infiltrated with glutaraldehyde. Some of the bullet-shaped particles observed in leaf extracts were surrounded by a continuous membrane and, therefore, should not be confused with the shorter, bullet-shaped, breakage artifacts reported by Lee (1968) and Ahmed *et al.* (1970). Assuming that the true morphology of the WSMV particles is bacilliform (245-250 x 75 nm), Sinha (1971) suggested ways in which enveloped bullet-shaped and long bacilliform particles might be formed *in situ.* However, he also noted that the possibility of the WSMV genome directing the formation of all three types of particles could not be ruled out.

Virus-plant interactions. The virus has been found causing a striate mosaic disease of wheat in Canada and the United States (Slykhuis, 1962, 1963; Timian, 1960; Hamilton, 1964; Slykhuis and Sherwood, 1964). The host range of WSMV is narrow; about 20 species of Gramineae are susceptible to infection (Slykhuis, 1962, 1963). Diagnostic species are wheat (*Triticum durum* Desf. cv. Ramsey), oat (*Avena sativa* cv. Victory), stink grass (*Eragrostis cilianensis* cv. Lutati), witch grass (*Panicum capillare*), barley (*Hordeum vulgare* cv. Vantage), and corn (*Zea may* cv. Gaspe Flint). The aforementioned variety of wheat also serves as a propagation and assay species. Seed transmission is not known to occur, and attempts at transmission by dodder have been unsuccessful (Timian, 1964).

The fate of WSMV in infected wheat has been studied extensively by electron microscopy (Lee, 1964, 1967, 1970; Sinha, 1971; Vela and Lee, 1975). Virus particles can be seen in all parts of infected plants: leaves, sheaths, culms, awns, and roots. Particles occur in the cytoplasm, nucleoplasm (but not in the nucleolus), and perinuclear space of mesophyll cells of leaves and sheaths, cortical cells of culms, awns and roots, and phloem parenchyma and companion cells throughout the plant. Particles occurred singly, in small groups, and in large aggregates either membrane-bounded or free in the cytoplasm. Virus-containing, cytoplasmic invaginations into the nuclei of infected cells are not uncommon, and particles are routinely observed aligned with their longitudinal axes perpendicular to the nuclear membrane. Particles sometime occur free in the nuclear matrix, but most nuclear inclusions of virus are bounded by membranes. Studies suggest that both cytoplasmic and nuclear membranes may serve as sites for virus budding (maturation).

Virus-vector interactions. The leafhoppers *Endria inimica* (Say) and *Elyman virescens* (F.), an inefficient vector, are known transmitters of WSMV (Slykhuis, 1963; Sinha, 1970). Congenital passage does not occur (Slykhuis, 1963). All vector stages can acquire and transmit virus in a transstadial, circulative (propagative) manner. The incubation period in the vector varies from 4-6 to 22-24 days (Slykhuis, 1963). The minimum incubation period in the inefficient *E. virescens* is ca. 15 days (Sinha, 1970). The acquisition and inoculation thresholds are less than 1 min and ca. 15 min, respectively. After acquiring virus, insects usually retain inoculativity for several weeks or life, but some cease to transmit virus after about 5 weeks (Lee, 1963).

Using serial injection technique, Sinha and Chiykowski (1967) passed WSMV through seven successive groups of leafhoppers, *E. inimica.* The dilution end-point of the starting inoculum was 10^{-4}; the calculated dilution of the virus at the seventh passage was 10^{-13}. Paliwal (1968) reported a 1375 fold increase in virus concentration in insects between the 3rd and 8th day after virus acquisition; Sinha and Chiykowski (1969) showed a 5000 fold increase between days 1 and 7. Using infectivity bioassay, virus can be recovered from hemolymph, hemocytes, gut, salivary glands, fat body, brain and mycetomes of viruliferous *E. inimica*, but not from ovaries, testes or malpighian tubules (Sinha and Chiykowski, 1969). Virus was first recovered from the alimentary canal on the 2nd day following a 1-day acquisition-access feed, and from the salivary glands, hemolymph, and hemocytes on the 4th day. The concentration of virus in the gut peaked by day 8 and then decreased slightly between days 8 and 32. The virus concentration in the hemolymph, hemocytes and salivary glands plateaued in 6-8 days. In the case of injected insects, virus can be recovered from all the aforementioned tissues except the gut. Therefore, since virus multiplies in both plant-fed and injected insects, tissues other than the gut must also support virus synthesis (Sinha, 1973b). Infectivity bioassay experiments indicate that the concentration of virus per gram of tissue in viruliferous leafhoppers is about the same as in plants (Sinha and Behki, 1972).

Virions (mature particles only) have been observed in defined intranuclear inclusions, in perinuclear spaces, and in the cytoplasm in close proximity to infected nuclei of salivary gland cells in *E. inimica* (Bell *et al.*, 1978).

6.6.3.4 Cereal Chlorotic Mottle Virus (CCMV). Cereal chlorotic mottle is a disease affecting corn and a number of grasses in Australia. The causative agent of the disease is a rhabdovirus transmitted by the leafhopper *Nesoclutha pallida* (Evans). The virus has been isolated from infected plants, and virus particles have been observed in infected plants as well as in the brains and salivary glands of viruliferous insects (Greber, 1977; Grylls, 1978). Further studies are required to determine the relatedness of CCMV to other previously described phytorhabdoviruses.

6.6.3.5 Viruslike Particles. Lee (1965) observed rod-shaped particles measuring 300 x 30 nm in the salivary glands of the leafhopper *Endria inimica* (Say). Sinha (1973a) observed rod-shaped, viruslike particles (297 ± 26 x 35 ± 5 nm) in the cytoplasm of salivary gland cells of both nymphs and adults of *E. inimica.* In nymphs, the particles occurred in vacuolelike areas where they were stacked in groups of side-to-side aggregated particles or formed large crystals. In adults, the particles were sometimes observed packed in spherical, electron-dense bodies in the glands. Particles were also observed in extracts of salivary glands, but not in extracts of gut, brain, fat body, or ovary. The insects carrying these particles were apparently unable to transmit them to plants (Sinha, 1973a).

6.7 CIRCULATIVE PLANTHOPPER-BORNE VIRUSES

Planthopper-borne (Delphacidae) phytoreoviruses and rhabdoviruses multiply in both their plant and insect hosts.

6.7.1 Reoviridae

The planthopper-borne maize rough dwarf (MRDV), rice black-streaked dwarf (RBSDV), sugarcane Fiji disease (FDV), cereal tillering disease (CTDV), oat sterile dwarf (OSDV), and pangola stunt (PSV) viruses may all be tentatively listed as belonging to the family Reoviridae. Leafhopper and planthopper-borne phytoreoviruses appear to share similar fates in the cells of their plant and insect hosts. As discussed earlier, virions appear in the cytoplasm of infected host cells in four basic forms: in viroplasms, in defined phagocytic structures, in rows within tubular structures, and in microcrystalline arrays (Shikata, 1977).

The viroplasms or "virus factories" are not surrounded by limiting membranes and, therefore, are intimately associated with the surrounding cytoplasm and cytoplasmic organelles. The electron-opaque, viroplasmic matrices consist of granular and fine threadlike materials. The phagocytic structures appear in a later stage of infection, are membrane-bounded, and can often be seen to contain myelin figures. It is interesting to note that this morphology compares favorably with the virus-containing structures that Harris and associates (Harris, 1971, 1973; Harris and Bath, 1972; Harris *et al.*, 1974, 1975) described and later tentatively labeled as later-stage or secondary lysosomes in aphids exposed to pea enation mosaic virus (PEMV) (section 6.8.1.3). The tubular structures and microcrystalline arrays also occur during later stages of infection. The latter result from extensive virus assembly and the subsequent dense accumulations of virions in viroplasms, phagocytic structures, and vacuolated areas of the cytoplasm.

Maize rough dwarf virus and RBSDV cause similar diseases, have similar host ranges and insect vectors (Lovisolo, 1971; Harpaz, 1972; Shikata, 1974), and are serologically closely related (Luisoni *et al.*, 1973). The two viruses probably represent different strains of the same virus. Transovarial passage of MRDV has been recorded (Harpaz, 1972).

Initially, it appeared as though MRDV and RBSDV virions were about 60 nm in diameter and lacked surface projections. However, later observations of MRDV – virus obtained by certain purification procedures or fixed with glutaraldehyde prior to staining with PTA – revealed 75 nm virions with surface projections or spikes (Lesemann, 1972; Milne *et al.*, 1973). Milne and associates have proposed a model for MRDV in which an icosahedral outer capsid comprised of 92 capsomeres and bearing A spikes surrounds an inner capsid with B spikes (Shikata, 1977). Fiji disease virus and PSV appear to have particle morphologies similar to the MRDV model (Hatta and Francki, 1977; Giannotti and Milne, 1977).

In MRDV or RBSDV-infected host cells, one can discern particles of two sizes: a 75 to 85-nm particle with a dense, 50-nm core, and a smaller particle measuring ca. 55 nm in diameter (Shikata, 1977; Shikata and Kitogawa, 1977). The 75 to 85-nm particles occur at the periphery of viroplasms, in "crevices" within viroplasms, scattered in the cytoplasm outside of viroplasms, in tubular structures, and in microcrystalline arrays. Such particles correspond in size to the larger particles seen in fixed preparations, and presumedly represent mature, complete particles or virions. The smaller particles ("subviral particles" corresponding to the inner core of complete virions) occur scattered within viroplasms and are similar in size to the smaller particles observed in purified preparations of MRDV or RBSDV (Shikata, 1977). Shikata and Kitagawa (1977) recently reported on the intracellular localization and multiplication of RBSDV in plants and in the fat bodies (Fig. 5), salivary glands, intestines, and tracheoblasts of RBSDV-infected, planthopper vectors.

At least at the subviral particle (SVP) level, serological-relatedness studies indicate that OSDV, *Arrhenatherum* blue dwarf virus (ABDV) and *Lolium* enation virus (LEV) are strains of the same virus and are unrelated to other members of the fijivirus group such as MRDV and PSV (Milne and Luisoni, 1977; Milne and Lesemann, D.-E., 1978). In purified preparations, the SVP's of OSDV and MRDV are morphologically similar, but those of the former virus are more resistant to degradation. The electrophoretic patterns of the apparently identical, 10-segmented genomes of OSDV and ABDV (in 5% polyacrylamide gels) are different from those of other MRDV-like viruses such as the 10-segmented genomes of MRDV and PSV, but the spread of molecular weights is comparable (Boccardo *et al.*, 1978; Luisoni, *et al.*, 1978; Luisoni and Milne, 1978).

The leafhopper and planthopper-borne groups of phytoreoviruses produce similar but yet distinguishable pathological effects in the cells of their plant and insect hosts (Shikata, 1977, 1978a). Viroplasms in cells infected by leafhopper-borne phytoreoviruses are characteristically spherical in shape and of a size not exceeding that of the nucleus. Mature particles are harbored at the periphery of the viroplasm. And immature particles are rarely seen in the viroplasmic matrix. Contrariwise, viroplasms associated with the planthopper-borne group are large (often larger

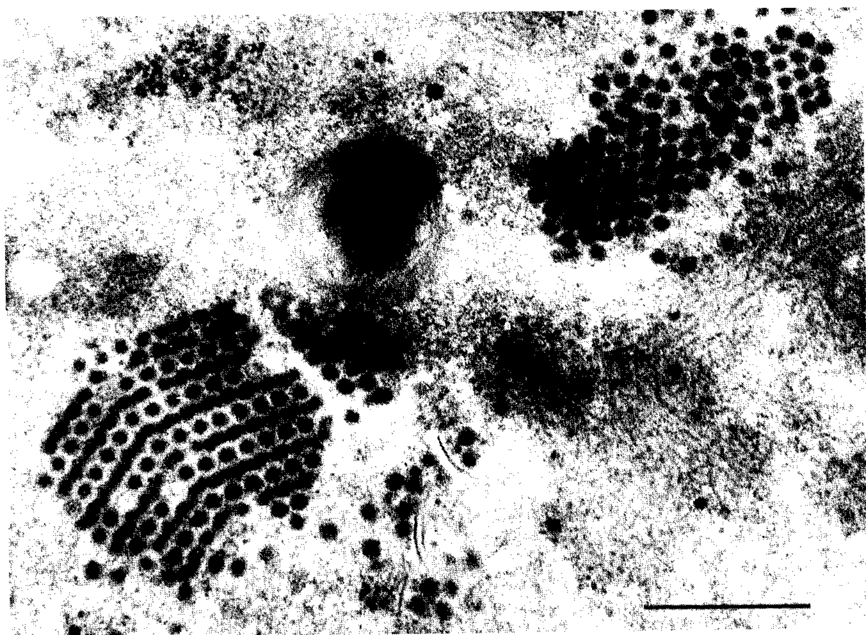


Fig. 5. Closely aggregated particles of rice black-streaked dwarf virus in the cytoplasm of a fat body cell of *Unkanodes albifascia*. Bar, 500 nm. (Courtesy of E. Shikata.)

than host-cell nucleus) amorphous structures with smaller, immature particles scattered in their electron-opaque, granular and fibrilar matrices, and larger, mature particles occurring peripherally or in electron-transparent crevices.

6.7.2 Rhabdoviridae

The planthopper-borne group of rhabdoviruses includes northern cereal mosaic (NCMV), maize mosaic (MMV), barley yellow striate mosaic (BYSMV), wheat chlorotic streak (WCSV; vector = *Laodelphax striatellus* Fallén), bobone disease (BDV; vector = *Tarophagus proserpina* [Kirk]; Gollifer *et al.*, 1977), and *Digitaria* striate mosaic (DSMV) viruses.

Particles of NCMV have been isolated from diseased plants and viruliferous planthoppers using differential and sucrose density-gradient centrifugation (Lu *et al.*, 1968). Five species of delphacid planthoppers are known to transmit NCMV (Kisimoto, 1973). Virus particles have been observed in the cytoplasm but not nuclei of infected plants and viruliferous insects (Shikata, 1973; Shikata and Lu, 1967; Shikata, 1978a). In infected plant cells, enveloped ("coated") particle accumulations usually occur in vesicles that are apparently derived from cisternae formed by the endoplasmic reticulum. Thinner rods, ca. 40 nm in diameter and of variable length (presumedly unenveloped nucleocapsids), occur in the cytoplasm of infected plant cells (Shikata and Lu, 1967). Nucleocapsid synthesis seems to occur in viroplasms in infected plant and vector host cells (Shikata,

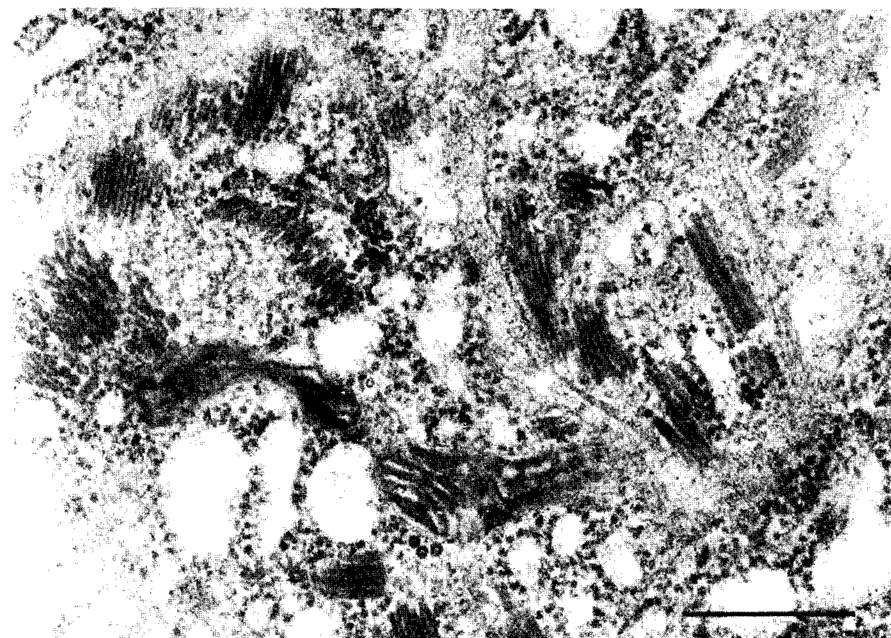


Fig. 6. Clusters of rod-shaped particles of northern cereal mosaic virus in the cytoplasm of a salivary gland cell of *Laodelphax striatellus*. Individual particles can be seen in a variety of sectioning angles ranging from transverse to longitudinal. Bar, 500 nm. (Courtesy of E. Shikata.)

1978a). Figure 6 shows accumulations of unenveloped NCMV nucleocapsids in a salivary gland cell of *Laodelphax striatellus* (Fallen). Nucleocapsids have been observed in the fat body, sometimes in association with viroplasms (Shikata and Lu, 1967; Shikata, 1978a). Enveloped particles have not been observed in vectors. It is not known whether the enveloped particles observed in plants obtain their envelopes via a budding process. Propagation in the vector has been demonstrated by serial passage of virus through successive groups of insects. And virus has been recovered from vector organs (gut and salivary glands but not fat body) using infectivity bioassay technique (Yamada and Shikata, 1969). Other vectors of NCMV include *Ribantodelphax albifascia* (Mats.), *Unkanodes sapporanus* (Mats.), and *Muellerianella fairmairei* Perris.

