# THE FATE OF PEA ENATION MOSAIC VIRUS IN ITS PEA APHID VECTOR, ACYRTHOSIPHON PISUM (HARRIS)

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY
KERRY FRANCIS HARRIS

1971

This is to certify that the

thesis entitled

THE FATE OF PEA ENATION MOSAIC VIRUS IN ITS PEA APHID VECTOR, ACYRTHOSIPHON PISUM (HARRIS)

presented by

Kerry Francis Harris

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Entomology

James E. Bath
Major professor

Date 9 Febr. 1971

O-169

ABSTRACT

THE FATE OF PEA ENATION MOSAIC VIRUS IN ITS PEA APHID VECTOR, ACYRTHOSIPHON PISUM (HARRIS)

By

Kerry Francis Harris

Previous nonmicroscopical studies of the vector-virus relationships of pea enation mosaic virus and its pea aphid vector have given every indication that PEMV does not multiply in its vector. The virus has been localized only in the gut lumen and fatbody cells in previous transmission electron microscopical studies. The main purpose of this research was to study the fate of PEMV in its primary vector, the pea aphid Acyrthosiphon pisum (Harris), using the ultrathin sectioning technique of transmission electron microscopy.

As others had great difficulty locating PEMV in the pea aphid (found only in the gut lumen and fatbody cells), this research employed a vector-virus combination that would presumably increase the titre of virus within the insect and thereby maximize the chances of locating the virus and studying its fate in various tissues. The New York PEMV strain (known to be efficiently transmitted by pea aphids) and a pea aphid strain from East Lansing, Michigan which was a proven very efficient PEMV vector were used as test virus and vector. Aphids were exposed to the virus for acquisition as first instars since nymphs are known to be much more efficient vectors than adults. Also, only 12

day old mechanically inoculated garden pea plants, Pisum sativum L. var. Midfreezer, showing the severest symptoms were chosen as virus source plants.

Newly-born aphid nymphs were allowed to feed on PEMV-infected pea plants for from 1 to 7 days. At 24-hour intervals during the 7 day period various developmental instars were removed from the source plants and processed for transmission electron microscopy. Whole aphids, as well as individually dissected and excised organs, were fixed in cold (ca. 12°C) 6% glutaraldehyde in 0.1M Sorensen's phosphate buffer solution with 0.2M sucrose, and postfixed in cold 1% osmic acid in 0.1M phosphate buffer for at least 4 hours or overnight in the refrigerator at 12°C. Specimens were then dehydrated in ethanol series and embedded in Spurr's low viscosity epoxy resin medium. Ultrathin sections of embedded specimens were prepared and examined in a Philips EM-300 transmission electron microscope. The alimentary canal, salivary system, fat body, blood, central nervous system (brain and subesophageal ganglionic mass), reproductive system, eye tissues, muscle, and mycetome were among the organs and tissues studied.

This research provided electron microscopical evidence for the multiplication of PEMV in its pea aphid vector. The major site of virus accumulation and assembly is within the cytoplasm of infected epithelial cells of the midgut. The infection was more pronounced in the stomach and the anterior portion of the intestine than in the posterior portion of the intestine. Infection of the midgut epithelium was rapid. First instar nymphs which had been allowed a 24-hour acquisition access period contained midgut epithelial cells in various

stages of infection from the earliest to the latest. Foregut and hindgut epithelial cells were not invaded by PEMV.

Viroplasm-like areas detected within the cytoplasm of midgut cells are suspected of being the actual loci for virus assembly. Also, the nuclei of midgut cells may contribute to an increase in virus during later stages of the infection process. PEMV virions were also seen in the cytoplasm of muscle fiber cells in the midgut sarcolemma, the fat body, the blood, and, in one instance, in the cytoplasm of a follicular cell. The presence of viroplasm-like areas in the cytoplasm of muscle fiber cells, plasmatocytes, and spherule cells, and the localization of aggregates of PEMV virions in electron-dense structures in the cytoplasm of granular hemocytes and cells of the fat body suggest that limited viral assembly may also occur in one or more of these cell types.

The small number of blood cells in which PEMV was detected indicates that this tissue is probably an inefficient transporter of virions to other organs and tissues. No virions were observed in blood cells after the 3rd instar stage. Virus multiplication in the blood, if it occurs, is apparently limited and not capable of increasing or perhaps even of maintaining virus titre in the blood tissue.

Brief descriptions of the internal morphology and ultrastructure of aphid organs and tissues are included; also, the transmission electron microscopical data are discussed in view of previous non-microscopical studies on the fate of PEMV in the pea aphid.

# THE FATE OF PEA ENATION MOSAIC VIRUS IN ITS PEA APHID VECTOR, ACYRTHOSIPHON PISUM (HARRIS)

By

Kerry Francis Harris

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Entomology

1971

TO KATHRYN

## ACKNOWLEDGMENTS

This research was supported in part by a United States Public Health Service Predoctoral Fellowship from the National Institutes of Health and by a Research Traineeship from the National Science Foundation.

I express my appreciation to the faculty of the Department of Entomology at Michigan State University, and especially to Dr. Gordon E. Guyer, Chairman of the Department, for making available to me every research facility required. I am also indebted to Dr. Guyer for granting financial assistance in this period.

I am particularly grateful to my thesis director, Dr. James E. Bath, for his valuable criticism and guidance throughout the course of this research. Special thanks are due the members of the graduate committee, Drs. James W. Butcher, Gary R. Hooper, Gordon C. Spink, and Frederick W. Stehr for many helpful exchanges of information and ideas.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

# INTRODUCTION

Insect-transmitted viruses are responsible for many of our most devastating plant diseases. The elimination of their insect vectors would greatly diminish the importance of these diseases and would threaten the very existence of many, particularly those which rely exclusively on insects for their dissemination and inoculation to host plants. The pesticidal control of insects which cause direct injury to plants is far less difficult than the control of vectors. The former is accomplished by reducing their number to a point where the resulting damage is tolerable. However, to control a vector, a pesticide must be applied before an infestation occurs, and should kill each new arrival before it feeds. Unfortunately, conventional pesticides have usually not been effective in controlling the spread of viruses among our food crops. The fundamentals of vector-virus relationships must be learned before efficient and safe methods of crop disease control can be developed.

For many years it was thought that insects responsible for the transmission of plant and animal viral diseases served only as passive carriers, and were not themselves affected by the viral agents. Vector-virus relationship studies have been conducted by relatively few researchers. Yet these studies have presented evidence of several kinds which have greatly modified the concept of the insect vector as simply a "flying needle." Insect-borne plant viruses are proving to

be among the most complex and least understood of the plant viruses. The insect vectors may represent the original hosts of some, if not all plant viruses. Several viruses have been shown to multiply in their insect vectors as well as in their plant hosts. Some are known to be transmitted via their vector's eggs to progeny. Indeed, a few of these "inheritable" viruses could probably survive indefinitely in their vectors in the absence of their plant hosts. Most interesting of the results of vector-virus relationship studies in recent years has been the steadily increasing evidences of viruses which produce detectable and sometimes pathological effects on their insect vectors. Cytological and metabolic changes, premature death, and sterility or reduced fecundity are but a few of the effects which have been noted.

The main purpose of this study was to apply ultrathin sectioning technique and transmission electron microscopy (TEM) to a study of the relationship between pea enation mosaic virus (PEMV) and its pea aphid vector Acyrthosiphon pisum (Harris). Except for the fact that it is readily mechanically inoculable (Osborne 1935, 1938), PEMV is quite representative of the persistent type of transmission. In spite of much experimentation and theorizing by numerous researchers, there is still considerable confusion of thought concerning the interactions between plant-pathogenic viruses and aphid vectors, particularly in the group of so-called persistent (Watson and Roberts 1939), circulative (Kennedy, Day, and Eastop 1962) viruses.

While PEMV has not commanded as much attention from plant pathologists as other more devastating viruses, it has shown considerable promise as a tool in vector-virus-plant relationship studies. Such studies are important from both entomological and virological

points of view, as many of the virus diseases are dependent on arthropod vectors for dissemination. Information on vector-virus relationships at an organ, tissue, cellular, and subcellular level should give a more complete understanding of the factors which enable insects to function as vectors. Knowledge of what makes a vector could provide the basis for the development of techniques aimed at "unmaking" them--methods of changing efficient vector populations into inefficient or possibly even into non-vector populations.

# REVIEW OF THE LITERATURE

## MECHANISM OF PLANT VIRUS TRANSMISSION BY INSECTS

The suborder Homoptera includes more than 80% of the known species of insect vectors of plant virus diseases (Heinze 1959, Ossiannilsson 1966). Of these diseases, 40% are transmitted by the Auchenorrhyncha and 60% by the Sternorrhyncha. Leafhoppers (Cicadellidae), with 109 vector species, are the most important vectors of plant viruses in the Auchenorrhyncha (Neilson 1962), followed by the Cercopidae with 10 vector species, and the Flatidae with 1 (Carter 1962). Leafhoppers constitute the largest group of biological-transmitters of plant viruses. In the Sternorrhyncha, the aphids represent the largest group of arthropod transmitters of plant viruses with more than 180 vector species. Other sternorrhynchous vectors include 17 species in the Coccoidea, 9 in the Aleyrodoidea, and 1 in the Psylloidea (Carter 1962; Kennedy et al. 1962; Ossiannilsson 1966).

Watson and Roberts (1939) distinguished two modes of plant virus transmission by insects, and categorized them as either nonpersistent or persistent depending on the length of virus retention by the vector. Later, Sylvester (1956) introduced the term semipersistent to describe a third category of viruses, such as beet yellows, which were retained by their vectors for relatively brief periods, but yet much longer than the nonpersistent group. This system did not clearly define vector-virus relationships, and clear boundaries between the 3 categories

could not be established. Watson (1960) therefore proposed a more satisfactory system in which viruses were categorized as internal or external based on their fate in the vector rather than on length of retention. Internal viruses included those which were ingested, passed through the intestine to the hemolymph, then to the salivary glands where the virus could be reinjected, via the insect's saliva, into plant tissue during subsequent feedings. In addition, vectors of these internal viruses retained infectivity through molts. External viruses were carried on the stylets and did not pass through the vector; also, infectivity was lost through the molting process. Later Kennedy et al. (1962) adopted Black's (1959) term, circulative, and the term stylet-borne. Circulative viruses are those transmitted by the so called "latent vectors" of Day and Irzykiewicz (1953, 1954) while "direct vectors" transmit the stylet-borne viruses. All of the nonpersistent and most of the semipersistent viruses, such as cauliflower mosaic (Day and Venables 1961; Orlob and Bradley 1961), are now encompassed by the stylet-borne group which includes all viruses carried by the vector's stylets. Most of the persistent and some of the semipersistent viruses fit the circulative group which includes all viruses transmitted via the circulatory system of the vector. The term propagative further categorizes those circulative viruses which are known to multiply in their insect vectors. This new nomenclature implies a profound understanding of the mode of transmission. It is perhaps best to continue to describe some viruses according to the old terminology (Watson and Roberts 1939; Sylvester 1956), until a more complete understanding of their transmission is obtained. For many of the semipersistent viruses, such as beet yellows and many of the viruses infecting strawberry, it

has not been possible to determine whether transmission is stylet-borne or circulative (Frazier 1966a, 1966b; Frazier and Posnette 1958; Mellor and Fitzpatrick 1951; Prentice 1952; Prentice and Harris 1946). Ultraviolet irradiation and formalin treatment of aphid stylets failed to give conclusive results with beet yellows virus (Bradley and Sylvester 1962; Sylvester and Bradley 1962). When such treatments reduced transmission, aphid feeding on test plants was likewise decreased. Those aphids which fed normally showed little, if any, reduction in transmission. Work by Sylvester (1962) and Heinze (1959c) failed to demonstrate transstadial passage of beet yellows virus. Convincing evidence against transstadial passage of either strawberry mottle virus or strawberry vein clearing virus has been presented by Frazier (1966b).

Most leafhopper-borne viruses are propagative, a few circulative, and possibly one is stylet-borne (Ling 1966, 1969). The leafhopper-borne viruses, both the circulative and propagative groups, have been the subject of comprehensive discussions and extensive reviews by Bawden (1964), Black (1953b, 1953c, 1954, 1959, 1962), Carter (1962), Leach (1940), Ling (1969), Maramorosch (1954, 1955, 1959, 1960, 1963, 1964), Maramorosch, Shikata, and Granados (1969), Shikata and Maramorosch (1969), Smith and Brierley (1956), Smith (1958, 1965), and Storey (1939). Eighty-four aphid-borne viruses are stylet-borne, thirty are circulative, the status of about forty-five is uncertain (Bath 1964; Kennedy et al. 1962), and only three have been shown to propagate in their vectors--potato leafroll (Stegwee and Ponsen 1958), lettuce necrotic yellows (O'Loughlin and Chambers 1967), and sowthistle yellow vein (Duffus 1963; Hackett et al. 1968; Peters and Black 1970; Peters and Kitajima 1970; Richardson and Sylvester 1968; Sylvester

1969a; Sylvester and Richardson 1969, 1970). Several plant viruses such as rice dwarf, rice stripe, hoja blanca, clover club leaf, European wheat striate mosaic, wound tumor, and potato yellow-dwarf, have been reported to be transmitted transovarially in their insect vectors (Administración de Estabilización del Arroz 1959; Black 1948, 1953a; Everett and Lamey 1969; Fukushi 1933, 1939, 1969; Nasu 1963, 1965, 1969; Shinkai 1962; Slykhuis and Watson 1958). To date, potato leafroll (Miyamoto and Miyamoto 1966) and sowthistle yellow vein (Sylvester 1969a) are the only known cases of transovarial passage of aphid-borne, plant-pathogenic viruses.

Stylet-borne viruses are, by definition, those carried at the tips of the aphids' stylets (Kennedy et al. 1962). That so many perplexing findings could result from such a seemingly simple virus-vector relationship amply testifies to our lack of knowledge about the factors involved in the transmission of these viruses which have been reviewed by Bradley (1961, 1964), Carter (1961), Chalfont (1959), Kennedy (1960), Kennedy et al. (1962), Leclant (1968), Maramorosch (1963), Pirone (1969), Posnette (1960), Rochow (1963), Smith (1957, 1958, 1965), Sylvester (1958, 1961, 1962, 1969b), and Watson (1960).

## CHARACTERIZATION OF PEA ENATION MOSAIC VIRUS

Pea enation mosaic virus (PEMV) is one of the most serious diseases of garden pea Pisum sativum L. It has occasionally been of economic importance, affecting peas in New York (Schroeder and Barton 1958), Oregon (McWhorter and Cook 1958), California (Simons 1954), Wisconsin (Ruppel and Hagedorn 1963b) and probably occurs in trace amounts wherever peas are grown in the U.S.A. Six species of aphids,

the potato aphid Macrosiphum euphorbiae (Thomas), green peach aphid Myzus persicae (Sulz.), ornate aphid M. ornatus (Laing), foxglove aphid Acyrthosiphon solani (Kaltenbach), pea aphid A. pisum, and Aulacorthum solani (Kalt.) have been shown to be experimental vectors of PEMV (Bath 1964; Bath and Chapman 1966, 1967, 1968; Bath and Tsai 1969; Chaudhuri 1950; Hinz 1968; Nault 1967; Osborn 1935, 1938; Simons 1954; Tsai 1967, 1969). Host range studies of PEMV have been conducted by a number of workers, and experimentally susceptible plants include six varieties of alfalfa Medicago sativa L., crimson clover Trifolium incarnatum L., ladino clover T. repens L., wild white clover T. repens L., bur clover Medicago hispida Gaertn., alsike clover Trifolium hybridum L., white sweet clover Melilotus alba Desr., yellow sweet clover M. officinalis (L.) Lam., common vetch Vicia sativa L., hairy vetch V. villosa (Roth), broadbeam V. faba L., rough peavine Lathyrus hirsutus L., sweet pea L. odoratus L., perennial pea L. latifolius, bean Phaseolus valgaris L. var. Corbett Refugee, soybean Soya max L. var. Midwest, garden pea Pisum sativum L., field pea P. sativum var. arvense Poir, Chenopodium album L., C. quinoa L., Galactia sp., and numerous others (Ainsworth 1940; Chaudhuri 1950; Hagedorn and Walker 1954; Hagedorn, Layne, and Ruppel 1964; Izadpanah and Shepherd 1966a; Johnson and Jones 1937; McEwen and Schroeder 1956; Osborn 1935, 1938; Pierce 1935; Simons 1954; Stubbs 1937). Local lesion hosts have been reported by Bozarth and Chow (1965), Hagedorn et al. (1964), Izadpanah and Shepherd (1966a), and Ruppel and Hagedorn (1963b).

Pea plants infected with PEMV initially show chlorotic or translucent spots on the leaves; later quite diagnostic blister- or ridge-like

pseudoenations and true lamina-like enations appear primarily on the underside of leaves and stipules. Giant, stipitate, laminate enations, primarily at the nodal regions of the stems and in close proximity to the stipules have been observed by Ruppel and Hagedorn (1963a). Infected plants are malformed and stunted and bear fruit which is distorted, undersized, and undesirable as a market commodity. 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 areas were frequently observed.

Shikata and Maramorosch (1965a, 1966b) and Shikata, Maramorosch, and Granadas (1965, 1966) have reported on the electron microscopy of PEMV in cells of infected plants. Numerous virus particles about 28 nm in diameter, occurred in the nuclei, cytoplasm, and central vacuoles of necrotic and nonnecrotic pea leaf and pod enations. The virus first appeared in plant cell nuclei. Dip preparations of crude plant sap from PEMV-infected plants contained spherical particles ca. 30 nm in diameter, the morphology of which corresponded with that of virions in infective purified preparations (Bustrillos 1964).

Considerable progress has been made in the purification and characterization of some of the circulative and propagative plant viruses of leafhoppers and aphids (Brakke 1969; Suzuki 1969). Circulative aphid-borne viruses which have been obtained in sufficient amounts and states of purity for extensive morphological and physicochemical characterization include potato leafroll (Day and Zaitlin 1959; Peters 1965, 1967a, 1967b; Peters and Van Loon 1968), barley yellow dwarf (Rochow and Brakke 1964), lettuce necrotic yellows

(Crowley, Harrison, and Francki 1965; Harrison and Crowley 1965), sowthistle yellow vein (Peters and Kitajima 1970), and PEMV (Bozarth and Chow 1966, 1968; Gibbs, Harrison, and Woods 1966; Izadpanah and Shepherd 1966b; Shepherd, Wakeman, and Ghabrial 1968).

Pea enation mosaic virus is a small polyhedral virus. Its reported diameter ranges anywhere from 22 to 37 nm depending on the technique used to isolate the particles. Particle diameters which have been reported from purified preparations include 20 nm by Bustrillos (1964), about 30 nm by Gibbs et al. (1966), 22 to 24 nm by Bozarth and Chow (1966), and 36 ± 2.5 nm by Izadpanah and Shepherd (1966b). The estimate of Izadpanah and Shepherd was inaccurate due to metal shadowing and partial flattening of the air-dried, purified preparation. Shikata and Maramorosch (1965a) and Shikata et al. (1965, 1966) reported earlier measurements of 30 to 35 nm, and later measurements of ca. 28 nm and 24 to 27 nm from particle aggregates and crystals, respectively, in ultrathin sections of plants and aphids.

Like several other small polyhedral viruses which have been purified in recent years (Agrawal 1964; Bancroft 1962; Bancroft and Kaesberg 1960; Diener and Schneider 1966; Gibbs et al. 1966; Izadpanah and Shepherd 1966b; Markham and Smith 1949; Mazzone, Incardona, and Kaesberg 1962; Rice et al. 1955; Schneider and Diener 1966; Semanick and Bancroft 1964; Sinclair, Geil, and Kaesberg 1957; Stace-Smith, Reichmann, and Wright 1965; Tromans and Horne 1961; Yamazaki, Bancroft, and Kaesberg 1961; Yamazaki and Kaesberg 1961), PEMV has been found to occur in concert with an "extra-virus" component; however, no fraction containing "empty" particles has yet been found (Gibbs et al. 1966; Izadpanah and Shepherd 1966b). Thus far, all extra-virus components

have been found to have the same amount of protein and the same dimensions (Breedis, Berwick, and Anderson 1962; Gibbs et al. 1966; Markham and Smith 1949; Semancik and Bancroft 1964; Sinclair et al. 1957; Stace-Smith 1966; Stace-Smith et al. 1965; Tromans and Horne 1961; Yamazaki and Kaesberg 1961) and diffusion rate (Yamazaki and Kaesberg 1961) as the major virus fraction which is the only infectious entity. Both bands from purified preparations of PEMV were found to be infectious by Izadpanah and Shepherd (1966b) and Gibbs et al. (1966). However, Bozarth and Chow (1966) have presented convincing evidence that infectivity resides only with the lower component. With possibly one exception (Agrawal 1964), all extra-virus components have less ribonucleic acid (RNA) and sediment at a slower rate than the major component. The upper or extra-virus component of PEMV has been shown to contain 18% RNA (Bozarth and Chow 1966), or about two-thirds of the RNA of the infective particle (Shepherd and Ghabrial 1966; Shepherd et al. 1968).

Shepherd et al. (1968) characterized PEMV as having four sedimenting components; the two components that sedimented most rapidly were aggregates of the major virus component. The virus contains 2.48% phosphorus and 28-30% RNA. The nucleic acid consists of 24.1% adenylic acid, 26.1% quanylic acid, 24.0% cytidylic acid, and 25.8% uridylic acid. The single structural protein of the virus has a relatively high content of basic amino acids and is comprised of approximately 199 amino acid residues and has a molecular weight of 21,800.

Ruppel and Hagedorn (1963b) have studied the physical properties *in vitro* of five PEMV isolates under standardized conditions. All isolates remained infective longer *in vitro* and were less tolerant to

dilution than has generally been reported. Longevity ranged from 12 to 6 days depending on the isolate. Dilution tolerances for all isolates fell between 1:800 and 1:1,000. The thermal inactivation of four isolates occurred between 66-68°C and that of the fifth between 64-66°C.

PEMV is transmitted by the pea aphid Acyrthosiphon pisum in a circulative manner (Bath and Chapman 1968; Chapman and Bath 1968; Chaudhuri 1950; McEwen, Schroeder, and Davis 1957; Nault, Gyrisco, and Rochow 1964; Osborn 1935; Simons 1954; Sylvester 1965; Sylvester and Richardson 1966b), and is completely dependent on the aphid for transmission in the field. Similarities and differences between circulative, persistent viruses and other groups of arthropod-borne viruses have been reviewed by Maramorosch (1964) and Smith (1965). Vector-virus relationships in the case of PEMV are quite representative of those found with other aphid-borne circulative viruses; the only exception being that, like lettuce necrotic yellows virus, PEMV is quite readily mechanically inoculable (Osborne 1935, 1938). Transstadial passage of PEMV inoculativity has been demonstrated (Osborne 1935; Nault et al. 1964). Virus-free aphids injected with hemolymph from aphids reared on infected pea plants have been shown to transmit PEMV to healthy plants (Nault et al. 1964; Richardson and Sylvester 1965; Schmutterer 1969; Schmutterer and Ehrhardt 1964). Richardson and Sylvester (1965) have compared infected plant extracts, hemolymph from viruliferous aphids, and honeydew excreted by aphids feeding on virus-infected plants as sources of inoculum and found that injection of honeydew into nonviruliferous aphids produced the highest rate of PEMV transmission. The injection technique has also been used with

the circulative aphid-borne viruses barley yellow dwarf (Mueller and Rochow 1961; Rochow and Pang 1961), potato leafroll (Stegwee and Ponsen 1958), and sowthistle yellow vein (Sylvester and Richardson 1969). Finally, Shikata et al. (1966) have reported virions of PEMV in the gut lumen and cell cytoplasm of the fat body of viruliferous aphids. This was considered as direct evidence of infection of the aphid by a plant-pathogenic virus.

