TRANSMISSION OF TWO PEA ENATION MOSAIC VIRUS ISOLATES BY THE PEA APHID, ACYRTHOSIPHON PISUM (HARRIS)

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ABSTRACT

TRANSMISSION OF TWO PEA ENATION MOSAIC VIRUS ISOLATES BY THE PEA APHID, ACYRTHOSIPHON PISUM (HARRIS)

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The pea aphid, Acyrthosiphon pisum (Harris), was used to compare the transmissibility of a New York and a California isolate of pea enation mosaic virus. In all but two tests, the New York isolate was more efficiently transmitted than the California isolate. No differences in physical properties could be detected although the lack of a suitable local lesion host hindered a precise study of these properties.

First instar pea aphids were 19.3, 36.9, 31.1, 37.0 and 14.1% more efficient in acquisition of the New York than California isolate at 1, 2, 4, 8 and 24 hr, respectively. Acquisition trials with the adult stage produced even wider differences in the two isolates, but efficiency in both cases was lower than with the nymphs.

Inoculation efficiency tests with adult pea aphids revealed that the New York isolate was transmitted with about twice the efficiency of the California isolate during probes of 5 and 10 min. One min probes produced no difference between the two isolates.

A 9-hr acquisition period for first instars resulted in average Latent Period$_{50}$'s (LP$_{50}$) of 19.5 and 31.5 hr for the New York and California isolates, respectively. Five trials with the adult after 24 to 48-hr acquisition periods were unsuccessful, as the California

isolate was not transmitted; the New York isolate had an average LP_{50} of about 60-hr.

The first two retention tests produced expected results--the California isolate was not transmitted in one test and very inefficiently in the other. Two final tests, however, showed the California isolate to be retained longer and with a shorter LP_{50} than the New York isolate.

Possible reasons for the unexpected shift in isolate transmission efficiency during the final two tests of this study are discussed. But before these two isolates are referred to as strains on the basis of aphid transmission variation additional retention and latent period trials will be made.

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INTRODUCTION

Pea enation mosaic virus (PEMV) was first described as a disease of pea by Osborn (1935); however, the disease has only sporadically been economically important. Incidences as high as 90% in commercial and experimental plantings have occurred (Schroeder 1951, and McEwen and Schroeder 1956), but while it occurs throughout the pea-growing areas of this country it is usually only present in trace amounts.

Pea plants infected with PEMV initially show chlorotic and trans-plant spots on the leaves; later the lower epidermis of the foliage proliferates to form blisters or enations. These enations are the most diagnostic symptom. Occasionally large or giant enations are formed on the stems and stipules. Plants infected with PEMV become stunted and bear fruit of poor quality and quantity.

Vector studies with PEMV probably are not warranted on the basis of ultimate PEMV control in pea fields; however, this virus is more important as a tool for obtaining detailed information on the vector-virus relationships of the circulative (persistent) aphid-borne group to which it belongs. While much has been done in this area, there is still much variation in the transmission characteristics of PEMV between laboratories. Bath and Chapman (1966) showed that pea aphid strains exist that vary grossly in their efficiency of PEMV transmission and pointed out that a standard pea aphid strain must be used throughout a study.

This study was conducted to test the hypothesis that the virus also has strains that are transmitted with varying efficiency by a standard vector-strain. To test this hypothesis, 2 PEMV isolates were compared directly as to efficiency of pea aphid acquisition, inoculation and retention and to length of latent period. Additionally the physical properties of each strain were determined.

REVIEW OF LITERATURE

Aphids represent the largest group of arthropod vectors of plant viruses. Classically the plant viruses were categorized, on the basis of the length of virus retention in or on the vector, as either non-persistent or persistent (Watson and Roberts 1939). But these 2 groups were not mutually exclusive; hence, an intermediate group--the semi-persistent viruses--was created (Sylvester 1958). Even this system did not provide clear-cut criteria for vector-virus relationships, as boundaries could not be established between the 3 categories. Kennedy, Day and Eastop (1962) set up a revolutionary system based on the relationship of the virus to the vector. In this scheme, viruses are categorized as (1) stylet-borne, (2) circulative, or (3) propagative. The first group includes all viruses that are carried on the vector's stylets; all of the nonpersistent and most of the semipersistent viruses now are encompassed by this stylet-borne group. Those viruses that are transmitted via the circulatory system of the vector fit the circulative group; most of the persistent and some of the semipersistent aphid-borne viruses fit this group. Viruses that multiply within the vector constitute the third group. Only 1 aphid-borne virus, potato leafroll, is known to propagate in its vector (Stegwee and Ponsen 1958); however, most of the leafhopper-borne viruses are propagative, a few circulative and none stylet-borne.

The virus (pea enation mosaic) studied in this thesis project is transmitted by the pea aphid, _Acyrthosiphon pisum_ (Harris), in a circulative manner (Osborn 1935; Chaudhuri 1950; Simons 1954; McEwen, Schroeder and Davis 1957; Black 1959; Schmidt 1959; Kennedy et al 1962; Nault, Gyrisco and Rochow 1964; Schmutterer and Ehrhardt 1965; and Bath 1964). It also is transmitted by the green peach aphid, _Myzus persciae_ (Sulzer), and the potato aphid, _Macrosiphum euphorbiae_ (Thomas), (Chaudhuri 1950, Osborn 1938a, Simons 1954, and Bath 1964).

The following review will show the relationship of pea enation mosaic virus (PEMV) to the other circulative, aphid-borne plant viruses. Transmission characteristics included in this review are: vector specificity, efficiency of virus acquisition, length of latent periods in the vector, length of virus retention in the vector, and rate of virus inoculation into plants.

Vector specificity.--Vector specificity is usually more pronounced in the circulative group than in the stylet-borne group. Only one or a few insect species are usually found to be vectors of circulative viruses. This specificity is most distinct in the case of barley yellow dwarf virus (BYDV), as at least 3 vector-specific strains are known in New York (Rochow 1961a, 1961b). One isolate is transmitted only by the English grain aphid, _Macrosiphum granarium_ (Kirby), another by the apple grain aphid, _Rhopalosiphum fitchii_ (Anderson), and the third only by the corn leaf aphid, _R. maidis_ (Fitch). Similar specificity also is found with Washington isolates of BYDV (Bruehl 1958, Toko and Bruehl 1956, 1957 and 1959).

Specificity was extended to the biotype level of an aphid species when a BYDV inactive clone of the greenbug, _Schizaphis graminum_ (Rondani), was collected in Florida, whereas greenbug collections from Wisconsin and Illinois were efficient vectors (Rochow 1960a). Another example of biotype specificity was encountered in the transmission of 4 isolates of strawberry vein banding virus (SVBV) by *Chaetosiphon fragaefolii* (Cockerell), the strawberry aphid (Frazier 1960). A typical clone of this aphid transmitted all 4 isolates of SVBV, whereas an atypical clone transmitted only 3 of the 4 isolates. Frazier suggested that the 2 *C. fragaefolii* clones may represent 2 species.

A similar situation was uncovered for PEMV and the pea aphid (Bath 1964). One strain of pea aphid failed to transmit a California isolate of PEMV but did transmit a New York isolate of PEMV. A second aphid strain efficiently transmitted both isolates.