Maize mosaic virus (MMV) has been localized in infected plants and viruliferous planthoppers, *Peregrinis maidis* Ashmead, by electron microscopy (Herold *et al.*, 1960; Herold and Munz, 1965). Cytoplasmic inclusions of MMV particles occur in epidermal, palisade and parenchyma cells of MMV-infected corn leaves. Particles appear to bud at the inner lamella of the nuclear membrane and accumulate in the perinuclear space (Herold et al., 1960; Herold, 1972). In infected salivary gland and intestinal cells of *P. maidis*, particles can be seen singly and in groups in the perinuclear space as well as in tubules and cisternae of the endoplasmic reticulum (Herold and Munz, 1965; Herold, 1972). In the vector, both nuclear and cytoplas-

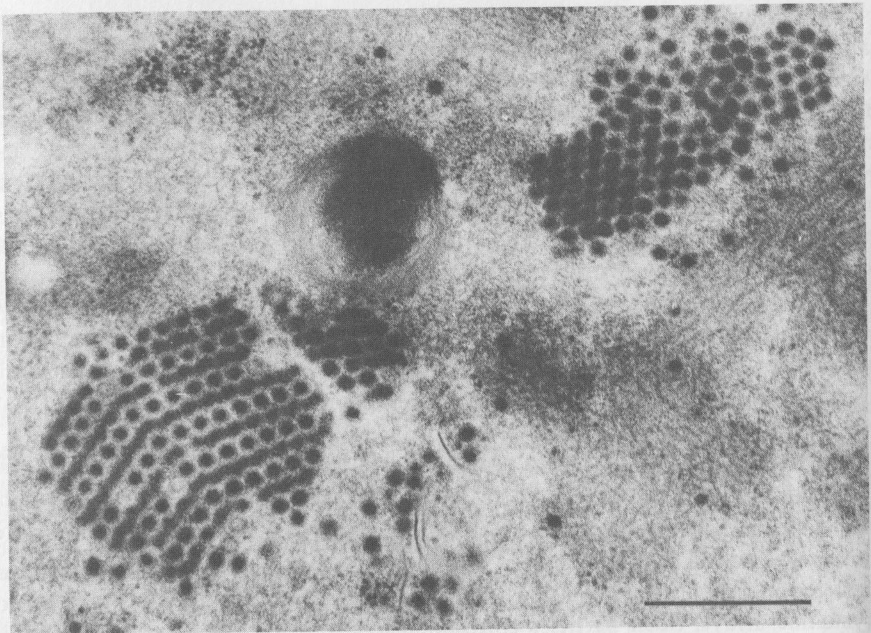


Fig. 5. Closely aggregated particles of rice black-streaked dwarf virus in the cytoplasm of a fat body cell of *Unkanodes albifascia.* Bar, 500 nm. (Courtesy of E. Shikata.)

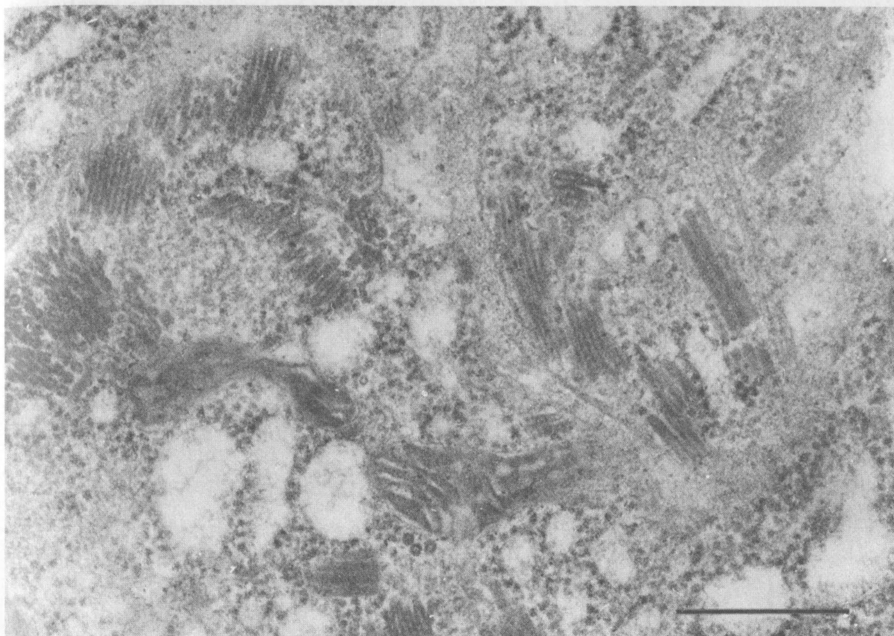


Fig. 6. Clusters of rod-shaped particles of northern cereal mosaic virus in the cytoplasm of a salivary gland cell of *Laodelphax striatellus*. Individual particles can be seen in a variety of sectioning angles ranging from transverse to longitudinal. Bar, 500 nm. (Courtesy of E. Shikata.)

mic membranes may serve as sites for virus maturation by budding. The incubation period in the vector may be as brief as 4 days but is usually longer (Carter, 1948; McEwen and Kawanishi, 1967).

Accumulations of barley yellow striate mosaic virus (BYSMV) particles (330 x 45 nm) have been observed in the cytoplasm of infected plant cells (Conti, 1969; Conti and Appiano, 1973). Cytoplasmic membranes appear to serve as budding sites for the envelopment of nucleocapsids as they emerge from membrane-bounded viroplasms.

Partially purified preparations from BYSMV-infected barley contain particles measuring 320-330 nm in length and ca. 60 nm in width and having a helically arranged nucleocapsid with a pitch of 4.4 nm. Up to 90% of *L. striatellus* planthoppers can be rendered inoculative by injection with partially purified virus. The vector is reported to transmit BYSMV to 27 of 46 species of Gramineae. The virus is not transmissible by sap inoculation or through seed of wheat. The propagative nature of BYSMV is confirmed by persistent transmission, transovarial passage, and electron microscopic evidence of virus multiplication in insect tissue (Conti *et al.*, 1978).

Digitaria striate mosaic virus (DSMV) infects cereals and grasses in Australia (Greber, 1972). The virus, presumedly a rhabdovirus, is transmitted by the delphacid *Sogatella kolophon* (Kirkally). Virus particles have been found in infected plants and also in the brain and salivary glands of the vector (Greber, 1976).

6.7.3 Hoja Blanca Virus (HBV)

Hoja blanca is one of the most destructive rice diseases in the Western Hemisphere (Everett and Lamey, 1969). The causative agent of hoja blanca, apparently a virus, is transmitted in a circulative manner by the delphacids *Sogatodes oryzicola* (Muir) and *S. cubanus* (Crawford). Its incubation period in insects is ca. 28-31 days; and, once having acquired virus, insects generally retain inoculativity for life. Gâlvez (1968) detected a high incidence of transovarial passage in *S. oryzicola.* These transmission characteristics suggest that HBV multiplies in its planthopper vectors. Jennings and Peneda (1970) reported a reduction in the fertility and longevity of HBV-carrying planthoppers, *S. oryzicola.*

There are two theories regarding the morphology of the HBV virion. According to Herold *et al.* (1968), the particle is isometric and ca. 42 nm in diameter. Shikata and Gâlvez-E (1969), on the other hand, consider the particle to be long, flexuous, threadlike, and ca. 8-10 nm in diameter. Such particles were seen in the nuclei and cytoplasm of cells (including the epidermis, palisade cells, spongy parenchyma, phloem, and vessels) in HBV-infected rice leaves, and also in the gut lumen and gut epithelial cells (cytoplasm) of viruliferous insects. Similar particles were never observed in sections of non-HBV-exposed plants and insects. Data from preliminary purification attempts also suggested that the threadlike particles observed in in-

fected plants and viruliferous insects were indeed virions of HBV (Shikata and Gâlvez-E, 1969).

6.7.4 Viruslike Particles

Viruslike particles have been observed in two delphacid planthopper species, *Peregrinis maidis* (Ashmead) and *Javesella pellucida* Fabricius, that were not known to have had prior exposure to virus-infected plants. And the insects carrying these particles were apparently unable to transmit them to plants (Herold and Munz, 1967; Ammar *et al.*, 1970). Those observed by Ammar *et al.* (1970) in some tissues of *J. pellucida* were bacilliform and measured 65 x 30 nm. Herold and Munz (1967) observed polyhedral particles measuring 54 ± 9 nm in the cytoplasm of cells of the salivary glands, intestine, mycetome, fat body, ovary, and blood of *P. maidis* from Venezula. The particles occurred in the cytoplasm singly, in groups, free or in vesicles, and in hexagonally arranged crystals. Such particles were also observed in *P. maidis* from Hawaii, but only after the insects had been injected with suspensions of intestine from the Venezuelan planthoppers. The researchers, therefore, suggested that the particles represented a "*Peregrinis* virus" causing a latent infection in the insect.

6.8 CIRCULATIVE APHID-BORNE VIRUSES

Circulative, aphid-borne viruses can be separated into at least five distinct taxonomic categories or groups. The International Committee on Taxonomy of Viruses (ICTV) has assigned latinized names to two groups, the luteoviruses and rhabdoviruses, and recognizes a third, monotypic group, as yet unnamed, founded on pea enation mosaic virus (PEMV). Two additional groups based on potato leafroll virus and carrot mottle viruses, respectively, can be postulated. For additional information regarding ICTV's taxonomic grouping of plant and animal viruses consult Harrison *et al.* (1971), Fenner (1975/76, 1976), Shepherd *et al.* (1975/76) and Shepherd (1977).

Those circulative, aphid-borne viruses that have been studied in their vectors at the organ, tissue, or cellular level are, for convenience, here categorized as polyhedral viruses and rhabdoviruses (Rhabdoviridae).

6.8.1 Small Spherical or Polyhedral Viruses

Polyhedral viruses which have been localized in their vectors include barley yellow dwarf (BYDV), potato leafroll (PLRV), pea enation mosaic (PEMV), and beet western yellows (BWYV). There is no unequivocal evidence that any of these viruses multiply in their vectors; and for one, BYDV, there is convincing evidence that it does not. Miyamoto and Miyamoto (1966, 1971) demonstrated transovarial passage of PLRV in *Myzus persicae* Sulz.

6.8.1.1 Barley Yellow Dwarf Virus (BYDV). The ICTV has assigned BYDV as the type virus for the luteovirus group of aphid-borne plant viruses (Shepherd *et al.*, 1975/76; Shepherd, 1977). The sigla "luteo" comes from the Latin *luteus*

meaning yellow and refers to the yellowing produced in infected plants. Members of the luteovirus group are characterized by isometric particles (115-118 S and ca. 25 nm in diameter) containing single-stranded RNA of ca. 2.0 x 10^6 daltons, thermal inactivation points between 60 and 70°C, concentrations in plant sap of less than 100 μg/l (phloem-restricted), non-mechanical-transmissibility, and persistent transmission by aphid vectors, with virus strains having high degrees of vector specificity. Some members are serologically related. Also included in the group are beet western yellows virus (Duffus, 1972), soybean dwarf virus, and several vector-specific viruses which are biologically and physically similar to but not necessarily serologically related to barley yellow dwarf virus (designated as PAV, RPV, RMV, MAV, and SGV: Rochow, 1970a, 1970b). The RPV isolate is serologically distinct from the MAV and PAV strains.

More recently, Duffus (1977b) demonstrated the serological relatedness of the dwarfing and yellowing strains of soybean dwarf virus (SDV-DS and SDV-Y, respectively) from Japan with beet western yellows virus (BWYV) isolates from the United States and Europe, with beet mild yellowing virus (BMYV) from Europe, with turnip yellows virus (TuYV) from Europe, and with the RPV isolate of barley yellow dwarf virus (BYDV). A reciprocal relationship has been established between the RPV isolate of BYDV and beet western yellows virus (BWYV) (Rochow and Duffus, 1977). Indeed, the RPV isolate of BYDV appears to be more closely related to BWYV than to the PAV and MAV isolates of BYDV (Duffus and Rochow, 1978). Luteoviruses are characterized by restriction of virus to the phloem, very low titers of virus in preparations from diseased plants, and non-mechanical-transmissibility. Other possible members of the luteovirus group include beet mild yellowing, turnip yellows, banana bunchy top, bean leaf roll, carrot red leaf, cotton anthocyanosis, and filaree red leaf viruses (Shepherd *et al.*, 1975/76).

The genome of BYDV consists of a single component of single stranded RNA with a molecular weight of 2.0 x 10^6 daltons. Virions are isometric in shape (possibly octahedral: Israel and Rochow in Rochow, 1970a) with a sedimentation coefficient (S20, w) of 115-118 S and a diameter of 30 nm in shadowed preparations (Rochow and Brakke, 1964), 24 nm in thin sections of plant host tissue (Jensen, S., 1969), and 20 nm in negatively stained virus preparations (Rochow, 1970b). In plants, the particles appear to be confined to the phloem (Jensen, S., 1969). The thermal inactivation point (10 min) in crude sap and in partially purified preparations is 65-70°C (Heagy and Rochow, 1965). The concentration of BYDV is usually 100 μg or less per liter of plant sap (Rochow, 1970a; Rochow *et al.*, 1971).

Reported aphid vectors of BYDV include *Macrosiphum (=Sitobium) avenae* F., *Macrosiphum granarium* (Kirby), *Metopolophium dirhodum* (Walk.), *Neomyzus circumflexus* Buckt., *Rhopalosiphon annuae* (Ostl.), *R. maidis* (Fitch), *R. padi* L., *R. prunifoliae* (Fitch), and *Schizaphis graminum* Rond.

Fate in plant. Electron microscopical examination of BYDV-infected oat leaves has revealed high concentrations of virions in phloem cells. Virus particles have not been detected in any other cells of infected plants (Jensen, S., 1969; Paliwal and Sinha, 1970). This apparent restriction of virus to the phloem would account for the low yields of virus from extracts of whole infected plants (usually less than 100 μg/1).

Fate in vector. Miller (1962) reported that BYDV-inoculative English grain aphids, *Macrosiphum granarium* (Kirby), consumed 14% less oxygen, developed faster from 1st instar to adult stage, lived longer, and were more fecund than nonviruliferous controls.

Attempts to locate BYDV in cells of viruliferous aphids by electron microscopy have been unsuccessful (Paliwal and Sinha, 1970; Rochow, 1977b). Viruslike particles have been observed in the gut lumina of BYDV-exposed aphids; however, positive identification of these particles as BYDV virions was not possible since similar ones were also occasionally seen in the guts of non-BYDV-exposed aphids (Paliwal and Sinha, 1970).

The fate of BYDV in its vector has been studied at the organ level using infectivity bioassay and serological techniques (Paliwal and Sinha, 1970). By using the precipitin ring test, BYDV antigen could be detected in the gut and hemolymph but not in the salivary glands and brains of viruliferous aphids. For infectivity bioassay, guts, salivary glands, and brains were excised from aphids previously allowed an acquisition-access feeding period of 4 days on BYDV-infected source plants. One hundred organs of each kind were individually pooled and used to prepare extracts for intrahemocoeleic injection into recipient, nonviruliferous test aphids. Injected aphids were then placed in groups of 5 on healthy oat plants for 4 days to test for inoculativity. In this way, virus was detected in the gut and salivary glands but not in brains of viruliferous aphids. Inocula prepared from hemolymph taken from viruliferous aphids were also infective. In terms of relative infectivity, guts were shown to be the best and salivary glands the poorest sources of inocula.

Increasing concentrations of virus in the gut with increasing acquisition-access feeding periods demonstrated that for BYDV, as for several other circulative viruses (O'Loughlin and Chambers, 1967; Shikata and Maramorosch, 1967a; Sylvester and Richardson, 1970; Harris and Bath, 1970, 1972; Harris *et al.*, 1975), the gut appears to be a major site for virus accumulation in the vector. Assays of guts at various time intervals following a 6-hr acquisition-access feeding period indicated that the concentration of virus remained about the same between 6-24 hr and then progressively declined up to 96 hr (Paliwal and Sinha, 1970). As was the case for two leafhopper-borne circulative viruses, wound tumor (WTV) and wheat striate mosaic (WSMV) viruses (Shikata and Maramorosch, 1967a; Sinha, unpublished data, in Paliwal and Sinha, 1970), no virus was detected in the guts of abdominally inoculated aphids, thus suggesting that gut permeability to BYDV is unidirectional. Contrariwise, PEMV has been observed in the stomach lumina of hemocoeleically injected pea aphids (Harris, 1974a, 1974b, 1974c; Harris *et al.*, 1975).

Multiplication in Vector? Barley yellow dwarf virus does not appear to multiply in the vector for the following reasons (Paliwal and Sinha, 1970): (1) BYDV

cannot be transmitted from aphid to aphid using serial-injection techniques (also see Rochow, 1969b); (2) viruliferous aphids separated from a continuing source of virus gradually lose their inoculativity; (3) retention time and transmission efficiency are dose-dependent, both for injected and source-plant fed insects; (4) bioassay tests indicate that virus does not multiply in the gut; and (5) aphids that are rendered viruliferous by injection with virus retain inoculativity for shorter time periods than those which acquire virus *per os*. However, as noted by Paliwal and Sinha (1970), these negative data, however convincing, do not preclude the possibility of limited multiplication in certain vector tissues.

Lack of multiplication in the vector or low-level multiplication in a selected tissue(s), and/or invasion of only a limited number of vector tissues by acquired virus might explain why small, isometric, aphid-borne viruses such as BYDV, PEMV (Harris, 1971, 1973, 1974a, 1974b, 1974c, 1975; Harris and Bath, 1972; Harris *et al.*, 1974, 1975), and PLRV (Moericke, 1963; Peters, 1971; Ponsen, 1972) have proved far less amenable to *in situ* study in aphids by electron microscopy than have the propagative, phytorhabdoviruses, i.e. sowthistle yellow vein (Richardson and Sylvester, 1968; Sylvester and Richardson, 1970), lettuce necrotic yellows (O'Loughlin and Chambers, 1967), strawberry crinkle (Richardson *et al.*, 1972) and broccoli necrotic yellows (Garrett and O'Loughlin, 1977) viruses. Electron microscopical studies of BYDV-injected aphids (Harris *et al.*, 1975), aphid cell culturing (Adam and Sander, 1976; Matisova and Valenta, 1977), and immunofluorescent antibody (Matisova and Valenta, 1975) techniques might be useful in future studies of BYDV-vector interactions at the tissue and cellular level.

Specificity. Like most circulative viruses, BYDV is not mechanically transmissible. And, like most luteoviruses, purification from infected plants yields very low titers of virus. Both of these characteristics handicap researchers interested in studying vector-virus-plant interactions. However, partially purified virus is stable and can be concentrated. Additionally, BYDV isolates show a high degree of vector specificity and are strongly immunogenic. The relative specificity of vector-virus isolate combinations remains the same whether virus is acquired by aphids by feeding on infected plants, by feeding through membranes on crude plant extracts or purified virus suspensions, or by intrahemocoeleic injection with virus. These latter characteristics make detailed studies of the specificity phenomena of aphid transmission of BYDV possible (Rochow, 1969b, 1977a; Rochow *et al.*, 1975; Rochow and Gill, 1978).