## CRITERIA USED TO CLASSIFY VIRUSES AS CIRCULATIVE OR PROPAGATIVE

Since the transovarial passage of rice dwarf virus from generation to generation of Nephotettix cincticeps (Uhler) (Deltocephalidae) was first reported by Fukuski (1933, 1934), pathologists have been greatly concerned with the question of whether viruses multiply in their insect vectors. In 1952, Maramorosch applied the serial passage technique to show multiplication of a plant virus in its vector. The same technique has been used to demonstrate the multiplication of wound tumor, potato leafroll, and sowthistle yellow vein viruses in their insect vectors (Black and Brakke 1952; Stegwee and Ponsen 1958; Sylvester and Richardson 1969). Transovarial passage technique (Black 1953a; Fukushi 1933, 1934, 1940, 1969; Miyamoto and Miyamoto 1966; Shinkai 1954, 1958, 1962; Sylvester 1969a; Yamada and Yamamoto 1954, 1955, 1956) and serial passage technique (Maramorosch 1952) have frequently been applied to prove virus propagation in many leafhoppers and a few aphids. Purified virus preparations have facilitated the use of immunological techniques for the detection of virus in host plants and insect vectors (Nagaraj, Sinha and Black 1961; Reddy and Black 1966; Sinha 1965, 1969; Sinha and Black 1963; Sinha and

Chiykowski 1967; Sinha, Reddy, and Black 1964), and have also simplified electron microscopy of ultrathin sections of vectors for the detection and localization of virus particle clusters in the tissues of viruliferous leafhoppers (Fukushi 1969; Fukushi and Shikata 1963; Fukushi, Shikata, and Kimura 1962; Fukushi et al. 1960; Nasu 1965, 1969; Shikata 1966; Shikata and Maramorosch 1965d; Shikata et al. 1964) and aphids (O'Loughlin and Chambers 1967; Richardson and Sylvester 1968; Shikata et al. 1965, 1966). The results of these electron microscope studies have provided unquestionable evidence for the multiplication of plant viruses in insect vectors--would tumor virus, rice dwarf virus, and lettuce necrotic yellows virus being excellent examples. The rate of virus increase in an insect has been measured by assaying the soluble antigen (Whitcomb and Black 1961). Cross protection between strains of virus in the vector (Kunkel 1955), effect of heat on incubation period (Kunkel 1937, 1941), influence of temperature on incubation period (Duffus 1963; Heinze 1959; Maramorosch 1950; Osborne 1935), effect of dosage (Day 1955; Duffus 1963; Maramorosch 1950), and the effect of volume on incubation (Maramorosch 1953) have also been cited as methods of predicting but not proving virus circulation or propagation in insects.

Bath (1964) and Tsai (1967) have presented extensive reviews of the criteria used to characterize viruses, especially the aphid-borne ones, as circulative or propagative. The criteria discussed included: vector specificity, speed of acquisition and rate of virus intake, speed of virus inoculation into plants, effect of vector starvation on transmission, length of latent or incubation periods in the vector, and length of virus retention or persistence. Since the findings of this

electron microscopy research cannot be viewed in isolation, but must be related to previous non-microscopic data, these traits will now be discussed, but only as they pertain to the vector-virus relationships of PEMV.

Vector Specificity Viruses of the circulative and propagative groups generally exhibit high vector specificity compared to those of the stylet-borne group, and are usually transmitted by only one or a few insect species. Eight species of aphids (previously listed) have been reported as vectors of PEMV.

Bath (1964) and Bath and Chapman (1966) found that aphid species, as well as pea aphid strains, vary greatly in their ability to transmit PEMV. Hinz (1963, 1966b) compared 6 bionomical races of the green peach aphid Myzus persicae as vectors of PEMV. He found that the strains could be divided into groups with high transmission efficiencies (90-95%), middle efficiencies (45-70%), and those only incidentally able to transmit. Nault (1967) found that the green peach aphid and the potato aphid Macrosiphum euphorbiae (Thomas) were better vectors of PEMV than the foxglove aphid Acyrthosiphon solani.

Bath (1964) and Bath and Chapman (1967) have reported that PEMV vector specificity can be due to the virus strain being transmitted and not to the aphids. One strain of the pea aphid A. pisum failed to transmit a California isolate of PEMV, but did transmit a New York isolate. Also, both isolates were found to be transmitted efficiently by a second pea aphid strain. Later, Bath and Tsai (1969) used the pea aphid to separate two strains of PEMV on the basis of transmission. The New York strain had a shorter latent period, remained longer in an

inoculable form, and was transmitted more efficiently by the aphid than the California strain.

Virus Acquisition Vectors of circulative and propagative viruses generally require longer acquisition periods than those of stylet-borne viruses. Early work by Chaudhuri (1950) with PEMV indicated an acquisition threshold of 1 to 2 hours for pea aphid adults. Simons (1954), working with adult pea aphids Acyrthosiphon pisum, also found the threshold to lie between 1 and 2 hours. The effect of acquisition feeding time on rate of PEMV pick up was almost linear. Sylvester (1965) and Sylvester and Richardson (1966b) noted that increasing the temperature at which PEMV was acquired increased the proportion of insects that acquired the virus. According to Ehrhardt and Schmutterer (1965), the minimum acquisition feeding period for adult A. pisum to become viruliferous was 2 hours, whereas nymphs acquired PEMV more efficiently than adults and required minimum feeding periods of 15 minutes. Heinze (1959b) demonstrated that Acyrthosiphon onobrychis could acquire PEMV in as little as 1 hour. Bath (1964) worked with a highly efficient A. pisum strain and reduced the acquisition threshold to 5 minutes or less. Using 20 to 60 second acquisition probes, Nault et al. (1964) found that none of 800 pea aphids transmitted PEMV during 24 hour test feedings.

Pea aphid nymphs acquire PEMV more efficiently than adults (Bath 1964; Bath and Chapman 1968; Ehrhardt and Schmutterer 1965; Tsai 1967). First instar nymphs and adults of A. pisum could acquire PEMV in 5 minutes or less and 10 minutes or less, respectively. These differences decreased with increases in the length of feeding periods and were almost nonexistent after a 24 hour feeding period (Bath and Chapman 1968).

Bath and Chapman (1966) compared the relative acquisition efficiencies of the pea aphid Acyrthosiphon pisum, the potato aphid Macrosiphum euphorbiae, and the green peach aphid Myzus persicae, through the use of 2 to 4 strains of each species. Pooled data from all test strains ranked the 3 species in the following order of descending acquisition efficiencies: M. persicae (0.5 hours or less), Macrosiphum euphorbiae (0.5 - 1 hour), Acyrthosiphon pisum (1 hour or less). Tsai (1967) compared the ability of adults and first instar nymphs of A. pisum to acquire New York and California isolates of PEMV. First instar pea aphids were 19.3, 36.9, 31.1, 37.0 and 14.1% more efficient in the acquisition of the New York than the California isolate at 1, 2, 4, 8 and 24 hour acquisition feedings, respectively. Acquisition trials with the adults produced even wider differences in the two isolates, but efficiency in both cases was lower than with the nymphs.

Using PEMV-infected broadbean Vicia faba as a source plant, Hinz (1966b) found that aphids which fed on the three youngest leaves acquired the virus best and gave transmission efficiencies of from 90 to 100%.

Plant Inoculation Relatively short feeding times (10 to 20 minutes) are usually required for aphid inoculation of circulative viruses into plants. Simons (1954) found the inoculation threshold period for adult pea aphids A. pisum to be between 15 and 20 minutes. The effect of test feeding on rate of transmission was logarithmic. Later, work by McEwen et al. (1957) reduced the threshold to 5 minutes. Inoculation trials with aphid strains which were highly efficient in PEMV acquisition revealed that the pea aphid A. pisum and the potato

aphid Macrosiphum euphorbiae were capable of inoculating pea in 1 minute or less; whereas the green peach aphid Myzus persicae required 5 minutes or less, but too few trials were conducted at 1 minute to rule it out. Increases in inoculation probing time from 15 to 60 minutes had little or no effect on Acyrthosiphon pisum and Myzus persicae, but the transmission efficiency of Macrosiphum euphorbiae increased exponentially (Bath 1964; Bath and Chapman 1966; Bath and Chapman 1968). Aphids have been shown to transmit PEMV to pea during test feedings of 20 - 60 seconds (Nault et al. 1964), 15 seconds or less (Nault 1967), and as short as 7 seconds (Nault and Gyrisco 1966). Increase in the rate of transmission increased during probes from 1 to 5 minutes (Nault and Gyrisco 1966). Ehrhardt and Schmutterer (1964) reported that A. pisum could infect Vicia faba plants after only a 5 second probe, and also found that transmission efficiency increased during probes of from 1 to 5 minutes.

Numerous workers have reported that nymphs have substantially higher transmission efficiencies than adults (Bath 1964; Bath and Chapman 1968; Ehrhardt and Schmutterer 1965; Heinze 1959b; Hinz 1966b; Nault et al. 1964; Tsai 1967). Ehrhardt and Schmutterer (1964), while comparing the inoculative ability of different aphid forms, found that winged adults transmitted virus to a lesser extent than wingless forms. Also, the last larval stage of winged forms transmitted PEMV much better than winged adults.

The brief inoculation threshold for PEMV led Bath (1964) and Nault et al. (1964) to suggest that it was not necessary for the aphid's stylets to reach the phloem for inoculation to occur. Subsequent light microscopy observations of salivary sheath saliva left behind in pea

leaves subsequent to probes by aphids revealed that during short probes of 27 seconds or less in duration, the aphid's stylets do not penetrate beyond the epidermis. This showed that the pea aphid could inoculate PEMV to the epidermis and to the interveinal and veinal parenchyma of the pea leaf (Nault and Gyrisco 1966). Studies of the salivary sheath by Tsai (1969) gave similar results. Light microscopy showed that aphids probed intercellularly. It was therefore postulated (Nault and Gyrisco 1966) that during superficial probes virus was inoculated into cells through plasmodesmata broken by the piercing stylets. However, in a recent electron microscope study, Lopez-Abella and Bradley (1969) found that five of ten salivary sheath paths showed a break in one of the cell walls through which saliva entered the cell. Presumably these breaks were caused by some part of the stylets piercing the wall to allow the aphid to sample the cell contents, and it may be then that virus is acquired or transmitted.

PEMV transmission is also dependent on the food plants, source plants, and test plants used (Hinz 1969). Tsai (1969) and Tsai and Bath (1970) have studied many of the factors affecting the inoculation phase of PEMV transmission. Transmission efficiency was not only significantly affected by the site of inoculation probing on the pea plant, but also by the age of the pea plant. A 24 hour preinoculation treatment at 10°, 20°, and 30°C had no significant effect on the transmission efficiency of the vector. Postinoculation temperature did affect efficiency of PEMV transmission, as 67% of the plants held at 24 - 32°C after inoculation developed symptoms, compared to only 30% of the plants held at 30 - 44°C.

McLean and Weigt (1968) have developed an electronic measuring system to record aphid salivation and ingestion. McLean and Kinsey (1967, 1968a, 1968b, 1969) and Hodges and McLean (1969) have recently used this device to relate aphid salivation and ingestion to virus transmission and acquisition, respectively.

Transmission Threshold Smith (1931) designated the transmission threshold as the total time elapse from the start of acquisition feeding until the end of successful plant inoculation with the virus. For PEMV Chaudhuri (1950) reported thresholds for Acyrthosiphon pisum from 6 hours to 4 days. Ehrhardt and Schmutterer (1964, 1965) stated that the threshold ranged from 30 hours to 13 days and from 18 hours to 5 days for young adults and young nymphs of A. pisum, respectively. In experiments with single infectious aphids, in which test plants were changed daily, A. onobrychis (B.d.F.) transmitted PEMV in one case after the 19th transfer (Heinze 1959b). Plants 20 to 24 remained healthy. Altogether, the aphid infected 16 test plants.

Effects of Starvation on Transmission Improved vector efficiency following preacquisition starvation is characteristic of stylet-borne viruses (Watson and Roberts 1939). Preacquisition starvation apparently does not affect PEMV transmission. Also, while vector efficiency of most stylet-borne viruses is decreased following postacquisition starvation, Simons (1954) studied four aphid species and found that starvation periods of up to 24 hours produced no effect on transmission. The effect of postacquisition starvation is apparently eliminated by the long retention of circulative viruses in their vectors. Tsai (1969) found changes in vector efficiency in aphids which were submitted to different preinoculation starvation periods. A period of 4 - 8 hours

fasting resulted in significantly higher transmission than did that of 16 - 20 hours.

Latent Period Reported latent periods for PEMV vary considerably depending on the virus isolate and the aphid species, strain, or stage tested, as well as the laboratory making the estimation. Published latent periods in Acyrthosiphon pisum adults include: 24 - 48 hours (Osborn 1935), 6 - 26 hours (Chaudhuri 1950), 25 - 29 hours (Simons 1954), less than 24 hours (McEwen et al. 1957), 14 - 70 hours (Sylvester and Richardson 1966b), and 27 hours - 10 days (Ehrhardt and Schmutterer 1965). A latent period between 16 and 24 hours has been reported for A. onobrychis (Heinze 1959b), between 12 and 20 hours for Macrosiphum euphorbiae (Osborn 1938), and between 14 and 18 hours for Myzus persicae (Bath 1964)

The latent period of PEMV is shorter in nymphs than adults. Ehrhardt and Schmutterer (1965) found the circulation period in Acyrthosiphon pisum nymphs ranged between 18 hours and 4 days, compared to between 27 hours and 10 days for adults. The pea aphid A. pisum showed mean latent periods of 30.0 hours and 56.8 hours for nymphs and adults, respectively (Simons 1954). With A. pisum the minimum adult latent period was 25 to 29 hours compared with 16 to 20 hours for first instar nymphs (Simons 1954). It was suggested that differences between nymphal and adult latent periods were pseudo-differences resulting from the greater efficiency of nymphs as vectors (Simons 1954). Bath (1964) indicated that the latent period of PEMV in first instar nymphs of A. pisum was 8 to 10 hours, and that mean latent periods increased in length with each successive instar for both Acyrthosiphon pisum and Macrosiphum euphorbiae. Bath (1964) postulated that the nymph's

advantage over the adult in virus acquisition gave it a greater chance to achieve a virus-contaminated saliva in the shortest time. Chapman and Bath (1968) have reported variations in the latent period of PEMV among aphid species, strains, and stages. The potato aphid M. euphorbiae revealed a PEMV latency gradient of 19.5, 24.6, 25.7, and 41.4 hours (arithmetic means) from first to fourth instars, respectively. The pea aphid demonstrated a similar latent period gradient--13.2, 23.1, 28.0, 36.0, and 40 hours from 1st instar to adult, respectively.

Sylvester (1965) postulated that the variance of the $LP_{50}$ of PEMV in A. pisum at any given temperature was probably related to the dose of the virus acquired. The $LP_{50}$ at 10°C tended to be twice as long as that at 20°C, but that at 20°C was not double that at 30°C. The median latent period decreased from 70 hours at 10°C to 25 and 14 hours at 20° and 30°C, respectively. There was evidence that the efficiency of virus transmission was negatively correlated with the length of the latent period.

Bath and Tsai (1969) found that a New York isolate of PEMV had a shorter latent period, remained inoculable longer, and was transmitted more efficiently by the pea aphid A. pisum than a California isolate. A 9 hour acquisition period for first instars resulted in average $LP_{50}$'s of 19.5 and 39.5 hours for the New York and California isolates, respectively. The New York isolate had an average $LP_{50}$ of about 60 hours in adults. The California isolate was not transmitted by adults (Tsai 1967).

Virus Retention The circulative and propagative viruses are usually retained for days to weeks in their vectors, whereas stylet-borne viruses generally have retention periods in minutes. Osborn

(1935) showed that PEMV could be retained in the pea aphid for periods up to 29 days. In the case of individual aphids, as transmission continued over several weeks of daily transfers, there were many more infections present in the first 10 days of transfer than in the following 10 days of a series. Chaudhuri (1950) found that PEMV was retained in Myzus persicae and Acyrthosiphon pisum for more than 140 hours. Simons (1954) presented evidence indicating a positive correlation between the length of retention and the length of the acquisition feed. The mean retention periods for PEMV in pea aphids A. pisum were 11.0, 14.6, and 15.0 days following acquisition feeds of 24, 48 and 96 hours, respectively. Ehrhardt and Schmutterer (1965) similarly noted the positive correlation between length of acquisition feeds and virus retention, and also noticed a marked influence of the last molting of A. pisum on retention, as most of the aphids loss their infectivity rather soon after it. Bath (1964) recorded a retention period in A. pisum of 29 days.

Heinze (1959b) found that pea aphids kept at 31.5°C after acquisition lost their infectivity after the 5th day. Infectious aphids held at -6°C for 6 days were no longer infective when transferred to test plants at room temperature. Temperature effects on retention of PEMV in A. pisum were also studied by Sylvester and Richardson (1966b). The weighted mean period of retention varied from 29.5 days at 10°C to 13.7 and 4.3 days at 20° and 30°C, respectively.

In tests on retention of PEMV by molting aphids, Nault et al. (1964) found that all of the viruliferous aphids retained the virus through at least one molt; 11 of 16 aphids transmitted virus after 3 molts. Sylvester (1967) checked 4 isolates for retention of inoculativity

in the transmission of PEMV. The period of time that pea aphid vectors A. pisum retained the capacity to transmit PEMV to sweet pea seedlings Lathyrus odoratus in serial transmission experiments varied with the virus isolate used. The decline in the transmission rate with the more persistent isolates of the virus occurred with a similar decline in the rates of reproduction and excretion, and presumably reflected a general lessening in the feeding activity of aging vectors. The mean retention periods varied from 12.9 to 10.9 days for the most persistent isolate, to lows of 7.1 and 5.4 for 2 of the least persistent isolates, respectively. Bath and Tsai (1969) found that a New York PEMV isolate was retained longer in the pea aphid than a California isolate. The mean retention time for the New York isolate was about 5.7 days.

## PEMV--CIRCULATIVE OR PROPAGATIVE?

The question of whether or not PEMV multiplies in its vector remains unresolved. The factors for and against PEMV multiplication in its vector have recently been summarized by Sylvester (1969b). Transstadial passage; the presence of a latent period, the length of which is a function of (a) dose of inoculum (whether by feeding or injection), (b) ambient temperature, and (c) vector age at the time of acquisition; relatively long persistence of vector inoculativity; retention of inoculativity, independent of the presence of detectable virus in the alimentary canal; and 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 used to support the hypothesis that PEMV is propagative in the aphid vector. Experimental results not favoring

the propagative hypothesis include the fact that the vectors transmission efficiency gradually declines following acquisition; its inoculative capacity is positively correlated with the dose of inoculum; vectors can be "recharged" (Sylvester and Richardson 1966a) by additional acquisition feedings after their transmission efficiency has declined; and that the virus cannot be maintained within a vector population by serial passage of hemolymph from one insect to another. Evidence available to date would perhaps support limited multiplication as a tentative hypothesis. Such a hypothesis would best explain the temperature-sensitive latent period, as well as the relatively prolonged period of inoculativity retention. Failure to maintain transmission efficiency is a weak argument against virus multiplication since this also occurs with the propagative potato leafroll virus (Stegwee and Ponsen 1958). That inoculative capacity is a function of the dose of inoculum is also an indicisive argument against multiplication, as this is also the case with potato leafroll virus (Kirkpatrick and Ross 1952; Heinze 1959c); although, Day (1955) did not get such results. Limited multiplication or localized infection could account for the dose-related transmission efficiency (Black 1953b). Recharging could be due to a temporary increase in the hemolymph titre from virus absorbed from the gut; an explanation used by Stegwee (1961) to explain the same phenomenon with potato leafroll virus. Failure to maintain infectivity through serial passage is perhaps the strongest argument against multiplication. However, injection of hemolymph may be a poor method for virus inoculation as compared to acquisition via the alimentary canal.

The site of PEMV multiplication in the aphid, if it exists, is not known. The high efficiency of acquisition and the brevity of the latent period, especially in young nymphs, would suggest a tissue system of a developing or regenerative nature. The brief latent period might also indicate rapid multiplication of a localized nature. Sylvester and Richardson (1966b) found no differences in longevity or reproductive capacity between viruliferous and nonviruliferous aphids, possibly indicating a nondegenerative multiplicative process, or multiplication in a non-vital vector tissue, or one which is being continually renewed or enlarged.

## STUDIES OF INSECT-BORNE VIRUSES IN SITU

Recent advances in electron microscopy, serology, and insect tissue culture techniques have greatly enhanced the identification and localization of insect-borne, plant-pathogenic viruses in vector tissues. Most of the work on the localization of plant viruses in insect vectors has been with leafhopper-borne viruses. The first electron micrographs of a circulative virus in plants and insects were by Fukushi et al. (1960) who demonstrated the presence of rice dwarf virus in the cytoplasm of virus-carrying Nephotettix cincticeps leafhoppers, as well as in diseased rice plants. The wound tumor virus was localized in plant tumors and in leafhopper vectors by Shikata et al. (1964), and the invasion of vector tissues has been studied in great detail. The only leafhopper-borne virus which doesn't seem to circulate in its vector is the tungro virus (Ling 1966) transmitted by Nephotettix impicticeps (Ishihara). Ling (1966, 1969) reported that leafhoppers could transmit the virus after acquisitions and inoculation feeding periods of only

1 hour each. Moreover, unlike other leafhopper-borne viruses, the infective nymphs failed to transmit the virus after molting into adults. Ling suggested that tungro virus may be stylet-borne and "nonpersistent" in its vector.

Among the circulative aphid-borne viruses, potato leafroll (Stegwee and Ponsen 1958), lettuce necrotic yellows (O'Loughlin and Chambers 1967), and sowthistle yellow vein (Duffus 1963; Hackett et al. 1968; Peters and Black 1970; Peters and Kitajima 1970; Richardson and Sylvester 1968; Sylvester 1969a; Sylvester and Richardson 1969, 1970) have been shown to propagate in their vectors. To date, potato leafroll (Miyamoto and Miyamoto 1966) and sowthistle yellow vein (Sylvester 1969a) are the only known cases of transovarial passage of aphid-borne, plant-pathogenic viruses. PEMV (Shikata and Maramorosch 1966b; Shikata et al. 1966), lettuce necrotic yellows (O'Loughlin and Chambers 1967; Chambers, Crowley, and Francki 1965), and sowthistle yellow vein (Richardson and Sylvester 1968, 1970) have been localized in the tissues of their aphid vectors as well as in infected plants using ultrathin sectioning technique and transmission electron microscopy. In the discussion that follows, these aphid-borne propagative viruses and one of the most comprehensively studied leafhopper-borne viruses, wound tumor virus, will be considered in greater detail with special emphasis on their localization in vector tissues.

Wound Tumor Virus Black (1945) proposed the name wound tumor virus (WTV) for the causative agent of a clover disease discovered by him and originally called clover big vein disease (Black 1944). WTV morphology has been studied extensively using the negative staining technique and transmission electron microscopy. The virus is

icosahedral in shape, ca. 60 nm in diameter, and has a capsid containing 92 capsomers, ca. 7.5 nm in diameter, numbering 4 along an edge, with smaller units of capsomeres of ca. 2.5 nm regularly packed (Bils and Hall 1962; Shikata and Maramorosch 1965d; Streissle and Granados 1968). Black and Markham (1963) and Gomatos and Tamm (1963a) have reported on the chemical composition of WTV. Approximately 20% of the virus content is double-stranded ribonucleic acid (RNA) as indicated by determination of the nucleic acid and protein content, ultraviolet absorption curve, and specific volume of the virus.

WTV was first recovered from one species of leafhopper collected in the Washington, D.C. area. It has subsequently been found to experimentally infect two additional related species of leafhoppers. Thus far, Agallia constricta (VD.), A. quadripunctata (Provancher), and Agalliopsis novella (Say) are the only three agallian species of leafhoppers known to be susceptible to infection. The virus causes an experimental disease in many species of plants belonging to various families, but its economic importance is as yet undetermined as it has not been observed to cause disease in nature. The virus produces a systemic infection in many plant hosts and is often accompanied by irregular vein enlargement, wart-like leaf enations, and root and stem tumors. The International Committee on Nomenclature of Viruses has placed WTV in the same group of double-stranded RNA viruses as reoviruses (Maramorosch 1966; Wildy et al. 1967). Similarity in size, shape, number and arrangement of capsomeres, double-stranded RNA, and the guanine-cytosine/adenine-uracil base ratios of the RNA are all factors relating WTV to reoviruses of man and a wide variety of lower animals. The similarities between WTV and reoviruses have been the subject of

numerous investigations (Gamez, Black, and MacLeod 1967; Gomatos and Tamm 1963a, 1963b; Rosen 1968; Streissle and Maramorosch 1963). Whether these similarities are due to a phylogenetic relationship or to parallel evolution from different phylogenetic sources is not known (Gamez et al. 1967; Maramorosch 1966).

Leafhopper nymphs, as well as adult males and females, can transmit the virus (Maramorosch 1950). Insects can be made infective by feeding on diseased plants, or by needle inoculation with infective plant extract or insect hemolymph. Once acquired by feeding on diseased plants, the virus undergoes a 13 - 15 day incubation period before the vector is able to transmit the disease (Maramorosch 1950; Maramorosch, Brakke, and Black 1949). Multiplication of wound tumor virus (WTV) in leafhopper vectors has been demonstrated by electron microscopy (Granados, Hirumi, and Maramorosch 1967; Granados, Ward, and Maramorosch 1968; Hirumi, Granados, and Maramorosch 1967; Maramorosch, Shikata, and Granados 1969; Maramorosch, Shikata, Hirumi, and Granados 1968, 1969; Maramorosch et al. 1965; Shikata and Maramorosch 1965b, 1965c, 1965d, 1967a, 1967b, 1969; Shikata et al. 1964) and serial injection technique (Black and Brakke 1952). Electron microscopy has revealed the presence of virions in the cytoplasm of fat body, malpighian tubules, epidermis, trachea, muscle, mycetome, gut, salivary glands (Maramorosch et al. 1965; Shikata and Maramorosch 1965d; Shikata et al. 1964), nervous system (Hirumi et al. 1967), and certain types of hemocytes (Granados et al. 1968). In addition, serology has indicated that virus may also be present in the ovaries (Sinha 1968). WTV is occasionally passed transovarially to progeny of infected females, and transovarial passage

can be increased considerably by selection and breeding (Sinha and Shelley 1965).

