SOME FACTORS AFFECTING THE TRANSMISSION OF PEA ENATION MOSAIC VIRUS BY THE PEA APHID, ACYRTHOSIPHON PISUM (HARRIS), WITH EMPHASIS ON THE INOCULATION PHASE OF THE TRANSMISSION CYCLE

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Some factors affecting the transmission of pea enation mosaic virus by the pea aphid, Acyrthosiphon pisum (Harris), with emphasis on the inoculation phase of the transmission cycle

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ABSTRACT

SOME FACTORS AFFECTING THE TRANSMISSION OF PEA ENATION MOSAIC VIRUS BY THE PEA APHID, ACYRTHOSIPHON PISUM (HARRIS), WITH EMPHASIS ON THE INOCULATION PHASE OF THE TRANSMISSION CYCLE

By

James Hsi-cho Tsai

Adult pea aphids, Acyrthosiphon pisum (Harris), previously proved to be an efficient vector of the New York strain of pea enation mosaic virus (PEMV) were used to investigate some of the factors that affect vector efficiency.

Transmission efficiency was not only significantly affected by the site of inoculation probing on the pea plant but also by the age of the pea plant. Transmission to the lower surface of the petiole was significantly more efficient than to the upper surface of the leaf, the upper surface of the petiole and lower surface of the leaf during 1, 5 or 10-min inoculation probing periods. Transmission to the lower surface of the petiole, the terminal bud and the stem was more efficient than to the upper surface of the leaf. Transmission to plants in the pre-leaf (sprout) stage was significantly more efficient than to those in the one-leaf stage. The youngest leaf on a 2-leaf plant was significantly more suited for disease transmission than was the oldest leaf.

The efficiency of PEMV transmission was tested at temperature of 10°, 20° and 30°C. The latent period (expressed as LP_{50}) at 10°C was

twice as long as that at 20°C, but that at 20°C was equal to that at 30°C. The mean transmission efficiency resulting from 2-min inoculation probes by individual insects which acquired the virus and completed their latent period at 20°C was 77.5%. Insects that acquired the virus at 30° and 10°C and completed their latent period at 20°C were about 70% efficient. Insects that acquired the virus and completed their latent period at 10°C was about 60% efficient. Whereas the insects that acquired the virus and completed their latent period at 30°C was only 13% efficient.

A 24-hr pre-inoculation treatment at 10°, 20° and 30°C had no significant effect on the transmission efficiency of the vector.

Post-inoculation temperature affected the efficiency of PEMV transmission, as 67% of the plants held at 24-32°C after inoculation developed symptoms, whereas only 30% of the plants held at 30-44°C developed symptoms.

Acquisition periods of 4, 8 and 24 hr resulted in no significant difference in the length of LP_{50} (32-48 hr). But the transmission efficiency of the 24-hr acquisition period groups was significantly higher than the 4- and 8-hr groups and the 8-hr acquisition period also resulted in higher transmission efficiency than the 4-hr groups.

Temperature appeared to greatly affect the life-cycle of insect. The average nymphal stage at 10°, 20° and 30°C was 25.8, 6.2 and 4.8 days, respectively; the mean adult stage at the 3 temperatures was 9.9, 23.2 and 7.4 days, respectively. The mean number of progeny produced by each female at the 3 temperatures was 5.6, 49.8 and 8.2, respectively.

The influence of temperature on the probing behavior of the insect was investigated at 10°, 20° and 30°C. Increases in temperature

caused an increase in the frequency of short 'test' probes prior to the initiation of a long phloem-seeking probe.

Histology of the pea aphid probes on various sites of the pea plant was extensively investigated. Of the 99 single 10-min probes made on 7 sites of the pea plant, only 50 tissues were found to have salivary sheath in section. Twenty of 72 probes during 5 min period contained the sheath in section, whereas only 35 out of 110 1-min probes found the sheath in section. All sheaths but 5 penetrated intercellularly in the plant tissues, only 16 sheaths ended in phloem of the leaf and the petiole in 10 min probes and 1 sheath reached the phloem of the lower leaf in a 5-min probe. The length of sheaths deposited on the 7 tested sites of the pea plant was dependent on the duration of the probes; the average length of sheath was 1.78, 1.16 and 0.37 mm in 10, 5 and 1 min probes, respectively. However, the transmission efficiency on the 7 sites of the pea plant did not appear to be a function of the depth of stylet penetration.

The length of pre-inoculation starvation affected the vector efficiency. A period of 4-8 hr fasting resulted in significantly higher transmission than did that of 16-20 hr.

SOME FACTORS AFFECTING THE TRANSMISSION OF PEA ENATION MOSAIC VIRUS BY THE PEA APHID, ACYRTHOSIPHON PISUM (HARRIS), WITH EMPHASIS ON THE INOCULATION PHASE OF THE TRANSMISSION CYCLE

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INTRODUCTION

Pea enation mosaic virus (PEMV) has been and is being used in several research laboratories as a vehicle for elucidating the vector-virus-plant relationships of circulative, aphid-borne plant viruses, yet unfortunately there are still many variations in interlaboratory results of transmission tests. Additionally, very little regard is paid to the inoculation phase of transmission experiments; i.e., test insects are routinely placed on test plants for long inoculation (assay) periods with little attention paid to the site or age of plant.

Some very obvious possible sources of variation have not, or only partially, been investigated. Nault and Gyrisco (1966) showed that the inoculation of PEMV to various tissues of the pea leaf by the pea aphid, Acyrthosiphon pisum (Harris), yielded tremendous differences in transmission efficiency and pointed out that the feeding processes of the vectors were important in the acquisition and inoculation of plant viruses. Sylvester and Richardson (1966) compared the transmission of PEMV by A. pisum at 10, 20 and 30°C and found that temperature exerted a significant effect on every phase of the virus transmission cycle.

Therefore, this study was designed to reveal more basic information about the sources of variation inherent in transmission tests with PEMV and the pea aphid. The following hypotheses were tested:
(a) transmission efficiency is significantly affected by the site of inoculation probing on the test plant and the age of the test plant;

(b) temperature affects the virus acquisition, latency and inoculation processes with the pea aphid. Included in these tests were: (a) a detailed study of the feeding process of a highly efficient A. pisum transmitter of PEMV on various parts of the pea plant during probing periods of various lengths; (b) the effects of 3 temperatures (10, 20 and 30°C) on the acquisition, latency and inoculation phases of transmission by the pea aphid. Additionally, the reproductive rate and generation time of the vector at 3 temperatures were investigated.

REVIEW OF THE LITERATURE ON THE FACTORS AFFECTING THE TRANSMISSION OF PLANT VIRUSES BY THEIR INSECT VECTORS WITH EMPHASIS ON THE TRANSMISSION OF PEA ENATION MOSAIC VIRUS BY THE PEA APHID

Pea enation mosaic virus (PEMV) is characterized as being in the circulative aphid-borne group (Osborn 1935, Chaudhuri 1950, Simons 1954; McEwen et al. 1957, Black 1959, Schmidt 1959, Kennedy et al. 1962, Nault et al. 1964, Ehrhardt and Schmutterer 1964 and 1965, and Bath and Chapman 1966). It long has been postulated that aphids must inoculate circulative viruses into the phloem. Recent studies have shown that PEMV can be transmitted to nonvascular tissues of the pea plant during single probes as short as 1 min or less (McEwen et al. 1957, Nault et al. 1964, Ehrhardt and Schmutterer 1964, Bath and Chapman 1966, and Nault 1967). While much has been done in this field, there is still rather limited information available for the explanation of inter-laboratory discrepancies which might be due to the effects of experimental factors. Thus the following review will emphasize the factors affecting the transmission of PEMV in relation to the other stylet-borne and circulative viruses and will include the effect on transmission efficiency of (1) inoculation to various parts of the pea plant, (2) age of test plant, (3) temperature during acquisition, latent period and inoculation; (4) the probing behavior of aphid at 3 temperatures, (5) the length of probe in relation to the length of sheath in tissues, (6) starvation in relation to inoculation, (7) temperature on the reproductive rate and longevity of the vector.

Effect of inoculation to various parts of the pea plant.--It has been shown that leaves of the same plant differed as virus sources for insects (Kirkpatrick and Ross 1952, Storey 1928, Watson 1936, and Sylvester 1956). Bradley (1962) proved that different areas of a tobacco leaf also vary as sources of virus. By using radiophosphorus, Day and Irzykiewicz (1953) found that Myzus persicae (Sulzer) ingested more material per unit of time from the lower than from the upper surface of the leaf of Brassica chinensis L. Other experiments (MacKinnon 1962) showed that leaves cut from upper, intermediate and lower parts of infected Physalis floridana (Rydb.) differed as sources of potato leaf roll virus (PLRV) and turnip latent virus (TLV) for aphids. Furthermore, he found only slight differences in the transmission of PLRV from midvein, secondary veinal and interveinal areas, but TLV transmission efficiencies were 8% from midveins, 18% from secondary veins, and 14% from interveinal areas (MacKinnon 1963a).