Rochow and associates' research centers around two very specific aphid-virus isolate combinations. One isolate, RPV, is transmitted efficiently by *Rhopalosiphum padi* L. but very rarely by *Macrosiphum avenae* F. A second isolate, MAV, is transmitted by *M. avenae* but not by *R. padi*. However, the specificity of the systems can be manipulated. For example, *R. padi* will transmit MAV from plants infected with both RPV and MAV isolates. This simple experiment emphasizes two crucial factors in vector specificity: the importance of viral coat protein and the role of transcapsidation (heterologous encapsidation or genomic masking) in dependent transmission from mixed infections (Rochow, 1970b; Rochow *et al.*, 1975; Rochow, 1977a; Rochow and Gill, 1978). *R. padi* transmits MAV (genotype) from doubly-infected plants because in these plants some MAV nucleic acid is transcapsidated and thus masked from the "nonvector," *R. padi*, in the guise (phenotype) of "transmissible" RPV coat protein. These heterologously encapsidated particles function like RPV in *R. padi* but like MAV in the plants to which *R. padi* inoculates them, because of their MAV nucleic acid content.

Since MAV and RPV are serologically distinct viruses, transcapsidation is also demonstrable using membrane feeding and serological techniques. Transmission of either isolate or mixtures of the two (each prepared from singly-infected plants) by its specific vector is blocked when membrane-fed, virus-source suspensions are treated with homologous antisera (Rochow, 1970b). This being the case, heterologously encapsidated MAV virions (MAV genome in RPV capsid) ought not to be affected by treatment with MAV antiserum. Therefore, if heterologous encapsidation does occur and is responsible for the transmission, *R. padi* should still be able to transmit MAV that it acquires by feeding through membranes on virus suspensions prepared from doubly-infected plants and pretreated with MAV antiserum. And it does. For a more detailed discussion of the many lines of evidence for heterologous encapsidation, consult Rochow *et al.* (1975) and Rochow (1977a).

Until recently, researchers have focused on the vector gut as the major contributor to the specificity phenomena associated with persistent transmission: an emphasis derived for the most part from Storey's (1933) early research on the effect of gut puncturing on vector specificity in the transmission of maize streak virus by the leafhopper *Cicadulina mbila* Naude. But this does not account for BYDV specificity. Both RPV and MAV isolates pass through the guts of their respective nonvectors and arrive in the hemocoele in a potentially infectious titer (Rochow and Pang, 1961). Furthermore, noncompatible vector-virus combinations are not altered by gut puncturing or by abdominal injection of nonvectors with purified preparations of virus (Rochow, 1969a; Rochow and Pang, 1961). These and numerous other considerations led Rochow *et al.* (1975) to postulate that BYDV specificity is based on interactions of virus capsid protein with membranes of the aphid's salivary glands. Compatible vector-virus combinations allow for passage of virus through the glands, whereas noncompatible ones do not. I (Harris, 1975, 1977a; see also section 6.8.1.3, *Specificity*) have proposed a similar specificity mechanism for aphid-PEMV transmission systems based on data from my electron microscopical studies of PEMV in compatible and noncompatible vector-virus combinations.

In addition to those references already cited, the reader may wish to consult the following articles for detailed information on the purification, intrinsic properties, serology, aphid transmission, and vector-virus and virus-vector specificity characteristics of BYDV: Rochow, 1959, 1960a, 1960b, 1961, 1965, 1969a; Mueller and Rochow, 1961; Gill, 1970; and Aapola and Rochow, 1971. The best reviews of the vector-virus relationships of BYDV are found in Rochow, 1969b, 1977a, and Rochow *et al.*, 1975.

6.8.1.2 Potato Leafroll Virus (PLRV). Virions of PLRV are isometric in shape and measure about 24 nm in diameter. The thermal inactivation point (about 70°C) and dilution end-points (about 10^{-4}) are the same in both aphid and sap extracts. Longevity *in vitro* is about 4 days at 2°C in plant sap and 12-24 hr at 25°C in aphid extracts (Murayama and Kojima, 1965; Peters, 1967b, 1970). The virus is not normally sap transmissible, has a narrow host range, and is transmitted by about ten aphid species (Kennedy *et al.*, 1962). *Myzus persicae* Sulz. is the most efficient vector, with nymphs being more efficient vectors than adults. It is transmissible by dodder but not through seed. Plant hosts are mainly Solanaceae, but some nonsolanaceous plants are susceptible (Natti *et al.*, 1953). Virus strains can be distinguished on the basis of symptomatology in *Physalis floridana* (Webb *et al.*, 1952; Peters, 1970; Shepherd, 1977). Information on aphid transmission of potato leafroll virus is available in papers by Elze (1927), Smith (1929, 1931), MacCarthy (1954), Day (1955), MacKinnon (1963), Miyamoto and Miyamoto (1966, 1971), Clark and Ross (1964), Peters and van Loon (1968), Ponsen (1970), Wright *et al.* (1970), and MacKinnon (1972).

Potato leafroll virus has been purified both from plants and viruliferous aphids (Peters, 1967a, 1967b; Kojima *et al.*, 1968, 1969). However, until recently, very little was known about the properties or composition of PLRV virions other than their morphology. Potato leafroll virus was formerly considered a possible member of the luteovirus group. However, Duffus and Gold (1969) were unable to detect any serological relatedness between PLRV and beet western yellows virus (BWYV) using infectivity neutralization and membrane feeding techniques. Also, Sarkar and associates (Sarkar and Blessing, 1973; Sarkar and Kaus, 1974; Sarkar, 1976) have provided convincing evidence that PLRV contains DNA. This latter characteristic definitely indicates that it does not belong with RNA-containing viruses, and it can be postulated that the ICTV will place PLRV in a group of its own (Shepherd, 1977). Few other viruses of higher plants, such as cauliflower mosaic virus (Shepherd *et al.*, 1968b), carnation etch ring virus (Fujisawa *et al.*, 1972), and dahlia mosaic virus (Fukisawa *et al.*, 1974), are known to contain DNA rather than RNA genomes. Sarkar (1976) isolated PLRV nucleic acid from purified virus preparations by treatment with 0.5N perchloric acid at 70°C and quantitative estimation of the products (DNA: protein ratio of 40:60, w/w), and by a more protected extraction with a mixture of phenol and sodium dodecyl sulfate. Nucleic acid extracted by the latter procedure is resistant to RNase, sensitive to DNase, and possesses a cooperative-type melting profile (Tm = 87.4 in 0.1 M NaCl, 0.015 M sodium citrate), a buoyant density of 1.689 in CsCl solution, and a molecular weight of 0.56 x 10^6. All of which indicates that the nucleic acid of PLRV is a double-stranded DNA (Sarkar, 1976).

Localization in infected plants. Electron microscopical studies of PLRV in infected plants suggest that virus is restricted to the phloem (Kojima *et al.*, 1968, 1969).

Localization in vector. Ponsen (1972) has studied the fate of PLRV in *M. persicae* using an infectivity bioassay procedure in which whole bodies as well as various organs, tissues, and fluids of viruliferous aphids were used to prepare inocula for injection into non-virus-exposed test aphids. Injected aphids were then placed on test plants to check for inoculativity. In this way, virus was recovered from larvae, intact guts, honeydew, cornicle secretions, and hemolymph. Negative results were obtained when inocula were prepared from guts without contents, salivary glands, integument (including connective tissue cells, pericardial cells, dorsal vessel, muscles, and tracheae), mycetocytes, or from ovaries plus embryos. Test aphids were not inoculative if they were injected with intact gut or honeydew inocula from viruliferous aphids which were previously allowed 1-2 day feeding periods on immune Chinese cabbage seedlings. However, hemolymph and cornicle-secretion inocula from such aphids maintained their infectivity (Ponsen, 1972).

Attempts to trace PLRV in the vector by electron microscopy have met with limited success. Electron-dense particles measuring ca. 23 nm in diameter were observed in a degenerating fat cell of single viruliferous larva. Whether or not the particles represented PLRV virions is not known (Ponsen, 1972). Aphids can be rendered viruliferous by abdominal injection with purified virus. Electron microscopic studies of injected aphids might facilitate its localization in the vector (Harris, 1974a, 1974b, 1974c; Harris *et al.*, 1975). Aphid cell culturing (Adam and Sander, 1976) and aphid-applied fluorescent antibody techniques (Matisova and Valenta, 1975) have also been perfected to the point where they might be useful in PLRV-vector interaction studies.

Multiplication in vector? Miyamoto and Miyamoto (1966) demonstrated transovarial passage of PLRV in *M. persicae*: a first for a polyhedral, aphid-borne virus. When viruliferous adult aphids were maintained on excised leaves of immune Chinese cabbage, *Brassica pekinensis*, small numbers of viruliferous larvae were detected in the 2nd and 5th generations. Because of the duration of the transovarial passage and the few generations involved, transovarial passage cannot be used to demonstrate PLRV multiplication in the vector.

Vago (1958) reported the presence of hypertrophied fat cells which coincided with deformation of the nuclei in viruliferous *M. persicae*; however, Ponsen (1972) observed the same phenomena in nonviruliferous aphids and considered them to be representative of a normal degeneration process in fat cells. Schmidt (1959) reported more stellate nuclei in the fat body of viruliferous adults than in nonviruliferous ones, but this observation may have resulted from differences in the ages of his viruliferous versus nonviruliferous aphids (Ponsen, 1972). Rutschky and Campbell (1964) studied aphids, *Macrosiphum granarium (=Macrosiphum [Sitobion] avenae* F.), of the same age and did not note significant differences in the total number of stellate nuclei in BYDV-inoculative versus noninoculative insects.

Ponsen (1969) compared the oxygen consumption, longevity, and fecundity of viruliferous and nonviruliferous *M. persicae* and concluded that PLRV does not affect the biology of vector. Ehrhardt (1960) concluded that PLRV does have an effect on the vector's rate of oxygen consumption. Nonviruliferous control aphids, *Myzus persicae*, fed on non-PLRV-infected *Physalis floridana* or Chinese cabbage (PLRV-immune) consumed 2.95 milliliters of oxygen per gram of aphids per hour.

In contrast, test aphids showed a slight reduction in oxygen consumption during an 8-hr acquisition-access feeding period on PLRV-infected *P. floridana*; and, thereafter, oxygen consumption decreased significantly with time until, after about 30 hr, it finally leveled off at a rate 30% below the starting value. Ehrhardt pointed out that the leveling off after 30 hr of gradual reduction coincided with the completion of the incubation (latent?) period in the vector.

The most convincing evidence for multiplication of PLRV in its vector comes from serial-injection experiments. Using hemolymph as inoculum, Stegwee and Ponsen (1958) reported carrying virus through as many as 15 successive aphid-to-aphid passages in which injected, recipient aphids were kept on immune Chinese cabbage. After the 15th passage the theoretical dilution of the virus was estimated as 10^{21}, which is many times over the 10^{-4} dilution end point of virus in hemolymph. However, these data conflict with others (Harrison, 1958) which indicate that the virus titer in viruliferous aphids rapidly decreases to a nondetectable (by infectivity bioassay) level when aphids are allowed to feed on immune plants. It therefore appears that additional information is needed before the question of multiplication can be resolved.

6.8.1.3 Pea Enation Mosaic Virus (PEMV). The International Committee on Taxonomy of Viruses (ICTV) has placed PEMV in a monotypic group of its own (Harrison *et al.*, 1971; Shepherd, 1977). Plant hosts include many legumes but few plants in other families. Local-lesion, chenopodiaceous, assay species include *Chenopodium album, C. amaranticolor*, and *C. quinoa* (Ruppel and Hagedorn, 1963b, Hagedorn *et al.*, 1964; Izadpanah and Shephard, 1966a). Pea enation mosaic virus causes one of the most serious diseases of garden pea, *Pisum sativum* L., and is widely distributed in northern temperature regions. Occasional outbreaks of economic importance have been reported in New York, Oregon, California, and Wisconsin. Six species of aphids, *Macrosiphum euphorbiae* Thos., *Myzus persicae* Sulz., *M. ornatus* (Laing), *Acrythosiphon solani* (Kalt.), *A. pisum* (Harris), and *Aulacorthum solani* (Kalt.) have all been shown to be experimental vectors of PEMV.

Infected pea plants initially show chlorotic or translucent spots on the leaves. Later, quite diagnostic blister or ridgelike pseudoenations and true laminalike enations appear primarily on the underside of leaves and stipules. Giant, laminate enations, primarily at the nodal regions of the stems in close proximity to the stipules, have been observed by Ruppel and Hagedorn (1963a). Infected plants are malformed and stunted and bear distorted, undersized, nonmarketable fruit. Anatomical studies by McWhorter (1949, 1950, 1965) revealed nuclear changes in cells of infected plants. Hyperplasia and hypertrophy of vascular bundles as well as necrosis of the mesophyll were frequently observed. The electron microscopy of PEMV in infected plants will be discussed shortly.

Unlike most circulative aphid-borne viruses, PEMV, like lettuce necrotic yellows virus, is sap transmissible and occurs in both superficial and deep tissues of infected plants. It is not known to be seed-borne. Purification from infected plants yields 5-100 mg or more of virus per liter of sap, depending on the virus isolate. The broader distribution of virus in the plant is also reflected by unusually brief acquisition and inoculation thresholds. Acquisition and inoculation thresholds as brief as 5 min and 7 sec, respectively, have been reported. Thus, it would appear that aphids can occasionally inoculate PEMV into epidermal cells during brief probes. Moreover, Toros *et al.* (1978) have recently demonstrated that, when compared with intervening mesophyll tissue, the phloem is apparently an inefficient, if not nonsusceptible, site for aphid inoculation of plants with PEMV.

All instars can transmit virus, but larvae are more efficient vectors than adults. Transmission efficiency varies with different vector species and biotypes. And the efficiency of a single vector species will vary depending on the food, virus-source, and test plants used and on the location on the virus-source or test plant from which or into which virus is acquired or inoculated, respectively. Estimates of the transmission threshold range from 6 hr to 13 days; the threshold being shorter for nymphs than adults. The latent period in the vector is temperature-dependent and also varies considerably with the virus isolates and aphid species, biotypes, or stages tested. Published latent periods range from 6-70 hr. A latency gradient (briefer to longer) has been demonstrated from 1st instars to adults.

Viruliferous aphids remain inoculative following ecdysis (transstadial passage). Retention time varies depending on the vector species, virus isolate, vector age at the time of acquisition, length of acquisition feed, ambient temperature, number of molts following acquisition, and so on (Osborn, 1935; Chaudhuri, 1950; Simons, 1954; Heinze, 1959; Nault *et al.*, 1964; Ehrhardt and Schmutterer, 1965; Sylvester and Richardson, 1966b; Sylvester, 1967). In general, aphids may retain inoculativity for from a few days to as long as 4 weeks, depending on the experimental design and conditions. Sylvester and Richardson (1966a) found that aphids declining in their rate of transmission could have their inoculative potential at least partially restored if given an additional acquisition-access feeding period on infected plants.

Aphids can be rendered viruliferous by feeding on infected plants or through membranes on suspensions of partially purified virus (Thottappilly *et al.*, 1972; French *et al.*, 1974), and by abdominal injection with infectious plant extract, hemolymph, honeydew, or partially purified virus (Nault *et al.*, 1964; Richardson and Sylvester, 1965; Schmutterer and Ehrhardt, 1974; Schmutterer, 1969; Harris, 1976a-c, 1975; Harris *et al.*, 1975). Richardson and Sylvester (1965) compared crude plant extract, hemolymph, and honeydew as sources of inocula and found that injection of nonviruliferous aphids with honeydew resulted in the highest rate of PEMV transmission – a not too surprising finding when one considers the large accumulations of virions observed in the gut lumina of inoculative insects (Harris, 1973, 1974a-c; Harris and Bath, 1972; Harris *et al.*, 1975).

PEMV: purification and intrinsic properties. Pea enation mosaic virus has a diffusion coefficient (D20, w) of ca. 1.89 x 10^{-7} cm2/sec (Bozarth and Chow, 1966), a buoyant density in CsC1 of 1.42 g/cm^3, an absorbance of 7.5 at 260 nm (1 mg/ml, 1 cm light path) (Shepherd, 1970), a thermal inactivation point (10 min) of ca. 65°C, a dilution end-point of ca. 10^{-4}, and a longevity *in vitro* at 20°C of 3-12 days depending on the isolate (Pierce, 1935; Osborn, 1935; Ruppel and Hagedorn, 1963b). For some isolates, yields as high as 0.1-0.3 mg of virus/g of leaf material are not unusual when infected young pea plants are harvested 10-12 days after ino-

culation, thus making it possible to study vector-virus interactions using partially purified virus.

Numerous purification schemes have been published: Bozarth and Chow, 1966; Gibbs *et al.*, 1966; Izadpanah and Shepherd, 1966b; Shepherd *et al.*, 1968a; Musil *et al.*, 1970; Gonsalves and Shepherd, 1972; Thottappilly *et al.*, 1972; Volvas and Rana, 1972; French *et al.*, 1973, 1974; Hull and Lane, 1973, and Mahmood and Peters, 1973. In most cases, PEMV has been purified from infected plants, usually garden pea; however, purifications from tobacco protoplasts (Motoyoshi and Hull, 1974) and aphids (French *et al.*, 1973) have also been reported. The feeding behavior of aphids that are fed through membranes on partially purified virus is significantly affected by the solvents and buffers used in the purification procedure (French *et al.*, 1974). Hull (1977a) recently reviewed the literature pertaining to the purification and intrinsic properties of various PEMV isolates and variants.

Pea enation mosaic virus is an RNA-containing virus with isometric particles. Estimates in the literature of particle diameter range from 20-36 nm. Size estimates seem to be largely dependent on the treatment of virions prior to measurement and the medium in which they are measured: i.e., in ultrathin sections of plants or aphids; in plant cell nucleplasm; scattered or in microcrystals in cytoplasm; or in prefixed or nonfixed, negatively stained or shadowed, purified or leaf-dip, preparations. The diameter of sectioned virions in microcrystals in plants is 24-27 nm (Shikata *et al.*, 1966). However, measurements of interparticle distances in crystalline arrays in cells are usually higher than expected, presumably because cellular components become trapped within the arrays (Hatta, 1976). The diameter may also vary depending on the embedding medium used to prepare specimens for ultrathin sectioning (Harris and Bath, 1972; Harris *et al.*, 1975). Harris *et al.* (1975) reported a diameter of 20.7 ± 1.3 nm for ultrathin-sectioned, Spurr's-medium-embedded virions both in aphid tissues and in partially purified preparations obtained by sucrose, density-gradient centrifugation. Isolated virions fixed with formalin prior to being stained with phosphotungstic acid and micrographed in the electron microscope measure 22-24 nm (Farro and Vanderveken, 1969; Farro and Rassel, 1971). Bozarth and Chow (1966) observed projections on PEMV virions, but these may have been artifacts produced by negative staining (Farro and Rassel, 1971).