Fatbody tissues of viruliferous leafhoppers were found to contain the largest accumulations of WTV, often in the form of microcrystals (Maramorosch, Shikata, Hirumi, and Granados 1969). Crystalline arrangements were also seen in muscle cells, in the gut (Shikata and Maramorosch 1965), in hemocytes (Granados et al. 1968), and rarely in the salivary glands (Maramorosch, Shikata, Hirumi and Granados 1969). Electron microscopy of ultrathin sections of insects that acquired virus by feeding or by injection (Shikata and Maramorosch 1967a, 1967b) revealed numerous sites of WTV multiplication. WTV assembly was shown to occur within aggregates of finely textured, electron-dense material in the cytoplasmic matrix of the cell (Maramorosch 1970). These electron-dense areas or viroplasms corresponded in appearance with the "virus factories" described in several RNA viruses such as polio (Dales, Eggers, Tamm, and Palade 1965), mengo (Dales and Franklin 1962), reo (Dales, Gomatos, and Hsu 1965), and others. The appearance of assembly sites or viroplasms in leafhopper vector cells or in the cells of infected plants was always followed by the formation of complete virus particles first at the periphery and later within the entire viroplasm. The detection of viroplasms by electron microscopy thus provided a technique for the precise localization of virus assembly sites at a subcellular level. Viroplasms were found in gut cells, fat body, muscles, malpighian tubules, trachea, salivary gland, central nervous system, blood cells, and even in epidermal cells. Thus it became apparent that numerous vector tissues were capable of supporting WTV multiplication. The presence of viroplasms in the hemocytes

(Granados et al. 1968) indicated that blood acted not only as a carrier of virus to various sites, but also as a source of fresh virus. The electron microscopy of the sequential infection of leafhopper vector tissues following oral acquisition of WTV by feeding on infected plants or by abdominal injection with infective plant extract has been studied by Shikata and Maramorosch (1965d, 1967b) and recently reviewed by Maramorosch, Shikata, and Granados (1969) and Maramorosch et al. (1965). That the virus multiplies in and systematically invades its leafhopper vector has also been shown by serological methods, particularly by fluorescent antibody technique (Black and Brakke 1954; Nagaraj et al. 1961; Reddy and Black 1966; Sinha 1965, 1967, 1969; Sinha and Black 1962, 1963; Sinha and Reddy 1964; Sinha et al. 1964; Whitcomb and Black 1959, 1961). Electron microscopy has also demonstrated that cytopathic changes occur in the cells of the nervous system, fat body, and gut of the viruliferous leafhopper (Hirumi et al. 1967; Maramorosch, Shikata, Hirumi, and Granados 1969; Shikata and Maramorosch 1967b).

Aphid-borne Propagative Viruses Evidence has been presented that at least three aphid-borne circulative viruses are also multiplicative in at least one of their aphid vectors, viz., potato leafroll virus (PLRV), lettuce necrotic yellows virus (LNYV), and sowthistle yellow vein virus (SYVV). PLRV has been serially passed among successive groups of virus-free green peach aphids Myzus persicae, using hemolymph from the vectors (Stegwee and Ponsen 1958). Electron microscopy was used to demonstrate a general cytoplasmic infection of the aphid Hyperomyzus lactucae (L.) by LNYV (O'Loughlin and Chambers 1967). Two types of particles were found in aphids that acquired LNYV from infected sowthistle, Sonchus oleraceus L. One type was identical to

those observed in infected plant cells and in purified LNYV preparations, and the other was similar but lacked an outer coat. No such particles were found in sections of healthy control aphids. In viruliferous aphids, characteristic particles were found in the cytoplasm of cells in the muscle, fat body, brain, mycetome, tracheae, epidermis, salivary glands, and alimentary canal. A few particles were also observed in the perinuclear space in a muscle cell. The large number of particles forming regular aggregates in the cytoplasm of the cells of these organs was interpreted as evidence for virus multiplication within the aphid.

Several studies have indicated the multiplication of SYVV in the aphid vector H. lactucae. Duffus (1963) indicated that SYVV had many transmission characteristics which were similar or identical to those first described for plant-pathogenic viruses known to be propagative in their leafhopper vectors, particularly the presence of a relatively long incubation period and efficient prolonged retention of inoculativity. Richardson and Sylvester (1968) found bacilliform particles similar to those found associated with the nuclei of infected sowthistle cells in the cytoplasm adjacent to nuclei in cells of the salivary tissue of aphid vectors reared on SYVV-infected plants. Particles not surrounded by a unit membrane were found in the nucleoplasm. Sylvester and Richardson (1969) were also successful in the efficient serial passage (six successive passages) of SYVV infection 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 sufficiently high, so that, if no virus multiplication had occurred, the volume inoculated would have contained less than one virus particle.

This strongly suggested that SYVV had multiplied in the aphid. In the same paper Sylvester and Richardson also presented evidence of a deleterious effect of SYVV infection on the longevity of the aphid vector--to date, the only such evidence for an aphid-borne circulative virus. Later, Sylvester (1969a) obtained evidence for a low amount of transovarial passage (one per cent among all larvae produced) of the SYVV to larvae of apterous viruliferous viviparae. Peters and Black (1970) were able to infect primary cell cultures of the aphid vector with SYVV. Samples of purified preparations were inoculated to cultures two days old. Infection of the cells could be demonstrated by direct fluorescent antibody staining. The first infected cells were found 37 hours after inoculation and the number of infected cells reached a maximum after 48 hours.

A most recent transmission electron microscopy study by Sylvester and Richardson (1970) has shown that the aphid Hyperomyzus lactucae can be systemically infected with SYVV. Particles were found in the nucleoplasm as well as the cytoplasm of cells of the brain, subesophageal ganglion, main and accessory glands of the salivary system, esophagus, ventriculus, ovaries, fat body, mycetome, and muscle. The initial site of infection was reported to be the stomach region of the midgut. No particles were found in the cells of the posterior portion of the intestine, hindgut, or in embryos. Infection of foregut cells was considered to be secondary. Evidence indicated that virions of SYVV were assembled in nuclei of cells.

Ruppel (1968) has found particles believed to be beet western yellows virus (BWYV) in the gut lumen and cellular cytoplasm of viruliferous green peach aphids Myzus persicae. Comparable particles were

not seen in the lumen or intestinal tissue of aviruliferous aphids. Electron micrographs of the particles revealed that they exhibited relative uniformity of shape, and their size, 25 - 30 nm, and shape coincided with that of purified virus. Once aphids acquire BWYV they are able to transmit it for life without further access to a virus source. However, further studies with electron microscopy and other approaches will be needed to determine if BWYV does indeed multiply within the green peach aphid.

Although no direct evidence has been presented for the multiplication of PEMV in its aphid vector Acyrthosiphon pisum, there has been considerable speculation by numerous researchers concerning the possible infection of the insects that transmit the virus. The electron microscopy of ultrathin sections of viruliferous pea aphids has revealed scattered electron-dense particles similar to those observed in infected plant cells within the lumen of the gut, and accumulations of these particles have also been detected in the cytoplasm of fatbody cells (Shikata and Maramorosch 1965a, 1966b; Shikata et al. 1965, 1966).

# MATERIALS AND METHODS

The New York strain of pea enation mosaic virus used throughout this research was originally obtained from D. J. Hagedorn of the Department of Plant Pathology, University of Wisconsin, Madison. This same strain has been employed in numerous earlier studies (Bath 1964; Bath and Chapman 1966, 1967, 1968; Bath and Tsai 1969; Chapman and Bath 1968; Ruppel and Hagedorn 1963b; Tsai and Bath 1970). The New York strain was preferred to a California strain of PEMV because it had previously been shown to have a shorter latent period, to remain longer in an inoculable form, and to be transmitted more efficiently by the pea aphid (Bath and Tsai 1969; Tsai 1967). In vitro cultures of this New York strain were maintained in desiccated conditions and stored over calcium chloride in a refrigerator at 5 to 6°C. An in vivo culture was maintained in pea plants by periodical insect or mechanical transfers.

The pea aphid, Acyrthosiphon pisum (Harris), used in this study was collected in an alfalfa field on the University Farm in East Lansing, Michigan in 1964 and was subsequently found to be a highly efficient vector of PEMV. Mr. Francis E. Giles confirmed the aphid's identity and specimens were deposited in the MSU Entomology Museum. One apterous adult was used as the basis for the ensuing colony. Broadbean Vicia faba L. was used as the host plant, and the aphid culture was maintained under controlled conditions in environmental growth chambers as

previously described by Tsai (1967). This combination of a readily transmissable virus isolate and a highly efficient aphid vector provided optimum conditions for PEMV localization in aphid tissues using ultrathin sectioning technique and transmission electron microscopy.

Garden pea, Pisum sativum L. var. Midfreezer, was used exclusively as both virus source plant and test plant. The techniques for obtaining test and source plants have been described by Tsai (1967). Seeds were evenly distributed in plastic pans containing vermiculite. The depth of vermiculite below and above the seeds was 1 inch and 1/2 inch, respectively, thus insuring uniform growth of the seedlings. After a 5 to 7 day interval, or once the seedlings were just visible above the vermiculite surface, they were individually transplanted into plastic pots containing a potting medium of a sterilized loam-sand-peat mixture. Immediately following transplantation, seedlings were used directly as test plants or mechanically inoculated for use as source plants. Inoculum for mechanical virus transfer was prepared by grinding the terminal leaves of pea plants showing severe symptoms in a sterile mortar with a pestile. This extract was squeezed through cheesecloth to remove excess fibrous materials and inoculated without dilution onto wet, carborundum-dusted seedlings using a wooden pot label as an applicator. The freshly inoculated seedlings were grown in an isolated greenhouse room. On the 12th day following inoculation, plants showing the most severe symptoms were chosen as source plants.

Aphids used in experimentation were gathered from broadbean by gently tapping the host plant over a plastic Petri dish. Hundreds of aphids were easily collected in minutes. These aphids were then anesthesized with carbon dioxide, and under a stereomicroscope only

newly-born 1st instar nymphs were picked up with a size 00 sable hair brush and transferred into another plastic Petri dish for later use. The newly-born nymphs were detectable on the basis of size and by their "square-shaped" abdomens. The abdomens of older nymphs terminated in the more rounded or pointed fashion typical of later instars and adults.

Virus acquisition was accomplished by placing groups of nymphs onto the terminal portion of 12 day old source plants which showed the severest symptoms. The feeding area available to the aphids was limited by inserting the plant area of optimum virus concentration through a 1 inch hole in an elevated platform. The platform hole was covered by fitting a piece of filter paper around the stem so that only the most desirable acquisition tissues were above the filter paper and platform. A glass lantern chimney with a screened top was placed over the plant, and the circumference of its open base pressed the filter paper tightly against the platform. Any aphids that fell landed on the filter paper and could then return to the source plant. Control aphids were treated in the same manner, only they were confined to 12 day old healthy pea plants.

Source plants were kept in an insect rearing room under controlled lighting conditions (12 hour photoperiods) at 26°C during the acquisition access feedings. Aphids were allowed acquisition access periods of from 1 to 7 days. This schedule insured that viruliferous aphids of each developmental stage from 1st instar nymphs to adults would be included in the transmission electron microscopy research. At 24 hour intervals during the 7 day period aphids were removed from the source plant with a brush and placed individually into 12 x 35 mm (1/2 dram) shell vials. The vials were corked and each aphid given a

number which was to identify it throughout the experiment. Each aphid was removed from its vial and placed onto an individual healthy pea seedling for a 15 to 30 minute inoculation access period. A wooden pot label bearing the same number previously assigned to the aphid was used to identify each of the test plants. Aphids were reclaimed and test plants were then sprayed with Naled and transferred to an insect-proof greenhouse room for eventual symptom development. The room was fumigated on a 7 to 10 day schedule with Sulfotepp to guard against contaminant insects entering the room. Test plants were checked every 5 to 7 days for symptom development over a period of at least 1 month before being discarded. Test plants for control aphids were treated in a similar manner and kept isolated from test plants fed upon by viruliferous aphids. Test plants were employed in order that the tedious process of ultrathin sectioning and staining would not be wasted on aphids which had not transmitted PEMV and were therefore perhaps less likely to contain virus particles in sufficiently high titre for detection in the transmission electron microscope (TEM).

Following the 15 to 30 minute inoculation access feeding, aphids were removed with a brush from their respective test plants and prepared for study in the TEM. Whole aphids as well as dissected and excised organs and tissues were processed for electron microscopy. Intact aphid intestines were readily obtained by the method described by Kikumoto and Matsui (1962). Specimens were placed in cold (12°C) 6% glutaraldehyde in 0.1M Sorensen's phosphate buffer solution at pH 7.2 with 0.2M sucrose for 90 minutes. With whole aphids, the insects were gripped by an appendage using finely tipped forceps and submerged in a drop of the cold glutaraldehyde fixative placed in an open Petri

dish. Under a stereoscope the aphid's legs and antennae were removed from their sockets using 2 pairs of finely tipped forceps. These openings facilitated the rapid penetration of the fixative into both head and abdomen. The aphid was then returned to its vial which had previously been filled to a depth of approximately 1 cm with fresh, cold, glutaraldehyde fixative. Gentle agitation of the vial was usually required in order to sink the aphid to the bottom of the solution.

After primary fixation in glutaraldehyde, samples were given three 30 minute washings in 0.1M Sorensen's phosphate buffer and post-fixed in cold 1% osmic acid in 0.1M phosphate buffer for 4 hours or overnight in the refrigerator at 12°C. Fixation was followed by dehydration in a graded series of ethanol (25, 50, 75, 95, 100%), 15 minutes in each followed by at least 3 additional 30 minute changes in the 100%. The aphids were then infiltrated with Spurr's (1969) epoxy resin embedding medium (Electron Microscopy Sciences, Fort Washington, Pa.). The "E" modification was used since this gave a medium of even lower viscosity than the standard medium and was therefore preferable for infiltration of whole aphids. While the embedding medium was prepared as described (Spurr 1969), the infiltration procedure was slightly modified. Following dehydration, the last change of 100% ethanol was replaced by a 1 : 1 mixture of embedding medium and 100% ethanol. The specimens remained overnight in this mixture in corked shell vials within a desiccator. Specimens were then held overnight in 75% embedding medium, followed by another overnight stand in 100% embedding medium. Finally, the vials were removed from the desiccator and placed, with corks removed, into a vacuum desiccator and held under

vacuum for 30 minutes to insure complete infiltration by the epoxy resin mixture.

To embed, specimens were individually transferred with wooden applicators into oven-dried, flat embedding molds (Ladd Research Industries, Inc., Burlington, Vt.) filled with fresh 100% embedding medium from the same batch used for infiltration. A single stock solution of embedding medium, used throughout the infiltration and embedding procedure, was maintained in a glass-stoppered flask in a desiccator at room temperature. Whenever the stock solution was exposed to the atmosphere, the air within the flask was replaced with freon gas, "Blast Off" (Ladd Research Industries, Inc.), before closing. The advantages of the flat, silicone rubber, embedding molds used have been described by Rockwell et al. (1966). Penciled, oven-dried, paper labels were included in the blocks with the specimens and identified them by number. Whole aphids were oriented with their heads towards the "pre-trimmed" ends of the blocks and the molds placed in the desiccator for an overnight stand. The following day, after a final check for proper specimen orientation, the molds were placed in an oven for polymerization and cured at 70°C for at least 16 hours.

Ultrathin sections of whole aphids, as well as of individually embedded organs, were cut with a diamond knife on a Porter-Blum MT-2 ultra-microtome and placed directly onto 300-mesh nickel grids or onto formvar coated 75 mesh grids. As soon as possible after sectioning, sections were stained for 45 minutes in a freshly prepared saturated solution of uranyl acetate in 25% methanol and 70% ethanol in a 1 : 1 ratio. Grids were totally immersed in droplets of the staining solution placed on "Parafilm" and covered with a Petri dish bottom to

exclude air contaminants and prevent excessive evaporation. Grids were then thoroughly rinsed with methanol-ethanol solution, followed with distilled water. Next, grids were submerged for 5 minutes in droplets of lead citrate staining solution and washed with 0.02N NaOH, followed by distilled water. The lead citrate stain was prepared by slowly adding ca. 0.5 ml of 10N NaOH to a solution of 0.2 gm of lead citrate in 50 ml of boiled, double-distilled water with vigorous shaking. Upon addition of the NaOH, the milky colored lead citrate solution became clear. The pH of the solution was not allowed to go over 12. The rinsing phases of the staining processes were accomplished by washing both surfaces of the grids with the appropriate solutions squirted from polyethylene squeeze bottles.

Observations were made with a Philips EM - 300 transmission electron microscope (TEM) using Kodak fine grain positive 70 mm X 30.48 m roll film, Kodak electron microscope 8.3 X 10.2 cm (3 1/4 X 4 inches) cut film with Estar thick base, and Kodak 8.3 X 10.2 cm electron image plates.

# RESULTS

## PRELIMINARY INVESTIGATIONS

Virus particles were first localized in ultrathin sections of infected plant tissue, and examined in purified preparations. In the plant tissue, dense aggregates and occasional crystalline masses of PEMV particles (size: 26 - 30 nm) were detected in the cytoplasm of infected plant cells in necrotic and nonnecrotic pea leaf enations. PEMV virions were also localized in the nuclei of infected plant cells. The findings in infected plants and the size and morphology of PEMV virions observed in pure preparations agreed with previous reports (Bozarth and Chow 1966; Shikata and Maramorosch 1966b; Shikata et al. 1965, 1966).

As mentioned previously, all aphids were given test feedings on individual healthy pea seedlings before being processed for electron microscopy. Only 2 of 82 aphids from PEMV-infected source plants failed to transmit PEMV. This high transmission efficiency of 97.5% can be attributed to the pairing of a highly efficient aphid vector with a readily transmissible virus strain. None of the 50 control aphids transmitted PEMV to test plants.

Approximately equal numbers of each developmental instar were processed. With both viruliferous aphids and healthy controls, at least five specimens from each instar were included in this research. In addition, before searching for PEMV in viruliferous aphids,

thousands of sections of whole control aphids, as well as of individually embedded organs, were carefully studied in order to gain proficiency in tissue identification. I did not observe particles of PEMV at any time during this preliminary study of control material. Unless otherwise stated, the results of this research apply equally to all developmental stages of the pea aphid.

## INTERNAL MORPHOLOGY OF THE PEA APHID

Even though aphids are the major vectors of plant viruses, studies of their internal morphology are few. The small size of aphids and the consequent difficulty in working on them are perhaps partly responsible for this scarcity of information. A classic paper by Weber (1928) on the black bean aphid, Aphis fabae Scop., has become a standard reference. Davidson (1913) and Weber (1928) cite earlier fragmentary accounts of aphid gut morphology. Forbes (1964) published a comprehensive study of the morphology, histology, and fine structure of the gut of the green peach aphid, Myzus persicae (Sulz.), using both light microscopy and transmission electron microscopy. Recently, Forbes and MacCarthy (1969) reported on the morphology of the Homoptera, with emphasis on virus vectors. These last two references were used extensively during the initial phases of this research.

I dissected numerous pea aphids under the stereoscope in order to become familiar with the relative positioning of the various organs and tissues. Knowledge of the size and positioning of organs made it possible to immediately trim blocks of embedded whole aphids to points of interest. It became apparent, for example, that trimming a block to a point just in back of the prothoracic coxae would yield sections

through the anterior portion of the stomach region of the midgut. The relative positionings in the aphid of the organs included in this research have recently been illustrated by Sylvester and Richardson (1970). Similarly, the identification of organs and tissues in cross sections of whole aphids was made easier by first examining sections of individually embedded organs and tissues. In addition, sections of whole aphids were placed on formvar coated 75 mesh grids and viewed in the transmission electron microscope at a scanning magnification of X220. Observations on the internal morphology and cellular ultrastructure of the pea aphid, Acyrthosiphon pisum, were included in this research.

## MORPHOLOGY OF THE ALIMENTARY CANAL AND ITS ROLE IN THE UPTAKE, DISTRIBUTION, AND MULTIPLICATION OF PEA ENATION MOSAIC VIRUS IN THE APHID VECTOR

Since there is lack of uniformity in naming the parts of the alimentary canal of the Homoptera, the terminology used here is that of Forbes (1964) and Forbes and MacCarthy (1969). The term alimentary canal is used to include the food canal in the maxillary stylets, the sucking pump, and the gut. The gut is divided into foregut, midgut, and hindgut. The midgut is further divided into stomach and intestine.

Morphology of the Food Canal The alimentary canal begins as the food canal within the maxillary stylets. Interlocking ridges and grooves hold the maxillary stylets closely together, and thus between them is formed the food canal anteriorly and the salivary canal posteriorly. The fine structure of aphid stylets has been studied by van Hoof (1957). The food canal is considerably larger than the salivary canal, and tapers from about 1.5 μm in diameter near the head to 0.35 μm near the tip (Forbes and MacCarthy 1969).

Absence of PEMV in the Food Canal Ultrathin sections of whole aphids taken in the head and prothoracic regions yielded cross sections through the mouthparts as well. Unfortunately, intact sections of the maxillary stylets, particularly in the distal portion, were difficult to obtain. Also, sections usually separated under the electron beam in areas of contact between integument and embedding medium.

Examination of sections from viruliferous aphids as well as from aviruliferous controls which remained intact under the electron beam for reasonable lengths of time demonstrated that no particles resembling PEMV were present in either the food or salivary canals.

Morphology and Fine Structure of the Foregut The food canal opens into a tubular functional mouth which then leads into the lumen of the sucking or cibarial pump. The pump arcs dorsad between the circumesophageal connectives and joins the foregut at the transverse tentorial bar. The pump is ca. 30 μm in diameter distally and 15 to 20 μm at its juncture with the foregut (Forbes and MacCarthy 1969). The unspecialized foregut connects the sucking pump and the stomach region of the midgut.

The wall of the foregut consisted of squamous epithelial cells with overlapping edges (Fig. 1). The nuclei were ovoid and occupied most of the central part of the cell. The free surface of the epithelial cells bore sparse cytoplasmic protrusions or microvilli. Some were long and cylindrical while others were irregular. Their bounding membrane was clearly part of the cell membrane. Where two epithelial cells came into contact the limiting membranes were separated by a clear space of fairly uniform width. The foregut was enveloped by a

tunica propria and lined by a longitudinally folded intima. The intima was free from the surface of the epithelial cells and usually compressed by preparative methods in the center or to one side of the lumen. In its natural state, the intima would probably be forced against the free surfaces of the epithelial cells by ingested sap passing along the foregut. The intima was found to be an intact structure, completely surrounding the ingested material and thus preventing it from intimately contacting the cell surface. Ingested materials were never found in the space between the free cell border and the intima (Fig. 1).

PEMV in the Foregut Lumen A few small, darkly stained particles similar in size to PEMV were found sparsely distributed within the region of the foregut lumen defined by the intima. A critical study of these electron-dense particles was not possible since ingested materials were tightly compressed, and therefore quite electron-dense, within the lumen. The particles were found in the foregut lumens of viruliferous aphids from first instars to adults, and were never detected in control aphids. The particles probably represented PEMV virions which had been ingested along with sap when the aphids fed on PEMV-infected source plants. Later observations of particles in the stomach and intestine regions of the midgut, as well as in the hindgut, supported this hypothesis. A preliminary search for virus particles in the cytoplasm and nuclei of foregut epithelial cells gave negative results. These initial observations were later reaffirmed when foregut epithelial cells were reexamined following the finding of virus particles in cells of the stomach and intestine.

PEMV in the Stomach Lumen The search for PEMV in the midgut resulted in the localization of large concentrations of electron-dense,

"spherical" particles in the stomach lumen. The particles were densely aggregated in a few areas of the lumen, and scattered in others (Fig. 2). The particles tended to aggregate along the peripheral margins of ingested food materials (Figs. 2-4). No comparable particles were ever seen in aviruliferous control aphids. With both viruliferous aphids and virus-free controls, at least five specimens from each developmental instar were included in the sample. The particles exhibited relative uniformity of shape, and their size, 24 - 27 nm, and shape coincided with that of purified virus (Bozarth and Chow 1966), and virus observed in ultrathin sections of plants and aphids (Shikata et al. 1966). The particles were therefore assumed to be virions of PEMV. The particles were polygonal in shape, with the hexagonal profile being predominant. However, a few exhibited a distinct pentagonal profile (Fig. 4). Bozarth and Chow (1966) have reported finding pentagonal particles in purified preparations. PEMV virions in the lumen did not stain equally. Some were quite electron-dense, others less so, and still others appeared almost electron-transparent (Fig. 4).

Morphology and Fine Structure of the Foregut The foregut connects with the sac-like stomach of the midgut. The stomach leads to the tubular intestinal region of the midgut which folds upon itself several times before enlarging and joining the straight, thin-walled, much-enlarged hindgut. There is no filter chamber or Malpighian tubules.

