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Title: Aphid Age and In-Vector Virus Concentration as Factors Influencing Pea Enation Mosaic Virus Transmission by the Pea Aphid.

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The relationship between pea enation mosaic virus (PEMV) and aging pea aphids was studied. Although this research initially emphasized the relationship between the decline in the rate of PEMV transmission and the concentration of PEMV within pea aphids, aphid age was found to be the principle factor influencing transmission. Enzyme-linked immunosorbent assays (ELISA) were used to assay the concentration of virus within aphid bodies.

Aphid age at the time of virus acquisition significantly influenced the maximum rate of transmission attained and the rate of transmission decline by aging aphids. Following a 24-hr virus acquisition treatment, 1-day-old aphids achieved the highest transmission rate (95%) and the slowest decline in their rate of transmission of 4 age groups tested. Eight-day-old aphids had a maximum transmission rate of 50% and a more rapid decline in

transmission. Thirteen-day-old aphids had a maximum rate of only 5% while 23-day-old aphids did not transmit at all.

The relative feeding rate of aphids 8, 13, 18, and 23 days old was measured indirectly by assaying the virus concentration in aphids immediately following a standard 24-hr virus acquisition treatment. The 18- and 23-day-old aphids fed significantly less than 8- and 13-day-olds.

The concentration of PEMV was monitored periodically in both whole and dissected aphids during 16 day test periods following a 24-hr virus acquisition treatment. In the whole insect study, 1-day-olds initially acquired A_{405} = 0.06 or 2.3 ng of PEMV while 8-day-olds acquired A_{405} = 0.44 or 20 ng of PEMV per aphid. The average concentration of PEMV recorded from individuals in the 1-day-old group remained constant during the 16-day test period while in the 8-day-old group it decreased significantly. When aphids from the 8-day-old group were dissected immediately after completion of the virus acquisition treatment, the virus concentration was found to be higher in the gut than in the body region. The concentration in the gut, however, declined rapidly while in the body it remained constant during the 16-day post-virus acquisition test period.

Decline in the virus transmission rates by aging aphids was not explained by the virus concentration within aphids. In fact, transmission of PEMV by individual aphids was independent of the virus concentration within individual aphids. Therefore, decline in the transmission rate is

more likely dependent on the physiological realtionship between PEMV and key processes within vectors, such as, entry of virions into the salivary system. These processes may be slowed by vector aging.

Aphid Age and In-Vector Virus Concentration as Factors Influencing Pea Enation Mosaic Virus Transmission by the Pea Aphid

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INTRODUCTION

Pea enation mosaic virus (PEMV) is a virus disease of garden pea (Pisum sativum L.), and several other leguminous plants. It is transmitted by several aphid species in a persistent manner (Sylvester 1956).

The relationship between PEMV and its aphid vectors, primarily the pea aphid (Acyrthosiphon pisum, Harris), has been intensively studied (Osborn 1935, Simons 1954, Sylvester 1966, Bath and Chapman 1968, Bath and Tsai 1969, and Thottappilly et al. 1972). Yet, a complete understanding of the biological relationship between PEMV and the pea aphid is lacking. For example, it is not known why the transmission rate of PEMV by the pea aphid to garden pea declines with increasing age of the vector. This decline in inoculativity is more rapid for aphids which acquired virus as adults than for those which acquired virus as nymphs (Sylvester and Richardson 1966b). Sylvester and Richardson (1966a) hypothesized that this decline in transmission rate was a result of vector aging. Later, they hypothesized that the decline in the transmission rate was not dependent upon the concentration of virus acquired by an aphid during feeding but upon the age of vector at the time of acquisition (Sylvester and Richardson 1966b).

I chose for this thesis research to investigate this transmission decline phenomenon in aging vectors, using the pea aphid and PEMV as the virus-vector components. This research focused on the post-virus acquisition period of the vector's life and utilized two age-specific virus bioassays. First was a conventional plant to aphid to plant transmission test monitoring the inoculativity of specific aphids. Second was a quantitative virus assay utilizing a serological technique (enzyme-linked immunosorbent assay--ELISA). These bioassays complemented each other in this investigation of the transmission decline phenomenon.

As a result of this research, new insights into the biological relationship of this virus and its vector were obtained. These will increase our basic knowledge of this specific virus-vector relationship which, in turn, will help us to better understand persistent plant virus transmission by aphids in general.

REVIEW OF LITERATURE

Pea enation mosaic virus (PEMV) is a virus disease of garden pea (Pisum sativum L.) and other leguminous plants. Symptom expression varies among leguminous plant species. Symptoms on garden pea usually include stunting, chlorotic spots and malformations, but the degree of such symptoms varies. The best diagnostic symptom consists of ridges (enations) on the underside of leaves caused by tissue proliferation especially along veins and bordering lucid flecks (McEwen et al. 1957). Serological testing also provides a good means for identifying PEMV infected plants.

Five aphid species were recorded as PEMV vectors (Kennedy et al. 1962). In the field, the pea aphid is the most important vector of PEMV. However, the efficiency of transmission differs among aphid species, biotypes and virus isolates. The potato aphid (Macrosiphon euphorbiae (Thomas)) and the pea aphid (Acyrthosiphon pisum (Harris)) were found to have the same transmission efficiency (Simons 1954), while the foxglove aphid (Acyrthosiphon solani (Kaltenbach)) was shown to be an inefficient vector (Nault 1967). Bath and Chapman (1966) found no significant difference among biotypes of the potato aphid and the green peach aphid (Myzus persicae (Sulzer)) in transmitting several PEMV isolates. However, the pea aphid showed differences among biotypes in transmitting PEMV isolates. Later, the pea aphid was used to separate isolates of PEMV

(Bath and Chapman 1967, and Bath and Tsai 1969).

Two classification schemes have been used to categorize vector-virus relationships. Kennedy et al. (1962) classified insect-plant virus relationships based on a mechanism of transmission scheme, i.e., stylet-borne and circulative viruses. Sylvester (1956) previously had classified vector relationships based on the length of virus retention by the vector, i.e., nonpersistent, semipersistent and persistent. The stylet-borne viruses listed by Kennedy et al. (1962) included both the nonpersistent and semipersistent examples mentioned by Sylvester (1956). Whereas, the circulative viruses were identical to the persistent viruses of Sylvester. Nonpersistent (Stylet-Borne) viruses differ in many respects from persistent (Circulative) viruses while semipersistent viruses have characteristics of both. This thesis deals with PEMV which has a persistent relationship to its pea aphid vector.

Persistently Transmitted Plant Viruses

Plant viruses in the persistent category have several characteristics in common. The following applies to both aphid and leafhopper vectors but aphid vectors will be emphasized. Aphids acquire these viruses only during extended feeding periods on infected plants. The minimum acquisition-access period (AAP = the time interval during which an aphid is allowed to feed on an infected plant) for these viruses may be several hours depending upon the

virus. The actual feeding (sap ingestion) period during an AAP is generally not known. Persistent viruses are not acquired during feeding probes of short duration as are nonpersistent viruses. Also, preacquisition starvation of aphids has no effect on the rate of transmission. There is a positive correlation between the number of aphids rendered viruliferous, the rate of transmission and the length of the AAP (Simons 1954). There is a latent period (LP = the period of time between the start of an AAP until subsequent feedings on healthy plants produce infections) for all persistent plant viruses. This is the time course during which ingested virus circulates in the vector and enters into the salivary system. It is then available for injection into plants along with saliva during feeding. The minimum inoculation period (IP = the time interval required for viruliferous aphids feeding on healthy plants to inoculate sufficient virus to produce an infection) may be several hours for persistent viruses. To a certain point, there is a positive correlation between the length of the IP and the rate of transmission.

