A PARTIAL CHARACTERIZATION OF *Acyrthosiphon pisum* (Harris) STRAINS EFFICIENT AND INEFFICIENT IN THE TRANSMISSION OF PEA ENATION MOSAIC VIRUS

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ABSTRACT

A PARTIAL CHARACTERIZATION OF Acyrthosiphon pisum (Harris) STRAINS EFFICIENT AND INEFFICIENT IN THE TRANSMISSION OF PEA ENATION MOSAIC VIRUS

by E. Chukwuemeka Igbokwe

Pea aphids, Acyrthosiphon pisum (Harris), collected from 18 sites in Michigan and Wisconsin were established separately on garden pea in the laboratory and tested to determine their efficiency of transmitting two isolates of pea enation mosaic virus (PEMV), a circulative (persistent) aphid-borne plant virus. The most efficient and inefficient aphid strains -- origin: East Lansing, Michigan (Clone EL) and Wisconsin Alumni Research Foundation (Clone WARF), respectively -- were selected as test insects for an initial characterization of the determinants of vector efficiency.

Both of the virus isolates (origin: California and New York) were most efficiently acquired by aphids of Clone EL. Following a 48-hour acquisition-access period, 34.6% of Clone EL adult aphids transmitted the California isolate whereas none of Clone WARF became infectious; the New York isolate was transmitted with 47.4 and 15.6% efficiency by adults of Clones EL and WARF, respectively. First-instar aphids of Clone EL transmitted both virus isolates with 100% efficiency after a 48-hour acquisition-access period, but Clone WARF was only 41.7 and 52.9% effective in the transmission of the California and New York isolates, respectively.

The effects of temperature on acquisition and inoculation were tested, but the results were inconclusive. In general, acquisition of the California isolate of PEMV was enhanced at 55 F, in contrast to 75 F, by both aphid strains; Clone WARF was an occasional vector at 55 F but never at 75 F when the aphid was reared on pea. Acquisition from the New York isolate was higher at 75 than at 55 F for Clone E1 whereas the converse occurred with the WARF clone. Temperature appeared not to affect the inoculation phase.

Acquisition also was affected by the plant species upon which the test aphids were reared. While Colone EL aphids reared on pea did not transmit California PEMV at 75 F, 11% reared on common vetch became vectors. In general, common vetch was conducive to the best transmission by both aphid strains; broad bean was intermediate and pea the poorest.

Efficiency, as established PEMV transmission, was reversed when the two aphid strains were tested with a stylet-borne virus, bean yellow mosaic virus. Fifty-seven to 60 sec probes by adult aphids on the upper surface of the pea leaf resulted in 13.7% transmission by Clone EL and 42.3% by Clone WARF -- lower surface: 12.8 and 31.7%, respectively; stipule: 7.3 and 17.4%, respectively; and stem: 4.0 and 15.6%, respectively.

Other characters of difference between the two aphid strains were:

(1) body size: Adults and third-instars of Clone EL were smaller than those of Clone WARF. No difference was detected in the sizes of the first-instars; the other stages were not measured.

(2) chromoreaction: When subjected to 95% ethyl alcohol individuals of Clone WARF changed color more readily than Clone EL.

(3) fecundity: The reproductive rate of Clone WARF was higher than that of Clone EL and was influenced by the host plant on which the test was conducted.

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INTRODUCTION

Inherent variability in the efficiency of insects to transmit plant viruses is known to occur within and among vector species. This is true of the pea aphid, Acyrthosiphon pisum (Harris), which has strains and biotypes that differ widely in their efficiency of transmitting the circulative pea enation mosaic virus and the stylet-borne bean yellow mosaic virus. So far, very little research has been conducted into the nature of the factors responsible for such efficiency or inefficiency.

It was in the hope of characterizing, as much as possible, the precise nature of such intraspecific variability in the transmission of a circulative aphid-borne plant virus that this research was initiated. Two isolates of pea enation mosaic virus were used as "tools" for the comparison of an inefficient and an efficient pea aphid-vector clone. Transmission, physical and physiological characteristics of each clone were compared.

Long-range objectives of the research program of which this thesis is an initial contribution are aimed at insect-virus control. Knowledge of the naturally-occurring vector inefficiencies may well be incorporatable into the development of commercially feasible control agents. Such agents may be entirely safe to man and insects as they would not have to be insecticidal. Agents would only have to inhibit the circulation or development of the virus in the insect's body.