Virus acquisition.--Longer acquisition periods generally are required by vectors of circulative and propagative viruses than of stylet-borne viruses. Barley yellow dwarf virus (BYDV) was acquired by *R. fitchii* and *M. granarium* in 10 min (Allen 1957) whereas Toko and Bruehl (1959) could not achieve infectivity with these 2 species until at least a 24 hr acquisition period was allotted. Potato leafroll virus (PLRV) was rarely acquired by *M. persicae* in less than 8 hr (Klostermeyer 1953). Later this threshold was found to be 2 hr and transmission efficiency increased steadily as acquisition time was increased to 100 hr (MacCarthy 1954). Early work with PEMV indicated an acquisition threshold of 1 to 2 hr for *A. pisum* adults (Chaudhuri 1949, Simons 1954), whereas later work with a highly efficient *A. pisum* strain reduced the threshold to 5 min or less (Bath 1964). Pea aphid nymphs have been shown to acquire PEMV more efficiently than adults (Bath 1964 and Ehrhardt and Schmutterer 1964), particularly during short acquisition periods.

Plant inoculation.--Inoculation of circulative viruses into plants by aphids usually requires 10 to 20 min. Simons (1954) reported a 15 to 20 min threshold for PEMV and *A. pisum* adults, whereas later work has decreased this threshold to 5 min (McEwen et al 1957) and to 1 min or less (Bath 1964, Nault et al 1964, Ehrhardt and Schmutterer 1964, and Nault and Gyrisco 1966).

The 10 to 20 min threshold for most of the circulative group has been explained as the minimum time required for the aphid stylets to reach the phloem and permit inoculation via the saliva (Roberts 1940 and Simons 1954). Rapid inoculations of PEMV led Bath (1964) and Nault et al (1964) to suggest that inoculations were being made into the nonvascular tissues of the host plant. Nault and Gyrisco (1966) have shown that the pea aphid can inoculate PEMV to the epidermis, and to the interveinal and veinal parenchyma of the pea leaf.

Transmission threshold.--Transmission thresholds for circulative and propagative viruses are always longer than the sum of acquisition and inoculation thresholds, as the virus enters a latent period after acquisition and before inoculation. During this latency the virus is in journey to the salivary glands and may or may not be increasing in titre through propagation.

The transmission threshold for potato leafroll virus (PLRV) in *M. persicae* was initially reported to be 54 hr (Smith 1931), but later it was reduced to 12 hr (MacCarthy 1954), 1.5 hr (Kirkpatrick and Ross 1952), 30 min (Meester-Manger Cats 1956) and 20 min (Klostermeyer 1953). A threshold for BYDV as short as 6 hr was recorded for *R. fitchii*, but usually 24 to 48 hr was required (Orlob, Arny and Medler 1961). In the case of PEMV the variations in transmission thresholds for A. pisum have been reported from 6 hr to 4 days (Chaudhuri 1949). Ehrhardt and Schmutterer (1964) stated that the transmission threshold for PEMV in young adults of A. pisum ranged from 30 hr to 13 days and from 18 hr to 5 days for young nymphs.

Latent or incubation periods.--It has been demonstrated that most of circulative and propagative viruses possess a definite latent or incubation period in their vectors. The term latent period is used if the virus is non-propagative, and it is referred to as the length of time necessary for the journey of the virus through the vector's circulatory system to the salivary glands. If this time is required for multiplication of the virus to an infectious titre, it is termed an incubation period.

The lengths of the latent or incubation period vary considerably between viruses, vectors and laboratories. The incubation period of PLRV was first reported as 24 to 48 hr in M. persicae (Elze 1927); later it was found to be 9.5 to 120 hr in the same vector (MacCarthy 1954). Latent periods for PEMV in adult A. pisum have been reported at 24 to 28 hr (Osborn 1935), 25 to 55 hr (Simons 1954), 6 to 26 hr (Chaudhuri 1950), 24 hr (McEwen et al 1958), 16 to 24 hr (Heinze 1959a) and 14 to 70 hr (Sylvester and Richardson 1965). The latent period of PEMV in other vector-species was demonstrated as 12 to 20 hr in M. euphorbiae (Osborn 1938), and 14 to 18 hr in M. persicae (Bath 1964).

Nymph and adult aphids do not necessarily have the same latent period for a given virus. With PEMV and A. pisum, the minimum adult latent period was 25 to 29 hr in contrast to first instar nymphs: 16 to 20 hrs (Simons 1954). Similarly Ehrhardt and Schmutterer (1964) indicated that latent period of PEMV in young A. pisum adults was 27 hr to 10 days, whereas in young nymphs it was set at 18 hr to 4 days. Recent work by Bath (1964) indicated that the latent period of PEMV in first instar nymphs of A. pisum was 8 to 10 hr; mean latent periods increased in length with each increase in instar number for both A. pisum and M. euphorbiae. Nymphs were believed to be more efficient vectors than adults for they have higher metabolic rates--a factor that could govern the speed of the journey of the virus to the saliva. Bath (1964) believed that the nymph's advantage over the adult in virus acquisition gives it a greater chance to achieve a virus contaminated saliva in the shortest length of time.

Virus retention.--The circulative and propagative viruses are retained much longer in their vectors than are stylet-borne viruses. PEMV was retained in M. persicae and A. pisum for more than 140 hr (Chaudhuri 1949); Osborn (1935) showed that PEMV was retained for periods up to 28 days in A. pisum. Other PEMV retention records in A. pisum are 5 to 6 days (Heinze 1959) and 29 days (Bath 1964). At 30 and 10° C retentions were 4.2 and 14.3 days, respectively (Sylvester and Richardson 1965). Simons (1954) showed that the length of PEMV retention increased as length of acquisition feeding increased for A. pisum (Simons 1954) and over a 20 day period the number of daily transmissions from a given number of insects decreased. Thus, he concluded that this virus does not multiply in the vector. Failures in attempts to transfer hemolymph from donor viruliferous aphids to a series of recipient healthy aphids, indicated that multiplication does not occur (Heinze 1956). Schmidt (1959) and Nault et al (1964) showed that only limited transmission could be obtained by injection of hemolymph from viruliferous *A. pisum* to healthy pea aphids.

Success in serially transferring PLRV in *M. persicae* allowed Stegwee and Ponsen (1958) to assert that PLRV does multiply in that vector. No other virus has been shown to multiply in an aphid species.

MATERIALS AND METHODS

The 2 isolates of pea enation mosaic virus used in this study were the same as used by Bath (1964); one isolate originated in California and the other in New York. A stock culture of each isolate was maintained *in vitro* and *in vivo*. The *in vitro* culture was in desiccated conditions and stored over calcium chloride in a refrigerator (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. Its identity was confirmed by Mr. Francis E. Giles and specimens were deposited in the MSU Entomology Museum. One apterous adult was used as the basis for the ensuing colony. This culture was reared in cages constructed of wood with Lumite saran-screened sides and top; entrance to the cage was through a vertically sliding pane of glass. The size of cage was 15 x 15 x 18 inches.

The colony was reared on broad bean (*Vicia faba* L.), an excellent greenhouse host plant, for it gave rise a uniformly large pea aphid adults with high fecundity. The large adult was particularly useful in inoculation tests, as the large proboscis made timing of the inoculation probes much more accurate and easier than would have been possible with a smaller adult.

Garden pea (*Pisum sativum* L.) variety Perfected Wales was used exclusively as the virus-source and test plant. The seeds were germinated in a plastic pan and covered to a uniform depth with vermiculite in order to obtain a uniform growth of the seedlings. The seedlings were transplanted, a week after the seeds were sown, into plastic pots (diam: 2 inches; height: 2-1/4 inches); a sterilized loam-sand-peat mixture was used as the potting media. The test plants were used in experiments 2 to 3 days after transplantation--at the 2 to 3 leaf stage.