Persistent viruses are retained by their aphid vector for a long period of time and are not lost during ecdysis. Like several of the leafhopper transmitted viruses, a few persistent aphid-borne plant viruses have been reported to multiply in their aphid vector. For example, potato leafroll virus (PLRV) was reported to multiply in the green peach aphid (Stegwee and Ponsen 1958). They found that

aphids were still rendered viruliferous after 15 serial passages of hemolymph via intrahemocoelic-injection using an aphid to aphid (donor-recipient) system. The dilution of the original hemolymph sample after 15 passages was approximately 10^{-21}. They had shown that the dilution end point for rendering a recipient aphid viruliferous using hemolymph from a viruliferous aphid donor was 10^{-3}. They concluded that PLRV must multiply in the aphid vector. However, Harrison (1958) and Eskandari et al. (1979) failed to render recipient aphids viruliferous after the first hemolymph passage which leaves the question of PLRV multiplication unresolved.

Other characteristics of persistent viruses include a high degree of vector-virus specificity. That is, persistent viruses tend to have only a few aphid vector species. Also, most persistent viruses are not transmitted by sap inoculation primarily because they are phloem- or xylem-limited in their host plant.

There are several lines of evidence indicating that persistent plant viruses circulate in the vector's body. An aphid will be rendered viruliferous if it is injected with hemolymph obtained from a viruliferous donor aphid (Mueller and Rochow 1961). Also, virions of persistent viruses have been visualized in various body parts of their vectors by electron microscopy (Shikata et al. 1966). The virus is ingested during sap feeding and passes through permeable gut tissue into the hemolymph. In the hemolymph,

virions flow to the salivary glands (Harris et al. 1975). Virions are assumed to be injected into plants via saliva during vector feeding. However, Harris et al. (1975) hypothesized a possible regurgitation transmission mechanism where virions held in the midgut of the aphid are inoculated into plants when mid- or foregut contents are regurgitated during feeding.

The Persistent Relationship of PEMV to Aphids

In contrast to most persistent viruses, PEMV is readily transmitted by sap inoculation (Osborn 1938). For it infects epidermal as well as phloem tissue. The degree of vector specificity is also less than other persistent viruses. Because several aphid species are recorded as PEMV vectors.

The retention of PEMV by its aphid vectors, as measured by the inoculativity of the aphids over time, has been intensively studied. Inoculativity (Sylvester and Richardson 1966a) is the ability of an aphid after being rendered viruliferous to produce infected plants during feeding or is an indirect measure of the presence of transmissible virus within an aphid. Osborn (1935) recorded that the pea aphid retained PEMV for 29 days, almost as long as the pea aphid's life. The length of the retention time depended upon the particular virus isolate studied (Bath and Chapman 1967, and Bath and Tsai 1969), retention

time has also been positively correlated with the length of the AAP on virus dosage (Simons 1954). Since the length of the AAP was assumed in these studies to be correlated with the quantity or dosage of virus acquired by the vector. Kyriakopoulou and Sylvester (1969) found a positive correlation of virus dosage (length of AAP) and the retention of inoculativity. Injection of pea aphids with different dosage levels of PEMV also indicated both an increased rate of transmission and a longer retention of inoculativity with increasing dosages of virus (Clarke and Bath 1973).

Localization and distributional studies of PEMV within the pea aphid using electron microscopy have been conducted by several investigators. In the pea aphid, Shikata et al. (1966) observed PEMV virions in the midgut lumen and losely scattered in the cytoplasm of gut epithelial and fat body cells. At four days post-acquisition of PEMV by pea aphids, Harris et al. (1975) found virions of PEMV concentrated in the midgut and hindgut lumens and in the cytoplasm of midgut muscle cells and of fat body cells. Virions were visualized in the salivary gland of aphids injected intrahemocoelically with PEMV or of aphids which had fed directly on infected plants. Harris et al. (1975) showed that a possible site determining specificity for PEMV may lay at the salivary gland membrane. He found that a nontransmissible PEMV isolate (CNT-PEMV) apparently could not enter into the salivary gland of the pea aphid while in a parallel experiment an aphid-transmissible isolate

(CAT-PEMV) was readily visualized within the salivary gland.

It has been speculated by several investigators that PEMV may multiply in its pea aphid vector (Sylvester 1969, and Harris and Bath 1972). PEMV probably multiplies in young nymphs (Harris and Bath 1972). However, conclusive evidence has not been obtained. The transmission phase of PEMV is influenced by temperature (Sylvester and Richardson 1966). They found that the latent period (LP) was approximately 70, 25 and 14 hours at 10, 20, and 30°C, respectively. Clarke and Bath (1973) found that the LP for PEMV within the pea aphid was dosage dependent, LP was negatively correlated with dosage. These results seem to indicate virus multiplication. However, other results indicate that virus retention is correlated with virus dosage (Simons 1954, Kryakopoulou and Sylvester 1969, and Clarke and Bath 1973) leaves doubt as to multiplication occurring. The serial passage technique (Maramorosh 1952) is the accepted proof of virus multiplication (Sylvester 1969) and it has not been successful for PEMV and the pea aphid. Clarke and Bath (1973) found only the first passage of hemolymph to produce viruliferous aphids and that subsequent passages failed to produce viruliferous aphids.

Nymphs versus Adults in Transmitting PEMV

In all respects, nymphs of the pea aphid are more efficient vectors of PEMV than adults (Simons 1954).

Efficiency is expressed normally as the number of insects rendered viruliferous per unit effort, i.e., length of acquisition time or the number of plants infected by a group of insects in a unit effort of time, i.e., inoculation efficiency. Thottappilly et al. (1972) found that if aphids acquired purified virus across a Parafilm membrane, nymphs were 100% efficient in transmission after a 1-hr AAP. Adults, on the other hand, given a 24-hr AAP were only 26.7% efficient in acquisition. Osborn (1935) estimated the latent period of PEMV in the pea aphid to be about 28 hours. Chapman and Bath (1968) determined the minimum latent period for nymphs of the pea aphid to be 6-8 hrs (LP50 = 20.7 hrs) and for adults 23 hrs (LP50 = 60.6 hrs). The difference between the length of the latent period in nymphs and adults probably is influenced by the rapid growth rate of nymphs. Bath and Chapman (1968) also showed that nymphs were more efficient inoculators of PEMV than adults. However, with increasing inoculation access periods (IAP), the efficiency of transmission by both nymphs and adults increases.

Retention of Inoculativity versus Virus Concentration

The rate of transmission of PEMV by individual pea aphids tends to fluctuate during a vector's life and generally declines with age. When adults were given a 24-hr AAP, the rate of transmission was low during the

first two days after the AAP. The rate of transmission increased to a maximum and then rapidly declined. This pattern of transmission differed from nymphs. For nymphs, the rate of transmission was high from the first day after the AAP and reached a maximum; the high transmission rate was maintained for a longer period than for adults. The transmission rate declined with age but the decline was more gradual than for adults (Sylvester and Richardson 1966b). Therefore, pea aphids given an AAP as 1st instar nymphs infected more plants than adults given an AAP, thus their retention of inoculativity was longer.