LITERATURE REVIEW

Inherent intraspecific variability among insect vectors of plant viruses has been recognized for nearly 40 years but few reports of attempts to explain or characterize the variability have been published. This review surveys the literature in that area and in the areas of (1) aphid transmission of the stylet-borne and the circulative or propagative viruses, with emphasis on 2 viruses used in this research, bean yellow mosaic and pea enation mosaic viruses; (2) temperature effects on transmission efficiency; (3) aphid- and virus-host plant variability; and (4) biological inhibition of plant virus infection.

Intraspecific variability among insect vectors.--

Intraspecific variability is known to occur among aphids, leafhoppers and other sap-sucking insects that are vectors of plant viruses. This variability involves the occurrence of stages, forms, individuals, strains and biotypes that differ in their efficiency of transmitting these viruses.

All of the leafhopper examples of intraspecific variability are in the circulative or propagative group of plant viruses. Cicadulina mbila (Naude) is an effecient vector of the maize streak virus and individuals of this vector species have been observed to be incapable of transmitting the virus (Storey 1928); among individuals that transmitted, more males than females were vectors. Storey (1932) established that genetic differences were responsible for such a variability.

Individuals of the rice leafhopper, Nephottetix apicalis Motsch var. cinticeps Uhl., varied widely in their ability to transmit the rice-dwarf disease virus, and some individuals failed in all developmental stages to transmit (Fukushi 1934). Variability occurred among the sugarbeet leafhopper, Circulifer tenellus, and the clover leafhopper, Aceratagallia sanuinolenta, in the transmission of the curly top and the potato yellow dwarf viruses respectively, but neither species had individuals that could not transmit at all (Bennett and Wallace 1938; Black 1938). More individuals of the planthopper, Sogota orizicola Muir, failed to transmit the hoja blanca disease virus of rice and among vectors there were differences in transmission efficiency (Galvez et al 1960).

Intraspecific variability among aphid vectors has been detected with both circulative and stylet-borne plant viruses. A persistent yellows virus of spinach was transmitted differentially by clones of the green peach aphid, Myzus persicae (Sulzer), (Stubbs 1955a). In a more extensive study involving 100 strains of 6 species of aphid vectors, Bjorluig and Ossiannilsson (1958) established inherent strain differences for M. persicae, Aphis faba (Scopoli) and Myzus ascalonicus (Doncaster) in their efficiency of transmitting two persistent viruses, beet yellows mosaic and potato leafroll. Williams and Ross (1957) reported inherent clonal differences in the transmission of potato leafroll virus by M. persicae whereas Day (1955) reported the absence of such difference in the same vector-virus combination. Isolates of the persistent barley yellow dwarf virus were differentially transmitted by clones of the greenbug, Schizaphis graminum (Rondani), and morphological differences in the tip of aphid rostrum were thought to

be involved in differences in transmission efficiency of aphid clones (Rochow 1960a).

Three additional circulative plant viruses have been reported to be transmitted with varying efficiency by aphids. Frazier (1960) reported differential transmission of different strains of the strawberry vein-banding virus by clones of the strawberry aphid, Chaetosiphon fragaefolii (Cockerell) with one clone unable to transmit the virus and morphologically different from the other clones. Bath and Chapman (1966) reported significant differences in acquisition efficiency by strains of the pea aphid, Acyrthosiphon pisum (Harris) in the transmission of a California isolate of the circulative pea enation mosaic virus. Strains of the African black groundnut aphid, Aphis craccivora (Koch), were found to vary inherently in their efficiency of transmitting the groundnut rosette virus, with one strain unable to transmit the virus (Storey and Ryland 1955).

Among the stylet-borne plant viruses, Potato virus C was not transmitted by most individuals of M. persicae (Bawden and Kassanis 1947). Clones of the cotton aphid, Aphis gossypii (Glover), and the green peach aphid were found to vary considerably in their efficiency of transmitting the southern cucumber mosaic virus on pepper (Simons 1958, 1959). Sohi and Swenson (1964) isolated two biotypes of the pea aphid, Acyrthosiphon pisum (Harris), which differed not only in their efficiency of transmitting bean yellow mosaic virus on sweet pea, Larynthus odoratus L., but also in their body size and fecundity.