Sable hair brushes (size 00) were utilized to manipulate test aphids in the various experiments. Insects in feeding position were freed in a few seconds by "tickling" the abdomen with the brush hairs.

For tests that required the use of 1st instar nymphs, a number of mature adults were placed on a host plant the previous evening and progeny were collected the following morning. These nymphs were of mean age 6 hr.

The terminal foliage of infected young pea plants with severe symptoms was used exclusively as the source for virus acquisitions. To insure that test insects fed only on that area, the terminal portion was inserted through a 1-inch hole in an elevated platform. A piece of filter paper was fitted around the stem in such a way that the platform hole was covered and only the desirable tissues for acquisition were above the filter paper and platform. A glass lantern globe (with a screen-enclosed top) was placed on the platform and over the plant. Aphids that fell from the plant landed on the filter paper and could easily crawl back onto the source plant.

Since 2 virus isolates were compared in this work, all tests were set up to remove as much variability as possible between the 2 isolates. The major source of variability existed between source plants of the 2 isolates, thus, care was taken to select source plants that were of equal age and symptom expression. Symptom severity is not an absolute measure of the virus titre in a plant but it is a rapid, visual method for an approximation on a comparative basis.

During the inoculation phases of most tests (except the precisely-timed inoculation probe experiments), each aphid was confined to its test plant with a small cage (diam: 2.25 inches; height: 6 inches) constructed from cylindrical extruded buterate tubing. The top and 3 one-half inch ventilation holes on the side were covered with a fine nylon cloth.

Short acquisition and inoculation periods were timed with a stop-watch. Timings were initiated as soon as the aphid stylets were in probing position against the plant surface and when the aphid was motionless. If the insect made a brief (10 to 20 sec) test probe prior to the start of a desired longer probe, that plant was discarded. Thus, the inoculation probes were of a precise length and no other probes, no matter how short, were made on that particular test plant.

The insect phases of all tests were conducted in a Sherer-Gillett model 512-37 Growth Chamber. Temperature was controlled at 22° C $\pm$ 2° whereas relative humidity varied from 50 to 60%. The lack of adequate growth chamber space and the magnitude of most experiments necessitated moving the test plants to a greenhouse room after the insects were removed. All plants were sprayed with naled soon after the insect phase was completed to guard against possible escapes and contaminations. The greenhouse room was fumigated on a 7 to 10 day schedule with sulfotepp.

Inoculum for mechanical virus transfers was prepared by grinding the infected terminal parts of peas, showing severe symptoms, in a sterile mortar with a pestle. This extract was filtered through 4 layers of cheesecloth to remove fibrous materials. A glass spatula was used to inoculate the leaves of carborundum-dusted plants.

Physical property tests utilized infected plant sap prepared in the same manner as for mechanical transfers. All of the source plants were mechanically inoculated and used for extractions 10 to 12 days later. Plants that showed the most severe symptoms were selected as sources for each isolate.

EXPERIMENTS AND RESULTS

Acquisition

Adult and first instar pea aphids were used to compare the acquirability of the 2 PEMV isolates. Test insects were routinely starved 1 to 2 hr; then transferred to a source plant (1 source per isolate was used for each insect stage) for acquisition periods of 1, 2, 4, 8, and 24 hr. At the end of each acquisition period, a group of 12 to 20 aphids was removed from each source plant and caged singly on young pea seedlings. Then they were placed in the growth chamber for a 7-day inoculation period. During this period, aphid mortality was checked daily; plants on which an aphid died were discarded unless they developed symptoms. All test plants were sprayed with naled before they were moved into the greenhouse for virus incubation; each test was terminated a month later.

In a direct comparison of first instar versus adult acquisition of the 2 isolates, both stages transmitted the New York isolate with significantly greater efficiency than the California isolate (Table 1). As expected, the first instar was more efficient than the adult with either isolate. The first instar was selected as the test stage for extensive isolate comparisons of acquisition efficiency, as too few successes were attained with the adult--even with the New York isolate only a 44.4% efficiency was attained after 24 hr.

Eight tests were conducted between August 30, 1965 and April 24, 1966; both isolates were used in each test (Table 2). Significant differences were obtained between the 2 isolates at each of the tested acquisition periods, with a maximum difference (37%) demonstrated at 8 hr. The difference at 24 hr was only 14.1%, but the New York isolate was acquired at peak efficiency at about 8 hr and showed no increase in efficiency with an increased acquisition period. These differences would have been even greater had the adult been used as the test insect.

TABLE 1.--Acquisition of New York and California pea enation mosaic virus isolates by first instar and adult pea aphids

Aphid stage and virus isolate	Transmission[a] after specified acquisition periods (hr)				
	1	2	4	8	24
First instar					
New York	3	8	8	15	16
California	3	2	7	8	10
Adult					
New York	2	1	4	3	8
California	0	0	1	1	3

[a]Number of transmissions per 18 attempts.

TABLE 2.--Acquisition of New York and California pea enation mosaic virus isolates by first instar pea aphids

Test no. and date	Transmission[a] after specified acquisition periods (hr)				
	1	2	4	8	24
New York PEMV					
1. Aug. 30, 1965	2/8	6/10	7/10	10/10	--
2. Sept. 1, 1965	3/10	9/12	12/12	12/12	12/12
3. Sept. 7, 1965	2/10	5/12	10/11	10/10	11/13
4. Dec. 23, 1965	3/12	4/12	8/12	10/10	16/18
5. Dec. 26, 1965	3/12	5/12	4/12	7/12	14/18
6. Apr. 3, 1966	4/12	10/12	12/12	11/12	12/12
7. Apr. 11, 1966	14/20	16/20	16/20	20/20	20/20
8. Apr. 24, 1966	3/18	8/18	8/18	15/18	16/18
Total	34/100	63/108	77/107	95/104	101/111
Percent	34.0	58.3	71.9	91.3	90.9
California PEMV					
1. Aug. 30, 1965	1/4	6/8	5/9	6/9	--
2. Sept. 1, 1965	3/12	4/12	9/10	8/12	9/11
3. Sept. 7, 1965	2/12	3/11	7/10	8/10	10/11
4. Dec. 23, 1965	1/12	4/10	4/12	8/12	15/18
5. Dec. 26, 1965	2/12	0/12	3/12	3/12	14/18
6. Apr. 3, 1966	1/12	0/12	7/12	10/12	9/12
7. Apr. 11, 1966	2/20	3/20	0/20	6/20	16/20
8. Apr. 24, 1966	3/18	2/18	7/18	8/18	10/18
Total	15/102	22/103	42/103	57/105	83/108
Percent	14.7	21.4	40.8	54.3	76.8

[a]Numerator = number of infections; denominator = number of trials.

Inoculation

Adult pea aphids that had spent their life on PEMV-infected pea were used exclusively as test insects in the comparison of the 2 isolates. Since the pea aphid is at least 7 days old when it reaches adulthood, most of them had the capability of being infectious as the latent period is usually completed in less than 7 days.

All test aphids were routinely starved 1 to 2 hr prior to their use in inoculations; these aphids were held singly in petri dishes during that time when they were not on a test plant. Inoculation periods of 1, 5 and 10 min were used. Each insect was used for each of the 3 inoculation periods; then, it was placed on a 4th plant, which served as a check as to whether the insect was indeed infectious, for 3 to 4 hr. If the insect failed to infect any of the test plants, it was judged as non-infectious and the plants it probed upon were discarded. Thus, only the performances of truly infectious individuals were recorded.