Sylvester and Richardson (1966b) hypothesized that the age of the vector at the time of virus acquisition was the key factor influencing retention of inoculativity not the virus concentration acquired during feeding. Sylvester (1969) concluded that the length of actual PEMV retention by the pea aphid, i.e., virus held within the vector's body, cannot be determined by retention of inoculativity testing. Sylvester and Richardson (1966b) attempted to "recharge" viruliferous adult aphids with virus by giving them a second 24-hr AAP, seven days after completion of a first 24-hr AAP. The first AAP was given when the aphids were 1st instars. The second AAP was given at about the time the decline in inoculativity was known to occur. The rate of transmission of the "recharged" adults increased for a few days but ultimately they did not transmit significantly longer than a parallel set of nonrecharged adults. Clarke

and Bath (1973) found that the retention of inoculativity of PEMV was dosage dependent for pea aphids given an intrahemocoelic injection as early instars. Failure to significantly increase the retention of inoculativity of adult viruliferous pea aphids by recharging seems to contradict evidence of a positive correlation between virus dosage and retention of inoculativity.

Sylvester and Richardson (1966) did determine that the first acquisition of virus during the recharging experiment was an independent event and did not interfere with the ability of the aphids to acquire a subsequent "charge" of virus during the second AAP. Therefore, they concluded that the decline in inoculativity even in recharged adults was the result of an aging factor in adult aphids.

Methods Used to Study Virus-Vector Relationships

Various methods have been used to study the biological relationships between plant viruses and their insect vectors. Inoculativity assays (conventional aphid to plant transmission experiments) have been used for many years to study the various aspects of insect transmission, i.e., acquisition, inoculation and retention. With the development of virus purification technology more quantitative assays have been possible. Various dosages of purified virus have been fed to aphids across parafilm membrane systems (Thottappilly *et al*. 1972). This method provided

more control of virus dosage acquired by the aphid than did varying the length of the AAP on infected plants. Electron microscopy has been used to detect and to study virus distribution in both plants and insect vectors (Shikata et al. 1966, and Harris et al. 1972, 1975). Intrahemocoelic-injection techniques also have been used to study the effect of virus dosage on transmission (Clarke and Bith 1973), virus multiplication (Stegwee and Ponsen 1958, and Harrison 1958) and distribution (Harris et al. 1975). Use of serological methods have provided a valuable tool for studying viruses. To increase the sensitivity of serological tests, various labels such as enzymes, fluorescent molecules and radioisotopes have been attached to antibodies. Sinha (1965) used a fluorescent antibody technique to study the distribution and accumulation of wound tumor virus in its leafhopper vector.

Enzyme-Linked Immunosorbent Assay

A new serological method to identify plant viruses was introduced by Clark and Adams (1977). This method previously had been used to identify animal and human pathogens (Voller et al. 1977). This method, enzyme-linked immunosorbent assay (ELISA), uses antibody labeled with an enzyme. The double sandwich method of ELISA (Voller et al. 1977) is most suited for plant virus research. This method is helpful for identifying plant viruses in plant material having low virus concentrations where other serological

methods were not successful (Casper 1978, and Thresh et al. 1977). ELISA has a detection sensitivity 150 times greater than latex tests and about 2000 times greater than a precipitin ring test (Koenig 1978). This method is not only useful in virus identification, but it also gives a quantitative measure of virus concentration. This added property proves to be useful in studying virus distribution and accumulation in plant parts (Casper 1978, and Converse 1978).

Application of this method to the study of virus-vector relationships was first made by Gera et al. (1978). They used an ELISA system for a nonpersistent virus, cucumber mosaic virus (CMV), and its vector the melon aphid (Aphis gossypii Glover). The quantity of transmissible CMV held by an aphid was 0.01 to 0.1 ng. The transmissible isolate was detected in the melon aphid after a one minute AAP, while a poorly transmissible isolate could not be detected. Clarke et al. (1980) used ELISA to detect potato leaf-roll, a persistently transmitted virus, in the green peach aphid (Myzus persicae (Sulzer)).

MATERIALS AND METHODS

Aphid Vectors

The pea aphid biotype EL_1 (Bath and Tsai 1969) reared on broad bean (Vicia faba L.) was used in this thesis research. This biotype is an efficient vector of the California aphid transmissible (CAT) isolate of PEMV. Only apterous aphids of known age were used in the following experiments. Test aphids were produced by placing viviparae on broad bean for a 12 to 16 hr nymph deposition period. First instars collected from these plants were designated as 1 day old. Groups of these aphids were either used immediately in an experiment or reared for designated intervals on broad bean until they reached a specific age required for a particular experiment. Therefore, all references to aphids in this thesis are based on their chronologic age, e.g., 1 day old, 8 days old, etc. Aphids 1 to 7 days old generally were developing instars (I-IV) and those 8 days and older were adults (viviparae). All aphids were maintained in a greenhouse at 20-25^{o}C with supplemental lighting producing a 16-hr photoperiod.

CAT-PEMV Isolate

The CAT-PEMV isolate was maintained during this research in garden pea (Pisum sativum L.) ca. Midfreezer by routine aphid transmission at 10 day intervals. Aphids were given a 24-hr AAP on 10-day infected pea plants and

these aphids were used to inoculate preleaf-stage peas for 48 hours. Ten-day CAT-PEMV infected peas were used as virus source plants in the various virus acquisition treatments. Peas in the preleaf-stage also were used as test plants in transmission experiments. Virus source plants as well as plants from various transmission experiments were maintained in a greenhouse at 20-25°C with supplemented lighting producing a 16-hr photoperiod. Virus transmission results, based on symptomatology, were recorded 20 days after aphid inoculation.

Virus Bioassay

The enzyme-linked immunosorbent assay (ELISA) was used as a bioassay method for detecting the concentration of CAT-PEMV in test aphids. Antiserum used in ELISA testing was produced by Clarke and Bath (1977). The procedure used polystrene microelisa plates (Dynatech Lab., Alexandria, VA) as described by Clark and Adams (1977). The gamma globulin fraction of CAT-PEMV antiserum used to coat microelisa plates was adjusted to 1 µg/ml. Alkaline phosphatase (Sigma Chem. Co., St. Louis, MO) conjugated to CAT-PEMV gamma globulin was used at a 1:500 dilution in the bioassays (Clark and Adams 1977).

The procedure for ELISA testing was as described by Clark and Adams (1977) with the modifications of Clarke et al. (1980). ELISA testing consisted of a series of incubation periods with different ELISA reagent separated

by washing steps (Voller et al. 1977). All buffers in this procedure contained sodium azide (NaN_3) as a preservative. The buffers were as follows:

Phosphate buffered saline (PBS) = 8.0 g NaCl, 0.2 g KH_2PO_4, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g KCl, 0.2 g NaN_3, and distilled water to make up 1 and adjusted to pH 7.4.

PBS Tween = 0.5 ml Tween 20 in 1 l. PBS. Tween is a wetting agent to prevent nonspecific absorption to the solid phase of the microelisa plate. PBS Tween was used for washing microelisa plates.

Sample buffer = PBS Tween with 0.2% egg albumin and 2% polyvinyl pyrrolidone (PVP). This buffer was used for preparing test samples and also for diluting the enzyme-labeled gamma globulin. Egg albumin reduces background by keeping nonspecific uptake of reagent to a low level, while PVP absorbs phenolic material in samples.

Coating buffer = 1.59 g $NaCO_3$, 2.93 g $NaHCO_3$, 0.2 g NaN_3 in 1 l. distilled water adjusted pH 9.6. This buffer was used for diluting the gamma globulin.

Substrate buffer = 97 ml diethanolamine, 0.2 g NaN_3 with distilled water added to 1 l., pH 9.8. This was used in preparing a 1 mg/ml solution of p-nitrophenyl phosphate as the enzyme substrate.