Vector efficiency in the transmission of several circulative viruses is known to vary among the developmental stages of various insect vector species. The larval stages of all species of thrips

known to be vectors of the spotted wilt virus of tomato can acquire and transmit the virus but adult stages cannot (Sakimura 1962). A similar case has been reported for the planthopper, Perkinsiella saccharicida Kirk, in the transmission of the Fiji disease virus of sugarcane (Mungomery and Bell 1933), and for the leafhopper, Deltocephalus striatus L., in the transmission of the winter wheat mosaic virus in Russia (Zazhurilo and Sitnikova 1941). Adults of the planthopper, Delphacodes pellucida Fabr., acquired and transmitted differentially the European wheat striate mosaic virus with greater difficulty than do the nymphal stages (Sinha 1960). No cause for the preceding cases have been established. Young nymphs of the aphid, Macrosiphum geranicola (Hille Ris Lambers) acquired and transmitted the circulative filaree red-leaf virus more efficiently than the adults (Anderson 1951); the same phenomenon has been reported for the pea aphid as vectors of pea enation mosaic virus (Simons 1954, Bath 1964, Ehrhardt and Schmutterer 1965). These latter cases have been attributed to the smaller size and higher metabolic rate of the nymphs as compared to the adult. Transmission efficiency as determined for the fourth-instar alatiform nymphs and apterous adults of the green peach aphid was the same in the transmission of the stylet-borne bean yellow mosaic virus (Swenson 1962).

In addition to vector variation in plant virus transmission, other types of intraspecific variation in aphids have been recorded. Biotypes and strains of the pea aphid have been recognized and characterized on the basis of inherent differences in size, weight, reproductive physiology and relative damage to both resistant and susceptible aphid host plants (Harrington 1943, 1945; Cartier 1959, 1963).

Differences have occurred in the degree and nature of damage to varieties of certain small grains among collections of the greenbug, S. graminum (Dahms 1948) and in the responses of strains of the green peach aphid to malathion (Shirck 1960).

Aphid and virus host plant variability in relation to virus transmission by insects.--

The species and variety of aphid--or virus--host plant are known to influence the efficiency of aphid transmission of plant viruses (Swenson 1963). The efficiency of the green peach aphid in transmitting the stylet-borne cucumber mosaic virus was reported to vary with the species of aphid-host plant (Hoggan 1931). A similar case also is true for clones of the green peach aphid and the cotton aphid in the transmission of a circulative yellows virus of spinach (Simons 1959) and the stylet-borne cucumber mosaic virus (Stubbs 1955a), respectively. Inoculation efficiency of the pea aphid was higher on bur clover, Melilotus hispida Gaertn., than on garden pea, Pisum sativum L., in the transmission of the circulative pea enation mosaic virus (PEMV) to these plants (Chaudhuri 1950; Bath and Chapman 1966). Various degrees of efficiency have been differently reported for the pea aphid in the transmission of PEMV on the same, and sometimes various, species of aphid--and PEMV--host plants (Chaudhuri 1950; Simons 1954; Ehrhardt and Schmutterer 1965; Bath and Chapman 1966). The stylet-borne potato virus Y was differentially transmitted from different varieties of potato used as virus source plants by the green peach aphid (Bawden and Kassanis 1946). Different varieties of dwarf horticultural bean varied in their susceptibility to bean yellow mosaic virus infection through inoculation by aphids (Adlerz 1959).

On the other hand, no differences in transmission efficiency occurred between clones of the green peach aphid reared on different species of host plant in the transmission of the apparently propagative potato leafroll virus (Day 1955; Stegwee and Ponsen 1958). Similarly no relationship was found to exist between aphid-host plant and variability in the transmission efficiency of strains of M. persicae, Aphis fabae and Myzus ascalonicus in the transmission of beet yellow mosaic and potato leafroll viruses (Bjorling and Ossiannilsson 1958).

Influence of temperature on virus transmission by insect vectors.--

Temperature influences virus transmission by insect vectors in several ways. The influence may be on (1) the susceptibility of the test plant to infection; (2) the virus itself; (3) the behavior of the insect vector at the time of virus transmission; or (4) on a combination of these (Kassanis 1957; Volk 1961; Swenson 1961). The circulative tomato spotted wilt virus which is transmissible by the onion thrips, Thrips tabaci (Lindeman), and the flower thrips, Frankliniella tritici (Fitch) was more pathogenic to french bean, Phaseolis vulgaris, and tobacco, Nicotiana glutinosa and Nicotiana tabacum, at a higher (20 C) than at a lower (15 C) temperature; at the higher temperature these plants were more susceptible to infection by this virus (Best 1936). Bean and pea plants were more susceptible to infection by bean yellow mosaic virus at a lower (18 C) than at a higher (27 C) temperature following inoculation of this virus to these plants by the green peach aphid (Swenson and Sohi 1961).