Four tests were conducted during an 8-day period with 20 insects from each virus-isolate colony. Thus, 80 insects were tested for each isolate, and of those 51 and 49 proved to be infectious for the New York and California isolates, respectively (Table 3). While both test groups were about 4% infectious during a 1 min inoculation probe, the New York isolate was transmitted with about twice the efficiency at 5 and 10 min as the California isolate.

A year prior to the isolate comparison tests (Table 3) I conducted 5 tests with only the New York isolate to compare the efficiency of 3rd instar and adult pea aphids--an area that needed additional study (Bath 1964). Third instars were 17.1% efficient during a 1 min probe whereas adults were only 6.5% efficient (Table 4). At 5 min the 3rd instars were only 1.5 times as efficient as adults, and almost no difference was detected at 10 min. These results indicate that increases in inoculation probing time produce increased transmission efficiency, at least during the 1 to 10 min range.

TABLE 3.--Transmission of New York and California pea enation mosaic virus isolates by adult pea aphids after various inoculation probing periods

Test no. and date	Transmission[a] after specified inoculation probing periods (min)		
	1	5	10
New York PEMV			
1. (Oct. 6, 1966)	0/14	6/14	6/14
2. (Oct. 10, 1966)	1/8	2/8	4/8
3. (Oct. 12, 1966)	1/12	3/12	6/12
4. (Oct. 14, 1966)	0/17	3/17	4/17
Total	2/51	14/51	20/51
Percent	3.9	27.4	39.2
California PEMV			
1. (Oct. 6, 1966)	0/13	4/13	2/13
2. (Oct. 10, 1966)	0/11	0/11	3/11
3. (Oct. 12, 1966)	2/15	1/15	4/15
4. (Oct. 14, 1966)	0/10	1/10	0/10
Total	2/49	6/49	9/49
Percent	4.1	12.2	18.3

[a]Numerator = number of infections; denominator = number of trials.

TABLE 4.--Transmission of the New York pea enation mosaic virus isolate by third instar and adult pea aphids after various inoculation probing periods

Test no. and date	Transmission[a] after specified inoculation probing periods (min)		
	1	5	10
Third instar			
1. (Aug. 11, 1965)	2/9	6/9	7/9
2. (Aug. 13, 1965)	0/9	4/9	6/9
3. (Aug. 17, 1965)	1/6	3/6	5/9
4. (Aug. 19, 1965)	0/6	3/6	6/6
5. (Aug. 25, 1965)	4/11	2/11	6/11
Total	7/41	18/41	30/41
Percent	17.1	43.9	73.1
Adult			
1. (Aug. 11, 1965)	2/11	6/11	8/11
2. (Aug. 13, 1965)	0/6	0/6	2/6
3. (Aug. 17, 1965)	0/6	3/6	5/6
4. (Aug. 19, 1965)	0/5	0/5	5/5
5. (Aug. 25, 1965)	0/3	0/3	2/3
Total	2/31	9/31	22/31
Percent	6.5	29.0	70.9

[a]Numerator = number of infections; denominator = number of trials.

Latent Period and Retention

When a test insect is given an acquisition period and transferred at specified intervals and along a series of test plants a simple means of determining virus latency and retention is afforded. The points at which the insect begins and stops transmitting the virus reflect the end of latency and retention, respectively. A long-term transferral of the vector constitutes a retention experiment whereas latent periods can be extracted from series of long or short duration (as only the first few hours or days after the end of acquisition are important). Thus, some of the following tests are varied in length, and often the intervals between transfers are irregular--particularly in tests designed for latency determinations.

Latency was measured as the length of elapsed time between the start of an acquisition period and the first transmission by that individual. The 2 PEMV isolates were compared on the basis of Latent Period_{50} (LP_{50}); i.e., when 50% of the test insects had completed latency (Sylvester 1965).

Two preliminary experiments were initiated on April 30 and May 3, 1966 with the 2 isolates and 2 pea aphid stages (adult and first instar). In these tests, the acquisition period was 6 hr. Six of 12 first instars transmitted the New York isolate in the first test and had an LP_{50} of 25.5 hr; second test--7 of 10 infected and LP_{50} 19.0 hr. On the other hand, none of 12 and 1 of 10 nymphs transmitted the California isolate in the first and second tests, respectively. Adult aphids were nearly inactive as only 2 of 22 transmitted each isolate in the 2 tests--too few for a LP_{50} determination. The actual data for these tests are presented in Tables A-1 and A-2 (Appendix).

Only first instar nymphs were used in a test started on May 9, 1966 since they appeared, from the first 2 tests, to be the stage most suited for isolate comparisons of latent period (Tables 5, A-3 and A-4). Twenty insects per isolate were tested in each of 2 replicates; the acquisition period was 9 hr. The New York isolate had a shorter latent period than the California isolate in both replicates--average LP_{50}'s of 19.5 and 31.0 hr, respectively.

TABLE 5.--Summary of latent period experiments for New York and California isolates of pea enation mosaic virus in the pea aphid[a]

Aphid stage and test date	Acq. period		Isolate: New York Infectivity[a]	New York LP_{50}	California Infectivity[a]	California LP_{50}	Raw data in table no.
1st Instar							
May 9, 1966	I	9	19/20	21.0	13/20	36.5	A-3
	II	9	19/20	18.0	17/20	26.5	A-4
Adult							
May 16, 1966		24	14/25	61.0	0/25	--	A-5
May 17, 1966		24	11/25	66.0	0/25	--	A-5
June 8, 1966		30	14/50	66.0	0/50	--	A-6
June 14, 1966		30	16/25	<54.0	0/25	--	A-6
June 16, 1966		48	18/28	<72.0	0/28	--	A-7

[a]Numerator = number of infections; denominator = number of trials.

Five additional latent period tests were initiated between May 16, 1966 and June 16, 1966 in hope of obtaining comparative isolate data for the adult stage (Table 5). Two of these tests utilized a 24 hr acquisition period (Table A-5), 2 a 30 hr period (Table A-6) and 1 a 48 hr period (Table A-7); however, in none of the tests was the California isolate transmitted. In contrast, the New York isolate demonstrated latent periods of about 60 hr. While LP_{50} values were not obtained for the California isolate with adults, the gross difference in infectivity obtained over the range of acquisition periods further emphasizes the difference between the 2 PEMV isolates.

To gather retention data, 4 serial transfer tests were continued until the insect died. An acquisition period of 20 hr was used in each test, and the insects were transferred daily. Transmission to the first plant in the series represented a possible minimum of almost 0 time and a maximum of almost 44 hr (20 + 24). The former value would arise from virus that was acquired at the end of the acquisition period and inoculated at the start of the insect's first inoculation period. If the virus was acquired and inoculated to the plant at the other extreme of times, the 44 hr possibility would exist. Thus, a mid-point value of 1-day was used to represent retention if the 1st plant became infected--2 days if the 2nd plant became infected and so on.

The first retention test (started June 20, 1966) was initiated with adult pea aphids and included 25 adults per isolate in each of 2 replicates. Again, the California isolate was not transmitted by adult pea aphids whereas 32 of the 50 aphids transmitted the New York isolate (Table 6). Retention in the 2 replicates was 9.1 and 9.3 days and corresponding LP_{50}'s were 86.0 hr and 81.0 hr.