Purified preparations of PEMV sediment as two nucleoprotein components, a faster sedimenting bottom component and a slower sedimenting middle one (Hull and Lane, 1973). The ratio of bottom to middle component varies with the virus strain or varient studied (Hull, 1977a). Estimates in the literature of the sedimentation coefficients (S20, w) range from 106 to 90 S for middle component and from 122 to 107 S for bottom component. Hull and Lane (1973) obtained S20, w values of 112 and 99 S, respectively, when bottom and middle components were separated before measuring. Purified PEMV preparations contain about 72% protein and 28% RNA (single-stranded) with base ratios of about 26% guanylic acid, 24% adenylic acid, 24% cytidylic acid, and 26% uridylic acid (Shepherd *et al.*, 1968a; Shepherd, 1970; Hull, 1977a). Middle and bottom component have

essentially the same percent nucleic acid content. An analysis of the available data on the infectivity of the two nucleoprotein components has led Hull (1977a) to conclude that the genome of PEMV is divided between two RNA species – RNA 1 of bottom component and RNA 2 of middle component. Unlike other divided genome viruses, the information for PEMV coat protein is in the larger piece of nucleic acid, RNA 1 of the faster sedimenting bottom component (Hull, 1977a). The coat protein of type PEMV electrophoreses as a single major band on SDS polyacrylamide gels. This single protein has a molecular weight of ca. 22,000 and consists of 199 amino acid units (Shepherd *et al.*, 1968a; Hill and Shepherd, 1972; Hull and Lane, 1973; Hull, 1977a). The relative molar ratios of the 18 amino acids represented have been determined (Shepherd *et al.*, 1968a). Possible models for the distribution of protein subunits in middle and bottom components have been reviewed by Hull (1977a).

Localization in plants by electron microscopy. Shikata *et al.* (1966) observed PEMV virions in dip preparations of crude plant sap from PEMV-infected plants and in ultrathin sections of diseased pea leaves and pods. Large accumulations of virus were seen in the cell cytoplasm of enations, and a few scattered particles occurred in cell vacuoles. Necrotic portions of leaf enations were densely packed with virions. In sections through nonnecrotic cells within enations, virions were characteristically arranged in the cytoplasm alongside the tonoplast, lining membranous structures in the cytoplasm, and sparsely scattered inside small vacuoles. Examination of sections through chlorotic portions of diseased pea leaves revealed virus microcrystals and scattered virions in the cytoplasm of cells.

In the early phases of their attempts to localize PEMV in infected plants, Shikata and associates concentrated almost exclusively on the cytoplasm of cells. However, in later experiments, nuclei were also carefully examined (Shikata and Maramorosch, 1966b). It was noted that a large number of the nuclei in diseased tissue stained unevenly and differently from nuclei in healthy controls. Cells with abnormal nuclei also contained smaller and fewer chloroplasts and mitochondria in their cytoplasm than did those with normal nuclei. High magnification micrographs revealed large masses of virions within the abnormal nuclei. Subsequently, by examining the nuclei of cells from tissues where chlorosis was only detectable with the aid of a X10 magnifying lens, it was possible to postulate the sequence of events in the infection of plant cells by PEMV. Virus first appears in plant cell nuclei. The nucleoli of cells in such early-stage, infected tissues are almost intact, but invasion and destruction of the more active parts of the abnormal nuclei have already begun. No virions are observable in the cytoplasm of cells in this infection stage. At a slightly later stage, the active part of the nucleus is partly destroyed and the nucleolus is almost completely taken over by virions. Eventually, the entire nuclear area, especially the portion previously occupied by the nucleolus, becomes packed with an almost solid mass of virions that are eventually leaked into the cytoplasm through the ruptured nuclear membrane. On the basis of these observations, it was concluded that PEMV first invades and multiplies in cell nuclei from which it is then released into the cytoplasm.

Localization in vector tissues and cells. Pea enation mosaic virus (PEMV) was the first of the aphid-borne, plant-pathogenic viruses to be seen in plants and insects by electron microscopy. Virions of PEMV were detected in the cytoplasm, central vacuoles, and nuclei of infected plant cells (Shikata and Maramorosch, 1966b) and in the fat body and gut lumina of viruliferous aphids (Shikata *et al.*, 1966).

Harris and associates later made more detailed electron microscopical studies of PEMV in aphids, *Acyrthosiphon pisum* (Harris), that were rendered viruliferous either by feeding on infected plants (Harris, 1971, 1973; Harris and Bath, 1972) or by direct abdominal injection with suspensions of partially purifed PEMV (Harris, 1974a-c, 1975; Harris *et al.*, 1975). In both instances, the researchers employed a highly efficient vector biotype-virus strain combination that would presumably maximize the chances of finding virus and determining its fate in various vector tissues. It was hoped that overloading the vector's system with large amounts of injected virus would help to elucidate the role of the electron-dense, sometimes viroplasmlike, viral inclusions observed in cells of aphids that acquired virus *per os*, and why researchers had thus far been unable to detect virions in the salivary systems of aphids which assumedly transmit the virus in a circulative manner.

Observations of plant-fed and injected aphids revealed that PEMV invades a number of vector organs, tissues, and cells (Harris and Bath, 1972; Harris *et al.*, 1975). Virions were observed in midgut and hindgut lumina, midgut epithelial cells, basal laminae and muscle cells in the tunica propria of the midgut, connective tissue cells, fat cells, basophilic mesodermal cells, and salivary glands. Organs and tissues in which PEMV was not observed include the hypodermis, mycetome, ovaries, tracheal system, foregut and hindgut epithelium and musculature, skeletal musculature, eye tissues, and nervous system. The following hypothetical, sequential account of the fate of PEMV in the vector is presented as a logical explanation for the thousands of static images of events observed in the same cell or in different cells of the same aphid, or in cells of more than a hundred different aphids examined by me over ca. an 8-yr period.

Foregut epithelial cells are devoid of detectable virus. An intact intima in this region of the gut apparently prevents ingested virus from contacting these cells; ingested materials do not occur in the space between their free cell borders and the intima. Enormous concentrations of virions occur in the stomach (Fig. 7) and hingut lumina of aphids allowed only 24-hr acquisition-access feeding periods. Whether this virus represents solely ingested virus that is concentrated in the stomach by the removal of water (Moericke and Mittler, 1965; Treherne, 1967) or, in part, progeny particles released from infected stomach epithelial cells is uncertain (Harris, 1971, 1973, 1974a-c; Harris and Bath, 1972; Harris *et al.*, 1975). The high concentrations of virus in the gut explains the excellence of honeydew as inoculum for injection of nonviruliferous aphids (Richardson and Sylvester, 1965).

The stomach and posterior intestine are the first areas of contact between ingested PEMV virions and an absorbing tissue in the vector. Very little is known

about how arboviruses or possibly their nucleic acids enter or leave the guts of their vectors or hosts (Maramorosch and Shope, 1975). In the case of PEMV, particles in the gut lumen can be observed in the vicinity of or in contact with the microvillous borders of intestinal epithelial cells. Virions also occur just inside the microvilli of cells which appear to be otherwise devoid of virus. There are no discernible differences between these particles and those outside the microvilli; nor are there evidences of plasma membrane-virion interactions that might be interpreted as suggesting viral entry by a process analogous to viropexis or phagocytosis. These electron microscopical data could be interpreted as suggesting that PEMV virions enter gut epithelial cells by direct penetration.

It has been suggested that the very dense ultrastructure of the basal laminae of the gut (Ossiannilsson, 1961) and salivary glands (Wohlfarth-Bottermann and Moericke, 1960) would prevent virus from entering or leaving the hemocoele in the form of virions. However, PEMV virions have been observed in the basal laminae of both the salivary glands (Harris, 1974a, 1974b, 1974c, 1975; Harris *et al.*, 1975) and intestine (Harris, 1977c) of its pea aphid vector. Lettuce necrotic yellows virus has also been localized in the basement membranes of both the hypodermis and midgut epithelium of its aphid vector (O'Loughlin and Chambers, 1967).

Nymphs allowed 24-hr acquisition-access feeding periods contain midgut cells with no visible virions, some with just a few particles, and still others with large

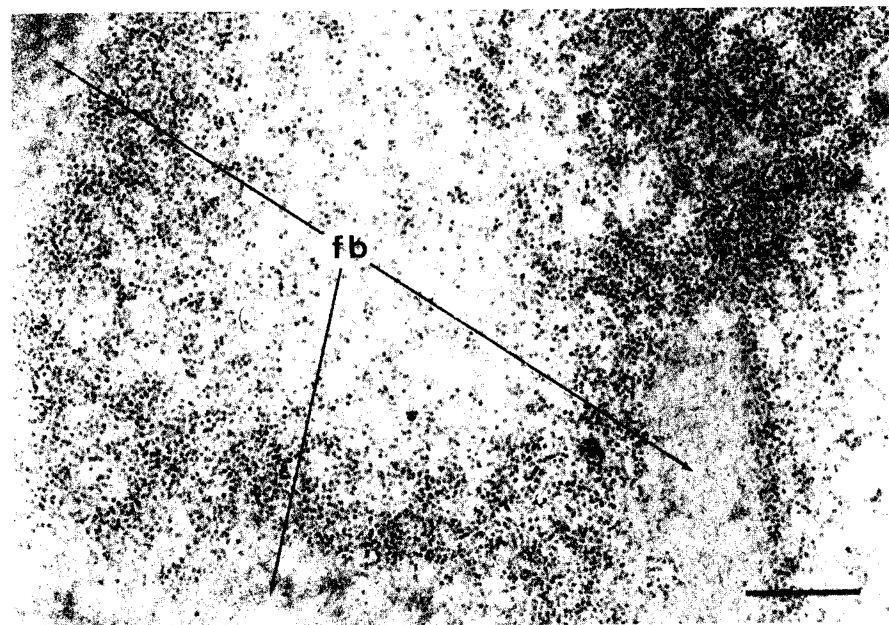


Fig. 7. Pea enation mosaic virus (PEMV) virions in the stomach lumen of a viruliferous pea aphid, *Acyrthosiphon pisum.* Most particles are aggregated along the peripheral margins of food boli (*fb*). The electron-dense area in the top right is composed almost entirely of virions. Bar, 500 nm.

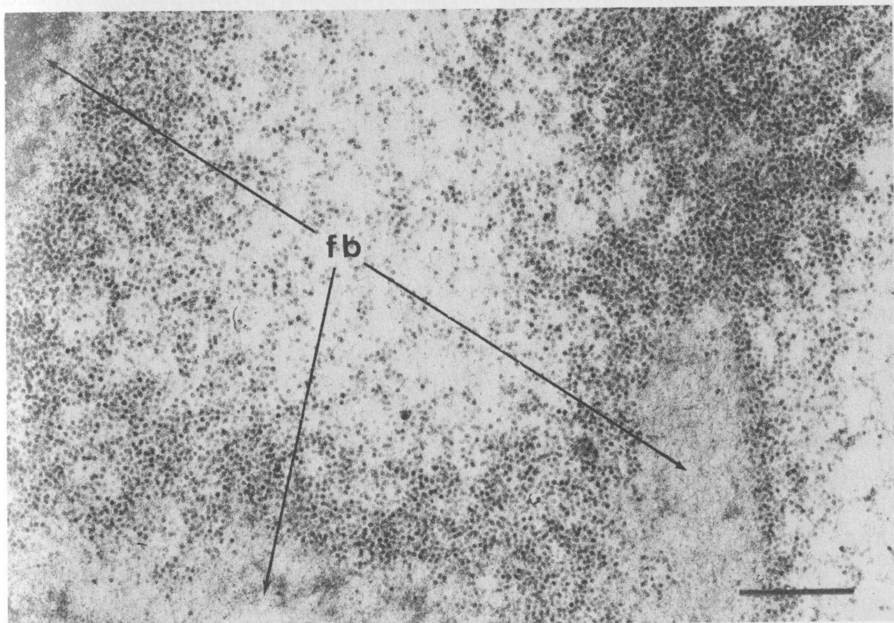


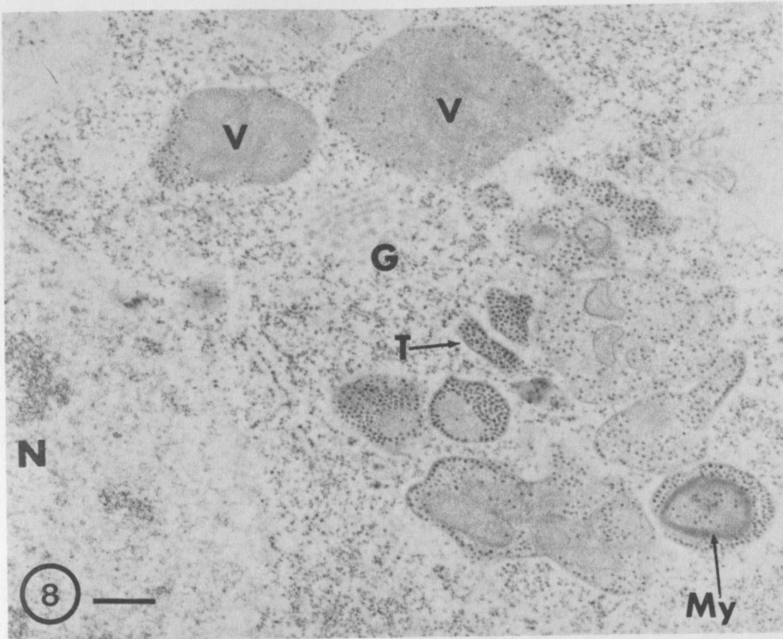
Fig. 7. Pea enation mosaic virus (PEMV) virions in the stomach lumen of a viruliferous pea aphid, *Acyrthosiphon pisum*. Most particles are aggregated along the peripheral margins of food boli (*fb*). The electron-dense area in the top right is composed almost entirely of virions. Bar, 500 nm.

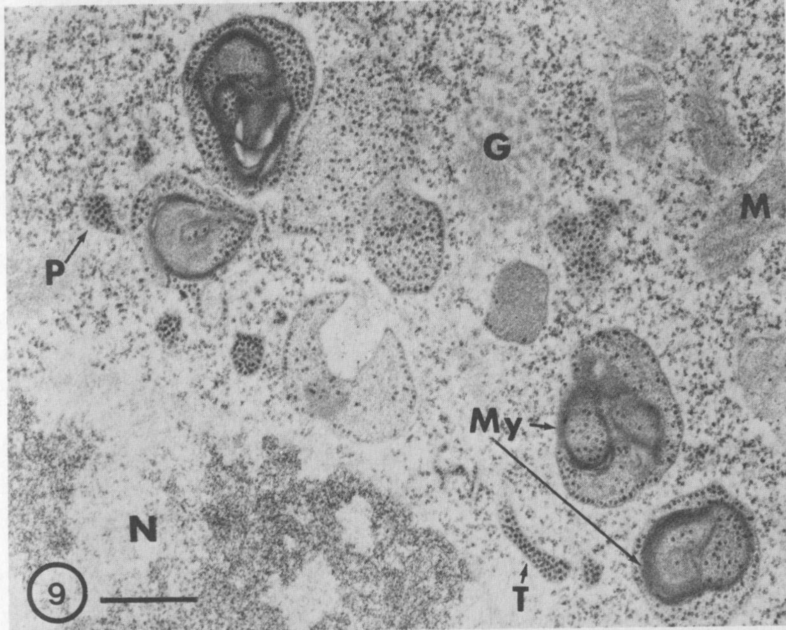
accumulations of PEMV. Similar concentrations of virus occur in the midgut, especially in stomach cells, of later nymphal stages and adults. The occurrence of detectable virus is accompanied by the appearance of defined, electron-dense, phagocytic structures and viroplasmlike areas in the cytoplasm. Virions appear first at the periphery of the viroplasmlike areas, and later throughout their electron-dense matrices. At a later stage, or at least in cells containing high concentrations of PEMV, virions occur throughout the cytoplasm in viroplasmlike areas and in the defined, electron-dense, structures (phagolysosomes?). The latter structures are partially or completely surrounded by a unit membrane and vary considerably in size, shape, and electron-density. Virus accumulations in them range from sparse and scattered to closely aggregated. These same membrane-bounded structures often enclose one or more myelinlike figures which themselves often contain virus (Figs. 8 and 9). Virions also occur in tubular structures in the cytoplasm. The tubes apparently originate as processes from the membrane-bounded viral inclusions (Fig. 9).

Very rarely, virions are seen in the nuclei of midgut cells which contain large amounts of virus in their cytoplasm (Harris and Bath, 1970, 1972). The nuclear membranes of such cells are completely degenerated in some areas and, where present, are indistinct, widely separated, and deformed. Because of the disintegrating nuclear membranes, there is no longer a distinct separation of cell cytoplasm from nucleoplasm. Whether the virus observed in the nuclei represents progeny particles produced in the nucleus, progeny particles produced in the cytoplasm that have moved into the nucleus, or assimilated, ingested virions is not known.

Virus which traverses the midgut epithelium and tunica propria is then circulated throughout the hemocoele, presumably by the hemolymph. In the hemocoele, virions invade the salivary system and certain cells of mesodermal origin, including connective tissue cells, fat cells, and "basophilic" mesodermal cells (Harris, 1974a-c, 1975; Harris *et al.*, 1975). It should be noted that Harris and associates (Harris and Bath, 1972; Harris *et al.*, 1974) and others have previously mistakenly referred to connective tissue cells as hemocytes (Harris, 1974a-c, 1975; Harris *et al.*, 1975). Virions appear to enter mesodermal cells by phagocytosis. In connective tissue cells, phagocytized secondary symbiotes and virions share similar fates (Harris, 1974a, 1974b; Harris *et al.*, 1975). Virions enter connective tissue cells (and assumedly fat cells and "basophilic" mesodermal cells as well) in endocytic vacuoles. These vacuoles, (hetero-)phagosomes, later combine with primary or secondary lysosomes that may themselves already contain virions to form phago- or secondary lysosomes (Figs. 10 and 11).