A test on the preference of region of the plant probed by variously treated M. persicae (Sylvester and Richardson 1963) illustrated that highest numbers of probes were made on the lower surface of the cotyledon, then followed in order by the upper cotyledon surface, stem, petiole and newly-emerging leaf bud. Swenson (1962) found that M. persicae, which probed the upper surface of the Pisum sativum L. leaf, transmitted bean yellow mosaic virus (BYMV) to fewer plants than those that probed on the lower surface.

On the other hand, not all evidence supported the difference in the probing area of the test plant. Sylvester (1955) reported lettuce mosaic virus transmission by M. persicae was not affected by inoculations to the upper and lower surface of leaf or cotyledon of lettuce seedling.

Earlier studies on Brassica nigra virus transmission by M. persicae (Sylvester 1953) revealed little differences in susceptibility of mustard (Brassica juncea C. et C.) seedlings, among the lower and upper surface of cotyledon, cotyledon petiole, leaf petiole, the upper and lower surface of the newly emerging leaf. He found that only the leaf petiole was apparently more susceptible to this virus. In the transmission of PEMV, inoculation to veinal areas was found to be twice or more as effective as inoculation to interveinal areas (Nault and Gyrisco 1966).

Age of the test plant.--Combinations of age and area of the plant affecting the transmission efficiency of plant viruses have received considerable attention from many workers. Swenson (1963) observed that the susceptibility of leaves of Chenopodium amaranticolor to inoculation of bean yellow mosaic virus decreased with increasing age on the same plant. He tested the transmissibility of the same virus by M. persicae and found that the differences in transmission were associated with the age of the inoculated leaves rather than the age of the whole plant (Swenson 1968). In transmission of Brassica nigra virus by M. persicae, the effect of age of mustard test plants from 1-6 weeks were approximately equal in susceptibility (Sylvester 1953), but later work (Sylvester 1955) with lettuce mosaic virus transmitted by M. persicae showed that lettuce (Lactuca sativa L.) test plants 1-5 weeks of age resulted in more infections in the older plants than in the younger. Watson (1936) also noted that the differences in susceptibility to inoculation of virus Hy. III by M. persicae occurred between leaves of different ages on the same plant. Recent work with the tristeza virus

transmission by the melon aphid, Aphis gossypii Glover revealed that young Citrus aurantifolia (Christm.) plants were equal to older plants as indicator plants (Norman et al. 1967).

Effect of temperature on acquisition, latent period and inoculation.--Early studies on the effects of temperature on virus transmission were based entirely on mechanical inoculation to the local-lesion hosts (Kassanis 1957, Sinha 1960, Lindner et al. 1959, and Hagedorn and Hanson 1957). However, the available information shows that plant susceptibility to virus is not necessarily the same with mechanical and aphid transmission (Swenson 1963 and 1968).

So far, very little information is available concerning the effects of temperature on virus acquisition by aphids. Simons (1966) made a comparative study on the transmission of potato virus Y and cucumber mosaic virus by the green peach aphid, the cotton aphid and the green and pink forms of potato aphid given 1 probe, 5 min and 15 min acquisition access feeding at 50, 70 and 90°F. The results showed that the highest transmission efficiency was achieved at 70°F, then followed by 50 and 90°F. Sylvester (1964) made an extensive study on the transmission of cabbage mosaic virus by M. persicae at 40, 50, 60, 70, 80 and 90°F and found that the most efficient acquisition was in the range of 60°-80°F.

Recent studies on the effects of temperature on the acquisition of PEMV by the pea aphid indicated that most of the reduction in virus acquisition occurred at 10°C with acquisition access periods of more than 3 hr (Sylvester and Richardson 1966).

The latent period of PEMV in A. pisum varied with the strain, form and age of the test aphids (Chaudhuri 1950, McEwen et al. 1957, Osborn 1935, Simons 1954, and Chapman and Bath 1968), and no reference was made regarding the environmental factors affecting the latency. Severin (1921) was the first to show that the sugar beet curly-top virus had the shortest incubation period in its leafhopper at 100°F. Later Storey (1928) found differences in incubation periods of maize streak virus in Cicadula mbila Naude held at 4 different temperatures.

Maramorosch (1950) found that the minimum incubation period of the wound-tumor virus in Agallia constricta was 14 and 30 days at 26 and 16°C, respectively. Duffus (1963) demonstrated that the extremely long latent period of sowthistle yellow vein virus in Amphorophora lactucae L. could be greatly altered by changes in temperature; the shortest latency was 8 days at 25°C and the longest was 46 days at 5°C.

The latent period of PEMV was found to be shortest at 80 to 90°F in the pea aphid (Osborn 1935). In 1965, Sylvester proposed a method to estimate the LP_{50} value and found that the LP_{50} of PEMV in A. pisum decreased from 70 hr at 10°C to 25 and 14 hr at 20 and 30°C, respectively.

The influence of temperature was not only observed in the acquisition and latent period phases but also in the inoculation phase. Raising the temperature from 25 and 30 to 35°C resulted in the loss of potato leaf-roll virus infectivity in M. persicae (Stegwee 1960). A similar phenomenon was observed in the case of wound-tumor virus in A. constricta which completed its incubation period at 26°C and lost its transmitting ability almost completely at 30°C (Maramorosch 1950).

Cockbain et al. (1963) tested the infectivity of aphid alatae and apterae at different temperatures. He found that the rate of decrease in infectivity of sugar-beet mosaic virus and pea mosaic virus in Aphis fabae and M. persicae, respectively, was accelerated as the temperature rose, and in fact the majority of the vectors lost infectivity after 30 min at above 30°C. Also, Sylvester (1964) reported that the rate at which inoculativity of cabbage mosaic virus by M. persicae declined during fasting periods was greater at 30°C than at 10 or 20°C, but the efficiency of inoculation increased with increasing temperature over the entire range of 40-90°F. Later Sylvester and Richardson (1966) tested A. pisum with PEMV at 10, 20 and 30°C, and concluded that the effect of temperature on inoculation was almost entirely associated with reduced transmission efficiency during inoculation access periods up to 30 min at 10°C.

Effects of acquisition time on inoculation efficiency and latent period.--Kassanis (1952a) showed that the efficiency of transmission of potato leaf-roll virus by M. persicae given 2, 4, 8 and 24 hr acquisition access time and 2 days on test plants was 13.3, 23.3, 43.3 and 66.6%, respectively. However, in the transmission of sowthistle yellow vein virus by the sowthistle aphid Duffus (1963) showed that latent period and retention of virus by this aphid was independent of length of the acquisistion feeding. MacKinnon (1964) used potato leaf-roll virus and turnip latent virus transmission by M. persicae to prove that an increase in acquisition access time resulted in a noticeable short latent period and high transmission efficiency. Studies on the transmission of PEMV by Simons (1954) and Ehrhardt and Schmutterer (1965)

showed that longer acquisition access time resulted in a longer retention period in its vectors than did short acquisition periods. Chapman and Bath (1968) tested PEMV in three of its aphid vectors and indicated that the shortest latent periods were found in the vectors most efficient in acquiring PEMV.

Temperature affects on the probing behavior of aphid.--Simons (1966) reported that 94% of the cotton aphids, Aphis gossypii Glover, in a test probed longer than 30 sec at 50°F, whereas at 90°F only 5% probed longer than 30 sec. Sylvester (1964) used M. persicae to test the probing behavior at 40-90°F and found that the time required by the aphids to begin probing decreased with increasing temperature reaching a minimum in the 70-90°F range. The length of probe minimized at 70°F and above, and the frequency for 1 min or longer probes increased as the temperature varied on either side of 70°F.

Recently, McLean and Kinsey (1968) conducted a detailed study regarding the effect of temperatures on probing behavior of A. pisum, and found that at temperatures between 25 and 30°C they ingested fluid from seive elements for longer periods and probed for longer time.