In the experiments that followed, the acquisition period was given to first instar pea aphids. Further use of adults was rejected

TABLE 6.--Retention of the New York pea enation mosaic virus isolate by pea aphids given a 20 hr acquisition period as young adults (June 20, 1966)

Class no.	Obser-vations	Transmission during daily serial transfers[a]														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Replicate 1																
1	1	+	+	+	+	+	+	+	-	-	+	-	-	-	D	
2	1	-	+	+	+	+	+	+	-	+	-	+	-	D		
3	1	-	+	+	+	+	+	+	+	-	+	-	-	D		
4	2	-	+	+	+	+	+	+	+	+	+	D				
5	1	-	+	+	+	+	+	+	+	-	-	-	-	-	D	
6	1	-	+	+	+	+	-	-	-	-	-	D				
7	1	-	-	+	+	+	+	+	+	-	+	-	D			
8	1	-	-	+	+	+	+	+	+	-	-	-	D			
9	1	-	-	-	+	+	-	-	-	-	-	+	-	-	D	
10	1	-	-	-	+	-	-	+	-	-	-	+	-	D		
11	1	-	-	-	+	+	-	+	-	-	+	+	-	D		
12	1	-	-	-	+	-	+	-	-	-	-	-	D			
13	1	-	-	-	+	+	+	-	+	D						
14	1	-	-	-	-	-	-	+	-	-	-	D				
15	1	-	-	-	-	-	-	-	+	-	-	-	-	D		
16	1	-	-	-	-	-	-	-	-	-	+	+	-	-	D	
17	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D
18	4	-	-	-	-	-	-	-	-	-	-	-	-	D		
19	3	-	-	-	-	-	-	-	-	-	-	D				

TABLE 6--Continued

Class no.	Obser- vations	Transmission during daily serial transfers[a]														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Replicate 2																
1	1	+	+	+	+	+	+	+	+	+	+	+	-	-	D	
2	1	+	+	+	+	+	+	+	+	+	+	-	-	-	D	
3	1	+	+	+	+	+	-	-	-	-	+	-	-	D		
4	1	-	+	+	+	+	+	+	+	+	+	+	+	D		
5	1	-	+	+	+	+	+	+	+	+	+	+	-	-	D	
6	1	-	+	+	-	-	+	+	+	+	+	-	-	D		
7	1	-	+	+	+	+	+	-	-	+	-	-	-	D		
8	1	-	-	+	+	-	+	-	-	-	+	+	-	D		
9	1	-	-	+	+	+	+	+	+	+	-	-	-	-	D	
10	1	-	-	-	+	+	-	+	-	-	-	-	-	-	D	
11	1	-	-	-	+	+	-	-	-	-	-	-	-	D		
12	1	-	-	-	-	+	+	+	+	+	-	-	-	D		
13	1	-	-	-	-	+	+	+	-	-	-	-	D			
14	1	-	-	-	-	-	-	-	+	-	-	-	D			
15	1	-	-	-	-	-	-	-	-	-	-	+	D			
16	2	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
17	4	-	-	-	-	-	-	-	-	-	-	-	-	D		
18	2	-	-	-	-	-	-	-	-	-	-	-	D			
19	2	-	-	-	-	-	-	-	-	-	D					
20	1	-	-	-	-	-	-	-	-	D						

[a]Infection (+); no infection (-); insect died on previous test plant (D).

as almost no success had been obtained with them in any of the foregoing research comparisons.

A test initiated on July 5, 1966 was only moderately successful (Tables 7 and 8). Only 2 of 46 individuals transmitted the California isolate at any time in the test (one retained it for 14 days, the other 2 days). The New York isolate was retained an average of 6.6 days and had an LP_{50} of 39.5 hr.

At this point, all experiments were held for incubation in a new greenhouse. This is mentioned because the "breaking-in" period for this house occurred at the hottest time of the summer and temperatures occasionally got out-of-hand in the house (above 100° F) during August and September.

The last 2 tests (initiated August 2, 1966 and September 13, 1966) each involved 51 aphids per isolate (Tables 9, 10 and 11). Both tests produced peculiar, or at least unexpected, results. The California isolate was transmitted more efficiently than the New York isolate for the only times in this research project (infectivity: 48 and 40 out of 51, respectively, in 1 test and 36 and 30 out of 51, respectively, in the other test). Additionally the former isolate was retained longer than the latter--7.58 and 6.25 versus 4.59 and 4.23 days in the 2 tests.

Another peculiarity was observed in the August 2 test (Tables 9 and 10). Whereas the New York isolate was transmitted with an expected degree of regularity from day to day, vectors of the California isolate frequently skipped many days between their next-to-last final transmissions.

TABLE 7.--Retention of the New York pea enation mosaic virus isolate by pea aphids given a 20-hr acquisition period as first instar nymphs (July 5, 1966)

Class no.	Obser- vations	Transmission during daily serial transfers[a,b]																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	D
2	1	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-
3	1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
4	2	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	1	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	D	
6	3	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1	+	+	+	D																
8	1	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
10	2	+	-	-	-	-	-	-	-	-	-	-	D								
11	1	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	D
12	1	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
13	1	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	1	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
15	1	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D
16	1	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	D
17	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	D					
19	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D
22	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
23	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D			
24	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D				
25	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D					
26	1	-	-	-	-	-	-	-	-	-	-	-	-	-	D						
27	1	-	D																		

[a]Infection (+); no infection (-); insect died on previous test plant (D).

[b]Insects of classes no. 2, 3, 4, 13, 17, and 20 were dead at the 21st transfer; the remaining insects were alive when the test was terminated after the 24th transfer. No transmissions occurred between the 20th and 24th transfers.

TABLE 8.--Retention of the California pea enation mosaic virus isolate by pea aphids given a 20-hr acquisition period as first instar nymphs (July 5, 1966)

Class no.	Observations	Transmission during daily serial transfers[a,b]																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
2	1	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D
6	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D				
8	4	-	-	-	-	-	-	-	-	-	-	-	-	-	D						
9	2	-	-	-	-	-	-	-	-	-	-	-	-	D							
10	1	-	-	-	-	-	-	-	-	-	-	-	D								
11	1	-	-	-	-	-	-	-	-	-	D										
12	1	-	-	-	-	-	-	-	-	D											
13	1	-	-	-	-	-	-	D													
14	1	-	-	-	-	-	D														
15	1	-	-	-	-	D															
16	2	-	-	-	D																
17	3	-	-	D																	

[a]Infection (+); no infection (-); insect died on previous test plant (D).

[b]Test terminated after the 24th transfer; insects of classes 1 to 4 were still alive at that time.

TABLE 9.--Retention of the New York pea enation mosaic virus isolate by pea aphids given a 20-hr acquisition period as first instar nymphs (August 2, 1966)

Class no.	Obser-vations	Transmission during daily serial transfers[a,b]																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
2	1	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3	1	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	1	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	D	
5	1	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	2	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	1	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	1	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	1	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	2	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	D			
12	1	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D
13	1	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
14	1	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	6	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	1	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	2	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	2	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D
19	1	+	+	-	D																
20	1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D		
21	1	+	-	-	D																
22	1	+	D																		
23	1	-	+	+	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
24	2	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	2	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
26	1	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D
27	1	-	+	+	-	-	-	-	-	-	-	-	-	-	D						
28	2	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D
31	1	-	-	-	-	-	-	-	-	-	-	-	-	-	D						
32	1	-	-	-	-	D															

[a]Infection (+); no infection (-); insect died on previous test plant (D).

[b]Test terminated after 24th transfer; no additional mortality or transmission was recorded beyond the 20th transfer.