Changes in temperature at which aphids were reared and tested for transmission of a strain of potato virus Y influenced aphid behavior and transmission efficiency; transmission efficiency of three species of aphids, Macrosiphum solanifolii, Doralis frangulae and Aphis nasturti, reared at room temperature increased gradually as the transmission test temperature increased from 9 to 24 C but decreased at 28 C (Volk 1961). While the transmission efficiency of these three species of aphid vectors was not influenced when aphids were reared at a temperature of between 13 and 20 C, it was found to decrease when aphids were reared at 25 C; prolonged acquisition periods were unable to reverse the effect of low temperatures of aphid rearing and transmission tests (Volk 1961).

Sylvester and Richardson (1966) compared the efficiency of pea enation mosaic virus transmission by nymphs of the pea aphid at 10, 20 and 30 C and established that:

(a) the influence of temperature was more on virus acquisition within a range of 1.5 to 24 hr of acquisition-access time than on inoculation in a range of 15 to 240 min of inoculation-access time, by early instar nymphs, with the most reduction in virus acquisition occurring at 10 C for acquisition-access time in excess of 3 hr,

(b) the median latent period (LP_{50}) decreased with increasing temperature, being 70 hr at 10 C, 25 hr at 20 C and 14 hr at 30 C,

(c) the period of retention of inoculativity, the same in adult apterae as in alatae, decreased with increasing temperature, being 29.5 days at 10 C, 13.7 days at 20 C and 4.3 days at 30 C.

The transmission characteristics of pea enation mosaic virus and bean yellow mosaic virus.--

Pea enation mosaic virus (PEMV) causes a systemic disease of several leguminous plants (Osborn 1938; McEwen and Schroeder 1956; McEwen et al 1965), and the virus can induce local lesions in certain hosts (Bozart and Chow 1965; Hagedorn, Layne and Ruppel 1964). PEMV-susceptible host plants have been reported among alfalfa, clover, bean, vetch, medics and peas (McEwen and Schroeder 1957; Ainsworth 1940; Chaudhuri 1950; Hagedorn and Walker 1954). Five strains of PEMV were isolated and characterized and found to differ in physical properties, host symptomatology and reaction of resistant and susceptible hosts to these isolates (Ruppel and Hagedorn 1963). This virus is sap transmissible but no evidence indicates seed transmission (Smith 1957).

PEMV virus is circulatively transmitted by several aphid vectors, namely the pea aphid, the green peach aphid, the potato aphid, Macrosiphum euphorbiae (Thomas) and the ornate aphid, Macrosiphum ornatus (Laing) (Chaudhuri 1950; Simons 1954). In the transmission of a New York isolate of PEMV on pea by the pea aphid, the minimum acquisition threshold period was found to be 2 hours, the inoculation threshold period 5 minutes or less, with a total transmission threshold period of 24 hours (Nault et al 1964). The acquisition threshold periods for a California isolate of this virus were found to vary with different aphid vector species, with a minimum threshold of 0.5 to 1 hour; an inoculation threshold of 1 minute or less was recorded for the pea and potato aphids in the transmission of both California and New York isolates (Bath and Chapman 1966). Simons (1954) recorded an

acquisition threshold period of between 1 and 2 hours and an inoculation threshold of at least 20 minutes.

Pea enation mosaic virus must undergo a latent period in its vectors before it can be inoculated to plants; this period has been determined by several workers. Osborn (1935) recorded a latency of 24 to 48 hours in pea aphid adults whereas the same period has been later measured at 6 to 20 hours (Chandhuri 1950), 25 to 29 hours (Simons 1954), less than 24 hours (McEwen et al 1957) less than 23 hours (Bath 1964), and 24 hours to 10 days (Ehrhardt and Schmutterer 1965). A latent period as short as 14 to 18 hours was found by Bath (1964) in M. persicae. First-instar pea aphid nymphs also have shorter latent periods than adults (Simons 1954; Bath 1964). McEwen et al (1957) and Simons (1954) agreed that for all circulative and propagative viruses no additional latent period was necessary before transmission could take place provided acquisition was prolonged sufficiently. Osborn (1935) recorded a 29 day period of retention of inoculativity by the pea aphid.