Figure 5 shows a cross section of the intestinal region of the aphid midgut. The midgut epithelium consisted of a single layer of cells which rested upon a connective tissue sheath or tunica propria. This investing sheath consisted of the basement cell membrane of the

epithelial cells, muscle fiber cells, and tracheoblasts. Wigglesworth (1956) reported that perhaps other connective tissue is laid down by amoebocytes. Isolated, circular, muscle fiber cells occurred around the midgut. The myofilaments were not grouped to form myofibrils, but were arranged in a continuous lattice. The muscle fiber cells were bound to the midgut by the fusion of the basement membrane of the sarcolemma with that of the midgut epithelial cells. No longitudinal muscle cells were observed. The basal cell membrane was thrown into many intracellular folds, or septa, which formed compartments in the basal region of the midgut cells. There was considerable anastomosis between adjacent folds, but the infolding and consequent compartmentation was not as extensive as at the free border of these cells (Fig. 5).

The cytoplasm of the midgut cells contained a well-developed endoplasmic reticulum, and mitochondria occurred throughout but were especially abundant adjacent to the free cell border and in the compartments formed by the infolded basal cell membrane. "Lipoid spheres," large electron-dense inclusions with an irregular outline, were also observed in the cytoplasm of midgut cells. Waterhouse and Wright (1960) reported similar spheres in the midgut cells of Lucilia larvae. It was not possible to assess the role of these spheres from their appearance in electron micrographs alone. Some probably represented either pinocytosis vesicles or excretory products. The spheres with concentric lamination (Fig. 5) appeared similar to those described by Wigglesworth and Salpeter (1962) as mineralized deposits in the Malpighian tubules of Rhodnius. The contents of some of the lipoid spheres occasionally "melted" under the electron beam, thus producing

holes in the section (Figs. 5 and 23). Areas thought to represent the Golgi complex were also noted in the cells of midgut sections (Figs. 11 and 13).

A striated border is almost universal for the lumen surfaces of insect midgut epithelia. The striated borders of stomach and intestine cells were found to be similar, except that intestine cells bore relatively more microvilli at the surface (Fig. 5). The midgut striated border was elaborately folded and anastomosis was frequent, thus producing a border of microlabyrinths and microvilli (Figs. 5-9).

PEMV in the Midgut Lumen and in Association with the Microvillous Borders of the Stomach and Intestine Virions of PEMV were highly concentrated in the midgut lumen, especially in the large lumen area of the stomach (Figs. 2-4). Particles were also found scattered, as well as in small aggregates, in the vicinity of the microvillous borders of the stomach and intestine. Individual virions were detected in and around the gut microvilli (Figs. 6-9), but actual sites of viral entry into (or exit from) the midgut cells were not observed. I was unable to discern any differences between virions just outside of the microvillous border and those just within.

PEMV in Infected Midgut Epithelial Cells Careful examination of viruliferous aphids revealed that many of the epithelial cells were indeed infected with PEMV. The infection was most intense in the stomach and the anterior portion of the intestine. Far fewer cells in the posterior portion of the intestine were found to contain PEMV. Both the invasion and the infection of this tissue were rapid. Newly-born first instar nymphs which were allowed a 24 hour acquisition access period on infected pea plants contained cells in various stages of

infection, from the earliest to the latest. Similar findings were observed in the midguts of later instars and adults. Infected cells were detected in every viruliferous aphid examined, but were never observed in aviruliferous controls.

The occurrence of detectable virus particles in midgut cells was preceded by the appearance of finely textured, electron-dense areas within the cytoplasmic matrix. These areas corresponded in appearance and function with the "virus factories" or viroplasms described in would tumor virus infected plant and insect host cells (Maramorosch 1970; Shikata and Maramorosch 1967a). Viroplasms were found only in viruliferous aphids, and not in virus-free controls. The detection of viroplasms by electron microscopy provided a technique for the precise localization of sites of virus assembly within cells. In the early stage of infection, viroplasms first appeared in the apical part of the cell, just beneath the striated border, in close proximity with the many mitrochondria concentrated in this region (Fig. 10). Viroplasms next appeared in the basal portion of the cell near the convoluted basal cell membrane where mitochondria were also highly concentrated. The appearance of viroplasms was followed by the formation of virus particles at the periphery, and later within the whole viroplasm. Mitochondria were never found to contain virions.

In a later stage of infection, virus particles occurred throughout the cell cytoplasm in defined electron-dense structures, in tube-like structures, and free in the cytoplasm (Fig. 11). The defined structures were partially or completely surrounded by a unit membrane, varied in size, shape, and electron density, and virus accumulations within ranged from sparse and scattered to closely aggregated. These

structures frequently enclosed one or more multimembranous or myelin-like figures which themselves often contained virions (Figs. 11-13). Virus particles were occasionally observed within processes from the periphery of larger viral structures (Fig. 11). These processes were often elongated and extended through the cytoplasm to form tubelike structures (Fig. 11). Tubular structures measuring several viral diameters across are shown in longitudinal and cross sections in Figure 13. The tubes were sometimes quite narrow and contained virions arranged in a single row. Aggregates of PEMV particles in electron-dense matrixes within the cytoplasm probably represented the remains of disintegrating mature viroplasms and other defined viral structures (Figs. 11-13). Another feature of Figures 11 and 13 is the presence of a Golgi complex in close proximity to viroplasms and other viral structures; this was a common observation.

Virions were also located within electron-dense, lysosome-like, inclusion bodies (Figs. 11 and 12). In Figures 14 and 15, virus particles appear enclosed in defined cytoplasmic structures similar to those described by Shikata et al. (1966) in fatbody cells of viruliferous pea aphids. Very few virions are scattered loosely in the cytoplasm. Figure 16 shows an accumulation of particles in the cytoplasm of a midgut epithelial cell. Viroplasms in different developmental stages could usually be found in the cytoplasm of cells containing the numerous other types of viral inclusions (Fig. 13).

In rare instances, PEMV was detected within the nuclei of infected midgut cells (Figs. 17-19). Changes in the ultrastructural integrity of these cells and the high concentrations of virus particles in their cytoplasm were indicative of advanced infection. A detailed

discussion of the cytopathological changes accompanying infection of midgut cells by PEMV will be presented in a later section. A few virions were scattered within the nuclear and nucleolar matrixes, but most were concentrated around the periphery of the nucleolus. These virions were not very electron-dense and possibly represented partially degraded particles or particles with an incomplete complement of nucleic acid. The electron-lucid nature of both the cytoplasm and nucleoplasm indicated a severe depletion of cellular materials. PEMV was not found in the nuclei of cells of any other aphid tissue.

PEMV in the Hindgut Lumen. Particles of PEMV were found highly concentrated in the lumen of the hindgut (Figs. 20-22). As in the midgut, virions tended to aggregate along the periphery of ingested food materials; but, unlike the situation in the midgut, virions were seldom found in contact with the microvillous border (Figs. 21 and 22). Infection of gut epithelial cells by PEMV was limited to the stomach and intestine regions of the midgut. Hindgut epithelial cells were found to be free of virions. PEMV particles were concentrated in the hindgut lumen of every viruliferous aphid examined, from first instar to adult, but never in control insects.

The epithelial cells of the hindgut were so thin that often the nuclei characteristically projected into the lumen (Fig. 21). The free borders showed a few cytoplasmic protrusions or rudimentary microvilli, and mitochondria and the endoplasmic reticulum were relatively sparse and evenly distributed (Figs. 21 and 22). The basal cell membrane of the hindgut epithelial cell was quite simple compared to the much convoluted membrane of the midgut (Fig. 21).

Cytopathic Changes in PEMV-infected Midgut Epithelial Cells

The cytoplasm of uninfected midgut cells contained a large number of ribosomes and typical metazoan mitochondria. Healthy cells also possessed typical nuclei with intact nuclear membranes (Fig. 23). Viruliferous aphids showed cytopathic changes in infected epithelial cells of both the stomach and intestine. The ultrastructural integrity of the cytoplasm of these cells was changed due to the depletion of ribosomes (Figs. 11-15). In addition, the membrane structures of cytoplasmic organelles such as the endoplasmic reticulum, nuclear membrane, Golgi apparatus, and mitochondria degenerated and became quite indistinct (Figs. 11-15 and 19). Eventually both the cytoplasmic matrix and the nuclear substance were changed into homogenous fine granules (Figs. 17-19). Whenever these ultrastructural changes were observed, virions were found in these and often in adjacent cells. No such changes occurred in control insects. In Figure 19, the inner and outer units of the nuclear membrane have degenerated completely in one area and, where present, are indistinct, widely separated, and deformed.

The overall electron opacity of the cytoplasm and nucleoplasm of an infected cell was noticeably decreased due to the depletion of ribosomes and nuclear materials, respectively. Thus a relative electron lucidity, as well as the presence of viroplasms and other defined viral structures in the cytoplasm, served as convenient methods of detecting infected midgut cells.

PEMV IN MUSCLE

Muscle fiber cells surrounding the midgut were invaded by PEMV. Virions were observed within the sarcoplasm in discrete electron-dense areas (Figs. 24 and 25) and occasionally in tubular structures. No virus particles were seen in muscle fiber cells surrounding the foregut and hindgut. Also, virions were not observed in large skeletal muscles.

PEMV IN DEFINED CYTOPLASMIC STRUCTURES WITHIN THE FAT BODY

Electron micrographs of ultrathin sections through fatbody tissues of viruliferous aphids revealed clusters of PEMV virions. The particles were mainly found within defined structures in the cell cytoplasm (Figs. 26-28). Occasionally a few particles were found loosely scattered in the cytoplasm in close proximity to the defined structures. The structure in Figure 26 is quite similar to the type of inclusion described by Shikata et al. (1966) in the fat body of aphids which had fed on PEMV-infected pea plants. Other interesting aspects of Figure 26 include the lamellar or myelin-like figure and the process by which a few particles are separated from the main viral structure. These processes sometimes extended through the cytoplasm to form tubular structures. Fatbody cells often contained numerous defined viral structures (Figs. 27 and 28) similar to some observed in the midgut (Figs. 14 and 15). Occasionally, when two or more such structures occurred in the cytoplasm in close proximity to one another, it was possible to locate an ultrathin section in a series of sections which demonstrated an area of union between their electron-dense matrixes (Fig. 27). One to several multimembranous figures which themselves contained virions were usually included in the larger electron-opaque

structures (Figs. 26-28). Degenerative, indistinct mitochondria were occasionally observed in close proximity to the viral structures within fatbody cells (Figs. 26 and 28). However, severe ultrastructural changes, such as those detected in infected midgut cells, were not observed. Examination of fatbody tissue did not reveal the presence of typical viroplasms. Fatbody cells which contained PEMV virions were found in all the developmental stages of viruliferous aphids from first instars to adults. No virus particles or corresponding electron-dense structures were observed in the fat body of control aphids.

PEMV IN HEMOCYTES

Three types of blood cells were identified in the blood tissue of the pea aphid. Using the terminology suggested by Jones (1962), these were plasmatocyte-like cells, spherule cells, and granular hemocytes. Plasmatocytes were easily the most frequently observed of the three types and usually appeared stretched out over relatively large areas of the hemocoel (Figs. 31 and 34). Granular hemocytes were numerous and were often found either in contact with or in close proximity to the basement membrane of the gut, ovarioles, the tracheal system, and epidermis (Figs. 5, 31, 34, and 35). Spherule cells were less frequently observed in the hemolymph.

Electron microscopy of ultrathin sections through plasmatocytes, spherule cells, and granular hemocytes suggested that all three cell types were only occasionally invaded by PEMV. Virions were found in the hemocytes of first, second, and rarely third instar nymphs, but never in the hemocytes of later instars or adult viruliferous pea

aphids. Virus particles were only observed in the cytoplasm of hemocytes, and were never detected free in the hemolymph.

In plasmatocytes and spherule cells, virions occurred in the cytoplasm within electron-dense areas (Figs. 29 and 30) consisting of tightly packed filamentous and granular elements. The similarity of these electron-dense areas to known loci of wound tumor virus assembly suggested that PEMV may multiply in these blood cells. In Figure 30 a few particles appear to be leaving (or entering) the viroplasm-like area. Corresponding viroplasm-like areas were not observed in granular hemocytes.

The localization of virions in granular hemocytes was less frequent than in other blood cell types. Virions were detected in two types of structures in the cytoplasm. One type consisted of an electron-dense matrix interrupted in a random manner by areas of relative electron lucidity. The electron-lucid areas appeared splinter-like in cross section. This type of structure is shown in Figures 31-33. Virus particles were found scattered throughout the electron-dense areas, and rarely formed microcrystals near the periphery of the structure. The second type of viral structure observed in granular hemocytes was similar to the first, but differed in being more electron-dense and in having the electron-transparent areas subdivided by septa or membranous partitions (Figs. 34 and 35). These structures often occurred in close proximity to the nuclei of granular hemocytes (Figs. 35-37) and appeared to arise from very dense cytoplasmic inclusion bodies by a partitioning process. Several of these bodies in Figure 36 are near the nucleus, which appears somewhat indented by their formation. The nucleus of the granular hemocyte in Figure 37 also

appears slightly indented by the septate, electron-dense structure. A few of the PEMV virions within the structure are loosely scattered, but many have formed strings of particles between and along the septa (Fig. 38). PEMV virions or similar inclusion bodies were not found in blood cells of control aphids.

MORPHOLOGY OF THE CENTRAL NERVOUS SYSTEM

The central nervous system of the pea aphid was found to be similar to that described for the leafhopper Agallia constricta (Gil-Fernandez and Black 1965; Hirumi et al. 1967). The supraesophageal ganglion or brain is composed of proto-, deuto-, and tritocerebral lobes. The protocerebrum consists of a median region and lateral optic lobes. Extensive cephalization of the ventral ganglia has resulted in a single, large, ventral, subesophageal ganglionic mass. The ventral ganglion represents the fused subesophageal ganglion, pro-, meso-, and metathoracic ganglia, and the abdominal ganglia. In the posterior region of the tritocerebrum, circumesophagial connectives link the brain to the anterior of the subesophagial ganglionic mass. The central nervous system is concentrated in the head and prothoracic regions. Its position relative to other organs in the same area has been illustrated by Sylvester and Richardson (1970).

The search for PEMV virions in ultrathin sections through the brain and subesophageal ganglionic mass of viruliferous pea aphids yielded negative results. No virus particles were found in any of the cells comprising these organs. However, degenerating ganglion cells were occasionally observed in the subesophageal ganglionic mass (Fig. 39). These cells were observed in viruliferous aphids but were

also observed, though far less frequently, in ganglion cells of adult control aphids. The cells were distinguishable by their relative electron-lucidity. Membrane structures of these cells, especially the nuclear membrane, were indistinct (Fig. 39). While at this time a relationship between these degenerate cells and infection by PEMV appears doubtful, the possibility should not be entirely ruled out.

PEMV IN THE REPRODUCTIVE SYSTEM

The reproductive system of the pea aphid appeared as described by Uichanco (1924). It consisted of paired ovaries, each comprised of four telotrophic ovarioles. A succession of young egg cells or oöcytes and embryos in various stages of maturity is contained in each tapering ovariole.

Particles suspected of being virions of PEMV were localized within an ovariole of only one aphid. The particles were observed within an electron-dense inclusion body located in the cytoplasm of a follicular cell (Fig. 40). Similar inclusion bodies were rarely seen in follicular cells of other viruliferous aphids, but virus particles were not observed in their electron-dense matrixes. No such inclusions were found in the reproductive systems of control aphids. No virions were observed in embryos of viruliferous aphids.

Mycetome associated with the ovaries contained numerous symbionts. In the mycetome of one viruliferous aphid, an electron-dense structure was observed in the cytoplasm in close association with the nucleus of a mycetocyte (Fig. 41). The inclusion was similar to some of the viral structures observed in granular hemocytes, but did not contain virions of PEMV.

OTHER ORGANS

PEMV virions were not seen in the salivary system, central nervous system, mycetome, tracheal system, epidermis, or eye tissue of the pea aphid. However, this should not necessarily be considered as proof that PEMV does not multiply and/or accumulate in these organs.

The salivary system consists of a pair each of principal and accessory glands. The principal gland is bilobed and much larger than the single-lobed accessory gland. Ducts from the paired principal and accessory glands meet in the midline to form the common salivary duct which leads to the salivary syringe. The syringe empties into the salivary canal within the maxillary stylets. Figure 42 shows intracellular canaliculi and microtubules in an ultrathin section through salivary gland tissue.

# DISCUSSION AND CONCLUSION

## FACTORS INSTRUMENTAL IN THE LOCALIZATION OF PEMV IN THE VECTOR

Numerous techniques were employed in this investigation which together provided optimum conditions for PEMV localization in aphid tissues using ultrathin sectioning technique of electron microscopy. The choice of the aphid and PEMV strains used in the study was a most important factor. Numerous strains of the pea aphid had previously been screened on the basis of PEMV transmission efficiency (Tsai 1967). Subsequently, an East Lansing strain shown to be the most efficient vector was used exclusively in this research. Similarly a New York strain of PEMV was preferred to a California strain because it had previously been shown to have a shorter latent period, to remain longer in an inoculable form, and to be transmitted more efficiently by the pea aphid (Bath and Tsai 1969; Tsai 1967). Having chosen the best vector-virus combination, steps were taken to insure optimum uptake of virus by aphids during the allotted acquisition access periods.

Twelve day old pea plants showing the severest symptoms were used exclusively as source plants. The plant tissues available for aphid feeding were further limited to plant areas of optimum virus concentration. Only newly-born aphid nymphs were placed on the PEMV-infected source plants, and aphids were not removed from source plants until ready for processing for electron microscopy.

Success in detecting PEMV in the vector was also aided considerably by various preparative techniques. The basic methods were not new, but their adaptation to the search for virus afforded easy identification of vector tissues and PEMV virions. Preliminary dissections of numerous pea aphids under the stereomicroscope allowed me to become familiar with the morphology and relative positioning of the various organs and tissues of interest. This knowledge later greatly diminished the amount of time spent on the ultramicrotome, since I was thus able to immediately trim blocks of embedded whole aphids to points of interest. Moreover, the identification of areas within cross sections of whole aphids was made easier by first studying sections of individually embedded organs and tissues. Whole aphids (even adults) were embedded through the use of a low viscosity epoxy resin medium (Spurr 1969). Cross sections through whole aphids cut through numerous organs and tissues. This greatly increased the number of sections of various tissues examined during this investigation, and thereby increased the likelihood of observing virus. Having optimized the chances of virus being present in the vector, there remained the problem of recognizing the virus as virus when confronted with it.

Recognition of the virus in the vector was helped substantially by first studying PEMV virions in infected plant tissues and in purified preparations. This provided first hand information on the relative size and shape of PEMV particles. In addition, the interpretation of micrographs was greatly helped by the work of the numerous other researchers previously mentioned who have reported on the electron microscopy of plant-pathogenic viruses in leafhopper and aphid vector tissues. The <u>in situ</u> study of plant viruses in infected cells by

means of electron micrographs of ultrathin sections permits the precise localization of virions, provided these can be characterized morphologically. With small spherical virions such as PEMV this can present a formidable task, since cells normally contain numerous other submicroscopic spherical structures. Fortunately, in this investigation virions of PEMV were present in relatively large numbers and in easily recognizable structures within cells.

## IDENTIFICATION OF PARTICLES AS PEMV VIRIONS

The identification of the electron-dense particles found in the gut lumen, midgut epithelium, midgut sarcolemma, fat body, blood, and reproductive system of A. pisum as virions of PEMV was based on the fact that (1) the particles were only present in viruliferous aphids, (2) they exhibited relative uniformity of shape, (3) their size, 24 - 27 nm, and shape coincided with that of the purified virus (Bozarth and Chow 1966) and virus in ultrathin sections of plants (Shikata and Maramorosch 1966b), and (4) particles in the gut lumen and in the fat body, and the viral structures found in the cytoplasm of fatbody cells corresponded with those found by Shikata et al. (1966) in viruliferous pea aphids.

## PEMV IN THE GUT LUMEN

The scarcity of virions in the foregut suggests that a low titre of virus is taken in during the feeding process. Invasion of foregut cells by PEMV was probably prevented by the intact intima which is shed with each molt. Also, there is little evidence of any significant absorption in the foregut of insects (Treherne 1967).

The resolution of greatly enlarged electron micrographs of PEMV virions in the gut lumen did not reveal the ultrastructure (capsomeres) of the particles, but possibly did yield some information on the composition of individual particles. Virions in the lumen did not stain equally. Some were quite electron-dense, others less so, and still others appeared almost electron-transparent. While these variations in particle electron density may represent sectioning artifacts or virions in different stages of degradation, I consider it worthwhile to speculate on yet another explanation. It is primarily the ribonucleic acid (RNA) of small spherical viruses which picks up the electron stain; therefore, these density differences may, at least in part, reflect the relative nucleic acid content of the particles. Based on this assumption, the virions of the highest electron density would correspond to the lower component particles of purified preparations--particles with a greater RNA content. Similarly, less electron-dense virions would correspond to the upper component particles of purified preparations. The upper or "extra-virus" component of PEMV has been shown to contain 18% RNA (Bozarth and Chow 1966), or about 1/3 less RNA than the lower component (Shepherd and Ghabrial 1966; Shepherd et al. 1968). The few electron-lucid particles observed in the lumen were equal in size to electron-dense particles and may represent empty viral capsids or "ghosts."

Considering the low titre of virus ingested with plant sap, one might well wonder at the notable increase in virus titre observed within the midgut lumen, especially in the stomach region. Presumably, large numbers of progeny virions produced within infected midgut epithelial cells are eventually released into the lumen. Also,

ingested material (such as virus particles) may be concentrated and gradually accumulated (Moericke and Mittler 1965) in the stomach by the removal of water (Treherne 1967). Virions entering the midgut lumen with ingested sap, or by release from infected midgut epithelial cells, presumably are absorbed, reabsorbed, degraded, or excreted in the honeydew. The finding of PEMV in the midgut lumen could have been expected in view of the retention of PEMV-infectivity through molts (Nault et al. 1964). Transstadial passage requires that a virus be present in the midgut (Smith 1965). Also, the high concentration of virus particles in the hindgut lumen is not surprising. Several workers have mechanically transmitted PEMV to aphids by using insect honeydew as inoculum. Richardson and Sylvester (1965) have tested infected plant extracts, hemolymph from viruliferous aphids, and honeydew excreted by aphids feeding on virus-infected plants as sources of inoculum and found that of the three the use of honeydew as inoculum produced the highest rate of PEMV transmission. That the hindgut epithelium is not infected by PEMV can probably be attributed to the presence of an intima which is shed with each molt, as well as to the functioning of the hindgut in excretion (Gersch 1942).

## MULTIPLICATION OF PEMV IN MIDGUT EPITHELIAL CELLS

This research was not intended to present a description of the sequential invasion of aphid tissues. Since the invasion and infection process is quite rapid, the developmental cycle of infection would be more profitably studied by examining aphid tissues at various time intervals during the latent period of PEMV within the vector. Serological techniques with fluorescent (Sinha 1965) or isotopically

labeled antibody (Langenberg and Schlegel 1967) should also prove useful in such studies. However, it appears obvious from this research that infection is initiated in the stomach and intestinal regions of the midgut. The principal function of the insect midgut is digestion and absorption of nutrients (Waterhouse and Day 1953; Wigglesworth 1953). The stomach, therefore, is the first area of contact between ingested virions of PEMV and an absorbing tissue.

The method of viral entry into midgut epithelial cells could not be determined on the basis of the electron micrographs examined. Workers such as Ossiannilsson (1961) cannot accept the idea of the passage of whole plant virus particles through the gut wall in aphid vectors, and are looking for pores or ducts in the gut wall to explain the passage of virus from gut lumen to hemocoel. However, considering what has been written on the subject, a physical process such as pinocytosis (Forbes 1964) or phagocytosis seems to be a much more reasonable mechanism to explain passage of virus particles through cells. Indeed, tobacco mosaic virus has been shown to enter amoebae by pinocytosis (Holter 1959).

That virus multiplication does occur in the stomach and intestinal epithelium is indicated by the detection of cells which contain large numbers of PEMV in viroplasm-like areas and in defined cytoplasmic structures. Such cells are frequently bounded on either side by cells in which no virions are detectable. If all the virus in the infected cells was derived from virions ingested with plant sap, it appears improbable that regular aggregations would occur in only a few cells of a tissue containing many cells of a similar type. Also, the assembly of virions in infected cells and their subsequent release

into the midgut lumen would explain why virus particles are highly concentrated in the lumen of the stomach, intestine, and hindgut, but very sparse in the lumen of the foregut. Finally, with several other insect-borne, plant-pathogenic viruses, virus multiplication in the alimentary canal appears to be limited to the midgut.