Length of probe in relation to depth and position of probe.--Mittler and Dadd (1963) studied the artificial feeding of M. persicae and found that with starvation, progressively greater numbers of aphids took up fluid and this was reflected by an increasing proportion of salivary deposits being found as salivary sheaths. Roberts (1940) used Myzus persicae and Myzus circumflexeus to study the penetration rates on tobacco; neither of them were found to reach the phloem after 5 min probing. Very few M. persicae penetrated the phloem in 15 min, and

only about 50% of probes were intracellular. Another example was shown by Esau et al. (1961) with M. persicae feeding on sugar-beet leaves. They examined over 150 penetrations and found that the depth of probe was dependent on the length of feeding and the probes were mostly intercellular. Bradley (1956) also used M. persicae to probe on tobacco leaves; less than 1 min of probing resulted in penetrations of 21μ or less and after 1-10 min probing the depth of stylet was found to be mostly between 21 and 100μ.

McLean (1964) attempted to relate depth of stylet penetration to the transmission of artichoke latent virus by M. persicae and A. pisum after 15 and 30 sec probes. He found that both species exposed stylets after such probes, but more of them exposed stylets after a 30 sec probe than after a 15 sec probe. McLean and Kinsey (1967) studied the probing behavior of A. pisum and found that in 5-min probes 12 out of 13 aphids reached only parenchyma tissue of Vicia faber L.; 1 reached bundle tissue. Whereas in 10 min probes, 10 out of 11 aphids reached phloem tissue. Nault and Gyrisco (1966) observed 50 short probes by pea aphids on pea leaves and found that short probes rarely penetrated beyond epidermis, and on longer probes the parenchyma was penetrated intercellularly, and a higher penetration rate was found in interveinal than veinal areas. Further studies (Nault 1967) on 3 aphid species given 15 and 60 sec probes on pea leaves showed that 15 sec probes were in the epidermis only and 60 sec probes were in the parenchyma.

Effect of pre-inoculation starvation on transmission efficiency.--The preacquisition fasting could greatly enhance the stylet-borne virus transmission by the aphids (Chaudhuri 1950; Kvicala 1947; Sylvester 1949,

1950 and 1954; and Watson 1938). Little information is available on the effect of pre-inoculation fasting period of the insects.

Day and Irzykiewicz (1953) using radiophosphorus to measure the effect of starvation times concluded that starvation from 30 min to 4 hr did not affect the amount subsequently ingested by M. persicae during a 30-min feeding period. Simons (1954) found that post-acquisition starvation for periods up to 24 hr produced no effect on transmission efficiency of PEMV by A. pisum.

Temperature affects on the reproductive rate and longevity of the vector.--The biology of A. pisum has been studied by many early workers and it varied with biotypes, environmental factors and host plants (Davis 1915, Smith and Davis 1926, Campbell 1926, Evans and Gyrisco 1956, and Markkula 1963). Dahms and Painter (1940) noted that under 80°F the lower the temperature the lower the rate of reproduction in A. pisum. Duffus (1963) found that the average longevity of the sowthistle aphid was 54.4, 42.4, 22.6, 16.6 days at 5, 15, 25, and 30°C and the average number of nymph reproduced per day per adult was 1, 2.1, 4.1, 3.3, respectively. Isaak et al. (1963) compared the reproduction rate of A. pisum at 55, 70 and 85°F and concluded that all test clones reproduced higher at 70°F than at the other 2 temperatures. Sylvester and Richardson (1966) found that the average reproductive rate of A. pisum at 10, 20 and 30°C was 41.23, 81.38 and 3.35 nymphs per life, respectively. A wide range of temperature (5, 10, 15, 20, 25, 30 and 35°C) was tested by McLean and Kinsey (1968) and revealed that A. pisum produced more offspring at 25-30°C than aphids at the other 5 temperatures.

MATERIALS AND METHODS

The New York strain of pea enation mosaic virus was used throughout this study and was maintained *in vitro* and *in vivo* as described by Bath and Chapman (1966). The pea aphid biotype that was used originated from a single, apterous female that was collected from alfalfa in East Lansing and subsequently found to be an efficient vector of PEMV. Broad bean served as the host plant for aphid cultures that were maintained as previously described (Tsai 1967).

Techniques used for acquisition, inoculation and latent period tests as well as those for test plant culture and insect transfers were previously described (Bath and Chapman 1966, 1967). The aphid aspects of all experiments were conducted in environmental growth chambers (Sherer-Gillett) at prescribed temperatures, about 50% RH and 12-hr photoperiods. Unless otherwise stated insects were transferred and held at 20°C. Most experiments were arranged in factorial designs and evaluated with an analysis of variance and Duncan's Multiple Range Test.

For histological studies, vectors were treated in the same manner as in inoculation experiments. They were allowed single probes of certain lengths on the abaxial surface of various parts of the pea plant. Probes were observed with a hand lens at 15X and timed with a stopwatch, the probed tissues were marked with a dot of India ink and cut out in 2 mm square pieces, and placed in vials of FAA fixative.

Then they were transferred through butyl alcohol series for dehydration. Tissues were embedded in paraffin and sectioned with a rotary microtone at a thickness of 12-15μ. The sections were stained with 1% aqueous safranin solution and fast green in 95% ethyl alcohol (Sass 1958). The sections were examined microscopically to determine the length and path of the salivary sheath.

EXPERIMENTS AND RESULTS

1) The influence of probing site and plant age on the efficiency of PEMV transmission.--Inoculation probing periods of 1, 5, and 10 min on test plants in the pre-leaf stage and on 6 sites of plants in the one-leaf stage were alloted to young, adult pea aphids to test the influence of probing site and plant age on the efficiency of PEMV transmission.

Inoculative aphids were obtained by placing first-instar nymphs on PEMV-infected pea plants and allowing them to feed for 7-8 days. Aphids were starved 4-8 hr prior to each trial in order to enhance consistent probing behavior which would provide probes of desired duration. Plants which received shorter than desired probes or probes on other than the desired sites were discarded. Each test insects was used for only 1 test probe, then it was placed on another healthy plant for a 2-4 hr period to ascertain whether or not the insect was infectious at the time of test.

Trials were conducted on 59 occasions over a 5-month period; all trials were made at 20°C. Each trial involved 25-30 aphids and 1 of the 21 probe treatments. Forty to 91 probes by infectious aphids were made for each probe-treatment.

Transmission of the virus to the plant in the pre-leaf stage was significantly (at the 5% level) more efficient than to those in the one-leaf stage (Table 1). Significant differences occurred between

Table 1.--Percent transmission of pea enation mosaic virus by inoculative pea aphid adults given 1, 5, and 10 min inoculation probing periods at various sites on test plants in the pre-leaf and one-leaf stages.

IPP[a] (min)	One-leaf stage[c]						Pre-leaf stage[c]	Avg effect of IPP[b]
	Upper leaf	Upper petiole	Lower leaf	Stem	Terminal bud	Lower petiole		
1	14.0	16.1	20.0	24.6	31.4	35.2	50.5	27.4a
5	21.4	19.5	27.8	41.3	37.5	42.9	77.1	38.2a
10	17.1	40.0	31.5	32.7	37.8	50.0	87.5	42.4a
Avg effect of site or stage[b]	17.5	25.2	26.4	32.9	35.6	42.7	71.7	

[a]IPP = Inoculation Probing Period.

[b]Means flanked by a common letter or underscored by a common line are not significantly different at the 5% levels; there was no significant interaction.

[c]40-91 trials were made at each treatment. Specific results are presented in Table A-1.

several of the sites on the one-leaf stage test plant. Transmission to the lower surface of the petiole was significantly more efficient than to the upper surface of the leaf, the upper surface of the petiole, and the lower surface of the leaf. Likewise transmission to the lower surface of the petiole, the terminal bud and the stem was more efficient than to the upper leaf surface. No significant differences were detected between the efficiency of transmission on the upper surface of petiole, the lower surface of leaf, the stem and the terminal bud. No interactions at 5% level were detected between inoculation probing period, test plant stages or probing sites on the one-leaf stage. No significance was detected in avg influence of probing time on transmission efficiency.

Another experiment was conducted to compare the efficiency of PEMV transmission to the first (oldest) and second leaf of plants in the two-leaf stage. A 5-min inoculation probing period was alloted to each inoculative pea aphid. Eighty infectious pea aphids were treated on each of the two leaves; this test revealed that transmission efficiency to the second leaf was 36%, whereas only 27% transmission occurred to the first leaf. These results were significant at 5% level and were analyzed with a paired t-test.

2) Influence of temperature on length of aphid latent period in virus transmission.--The efficiency of PEMV transmission was tested at temperatures of 10, 20, and 30°C; parameters measured were length of the latent period and transmission efficiency. A large number of first-instar nymphs were divided into 3 groups and given a 24-hr acquisition-access period (AAP) at one of the 3 temperatures. After that period, insects were removed from the virus source plant, caged

singly on pea seedlings, and transferred daily to healthy test plants for 1 week. To test the effect of temperature during the latent period and subsequent inoculation period, one half of the insects in each group was left in the same condition under which they acquired viruses while the other half was held at 20°C for the duration of the test. Each subgroup contained 30 to 36 aphids. At the end of the serial transfers, the test insects were starved 2-4 hr and used to determine the influence of the above temperature regimes on the efficiency of PEMV transmission during a 2-min inoculation probe.