In addition to virions and/or secondary symbiotes, secondary lysosomes may contain autophagic materials as well as myelin figures. The presence or absence of myelin figures depends on the amount of undigested lipids the lysosomes contain in their matrices (DeDuve and Wattiaux, 1966). Lysosomes of both heterophagous and autophagous lines can combine to form large membrane-bounded telolysosomes, or they can individually progress to the dark staining residual body





Figs. 8 and 9. PEMV virions in viroplasmlike areas (Fig. 8, *V*) and in defined structures in the cytoplasm of affected, midgut, epithelial cells of viruliferous pea aphids. Defined viral inclusions, presumedly secondary lysosomes, are partially or completely surrounded by a membrane and many contain myelinlike figures (*My*). Processes (Fig. 9, *P*) from these defined viral inclusions sometimes appear to extend through the cytoplasm in the form of tubes (*T*). Note that the membrane structures of the endoplasmic reticulum, nucleus (*N*), Golgi apparatus (*G*), and mitochondria (*M*) are deteriorated and indistinct. Bars, 500 nm.

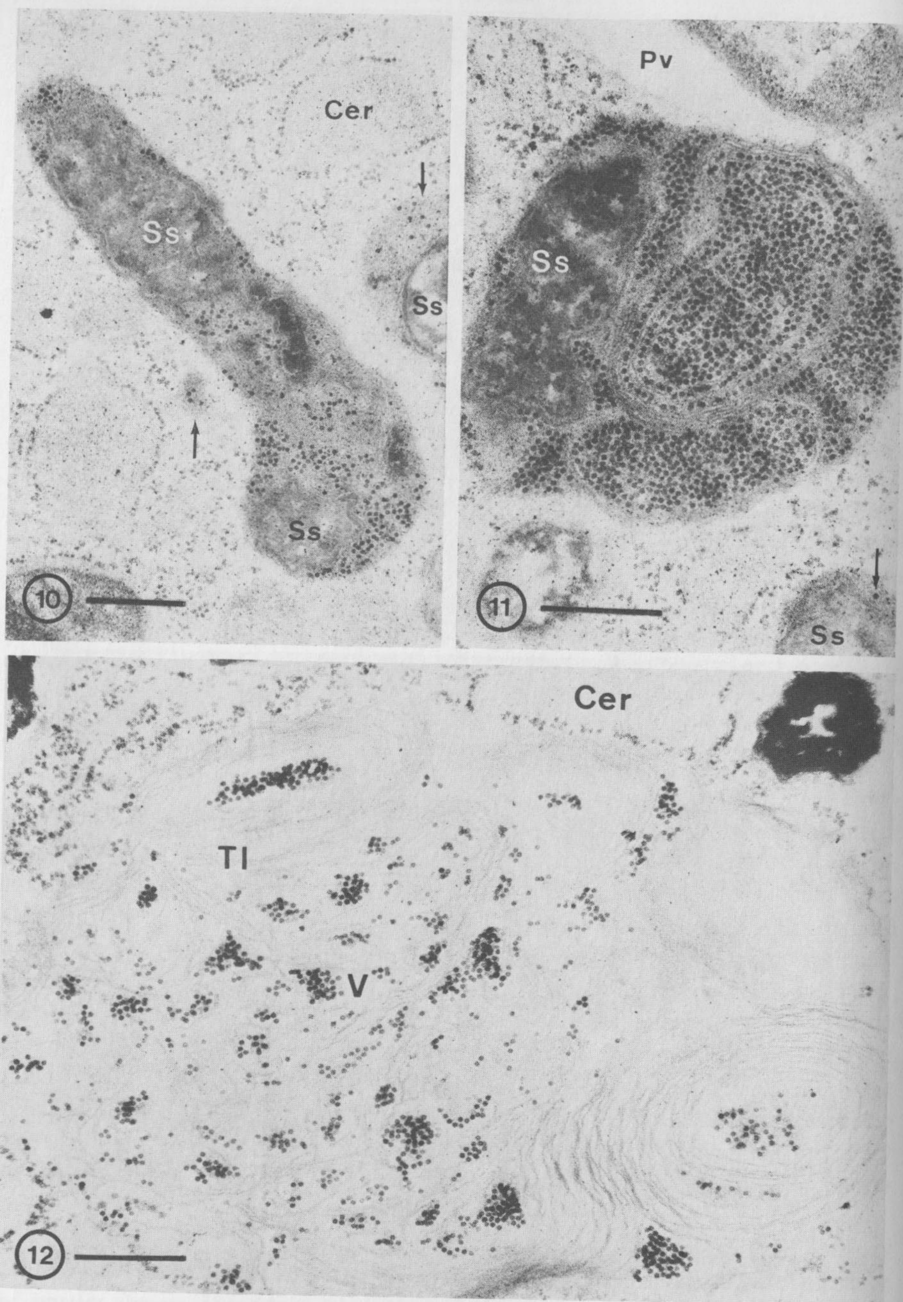


Fig. 10 and 11. PEMV virions and partially digested secondary symbiotes (*Ss*) in electron-dense, secondary lysosomes in the cytoplasm of a connective tissue cell of a viruliferous pea aphid. *Arrows* point to virions in electron-dense areas adjacent to the secondary lysosomes. *Cer* (Fig. 10), cisternal endoplasmic reticulum. *Pv* (Fig. 11), phagocytic vacuole. Bars, 500 nm.
Fig. 12. Large accumulations of PEMV virions (*V*) in a telolysosome (*Tl*) in the cytoplasm of a connective tissue cell of a viruliferous pea aphid. *Cer*, cisternal endoplasmic reticulum. Bar, 500 nm.

and finally the whorled, myelin-figure stage. A telolysosome containing a large accumulation of PEMV virions is shown in figure 12. Whether or not entry into the phagosome-lysosome system (vacuolar apparatus) of cells represents a "dead end" for PEMV virions is an open question. Following viropexis, several types of virus particles are known to suffer a certain degree of proteolytic uncoating – presumably in lysosomes – before entering the cytoplasm in the form of an infective nucleic acid (Dales and Choppin, 1962; Dales, 1963; Joklik, 1964a, 1964b; David-Ferreira and Manaker, 1965). In lysosomes, PEMV virions are very "darkly stained" or electron-dense; but they maintain a distinct profile, even in late-stage secondary lysosomes and telolysosomes.

For the many reasons discussed by Harris *et al.* (1975), the membrane-bounded viral inclusions observed in the cytoplasm of midgut epithelial cells, fat cells, connective tissue cells, and basophilic cells of both plant-fed and injected aphids can be tentatively identified as secondary lysosomes.

Pea enation mosaic virus is the first and only polyhedral, aphid-borne virus localized in the salivary glands of its vector by electron microscopy (Harris, 1974a-c, 1975; Harris *et al.*, 1975). The salivary system of the pea aphid is shown in figures 13 and 14. To date, virions have not been seen in any part of the primary glands other than the basal laminae that surround these bilobed organs. Virions are more concentrated in the basal laminae of the accessary glands (Harris, 1974a-c, 1975; Harris *et al.*, 1975), and they occur in the labyrinth of cisternae formed by extensive infolding and anastomosing of the plasma membrane of accessory gland cells (Figs. 15 and 16). The greater concentration of virions in the basal lamina of accessory glands and the inclusion of particles in their plasma-membrane cisternae suggest membrane flow as the process by which virions are transported from hemocoele to salivary duct lumina (Pease, 1956). It is interesting to note that watery saliva originates from the accessory glands; also, aphids have been observed ejecting watery, nongelling saliva during prolonged periods of ingestion (Harris and Bath, 1973).

Multiplication in vector? Transstadial passage; the presence of a latent period; relatively long persistence of vector inoculativity; retention of inoculativity, independent of the presence of detectable virus in the alimentary canal; and the fact that ambient temperature exerts an effect on both retention of inoculativity and the duration of the latent period of the same order of magnitude expected with living systems could all be interpreted as suggesting, but not proving, PEMV multiplication in the vector. Transmission data not favoring propagation include the fact that vector transmission efficiency gradually declines following acquisition; vector inoculative capacity is positively correlated with the dose of inoculum; vectors can be "recharged" by additional acquisition-access feeding periods; and, vector inoculativity cannot be maintained by serial passage of hemolymph from one insect to another. Failure to maintain inoculativity through serial passage is perhaps the strongest argument against multiplication. However, injection of hemolymph may be a poor method of virus acquisition compared to acquisition *per*

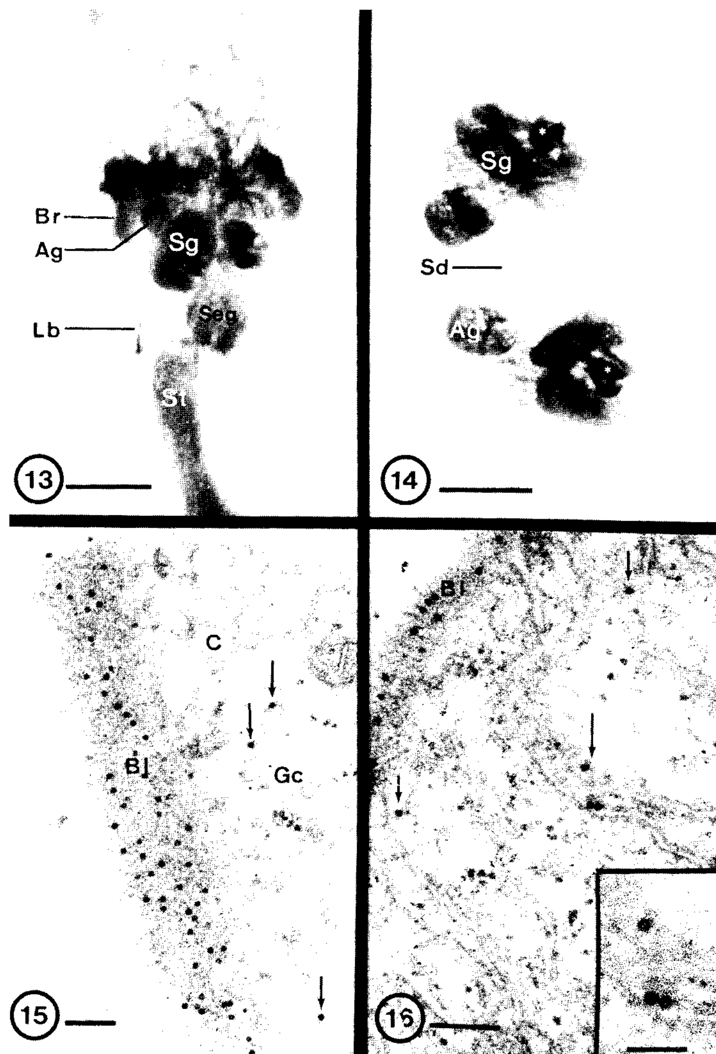


Fig. 13. Light micrograph showing the salivary system in relation to other organs of the pea aphid. *Ag*, accessory gland; *Br*, brain; *Lb*, labium; *Seg*, subesophageal ganglion; *Sg*, primary salivary gland, *St*, stomach. Bar, 20 μm.

Fig. 14. Light micrograph of an excised salivary system of a pea aphid. *Ag*, accessory gland; *Sd*, common salivary duct; *Sg*, primary salivary gland; *asterisks*, myoepithelioid cells. Bar, 10 μm.

Fig. 15. Accumulation of PEMV virions in the basal lamina (*B1*) of an accessary salivary gland from a viruliferous pea aphid. Note that three particles (*arrows*) have moved into the labyrinth of cisternae (*C*) formed by extensive infolding and anastomosing of the basal plasma membrane of the gland cell (*Gc*). Bar, 200 nm.

Fig. 16. PEMV virions in the basal lamina (*B1*) and plasma-membrane cisternae (*arrows*) of an accessory salivary gland from a viruliferous pea aphid. The three virions near the center of the micrograph are shown in higher magnification in the inset. Bars, 200 nm (Fig. 16) and 100 nm (inset).

os. Limited multiplication in a selected tissue(s), such as the gut epithelium, could explain the negative data mentioned above.

Many data from electron microscopical studies of viruliferous aphids could be interpreted as suggesting, but not proving, multiplication in the vector. For example, both Harris and Bath (1972) and Seryczynska and Wegorek (1972) have reported pathological changes in the ultrastructure of midgut epithelial cells in PEMV-inoculative pea aphids. The viroplasmlike areas observed in gut and connective tissues cells are also suggestive of PEMV propagation (Harris and Bath, 1972). Harris *et al.* (1975) suggested that the viroplasmlike areas, like the membrane-bounded viral inclusions in these cells, were probably lysosomes. However, this interpretation may have been premature. The morphologies of these two types of viral inclusions are different. The defined, electron-dense structures are membrane-bounded and frequently contain myelin figures typical of later-phase phagolysosomes: two features notably absent in the viroplasmlike areas (Figs. 8, 9, and 17). Additionally, gut cells can be found that contain only one or a few viroplasmlike areas (not present in controls) in which virions are either not present or present in low numbers. Since such cells appear healthy otherwise, they may represent cells in the earliest phase of virus replication.

Kao (1975) observed more viroplasmlike areas in the midgut cells of aphids fed on an aphid-transmissible isolate of PEMV than in those of aphids fed on a non-aphid-transmissible variant. Granados *et al.* (1967) found that an inefficient vector of wound tumor virus (WTV), *Agalliopsis novella*, could not support WTV multi-

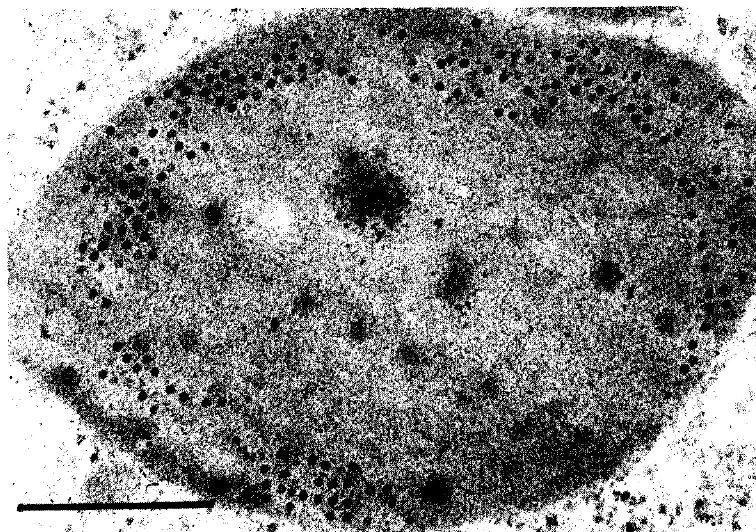
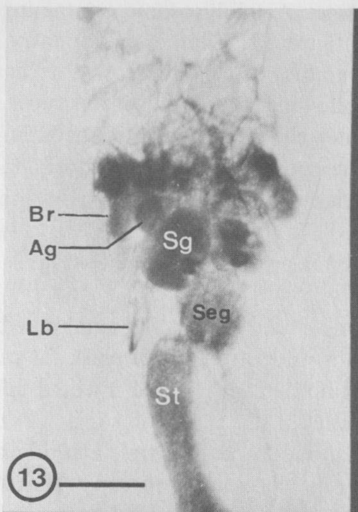


Fig. 17. PEMV virions in an electron-dense, viroplasmlike area in the cytoplasm of a connective tissue cell of a viruliferous pea aphid. Bar, 500 nm.


Br
Ag
Sg
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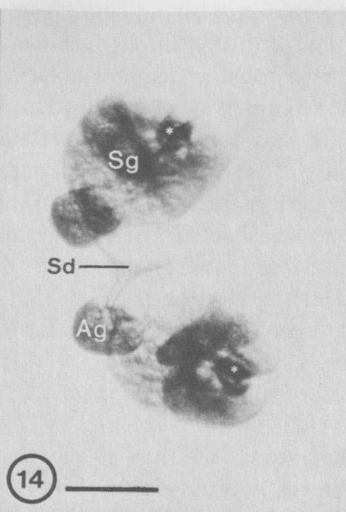
St
13


Sg
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Ag
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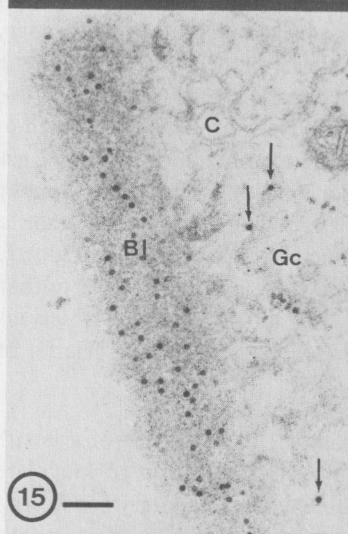
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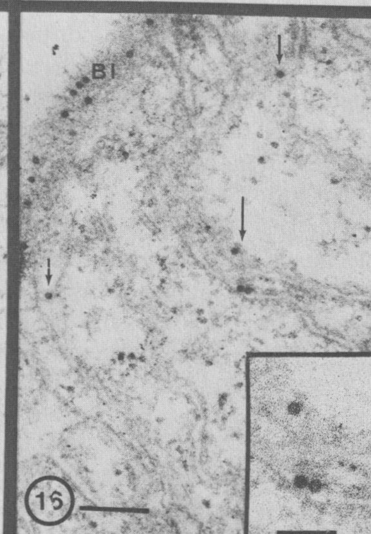
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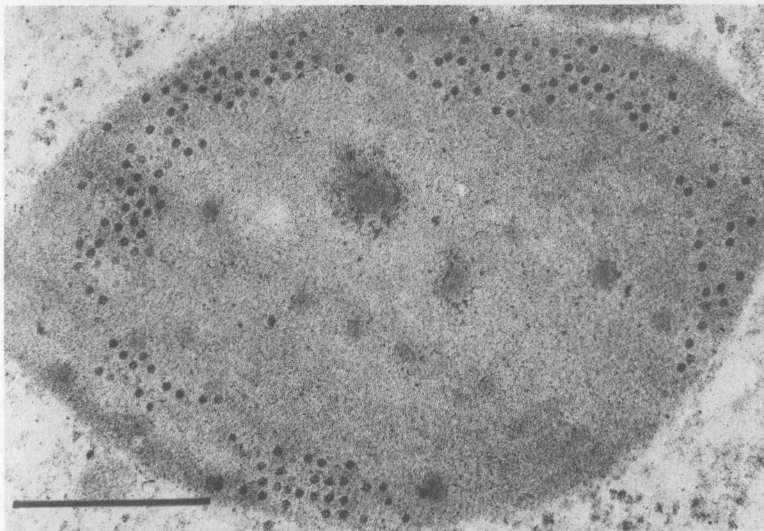


Fig. 17. PEMV virions in an electron-dense, viroplasmlike area in the cytoplasm of a connective tissue cell of a viruliferous pea aphid. Bar, 500 nm.

plication to the same extent as the more efficient vector, *Agallia contricta.* Cytoplasmic inclusions similar to those observed in PEMV-inoculative aphids also occur in WTV-infected leafhoppers. Maramorosch and associates (1969a) postulated that phagocytosis of WTV virions engulfed in defined electron-dense structures releases the virus genome which is then able to move to sites of virus assembly in the cytoplasm, i.e. viroplasms. The tracing of "hot" or "cold" labeled PEMV virions or RNA precursors in aphids using autoradiography and electron microscopy could help determine whether or not the same explanation is applicable to the PEMV-aphid system.