TABLE 10.--Retention of the California pea enation mosaic virus isolate by pea aphids given a 20-hr acquisition period as first instar nymphs (August 2, 1966)

Class no.	Obser- vations	Transmission during daily serial transfers[a,b]																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	+	+	+	+	-	+	-	+	+	+	-	-	-	-	+	-	-	-	-	-
2	1	+	+	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
3	1	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
4	1	+	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
5	1	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
6	1	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	D		
7	1	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	1	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	1	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	1	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	1	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	1	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D
14	1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D
15	1	+	-	+	-	-	-	D													
16	1	+	-	+	D																
17	1	-	+	+	+	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-
18	1	-	+	+	+	+	+	-	+	-	-	-	-	-	+	-	-	-	-	D	
19	1	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
20	1	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
21	1	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	D	
22	1	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	D	
23	1	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	1	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	4	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	1	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	1	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
28	1	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	
29	1	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-
30	1	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	D			
31	1	-	-	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	D		
32	1	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
33	1	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	D	
34	1	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	1	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	D	
36	1	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	D	
37	1	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	1	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	1	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	1	-	-	+	D																
41	1	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
42	1	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 10--Continued

Class no.	Obser-vations	Transmission during daily serial transfers[a,b]																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
43	1	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	D
44	1	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	D		
45	1	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
46	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	2	-	-	-	D																

[a]Infection (+); no infection (-); insect died on previous test plant (D).

[b]Test terminated after the 24th transfer; no additional mortality or transmission was recorded beyond the 20th transfer.

TABLE 11.--Retention of the New York and California pea enation mosaic virus isolates by pea aphids given a 20-hr acquisition period as first instar nymphs (Sept. 13, 1966)

Isolate and class no.	Obser- vations	Transmission during daily serial transfers[a,b]																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
New York isolate																				
1	2	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	4	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	1	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	1	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	3	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	1	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	6	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	1	+	+	+	-	-	D													
9	2	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	4	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	1	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	3	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	1	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	1	-	-	-	-	D														
16	1	-	-	-	D															
California isolate																				
1	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
2	2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
3	1	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
4	1	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-
5	1	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-
6	1	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
7	1	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
8	4	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9	4	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
10	1	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	1	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	1	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	1	+	+	+	-	+	-	-	-	-	-	D								
15	2	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	1	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	1	+	+	-	-	-	D													
19	1	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
20	1	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	4	-	-	-	-	-	D													
23	1	-	-	-	D															
24	1	-	-	D																

[a]Infection (+); no infection (-); insect died on previous test plant (D).

[b]Test terminated after 19th transfer.

Physical Properties

Previous studies to determine the physical properties of PEMV have been hampered by the lack of a suitable local lesion host (Johnson and Jones 1937; Ainsworth 1940; Pierce 1935; Osborn 1938; and Ruppel and Hagedorn 1963). Recent reports have indicated that Chenopodium album (Hagedorn, Layne and Ruppel 1963); broad bean, Vicia faba (L.), and pea var. Perfection (Bozarth and Chow 1965); and Galactia spp. (Izadpanah and Shepherd 1965) react with local lesions when inoculated with PEMV.

In this study, each of the above species was tested for suitability as local lesion hosts for the New York and California PEMV isolates. Each was rejected as either no local lesions resulted or success was irregular. Thus, as others before were forced to do, I had to resort to a systemic host (pea var. Perfected Wales) for physical property determinations. With this method a number of test plants are inoculated with each treatment, and the number of successes is used as the quantitative measure of the property being tested. It is an undesirable technique as 1 virus particle or a million may be present in an inoculum that produces an infected plant and the researcher has no way of knowing it. With local lesions, each lesion represents a virus infection, and the number of lesions is directly proportional to the virus titre of the inoculum.

Longevity in vitro experiments were conducted with undiluted sap extracts that were stored in sterile, stoppered serological test tubes at 22° C. This sap was inoculated to pea seedlings soon after it was extracted from the plants and daily thereafter. Test plants were planted on 2-day intervals to assure that all plants in the series were within 2 days of the same age.

Both isolates were tested on 4 occasions and were highly infectious at the start of the aging period (Table 12). Only low viability was exhibited after 24 hr (4.5% for the California isolate and 9.0% for New York), whereas neither isolate was infectious when tested at 48 hr. Thus, the resistance to aging period for both isolates was 24 to 48 hr.

TABLE 12.--Effect of aging *in vitro* on infectivity of extracts from pea infected with pea enation mosaic virus isolates from New York and California[a]

	Resistance to aging (days) at 22° C[b]				
Isolate	0	1	2	3	4
California	61/63	5/110	0/120	0/110	0/100
New York	62/63	10/100	0/120	0/110	0/100

[a]Results of 4 replicates.

[b]Numerator = number of infections; denominator = number of trials.

Johnson and Jones (1937) reported an aging value of under 3 hr for PEMV. A 4-day resistance to aging was obtained by Osborn (1938), and Ruppel and Hagedorn achieved 6 to 12 agings among the 5 isolates they tested.

Dilution end-points were determined in the following manner: sap extracts were initially inoculated to pea seedlings; then dilutions of 1:5, 1:10, 1:20, 1:40, 1:80, 1:100, and 1:1000 were made with distilled water and inoculated. Both isolate-extracts became diluted beyond infective levels between 1:100 and 1:1000 (Table 13). The differences between isolates was almost nil.

TABLE 13.--Effect of dilution with distilled water on infectivity of extracts from pea infected with pea enation mosaic virus isolates from New York and California[a]

Test 1 Isolate	Dilution tolerance[b] 0	1:5	1:10	1:20	1:40	1:80	1:100	1:1000
California	15/15	15/15	15/15	14/15	15/15	13/15	8/15	0/15
New York	15/15	15/15	15/15	15/15	15/15	15/15	12/15	0/15

Test 2 Isolate	0	1:100	1:140	1:220	1:300	1:1000
California	26/28	9/43	22/55	19/50	12/45	0/45
New York	27/28	11/43	10/55	14/50	5/45	0/45

[a]Test 1 consisted of 1 replicate; test 2: 2 replicates.

[b]Numerator = number of infections; denominator = number of trials.

To narrow the dilution end-point down between 1:100 and 1:1000, another test was conducted; dilutions of 0, 1:100, 1:140, 1:220, 1:300 and 1:1000 were utilized. Again, both isolates reacted similarly to dilution, and both were diluted beyond infective levels between 1:300 and 1:1000 (Table 13).

The dilution tolerances obtained here agreed closely with those of Ruppel and Hagedorn (1963). Values of less than 1:100 (Johnson and Jones 1937) and 1:1000 to 1:10,000 (Osborn 1938) also have been reported.

Plant sap was heated at various temperatures in a water bath for 10 min to determine at what temperature viral inactivity occurred. The sap was confined in glass capillary tubes (diam: 2 mm; length: 15 cm) that had been drawn on 1 end. After the sap was carefully sucked into the capillary, the tube was inverted slightly to permit the sap to flow out of the drawn end. Then, that end was sealed over a burner flame. Care was taken not to inactivate virus by the heat of the sealing process.

Two capillary tubes (one per isolate) were strapped to the sensitive end of a laboratory thermometer and plunged into the water bath at the proper temperature. The water bath consisted of a 1000 ml beaker full of water; it was situated on a magnetic stirrer-equipped hot plate. Frequent movement of the thermometer and attached capillaries kept the temperature within 1^{o} C of the desired reading. Test temperatures were 22 (Room), 48, 50, 52, 54, 56, 60, 64, 68 and 72^{o} C.