Work by Shikata and Maramorosch (1967a) has shown that leafhopper midgut epithelial cells, especially of the filter chamber, serve as the earliest and one of the major sites of wound tumor virus multiplication in the leafhopper Agallia constricta. Sylvester and Richardson (1970) have shown that the stomach and anterior intestinal epithelium of the aphid Hyperomyzus lactucae is the initial site of sowthistle yellow vein virus multiplication in the vector. The hindgut epithelium was not infected. Infected foregut cells were observed, but these were thought to have resulted from a secondary invasion. The stomach and intestinal cells of H. lactucae are also suspected as serving as the initial site of lettuce necrotic yellows virus (LNYV) multiplication (O'Loughlin and Chambers 1967). Moreover, Ruppel (1968) has presented evidence that high concentrations of beef western yellows virus occur in the intestinal lumen and cellular cytoplasm of viruliferous green peach aphids. Finally, maize mosaic virus is suspected of developing in the endoplasmic reticulum of midgut epithelial cells of Peregrinus maidis (Delphacidae) (Herold and Munz 1965).

The exact site of virus assembly in the midgut epithelial cells cannot yet be designated with certainty. Virus particles were present within electron-dense areas which corresponded in appearance with the "virus factories" or viroplasms described in wound tumor virus infected plant and insect host cells (Maramorosch 1970; Shikata and Maramorosch

1967a), and in several animal RNA virus infections such as polio (Dales, Eggers, Tamm, and Palade 1965), mengo (Dales and Franklin 1962) and reo (Dales, Gomatos, and Hsu 1965). Similarity to previously described viroplasms cannot in itself be considered as proof that the electron-dense structures observed in PEMV-infected aphids are, in fact, the virus factories of PEMV. However, cells could be found which contained only one or a few viroplasm-like areas in which virions were either not present or present in low numbers around the periphery of the electron-dense matrixes. Since such cells appeared quite healthy otherwise, they were considered representative of the earliest infection stage. Viroplasm-like areas were not present in virus-free controls. Yet, it is still perhaps best to refer to them as viroplasm-like areas, rather than viroplasms, until their primary position in the developmental cycle of infection can be confirmed by a study of insect tissues processed at various intervals during the PEMV latent period. It should also be noted that viroplasms have not been demonstrated in PEMV-infected plant tissues.

While present data suggests that the viroplasm-like areas are the most likely sites for viral assembly, whether this function is eventually shared with the various other defined viral structures seen in the cytoplasm of midgut epithelial cells in later stages of infection is not known. These structures could represent temporary storage areas or even "blind alleys" for particles assembled elsewhere. The intensification of membrane assemblage, as evidenced by the numerous myelin figures often contained in the viral inclusions, would tend to indicate an attempt by the tissue to isolate the virus particles. Particles in processes and tubular structures observed in midgut

epithelial cells, as well as in infected fatbody cells, probably represent virions in transit to or from viral inclusions. Shikata et al. (1966) reported tubular structures in PEMV-infected plant cells and aphid fatbody cells.

## PEMV IN THE NUCLEI OF MIDGUT EPITHELIAL CELLS

Virions found in the nuclei of infected midgut cells are thought to have been assembled within the nucleus because (1) the particles were in relatively high concentration and free within the nuclear matrix, (2) they tended to be concentrated in the area of the nucleolus, and (3) their presence in the nuclei was rare. These same points could be used as arguments against a hypothesis that the virions arrived in the nucleus through cellular movement of particles either absorbed from the gut lumen or assembled in the cytoplasm. If viral assembly does indeed occur within the nucleus, as well as in the cytoplasmic matrix, the present data suggests that such assembly may be limited to midgut cells in the final stage of infection. But again, it must be emphasized that a step-by-step invasion of cells could not be followed accurately by the methods employed.

Shikata et al. (1965, 1966) have reported on the electron microscopy of PEMV in cells of infected plants. PEMV virions first appeared in the nuclei of infected cells, often in high concentration (Shikata and Maramorosch 1966b). The virions seemed to multiply rapidly, destroying the nucleolus and taking over the more active part of the nucleus. It was suggested that virions from the nucleus entered the cytoplasm through broken nuclear membranes. Also, Sylvester and Richardson (1970) have recently presented evidence that sowthistle

yellow vein virus (SYVV) is assembled in cell nuclei of the aphid Hyperomyzus lactucae. Lettuce necrotic yellows virus, an aphid-borne propagative virus with striking similarities to SYVV, has veen observed in the perinuclear space in a muscle cell of H. lactucae (O'Loughlin and Chambers 1967).

## ROLE OF VECTOR BLOOD IN THE TRANSMISSION PROCESS

Since Storey (1933) first demonstrated that blood of viruliferous insect vectors contained infective virus, numerous circulative plant viruses have been detected in the hemocytes of their insect vectors. It is assumed that the blood cells play an important role in the distribution of virus to various internal organs of the vector. Granados et al. (1968) have presented evidence for the multiplication of wound tumor virus (WTV) in leafhopper plasmatocytes and spherule cells. No WTV virions were observed in granular hemocytes. However, Sylvester and Richardson (1970) were unable to find SYVV in the hemolymph or hemocytes of the aphid, and suggested that the infection of the foregut, salivary system, ovaries, fat body, mycetome, and muscle resulted from the close association of these organs to one another and to the infected stomach and intestine of the vector.

The finding of PEMV in aphid blood cells was not unexpected. Since virus-free aphids injected with blood from aphids reared on infected pea plants had previously been shown to transmit PEMV to healthy plants (Nault et al. 1964; Richardson and Sylvester 1965; Schmutterer 1969; Schmutterer and Ehrhardt 1964), aphid blood promised to provide a good source of PEMV for electron microscopy. The presence of virions in plasmatocytes, spherule cells, and granular hemocytes suggests that

these cells may distribute PEMV within the pea aphid. Furthermore, the observance of particles in viroplasm-like areas within the cytoplasm of plasmatocytes and spherule cells indicates that these cells may support PEMV multiplication. Whether or not PEMV multiplication occurs in granular hemocytes is not known. Microcrystal formation apparently results from high concentrations of virions, but does not necessarily designate a site of virus multiplication. Granular hemocytes were frequently observed in contact with the basement membrane of the midgut, and virions may have entered the cells at this time. Granular hemocytes have been reported to be phagocytic to varying extents (Jones 1956; Yeager 1945).

The small number of blood cells in which PEMV was detected indicates that this tissue is probably an inefficient transporter of virions to other organs and tissues. No virions were observed in blood cells after the third instar stage. Therefore, virus multiplication, if it occurs, is apparently limited in scope and not capable of increasing or perhaps even of maintaining the virus titre in the blood. The low blood titre would presumably result in very little virus being carried to the salivary glands, and thus would explain why virions could not be localized in the salivary system which, in turn, also explains the erratic transmission record of the aphid vector, particularly during its adult stage.

PEMV virions produced in the midgut epithelium are the major source of virus for hemocytes. However, virus particles must first pass through the gut wall. The observed decrease in the blood titre of PEMV with each successive instar can be explained on the basis of a gradual decrease in the gut permeability to virus particles. Decreases

in gut permeability to virus with increases in vector age have been shown for several vectors of plant-pathogenic viruses (Bald and Samuel 1931; Merril and Ten Broeck 1965; Sinha 1960, 1963; Slykhuis and Watson 1958; Storey 1933; Watson and Sinha 1959; Zazhurilo and Sitnicova 1941).

The brief latent period of PEMV is quite understandable when one considers the rapidity with which PEMV invades and multiplies in the midgut cells. In addition, the gut permeability factor would explain why (1) nymphs acquire and transmit PEMV more efficiently than do adults, (2) the latent period is shorter in nymphs than in adults, and (3) the length of the latent period is a function of the dose of inoculum and the vector age at the time of acquisition.

Previous experimental results not favoring the hypothesis that PEMV is propagative in its vector include the fact that the vector's transmission efficiency gradually declines following acquisition; vectors can be "recharged" by additional acquisition feedings after their transmission efficiency has declined; and that PEMV cannot be maintained within a vector population by serial passage of hemolymph from one insect to another. Failure to maintain transmission efficiency is a weak argument against multiplication since this also occurs with the propagative potato leafroll virus (Miyamoto and Miyamoto 1966; Stegwee and Ponsen 1958). Also since virions produced in the gut epithelium are apparently the only continuing supply of PEMV for the blood (and hence the salivary glands), one would expect a gradual decline in the transmission efficiency as fewer and fewer virions are able to penetrate the gut wall. Furthermore, as aphids feed on healthy plants, virions in the gut lumen are continually flushed through by

virus-free sap. This could presumably cause a gradual decline in the number of PEMV-infected midgut cells. O'Loughlin and Chambers (1967) could find no virions of lettuce necrotic yellow virus in the lumen of the alimentary canal, when viruliferous Hyperomyzus lactucae aphids were allowed a 7-day acquisition access period on healthy test plants prior to fixation. "Recharging" can be explained by a temporary increase in the hemolymph titre from virus absorbed from the gut; an explanation used by Stegwee (1961) to explain the same phenomenon with potato leafroll. Indeed, Sylvester and Richardson (1966b) noted that the duration of the retention period of PEMV in recharged aphids was a function of the age of the insect at the time of acquisition rather than the length of the acquisition access period. This again underscores the hypothesis of gut permeability to PEMV virions decreasing with increasing vector age. Finally, failure to maintain infectivity through serial passage was perhaps the strongest argument against multiplication. However, while this may stand as an argument against significant virus multiplication in the blood tissue, it can hardly be used to disprove either limited multiplication in the vector or infection of a localized nature (such as in the midgut epithelium). It isn't surprising that attempts at serial passage of PEMV have ended in blind transfers, when one considers the low virus titre in the blood, especially in later instars and adults. Hemolymph is a very poor source of inoculum when compared to honeydew (Richardson and Sylvester 1965). Schmutterer and Ehrhardt (1964) showed that healthy aphids injected with hemolymph from viruliferous aphids demonstrated the highest transmission efficiency when allowed inoculation access feeding times of 96 hours or longer. This could represent the time required for PEMV

virions to penetrate through the gut wall, infect midgut cells, again penetrate the gut wall, and be carried by the blood to the salivary glands in a sufficiently high titre for successful inoculation. However, Shikata and Maramorosch (1967) found no virus in the gut epithelium of abdominally inoculated leafhoppers, which suggests that gut permeability to virus is a one way affair. Potato leafroll virus (PLRV) is also poorly transmissable by injection. It is interesting that PEMV and PLRV have in common the characteristics of a very short latent period, small particle size, and relatively high stability.

## PEMV--A PROPAGATIVE PLANT-PATHOGENIC VIRUS

This investigation has shown that PEMV multiplication can occur in the pea aphid vector Acyrthosiphon pisum. The major site of viral assembly is the cytoplasm of infected midgut epithelial cells. The nuclei of midgut cells may also contribute to an increase in virus during later stages of the infection process. Viroplasm-like areas observed within the cytoplasm of these cells are suspected of being the actual loci for virus assembly. PEMV virions were also detected in the cytoplasm of cells of the midgut sarcolemma, the fat body, the blood, and, in one instance, in the cytoplasm of a follicular cell. The presence of viroplasm-like areas in muscle fiber cells, plasmatocytes, and spherule cells, and the localization of aggregates of PEMV in electron-dense structures within granular hemocytes and cells of the fat body suggest that limited viral assembly may also occur in one or more of these cell types.

The results of this transmission electron microscope research are supported by the data of previous nonmicroscopic studies.

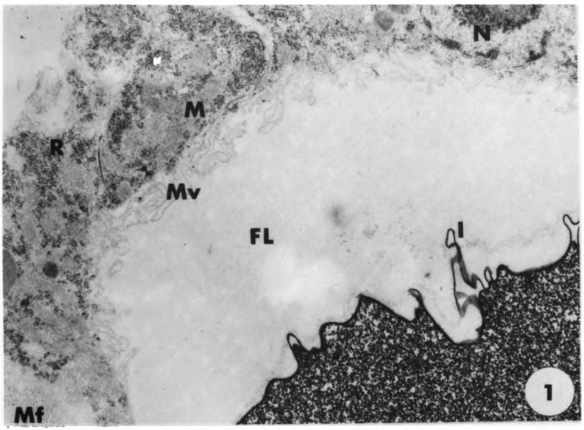
Transstadial passage; the presence of a latent period; relatively long persistence of vector inoculativity; and that ambient temperature exerts an effect on both retention of inoculativity and the duration of the latent period of the same order of magnitude expected with living systems have all previously been used to support the hypothesis that PEMV is propagative in the aphid vector.

It is expected that further investigations will reveal the presence of PEMV in other organs of the vector, particularly in the salivary glands. Further studies of tissues processed at various intervals during the latent period should help toward a more complete understanding of the sequence of the invasion process. Fluorescent and isotopic labeling techniques should also prove helpful in this respect. Recent advances in aphid tissue culture (Peters and Black 1970) offer the possibility of further study of cells infected *in vitro*.

FIGURES

Fig. 1. Cross section through the foregut. Magnification: X12,000. Nuclei(N) are ovoid and occupy most of the central part of cells. Epithelial cells with overlapping edges contain numerous ribosomes(R) and mitochondria(M), and bear sparse microvilli(Mv) at the free border surrounding the intima(I) and foregut lumen(FL). A portion of a muscle fiber cell(Mf) can be seen in the lower left corner of micrograph.

Fig. 2. PEMV virions in stomach lumen. Magnification: X38,000. Particles aggregate along the peripheral margins of ingested food(IF) materials.


N
M
R
Mv
FL
I
Mf
1

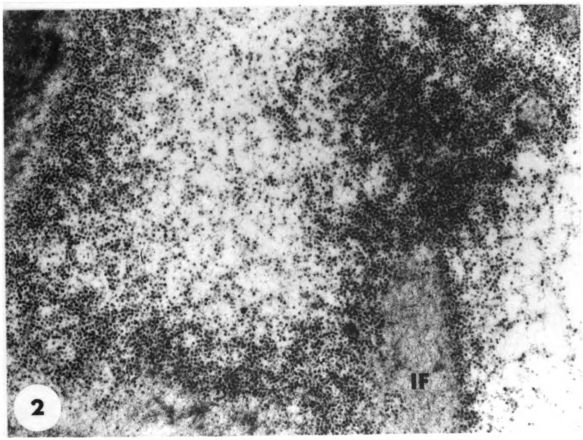
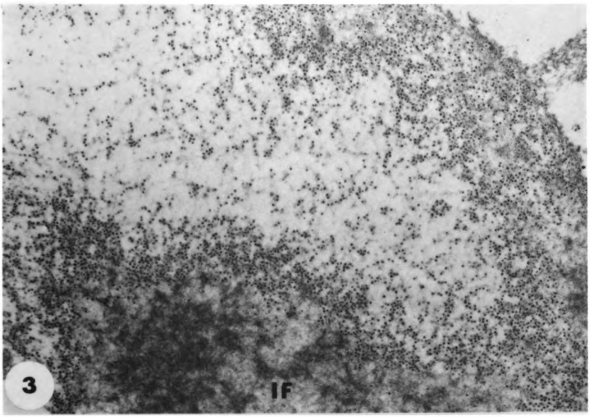

IF
2

Fig. 3. PEMV virions in stomach lumen. Magnification: X27,000. Particles are loosely scattered in some areas, but tend to aggregate along the peripheral margins of ingested food(IF) materials.

Fig. 4. High magnification of PEMV virions in Figure 3. Magnification: X70,000. Virions surrounding ingested food(IF) are not stained equally. Some are quite electron-dense(a), others less so(b), and still others appear almost electron-transparent(c). The hexagonal profile is predominant. However, a few particles exhibit a pentagonal profile(d).


3
IF

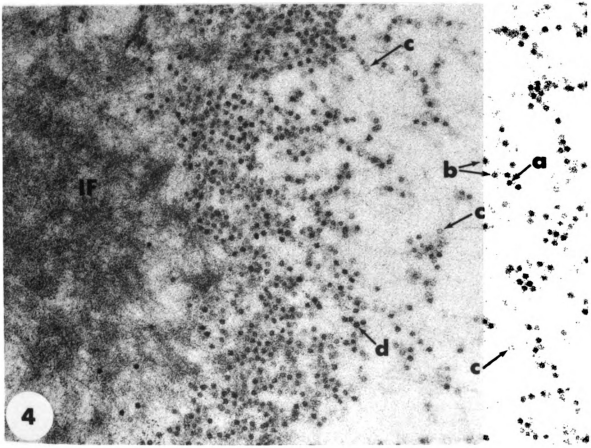
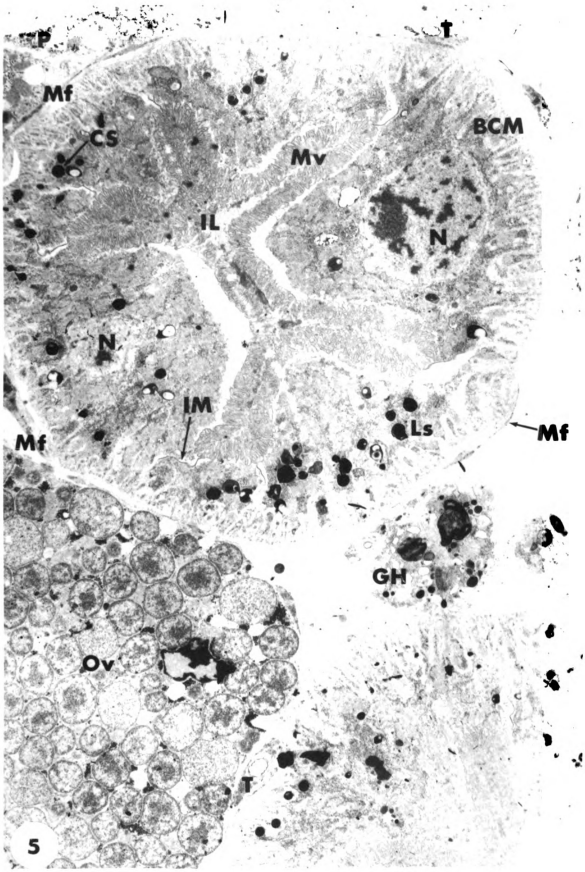
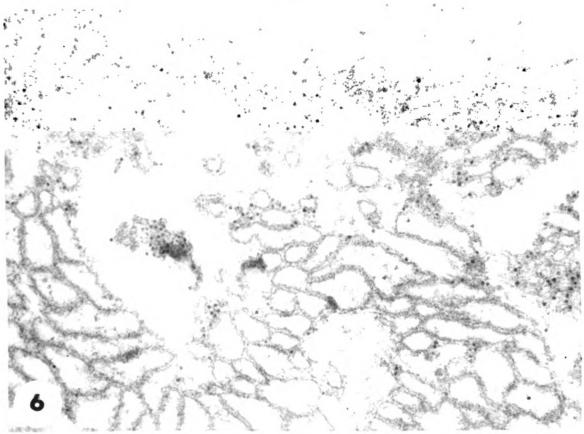

4
IF
c
b
a
c
d
c

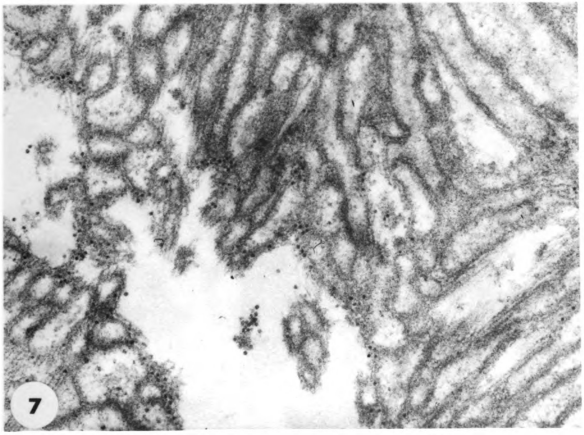
Fig. 5. Cross section of intestinal region of viruliferous aphid. Magnification: X4,000. The "melting" of lipoid spheres(Ls) has made holes in the section. BCM, Basement cell membrane. CS, Concentrically laminated sphere. GH, Granular hemocyte. IL, Intestinal lumen. IM, Intercellular membrane. Mf, Muscle fiber cell. Mv, Microvilli. N, Nucleus. Ov, Ovary. P, Plasmatocyte. T, Tracheoblast.


P
t
Mf
CS
Mv
BCM
IL
N
N
IM
Mf
Ls
Mf
GH
Ov
T
5

Figs. 6 and 7. PEMV virions associated with the microvillous borders of the stomach and intestine, respectively. Magnifications: X43,000 and X50,000, respectively.

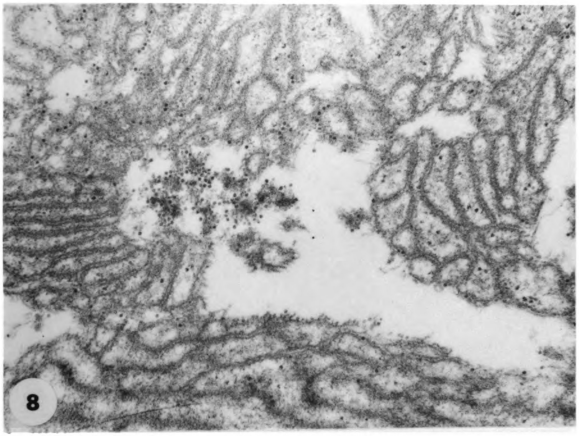


6



7

Figs. 8 and 9. PEMV virions associated with the microvillous border of the intestine. Magnifications: X43,000 and X27,000, respectively.


8

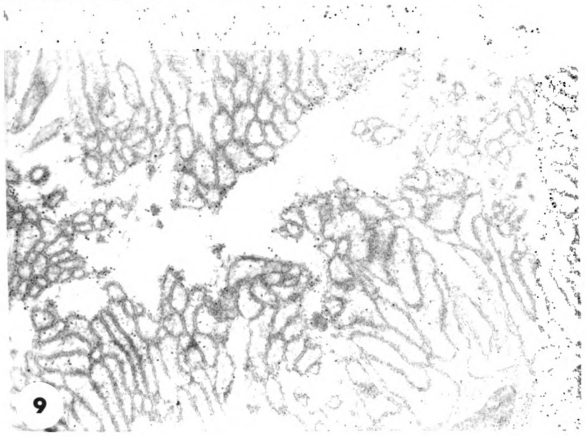
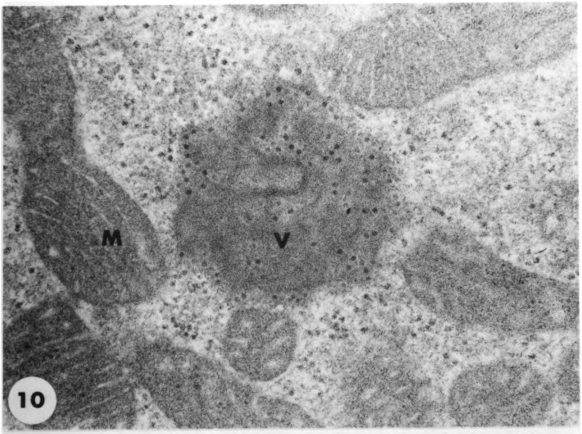

9

Fig. 10. Viroplasm in apical part of stomach epithelial cell. Magnification: X66,000. Viroplasm(V) is in close proximity to numerous mitochondria(M).

Fig. 11. Ultrathin section through infected stomach epithelial cell. Magnification: X32,000. The membrane structures of cytoplasmic organelles such as the nuclear membrane, Golgi complex(G), and Mitochondria(M) are indistinct. PEMV virions can be seen free in the cytoplasm and in various types of inclusion structures. The ultrastructural integrity of the cytoplasmic matrix is changed due to the depletion of ribosomes. a, Process from the periphery of larger viral structure. b, Virions in tubular structure. c, Virions in electron-dense matrix of disintegrating mature viroplasm. d, Electron-dense, lysosome-like, inclusion body. N, Nucleus.