Three replicates of this experiment (Table 2) revealed that the latent period $_{50}(LP_{50})$ in the 10°:10°C group (10°C during AAP and 10°C during serial transfers) was significantly longer (2 fold) than the 10°:20°C group. Since 90% of the insects that acquired viruses at 20° and 30°C completed their latent periods on the first test plant in the series, no latent LP_{50}s were determinable, but the latent period was much shorter than 48 hr.

Tests of the individual insects from the above experiments 1 week after the acquisition-access period, revealed that transmission efficiency was greatly affected by the temperature at which the insect had acquired the viruses and undergone the latent period (Table 2). The maximum mean transmission efficiency that resulted from the 2-min inoculation probe was obtained by insects that had been subject to the 20°:20°C regime (77.5%). Insects in the 10°:20°C and 30°:20°C regimes were about 70% efficient; whereas the 10°:10°C group was about 60% efficient and 30°:30°C group was only 13% efficient.

Table 2.--Summary of experiments to determine the effect of temperature on the latent period, acquisition and inoculation aspects of pea enation mosaic virus transmission by the pea aphid.[a]

Replicate	Temperature (°C) during: AAP	Latent period	LP_{50}	Infectivity[b]	Mean % transmission during a 2-min IPP 1 week after start of AAP[c]
1	10	10	70	100.0	60.0
	10	20	30	96.4	78.6
	20	20	<48	100.0	85.2
	30	30	<48	100.0	11.1
	30	20	<48	100.0	69.2
2	10	10	98	75.8	63.6
	10	20	45	96.8	83.9
	20	20	<48	100.0	80.0
	30	30	<48	100.0	22.7
	30	20	<48	100.0	86.2
3	10	10	127	26.5	54.8
	10	20	60	93.1	60.9
	20	20	48	100.0	68.6
	30	30	<48	100.0	0.0
	30	20	<48	100.0	56.7
4	10	10	--	--	45.5
	10	20	--	--	50.0
	20	20	--	--	67.9
	30	30	--	--	0.0
	30	20	--	--	62.1
5	10	10	--	--	50.1
	10	20	--	--	56.1
	20	20	--	--	60.0
	30	30	--	---	17.9
	30	20	--	--	54.8

[a]Infectivity = Percentage of insects that transmitted virus at any time during serial transfers. AAP = Acquisition access period; IPP = Inoculation access period; LP_{50} = Latent period 50.

[b]Each treatment consisted of 30-40 insects. Specific results are presented in Tables A-2 to A-4.

[c]Each treatment consisted of 27-40 infectious insects.

3) Effect of temperature on the inoculation phase of virus transmission.--To determine the influence of various temperatures on inoculation of PEMV to pea by the pea aphid, 3 groups of 6-7 day old insects that had spent their life on PEMV-infected plants were given a 24-hr pre-inoculation treatment of 10, 20 or 30°C, starved for 2-4 hr, and allowed to probe for 1 min on pea seedlings. Probes were made on specific sites on one-leaf stage plants and at random on plants in the pre-leaf stage.

No significant difference was detected at the 5% level in transmission efficiency that resulted from inoculation probes at the 3 test temperatures (Table 3). Additionally, all of the plant areas tested as sites of inoculation on 'one-leaf stage' plants were statistically inseparable on the basis of transmission efficiency. The pre-leaf stage, however, was significantly more suitable for virus transmission, regardless of temperature, than was the one-leaf stage.

4) Influence of acquisition period on length of latency and efficiency of virus transmission during a 2-min inoculation probe.--A test was conducted to determine the effect of acquisition feeding on latency and virus transmission during short inoculation probes. About 150 first-instar nymphs were used in each trial, and they were given a 4, 8, and 24 hr acquisition period on 2 leaves of a single virus source plant at 20°C. At the end of each acquisition period, a group of 50 nymphs was removed and placed singly on a test plant. They were moved to a new test plant in series after 3, 17, 24, 24, 24 and 24 hr, respectively.

Table 3.--Mean percent transmission of pea enation mosaic virus by inoculative pea aphid adults given a 1-min inoculation probing period at 3 temperatures at various sites on test plants in the pre-leaf and one-leaf stages.[a]

IPP[b] at: (°C)	One-leaf stage						Pre-leaf stage	Avg effect of temp[c]
	Upper leaf	Lower leaf	Upper petiole	Lower petiole	Stem	Terminal leaf		
10	20.0	17.4	24.0	26.9	23.1	16.7	38.1	23.7a
20	17.4	20.8	26.9	27.6	27.3	34.6	60.9	30.8a
30	25.0	27.3	25.9	22.2	26.7	26.1	44.0	28.2a
Avg effect of site or stage[c]	20.8	21.8	25.6	25.6	25.7	25.8	47.7	

[a]Each trial consisted of 15-30 infectious insects. Specific results are presented in Table A-5.

[b]IPP = Inoculation Probing Period.

[c]Means flanked by a common letter or underscored by a common line are not significantly different at the 5% level; there was no significant interaction.

Three replicates of this experiment (Table 4) revealed that there was no significant difference in the length of LP_{50} as related to acquisition period. Latent Period $_{50}$s of 31.5-35.5, 33.0-39.5, and 33.5-less than 48 hr resulted from AAPs of 4, 8 and 24-hr, respectively. Infectivity of the test insects was very high and ranged from 93-100% regardless of AAP.

Table 4.--Summary of trials to determine the effect of the acquisition access period (AAP) on the latent period, and the efficiency of pea enation mosaic virus transmission during a specified inoculation probing period (IPP) by the pea aphid.

Replicate	AAP (hr)	LP_{50}[a]	Infectivity[b]	Mean % transmission during 2-min IPP 1 week after start of AAP
1	4	35.5	84.6	50.0
	8	35.0	96.2	63.4
	24	<48.0	98.1	74.4
2	4	32.0	80.8	46.3
	8	39.5	94.2	55.8
	24	33.5	84.6	64.4
3	4	31.5	91.8	52.5
	8	33.0	95.8	61.9
	24	<48.0	96.0	79.1

[a]Fifty to 53 insects were tested at each AAP. Transmission data used to determine Latent Period$_{50}$ (LP_{50}) are presented in Tables A-6 to A-8.

[b]Infectivity = Percentage of insects that transmitted virus at any time during serial transfers.

Six to 8 days after the start of the acquisition period, all insects were given a 2-min inoculation probe on pea in the pre-leaf stage. The average transmission efficiency for the 3 replicates of insects that were given a 4, 8, and 24 hr acquisition period was 49.6,

60.3 and 72.6%, respectively. Duncan's multiple range test showed that the 24-hr acquisition period groups had a significantly higher transmission efficiency than did the 4- and 8-hr groups, and that the 8-hr acquisition period also resulted in higher transmission efficiency during short inoculation probes than did a 4-hr period.

5) Effect of postinoculation temperature on efficiency of PEMV transmission.--A small experiment was conducted to determine the effect of post-inoculation temperatures on the development of disease in the test plants. A group of 60 pea seedlings were inoculated by inoculative, adult aphids during a 2-min inoculation probing period at 20°C, and then divided into two subgroups. Thirty of the test plants were subsequently held in a greenhouse equipped with evaporative air-cooling where the temperature was 24-32°C; the remainder of the test plants were held in a greenhouse without air-cooling where the temperature ranged from 30-44°C.

Sixty-seven percent of the plants held at cool temperatures developed symptoms in contrast to only 30% infection in the plants held at high temperature.

6) Effect of temperature on the life history of the pea aphid.--Three groups of first-instar pea aphid nymphs were reared at 10, 20, and 30°C and caged singly on pea. The nymphal instars were counted through the recovery molted skin (exuviae); reproductive evaluation were made on a 24 hr nymphal-positional period. All daily observations were carried out until the death of insects.

I found that temperature had a tremendous effect on every phase of the insect life-cycle. The average nymphal and adult stages were

increased as the rearing temperature decreased. The average adult stage and the number of progeny produced by each female were much higher at 20°C than at 10 and 30°C (Table 5).

Table 5.--Effect of temperature on the life history of the pea aphid.