Both isolates were similarly affected by temperature as both were inactivated at 56 to 60^{o} C (Table 14). Differences between the isolates at lower temperatures were not significant. Ruppel and Hagedorn (1963) were likewise not able to separate isolates on thermal inactivation; their inactivity readings were 64 to 68^{o} C. Other thermal points for PEMV on record are: 40^{o} C or less (Johnson and Jones 1937), 55^{o} C (Ainsworth 1940), 56 to 58^{o} C (Pierce 1935) and 64 to 66^{o} C (Osborn 1938).

TABLE 14.--Thermal inactivation of extracts from pea infected with pea enation mosaic virus isolates from New York and California[a]

Test 1 Isolate	Thermal inactivation point (° C)[b]					
	Control	56	60	64	68	72
California	15/15	3/15	0/15	0/15	0/15	0/15
New York	15/15	1/15	0/15	0/15	0/15	0/15
Test 2 Isolate	**Control**	**48**	**50**	**52**	**54**	**56**
California	26/28	27/37	21/37	10/37	7/37	4/37
New York	27/28	36/37	37/37	16/37	10/37	1/25

[a]Test 1 consisted of 1 replicate; test 2: 2 replicates.

[b]Numerator = number of infections; denominator = number of trials.

DISCUSSION AND CONCLUSIONS

"Strains of a virus resemble one another in host range, symptomatology, physical properties, chemical composition, serological reactions, and particle morphology. They also differ from one another in these various ways. The differences among strains are, however, generally smaller than the differences among different viruses" (Price 1964).

Differences in physical properties, particularly thermostability, may not be real as they are influenced by virus concentration, pH of the solution, and presence of normal sap constituents (Price 1964). The following factors were included in Price's summary of sources of variation among strains of a particular virus: (1) virulence, or severity of symptoms produced; (2) antigenic properties; (3) chemical composition; (4) iso-electric points; (5) ultra violet absorption; (6) multiplication in host tissue at high temperatures and (7) insect-vector relationships.

Prominent among examples of virus strain separations on the basis of insect vector relationships are those with potato yellow dwarf virus (Black 1958), barley yellow dwarf virus (Rochow 1963), aster yellows virus (Kunkel 1955, and Maramorosch 1962) and corn stunt virus (Maramorosch 1958). Potato yellow dwarf and barley yellow dwarf viruses were shown to have vector specific strains, i.e., a particular species would transmit one strain but not another. Aster yellows virus (which is transmitted solely by insects) strains were separated on the basis of host susceptibility, severity of symptoms on a particular host, cross-protection in the vector and length of incubation period in the vector. Cross-protection was used to separate 2 corn stunt strains.

The question of whether strains now exist for pea enation mosaic virus may be superfluous, particularly if physical property data are weighed heavily. Those data compiled by Ruppel and Hagedorn (1963) coupled with the less extensive, but nevertheless comparative, data presented herein indicate strains do not exist. However, physical property studies must be taken lightly as pointed out by Price (1964). The lack of a comparison are the basis of local lesions certainly indicates that little weight can be placed on these PEMV studies.

Vector-virus relationships, however, have proved to be a sound criterion for the separation of strains. Until the final 2 tests were conducted in this research project, it was clear-cut that the New York and California isolates of PEMV were strains as judged by vector transmission characteristics. Bath (1964) already had shown that adults and first instars of a pea aphid strain from Arlington, Wisconsin were unable to transmit the California isolate and were efficient in the transmission of the New York isolate. The tests herein showed that the pea aphid could acquire and inoculate to plants the New York isolate with significantly greater efficiency than the California isolate. In each latent period test, the shortest LP_{50} was exhibited by vectors of the New York isolate. Only the retention tests, particularly the last 2 of 4 shadow acceptance of the hypothesis that the 2 isolates are strains of PEMV.

The first 2 retention tests produced data that supported the hypothesis, but none of 50 and only 2 of 46 insects transmitted the California isolate--a reflection of typically poor virus acquisition for that isolate. However, the final 2 tests showed a complete reversal of isolate superiority and the California isolate was acquired more efficiently and retained longer than the other isolate.

There are several possible explanations for a sudden shift in transmission characteristics--among them are:

(1) The 2 source plants used for the next-to-last test (August 2) were unknowingly switched, and the California data is indeed the New York data and vice versa. Source plants for the final test September 13) were obtained from the series of test plants in the previous test; thus, the 2 isolates would have remained switched.

(2) The California isolate is more suited for multiplication in plants at high temperatures (such as, existed in the greenhouse during the last 2 tests) than the New York isolate. Welton, Swenson and Sohi (1964) have made the only study of postinoculation temperature affects on insect-transmitted viruses; they found that a 2-day post-inoculation exposure to 30° C resulted in about twice as many bean yellow mosaic infected plants as at 15 to 24°. Although bean yellow mosaic virus is stylet-borne, it was tested in peas; thus, the information could be closely aligned to the PEMV situation.

(3) A mutant strain had infected the plant selected as the California isolate source plant for the August 2 test--a mutant that was more efficiently acquired and retained than the parent strain.

Rather than predict which of the above possibilities is most likely, additional work will be conducted beyond this thesis to ascertain under ideal greenhouse conditions (during the winter months) the retention characteristic for the 2 isolates. To eliminate the chance of a "possibility (1) error", new source plants will be developed from the original dehydrated (stored) cultures of each isolate.

An additional experiment will be conducted to test the possibility of high postinoculation temperature effects.

Completion of these tests should permit a final decision as to acceptance or rejection of the hypothesis that the 2 isolates are strains of PEMV. It is anticipated that the reason for the switch in characteristics that occurred in the last 2 tests will be better understood at that time.

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APPENDIX

TABLE A-1.--Latent period determination for New York and California pea enation mosaic virus isolates by serial transfers of first instar pea aphids after a 6-hr acquisition period (April 30, 1966 & May 3, 1966)

Date, isolate and class no.	Obser- vations	Transmission[a] at the indicated times after initiation of a 6-hr acquisition period[b]					
		8	20	32	44	56	68
April 30, 1966							
New York isolate							
1	1	-	+	+	+	+	+
2	3	-	-	+	+	+	+
3	2	-	-	-	+	+	+
4	6	-	-	-	-	-	-
California isolate							
1	12	-	-	-	-	-	-
May 3, 1966							
New York isolate							
1	1	+	-	+	+	+	+
2	2	-	+	+	+	+	+
3	1	-	+	+	+	+	-
4	1	-	-	+	+	+	+
5	1	-	-	-	+	+	+
6	1	-	-	-	-	+	-
7	3	-	-	-	-	-	-
California isolate							
1	1	-	-	-	+	+	+
2	9	-	-	-	-	-	-

[a]Infection (+); no infection (-).

[b]Inoculation periods on each of the 6 test plants in the series were 2, 12, 12, 12, 12 and 12 hr, respectively.

TABLE A-2.--Latent period determination for New York and California pea enation mosaic virus isolates by serial transfers of adult pea aphids after a 6-hr acquisition period (April 30, 1966 & May 3, 1966)

Date, isolate and class no.	Observations	Transmission[a] at the indicated times after initiation of a 6-hr acquisition period[b]					
		8	20	32	44	56	68
April 30, 1966							
New York isolate							
1	12	-	-	-	-	-	-
California isolate							
1	1	-	-	-	-	-	+
2	11	-	-	-	-	-	-
May 3, 1966							
New York isolate							
1	1	-	-	+	+	+	+
2	1	-	-	-	+	-	-
3	8	-	-	-	-	-	-
California isolate							
1	1	-	-	-	-	-	+
2	9	-	-	-	-	-	-

[a]Infection (+); no infection (-).