Although plants infected with PEMV required 7 to 9 days to develop disease symptoms, the virus became available to feeding aphids 3 to 5 days following initial infection of the plant (McEwen et al 1957). PEMV can be transmitted transtadially, that is it survives aphid moulting, and can be retrieved from the hemolymph of viruliferous vectors (Nault et al 1964). Nymphal pea aphid vectors may cease to transmit PEMV after their last ecdysis and between 20 to 30 C the last ecdysis may influence the retention of inoculativity by individuals that sustain transmission ability beyond the nymphal stage (Ehrhardt and Schmutterer 1965).

Simons (1954) suggested that viruliferous aphids had to reach the phloem, as is the case in virus acquisition, in order to infect a plant. Recent evidence of successful PEMV inoculation to a plant by pea aphids in 1 minute or less (Bath 1964; Nault et al 1964; and Ehrhardt and Schmutterer 1964), the knowledge that at least 5 minutes was required by a probing aphid to reach the phloem (Skotland and Hagedorn 1955) and the mechanical transmissibility of this virus have indicated that superficial probing by the aphid vector was enough to effect infection. Nault and Gyrisco (1966) have shown that PEMV can be inoculated to veinal and intervenal parenchyma and to the epidermis by pea aphids.

Like pea enation mosaic virus, bean yellow mosaic is a systemic virus disease of several legunimous plants and susceptible BYMV host plants have been found among clover, medics, pea, bean, vetch, lupines and gladiolus (McWhorter 1947; Hagedorn and Walker 1950; Hutton and Peak 1954; Goodchild 1956). Strains of BYMV differ in physical properties, symptomatology and virulence (Goodchild 1956). BYMV is stylet-borne and is transmitted by several aphid vectors varying in transmission efficiency. Species of aphids so far recorded as BYMV vectors are Macrosiphum euphorbiae (Thomas), Acyrthosiphon pisum (Harris), Myzus persicae (Sulzer), Aphis faba (Scop.), Therioaphis riehmi, Brevicoryne hedichyrsi and Rhopalosiphum fitchii (Rondani) (Swenson 1957; Sohi and Swenson 1964; Orlob and Arny 1960).

Several factors influence BYMV transmission by aphids. As recorded for the pea aphid these factors include variability within and among species of plants used as virus sources; the nutritional level which tends to alter plant susceptibility to infection through

aphid inoculation; the age and condition of aphid host plant as aphids reared on young healthy hosts transmit the virus with greater efficiency than those reared on old, unthrifty hosts; the concentration of virus in the virus source plant as determined by quantitative assay on BYMV local lesion host; the transmission test temperature which may influence aphid behavior, transmission efficiency and plant susceptibility to infection; and the method by which virus is maintained in the virus host plant (Corbett 1957; Adlerz 1959; Swenson and Sohi 1961; Swenson 1963). The efficiency of BYMV transmission by aphids has been found to decrease when the virus is consistently maintained in the virus host plant by mechanical inoculation as compared to its maintenance through insect transmission (Swenson 1963).

Swenson (1962) found that a starving green peach aphid could retain BYMV for at least 4 hours following acquisition feeding, and that transmission increased with increasing total probe period. He also observed that the efficiency of transmission decreased with increasing period of acquisition beyond a certain range of acquisition periods. Efficiency of BYMV transmission by the green peach aphid was constant and best within an acquisition period of 11 to 45 seconds but an acquisition period of 51 to 60 seconds reduced efficiency remarkably. Also, he established that at least 5 minutes of preacquisition starving of insects was sufficient to effect a high transmission efficiency; maximum efficiency was observed with a 10 to 15 minute preacquisition starving period.

Biological inhibition of plant virus infection.--

Various extracts from biological systems are known to inhibit

phytoviral infection when simultaneously inoculated with the plant virus to a susceptible virus host plant. Bawden (1954) described such extracts as "inhibitors of infection" to contrast them from another class of inhibitors, "the inhibitors of virus increase," which when applied on already infected plants, will decrease the rate of multiplication of viruses in such plants.