The first step in the ELISA procedure was to coat micorelisa wells with gamma globulin (1 µg/ml) diluted in coating buffer by adding 200 µl per well and incubating the plate at $4^{o}C$ overnight. The plate was then emptied and

washed by flooding wells with PBS Tween, letting the PBS Tween stand for 3 min, emptying, and repeating the process three times. The 200 µl of test samples were then added to assigned wells and incubated at 4°C overnight. The plate was then washed again as described above. Enzyme-labelled gamma globulin in sample buffer (200 µl/well) was then incubated at 37°C for 4 hours. The plate was again washed three times. Enzyme substrate, p-nitrophenyl phosphate, in substrate buffer was added (300 µl/well) and left to react at room temperature for 1 hour. The hydrolyzed substrate (p-nitrophenol) produced a yellow coloration, with its intensity directly proportional to the amount of virus in the sample. The enzyme reaction was stopped by adding 50 µl 3 M NaOH. The color intensity was measured in a Beckman 25 spectrophotometer at 405 mm visible light.

Preparation of Test Samples. Aphids used in ELISA testing were triturated in depression wells of Corning® spot plates. The test aphid was placed in a depression well containing 0.3 ml sample buffer. The end of a 10 x 75 mm test tube was used to triturate the aphid sample. A 200 µl aliquot of each test sample was then placed in a microelisa well.

Rate of Transmission by Variously Aged Aphids

One-, 8-, 13- and 23-day-old aphids were given a 24-hr virus acquisition treatment on CAT-PEMV infected pea. The

aphids from each group were placed on individual pea seedlings for a 24-hr inoculation period (IP). During the IP, each test plant was covered with a 2 inch diameter acetate cylinder with a saran screen top. Each aphid was transferred to a new pea seedling daily for a total 15 days or until death. After each inoculation, the test plants were fumigated with Vapona® insecticide and kept in a greenhouse to allow PEMV symptom development. The purpose of this experiment was to determine the rate of transmission of the variously aged aphids.

Concentration of CAT-PEMV in Variously Aged Pea Aphids

The concentration of CAT-PEMV in variously aged pea aphids was determined using ELISA bioassays. Aphids 8, 13, 18 and 23 days old were given a 24-hr virus acquisition treatment on infected peas. These aphids were individually bioassayed by ELISA immediately after completion of the virus acquisition treatment. The assay reading represented the accumulated virus concentration in the variously aged aphids after completion of the standard virus acquisition treatment and used as a relative measure of feeding rate between age groups.

Age-Specific Bioassay for CAT-PEMV Within Pea Aphids

One- and 8-day-old aphids were given a 24-hr AAP on CAT-PEMV infected plants. Individual weights of each

1- and 8-day-old aphid was recorded using a Mettler® H_{10} balance. Ten individuals from each age group were placed on individual pea seedlings for a 24-hr IP to assay the rate of PEMV transmission by each age group. After completion of the IP, these aphids were then individually bioassayed by ELISA. The remaining aphids from the two groups which were not assayed in the first post-acquisition day were kept in groups on separate broad bean plants and transferred to new plants at 2-day intervals until designated assay times of 5, 10, and 15 days post-virus acquisition. At these assay times, ten aphids from each age group were each given a 24-hr IP on test plants followed by individual ELISA bioassaying. Thereby age-specific assays of both virus transmission rate and virus concentration within pea aphids were obtained for two virus acquisition treatments.

Distribution of CAT-PEMV Within the Pea Aphid

Eight-day-old aphids were given a 24-hr virus acquisition treatment on infected peas. Five of these aphids were bioassayed by ELISA immediately after completion of virus acquisition with the remainder maintained on broad bean until designated age-specific bioassay times. Each aphid was dissected into head, gut and body regions for separate ELISA bioassays. Dissections viewed through a binocular microscope were performed on aphids held in

depression wells of a Corning® spot plate containing 0.05 ml of sample buffer. The gut region was removed by grasping the head (near the antennae) and the cauda with forceps and gently pulling. The head region with the gut remaining attached was placed in an adjacent depression well. The head was then separated from the gut region (foregut, midgut and the majority of the hindgut) and placed in a third well. Heads were pooled (five heads/sample) into a composite sample. After the three sample regions from each test aphid was triturated, 0.25 ml sample buffer was added to each to make a final volume of 0.3 ml/sample. The procedure was repeated at 5, 10, and 15 day post-acquisition with the aphids being 13, 18, and 23 days old, respectively.

RESULTS

Rate of Transmission by Variously Aged Aphids

The highest rate of transmission was attained by aphids given a 24-hr virus acquisition treatment at 1 day old (Figure 1). At 3 days old, their transmission rate was 85% with the highest rate (95%) occurring at 4 and 5 days old. At 6, 7, and 8 days old, the transmission rate declined gradually reaching 78% as new adults (8 days old). This rate was maintained by 9- and 10-day-old aphids but again declined rapidly with 16-day-old individuals having a transmission rate of 20%. In general, the decline in the transmission rate during the nymphal period was noticeably slower than that recorded for adults. During the nymphal period (days 2-8), the rate of decline was 17% while during the following 8 days as adults the decline was 58%.

In contrast, aphids given a 24-hr virus acquisition treatment at 8 days old had an initial transmission rate of only 50% at 10 days old (Figure 1). This rate was lower than for 10-day-old aphids which had acquired PEMV at 1 day old. Moreover, the decline in the transmission rate was more rapid than for aphids acquiring virus at 1 day old (Figure 1). At 16 days old, their rate of transmission was 5% and at 24 days old no transmission occurred. Further, aphids which initially acquired PEMV at 13 days old had a transmission rate of only 5% at 16 days old while those