Temp (°C)	No. of test insects	Mean nymphal stage (days)	Mean adult stage (days)	Mean no. of progeny/female	Mean longevity (days)
10	16	25.80	9.94	5.75	35.74
20	10	6.15	23.20	49.80	29.35
30	12	4.79	7.38	8.17	12.17

7) Histology of the pea aphid probe on various sites of the pea plant.--A group of adult pea aphids was removed from PEMV-infected pea plants and given 4-6 hours starvation period. At the end of fasting period, each insect was allowed a single probe on one of the 7 designated sites for either 1, 5 or 10 min in the same manner as allowed during virus transmission experiments. The tissue probed was excised from the plant, appropriately killed, desiccated, infiltrated with paraffin, embedded, sectioned, and stained.

Ninety-nine probes of 10-min duration were made but only 50 of the probed tissues revealed salivary sheaths upon microscopic observation. The 5 and 1-min probed tissues provided 29 and 35 salivary sheaths *in situ* per 72 and 110 attempts, respectively. All sheaths except 5 penetrated intercellularly in plant tissues. Intercellular penetration of the epidermis was marked by the formation of smooth salivary sheath, while penetration of the parenchyma was marked by the formation of

beaded sheath. Occasionally, the salivary flange was observed on the epidermal groove. Of the total 114 sheaths, only 16 sheaths ended in phloem of the leaf and the petiole in 10 minute probes and 1 sheath reached to phloem of the lower leaf in 5 min probe. However, the length of sheath was not proportional to the straight-line distance between the entry point of epidermis and the target phloem tissue.

Since the irregularity in distribution of phloem tissue in leaf and terminal bud, no unique standard could be used to evaluate whether or not the stylet reached to phloem. Thus only upper petiole, lower petiole, stem and 'pre-leaf stage' plant (=stem) which had a definite pattern of phloem distribution were included in the statistical analysis (Table 6). No significant difference in length of salivary sheath could be detected among any of the above tissues at the 5% level of significance. As they were compared with the transmission efficiency in Table 1; thus, transmission efficiency does not appear to be a function of the depth of stylet penetration. However, there was significant difference in length of salivary sheath with regard to the inoculation probing period--the average length of sheath was 1.78, 1.16 and 0.37 mm at probes of 10, 5 and 1 min, respectively.

8) Influence of temperature on probing behavior.--It was noted in the previous temperature experiment that insect behavior was adversely affected by the temperature. In low temperature, insects seldom initiated the probe within 3-5 minutes after they had been placed on the test plant. Whereas at high temperature, they made more short probes than long ones. Hence this experiment was solely designed to investigate the probing behavior in different temperatures. Three

Table 6.--Influence of inoculation probing period (IPP) and probing site on length of salivary sheath deposited by pea aphids.[a]

IPP min	Length (mm) of salivary sheath deposited in specified site: Upper petiole	Lower petiole	Stem	Pre-leaf stage plant	Avg effect of IPP[b]
1	0.36	0.29	0.41	0.39	0.37
5	1.43	1.10	1.22	0.87	1.16
10	1.96	1.89	1.35	1.92	1.78
Avg effect of site[b]	1.25	1.09	0.99	1.06	

[a]Figures in the table are in mm, a variety of measurements were made on each probe and tissue (see Table A-9).

[b]Means flanked by a common line are not significantly different at the 5% level, there was no interaction.

groups of adult aphids were subject to 10, 20 and 30°C for 24 hr and starved for 4-6 hr prior to each trial. Insects from each of the 3 temperature treatments were allowed probes of 1, 5 and 10 min. I found that the number of short probes (probes shorter than those desired) made before the end of the desired single probing duration was correlated to both temperature and the designated duration of probe.

Thirty to 50 aphids were observed during attempts to obtain 1-min probes at each of the 3 temperatures. At 10°C only 16.7% made 1 short probe prior to the end of test probe and none ever made more than 1 test probe. At 20°C 62% made no short probe, whereas 30 and 8% made 1 and 2 short probes, respectively. However, in the 30°C group, the percentage was almost evenly distributed in the 0-3 short probe range (Fig. 1).

Another group of 20-25 aphids was observed at 3 temperatures during attempts at 5-min probes (Fig. 1). In the 10°C group, the aphids demonstrated similar behavior as in the previous group, but it also showed the effect of probing duration, thus 50% made no short probe, 43% made 1 short probe, and 7.5% made 2 short probes prior to initiating a 5-min probe. At 20°C, 33% made no short probes; 42, 18 and 7% made 1, 2 and 3 short probes, respectively. Whereas in the 30°C group, the effect of the temperature and duration was more prominent; only 15% made no short probes--24, 27, 16, 8 and 8% made 1, 2, 3, 4, and 5 short probes, respectively.

Fifteen to 20 aphids were observed during attempts to make 10-min probes at each of the 3 temperatures (Fig. 1). At 10°C, 64% made no probes prior to the initiation of a 10-min probe; 23% made 1 short probe and 17% made 2 short probes. In the 20°C group, 33, 40, 15, 7 and 5% made 0, 1, 2, 3 and 4 short probes, respectively. At 30°C, only

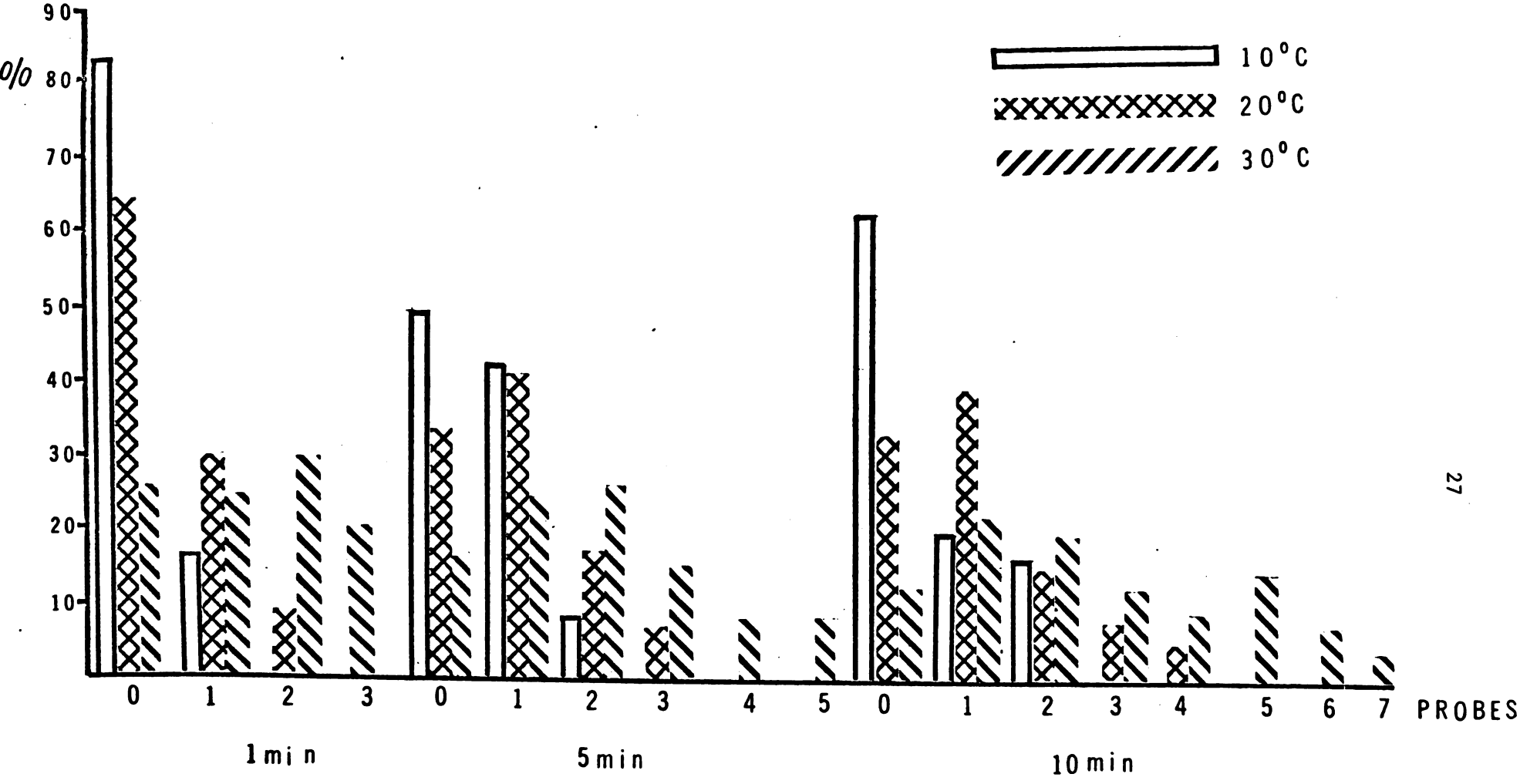

Fig 1. Frequency of short "test" probes prior to 1, 5, and 10 min probes at 10°, 20°, and 30°C. Each frequency was determined from the results of probing trials conducted on 2 occasions (see Tables A-10 to A-12).

about 13% made no short probe; 22, 20, 12, 8, 12, 8 and 5% made 1, 2, 3, 4, 5, 6 and 7 short probes, respectively.