M
V
10

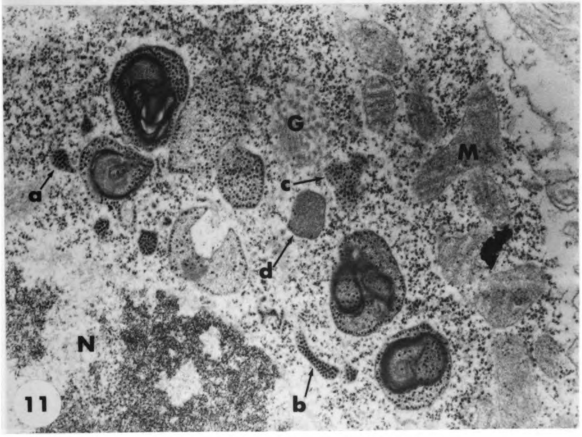
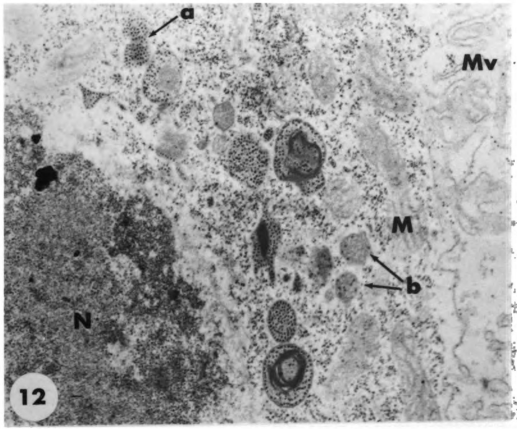
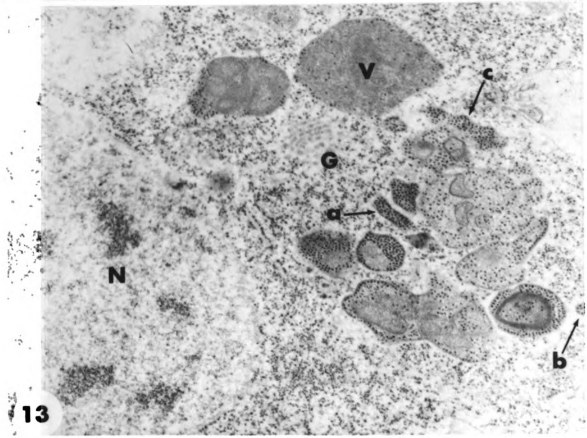

G
M
a
c
d
N
11
b

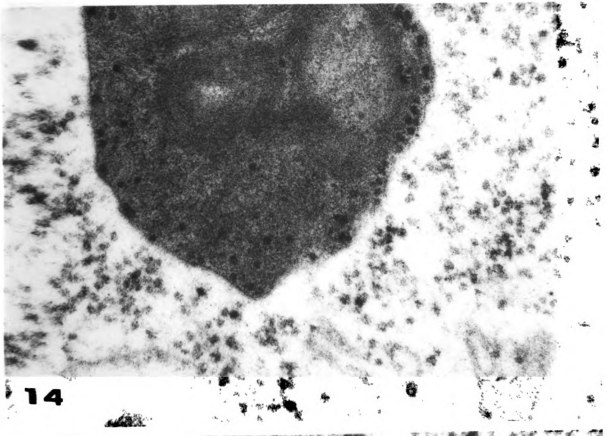
Fig. 12. Ultrathin section through infected midgut epithelial cell. Magnification: X25,000. Note the multimembranous figures enclosed within larger viral structures. Two deteriorated mitochondria are just visible in the upper left corner of the micrograph. a, Virions in electron-dense matrix of disintegrating mature viroplasm. b, Electron-dense, lysosome-like, inclusion bodies. IL, Intestinal lumen. M, Mitochondria. Mv, Microvilli. N, Nucleus.

Fig. 13. Another portion of the cell shown in Figure 12. Magnification: X25,000. a, PEMV virions in tubular structure. b, Cross section of tubular structure. c, Virions in electron-dense matrix of a deteriorating viral structure. G, Golgi complex. N, Nucleus. V, Viroplasm.


a
Mv
IL
M
b
N
12


V
c
G
a
N
b
13

Figs. 14 and 15. PEMV virions within defined cytoplasmic structures in infected stomach cells. Magnification: X99,000. Note the multimembranous, myelin-like figures.


14

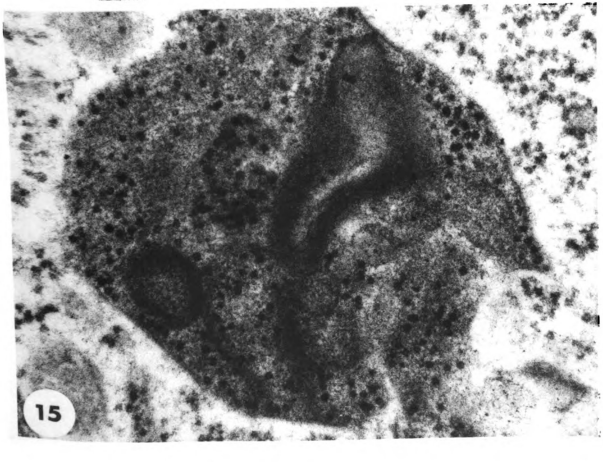
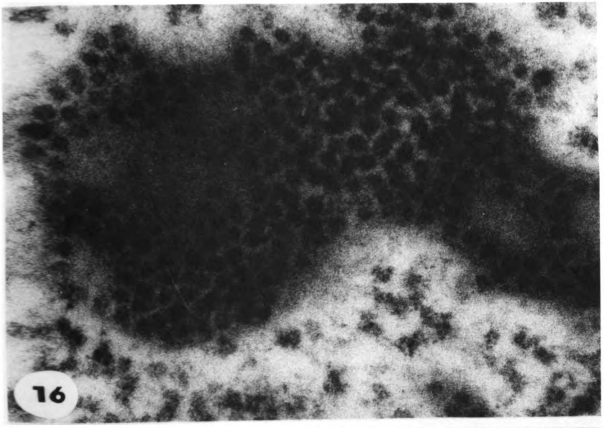
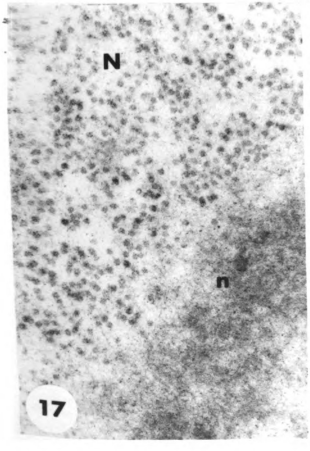

15

Fig. 16. Accumulation of PEMV virions in the cytoplasm of a midgut epithelial cell. Magnification: X189,000.

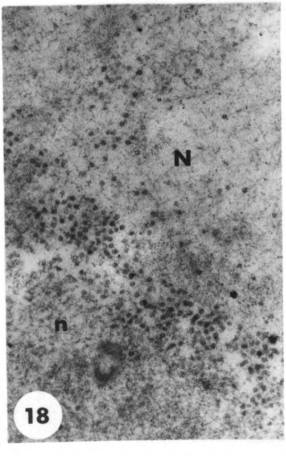
Figs. 17 and 18. PEMV virions in nuclei of infected stomach cells. Magnifications: X78,000 and X64,000, respectively. Note the homogenous, electron-transparent matrixes of the nucleus(N) and nucleolus(n). Virions were mainly found concentrated around the periphery of the nucleolus.



16



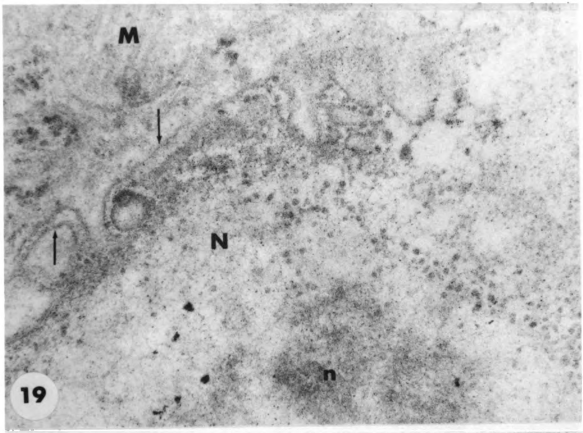

17




18

Fig. 19. PEMV virions in nucleus of infected intestinal cell. Magnification: X72,000. Note the deteriorated nuclear membrane(arrows) and mitochondrion(M). The cytoplasmic matrix and nuclear substance have been changed into homogenous fine granules. n, nucleolus.

Fig. 20. High concentration of PEMV virions in hindgut lumen. Magnification: X27,000.


M
N
n
19

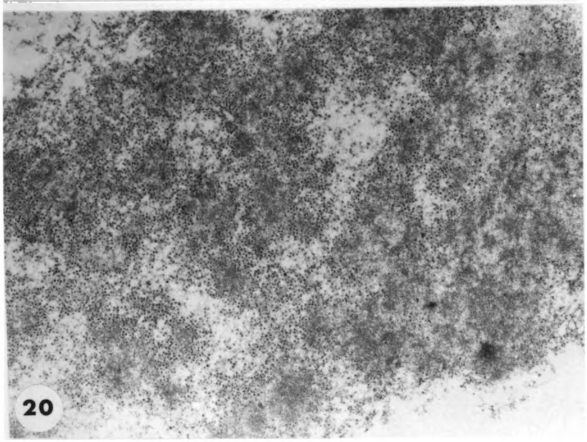
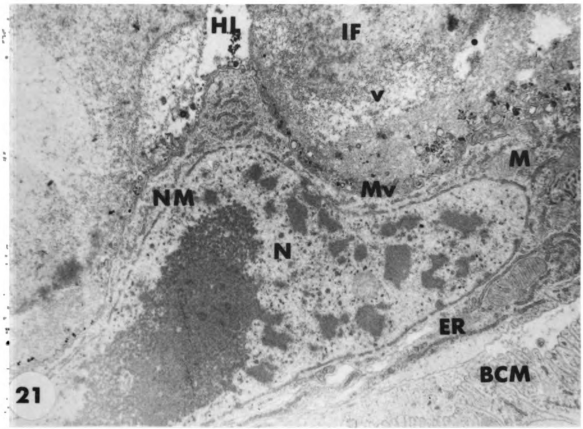

20

Fig. 21. Cross section through hindgut of viruliferous aphid. Magnification: X14,000. BCM, Basement cell membrane of midgut. ER, Endoplasmic reticulum. HL, Hindgut lumen. IF, Ingested food material. M, Mitochondria. Mv, Microvilli. N, Nucleus. NM, Nuclear membrane. v, Virions.

Fig. 22. PEMV virions in hindgut lumen. Magnification: X21,000. HL, Hindgut lumen. IF, Ingested food material. M, Mitochondria. Mv, Microvilli.


HL
IF
V
M
NM
Mv
N
ER
BCM
21

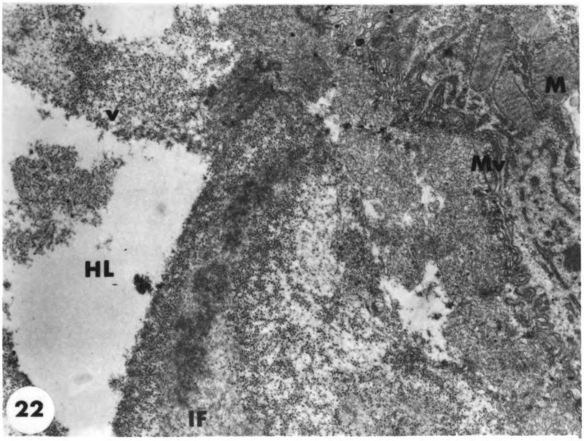
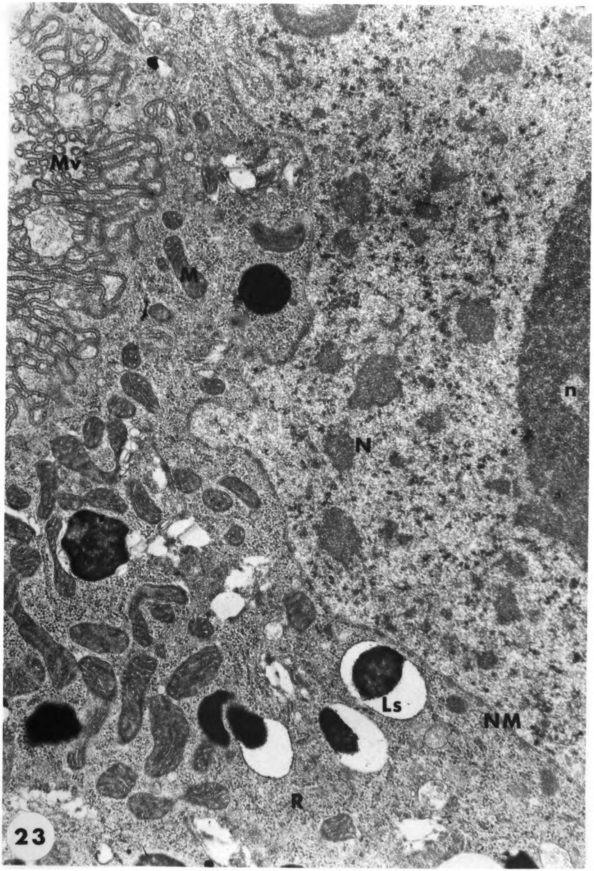
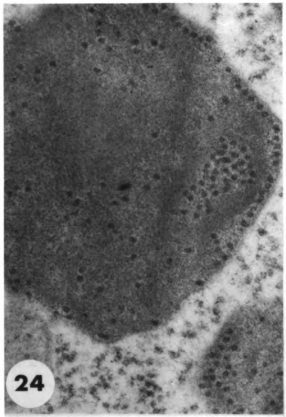

V
M
Mv
HL
22
IF

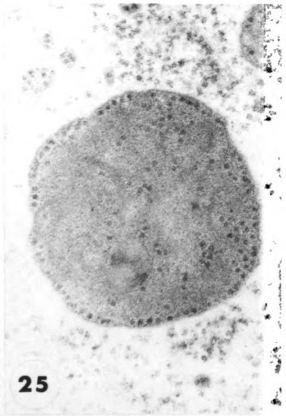
Fig. 23. Ultrathin section of healthy midgut epithelial cell. Magnification: X28,000. The "melting" of lipoid spheres(Ls) has made holes in the section. M, Mitochondria. Mv, Microvilli. N, Nucleus. n, Nucleolus. NM, Nuclear membrane. R, ribosomes.

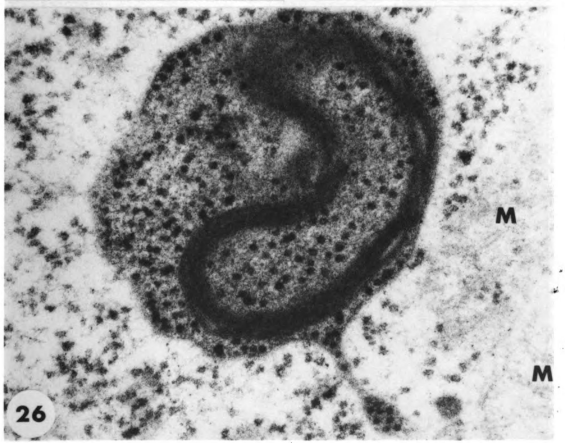

Mv
M
N
n
Ls
NM
R
23

Figs. 24 and 25. PEMV virions in electron-dense areas within the cytoplasm of muscle fiber cells. Magnification: X78,000 and X64,000, respectively.

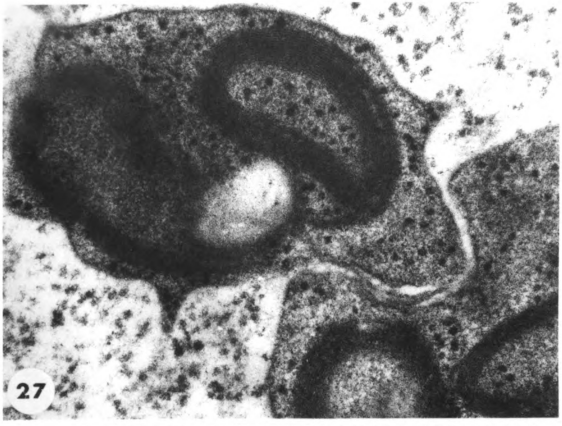
Fig. 26. PEMV virions in a defined structure within the cytoplasm of a fatbody cell. Magnification: X99,000. Note the deteriorated mitochondria(M), the myelin-like figure, and also the process by which some particles are separated from the main viral structure.


24


25


M
M
26

Figs. 27 and 28. PEMV virions in defined structures within the cytoplasm of fatbody cells. Magnification: X99,000. Note the myelin-like figures, and also the area of union between the electron-dense matrixes of the two viral structures in Figure 27. M, Mitochondrion.


27

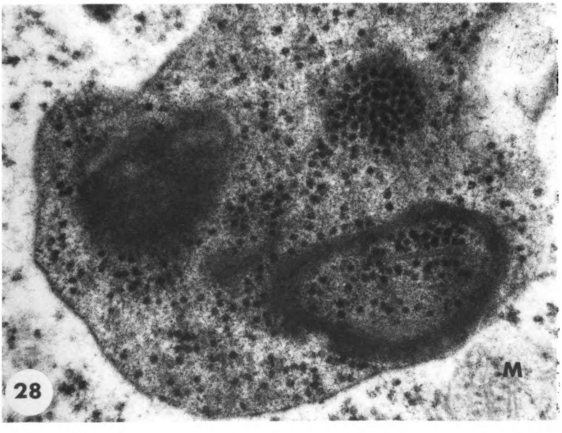
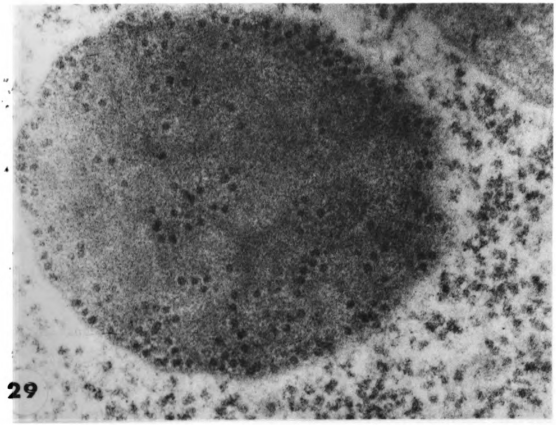

28
M

Fig. 29. Viroplasm-like areas within the cytoplasm of a spherule cell. Magnification: X99,000.

Fig. 30. Viroplasm-like area in a plasmatocyte. Magnification: X69,000. A few particles(arrow) appear to be leaving (or entering) the viroplasm(V). M, Mitochondria.


29

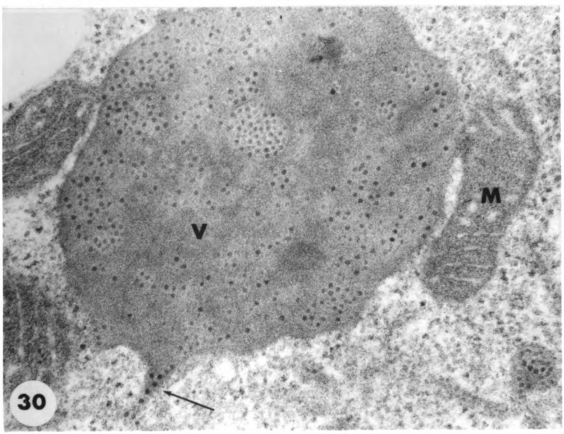
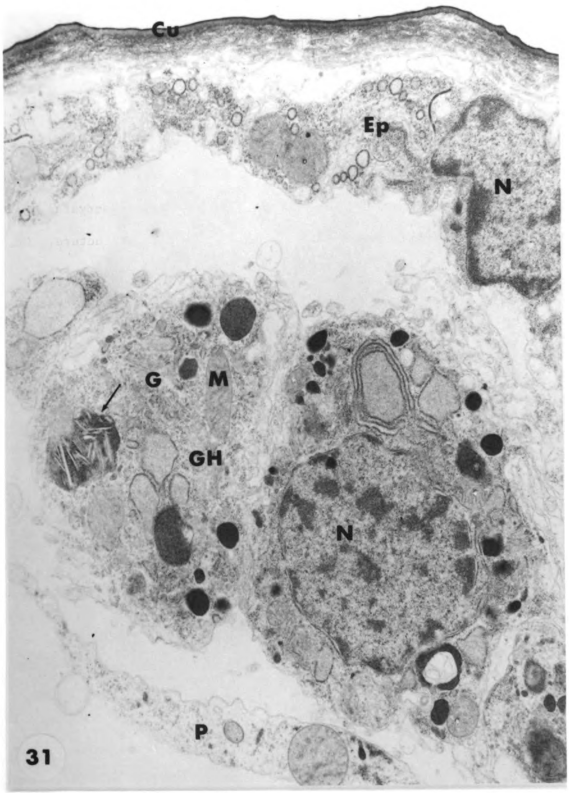
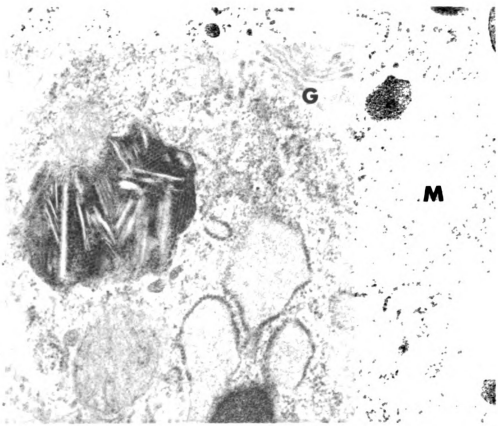

V
M
30

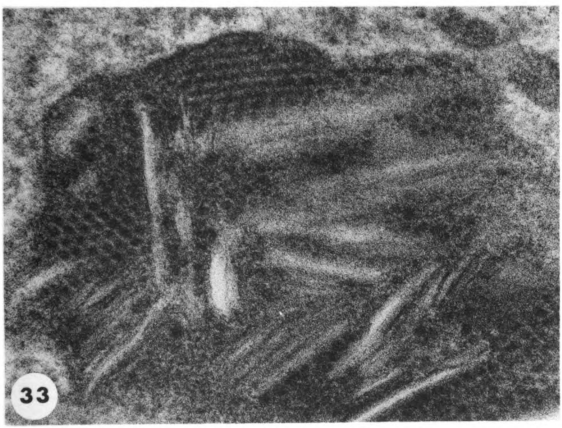
Fig. 31. PEMV virions within an electron-dense structure in a granular hemocyte. Magnification: X18,000. A virus microcrystal (arrow) is at the periphery of the electron-dense viral structure. Cu, Cuticle. Ep, Epidermis. G, Golgi complex. GH, Granular hemocyte. M, Mitochondrion. N, Nucleus. P, Plasmatocyte.


Cu
Ep
N
G
M
GH
N
P
31

Figs. 32 and 33. High magnifications of viral structure in Figure 31. Magnifications: X39,000 and 130,000, respectively. G, Golgi complex. M, Mitochondrion.



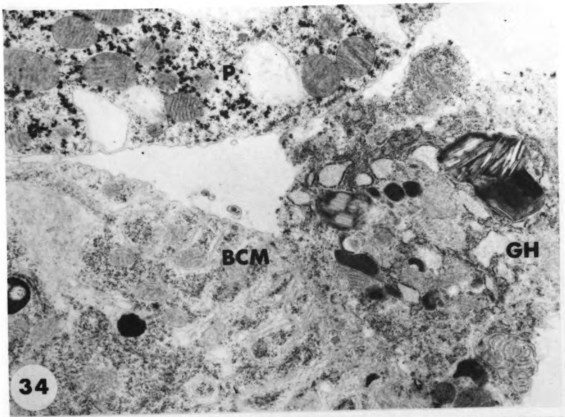

32



33

Fig. 34. Blood cell adjacent to midgut of viruliferous aphid. Magnification: X14,000. The granular hemocyte(GH) contains a septate, electron-dense structure. BCM, Basement cell membrane of midgut. P, Plasmatocyte.

Fig. 35. Blood cell adjacent to hindgut of viruliferous aphid. Magnification: X11,000. The granular hemocyte(GH) contains a septate, electron-dense structure in close proximity to the nucleus(N). Virions(v) are highly concentrated in the hindgut lumen(HL).


P
BCM
GH
34

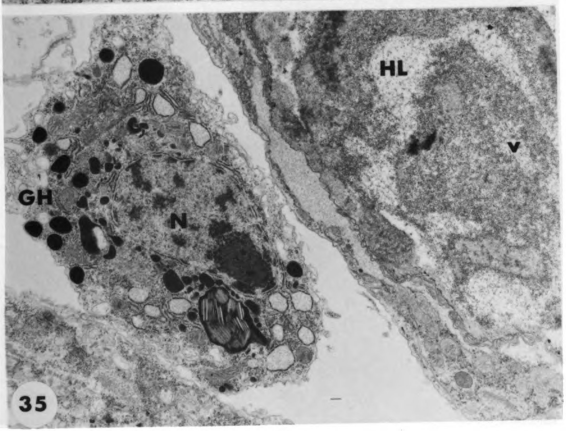
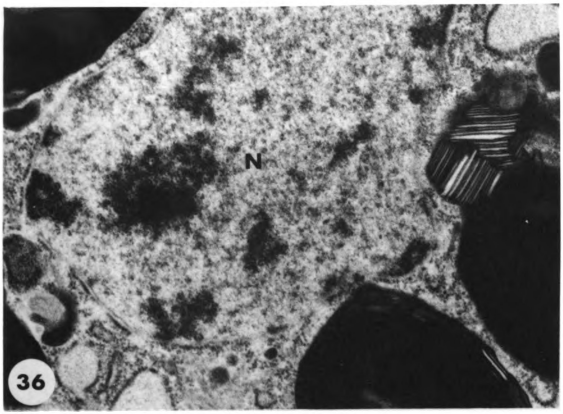

HL
V
GH
N
35

Fig. 36. Nucleus of granular hemocyte indented by the formation of a septate, electron-dense structure. Magnification: X31,000.

Fig. 37. PEMV virions within a septate, electron-dense structure in a granular hemocyte. Magnification: X23,000. The nucleus(N) of the granular hemocyte(GH) is apparently indented by the viral structure. M, Mitochondria. P, Plasmatocyte.