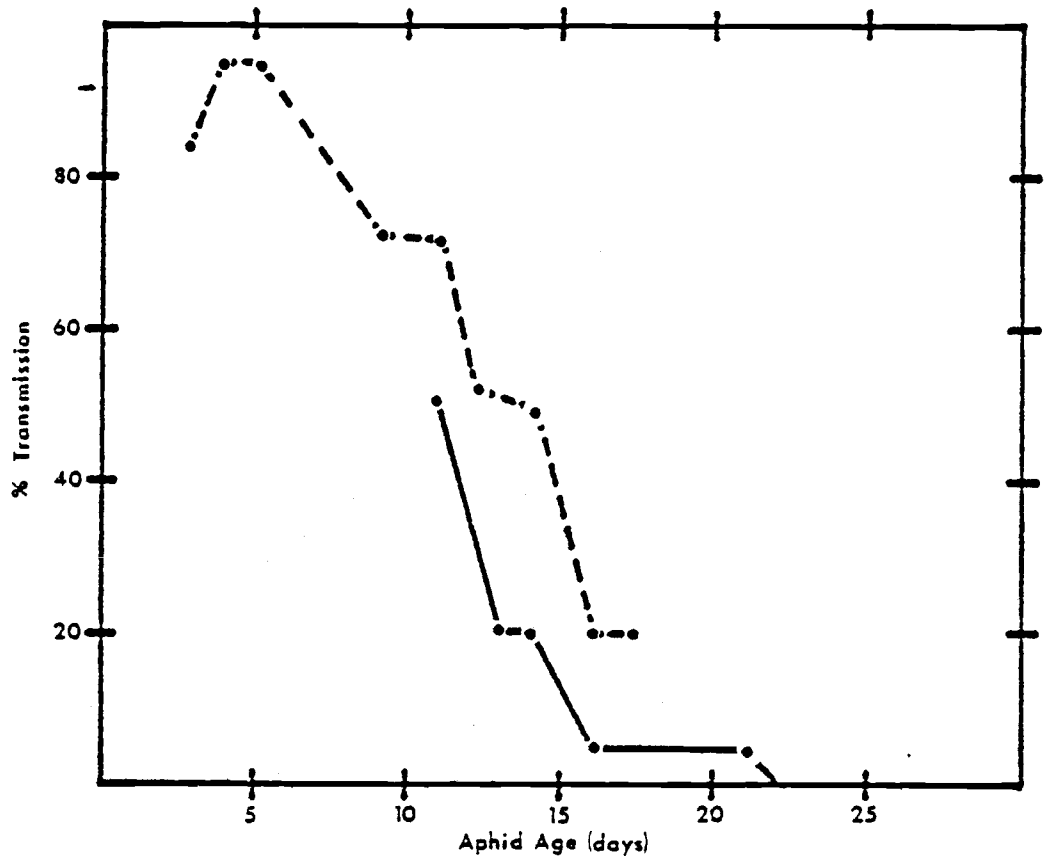

Figure 1. The transmission rate of pea enation mosaic virus (PEMV) by the pea aphid. One- (·-----·) and 8-day old (·———·) aphids were given a 24-hr virus acquisition treatment on CAT-PEMV-infected garden pea. Aphids were initially given a 48-hr inoculation period (IP) on a garden pea seedling followed by daily transfers to new pea seedlings at 24-hr intervals for 15 days or until death.

acquiring PEMV at 23 days old failed to transmit PEMV at all (Table 1).

Table 1. Periodic monitoring of the transmission rate of 4 different age groups of pea aphids following a 24-hr virus acquisition treatment on pea enation mosaic virus (PEMV) infected garden pea.

Aphid Age at Start of Virus Acquisition	Rate[a] of Transmission (%) at Indicated Age			
	3	11	16	24
1	85	72	20	-[b]
8	--	50	5	0
13	--	--	5	0
23	--	--	--	0

[a]% of aphids transmitting PEMV at the indicated age.

[b]Not recorded.

The rate of transmission was strongly influenced by the age of the aphid at the time of virus acquisition. For example, 11-day-old aphids which acquired PEMV at 1 day old had a transmission rate of 72% while 11-day-old aphids which acquired virus at 8 days old had only a 50% transmission rate (Table 1). At 16 days old, the transmission rate for aphids which acquired as 1, 8, and 13 days old was 20, 5, and 5%, respectively. No transmission was obtained from 24-day-old aphids that acquired PEMV as 8-, 13-, and 23-day-old individuals.

Concentration of CAT-PEMV in Variously Aged Pea Aphids

The concentration of CAT-PEMV was determined by ELISA testing of variously aged adult aphids following a 24-hr virus acquisition treatment (Table 2). Eight- and 13-day-old aphids had approximately the same concentration while 18- and 23-day-old aphids had a significantly lower concentration than 8- and 13-day olds. In about 25% of the 18- and 23-day-old aphids tested, no assayable virus was recorded (A_{405} = 0.00). On the other hand, assayable virus was found in all 8- and 13-day-old aphids tested following the 24-hr virus acquisition treatment.

Eighteen- and 23-day-old adults wandered more on virus source plants indicating a decrease in total feeding time. When 13- and 18-day-old adults were simultaneously observed during a 4-hr feeding period on pea plants, the 13-day-old aphids maintained a feeding posture an average of 172.5 minutes while the 18-day-old aphids only an average of 99.3 minutes. The decrease in the level of PEMV acquired by older aphids (18 and 23 days old) is probably a result of reduced feeding by these individuals. Sylvester (1967) found that the amount of honeydew excreted by older pea aphids declined with age indicating a decrease in their feeding rate.

Table 2. Concentration of pea enation mosaic virus (PEMV) in 4 age groups of pea aphids following a 24-hr virus acquisition treatment on infected garden pea.

Age of Aphid	Mean A_{405} Reading[a]
8	0.94
13	0.93
18	0.18
23	0.28

[a]Mean A_{405} value from ELISA testing of individual aphids LSD_{05} = 0.37.

Age-Specific Bioassay of CAT-PEMV Within Pea Aphids

Following a 24-hr virus acquisition treatment on PEMV-infected pea, 9-day-old aphids contained a higher concentration of PEMV than did 2-day-old pea aphids (Figure 2). In ELISA testing, the average A_{405} reading from 9-day-old aphids was 0.44 while the average reading from 2-day-old aphids was 0.05.

Differences in body size (wt) between these nymphs and adults probably accounts for this difference in initial virus uptake as reflected by different A_{405} readings. The average weight of 1- and 8-day-old aphids was 0.16 mg and 1.8 mg, respectively. The ratio of these body weights between 8- and 1-day-old aphids was about 11:1. The ratio

of the mean A_{405} readings from these aphids was of about the same magnitude, 9:1. Eulensen and Clarke (Unpublished) estimated that 1 ng of purified PEMV produced an A_{405} reading of about 0.02 in ELISA testing. Assuming that equivalent readings for PEMV can be obtained from in vitro and in vivo assays, 1- and 8-day-old aphids acquired 2.3 ng and 20 ng of PEMV, respectively, during the 24-hr virus acquisition treatment.

The virus concentration in 2-day-old aphids (A_{405} = 0.05) after completion of a 24-hr virus acquisition treatment was approximately the same as that in their 17-day-old cohorts which had been held during the interim on broad bean (Figure 2). At 7, 12, and 17 days old, A_{405} readings were 0.03, 0.06, and 0.07, respectively. Once PEMV was acquired by 1-day-old aphids the virus concentration remained constant for most of their life. In contrast, adult aphids (8 days old) given the same virus acquisition treatment maintained a significantly higher yet decreasing concentration of PEMV in their body during this monitoring period. Mean A_{405} readings from the adult aphids decreased from an initial A_{405} reading of 0.44 at 9 days old to 0.33 at 14 days old. This decline continued with 19- and 24-day-old aphids having even lower readings. The virus concentrations in the 19- and 24-day-old aphids were significantly different from the initial concentration at 9 days old but not significantly different from the concentration recorded at 14 days old ($P = 0.05$).