9) Influence of pre-inoculation starvation on efficiency of PEMV transmission.--During the course of this study, I found that the length of pre-inoculation starvation among the insects often varied with the number of insects involved in each trial. Thus 2 trials were conducted to determine whether or not the length of pre-inoculation fasting period had an effect on transmission efficiency of PEMV. Infectious insects were starved 0-2, 4-8, and 16-20 hr, then allowed to make a 2-min inoculation on pea seedlings at 20°C (Table 7). No differences were detected in probing behaviour, but the 4-8 hr fast produced significantly greater transmission efficiency than did the 16-20 hr fast--83.7 vs. 68.9%, respectively.

Table 7.--Transmission of pea enation mosaic virus by inoculative pea aphid adults given a 2-min inoculation probing period after various lengths of starvation at 20°C.[a]

Replicate	% transmission after specified pre-inoculation fasting periods (hr)			Avg effect of replicate[b]
	16-20	0-2	4-8	
1	68.2	73.9	86.4	76.17a
2	69.6	76.2	80.9	75.57a
Avg effect of fasting period[b]	68.9	75.1	83.7	

[a]Each treatment consisted of 21-23 infectious insects.

[b]Means flanked by a common letter or underscored by a common line are not significantly different at the 5% level; there was no interaction between replicate and fasting period.

DISCUSSION

In nature a variety of factors exist that could either independently or compoundly affect the transmission of virus by the insect vector, namely (a) susceptibility of the host plant, (b) specificity of virus, (c) efficiency of its vector under particular circumstances (d) state of the host plant and (e) the environment. Of the environmental factors, temperature seems to be the most important. Research has shown that temperature could greatly affect (1) the susceptibility of the test plant to infection (Kassanis 1952b, Sinha 1960, Welton et al. 1964), (2) the virus itself (Bawden 1964), and (3) the behavior of the insect vector (Volk 1961, Gemignani 1957, Sylvester 1964, Simons 1966, McLean and Kinsey 1967). Combinations of the above 3 factors also have been shown to affect virus transmission (Kassanis 1957, Volk 1961, Swenson and Sohi 1961).

Differences in the plant's susceptibility to the virus inoculation by the insect vector are not only due to the age of the test plant (Maramorosch 1950, Swenson et al. 1964) and different ages of leaves on the same plant (Watson 1936, Swenson 1968) but also due to different sites of inoculation (Swenson 1962, Sylvester 1953, Nault and Gyrisco 1966). Both of these cases hold true in the transmission of PEMV by the pea aphid as evidenced by the results presented in Table 1. Possible reasons for the differences in transmission efficiency related to the inoculation process may be (1) that different ages of the plant

have different nitrogen : carbon ratios (Bawden 1964). Higher nitrogen content may increase opportunities for virus synthesis, and thereby increase the chances of infections developing in a particular tissue; (2) that various tissue regions differ in their susceptibility to virus infection (Wildman 1959) and consequently the site of inoculation could influence the success of transmission; (3) that various regions of the plant possess different levels of phyto-inhibitors to virus infection. However in most cases, phyto-inhibitors were only recorded from the whole rather than from a specific region of the plant (Bawden 1964, Allard 1918, Kassanis and Kleczkowski 1948, Van der Want 1951); and (4) that plasmodesmata provide infection counts for virus transfer to the cytoplasm--presumed necessary for infection (Esau 1948, Mundry 1963). Those different tissues may differ in the number of plasmodesmata, thereby exposing different numbers of infection avenues. However, none of the foregoing explanations has been proved. A study of the biochemical components of various tissue regions and the structure of inter- and intra-cellular areas in those regions needs to be made before the nature of PEMV infection in the various host tissues can be fully explained.

Temperature is one of the most sensitive parameters in the environment commonly studied in biological processes; it often has been used as a controlled variable in work with aphid transmission of non-persistent viruses (Sylvester 1964, Simons 1966, Welton et al. 1964) and insect transmission of circulative and propagative (persistent) viruses (Maramorosch 1950, Sylvester 1964, Duffus 1963, Sylvester and Richardson 1966). Low temperature induces longer incubation (latent) periods in the vector than does high temperatures. This is true of

many circulative viruses in their insect-vectors (Osborn 1935, Duffus 1963, Sylvester 1964, Heinze 1959, Sylvester and Richardson 1966). In my work, the LP_{50} at 10°C was twice as long as that at 20°C, but that at 20°C was not double that at 30°C (Table 2). These results agree with those of Sylvester (1964) and Sylvester and Richardson (1966). I believe the most likely explanation for the variation in latency is that higher temperature stimulates higher metabolic rates in the insect-vector, consequently the speed that virus particles travel from the gut through the body of the insect to the salivary gland is also higher. However, the temperature studies revealed the following practical implications: (1) the LP_{50} varies with the temperature of the environment at which the insects are kept after the acquisition-access period--an explanation for the inter-experimental and inter-laboratory variations as the post-acquisition aspects of tests are often done in uncontrolled laboratory or greenhouse conditions; (2) in nature a mean difference of about 5-10°C may sometimes account for a severe or mild outbreak of virus diseases; (3) by raising or lowering the ambient temperature under controlled conditions, the latent period can be varied in order to suit the needs of any particular study.

A possible explanation for the low transmission efficiency that resulted from inoculation probes by pea aphids that were kept at 30:30°C (Table 2) could be that PEMV was nearly depleted in the salivary glands by the time of the inoculation probes. Since PEMV apparently does not multiply in the vector, high temperature would presumably result in both short latent and retention periods, as a high metabolic rate of the vectors at high temperature (30°C) should exhaust more virus particles within a certain length of time. Sylvester and Richardson

(1966) recorded that the weighted mean period of retention of inoculativity was only 4.3 days at 30°C. Though the insects used in the 5 tests that I conducted at 30:30°C (Table 2) showed that retention was longer than 4.3 days, but the inoculativity tended to decline in all 30:30°C groups. At high temperature (30°C), the titre of virus in the vector's salivary gland was probably relatively low at the time of inoculation tests (after 5 days). Two minutes of inoculation probing was probably too short to permit the secretion of an adequate amount of virus to cause infection whereas the check plants (a qualitative check on insect's infectivity) were inoculated for 2-4 hr and showed the expected increase in transmission (Table 2). That is, if the saliva is low in virus titre, short inoculation probes would result in low transmission efficiency in comparison with long probes. A long probe does not discern between low and high virus titres in saliva.

In aphid-vector studies with both stylet-borne and circulative viruses increased attention is being given to feeding behavior as it effects the probability of transmission (Mittler and Dadd 1963, MacKinnon 1963b, Sylvester 1949, 1964, Sylvester and Richardson 1963, McLean and Kinsey 1967, Simons 1966, Nault and Gyrisco 1966). Pea aphids often make one or more short probes before making a probe to the phloem elements (Mittler and Dadd 1963, Nault and Gyrisco 1966). Moreover, this phenomenon is most pronounced at high temperature (Gemignani 1957, Sylvester 1964, Simons 1966, McLean and Kinsey 1967). High temperature results in a decrease in length of probing time and in an increase in the number of short probes, which agrees with the experimental results in this study (Fig. 1). It is clear that aphid behavior is affected by temperature, and it is an important contributor to

mechanisms involved in the determination of vector efficiency. Theoretically, more probes would yield more salivary secretion and more salivary sheaths than would one long phloem-seeking probe. However, it is still difficult to explain the inter-laboratory descrepancies in vector efficiency, solely on the basis of behavioral hypotheses.

Length of latency has been shown to vary with aphid species, strains, stages, source plants, virus isolates and temperature (Chapman and Bath 1967, Osborn 1935, Ehrhardt and Schmutterer 1965, Sylvester 1965, Sylvester and Richardson 1965). It has been postulated that the amount of virus taken into the vector's body can directly influence the length of latent period, this leads to suggest that the latent period is dosage sensitive in persistent aphid-borne viruses (Day 1955, Duffus 1963, MacKinnon 1964, Sylvester 1965). In other words, there is an association between the efficiency of the vector and the length of the latent period (Sylvester and Richardson 1966). The experimental results obtained in this study (Table 4) clearly suggest that the latent period is not dosage sensitive and the efficiency of virus transmission is not associated with the length of the latent period. The possible hypothesis for supporting the experimental evidences could be that this highly efficient strain of the pea aphid vector has a very low acquisition threshold. Bath and Chapman (1966, 1967) established 5 minutes and 1 hour for first instar nymphs and adult A. pisum respectively. McLean and Kinsey (1964, 1965 and 1967) recorded that A. pisum could ingest fluids from subepidermis and mesophyll parenchyma of Vicia faba L. The acquisition-access times (4, 8 and 24 hours) in this study are probably much longer than the

acquisition threshold. Hence the latent period becomes insensitive to the dosage in this study.