N
36

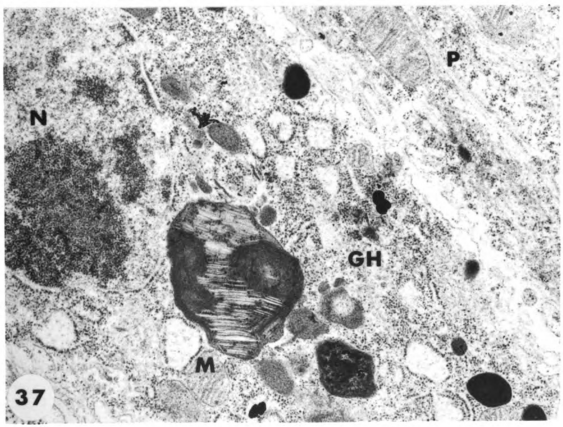
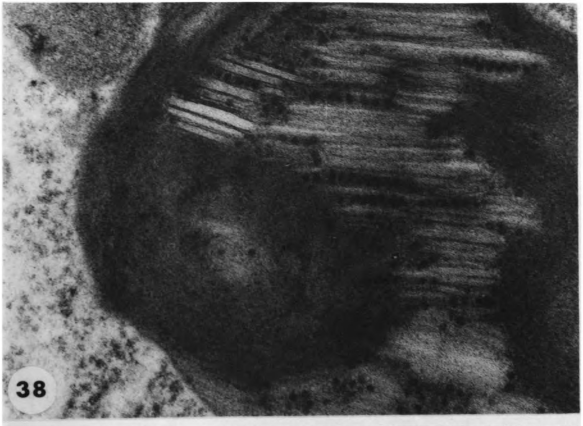

N
P
GH
M
37

Fig. 38. High magnification of viral structure in Figure 37. Magnification: X99,000. "Strings" of virions can be seen along and between the septa of the structure.

Fig. 39. Degenerating ganglion cell (note enclosed area) in the subesophageal ganglionic mass of a viruliferous aphid. Magnification: X11,000.


38

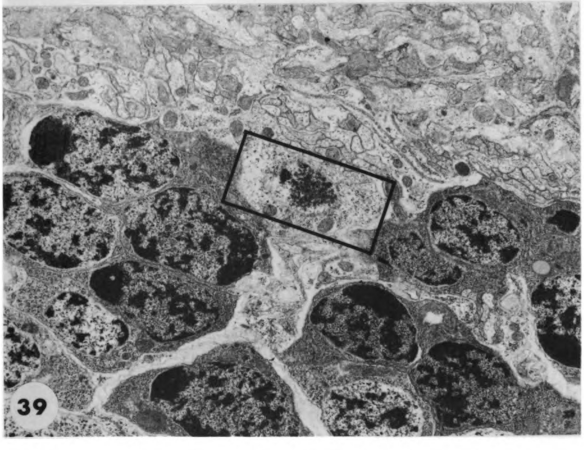
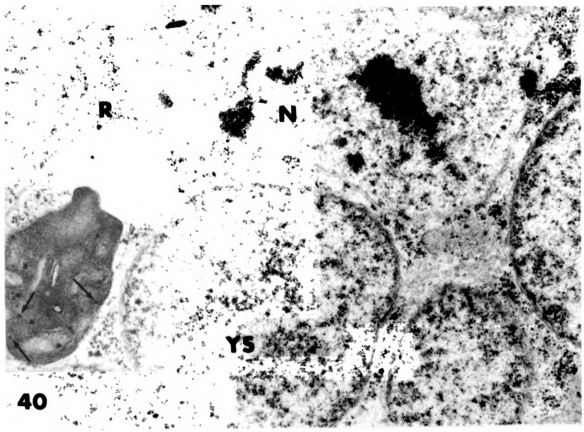
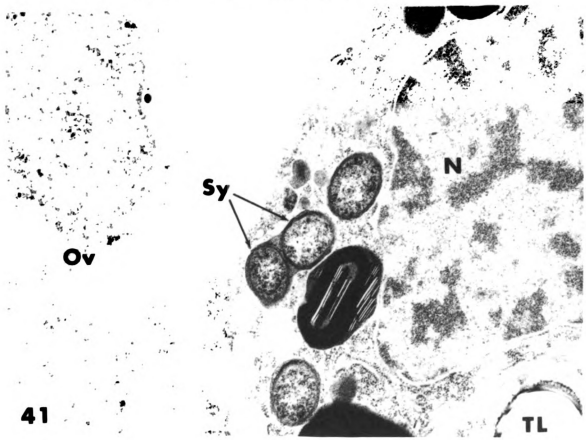

39

Fig. 40. PEMV virions in an electron-dense viral structure within the cytoplasm of a follicular cell. Magnification: X50,000. Smaller particles(arrows) may represent a viral developmental stage, or sectioning artifacts.

Fig. 41. Symbionts in the cytoplasm of a mycetocyte. Magnification: X25,000. An electron-dense structure without virions is adjacent to the nuclear membrane. N, Nucleus of mycetocyte. Ov, Ovary. Sy, Symbionts. TL, Tracheal lumen.

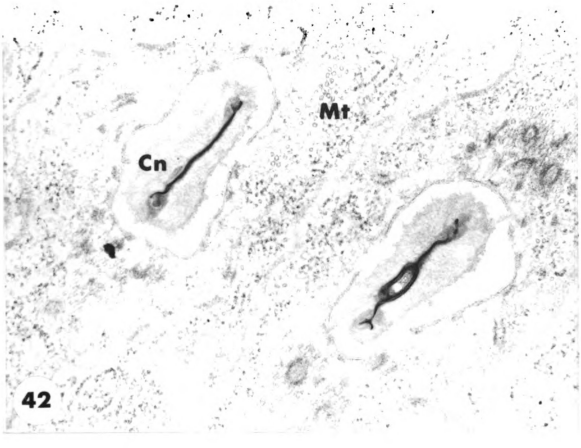



40




41

Fig. 42. Ultrathin section through salivary gland tissue of a viruliferous aphid. Magnification: X43,000. Cn, Intracellular canaliculi. Mt, Microtubules.


Cn
Mt
42

# LIST OF REFERENCES

LIST OF REFERENCES

Administración de Estabilización del Arroz. 1959. Informaciones de Interes General en Relacion con el Arroz. 12:20-21.

Agrawal, H. C. 1964. Identification of cowpea mosaic virus isolates. Mededel. Landbouwhogeschool Wageningen 64-5. 53 p.

Ainsworth, G. C. 1940. The identification of certain viruses found infecting leguminous plants in Great Britain. Ann. Appl. Biol. 27:218-227.

Bald, J. G. and G. Samuel. 1931. Investigations on "spotted wilt" of tomatoes. Australian, Commonwealth, Council Sci. Ind. Research Bull. 54.

Bancroft, J. B. 1962. Purification and properties of bean pod mottle virus and associated centrifugal and electrophoretic components. Virology 16:419-427.

Bancroft, J. B., and P. Kaesberg. 1960. Macromolecular particles associated with alfalfa mosaic virus. Biochim. Biophys. Acta 39:519-527.

Bath, J. E. 1964. Transmission of pea enation mosaic virus by green peach, potato, and pea aphids. Ph.D. thesis, University of Wisconsin. 124 p.

Bath, J. E., and R. K. Chapman. 1966. Efficiency of three aphid species in the transmission of pea enation mosaic virus. J. Econ. Entomol. 59:631-634.

1967. Differential transmission of two pea enation mosaic virus isolates by the pea aphid, Acyrthosiphon pisum (Harris). Virology 33:503-506.

1968. Influence of aphid stage on acquisition and inoculation phases of pea enation mosaic virus transmission. Ann. Entomol. Soc. Amer. 61:906-909.

Bath, J. E., and J. H. Tsai. 1969. The use of aphids to separate two strains of pea enation mosaic virus. Phytopathology 59:1377-1380.

Bawden, F. C. 1964. Plant viruses and virus diseases. 4th ed. Ronald Press, New York. 361 p.

Bils, R. F., and C. E. Hall. 1962. Electron microscopy of wound-tumor virus. Virology 17:123-130.

Black, L. M. 1944. Some viruses transmitted by agallian leafhoppers. Proc. Amer. Phil. Soc. 88:132-144.

1945. A virus tumor disease of plants. Amer. J. Botany 32:408-415.

1948. Transmission of clover club-leaf virus through the egg of its insect vector. Phytopathology 38:2.

1953a. Occasional transmission of some plant viruses through the eggs of their insect vectors. Phytopathology 43:9-10.

1953b. Transmission of plant viruses by Cicadellids. Advances Virus Res. 1:69-89.

1953c. Viruses that reproduce in plants and insects. Ann. N. Y. Acad. Sci. 56:389-413.

1954. Parasitological Reviews. Arthropod transmission of plant viruses. Exptl. Parasitol. 3:72-104.

1959. Biological cycles of plant viruses in insect vectors, p. 157-185. In F. M. Burnet and W. M. Stanley, (ed.), The viruses, biochemical, biological, and biophysical properties. Vol. 2. Academic Press, New York.

1962. Some recent advances on leafhopper-borne viruses, p. 1-9. In Karl Maramorosch (ed.), Biological transmission of disease agents. Academic Press, New York.

Black, L. M., and M. K. Brakke. 1952. Multiplication of wound-tumor virus in an insect vector. Phytopathology 42:269-273.

1954. Serological reactions of a plant virus transmitted by leafhoppers. Phytopathology 44:482.

Black, L. M., and R. Markham. 1963. Base-pairing in the ribonucleic acid of wound-tumor virus. Neth. J. Pl. Path. 69:215.

Bozarth, R. F., and C. C. Chow. 1965. Pea enation mosaic virus: a new local lesion host. Phytopathology 55:492-493.

1966. Pea enation mosaic virus: purification and properties. Contrib. Boyce Thompson Inst. 23:301-309.

1968. Pea enation mosaic virus: differential suspension of virus components in buffer and sucrose. Virology 36:506-508.

Bradley, R. H. E. 1961. Our concepts: on rock or sand?, p. 528-533. In Recent advances in botany. Univ. of Toronto Press, Toronto, Canada.

1964. Stylet-borne viruses, p. 148-174. In M. K. Corbett and H. D. Sisler (ed.), Plant virology. Univ. Florida Press, Gainesville.

Bradley, R. H. E., and E. S. Sylvester. 1962. Do aphids carry transmissible sugar beet yellows virus via their stylets? Evidence from ultra-violet irradiation. Virology 17:599-600.

Brakke, M. K. 1969. Isolation and purification of vector-borne plant viruses, p. 527-555. In, Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Brakke, M. K., A. E. Vatter, and L. M. Black. 1954. Size and shape of wound-tumor virus. Brookhaven Symp. Biol. 6:137-156.

Breedis, C., L. Berwick, and T. F. Anderson. 1962. Fractionation of Shope papilloma virus in cesium chloride density gradients. Virology 17:84-94.

Bustrillos, A. D. 1964. Purification, serology, and electron microscopy of pea enation mosaic virus. Ph.D. Thesis, Michigan State University.

Carter, W. 1961. Ecological aspects of plant virus transmissions. Ann. Rev. Entomol. 6:347-370.

1962. Insects in relation to plant disease. Wiley, New York. 705 p.

Chalfant, R. B. 1959. Transmission of cabbage viruses A and B by the cabbage and green peach aphids. Ph.D. Thesis, Univ. Wisconsin, Madison, 187 p.

Chambers, T. C., N. C. Crowley, and R. I. B. Francki. 1965. Localization of lettuce necrotic yellows virus in host leaf tissue. Virology 27:320-328.

Chapman, R. K., and J. E. Bath. 1968. The latent period of pea enation mosaic virus in three of its aphid vectors with emphasis on adult versus nymph comparisons. Phytopathology 58:494-499.

Chaudhuri, R. P. 1950. Studies on two aphid-transmitted viruses of leguminous crops. Ann. Appl. Biol. 37:342-354.

Crowley, N. C., B. D. Harrison, and R. I. B. Francki. 1965. Partial purification of lettuce necrotic yellows virus. Virology 26: 290-296.

Dales, S., H. J. Eggers, I. Tamm, and G. E. Palade. 1965. Electron microscopic study of the formation of poliovirus. Virology 26:379-389.

Dales, S., and R. M. Franklin. 1962. A comparison of the changes in fine structure of L cells during single cycles of viral multiplication, following their infection with the viruses of Mengo and encephalomyocarditis. J. Cell Biol. 14:281-302.

Dales, S., P. J. Gomatos, and K. C. Hsu. 1965. The uptake and development of reovirus in strain L cells followed with labeled viral ribonucleic acid and ferritin-antibody conjugates. Virology 25:193-211.

Davidson, J. 1913. The structure and biology of Schizoneura lanigera, Hausmann Part I. The apterous viviparous female. Quart. J. Micro. Sci. 58:653-701.

Day, M. F. 1955. The mechanism of the transmission of potato leaf roll virus by aphids. Australian J. Biol. Sci. 8:498-513.

Day, M. F., and H. Irzykiewicz. 1953. Feeding behavior of the aphids Myzus persicae and Brevicoryne brassicae, studies with radiophosphorous. Australian J. Biol. Sci. 6:98-108.

1954. On the mechanism of transmission of non-persistent phytopathologic viruses by aphids. Australian J. Biol. Sci. 7: 251-273.

Day, M. F., and D. G. Venables. 1961. The transmission of cauliflower mosaic by aphids. Australian J. Biol. Sci. 14:187-197.

Day, M. F., and M. Zaitlin. 1959. Infectivity and electron microscopy of extracts of Physalis floridana plants infected with potato leafroll virus. Phytopathol. Z. 34:83-85.

Diener, T. O., and I. R. Schneider. 1966. The two components of tobacco ringspot virus nucleic acid: origin and properties. Virology 29:100-105.

Duffus, J. E. 1963. Possible multiplication in the aphid vector of sowthistle yellow vein virus, a virus with an extremely long insect latent period. Virology 21:194-202.

Ehrhardt, P. and H. Schmutterer. 1964. Untersuchungen über die Beziehungen zwischen dem Enationenvirus der Erbse und seinen Vektoren. I. Ubertragungsversuche mit verschiedenen, an viruskranken Vicia faba-Pflanzen aufgewachsenen Blattlausarten. Z. Pflanzenkrankh. (Pflanzenpath.) Pflanzenschutz 71:381-394.

1965. Untersuchungen über die Beziehungen zwischen dem Enationenvirus der Erbse und seinen Vektoren. III. Das Übertragungsvermögen verschiedener Entwicklungsstadien von Acyrthosiphon pisum (Harr.) nach unterschiedlichen Saugzeiten an virusinfizierten Vicia faba-Pflanzen. Phytopathol. Z. 52:73-88.

Everett, T. R., and H. A. Lamey. 1969. Hoja blanca, p. 361-377. In Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Forbes, A. R. 1964. The morphology, histology, and fine structure of the gut of the green peach aphid, Myzus persicae (Sulzer) (Homoptera: Aphididae). Mem. Entomol. Soc. Can. No. 36. 75 p.

Forbes, A. R., and H. R. MacCarthy. 1969. Morphology of the Homoptera, with emphasis on virus vectors, p. 211-234. In Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Frazier, N. W. 1966a. Pseudo mild yellow edge: a new strawberry virus disease in the Fragaria virginiana indicator clone $M_1$. Phytopathology 56:571-572.

1966b. Nonretention of two semipersistent strawberry viruses through ecdysis by their aphid vector. Phytopathology 56: 1318-1319.

Frazier, N. W., and A. F. Posnette. 1958. Relationships of the strawberry viruses of England and California. Hilgardia 27: 455-513.

Fukushi, T. 1933. Transmission of virus through the egg of an insect vector. Proc. Imp. Acad., Japan. 9:457-460.

1934. Studies on the dwarf disease of rice plant. J. Faculty Agr. Hokkaido Univ. 37:31-164.

1939. Retention of virus by its vectors through several generations. Proc. Imp. Acad., Japan. 15:142-145.

1940. Further studies on the dwarf disease of rice plants. J. Faculty Agr. Hokkaido Univ. 45:83-154.

1969. Relationships between propagative rice viruses and their vectors, p. 279-301. In Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Fukushi, T., and E. Shikata. 1963. Localization of rice dwarf virus in its insect vector. Virology 21:503-505.

Fukushi, T., E. Shikata, and I. Kimura. 1962. Some morphological characters of rice dwarf virus. Virology 18:192-205.

Fukushi, T., E. Shikata, I. Kimura, and M. Nemoto. 1960. Electron microscope studies on the rice dwarf virus. Proc. Japan Acad. 36:352-357.

Gamez, R., L. M. Black, and R. MacLeod. 1967. Reexamination of the serological relationship between wound tumor virus and reovirus. Virology 32:163-165.

Gersch, M. 1942. Verteilung und Ausscheidung von Fluorescein bei Aphiden. II. Beitrag zur Exkretion bei Insekten. Z. Vergl. Physiol. 29:506-531.

Gibbs, A. J., B. D. Harrison, and R. D. Woods. 1966. Purification of pea enation mosaic virus. Virology 29:348-351.

Gil-Fernandez, C., and L. M. Black. 1965. Some aspects of the internal anatomy of the leafhopper Agallia constricta (Homoptera: Cicadellidae). Ann. Entomol. Soc. Am. 58:275-284.

Gomatos, P. J., and I. Tamm. 1963a. The secondary structure of reovirus RNA. Proc. Nat. Acad. Sci. USA 49:707-714.

1963b. Animal and plant viruses with double-helical RNA. Proc. Nat. Acad. Sci. USA 50:878-885.

Granados, R. R., H. Hirumi, and K. Maramorosch. 1967. Electron microscopic evidence for wound-tumor virus accumulation in various organs of an inefficient leafhopper vector, Agalliopsis novella. J. Invertebrate Pathol. 9:147-159.

Granados, R. R., L. S. Ward, and K. Maramorosch. 1968. Insect viremia caused by a plant pathogenic virus: electron microscopy of vector hemocytes. Virology 34:790-796.

Hackett, A. J., E. S. Sylvester, J. Richardson, and P. Wood. 1968. Comparative electron micrographs of sowthistle yellow vein and vesicular stomatitis viruses. Virology 36:693-696.

Hagedorn, D. J., R. E. C. Layne, and E. G. Ruppel. 1964. Host range of pea enation mosaic virus and use of Chenopodium album as a local-lesion host. Phytopathology 54:843-848.

Hagedorn, D. J., and J. C. Walker. 1954. Virus diseases of canning peas in Wisconsin. University Wisconsin Res. Bull. 185.

Harrison, B. D. 1958. Studies on the behavior of potato leafroll and other viruses in the body of their aphid vector Myzus persicae (Sulz.). Virology 6:265-277.

Harrison, B. D., and N. C. Crowley. 1965. Properties and structure of lettuce necrotic yellows virus. Virology 26:297-310.

Heinze, K. 1959a. Phytopathogene Viren und ihre Überträger. Duncker und Humblot, Berlin. 291 p.

1959b. Versuche über die Haltbarkeit des Enationenvirus der Erbse (scharfes Adernmosaik) in der Erbsenblattlaus (Acyrthosiphon onobrychis B.D.F.). Anz. Schädlingsk. 32: 123-124.

1959c. Versuche zur Ermittlung der Haltbarkeit des Blattrollvirus der Kartoffel und des virus der Vergilbungskrankheit der Rübe im Überträger. Arch. Virusforsch. 9:396-410.

Herold, F., and K. Munz. 1965. Electron microscopic demonstration of viruslike particles in Peregrinis maidis following acquisition of maize mosaic virus. Virology 25:412-417.

Hinz, B. 1963. Unterschiede in der Fähigkeit zur Übertragung von phytopathogenen Viren bei Blattlausrassen. Nachrichtenbl. Dtsch. Pflanzenschutzd. (Berlin) 17:173-176.

1966a. Beiträge zur Analyse der Vektoreignung einiger wirtschaftlich wichtiger Blattlausarten und -rassen. I. Versuche zur Ermittlung der Vektoreigenschaften für das Blattrollvirus der Kartoffel bei Rassen von Myzus persicae (Sulz.). Phytopathol. Z. 56:54-77.

1966b. Beiträge zur Analyse der Vektoreignung einiger wirtschaftlich wichtiger Blattlausarten und -rassen. II. Versuche zur Ermittlung der Vektoreigenschaften für das Enationenvirus der Erbse bei Rassen von Myzus persicae (Sulz.), Acyrthosiphon pisum (Harris) und Macrosiphum euphorbiae (Thomas). Phytopathol. Z. 56:123-140.

1968. Aulacorthum solani (Kalt.) - ein neuer wirksamer Überträger des Erbsenenationenvirus. Wiss. Z. Univ. Rostock, Math. -naturw. Reihe 17:391-393.

1969. Über den Einfluss der Blattlausfutterpflanze, der Infektionsquelle und der Testpflanze auf die Übertragung des Enationenvirus der Erbse durch Acyrthosiphon pisum (Harris). Arch. Pflanzenschutz. 5:245-249.

Hirumi, H., R. R. Granados, and K. Maramorosch. 1967. Electron microscopy of a plant-pathogenic virus in the nervous system of its insect vector. J. Virol. 1:430-444.

Hodges, L. R., and D. L. McLean. 1969. Correlation of transmission of bean yellow mosaic virus with salivation activity of Acyrthosiphon pisum (Homoptera: Aphididae). Ann. Entomol. Soc. Amer. 62:1398-1401.

Hoof, H. A. van. 1957. On the mechanism of transmission of some plant viruses. Koninkl. Ned. Akad. Wetenschap. Proc. Ser. C. 60:314-317.

Izadpanah, K., and R. J. Shepherd. 1966a. Galactia sp. as a local lesion host for the pea enation mosaic virus. Phytopathology 56:458-459.

1966b. Purification and properties of the pea enation mosaic virus. Virology 28:463-476.

Johnson, F. and L. K. Jones. 1937. Two mosaic diseases of peas in Washington. Jour. Agr. Res. 54:629-638.

Jones, J. C. 1956. The hemocytes of Sarcophaga bullata Parker. J. Morphol. 99:233-257.

1962. Current concepts concerning insect hemocytes. Am. Zoologist 2:209-246.

Kennedy, J. S. 1960. The behavioral fitness of aphids as field vectors of viruses. Rept. Commonwealth Entomol. Conf., 1960, London, 7th, 165-168.

Kennedy, J. S., M. F. Day, and V. F. Eastop. 1962. A conspectus of aphids as vectors of plant viruses. Commonwealth Inst. Entomol. London. 114 p.

Kikumoto, T., and C. Matsui. 1962. Electron microscopy of plant viruses in aphid midguts. Virology 16:509-510.

Kirkpatrick, H. C., and A. F. Ross. 1952. Aphid-transmission of potato leafroll virus to solanaceous species. Phytopathology 42:540-546.

Kunkel, L. O. 1937. Effect of heat on ability of Cicadula sexnotata (Fall.) to transmit aster yellows. Amer. J. Bot. 24:316-327.

1941. Heat cure of aster yellows in periwinkles. Amer. J. Bot. 28:761-769.

1955. Cross protection between strains of yellows-type viruses. Advances Virus Res. 3:251-273.

Langenberg, W. G., and D. E. Schlegel. 1967. Autoradiography with $^{125}$I-labeled antibodies as a means of localizing TMV antigen in plant cells. Virology 32:167-171.

Leach, J. G. 1940. Insect transmission of plant diseases. McGraw-Hill, New York. 615 p.

Leclant, F. 1968. Connaissances actuelles sur les pucerons dans leurs relations avec les maladies a virus des plantes. Ann. Épiphyties 19:455-482.

Ling, K. C. 1966. Nonpersistence of the tungro virus of the rice in its leafhopper vector, Nephotettix impicticeps. Phytopathology 56:1252-1256.

1969. Nonpropagative leafhopper-borne viruses, p. 255-277. In Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

López-Abella, D., and R. H. E. Bradley. 1969. Aphids may not acquire and transmit stylet-borne viruses while probing intercellularly. Virology 39:338-342.

Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9:409-414.

Maramorosch, K. 1950. Influence of temperature on incubation and transmission of the wound-tumor virus. Phytopathology 40: 1071-1093.

1952. Direct evidence for the multiplication of aster yellows virus in its insect vector. Phytopathology 45:59-64.

1953. Incubation period of aster yellows virus. Amer. J. Bot. 40:799-808.

1954. Biological transmission of plant viruses by animal vectors. Trans. N. Y. Acad. Sci. Ser. 2, 16:189-195.

1955. Multiplication of plant viruses in insect vectors. Advances in Virus Research 3:221-249.

1959. Leafhoppers (Cicadellidae) as vectors and reservoirs of phytopathogenic viruses. Homm. Vol. Savulescu. Annivers. 39: 421-443.

1960. Leafhopper-transmitted plant viruses. Protoplasma 52: 457-466.

1963. Arthropod transmission of plant viruses. Ann. Rev. Entomol. 8:369-414.

1964. Virus-vector relationship: vectors of circulative and propogative viruses, p. 175-193. In M. K. Corbett and H. D. Sisler (ed.), Plant Virology. Univ. Florida Press, Gainesville.