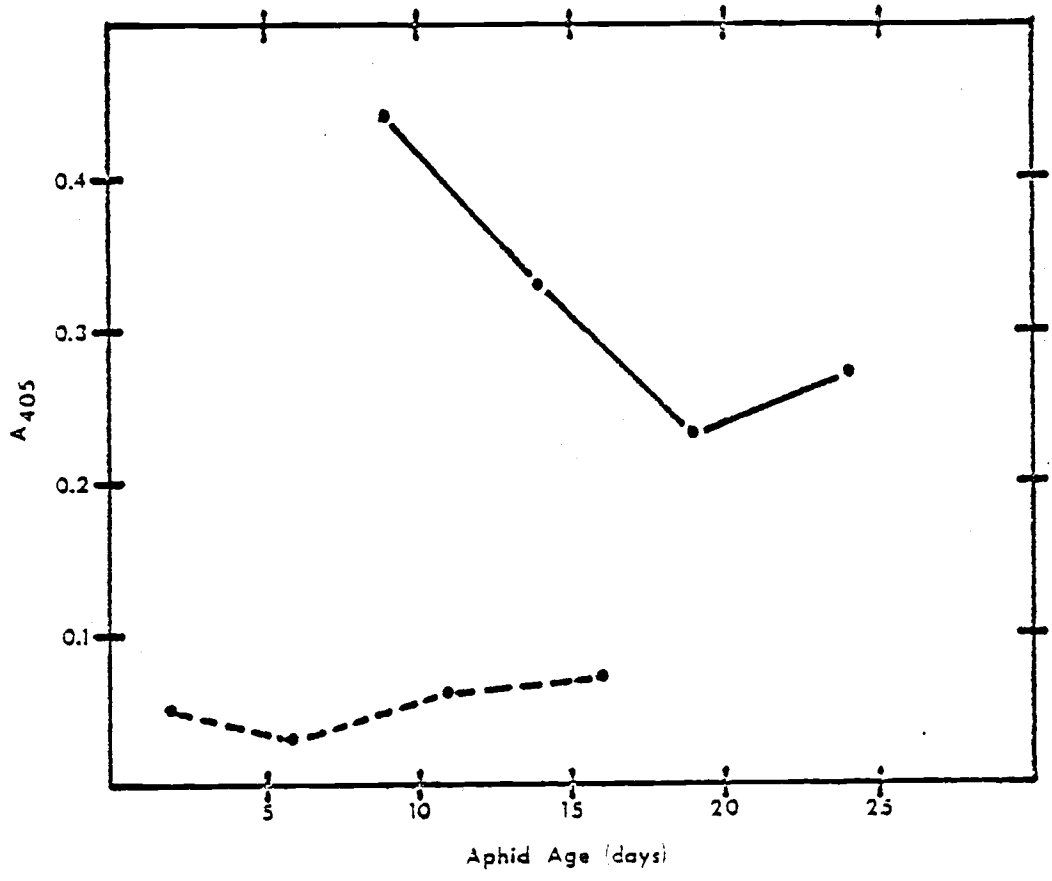

Figure 2. Age-specific concentration (A_{405}) of pea enation mosaic virus (PEMV) within pea aphids as determined by ELISA testing. A 24-hr virus acquisition treatment was given to two separate groups of aphids at either 1 day (·-----·) or 8 days old (·———·).

Observations on individual aphids indicated that transmitting aphids did not necessarily have a higher virus concentration than non-transmitting aphids. Aphids acquiring virus at 1 day old and transmitting at 2 days old had A_{405} readings ranging from 0.01 to 0.47 while non-transmitting aphids had A_{405} readings ranging from 0.00 to 0.20 (Table 3). The average A_{405} reading from transmitting aphids in this case was not significantly different from the mean A_{405} reading from non-transmitting aphids. This was true also at 7, 12 and 17 days old. In a parallel test, aphids acquiring virus at 8 days old and transmitting at 9 days old had A_{405} readings ranging from 0.12 to 0.42 while non-transmitting aphids had virus readings ranging from 0.09 to 1.66. In this case, there was a significant difference in the mean A_{405} readings between transmitting and non-transmitting aphids. In the other cases, however, 14, 19 and 24 days old, there was no significant difference between the mean readings of transmitting aphids and non-transmitting aphids. It was clear that transmission on a particular day by a viruliferous aphid was independent of the virus concentration within the aphid.

Table 3. Relationship of pea enation mosaic virus (PEMV) transmission by the pea aphid and the concentration of PEMV within the pea aphid assayed by ELISA.

Aphid Age at Acquisition[a]	Aphid Age at Transmission	Transmission Category[b]					
		TM			No TM		
		N[c]	Range[d]	Mean[e]	N	Range	Mean
1	2	10	0.01-0.47	0.09±0.04	10	0.00-0.20	0.05±0.02
	7	18	0.00-0.11	0.04±0.01	2	0.01	0.01±0.00
	12	12	0.01-0.08	0.04±0.01	8	0.00-0.10	0.03±0.01
	17	3	0.05-0.18	0.10±0.04	7	0.02-0.07	0.04±0.01
8	9	5	0.12-0.42	0.28[f]±0.06	15	0.09-1.66	0.47[f]±0.03
	14	5	0.05-0.52	0.22±0.09	15	0.03-1.28	0.35±0.10
	19	5	0.00-0.41	0.16±0.08	15	0.03-0.61	0.24±0.05
	24	1	0.26	0.26±0.00	8	0.11-0.46	0.27±0.04

[a] 24-hr virus acquisition treatment on PEMV infected garden pea.

[b] TM=aphids which transmitted PEMV, No TM=aphids which did not transmit PEMV during a 24-hr IP on a garden pea seedling.

[c] Number of aphids that were assayed by ELISA in each transmission category after completion of a 24-hr inoculation period (IP) on a healthy garden pea seedling.

[d] The range of A_{405} values from individual test aphids.

[e] Mean A_{405} readings from aphids ± standard error of mean. Unless otherwise indicated there was no significant difference between the mean of transmitting and non-transmitting aphids at the same test age (unpaired t test).

[f] These means were significantly different ($t_{cal.}$=2.37; $t_{05\ table}$=2.101 with df 18).

Distribution of CAT-PEMV Within the Pea Aphid

The highest concentration of PEMV was detected in the gut of 9-day-old aphids that had just completed a 24-hr virus acquisition treatment on PEMV infected peas (Figure 3). The mean concentration (A_{405}) from the gut was 0.41 compared to 0.08 from the body. The concentration in the gut was significantly higher than subsequent levels determined for 14-, 19- and 24-day-old aphids. On the contrary, the virus concentration in the body tended to increase with time. At 19 and 24 days old, the concentration (A_{405}) was 0.11 and 0.12, respectively, which was higher than the level in the body at 9 and 14 days old.

The high virus concentration detected in aphid guts immediately following completion of the acquisition treatment indicated that most of the virus within the vectors had not moved into the hemolymph. However, as the viruliferous aphids fed on healthy broad bean during post-acquisition rearing, the virus concentration in the gut decreased with time. Virus movement into the body of the vectors as evidenced by the increased PEMV level detected in the body and virus excretion in honeydew during subsequent feeding on healthy broad bean probably accounted for the majority of this decrease.

The virus concentration in the head region of the aphids was very low. The concentration from five aphids taken as a composite sample (A_{405}) was 0.03 immediately