For several of the circulative-propagative plant viruses, the anterior portion of the midgut (aphid stomach or leafhopper filter chamber) appears to be a primary focus of virus accumulation and multiplication (Herold and Munz, 1965; O'Loughlin and Chambers, 1967; Shikata and Maramorosch, 1967a; Sylvester and Richardson, 1970). PEMV-containing gut cells are frequently bordered by cells in which no virions are detectable. If all the virus in the affected cells is derived from virions ingested with plant sap, it appears improbable that regular aggregates would occur in only a few cells of a tissue containing many cells of a similar type. The assembly of virus in gut cells and its subsequent release into the gut lumen might explain how large accumulations of PEMV can persist in the stomach lumina of feeding aphids. Presumably, ingested virions would be flushed through the gut during feeding on healthy plants; however, I (Harris, 1977c) have observed high concentrations of virions in the gut lumina of aphids fed for 5-6 days on healthy plants. Virions also occur in high titer in the guts of PEMV-injected aphids fed for 2-5 days on virus-free, artificial feeding medium (Harris, 1974a-c; Harris *et al.*, 1975). Finally, Kao (1975) estimated that aphids subjected to a 3-day holding period on healthy plants contained more virions in their gut lumina than aphids that were processed immediately after virus acquisition. This, too, suggests that virus may have multiplied in the aphids during the holding period.

Recent advances in aphid cell culturing and aphid-applied fluorescent antibody technique may help to resolve the question of multiplication. Adam and Sander (1976) mixed partially purified suspensions of PEMV with cell suspensions prepared from embryonic tissues of *M. persicae* before seeding. After an incubation period of 38 hr, antigenic material specific for PEMV was detected in inoculated cells by applying fluorescein isothiocyanate (FITC)-labeled antibodies against PEMV. However, it was uncertain whether the antigenic material was due to virus multiplication or to accumulation of PEMV from the original inoculum. Matisova and Valenta (1975, 1977) have succeeded in eliminating unspecific fluorescence in whole aphids by first treating the FITC-labeled antiserum with aphid powder. Fluorescence was then detected in smears of viruliferous but not nonviruliferous aphids. Further improvements in the technique may allow for the precise localization of fluorescence in the vector or in vector cells.

Specificity. The vector gut can be a determinant of transmission specificity and efficiency for some vector-virus combinations. And decreased gut permeability to virus with increased vector age has been demonstrated (Bald and Samuel, 1931; Storey, 1932, 1933, 1939a; Zazhurilo and Sitnikova, 1941; Slykhuis and Watson, 1958; Watson and Sinha, 1959; Sinha, 1960, 1963). However, recent studies of two isometric, aphid-borne viruses, barley yellow dwarf virus and PEMV, indicate that the vector-virus and virus-vector specificity phenomena associated with some circulative transmissions are mediated by salivary gland-virus coat protein interactions. It appears that reciprocity between recognition sites on virus coat protein and salivary gland membranes is required for passage of virus through the salivary system (Harris, 1974a-c, 1975, 1977, 1978a). Slight variations in either the coat protein of viruses, virus strains or variants, or in the salivary membrane systems of vector species or biotypes could affect virus aphid-transmissibility as well as vector specificity and transmission efficiency by altering the permeability of the salivary glands to virions. Intervector variation in the ability of the salivary glands to allow virus passage and/or to support virus multiplication has been proposed to explain transmission-efficiency phenomena associated with circulative-propagative transmissions (Shikata and Maramorosch, 1965; Granados *et al.*, 1967; Behncken, 1973). Data in support of the salivary gland-viral capsid interaction hypothesis have already been discussed with respect to BYDV (section 6.8.1.1, *Specificity*); those pertaining to PEMV specificity are covered in the following paragraphs.

Direct supportive evidence comes from comparing the electron microscopy of PEMV in the salivary glands of aphids exposed either *per os* or by injection to a highly aphid-transmissible (T) California isolate of PEMV (CT) with that of aphids exposed to PEMV in similar fashion, but to a non-aphid-transmissible (NT) variant. The NT variant is here referred to as CNT since it was obtained by exposing the CT isolate to repeated sap transmission (Harris *et al.*, 1975). CT-exposed aphids transmit PEMV to test plants prior to being processed for electron microscopy; in all cases, virions can be seen in the basal laminae of the salivary system and in cisternae in the cells of the accessory glands. CNT-exposed aphids, on the other hand, do not transmit PEMV to plants once non-aphid-transmissibility of the variant is fully established. Nor can virions be seen in any part of their salivary system, not even when test aphids are subjected to a series of injections with concentrated suspensions of partially purified CNT (Harris, 1974a-c, 1975, 1977c; Harris *et al.*, 1975; Adam, 1977). Since PEMV appears to traverse the salivary system in the form of complete particles or virions (Harris, 1974a, Harris *et al.*, 1975), it seemed logical to postulate that salivary gland-CNT coat protein incompatibility prevents CNT from entering the glands (Harris, 1974a-c, 1975, 1977a, 1978a, 1978c).

It appears that salivary gland-coat protein interactions and not virus-gut interactions are responsible for PEMV-vector specificity phenomena because: (1) aphid membrane-feeding on or abdominal injection with partially purified CNT does not result in transmission, (2) virions occur in the connective tissue cells, fat body cells, and basophilic mesodermal cells of aphids exposed to CNT either *per os* or by injection, and (3) puncturing the gut of the vector before allowing it to feed on CNT-infected plants does not result in transmission (Harris, 1977c). Salivary

gland-coat protein interactions can also be manipulated by changing the salivary gland portion of the specificity system. When a comparative study was made of the fate of CT in highly efficient and inefficient vector biotypes of the pea aphid, it invariably took far less time to locate virus in the accessory glands of the efficient versus the inefficient transmitter. When ultrathin sections were taken approximately midway through the accessory glands of the two biotypes, the numbers of virions counted in the basal lamina of the efficient biotype were always many times greater than in the inefficient one (Harris, 1977c). Additionally, preliminary experiments (Harris, 1977c) indicate that gut-puncturing does not affect the transmission efficiency of the inefficient aphid biotype.

To explain the aforementioned data, Harris (1975) hypothesized that the CT isolate of PEMV actually represented a mixture of T and NT strains. Multiplication of NT-PEMV is favored over T-PEMV in the plant (French *et al.*, 1973; Hull, 1977a); therefore, T-PEMV would be gradually excluded from the mixture by a regime of consecutive, mechanical, plant-to-plant transmission. In contrast, the Wisconsin (Izadpanah and Shepherd, 1966b) and P-3 isolates (Gonsalves and Shepherd, 1972) presumably could represent pure T-PEMV isolates, because, over the years, despite repeated and prolonged periods of maintenance by sap inoculation, they have retained their aphid transmissibility. A T-NT mixture in the California PEMV isolate (CT) would be perpetuated via aphid transmission by "dependent transmission from mixed infections" (Rochow *et al.*, 1975; Rochow, 1977a). In such a mechanism, aphids can transmit CNT virions whose RNA's have been masked (heterologous encapsidation) with CT, "transmissible" coat protein, thus enabling them to "sneak through" the vector in the guise (phenotype) of CT particles. This hypothesis is supported by several lines of evidence. For example, when aphids are fed through membranes on mixtures of partially purified (from singly-infected plants) T and NT isolates, they only transmit T isolate to plants (Adam, 1977; Tsai *et al.*, 1978). The results are the same when test aphids are injected with similarly derived T-NT mixtures (Adam, 1977; Harris, 1977c). Moreover, aphids allowed sequential acquisition-access feeding periods on plants singly-infected with T and NT isolates, only transmit the T isolate. However, aphids can transmit both isolates from doubly-infected plants in which there is an opportunity for genomic masking (heterologous encapsidation) to occur (Adam, 1977; Tsai *et al.*, 1977). These same data could also be interpreted as negative, circumstantial evidence that heterologous encapsidation and, consequently, multiplication of *both* T and NT virus does not occur in the vector.

Non-aphid-transmissible (NT) and T isolates of PEMV are serologically related, but T isolates possess an extra serological determinant not found in NT isolates (Clarke and Bath, 1976; Adam, 1977). Upon electrophoresis in sodium dodecyl sulphate (SDS) polyacrylamide gels, the coat protein of NT-PEMV isolates forms a major band with a molecular weight of 22,000 daltons and a minor band with a molecular weight of 44,000. The minor band is considered to represent a dimer of the protein in the major band (Hull, 1977b). The electrophoretic pattern of T-PEMV isolates contains the aforementioned bands plus two, extra, minor bands

with molecular weights of 28.000 and 58,000 respectively. These two minor proteins may represent monomers and dimers of the same protein (Hull, 1977b). An analysis of the available data led Hull (1977a, 1977b) to conclude that the extra protein(s) was a normal constituent of T-PEMV.

Adam and associates (Adam, 1977; Adam *et al.*, 1978) made similar comparative studies on the structural, physicochemical, and biological properties of T and NT strains of PEMV. A non-aphid-transmissible wild type strain (PEMV-Wt) (Hull and Lane, 1973) was found to contain a single coat protein with a molecular weight of 17,000 daltons, whereas an aphid transmissible Tubingen strain (PEMV-Tü) (Adam and Sander, 1976) possesses the 17,000-dalton protein plus a second, larger protein with a molecular weight of 56,000 which is possibly the same as the 58,000 protein (dimer?) described by Hull (1977a, 1977b). They (Adam, 1977; Adam *et al.*, 1978) too considered the extra protein to be a normal constituent of the T-PEMV capsid. If this interpretation is correct, then T-PEMV bottom component should contain a larger RNA 1 to code for the larger protein. And it does. The molecular weight of RNA 1 from PEMV-Tü is ca. 1.2×10^5 daltons greater than that from PEMV-Wt (Adam, 1977; Adam *et al.*, 1978). Assuming a mean molecular weight of 1,020 for a triplet and 200 for an amino acid, the larger RNA 1 of the aphid-transmissible, Tübingen strain should contain sufficient additional information to code for a protein having a molecular weight of ca. 24,000 (Adam *et al.*, 1978) which correlates well with the 28,000-dalton, extra protein which Hull (1977b) reported in his aphid-transmissible PEMV isolates.

Each nucleoprotein component of NT-PEMV isolates forms a discrete band upon polyacrylamide gel electrophoresis, the separation being on the basis of size (Hull and Lane, 1973). However, the bottom component of T-isolates are reported to separate into particles in about 9 different size classes (Adam, 1977; Hull, 1977b; Adam *et al.*, 1978); Adam (1977) separated middle and bottom components of T-PEMV isolates before electrophoresis and found that the middle component also shows multibanding, with 10-11 different particle classes (Fig. 18). Multibanding of bottom component of T-PEMV (assumedly of middle too) can be explained by successive, equal, incremental replacements of the smaller, NT-coat protein by the extra, larger, coat protein associated with aphid-transmissible isolates (Adam, 1977; Hull, 1977a, 1977b; Adam *et al.*, 1978). As pointed out by Adam *et al.* (1978), an analogous process has been described for a mutant of the bacteriophage Qb (Radloff and Kaesberg, 1973). Adam *et al.* (1978) estimated the mean incremental difference in the diameters of particles from any two neighboring bands (of the multibanding bottom component of PEMV-Tü) to be 0.54 nm; virions in the slowest and fastest moving particle classes were estimated to measure 34 and 29 nm in diameter, respectively.

An analysis of the available data suggests that the second coat protein associated with aphid-transmissible PEMV strains is somehow responsible for aphid-transmissibility and accounts for the observed differential permeability of the vector's salivary system to CT and CNT isolates (Harris, 1974c, 1975; 1977a, 1978; Harris

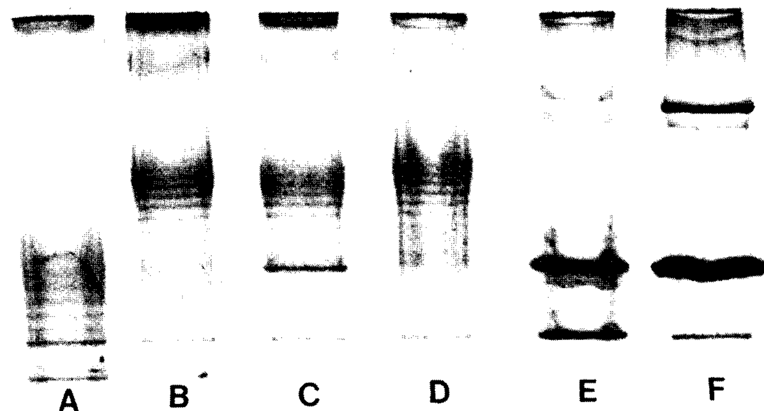


Fig. 18. Electrophoretic patterns of different strains and nucleoprotein components of pea enation mosaic virus. Purified virus and nucleoprotein components (middle and bottom components separated by three cycles of surcrose density-gradients) were loaded on a linear polyacrylamide gradient gel (2.5-10% acrylamide) and electrophoresed (240V const.; 24 hr; 4°C; pH 4.4; anode at top). After electrophoresis, the gels were stained with Coomassie brilliant blue. *A*, middle component of an aphid-transmissible, Tubingen strain (Adam and Sander, 1976; *B*, Tubingen strain, unfractionated; *C*, Tubingen strain, bottom component; *D*, unfractionated, aphid-transmissible, California isolate of PEMV (Harris *et al*, 1975); *E*, unfractionated, non-aphid-transmissible variant of PEMV derived from the aphid-transmissible California isolate (Harris *et al.*, 1975); *F*, unfractionated, non-aphid-transmissible, wild-type isolate (Hull and Lane, 1973; Hull, 1977a). (Courtesy of G. Adam.)

et al., 1975). Both Adam (1977, Adam *et al.*, 1978) and Hull (1977b) found a positive correlation between the loss of the multibanding property of T-PEMV isolates during successive mechanical transmission and the loss of aphid-transmissibility.

Adam *et al.* (1978) further demonstrated that non-aphid transmissible variants of PEMV-Tu that are produced by successive mechanical transmission lack the second coat protein (typical of T isolates) and have an RNA 1 with a molecular weight of 1.2 x 10^5 daltons less than that of the aphid-transmissible, parent isolate. It would be interesting to determine if the apparently "unalterable" (i.e. aphid-transmissibility is retained, even after repeated mechanical inoculation) Wisconsin and P-3 strains mentioned earlier contain the larger RNA 1 described by Adam and associates. Assumedly, one could isolate such unalterable variants from alterable isolates by subjecting the latter to a regime of plant-to-plant transmission using vectors only.

6.8.1.4 Beet Western Yellows Virus (BWYV). The ICTV has placed BWYV in the luteovirus group of circulative, aphid-borne viruses (Shepherd *et al.*, 1975/76; Shepherd, 1977). Serological relatedness to malva yellows, turnip yellows and beet

mild yellowing viruses has been demonstrated (Duffus, 1972; Duffus and Russel, 1972, 1975; Duffus, 1977b). A reciprocal relationship exists between BWYV and the RPV isolate of barley yellow dwarf virus (Rochow and Duffus, 1977; Duffus and Rochow, 1978). Beet western yellows virus is probably worldwide in distribution, and over 100 species in 21 dicotyledenous families are susceptible to various strains of the virus (Duffus, 1960). Infected plants typically exhibit stunting and interveinal yellowing of older or intermediate leaves, especially under high light intensity (Duffus, 1972).

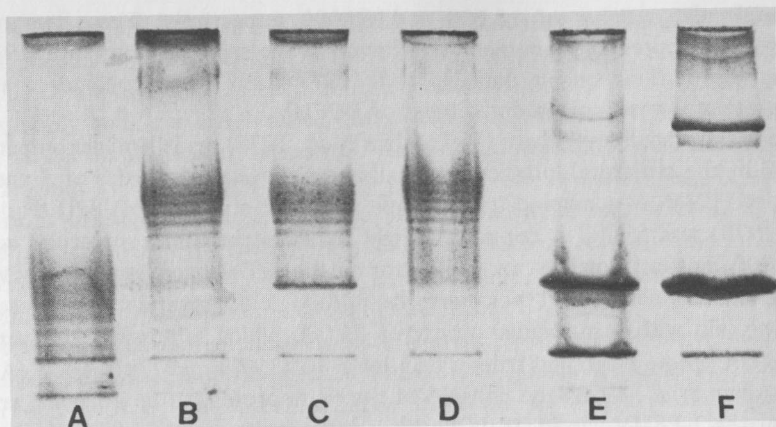
Commercially important host plants include sugar beet, red beet, spinach, lettuce, broccoli, cauliflower, radish, turnip, and flax. Susceptible and immune soybean cultivars have recently been reported (Duffus and Milbrath, 1977). Several diagnostic, propagation, and assay plant-host species have been reported (Duffus, 1972). Information on the epidemiology of BWYV can be found in a recent review by Duffus (1977a).

Eight species of aphids are known to transmit BWYV, the most important being *M. persicae*. Vectors may remain inoculative for over 50 days. Acquisition and inoculation thresholds are 5 min and 10 min, respectively, with a latent period in the vector of 12-24 hr. Transovarial passage and transmission through seed or by dodder are not known (Duffus, 1972).

Stability of virus in sap has been studied by feeding aphids through membranes on partially purified preparations. It has a thermal inactivation point (10 min) of ca. 65°C, a dilution end-point of 1/8, and a longevity *in vitro* of 16 days at 24°C (Duffus, 1972). Several purification schemes have been reported (Smith *et al.*, 1966; Gold and Duffus, 1967). Virions are isometric with a diameter of ca. 26 nm in ultrathin sections of infected plant tissue (Esau and Hoefert, 1972; Ruppel, 1968). Nothing is known about the intrinsic properties or composition of BWYV virions. Purification from plants yields low virus titers (phloem-restricted), but strong immunogenicity makes serological neutralization and membrane-feeding studies possible.