From a wide variety of plants, inhibitors (phytoinhibitors) of virus infection have been extracted, generally from all parts of a plant, and in most cases, have been identified (Bawden 1954). Among higher plants, pokeweed juice is known to contain a glycoprotein inhibitor which inhibits infection of healthy susceptible tobacco plants, but not of pokeweed itself, by cucumber mosaic virus (Allard 1918; Kassanis and Kleczkowski 1948). Extracts of rosaceous plants yield tannins which inhibit tobacco mosaic virus infectivity (Bawden and Kleczkowski 1948) and which are thought to be responsible for the inhibitory action of juice from most higher plants (Van der Want 1951). In several cases, phytoinhibitors have been shown to be virus and host specific (Kuntz and Walker 1948) but ineffective against the plant species that contain them (Bawden 1954). Among lower plants, the growth products of several bacteria and fungi yield extracts, for the most part polysaccharides or nonproteinous substances, which inhibit the infectivity of plant viruses such as tobacco mosaic virus (Gupta and Price 1950; Bawden and Freeman 1952).

Among enzymes, globin, trypsin and trypsinogen, but not pepsin, have been reported to inhibit infectivity of tobacco mosaic virus (Bawden 1954). Upon establishing that a constant amount of trypsin always produced a constant decrease in number of lesions when added

to varying concentrations of TMV, Stanley (1934) concluded that the trypsinic inhibitive effect must be on the host plant rather than on the virus which, however, is capable of combining with trypsin (Kleczkowski 1944). With potato virus X (PvX), however, Bawden and Pirie (1936) concluded that rather than affecting host plant susceptibility directly, trypsin affects the virus instead, combining with it to form a noninfective complex capable of dissociation upon dilution. Although uneffective against TMV infectivity, pepsin has been reported inhibitive to PvX infectivity (Bawden and Pirie 1936). On both TMV and PvX infectivities, pancreatic ribonuclease exerts equal inhibitive effect (Loring 1942).

A general inhibitive effect on the infectivity of several viruses has been established for normal sera of several animals; e.g., the inhibition of TMV infectivity by fresh normal rabbit serum (Mulvania 1926; Bawden 1954). Whereas normal sera are characterized by a property of nonspecificity for the virus inhibited, antisera (immune sera) are inhibition-specific against their respective antigenous viruses or related strains (Chester 1934; Bawden 1954). Besides insect juices (Black 1939) and animal sera, other biological fluids of animal origin have been found to inhibit phytoviral infection; such fluids include milk (Chester 1934; Johnson 1941), egg white (Johnson 1941) and the blood of fish and earthworms (Smith 1941).

Although by no means conclusive, experimental evidence points to two possible mechanisms of inhibition of phytoviral infection. One possibility is that the inhibitory action exerts effect on the virus host plant rather than on the virus itself, and this has been held true for trypsin (Stanley 1934), normal serum (Chester 1934), insect juice

(Black 1939) and the nonproteinous fungal inhibitors of Trichothecium roseum, trichothecin and a polysaccharide (Gupta and Price 1952; Bawden and Freeman 1952). Stanley (1934) and Chester (1934) based their conclusion on the observation that a constant concentration of inhibitor always produced the same degree of inhibitory effect irrespective of the virus concentration. In explaining the nature of inhibitory effect on the host plant, Stanley (1934) postulated an inhibitory interference by the inhibitor with the physiology of the host plant cells such that virus multiplication is impeded. Stanley's view was supported by Slagle et al (1952) who hypothesized that several inhibitors kill injured cells otherwise used as entry points by viruses. As an alternative explanation for the inhibitory effect on inoculated host plant, Bawden (1954) speculated on a possible interference of inhibitors with specific receptor or infection centers of the host cells that otherwise would combine with the viruses to accomplish infection. Although Black (1939) subscribed to this view on the ground that concentrated insect extracts damaged inoculated plants conspicuously, he nevertheless considered the possibility of inhibitory effect on the virus itself as he observed that the degree of inhibition of virus infectivity by insect juices was less with diluted rather than concentrated virus preparation. Bawden and Freeman (1952) suggested that since concentrated trichothecin visibly damages inoculated tobacco and French bean leaves, the inhibitory action must be related to some interference through injury with host plant metabolism so stimulating host plant cells to unusual activity that ultimately renders the viruses in the cells uninfective.