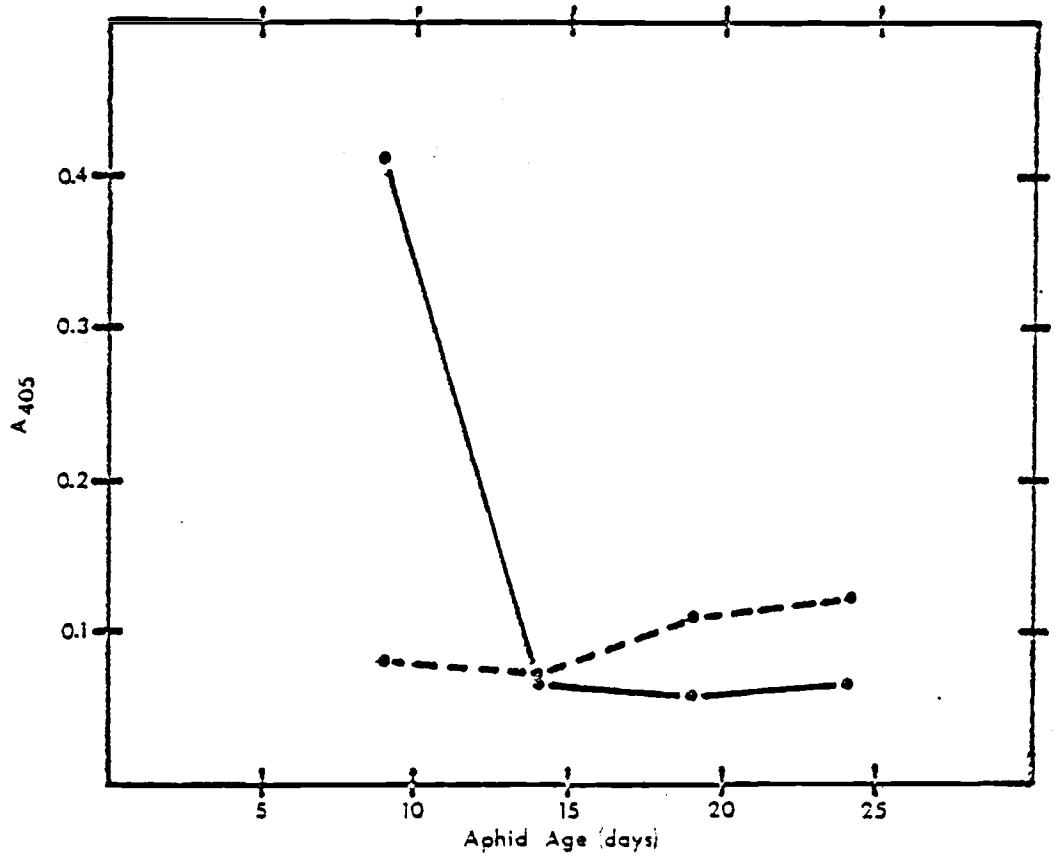

Figure 3. Age-specific concentration (A_{405}) as determined by ELISA testing of pea enation mosaic virus (PEMV) in aphids dissected into gut (·———·) and body (·-----·) regions. Eight-day-old pea aphids were given a 24-hr virus acquisition treatment on CAT-PEMV-infected garden pea. Aphids were reared on broad bean until age-specific dissection times.

after completion of the AAP. Afterwards in subsequent ELISA testing, virus was not detectable. Virus recorded in the heads may have been from virus-laden sap in the head portion of the alimentary canal. The salivary gland remains attached to the head during dissection and a portion of the virus may have been from this organ.

DISCUSSION

The maximum rate of PEMV transmission attained by the pea aphid in this research was dependent upon the age of the aphid at the time of virus acquisition. Maximum rates of transmission 95, 50 and 5% were achieved by 1-, 8- and 13-day-old aphids, respectively, given a 24-hr virus acquisition treatment (Figure 1 and Table 1). Twenty-three-day-old aphids treated similarly did not transmit. The highest transmission rate was achieved by the aphids within a few days after completion of the virus acquisition treatment. These results are similar to those reported elsewhere (Simons 1954, Sylvester and Richardson 1966b, and Bath and Chapman 1968).

When discussing the differences in transmission rates by nymphs and adults, Sylvester (1962) suggested the possibility that nymphs acquire more virus per unit of acquisition feeding time than do adults and thereby attain a higher virus concentration in their hemolymph. My results showed that this is not true. One- and 8-day-old aphids (newly-emerged adults) had about the same acquisition efficiency during a 24-hr virus acquisition treatment judging by the ratio of ELISA-assayable virus acquired, to the aphid's body weight. The initial virus concentration, expressed as nanograms of virus per milligrams of aphid, assayed from 2- and 9-day-old aphids immediately following the acquisition treatment, was approximately the same. It

appears that differences in transmission rates between nymphs and adults cannot be explained simply in terms of acquisition efficiency.

Virions must pass through the single layer of epithelial cells lining the alimentary canal in order to reach the hemocoel. Forbes (1964) stated that the most likely mechanism for virion entrance into epithelial cells was pinocytosis. Harris et al. (1975) reasoned that a direct penetration mechanism was more likely involved. The rate of virus penetration of the microvilli of epithelial cells would influence the rate of virion entry into the hemocoel. If such entry is influenced by or decreases with vector age, it would be a critical factor influencing transmission rates. Assuming, transmission rate is influenced by virion titer in the hemolymph.

The influence of gut permeability on virus transmission was demonstrated by Sinha (1965). He was able to change normally inefficient adult leafhopper vectors (Agallia constricta) of wound tumor virus (WTV) into efficient transmitters, similar to the nymphal stages, by puncturing the gut of adult leafhoppers just after completion of an AAP. He suggested that the inefficiency of the adults was due to decreased permeability of the gut to virions resulting in fewer wound tumor virions reaching the hemolymph and thereby fewer reaching the salivary gland. Once virus was allowed to pass into the hemolymph the adult leafhoppers had transmission characteristics similar to nymphs.

Differences in the transmission rates achieved by pea aphids 2, 9 and 14 days old (Table 1) could be explained by differences in gut permeability rates which would directly affect the titer of PEMV in the hemolymph. The initial PEMV concentration acquired by individual 8- and 13-day-old aphids during a 24-hr virus acquisition treatment was approximately the same (Table 2). This indicated that 13-day-old aphids had acquired sufficient virus to potentially transmit at the same rate as 8-day-olds. The majority of the PEMV detected in ELISA assays of 8-day-old aphids was recorded from the gut (Figure 3). Although not tested, it is assumed the virus detected in 13-day-olds was similarly distributed at completion of virus acquisition. One could speculate that decreased gut permeability in 13-day-old aphids accounts for differences in transmission rates. However, other factors are involved in this complex transmission cycle within aphids and will be discussed below.

Transmission of persistent plant viruses by aphids is dependent upon the presence of virus in the saliva of the vector. Bawden (1963) suggested that the hemolymph of aphids serves as a reservoir site for virus and that circulating virions pass from hemolymph into the salivary system intermittently and in small quantities. When pea aphids acquire PEMV at 8 days old, the gut and the body of these aphids contain ELISA-assayable virus for at least 16 days post-acquisition (Figure 3). At that time (24-day-old aphids), the virus content in the body was higher than that

in the gut. In assays to determine the transmissibility of circulating virions, nymphs (third instars) transmitted PEMV after being intrahemocoelically injected with hemolymph obtained from 12-, 15- and 18-day-old donor aphids given a 24-hr acquisition treatment at 8 days old. This indicated that circulating virions are aphid transmissible even though the aphids used as hemolymph donors in these experiments infrequently transmit during feeding on susceptible plants (Suharto and Clarke, Unpublished). This indicated that the rapid decline in the rate of PEMV transmission by 14-, 19- and 24-day-old aphids is not due to a lack of transmissible virus in the hemolymph but probably due to a lack of virus reaching the saliva. Bawden (1963) further stated that the virus concentration in the salivary system of vectors is readily exhausted during feeding and that virions pass into the salivary system intermittently rather than at a steady rate. This hypothesis is based on the observation that aging viruliferous aphids typically fail to transmit for one or two day periods during serial transmission experiments.

Harris et al. (1975) localized very low numbers of virions of PEMV in the salivary system of the pea aphid compared to other organs of the aphid. Virions were visualized in the basal lamina which surrounds the bilobed primary gland. A relatively higher concentration was observed in the basal laminae of the paired accessory glands and in the labyrinth of cisternae associated with

the plasma membrane of the accessory gland cells. When examining the basal laminae of other organs, i.e., neurilemma of the central nervous system, no virions were visualized indicating a special relationship of PEMV to the basal lamina of the salivary system. This supports Bawden's (1963) speculation of low level virus concentration in the salivary glands.

If the salivary system, particularly the accessory glands, like the epithelial cells lining the alimentary canal, become less permeable to virions in aging vectors, this change in permeability could explain the transmission decline phenomenon. If the hemolymph serves as a virus reservoir, the concentration of virions in the saliva would be determined both by the permeability of the salivary system basal lamina and membrane to the circulating virions in the hemolymph, and by the concentration of virions in the hemolymph. This salivary gland permeability factor parallels the gut permeability factor mentioned previously. Membrane flow has been suggested as a possible mechanism for virion entry into the accessory glands (Harris et al. 1975). Therefore, two structural barriers to virion movement may exist in the pea aphid, the gut epithelial cell and salivary system basal lamina, and membranes. Both of these may affect virus transmission and may be influenced by aphid age.