Fate in host plants. Infection is apparently confined to phloem tissues. Virions first appear in mature sieve elements, and virus then moves, assumedly via plasmadesmata, to adjacent phloem parenchyma cells. Particles are observed both in the nuclei and cytoplasm of infected cells, but virus multiplication is thought to occur in the nucleus (Esau and Hoefert, 1972).

Fate in vector. Ruppel (1968) observed high concentrations of particles, believed to be BWYV virions, in the gut lumen and cellular cytoplasm of viruliferous green peach aphids, *M. persicae*. The particles exhibited relative uniformity of shape; and their size, 25-30 nm, and shape coincided with that of purified virus. Comparable particles were not seen in the gut lumina or intestinal tissues of nonviruliferous control aphids. Once aphids acquire BWYV, they often remain inoculative for life without further access to a virus source. However, additional information is needed to determine if BWYV does indeed multiply in the vector.



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6.8.1.5 Viruslike Particles (VLP's). Peters (1965), using CsC1 density-gradient centrifugation technique, purified viruslike particles from extracts of both potato leafroll virus (PLRV)-free and PLRV-carrying green peach aphids, *Myzus persicae* (Sulz.). The VIP's were circular to hexagonal in profile and measured either 20 or 24 nm in diameter in negatively stained preparations; however, 29-nm VIP's were seen occasionally. The angular shadows cast by the VIP's in palladium-shadowed preparations indicated that they were polyhedral in shape. Similar VIP's were also purified from *Physalis floridana* Rydb. that were previously fed on by PLRV-free aphids, but not from extracts of non-aphid-exposed plants. On the basis of particle morphology and aphid transmissibility, Peters (1965) suggested that the VIP's represented latent plant viruses belonging to the circulative group.

6.8.2 Rhabdoviridae

Aphid-borne phytorhabdoviruses that have been localized in both their plant and insect hosts (circulative-propagative) are lettuce necrotic yellows (LNYV), sowthistle yellow vein (SYVV), strawberry crinkle (SCV), broccoli necrotic yellows (BNYV), and *Sonchus* yellow net (SYNV) viruses. These viruses are discussed in more detail in the following subsections of section 6.8.2. Other aphid-borne rhabdoviruses are parsley rhabdovirus (Tomlinson and Webb, 1974; Tomlinson, 1976, personal communication in Martelli and Russo, 1977), raspberry vein chlorosis virus (RVCV) (vectors = *Aphis idaei* v.d. G. and *Amphorophora rubi* Kalt.: Putz and Meignoz, 1972), lucerne enation virus (*Aphis craccivora* Koch), and carnation bacilliform virus (K. S. Milne, 1976, personal communication in Martelli and Russo, 1977). No vector has yet been recorded for *Gomphrena* virus (GV), but GV is suspected of being the same as or closely related to LNYV (Kitajima and Costa, 1966). Transovarial passage has been recorded for SYVV (Sylvester, 1969) and LNYV (Francki, 1973).

6.8.2.1 Lettuce Necrotic Yellows Virus (LNYV). Lettuce necrotic yellows virus virions are bacilliform in shape and measure ca. 227 x 66 nm. Serological relatedness to other rhabdoviruses has not been studied, but it resembles *Gomphrena* virus (GV), sowthistle yellow vein virus (SYVV), and broccoli necrotic yellows virus (BNYV) in particle structure, host range and mode of transmission (Kitajima and Costa, 1966; Hills and Campbell, 1968; Richardson and Sylvester, 1968; Duffus *et al.*, 1970; Peters and Kitajima, 1970). In *Nicotiana glutinosa* L., LNYV has a thermal inactivation point (10 min) of ca. 52°C, a dilution end-point of ca. 10^{-2}, and a longevity *in vitro* of 8-24 hr (Stubbs and Grogan, 1963). The virus has a narrow host range. In Australia, LNYV causes a serious disease of lettuce, and its distribution in South Australia appears to coincide with sowthistle, *Sonchus oleraceus* L., a symptomless weed host that also hosts the important aphid vector, *Hyperomyzus lactucae* L. Diagnostic, propagation, and assay hosts have been reported (Stubbs and Grogan, 1963; Francki and Randles, 1970). *H. carduellinus* has also been recorded as a vector of LNYV (Randles and Carver, 1970). Virus is sap-transmissible from infected lettuce or sowthistle to several

indicator plants, but to lettuce or sowthistle only with great difficulty. There is no evidence for seed transmission; transmission by dodder has not been tested. *N. glutinosa* and naturally infected lettuce are good sources for virus purification (Francki and Randles, 1970).

Fate in plants. In *N. glutinosa* and *S. oleraceus*, generally membrane-enclosed particles occur in the cytoplasm of mesophyll, epidermal hair cells, immature xylem, and sieve tubes (Chambers *et al.*, 1965; Chambers and Francki, 1966; Wolanski and Chambers, 1971). Pathological changes associated with symptom development include degeneration of nuclei, chloroplasts, and mitochondria, and loss of 70 S chloroplast ribosomes (Wolanski, 1969; Randles and Coleman, 1970). Wolanski and Chambers (1971) were able to divide multiplication of LNYV in *N. glutinosa* into two phases, a nuclear phase and a cytoplasmic phase. In the nuclear phase (5-7 days postinfection), perinuclear spaces resulted from a blistering of the outer nuclear membrane. First, spherical vesicles formed by budding from the inner nuclear membrane and, later, mature virions became localized within the perinuclear spaces. In the cytoplasmic phase of virus multiplication (day 7 postinfection onward) virions were found free in the cytoplasm and in association with viroplasmlike masses of granular and fibrilar material. Virus budding through the nuclear membrane was not observed.

Fate in vector. An electron microscopical study of LNYV in organs and tissues of LNYV-inoculative aphids, *H. lactucae*, led O'Loughlin and Chambers (1967) to conclude that LNYV systemically infects its aphid vector. Two types of particles were localized in viruliferous aphids that had fed on infected sowthistle, *S. oleraceus*. One particle type was identical to virions observed in infected plant cells and in purified LNYV preparations. The second type was similar but lacked an outer coat (unenveloped nucleocapsids). Neither of these particle types was observed in ultrathin sections of non-virus-exposed control aphids. In viruliferous aphids, unenveloped particles occurred in the cytoplasm of cells in muscle, fat body, brain, eye tissue, mycetome, tracheae, midgut, and salivary glands. Both enveloped ("coated") and unenveloped ("uncoated") nucleocapsids were observed in muscle cells and in midgut epithelial cells. Enveloped particles were also observed in the basement membranes of the hypodermis and midgut. In a single instance, enveloped particles were observed in the perinuclear space of an infected muscle cell.

6.8.2.2 Sowthistle Yellow Vein Virus (SYVV). Sowthistle yellow vein virus was first described in 1963 by Duffus as the causative agent of a vein-chlorosis disease of sowthistle, *Sonchus oleraceus* L., in California and Arizona. Virions are bacilliform in shape and measure ca. 230 x 100 nm in glutaraldehyde-fixed, negatively stained preparations (Peters, 1971), ca. 220 x 85 nm in ultrathin sections of infected plants or inoculative aphids (Richardson and Sylvester, 1968), and bullet-shaped and ca. 180 nm long in unfixed, negatively stained preparations (Peters and Kitajima, 1970). Upon electrophoresis in SDS-polyacrylamide gels, SYVV is found to contain four major and one minor structural polypeptides with

estimated molecular weights of 150,000, 83,000 (G), 60,000 (N), 44,000 (M1), and 36,000 (M2). Covalently bound carbohydrate has been detected in the 150,000 molecular weight species and the G protein (Ziemiecki and Peters, 1976b).

Schemes for purification of SYVV from infected sowthistle have been reported by Peters and Kitajima (1970) and Ziemiecki and Peters (1976a). Suspensions of purified virus retain infectivity for several days at 2°C, and the virus is strongly immunogenic (Peters and Black, 1970). Sylvester *et al.* (1968) were unable to establish serological relatedness to vesicular stomatitis virus (VSV). Recent tests reveal no cross neutralization between SYVV antiserum and PYDV (Liu and Black, 1978). Sowthistle yellow vein virus resembles LNYV in particle structure, host range, mode of aphid transmission, and vector specificity; however, its particle diameter is greater than that of LNYV and, unlike LNYV, it is not mechanically transmissible.

Sowthistle and lettuce, Compositae, are the only known plant hosts; each can serve as a diagnostic and propagation species. The virus has been found in the United States (California and Arizona), England, France and the Netherlands (Duffus, 1963; Duffus and Russell, 1969; Schultz and Peters, 1976). The aphid vector, *H. lactucae*, is useful in virus assay studies since it can be rendered viruliferous by feeding on infected plants or through membranes on virus suspensions, and by abdominal injection (Peters and Kitajima, 1970; Sylvester and Richardson, 1969). Virus suspensions can also be assayed in primary cell cultures of the vector by fluorescent-antibody staining technique (Peters and Black, 1970). Seed transmission is not known to occur; transmission by dodder has not been tested.

Fate in plants. Sowthistle yellow vein virus occurs primarily in the nuclei and rarely in the cytoplasm of parenchyma cells, developing xylem cells (resembles LNYV in this respect), and phloem cells in areas of infected sowthistle leaves showing chlorosis and vein-clearing but not in adjacent green areas (Lee and Peters, 1972). Virus appears to multiply in the nuclei of these cells, and particles infrequently seen in the cytoplasm probably represent virions released from the perinuclear space. In most infected cells, the perinuclear space is enlarged and often invaginated into the nucleus to accomodate large aggregates of virions. Some of the viral inclusions observed in the nucleoplasm are viroplasmlike in appearance and may represent centers for virus replication (Lee and Peters, 1972). Unenveloped nucleocapsids, such as those observed in the nuclei of vector cells (Sylvester and Richardson, 1970), have not been seen in plants. However, in plants, accumulations of virions in the perinuclear space and association of virions with the inner nuclear membrane are suggestive of nucleocapsid assembly within the nucleus and maturation (envelopment) on the inner lamella of the nuclear membrane. Seemingly bullet-shaped nucleocapsids are readily discernible inside enveloped particles in plants. A model for plant and animal rhabdovirus morphogenesis based on envelopment of a nucleocapsid bullet has been presented by Peters and Schultz (1975).

Fate in vector. Several lines of evidence unequivocally establish that SYVV multiplies in *H. lactucae* and also in an experimental, inefficient vector, *Macrosiphum euphorbiae* Thos. Duffus (1963) suggested multiplication in the vector and indicated that SYVV had many transmission characteristics in common with

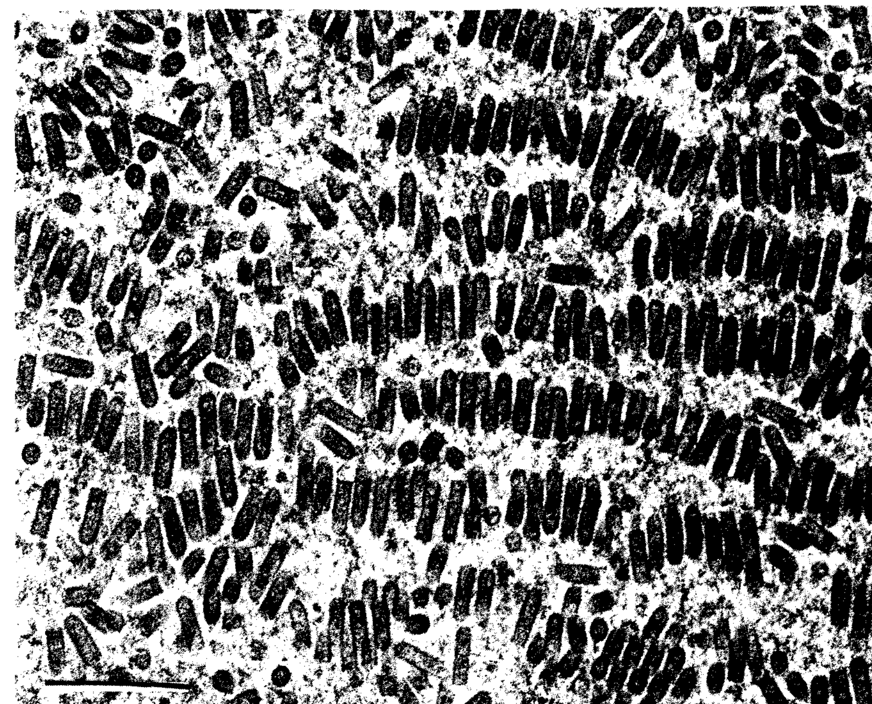

Fig. 19. Accumulation of uncoated sowthistle yellow vein virus (unenveloped nucleocapsids) in the nucleoplasm of a salivary gland cell of *Hyperomyzus lactucae*. In cross section, particles appear as thickened electron-opaque rings, with or without an electron-opaque core. Bar, 500 nm. (Courtesy E. Sylvester and J. Richardson.)

known circulative-propagative, leafhopper-borne viruses: most specifically, a relatively long incubation period in the vector and prolonged, efficient retention of vector inoculativity. In a preliminary study, Richardson and Sylvester (1968) noted that SYVV caused a nuclear infection in the salivary gland cells of *H. lactucae.* High concentrations of unenveloped particles or nucleocapsids were seen in the nucleoplasm of infected cells (Fig. 19). They (Sylvester and Richardson, 1969) also reported vector inoculativity following six successive passages of virus from aphid to aphid without access to an exogenous source of virus. By the fourth passage or third hemolymph transfer, the final dilution factor was already sufficiently high that, if virus multiplication had not occurred, the volume inoculated would have contained less than one virus particle. An increased mortality rate was obvious among *H. lactucae* vectors inoculated with serially passed SYVV-infective hemolymph. To date, this is the only such evidence for a deleterious effect of an aphid-borne virus on its vector (Sylvester and Richardson, 1969, 1971). More virulent, sometimes non-aphid-transmissible, SYVV variants, can be selected out by successive serial passage of SYVV isolate in the vector (Sylvester and Richardson, 1971).

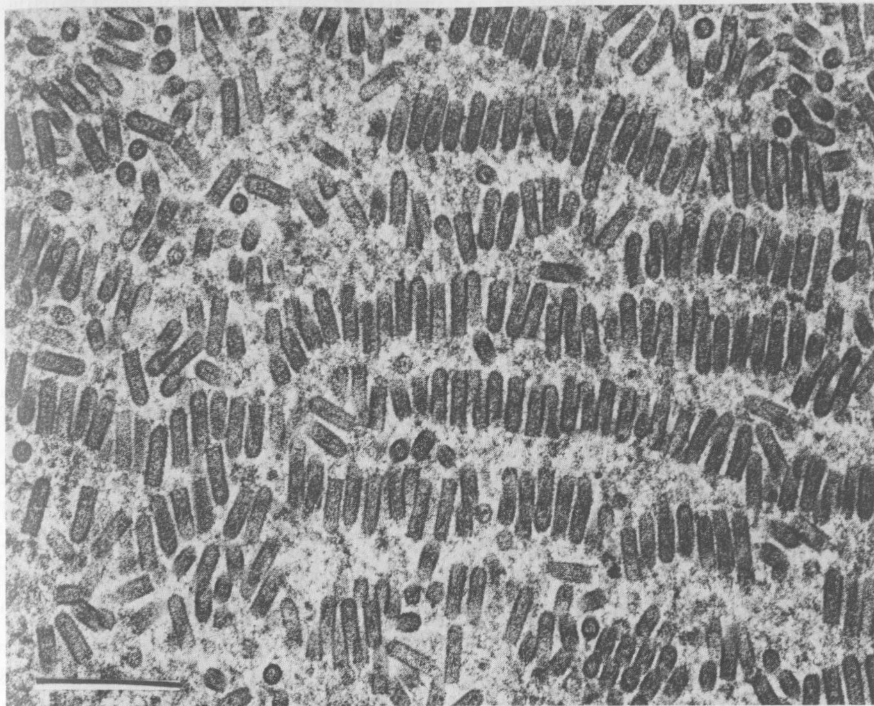

Fig. 19. Accumulation of uncoated sowthistle yellow vein virus (unenveloped nucleocapsids) in the nucleoplasm of a salivary gland cell of *Hyperomyzus lactucae*. In cross section, particles appear as thickened electron-opaque rings, with or without an electron-opaque core. Bar, 500 nm. (Courtesy E. Sylvester and J. Richardson.)

Similarly, recipient *M. euphorbiae*, an inefficient vector of SYVV, were inoculative following 16 interaphid passages of hemolymph (Behncken, 1973). Based on an estimated 100 fold dilution at each passage, this was equivalent to a 10^{-32} dilution of the original inoculum. Infectivity bioassay experiments indicated that hemolymph inoculum from viruliferous, plant-fed aphids lost infectivity after a 10^{-3} dilution. Behncken (1973) suggested that, in *M. euphorbiae*, transmission of SYVV is blocked by selective passage of virus particles from the hemolymph through the outer membrane of the salivary gland or by the inability of the salivary gland tissues to support virus multiplication or accumulation (section 6.8.1.3, *specificity*).

H. lactucae becomes systemically infected by SYVV (Sylvester and Richardson, 1970). Particles occur in the nucleoplasm as well as the cytoplasm of cells of the brain, subesophageal ganglion, main and accessory glands of the salivary system, esophagus, stomach, ovaries, fat body, mycetome, and muscle. The initial site of infection is the stomach region of the midgut. Infection of foregut cells is considered secondary. Sowthistle yellow vein virus nucleocapsids are apparently assembled in the nuclei of infected cells, and these are later enveloped by a process of budding through the inner nuclear membrane into perinuclear cisternae. The nuclear membrane disintegrates in later stages of infection, thus releasing masses of enveloped particles into the cytoplasm (Sylvester and Richardson, 1970). No particles are seen in the cells of the posterior intestine, hindgut, or embryos. However, a low level of transovarial passage (1% of all larvae produced) from apterous viruliferous ovoviviparae does occur (Sylvester, 1969).