[b]Inoculation periods on each of the 6 test plants in the series were 2, 12, 12, 12, 12 and 12 hr, respectively.

TABLE A-3.--Latent period determination for New York and California pea enation mosaic virus isolates by serial transfers of single first instar pea aphids after a 9-hr acquisition period (May 9, 1966--Replicate 1)

Isolate and class no.	Obser-vations	Transmission[a] at the indicated times after initiation of a 9-hr acquisition period[b]					
		21	33	45	57	69	81
New York isolate							
1	8	+	+	+	+	+	+
2	1	+	+	0			
3	10	-	+	+	+	+	+
4	1	-	0				
California isolate							
1	1	+	+	+	+	+	+
2	1	+	+	+	-	-	-
3	2	-	+	+	-	+	+
4	1	-	+	-	+	-	+
5	1	-	+	-	-	-	+
6	1	-	-	+	-	-	+
7	1	-	-	+	-	-	-
8	3	-	-	-	+	-	-
9	1	-	-	-	+	+	-
10	1	-	-	-	-	+	+
11	1	-	-	-	-	-	+
12	1	-	0				
13	5	-	-	-	-	-	-

[a]Infection (+); no infection (-); insect died on previous test plant or was lost in transfer (0).

[b]Inoculation periods on each of 6 test plants in the series were 12, 12, 12, 12, 12 and 12 hr, respectively.

TABLE A-4.--Latent period determination for New York and California pea enation mosaic virus isolates by serial transfers of single first instar pea aphids after a 9-hr acquisition period (May 9, 1966-Replicate 2)

Isolate and class no.	Observations	Transmission[a] at the indicated times after initiation of a 9-hr acquisition period[b]					
		21	33	45	57	69	81
New York isolate							
1	11	+	+	+	+	+	+
2	1	+	-	+	+	+	+
3	1	+	+	0			
4	4	-	+	+	+	+	+
5	1	-	+	-	-	-	-
6	1	-	-	+	+	+	+
7	1	-	0				
California isolate							
1	3	+	+	+	+	+	+
2	1	+	+	+	-	+	+
3	1	+	-	+	-	+	+
4	1	+	+	+	-	-	-
5	1	-	+	+	+	+	+
6	2	-	+	+	-	+	+
7	1	-	+	-	+	+	+
8	1	-	+	-	0		
9	1	-	+	-	0		
10	1	-	-	+	-	+	+
11	1	-	-	+	-	+	-
12	1	-	-	-	+	-	+
13	1	-	-	-	-	+	+
14	1	-	-	-	-	+	-
15	1	-	0				
16	2	-	-	-	-	-	-

[a]Infection (+); no infection (-); insect died on previous test plant or was lost in transfer (0).

[b]Inoculation periods on each of 6 test plants in the series were 12, 12, 12, 12, 12 and 12 hr, respectively.

TABLE A-5.--Latent period determination for New York and California pea enation mosaic virus isolates by serial transfers of adult pea aphids after a 24-hr acquisition period (May 16, 1966 and May 17, 1966)

Date, isolate and class no.	Observations	Transmission[a] at the indicated times after initiation of a 24-hr acquisition period[b]				
		48	72	96	120	144
May 16, 1966						
New York isolate						
1	1	+	+	+	+	-
2	1	+	-	+	+	+
3	1	+	+	-	-	+
4	1	+	-	+	+	-
5	2	-	+	+	+	+
6	2	-	+	+	-	+
7	1	-	+	+	+	-
8	1	-	+	0		
9	2	-	-	+	-	-
10	2	-	-	-	+	-
11	1	0				
12	-	-	-	-	-	
California isolate						
1	25	-	-	-	-	-
May 17, 1966						
New York isolate						
1	1	+	+	+	+	+
2	1	+	+	-	+	+
3	1	+	+	-	-	+
4	2	-	+	-	-	+
5	1	-	+	-	+	+
6	3	-	-	+	+	+
7	1	-	-	-	+	+
8	1	-	-	-	+	-
9	14	-	-	-	-	-
California isolate						
1	25	-	-	-	-	-

[a]Infection (+); no infection (-); insect died on previous test plant or was lost in transfer (0).

[b]Inoculation periods on each of 5 test plants in the series were 24, 24, 24, 24 and 24 hr, respectively.

TABLE A-6.--Latent period determination for New York and California pea enation mosaic virus isolates by serial transfers of adult pea aphids after a 30-hr acquisition period (June 8, 1966 and June 14, 1966)

Date, isolate and class no.	Observations	Transmission[a] at the indicated times after initiation of a 30-hr acquisition period[b]				
		54	78	102	126	150
June 8, 1966						
New York isolate						
1	1	+	-	-	-	+
2	1	+	-	-	-	-
3	1	+	0			
4	2	-	+	+	+	-
5	2	-	+	-	-	+
6	1	-	+	+	-	+
7	3	-	+	+	-	-
8	3	-	0			
9	2	-	-	0		
10	2	-	-	-	+	-
11	1	-	-	-	-	+
12	1	-	-	-	-	0
13	30	-	-	-	-	-
California isolate						
1	2	-	0			
2	1	-	-	0		
3	2	-	-	-	0	
4	45	-	-	-	-	-
June 14, 1966						
New York isolate						
1	3	+	+	+	+	+
2	1	+	-	+	+	+
3	1	+	+	+	+	0
4	2	+	+	0		
5	1	+	-	-	-	-

TABLE A-6--Continued

Date, isolate and class no.	Observations	Transmission[a] at the indicated times after initiation of a 30-hr acquisition period[b]				
		54	78	102	126	150
June 14, 1966						
New York isolate						
6	7	-	+	+	+	+
7	1	-	+	0		
8	2	-	0			
9	3	-	-	0		
10	4	-	-	-	-	-
California isolate						
1	6	-	0			
2	3	-	-	0		
3	2	-	-	-	0	
4	1	-	-	-	-	0
5	13	-	-	-	-	-

[a]Infection (+); no infection (-); insect died on previous test plant or was lost in transfer (0).

[b]Inoculation periods on each of 5 test plants in the series were 24, 24, 24, 24 and 24 hr, respectively.

TABLE A-7.--Latent period determination for New York and California pea enation mosaic virus isolates by serial transfers of adult pea aphids after a 48-hr acquisition period (June 16, 1966)

Isolate and class no.	Obser-vations	Transmission[a] at the indicated times after initiation of a 48-hr acquisition period[b]				
		72	96	120	144	168
New York isolate						
1	6	+	+	+	+	+
2	3	+	+	+	-	+
3	1	+	+	+	+	-
4	1	+	-	+	+	+
5	3	+	+	-	-	-
6	1	+	-	-	-	-
7	1	+	+	0		
8	1	+	0			
9	1	-	+	-	-	-
10	3	-	0			
11	2	-	-	0		
12	1	-	-	-	0	
13	4	-	-	-	-	-
California isolate						
1	2	-	0			
2	2	-	-	0		
3	1	-	-	-	0	
4	23	-	-	-	-	-

[a]Infection (+); no infection (-); insect died on previous test plant or was lost in transfer (0).

[b]Inoculation periods on each of 5 test plants in the series were 24, 24, 24, 24 and 24 hr, respectively.