The second possible mechanism is that the inhibitory effect is on the virus itself and this view, expressed by Bawden and Pirie (1936), Johnson (1941), Loring (1942), Takahashi (1946), Weitraub and Gilpatrick (1952), is based on the observation that many inhibitors such as the enzyme trypsin can interact and combine with viruses *in vitro*. Bawden (1954) explained that "in combining with the virus, the inhibitors block some groups on the virus particles and so prevent combination with some essential receptors (infection centers) in the host cell."

Insects in relation to inhibition of phytoviral infection.--

The homogenates of various insects are known to inhibit phytoviral infection strongly. This probably would account for the absence of infectivity in extracts from macerates of viruliferous insect vectors (Bawden 1954). An exception rather than the rule is the reported case of the infective power of juice from potato aphid, *Macrosiphum euphorbiae*, and *M. persicae*, which acquired "spinach blight" (cucumber mosaic virus) from an infected spinach plant (McClintock and Smith 1918).

While failing, like Allard (1918), to infect healthy cucumber through sap inoculation with juice from CMV infected pokeweed, Doolittle and Walker (1925) succeeded in transmitting the virus from diseased pokeweed plant to healthy cucumber plant by using the cucumber (cotton) aphid vector, *Aphis gossypii* Glover, and thus for the first time drew attention to the possibility that the action of substances present in plant juices and normally inhibiting phytoviral infection during sap transmission may be ineffective against insect transmission of such viruses. Whereas sap transmission of sugarbeet mosaic virus (Hoggan

1933) and CMV (Bhargava 1951) to healthy susceptible tobacco plants was impaired by inhibitory substances in the inoculum plant juice, aphid transmission with the green peach aphid, ornate aphid, M. ornatus (Laing), and potato aphid, was a success. The infectivity of the stylet-borne, henbane mosaic virus (Hyoscyamus virus 3) has been reported to be inhibited by juice from its vector, the green peach aphid (Hamilton 1935). The infectivity of TMV is decreased to various degrees by extracts from (1) three leafhopper species (the sugarbeet leafhopper, Eutettix tenellus Baker, the aster leafhopper, Macrosteles fascifrans (Stal), and the clover leafhopper, Aceratogallia sanguinolenta Prov.); (2) four aphid species (bean aphid, Aphis ruminis L., the pea aphid, the potato aphid, and the green peach aphid; and (3) one mosquito species, Aedes aegypti L. (Black 1939). The extract from the clover leafhopper alone has been shown to decrease infectivity of six different viruses in vitro--potato yellow dwarf, TMV, potato virus X, turnip mosaic, tobacco necrosis and tobacco ringspot I (Black 1939). Although Black (1939) associated some proteinous substance with the inhibitory action of juice from the clover leafhopper, no concrete inhibitory substance from insect macerates has been identified. Juice from insect caterpillars inhibits the infectivity of tobacco necrosis virus and because the inhibitory power of such juice is decreased, but not totally destroyed, by boiling, Smith (1941) concluded that more than one inhibitor may be present in an insect juice.

The ability of insects to transmit phytoviruses appears unrelated to the presence of inhibitory substances within insect vectors. Bawden (1954) suggests that "either the viruses in infected (plant) cells occur separate from these substances (phytoinhibitors), the two

being brought together only when the (plant) tissues are macerated, or that the insect vectors can separate the viruses from the (plant) inhibitors. There is no reason to assume that, while being transmitted by insects, viruses ever come into contact with substances that occur in extracts of macerated insects, and (insect) inhibitors are as effective in preventing infection of viruses that the insects transmit as of those they do not transmit. . .and it is obvious that many proteins and polysaccharides. . .no doubt account for the action of many biological fluids." So far the inhibitors present and active in insect macerates have not been shown to be inhibitively operative in the live insect vectors.

MATERIALS AND GENERAL METHODS

The vector, test plant and virus.--

Pea aphids were collected from various sites and used to initiate test clones which were started with a single agamic adult and colonized on garden pea, Pisum sativum L., variety Perfected Wales. Determinations were made by Dr. Henry Giles. Aphids were reared in saran-screened cages (15 x 15 x 18 inches) constructed of wood and with a vertically-sliding glass entrance. All rearing was done in a 12 x 60 ft "rearing-room" in which the temperature was about 75 F, relative humidity 60% and photoperiod 12 hr.