In studies concerned with the mechanism of transmission of plant viruses by aphids, there is a positive correlation between the length of the probing period and the length of the salivary sheath (Roberts 1940, Bradley 1956, Esau et al. 1961, McLean 1964, McLean and Kinsey 1967, Nault 1967). This holds true in the experimental results from this study (Table A-9). Nault and Gyrisco (1966) hypothesized that the stylet penetration rate was higher in inter-veinal areas than veinal areas. However, the data herein showed no association between the length of salivary sheath (length of probing time) and the transmission efficiency (Table 1). One possible explanation is that this highly efficient strain of pea aphid has a very low inoculation threshold. Nault et al. (1964), Bath and Chapman (1966, 1967), Nault and Gyrisco (1966) all proved that inoculation of PEMV to pea by the pea aphid could be accomplished in less than 1 min. Bath and Chapman (1966) showed that prolonged inoculation probing had little or no effect on the inoculation efficiency of pea aphid; with this I concur.

In histology trials at least 50% of the tissue sections failed to yield stylet sheath, regardless of the length of inoculation probing period. Skotland and Hagedorn (1955) and Nault and Bradley (1969) also obtained negative evidences, whereas Nault and Gyrisco (1966) observed 112 sheaths out of 156 probes but later found that all probes by the pea aphid were accompanied by secretion of sheath saliva on the surface of the pea leaf. While sectioning and staining processes may destroy or mask sheaths and lead to about a 50% recovery, it is also possible that sheaths are not made in every probe--even though sheath saliva

may be deposited on the surface. Proof of such a hypothesis would certainly alter current beliefs on aphid probing behavior.

The length of the various stages in the life cycle of pea aphid and the net reproduction rate per female vary significantly with the temperature. It is unanimously recorded that the optimum temperature for the pea aphid in terms of growth and productivity is near 20°C (Campbell 1926, Smith and Davis 1926, Dahms and Painter 1940, Isaak et al. 1963, Markkula 1963, Sylvester and Richardson 1966, Sylvester 1967, McLean and Kinsey 1967). Cartier (1957, 1959) and Harrington 1943, 1945) separated the biotypes of pea aphid on the basis of their reproductive rate and the size. The experimental results of this study showed that the same homogeneous population at different temperatures could be identified as different biotypes. Sylvester and Richardson (1966) recorded that the net reproductive rate and generation time of the infective aphids were similar to noninfective aphids. Thus the outbreak of this virus disease could become very serious in the 20°C zone.

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APPENDIX

DATA FROM THE SEVERAL EXPERIMENTS PRESENTED AND DISCUSSED IN THIS DISSERTATION

Table A-1.--Transmission of pea enation mosaic virus by inoculative pea aphid adults given 1, 5, and 10-min inoculation probing periods (IPP) at various sites on test plants in the pre-leaf and one-leaf stages.[a]

	One-leaf stage						
IPP (min)	Upper leaf	Upper petiole	Lower leaf	Stem	Terminal bud	Lower petiole	Pre-leaf stage
1	3/24	2/15	5/21	7/26	8/29	7/23	13/24
							12/25
	4/26	4/22	4/24	7/31	8/22	10/28	11/21
		5/31				8/20	10/21
Total	7/50	11/68	9/45	14/57	16/51	25/71	46/91
5	2/14	5/20	6/17	1/8	3/10	8/20	10/13
			4/13	9/22			
	3/15	3/21	3/12	4/13	9/17	10/22	17/21
			6/22				
	4/13		3/15	12/20	6/21		10/14
Total	9/42	8/41	22/79	26/63	18/48	18/42	37/48
10	2/21	11/27	6/18	7/21	4/16	12/26	17/20
			6/19		11/21		
	5/20	11/28	3/12	9/28	3/3	15/28	18/20
					10/30		
			3/8		0/4		
Total	7/41	22/55	18/57	16/49	28/74	27/54	35/40

[a]Numerator = number of infections; denominator = number of inoculative insects proved to be infectious.

Table A-2.--Determination of the latent period of pea enation mosaic by serial transfers of single pea aphids after a 24-hr acquisition-access period (AAP) at different temperatures--June 28, 1968.

Temp (°C)[a]	Class no.	Observations	Transmission[b] at the indicated times after initiation of a 24-hr AAP[c]			
			48	72	96	120
10:10						
	1	8	-	+	+	+
	2	3	-	+	-	+
	3	14	-	-	+	+
	4	5	-	-	-	-
10:20						
	1	19	+	+	+	+
	2	7	-	+	+	+
	3	1	-	-	+	+
	4	1	-	-	-	-
	5	2	-	-	D	D
20:20						
	1	26	+	+	+	+
	2	1	+	+	+	-
	3	1	+	+	+	D
	4	1	+	+	D	D
	5	1	-	+	+	+
30:30						
	1	26	+	+	+	+
	2	1	+	-	+	+
	3	2	-	+	+	+
	4	1	-	D	D	D
30:20						
	1	27	+	+	+	+
	2	1	+	+	D	D
	3	1	-	+	+	+
	4	1	-	+	+	-

[a]Temperature during AAP : Temperature during serial transfers.

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

[c]Inoculation probing periods on each of test plants in the series were 24, 24, 24 and 24-hr, respectively.

Table A-3.--Determination of the latent period of pea enation mosaic virus by serial transfers of single pea aphid after a 24-hr acquisition access period (AAP) at different temperatures--July 1, 1968.

Temp (°C)[a]	Class no.	Observations	Transmission[b] at the indicated times after initiation of a 24-hr AAP[c]				
			48	72	96	120	144
10:10							
	1	5	-	+	+	+	+
	2	7	-	-	+	+	+
	3	1	-	-	+	-	+
	4	14	-	-	-	+	+
	5	6	-	-	-	-	+
10:20							
	1	18	+	+	+	+	
	2	1	+	-	+	+	
	3	5	-	+	+	+	
	4	5	-	-	+	+	
	5	1	-	-	-	+	
	6	1	-	-	-	-	
20:20							
	1	32	+	+	+	+	
	2	8	-	+	+	+	
30:20							
	1	26	+	+	+	+	
	2	4	-	+	+	+	
30:30							
	1	22	+	+	+	+	
	2	6	+	+	+	-	
	3	2	-	+	+	-	

[a]Temperature during AAP : Temperature during serial transfers.

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

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

Table A-4.--Determination of the latent period of pea enation mosaic virus by serial transfers of single pea aphid after a 24-hr acquisition access period (AAP) at different temperatures--July 28, 1968.

Temp (°C)[a]	Class no.	Observations	Transmission[b] at the indicated times after initiation of a 24-hr AAP[c]					
			48	72	96	120	144	168
10:10	1	1						
	1	1	-	-	+	+	+	+
	2	1	-	-	+	-	+	+
	3	7	-	-	-	+	+	+
	4	20	-	-	-	-	+	+
	5	4	-	-	-	-	-	+
	6	3	-	-	-	-	-	-
10:20								
	1	6	+	+	+	+	+	
	2	31	-	+	+	+	+	
	3	1	-	+	+	-	+	
	4	3	-	-	+	+	+	
	5	3	-	-	-	+	+	
	6	2	-	-	-	-	-	
20:20								
	1	21	+	+	+	+	+	
	2	18	-	+	+	+	+	
	3	1	-	-	+	+	+	
30:20								
	1	32	+	+	+	+	+	
	2	1	+	+	-	+	+	
	3	1	+	+	D	D	D	
	4	2	-	+	+	+	+	
30:30								
	1	22	+	+	+	+	+	
	2	3	+	+	+	+	-	
	3	2	+	+	+	-	-	
	4	4	+	+	+	-	+	
	5	1	+	-	+	-	+	
	6	1	+	+	+	D	D	
	7	1	+	D	D	D	D	
	8	1	-	+	+	+	+	
	9	1	-	-	+	-	-	

[a]Temperature during AAP : Temperature during serial transfers.

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

[c]Inoculation feeding periods on test plants in the series were 24, 24, 24, 24, 24, and 24 hr respectively.