Peters and Black (1970) inoculated primary cell cultures of ovarian and embryonic tissues of *H. lactucae* with SYVV. Samples of purified virus preparations were added to 2-day old cultures. The first SYVV-infected cells, as demonstrated by direct fluorescent-antibody staining, were found 37 hr after inoculation. Their numbers increased to a maximum of as many as 1700 infected cells per single coverslip culture after 48 hr.

6.8.2.3 Strawberry Crinkle Virus (SCV).

Fate in plants. Electron microscopic studies indicate that strawberry crinkle virus (Zeller and Vaughan, 1932; Zeller, 1933) has a particle morphology similar to other phytorhabdoviruses (Fenner, 1975/76) such as LNYV (O'Loughlin and Chambers, 1967) and SYVV (Lee and Peters, 1972; Sylvester and Richardson, 1970). Enveloped and unenveloped bacilliform particles have been observed (in the cytoplasm only) in epidermal cells and in parenchyma cells near vascular bundles in petal tissue taken from SCV-infected strawberry, *Fragaria vesca* L., showing typical petal streak symptoms (Richardson *et al.*, 1972). Aggregations of both enveloped and unenveloped particles sometimes occurred in the matrices of cytoplasmic vesicles. Particles measured ca. 190-380 nm long and 69 ± 5 nm (enveloped) or 44 ± 1 nm (unenveloped) wide. A larger particle, possibly an artifact, consisting of four subunits in a central core was also observed (Richardson *et al.*, 1972).

Fate in vector. Like LNYV and SYVV, SCV appears to be circulative-propagative in its vector. Multiplication in the vector was suspected on the basis of earlier

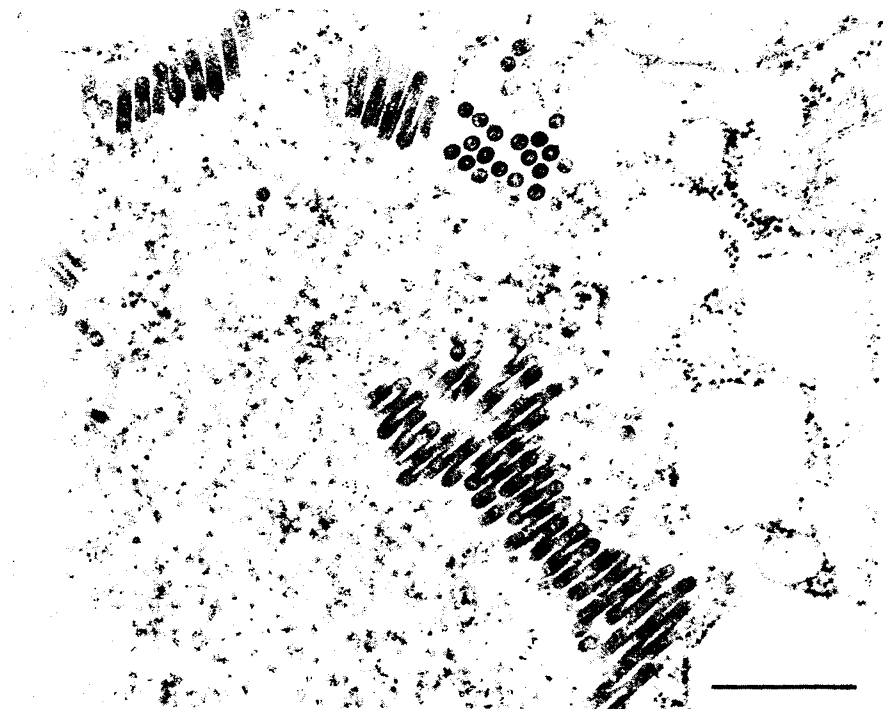

Fig. 20. Small aggregates of uncoated strawberry crinkle virus (unenveloped nucleocapsids) in the cytoplasm of a salivary gland cell of *Chaetosiphon jacobi.* Bar 500 nm. (Courtesy of J. Richardson and E. Sylvester.)

evidence that *Chaetosiphon jacobi* Hille Ris Lambers and *C. fragaefolii* (Cockerell) aphids that were fed on SCV-infected source plants were only inoculative following minimal latent periods of 10-14 days (Prentice, 1949; Prentice and Woollcombe, 1951; Engelbrecht, 1967; Frazier, 1968). This suspicion was later strengthened by electron microscopic observations of virus in tissues of *C. jacobi.* Unenveloped, bacilliform particles were localized in the primary and accessory salivary glands (Fig. 20), and in the subesophageal ganglia of SCV-inoculative aphids (Richardson, *et al.*, 1972). The particles, again occurring only in the cytoplasm, measured 190-380 nm in length and 42 ± 3 nm in diameter. Whether or not SCV systemically infects its vector in a manner similar to infection of *Hyperomyzus lactucae* by SYVV remains to be seen. In their preliminary study, Richardson *et al.* (1972) did not observe enveloped SCV particles in inoculative aphids; nor did they find evidence of infection of vector cell nuclei. Also, to date, there is not evidence of transovarial passage of SCV by *Chaetosiphon* spp.

The strongest evidence of SCV multiplication in aphids was provided by Sylvester *et al.* (1974) who maintained SCV through 6 consecutive passages by abdominal inoculation of *C. jacobi*. An estimated final dilution factor of more than 10^{-22}

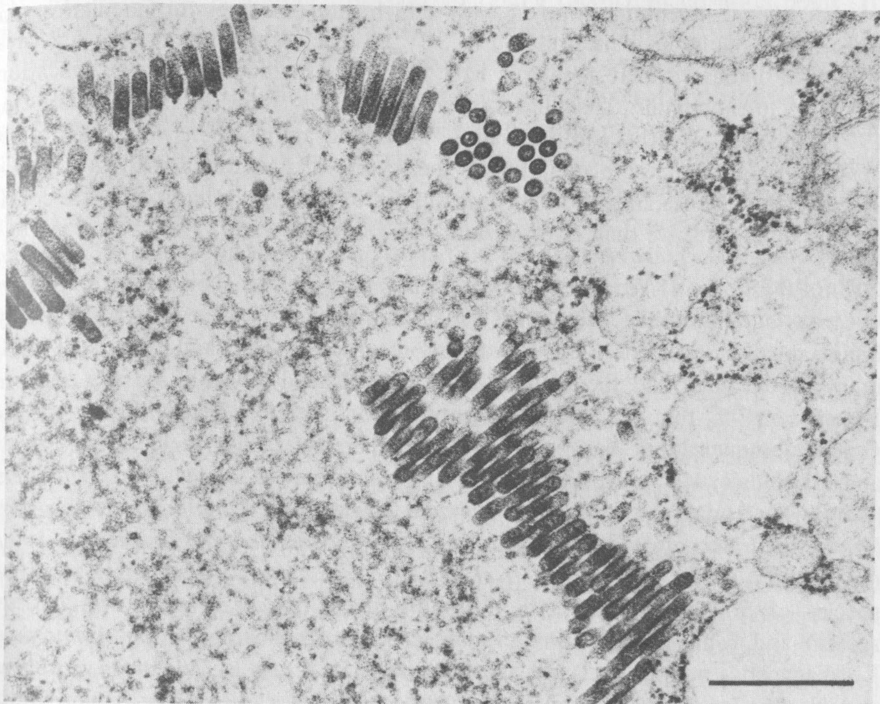

Fig. 20. Small aggregates of uncoated strawberry crinkle virus (unenveloped nucleocapsids) in the cytoplasm of a salivary gland cell of *Chaetosiphon jacobi.* Bar 500 nm. (Courtesy of J. Richardson and E. Sylvester.)

of the original inoculum, coupled with a high rate of successful inoculation (ca. 90%) and a stable incubation period of SCV in injected aphids (mean of 6.2 days at 25°C), indicated that the virus titer was being maintained by multiplication in the recipient aphids. The "head smear" technique (Sylvester and Richardson, 1971) was less effective in detecting SCV-infected aphids than were virus transmission trials using test plants. This is the reverse of that found with *H. lactucae* and SYVV where examination of head smears by electron microscopy was considerably more effective than transmission trials (Sylvester *et al.*, 1974).

6.8.2.4 Broccoli Necrotic Yellows Virus (BNYV). Broccoli necrotic yellows virus has a very limited host range and is not known to cause any economically important diseases in the field. It was first recorded by Hills and Campbell (1968) in England who found the virus in cauliflower-headed broccoli, *Brassica oleracea* L. var. *botrytis*, that was showing symptoms of infection by cauliflower mosaic virus. Subsequently, it has been reported in brassicas near Melbourne (Garrett and Martindale, 1973), Australia, and in brussel sprout, *B. oleracea* var. *gemmifera* (Tomlinson and Webb, 1974). *B. oleracea* (Dilleniidae: Cruciferae) is the only known naturally infected host plant, and the aphid *Brevicoryne brassicae* L. is the only known vector (Tomlinson *et al.*, 1972). Virus is sap-transmissible to a few species of Solanaceae and, with difficulty, to *B. oleracea* (cauliflower, brussel sprout, and cabbage). Susceptible solanaceous hosts include *Datura stramonium* L. (diagnostic, propagative, and assay species), *D. tatula* L., *Nicotiana glutinosa,* and *N. clevelandii* (diagnostic). Infected *B. oleracea* varieties either remain symptomless or develop initial mild veinclearing and slight leaf-rolling and then become symptomless (Campbell and Lin, 1972; Garrett and O'Loughlin, 1977). Seed transmission has not been detected, and transmission by dodder has not been tested (Lin and Campbell, 1972). Garrett and O'Loughlin (1977) found that their BNYV isolate was not sap-inoculable to the following: *Sonchus oleraceus* L. and *Lactuca sativa* L. cv Imperial Defiance, Henderson's 71 (Asteridae: Compositae); *Solanum tuberosum* L. cv Sebago (Solanaceae); *Zea mays* L. cv 10 Chief Hybrid (Liliatae: Gramineae), or *Plantago major* L. (Asteridae: Plantaginaceae).

Purification schemes using differential centrifugation, column chromatography on hydroxylapatite, and sucrose density-gradient centrifugation have been reported (Lin andCampbell, 1972; McLean and Francki, 1967). Purified preparations stained with uranyl acetate contain bacilliform particles measuring 275 x 75 nm. Bullet-shaped particles observed in potassium phosphotungstate-stained preparations are considered artifacts. The external and internal morphological features of particles in plants and aphids have also been studied (Lin and Campbell, 1972; Campbell and Lin, 1972; Garrett and O'Loughlin, 1977). Particles of BNYV have a buoyant density of 1.183-1.195 g/ml in potassium tartrate and a sedimentation coefficient ($S_{20, w}$) after column chromatography of 874 ± 41 S. Purified virus may contain some lipid in addition to its as yet uncharacterized RNA and protein moieties (Lin and Campbell, 1972). The virus is ether and butanol-sensitive and, in *D. stramonium* sap, has a dilution end-point between 10^{-3} and 10^{-4}, a longevity *in vitro* of 2 days or <24 hr at 4° and 23°, respectively, and a thermal inactivation point (10 min) of about 50°C (Lin and Campbell, 1972; Campbell and Lin, 1972).

The virus is poorly immunogenic, but BNYV-specific antiserum can be prepared (Lin and Campbell, 1972). Serological relatedness to other rhabdoviruses has not yet been established. Broccoli necrotic yellows virus closely resembles lettuce necrotic yellows virus (LNYV) in size, particle structure, and modes of transmission. However, it differs from the latter in host range, symptomotology, geographic distribution, and serological activity. Initial tests suggest that BNYV is serologically distinct from vesicular stomatitis, lettuce necrotic yellows, and barley yellow striate mosaic viruses (Lin, in Campbell and Lin, 1972). Additionally, unlike LNYV, there is no evidence for a BNYV nuclear phase in infected plant cells.

Fate in plants. No inclusion bodies appear to be associated with infection. Infected cells typically contain degenerate, swollen mitochondria with few cristae. In BNYV-infected *D. stramonium*, Hills and Campbell (1968) commonly observed virions in the cytoplasm of parenchyma cells. Particles in the cytoplasm were enclosed in membrane-bounded vesicles formed from the endoplasmic reticulum. All surfaces of the unit membrane defining these vesicles appear to serve as foci for BNYV accumulation and maturation (Hills and Campbell, 1968; Garrett and O'Loughlin, 1977). In infected cauliflower, virions are frequently observed in phloem parenchyma cells but only rarely in mesophyll cells. This apparent restriction of virus to the phloem parenchyma could explain the difficulty experienced in obtaining sap-transmission to and detecting virus in sap extracts of cauliflower (Garrett and O'Loughlin, 1977). Virions are often stacked side-by-side in single or double arrays within membrane-bounded vesicles in the cytoplasm of phloem parenchyma cells. Virions of BNYV have never been observed in the nuclei or perinuclear spaces, or at the plasma-membrane cell surfaces of infected plants.

Fate in vector. Like other aphid-borne, plant rhabdoviruses (e.g., LNYV, SYVV, and SCV), BNYV systemically infects its aphid vector, *B. brassicae*. Garrett and O'Loughlin (1977) localized BNYV particles in negatively stained hemolymph and in debris from squashed aphids that had been previously fed on BNYV-infected cauliflower. Electron microscopy of ultrathin sections of aphids that were exposed to BNYV in a similar fashion revealed that 8 of 10 aphids examined were systemically infected with BNYV. Virions were observed free in the hemolymph and in most organs and tissues of infected insects, with the notable exception of the gut epithelium and cells of developing embryos. Virions were occasionally seen in the stomach lumina of infected aphids and in muscle cells of the gut *tunica propria.* Some forms of virus localized in aphids were not observed in plants and vice versa (Garrett and O'Loughlin, 1977). The morphology of the particles in the gut lumen suggested that they were of aphid rather than plant origin. Although some virions were detected in the cytoplasm, they occurred more frequently and in much higher concentrations in the nucleoplasm of infected cells. Virions were observed in the nuclei of cells in the primary salivary glands and were especially numerous in nuclei

of primary mycetocytes. An analysis of the electron microscopical data suggests that the nucleus, not cytoplasmic vesicles, is the site of BNYV development and maturation in the aphid vector.

Virions in insect nuclei were usually enveloped in a single membrane. Passage of such particles through the nuclear membrane into the cytoplasm would presumably give rise to particles enclosed in a triple layer of membranes, and this type of particle was occasionally seen in the cytoplasm in the immediate vicinity of infected nuclei (Garrett and O'Loughlin, 1977). Accumulations of virions in perinuclear cisternae were not detected.

Based on the results of a series of transmission experiments, Garrett and O'Loughlin, 1977) hypothesized that the "aphid forms" of BNYV particles represent a host-specific variant of the virus found in plants. This hypothesis is deserving of further discussion in relation to the vector-transmissibility and specificity phenomena associated with insect-borne plant viruses. As discussed in section 6.8.1.3, there is evidence to suggest that some aphid-transmissible isolates of two isometric viruses, pea enation mosaic (PEMV) and barley yellow dwarf (BYDV) viruses, actually represent mixtures of aphid-transmissible and non-aphid-transmissible variants of their respective viruses. Slight differences in the genomes of variants in such a mixed "isolate" might make individual variants differentially suited for survival in either the plant host or insect vector. For example, the non-aphid-transmissible (NT) variant in a California isolate of PEMV can be separated from its aphid-transmissible counterpart by successive passage of the California isolate through plants using only sap inoculation. The NT variant is better suited for survival in the plant host. In nature, such mixed isolates of viruses could presumably be maintained via mixed infections (in the plant or insect vector) and the process of heterologous encapsidation (section 6.8.1.3).

Rochow (1977a) theorizes that we are only beginning to appreciate the prevalence and importance of dependent transmission in nature. Since heterologous encapsidation is especially common among the animal rhabdoviruses, insect-borne plant rhabdoviruses seem likely candidates for exhibiting the same process. For example, Sylvester and Richardson (1971) isolated several different strains of SYVV by serially passing a SYVV isolate through its aphid vector *H. lactucae*. Some of these strains may have represented variants which were present in and subsequently isolated from an initially mixed virus isolate. The apparently non-aphid-transmissible variant 15, which was highly infectious in aphids, could presumably be maintained in nature by the processes of mixed infections and heterologous encapsidation in plants and/or aphids.

6.8.2.5 Sonchus Yellow Net Virus (SYNV). This aphid-borne rhabdovirus was isolated in Florida from sowthistle, *Sonchus eleraceous*, and *Bidens pilosa* L. by Christie *et al.* (1974). Whereas SYNV shares some characteristics in common with SYVV and LNYV, its plant-host and vector ranges suggest that it is a previously undescribed member of the Rhabdoviridae. The only known vector of SYVV is *Aphis coreopsidis* (Thomas). The aphids *H. lactucae* – a vector of both SYNV and LNYV – and *Dactynotus* sp. are apparently unable to transmit SYNV.

Like SYVV, LNYV and GV, *Sonchus* yellow net virus infects lettuce and sowthistle, which are the only recorded host plants for SYVV. Unlike SYVV and like LNYV, SYNV is sap-transmissible, especially when the inoculum is stabilized by 0.5% sodium sulphite. Christie and associates (1974) reported that SYVV is mechanically transmissible to *Nicotiana* hybrid (*N. clevelandii* Gray X *N. glutinosa* L.), *S. oleraceous, B. pilosa, N. glutinosa* L., *N. clevelandii, Zinnia elegans* Jacq., and *Lactuca sativa* L., but not to *Datura stramonium* L. or *Gomphrena globosa* L. (hosts for LNYV and GV), Turkish tobacco (*N. tabacum* L.), *Chenopodium quinoa* Willd., and *C. amaranticolor* Coste and Reyn. This gives SYNV a recorded host range considerably wider than that of SYVV but narrower than that of either LNYV or GV (Stubbs and Grogan, 1963; Kitajima and Costa, 1966). As an assay species, *Nicotiana* hybrid proved to be several times more sensitive for SYNV than did sowthistle; however, sowthistle produced the most discrete local lesions of any plant tested. Symptomatology in both sowthistle and *Nicotiana* hybrid includes initial local lesion development followed by systemic expression.

Electron microscopy of leaf dips made from SYNV-infected leaves revealed "bullet-shaped" particles. In ultrathin sections of SYNV-infected sowthistle, the nucleoplasm of assumedly infected cells was found to contain inclusions composed of globular aggregates and crystalline arrays of bacilliform particles. Such inclusions are presumably representative of the one large to many smaller inclusions that can be seen by light microscopy in the nuclei of cells associated with areas of vein-clearing and local lesion development in epidermal strips from SYNV-infected leaves.

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