1966. Plant Viruses. In Symposium, 9th Internat. Congr. Microbiol. Moscow, p. 423-431.

1970. Insect infection caused by a plant tumor virus. World Rev. Pest Contr. 9:29-41.

Maramorosch, K., M. K. Brakke, and L. M. Black. 1949. Mechanical transmission of a plant tumor virus to an insect vector. Science 110:162-163.

Maramorosch, K., and E. Shikata. 1965. The fate of wound tumor virus in vectors and non-vectors: an electron microscopy study. Proc. U.S. - Japan Conf. Relationships between Arthropods and Plant-Pathogenic Viruses, Tokyo p. 70-72. U. S. - Japan Scientific Cooperation Program, Tokyo.

Maramorosch, K., E. Shikata, and R. R. Granados. 1969. The fate of plant-pathogenic viruses in insect vectors: electron microscopy observations, p. 417-431. In Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Maramorosch, K., E. Shikata, H. Hirumi, and R. R. Granados. 1968. Multiplication and cytopathology of a plant tumor virus in insects. Abstr. Symp. Neoplasia Invertebrate Primitive Vertebrate Animals. Smithsonian Institution, Washington, D.C.

1969. Multiplication and Cytopathology of a plant tumor virus in insects. Nat. Cancer Inst. Monogr. 31:493-507.

Maramorosch, K., E. Shikata, S. W. Orenski, and G. Streissle. 1965. Electron micrographic evidence for systemic wound tumor virus infection of an insect vector. Phytopathology 55:129.

Markham, R., and K. M. Smith. 1949. Studies on the virus of turnip yellow mosaic. Parasitology 39:330-342.

Mazzone, H. M., N. L. Incardona, and P. Kaesberg. 1962. Biochemical and biophysical properties of squash mosaic virus and related macromolecules. Biochem. Biophys. Acta 55:164-175.

McEwen, F. L. and W. T. Schroeder. 1956. Host range studies on pea enation mosaic cirus. Plant Dis. Reptr. 40:11-14.

McEwen, F. L., W. T. Schroeder, and A. C. Davis. 1957. Host range and transmission of the pea enation mosaic virus. J. Econ. Entomol. 50:770-775.

McLean, D. L., and M. G. Kinsey. 1967. Probing behavior of the pea aphid, Acyrthosiphon pisum. I. Definitive correlation of electronically recorded waveforms with aphid probing activities. Ann. Entomol. Soc. Amer. 60:400-406.

1968a. Probing behavior of the pea aphid, Acyrthosiphon pisum. II. Comparisons of salivation and ingestion in host and non-host plant leaves. Ann. Entomol. Soc. Amer. 61:730-739.

1968b. Probing behavior of the pea aphid, Acyrthosiphon pisum. III. Effect of temperature differences on certain probing activities. Ann. Entomol. Soc. Amer. 61:927-933.

1969. Probing behavior of the pea aphid, Acyrthosiphon pisum. IV. Effects of starvation on certain probing activities. Ann. Entomol. Soc. Amer. 62:987-994.

McLeon, D. L., and W. A. Weigt, Jr. 1968. An electronic measuring system to record aphid salivation and ingestion. Ann. Entomol. Soc. Amer. 61:180-185.

McWhorter, F. P. 1949. Pea enation mosaic, narcissus mosaic, and cancer. Phytopathology. 39:861.

1950. Neoplasms induced in plants by viruses. Biol. Colloq. Oregon State Chapter Phi Kappa Phi 11:14-15.

1965. Plant virus inclusions. Ann. Rev. Phytopathol. 3:287-312.

McWhorter, F. P., and W. C. Cook. 1958. The hosts and strains of pea enation mosaic virus. Plant Dis. Reptr. 42:51-60.

Mellor, F. C., and R. E. Fitzpatrick. 1951. Studies on virus diseases of strawberries in British Columbia II. The seperation of the component viruses of yellows. Can. J. Bot. 29:411-420.

Merril, M. H., and C. Ten Broeck. 1935. The transmission of equine encephalomyelitis virus by Aedes aegypti. J. Exptl. Med. 62: 687-695.

Miyamoto, S. and Y. Miyamoto. 1966. Notes on aphid-transmission of potato leafroll virus. Sci. Rptr. Hyogo Univ. Agric., Ser. Plant Protection 7:51-66.

Moericke, V., and T. E. Mittler. 1965. Schlucken Blattlaüse ihren eigenen Speichel? Z. Pflanzenkrankh. (Pflanzenpath.) Pflanzenschutz 72:513-515.

Mueller, W. C., and W. F. Rochow. 1961. An aphid injection method for the transmission of barley yellow dwarf virus. Virology 14:253-258.

Nagaraj, A. N., and L. M. Black. 1962. Hereditary variation in the ability of a leafhopper to transmit two unrelated plant viruses. Virology 16:152-162.

Nagaraj, A. N., R. C. Sinha, and L. M. Black. 1961. A smear technique for detecting virus antigen in individual vectors by the use of fluorescent antibodies. Virology 15:205-208.

Nasu, S. 1963. Studies on some leafhoppers and planthoppers which transmit virus diseases of rice plant in Japan. Bull. Kyushu Agr. Exp. Sta. 8:153-349.

1965. Electron microscopic studies on transovarial passage of rice dwarf virus. Japan J. Appl. Entomol. Zool. 9:225-237.

1969. Electron microscopy of the transovarial passage of rice dwarf virus, p. 433-448. In Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Nault, L. R. 1967. Inoculation of pea enation mosaic virus by the green peach, potato, and foxglove aphids. J. Econ. Entomol. 60:1586-1587.

Nault, L. R., and G. G. Gyrisco. 1966. Relation of the feeding process of the pea aphid to the inoculation of pea enation mosaic virus. Ann. Entomol. Soc. Amer. 59:1185-1197.

Nault, L. R., G. G. Gyrisco, and W. F. Rochow. 1964. Biological relationship between pea enation mosaic virus and its vector, the pea aphid. Phytopathology 54:1269-1272.

Nielson, M. W. 1962. A synonymical list of leafhopper vectors of plant viruses (Homoptera, Cicadellidae). U. S. Dep. Agr. ARS-33-74. 12 p.

O'Loughlin, G. T., and T. C. Chambers. 1967. The systemic infection of an aphid by a plant virus. Virology 33:262-271.

Orlob, G. B., and R. H. E. Bradley. 1961. Where cabbage aphids carry transmissible virus B? Phytopathology 51:297-399.

Osborn, H. T. 1935. Incubation period of pea mosaic in the aphid, Acyrthosiphon pisum (Harris) [= Macrosiphum pisi (Kalt.)]. Phytopathology 25:160-177.

1938. Studies on pea virus. I. Phytopathology 28:923-934.

Ossiannilsson, F. 1961. Negative evidence against passage of whole plant virus particles through the gut wall in aphid vectors. Nature, London 190:1222.

1966. Insects in the epidemiology of plant viruses. Ann. Rev. Entomol. 11:213-232.

Peters, D. 1965. The purification of viruslike particles from the aphid Myzus persicae. Virology 26:159-161.

1967a. The purification of potato leafroll virus from its vector Myzus persicae. Virology 31:46-54.

1967b. Potato leafroll virus, its purification from its vector Myzus persicae. Meded. L. E. B. Fonds No. 45. H. Veenman and Zonen, Wageningen, the Netherlands, 100 p.

Peters, D. and L. M. Black. 1970. Infection of primary cultures of aphid cells with a plant virus. Virology 40:847-853.

Peters, D., and E. W. Kitajima. 1970. Purification and electron microscopy of sowthistle yellow vein virus. Virology 41: 135-150.

Peters, D. and L. C. Van Loon. 1968. Transmission of potato leafroll virus by aphids after feeding on virus preparations from aphids and plants. Virology 35:597-600.

Pierce, W. H. 1935. The identification of certain viruses affecting leguminous plants. Jour. Agr. Res. 51:1017-1039.

Pirone, T. P. 1969. Mechanism of transmission of stylet-borne viruses, p. 199-210. In Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Posnette, A. F. 1960. Some aspects of virus spread among plants by vectors. Rept. Commonwealth Entomol. Conf., 1960, London, 7th, 157-161.

Posnette, A. F., and C. E. Ellenberger. 1963. Further studies of green petal and other leafhopper-transmitted viruses infecting strawberry and clover. Ann. Appl. Biol. 51:69-83.

Prentice, I. W. 1952. Resolution of strawberry virus complexes V. Experiments with viruses 4 and 5. Ann. Appl. Biol. 39: 487-494.

Prentice, I. W., and R. V. Harris. 1946. Resolution of strawberry virus complexes by means of the aphid vector Capitophorus fragariae. Theob. Annu. Appl. Biol. 33:50-53.

Reddy, D. V. R., and L. M. Black. 1966. Production of wound-tumor virus and wound-tumor virus soluble antigen in the insect vector. Virology 30:551-561.

Rice, R. V., G. D. Lindberg, P. Kaesberg, J. C. Walker, and M. A. Stahmann. 1955. The three components of squash mosaic virus. Phytopathology 45:145-148.

Richardson, J. and E. S. Sylvester. 1965. Aphid honeydew as inoculum for the injection of pea aphids with pea-enation mosaic virus. Virology 25:472-475.

1968. Further evidence of multiplication of sowthistle yellow vein virus in its aphid vector, Hyperomyzus lactucae. Virology 35:347-355.

Roschow, W. F. 1963. Variation within and among aphid vectors of plant viruses. Ann. N. Y. Acad. Sci. 105:713-729.

Roschow, W. F., and Brakke, M. K. 1964. Purification of barley yellow dwarf virus. Virology 24:310-322.

Roschow, W. F., and E. W. Pang. 1961. Aphids can acquire strains of barley yellow dwarf virus they do not transmit. Virology 15:382-384.

Rockwell, A. F., P. Norton, J. B. Caulfield, and S. I. Roth. 1966. A silicone rubber mold for embedding tissue in epoxy resins. Science Tools 13:9.

Rosen, L. 1968. Reoviruses. Virol. Monogr. 1:73-107.

Ruppel, E. G. 1968. Possible particles of beet western yellows virus in the intestines of viruliferous aphids. Phytopathology 58:256-257.

Ruppel, E. G., and D. J. Hagedorn. 1963a. Observance of giant stem enations on Pisum sativum infected with pea enation mosaic virus. Phytopathology 53:245.

1963b. Comparisons of pea enation mosaic virus isolates. Phytopathology 53:813-818.

Schmutterer, H. 1969. Versuche zur Übertragung des Erbsenenationenvirus durch Injektion virushaltiger Hämolymphe von einer Blattlausart oder-form in die andere. Z. Pflanzenkrankh. (Pflanzenpath.) Pflanzenschutz 76:257-263.

Schmutterer, H., and P. Ehrhardt. 1964. Untersuchungen über die Beziehungen zwischen dem Enationenvirus der Erbse und seinen Vektoren. II. Versuche zur Übertragung des Virus durch Injektion von Haemolymphe in verschiedene Aphidenarten. Phytopathol. Z. 50:80-85.

Schneider, I. R., and T. O. Diener. 1966. The correlation between the proportions of virus-related products and the infectious component during the synthesis of tobacco ringspot virus. Virology 29:92-99.

Schroeder, W. T., and D. W. Barton. 1958. The nature and inheritance of resistance to the pea enation mosaic virus in garden pea, Pisum sativum L. Phytopathology 48:628-632.

Semancik, J. S., and J. B. Bancroft. 1964. Further characterization of the nucleoprotein components of bean pod mottle virus. Virology 22:33-39.

Shepherd, R. J., and S. A. Ghabrial. 1966. Isolation and some properties of the nucleic acid and protein components of the pea enation mosaic virus. Phytopathology 56:900.

Shepherd, R. J., R. J. Wakeman, and S. A. Ghabrial. 1968. Preparation and properties of the protein and nucleic acid components of pea enation mosaic virus. Virology 35:255-267.

Shikata, E. 1966. Electron microscopic studies on plant viruses. J. Faculty Agr. Hokkaido Univ. 55:1-110.

Shikata, E., and K. Maramorosch. 1965a. Pea enation mosaic virus in plant and aphid hosts. Proc. U.S.-Japan Conf. Relationships between arthropods and plant-pathogenic viruses, Tokyo, p. 160. U.S. - Japan Scientific Cooperation Program, Tokyo.

1965b. Plant tumour virus in arthropod host: microcrystal formation. Nature 208:507-508.

1965c. Wound tumor virus in insect hosts inoculated parenterally. J. Appl. Phys. 36:2620.

1965d. Electron microscopic evidence for the systemic invasion of an insect host by a plant pathogenic virus. Virology 27: 461-475.

1966a. An electron microscope study of plant neoplasia induced by wound tumor virus. J. Nat. Cancer Inst. 36:97-116.

1966b. Electron microscopy of pea enation mosaic virus in plant cell nuclei. Virology 30:439-454.

1967a. Electron microscopy of wound tumor virus assembly sites in insect vectors and plants. Virology 32:363-377.

1967b. Electron microscopy of the formation of wound tumor virus in abdominally inoculated insect vectors. J. Virol. 1:1052-1073.

1969. Electron microscopy of insect-borne viruses in situ, p. 393-431. In Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Shikata, E., K. Maramorosch, and R. R. Granados. 1965. Electron microscopy of pea enation mosaic virus in ultrathin sections of plants and pea aphids. Phytopathology 55:1285.

1966. Electron microscopy of pea enation mosaic virus in plants and aphid vectors. Virology 29:426-436.

Shikata, E., S. W. Orenski, H. Hirumi, J. Mitsuhashi, and K. Maramorosch. 1964. Electron micrographs of wound-tumor virus in an animal host and a plant tumor. Virology 23:441-444.

Shinkai, A. 1954. Transovarial transmission of rice stripe virus in Delphacodes striatella Fall. Ann. Phytopathol. Soc. Japan 18:169 (Abstr.).

1958. Transovarial passage of rice dwarf virus in Inazuma dorsalis Motsch. (In Japanese) Ann. Phytopath. Soc. Japan 23:26 (Abstr.).

1962. Studies on insect transmissions of rice virus diseases in Japan. Bull. Nat. Inst. Agr. Sci. C14:1-112.

Simons, J. N. 1954. Vector-virus relationships of pea-enation mosaic and the pea aphid Acyrthosiphon pisum (Harris) [= Macrosiphum pisi (Kalt.)]. Phytopathology 44:283-289.

Sinclair, J. B., P. H. Geil, and P. Kaesberg. 1957. Biophysical studies of wild cucumber mosaic virus. Phytopathology 47: 372-377.

Sinha, R. C. 1960. Comparison of the ability of nymph and adult Delphacodes pellucida Fabricus, to transmit European wheat striate mosaic virus. Virology 10:344-352.

1963. Effect of age of vector and of abdomen punctures on virus transmission. Phytopathology 53:1170-1173.

1965. Sequential infection and distribution of wound-tumor virus in the internal organs of a vector after ingestion of virus. Virology 26:673-686.

1967. Response of wound-tumor virus infection in insects to vector age and temperature. Virology 31:746-748.

1968. Recent work on leafhopper-transmitted viruses. Adv. Virus Res. 13:181-223.

1969. Localization of viruses in vectors: Serological and Infectivity Tests, p. 379-391. In, Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Sinha, R. C., and L. M. Black. 1962. Studies on the smear technique for detecting virus antigens in an insect vector by use of fluorescent antibodies. Virology 17:582-587.

1963. Wound-tumor virus antigens in the internal organs of an insect vector. Virology 21:183-187.

Sinha, R. C., and L. N. Chiykowski. 1967. Initial and subsequent sites of aster yellows virus infection in a leafhopper vector. Virology 33:702-708.

Sinha, R. C., and D. V. R. Reddy. 1964. Improved fluorescent smear technique and its application in detecting virus antigens in an insect vector. Virology 24:626-634.

Sinha, R. C., D. V. R. Reddy, and L. M. Black. 1964. Survival of insect vectors after examination of hemolymph to detect virus antigens with fluorescent antibody. Virology 24:666-667.

Sinha, R. C., and S. Shelley. 1965. The transovarial transmission of wound-tumor virus. Phytopathology 55:324-327.

Slykhuis, J. T., and M. A. Watson. 1958. Striate mosaic of cereals in Europe and its transmission by Delphacodes pellucida (Fab.) Ann. Appl. Biol. 46:542-553.

Smith, K. M. 1931. Studies on potato virus diseases. IX. Some further experiments on the insect transmission of potato leaf-roll. Ann. Appl. Biol. 18:141-157.

1957. Arthropods as vectors and reservoirs of phytopathogenic viruses, p. 143-176. In C. Hallauer and K. F. Meyer (ed.), Vol. 4, Springer-Verlag, Vienna, Austria.

1958. Transmission of plant viruses by arthropods. Ann. Rev. Entomol. 3:469-482.

1965. Plant virus-vector relationships. Advances Virus Res. 11:61-96.

Smith, F. F., and P. Brierley. 1956. Insect transmission of plant viruses. Ann. Rev. Entomol. 1:229-322.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure Research 26:31-43.

Stace-Smith, R. 1966. Purification and properties of tomato ringspot virus and an RNA-deficient component. Virology 29:240-247.

Stace-Smith, R., M. E. Reichmann, and N. S. Wright. 1965. Purification and properties of tobacco ringspot virus and two RNA-deficient components. Virology 25:487-494.

Stegwee, D. 1961. Multiplication of plant viruses in their aphid vectors. Recent advances in botany. University of Toronto Press, Toronto. 533-535.

Stegwee, D., and M. B. Ponsen. 1958. Multiplication of potato leaf-roll virus in the aphid Myzus persicae (Sulz.). Entomol. Exper. Appl. 1:291-300.

Storey, H. H. 1933. Investigations of the mechanism of the transmission of plant viruses by insect vectors. I. Proc. Roy. Soc. (B)113:463-485.

1939. Transmission of plant viruses by insects. Bot. Rev. 5: 240-272.

Streissle, G., and R. R. Granados. 1968. The fine structure of wound-tumour virus and reovirus. Arch. ges. Virusforsch. 25: 369-372.

Streissle, G., V. Bystricky, R. R. Granados, and K. Strohmaier. 1968. Number of virus particles in insects and plants infected with wound tumor virus. J. Virol. 2:214-217.

Streissle, G. and K. Maramorosch. 1963. Reovirus and wound-tumor virus: Serological cross reactivity. Science 40:996-997.

Stubbs, M. W. 1937. Certain viruses of the garden pea, Pisum sativum. Phytopath. 27:242-266.

Suzuki, N. 1969. Purification of single and double-stranded vector-borne RNA viruses, p. 557-578. In Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Sylvester, E. S. 1956. Beet yellows virus transmission by the green peach aphid. J. Econ. Entomol. 43:789-800.

1958. Aphid transmission of plant viruses. Proc. Intern. Congr. of Entomol., Montreal, 1956, 10th, 3:195-200.

1961. Problems in the classification of plant virus transmission by aphids, p. 517-522. In Recent advances in botany, Univ. of Toronto Press, Toronto, Canada.

1962. Mechanisms of plant virus transmission by aphids, p. 11-31. In Karl Maramorosch (ed.), Biological transmission of disease agents. Academic Press, New York.

1965. The latent period of pea-enation mosaic virus in the pea aphid, Acyrthosiphon pisum (Harris) -- An approach to its estimation. Virology 25:62-67.

1967. Retention of inoculativity in the transmission of pea enation mosaic virus by pea aphids as associated with virus isolates, aphid reproduction and excretion. Virology 32: 524-531.

1969a. Evidence of transovarial passage of the sowthistle yellow vein virus in the aphid Hyperomyzus lactucae. Virology 38: 440-446.

1969b. Virus transmission by aphids -- a viewpoint, p. 159-173. In Karl Maramorosch (ed.), Viruses, vectors, and vegetation. Interscience Publishers, New York.

Sylvester, E. S., and R. H. E. Bradley. 1962. Effect of treating stylets of aphids with formalin on the transmission of sugar beet yellows virus. Virology 17:381-386.

Sylvester, E. S., and J. Richardson. 1966a. "Recharging" pea aphids with pea enation mosaic virus. Virology 30:592-597.

1966b. Some effects of temperature on the transmission of pea enation mosaic virus and on the biology of the pea aphid vector. J. Econ. Entomol. 59:255-261.

1969. Additional evidence of multiplication of the sowthistle yellow vein virus in an aphid vector -- serial passage. Virology 37:26-31.

1970. Infection of Hyperomyzus lactucae by sowthistle yellow vein virus. Virology 42:1023-1042.

Treherne, J. E. 1967. Gut absorption. Ann. Rev. Entomol. 12:43-58.

Tromans, W. J., and R. W. Horne. 1961. The structure of bacteriophage ØX 174. Virology 15:1-7.

Tsai, J. H. 1967. Transmission of two pea enation mosaic virus isolates by the pea aphid, Acyrthosiphon pisum (Harris). M.S. Thesis, Michigan State University. 54 p.

1969. Some factors affecting the transmission of pea enation mosaic virus by the pea aphid, Acyrthosiphon pisum (Harris), with emphasis on the inoculation phase of the transmission cycle. Ph.D. thesis, Michigan State University. 55 p.

Tsai, J. H., and J. E. Bath. 1970. Additional factors affecting the transmission of pea enation mosaic virus by the pea aphid. Ann. Entomol. Soc. Amer. 63:1723-1727.

Uichanco, L. B. 1924. Studies on the embryogeny and postnatal development of the aphididae with special reference to the history of the "symbiotic organ," or "mycetom." Philippine J. Sci. 24:143-247.

Waterhouse, D. F., and M. F. Day. 1953. Function of the gut in absorption, excretion, and intermediary metabolism, p. 331-349. In K. D. Roeder (ed.), Insect Physiology. Wiley, New York.

Waterhouse, D. F., and M. Wright. 1960. The fine structure of the mosaic midgut epithelium of blowfly larvae. J. Ins. Physiol. 5:230-239.

Watson, M. A. 1960. The ways in which plant viruses are transmitted by vectors. Rept. Commonwealth Entomol. Conf., 1960, London, 7th:157-161.

Watson, M. A., and F. M. Roberts. 1939. A comparative study of the transmission of Hyoscyamus virus 3, potato virus Y and cucumber virus 1 by the vectors Myzus persicae (Sulz.), M. circumflexus (Buckton) and Macrosiphum gei (Koch). Proc. Roy. Soc. (London) B127:543-576.

Watson, M. A., and R. C. Sinha. 1959. Studies on the transmission of European wheat striate mosaic virus by Delphacodes pellucida Fabricius. Virology 8:139-163.

Weber, H. 1928. Skelett, Muskulatur und Darm der schwarzen Blattlaus Aphis fabae Scop. mit besonderer Berücksichtigung der Funktion der Mundwerkzeuge und des Darms. Zoologica 28:1-120.

Whitcomb, R. F., and L. M. Black. 1959. Serological measurement of wound tumor soluble antigen in Agallia constricta. Phytopathology 49:554.

1961. Synthesis and assay of wound tumor soluble antigen in an insect vector. Virology 15:136-145.

Wigglesworth, V. B. 1953. The principles of insect physiology. 5th ed. Methuen, London.

1956. The haemocytes and connective tissue formation in an insect, Rhodnius prolixus (Hemiptera). Quart. J. Micr. Sci. 97:89-98.

Wigglesworth, V. B., and M. M. Salpeter. 1962. Histology of the Malpighian tubules in Rhodnius prolixus Stål (Hemiptera). J. Ins. Physiol. 8:299-307.

Wildy, P., H. S. Ginsberg, J. Brandes, and J. Maurin. 1967. Viruses-classification, nomenclature, and the International Committee on Nomenclature of Viruses, Progr. Med. Virol. 9:476-482.

Yamada, W., and H. Yamamoto. 1954. Transmission of rice stripe virus through the eggs of Delphacodes striatella Fall. Ann. Phytopathol. Soc. Japan 18:169. (abstr.)

1955. Studies on the stripe disease of rice plant. I. (in Japanese). Okayama Perfecture Agr. Exp. Sta. Special Bull. 52:93-112.

1956. Studies on the stripe disease of rice plant. III (in Japanese). Okayama Perfecture Agr. Exp. Sta. Special Bull. 55:35-56.

Yamazaki, H., J. Bancroft, and P. Kaesberg. 1961. Biophysical studies of broad bean mottle virus. Proc. Natl. Acad. Sci. U.S. 47: 979-983.

Yamazaki, H., and P. Kaesberg. 1961. Biophysical and biochemical properties of wild cucumber mosaic virus and of two related virus-like particles. Biochem. Biophys. Acta 51:9-18.

Yeager, J. F. 1945. The blood picture of the southern armyworm (Prodenia eridania). J. Agr. Res. 71:1-40.

Zazhurilo, U. K. and G. M. Sitnikova. 1941. Interrelations between mosaic disease virus of winter wheat and its vector. Deltocephalus striatus L. (In Russian.) Proc. Lenin Acad. Agr. Sci. U.S.S.R. 11:27-29.