Virions circulate freely in several sites within the pea aphid. These being the lumen of the alimentary canal,

the hemocoel and the salivary system. As of yet, there is no evidence that virions are chemically degraded in these sites or rendered nontransmissible. My results showed that high concentrations of PEMV are present in insects for at least 16 days post-acquisition (Figure 3). Virus maintained serological integrity, in the aphid, judging by the positive ELISA test results and also aphid transmissibility when circulating in the hemolymph as determined by intrahemocoelic injection assay. Sylvester (1965) showed that virus retained its aphid transmissibility even after being excreted in honeydew. No one has reported adverse effects of saliva on persistent plant viruses like PEMV. Therefore, degradation of circulating virions rendering them nontransmissible may not be a significant factor influencing transmission rates.

Harris et al. (1975) proposed a hypothetical sequence for the fate of virions in connective tissue cells in the pea aphid. He noted an excretory mechanism involving possible lysosomal digestion of circulating PEMV virions engulfed by mesodermal connective tissue cells. This process is similar to that described by Griffiths and Beck (1973) for secondary symbiotes which circulate in the pea aphid hemolymph much like virions. Apparently, virions which do not contact the salivary system and successfully enter the accessory or primary glands, may eventually suffer the fate of being digested and eliminated by this excretory mechanism.

One final point needs to be mentioned. That being changes in the feeding rate of aging viruliferous aphids. Sylvester (1967) emphasized that decreased feeding rates may be responsible for the decline in the transmission rate of aging vectors even though adequate virus is available for transmission. This subject was not directly studied in this thesis except for the assayable virus level experiment (Table 2) which showed that less assayable virus was present in 18- and 23-day-old aphid following a 24-hr acquisition treatment than in 8- and 13-day-old aphids. An important point is, however, that the 8- and 13-day-old aphids had approximately the same virus level indicating that each had fed enough to potentially be equivalent vectors. Yet, their maximum transmission rates were dramatically different, 50 and 5%, respectively. Sap ingestion (accumulation) rates and salivation rates during aphid feeding are certainly related and parallel each other. The rate of injection of virus-laden saliva ultimately directly determines the rate of transmission. More research is needed to quantify salivation rates for variously aged pea aphids to determine the influence of this parameter on the transmission rate decline phenomenon.

BIBLIOGRAPHY

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

Bath, J.E., and R.K. Chapman. 1967. Differential transmission of two pea enation mosaic virus isolates by the pea aphid, Acyrthosiphon pisum (Harris). Virology 33:503-506.

Bath, J.E., and R.K. Chapman. 1968. Influence of aphid stage on acquisition and inoculation phases of pea enation mosaic virus transmission. Ann. Entomol. Soc. Am. 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. (1963). Plant viruses and virus disease. 4th Ed. 355 p. The Ronald Press Comp., New York. 110-142.

Casper, R. 1978. Detection of potato leaf-roll virus in potato and in Physalis floridana by enzyme-linked immunosorbent assay (ELISA). Phytopathol. Z. 90:364-368.

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.

Clark, M.F., and A.N. Adams. 1977. Characteristics of microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. J. Gen. Virol. 34:475-482.

Clarke, R.G., and J.E. Bath. 1973. Transmission of pea enation mosaic virus by the pea aphid Acyrthosiphon pisum following virus acquisition by injection. Ann. Entomol. Soc. Am. 66:603-607.

Clarke, R.G., and J.E. Bath. 1977. Serological properties of aphid transmissible and aphid-nontransmissible pea enation mosaic virus isolates. Phytopathology 67:1035-1040.

Clarke, R.G., R.H. Converse, and M. Kojima. 1980. Enzyme-linked immunosorbent assay to detect potato leafroll virus in potato tubers and viruliferous aphids. Plant Disease 64:43-45.

Converse, R.H. 1978. Detection of tomato ring spot virus in red raspberry by enzyme-linked immunosorbent assay (ELISA). Plant Dis. Rep. 62:109-112.

Eskandari, F., E.S. Sylvester, and J. Richardson. 1979. Evidence for lack of propagation of potato leafroll virus in its aphid vector. Myzus persicae. Phytopathology 69:45-47.

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.

Gera, A.G., G. Lobenstein, and B. Raccah. 1978. Detection of cucumber mosaic virus in viruliferous aphids by enzyme-linked immunosorbent assay. Virology 86:543-545.

Griffiths, G.W., and S.D. Beck. 1973. Intracellular symbiotes of the pea aphid. Acyrthosiphon pisum. J. Insect. Physiology 19:75-84.

Harris, K.F., and J.E. Bath. 1972. The fate of pea enation mosaic virus in its pea aphid vector, Acyrthosiphon pisum (Harris). Virology 50:778-790.

Harris, K.F., J.E. Bath, G. Thottappilly, and G.R. Hopper. 1975. Fate of pea enation mosaic virus in PEMV-injected pea aphids. Virology 65:140-162.

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

Kennedy, J.S., M.F. Day, and V.F. Eastop. 1962. A conspectus of aphids as vectors of plant viruses. Commonwealth Inst. Entomol., London, pp. 1-14.

Koenig, R. 1978. ELISA in the study of homologous and heterologous reactions of plant viruses. J. Gen. Virol. 40:309-318.

Kyriakopoulou, Panayota F., and E.S. Sylvester. 1969. Vector age and length of acquisition and inoculation access period as factors in transmission of pea enation mosaic virus by the pea aphid. J. Econ. Entomol. 62:1423-1427.

Maramorosch, K. 1952. Direct evidence for multiplication of aster yellows virus in its insect vector. Phytopathology 42:59-64.

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.

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

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

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

Osborn, H.T. 1935. Incubation of pea mosaic in the pea aphid Macrosiphum pisi. Phytopathology 25:160-177.

Osborn, H.T. 1938. Studies on pea virus 1. Phytopathology 28:924-934.

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

Shikata, E., K. Maramorosch, and R. Granados. 1966. Electron microscopy of pea enation mosaic virus in plants and aphid vectors. Virology 29:426-436.

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

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

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

Sylvester, E.S. 1962. Mechanisms of plant virus transmission by aphids. In biological transmission of disease agent. K. Maramorosch (ed.). Academic Press, New York, pp. 11-31.

Sylvester, E.S. 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.

Sylvester, E.S. 1969. Virus transmission by aphids--A viewpoint. In Maramorosch K., Virus, Vectors, and Vegetation, Interscience Pub., pp. 159-174.

Sylvester, E.S., and J. Richardson. 1966a. Some effects of temperature on transmission of pea enation mosaic virus and on the biology of the pea aphid vector. J. Econ. Entomol. 59:255-261.

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

Thottappilly, G., J.E. Bath, and J.V. French. 1972. Aphid transmission characteristics of pea enation mosaic virus acquired from membrane feeding system. Virology 50:681-689.

Thresh, J.M., A.N. Adams, D.L. Barbara, and M.F. Clark. 1977. The detection of three viruses of hop (Humulus lupulus) by enzyme-linked immunosorbent assay. Ann. Appl. Biol. 87:57-65.

Voller, A., D.E. Bidwell, and A. Bartlett. 1977. The enzyme-linked immunosorbent assay. Flowline Press, Guernsey, U.K., 73 p.