Table A-5.--TRansmission of pea enation mosaic virus by inoculative pea aphid adults given a 1-min inoculation probing period (IPP) at 3 temperatures at various sites on test plants in the pre-leaf and one-leaf stages.[a]

Temp during IPP (°C)		One-leaf stage						Pre-leaf stage
		Upper leaf	Lower leaf	Upper petiole	Lower petiole	Stem	Terminal bud	
10		3/15	4/23	6/25	7/26	6/26	4/24	8/21
	%	20.0	17.4	24.0	26.9	23.1	16.7	38.1
20		4/23	5/24	7/26	8/29	6/22	9/26	14/31
	%	17.4	20.8	26.9	27.6	27.3	34.6	60.9
30		6/24	6/22	7/27	6/27	8/30	6/23	11/25
	%	25.0	27.3	25.9	22.2	26.7	26.1	44.0

[a]Numerator = number of infections; denominator = number of inoculative insects proved to be infectious.

Table A-6.--Determination of latent period of pea enation mosaic virus by serial transfers of single pea aphids after various acquisition access periods (AAP)--Aug. 12, 1968.

AAP (hr)	Class no.	Observations	Transmission[a] at the indicated times after initiation of the AAP[b]					
			7	24	48	72	96	120
4	1	12	-	+	+	+	+	+
	2	1	-	+	+	+	+	-
	3	15	-	-	+	+	+	+
	4	1	-	-	+	-	+	+
	5	5	-	-	-	+	+	+
	6	1	-	-	-	+	+	-
	7	2	-	-	-	+	-	-
	8	1	-	-	-	+	D	D
	9	1	-	-	-	-	+	+
	10	4	-	-	-	-	+	-
	11	1	-	-	-	-	-	+
	12	8	-	-	-	-	-	-
	13	1	-	-	-	-	D	D
8	1	8		+	+	+	+	+
	2	31		-	+	+	+	+
	3	1		-	+	+	-	+
	4	11		-	-	+	+	+
	5	2		-	-	-	-	-
24	1	49			+	+	+	+
	2	3			-	+	+	+
	3	1			-	-	-	-

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

[b]Inoculation feeding periods on each of test plants in the series were 3, 17, 24, 24, 24 and 24-hr, respectively.

Table A-7. Determination of latent period of pea enation mosaic virus by serial transfers of single pea aphids after various acquisistion access periods (AAP)--Aug. 26, 1968.

AAP (hr)	Class no.	Observations	Transmission[a] at the indicated times after initiation of the AAP[b]					
			7	24	48	72	96	120
4	1	1	+	+	+	+	+	
	2	14	-	+	+	+	+	
	3	1	-	+	-	+	+	
	4	13	-	-	+	+	+	
	5	6	-	-	-	+	+	
	6	7	-	-	-	-	+	
	7	10	-	-	-	-	-	
	8	1	-	D	D	D	D	
8	1	13		+	+	+	+	+
	2	16		-	+	+	+	+
	3	1		-	+	-	-	+
	4	14		-	-	+	+	+
	5	2		-	-	+	-	+
	6	1		-	-	+	D	D
	7	2		-	-	-	-	+
	8	3		-	-	-	-	-
	9	1		-	-	-	D	D
24	1	29			+	+	+	+
	2	1			+	-	+	+
	3	1			+	D	D	D
	4	8			-	+	+	+
	5	3			-	+	-	+
	6	1			-	+	-	D
	7	1			-	-	+	+
	8	8			-	-	-	-
	9	1			-	-	-	D

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

[b]Inoculation feeding periods on each of test plants in the series were 3, 17, 24, 24, 24 and 24 hr, respectively.

Table A-8. Determination of latent period of pea enation mosaic virus by serial transfers of single pea aphids after various acquisition access periods (AAP)--Sept. 6, 1968.

AAP (hr)	Class no.	Observations	Transmission[a] at the indicated times after initiation of the AAP[b]					
			7	24	48	72	96	120
4	1	17	-	+	+	+	+	+
	2	13	-	-	+	+	+	+
	3	2	-	-	+	+	+	-
	4	1	-	-	+	+	+	D
	5	8	-	-	-	+	+	+
	6	2	-	-	-	+	+	D
	7	1	-	-	-	-	+	+
	8	1	-	-	-	-	+	-
	9	4	-	-	-	-	-	-
	10	1	-	-	-	-	-	D
8	1	12		+	+	+	+	+
	2	2		+	+	+	+	-
	3	19		-	+	+	+	+
	4	2		-	+	-	+	+
	5	11		-	-	+	+	+
	6	1		-	-	+	+	-
	7	2		-	-	-	-	-
	8	1		-	-	-	D	D
24	1	40			+	+	+	+
	2	8			-	+	+	+
	3	2			-	-	-	-

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

[b]Inoculation feeding periods on each of test plants in the series were 3, 17, 24, 24, 24, 24 and 24 hr, respectively.

Table A-9.--Length of salivary sheath deposited in different parts of the pea plant by pea aphid during a 1, 5, and 10-min inoculation probing period.[a]

	10-min					5-min					1-min				
	A	B	C	N	n	A	B	C	N	n	A	B	C	N	n
Upper petiole	177.6±79.3	155.6±57.1	177.3±72.9	8	6	128.6±25.1	105.8±9.7	231.8±80.6	5	5	32.4±13.8	32.0±13.8	169.0±58.4	5	16
Lower petiole	169.8±55.0	151.6±43.0	157.0±30.1	5	5	99.0±12.5	94.0±9.6	203.5±32.5	4	5	25.8±13.2	25.8±13.2	270.0±85.3	9	12
Stem	121.9±42.8	100.3±18.6	391.6±153.6	7	2	110.0±53.7	90.0±38.2	295.0±120.2	2	1	37.0±16.5	36.0±15.1	595.0±189.9	3	8
Pre-leaf	172.8±71.1	152.8±57.5	581.3±196.9	8	9	78.6±41.8	75.2±39.7	429.0±109.8	5	17	35.5±7.6	34.3±6.7	787.5±234.3	4	6
Terminal bud	76.2±53.5	63.3±18.6	79.2±23.4	6	5	50.0±40.5	50.0±40.5	79.4±15.1	5	9	19.5±6.4	19.5±6.4	75.0±7.1	2	5
Lower leaf	128.1±50.3	120.9±45.0	128.0±69.7	9	8	79.0±25.0	70.4±23.5	119.6±38.9	5	3	30.3±9.0	30.3±9.0	137.5±31.4	6	13
Upper leaf	107.7±63.1	86.1±46.2	113.6±40.5	7	14	77.7±20.4	76.3±21.5	123.3±20.8	3	3	26.0±13.0	25.8±13.0	103.3±29.9	6	15

[a]Measurements were made at 450 magnification; 90 units = 1 mm; figures on right column of A, B, C are standard diviations. A = mean total length of the curved salivary sheath; B = mean shortest distance between the point of entry and the end of sheath; C = mean distance between the point of entry and the target phloem tissue; N = number of sheath found; n = number of slide not found with sheath.

Table A-10.--Influence of temperature on the behaviour of pea aphids prior to their completion of a 1-min test probe.

Temp (°C)	Replicate	No. of insects tested	No. of insects that made specified no. of short probes[a]			
			0	1	2	3
10	1	15	13	2	0	0
	2	15	12	3	0	0
20	1	25	15	8	2	0
	2	25	16	7	2	0
30	1	20	5	6	6	3
	2	20	5	4	6	5

[a]Probes shorter than 1 min.

Table A-11.--Influence of temperature on the behaviour of pea aphids prior to their completion of a 5-min test probe.

Temp (°C)	Replicate	No. of insects tested	No. of insects that made specified no. of short probes[a]					
			0	1	2	3	4	5
10	1	20	10	9	1	0	0	0
	2	20	10	8	2	0	0	0
20	1	20	6	8	4	2	0	0
	2	25	9	11	4	1	0	0
30	1	23	5	5	5	2	3	0
	2	22	2	6	7	5	1	0

[a]Probes shorter than 5 min.

Table A-12.--Influence of temperature on the behaviour of pea aphids prior to their completion of a 10-min test probe.

Temp (°C)	Replicate	No. of insects tested	No. of insects that made specified no. of short probes[a]							
			0	1	2	3	4	5	6	7
10	1	15	10	2	3	0	0	0	0	0
	2	15	9	4	2	0	0	0	0	0
20	1	20	5	10	3	1	1	0	0	0
	2	20	8	6	3	2	1	0	0	0
30	1	20	3	4	5	1	1	3	2	1
	2	20	2	5	3	4	2	2	1	1

[a]Probes shorter than 10 min.