Test plants were produced from pea seeds sown into vermiculite in plastic pans (11 x 7 1/2 x 2 1/2 inches). Following germination the young pea seedlings were transplanted into steamed soil (loam-peat-sand mixture) in small plastic pots (diam: 2 inches, height: 2 1/4 inches) and kept at 72-80 F in the greenhouse. Pea plants in the 2- or 3- leaf stage were used for all transmission tests.

The two test viruses, pea enation mosaic virus and bean yellow mosaic virus, were maintained in pea through frequent mechanical transfers. The former virus was obtained from the University of Wisconsin, Department of Plant Pathology whereas the latter was obtained from the Department of Botany and Plant Pathology, Michigan State University.

Acquisition tests.--

Insects were picked at random from their host plants, starved a specific time, and placed upon the terminal portion of virus-source plants that showed severe symptoms. Adults were exclusively agamic and apterous; first-instars were collected from adults 24 hr after the start of a larviposition period.

To insure that test insects fed on only the terminal portion of source plants, it 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 to cover the remaining portion of the hole and to expose only the desired tissues for virus acquisition. A glass lantern globe with a screened lid was placed over the plant tip and on the platform. Aphids that fell down from the plant were easily returned to the plant with a sable-hair brush or they returned on their own.

At the conclusion of a specific acquisition--access period the required number of aphids were gently prodded with a sable-hair brush (size 00) and transferred singly to young test seedlings for inoculation-access periods of usually 6 days. Aphids were confined to test plants by cylindrical cages (diam: 2 inches; length: 6 inches) constructed from buterate tubing; ventilation holes and the top were covered with a fine-mesh nylon. Inoculation-access periods were terminated by removing the cages and spraying all of the test plants with naled. Test plants were then placed in the greenhouse for the virus incubation period and subsequent symptoms. Final disease readings were usually made 30 days after the insects were removed; plants that died during the incubation period were discarded from records unless they had been noted as infected.

All insect phases of experimentation were conducted in environmental chambers (Sherer-Gillett Models CE 25-7 and CE 37-14). Environment was nearly constant at 75 F, 50 to 60% R.H. and 12-hr photoperiod.

EXPERIMENTS AND RESULTS

Isolation of pea aphid clones inefficient and efficient in the transmission of pea enation mosaic virus.--

Pea aphids were collected from alfalfa in five Michigan areas (Upper Peninsula, Cheyboygan, Alma, Holt and East Lansing) and used to initiate 18 clones; each clone was started with one apterous adult. An additional clone was obtained from the Wisconsin Alumni Research Foundation (WARF). All clones were colonized on pea.

Each clone was screened for efficiency of pea enation mosaic virus (PEMV) transmission to enable the selection of the best and poorest vector-clones. The "screen" experiment consisted of a 48-hr acquisition-access period on the California and New York PEMV isolates followed by a 6-day inoculation-access period; both phases were conducted at 75 F. All clones were not tested at the same time.

The New York isolate of PEMV was more efficiently transmitted by each of the 18 test clones than the California isolate (Table 1). A clone from East Lansing (EL) was the most efficient transmitter of both virus isolates (California: 31%; New York: 47%) whereas the clone from WARF was the poorest vector-clone (California: 0%; New York: 15%). The remaining 16 clones were intermediate in transmission efficiency. On the basis of these tests, Clones EL (East Lansing) and WARF were selected for further experimentation designed to characterize the nature of transmission efficiency and inefficiency.

Table 1.--Acquisition of the California and New York isolates of pea enation mosaic virus by 18 clones of the pea aphid.

Clone[b]	Transmission[a] after a 48-hr acquisition-access period: California PEMV		New York PEMV	
EL	14/45	(31.1)	23/49	(46.9)
WARF	0/45	(0.0)	7/48	(14.6)
Ht 1	3/17	(17.6)	5/15	(33.3)
Ht 2	5/28	(17.9)	8/30	(26.7)
Ht 3	1/19	(5.3)	4/14	(28.5)
Ht 4	6/46	(13.0)	9/42	(21.4)
Ch 1	2/14	(14.3)	3/15	(20.0)
Ch 2	2/19	(10.5)	3/15	(20.0)
Ch 3	3/20	(15.0)	5/25	(20.0)
Ch 4	3/28	(10.7)	5/30	(16.7)
Al 1	1/30	(3.3)	4/29	(13.8)
Al 2	1/29	(3.4)	3/26	(11.5)
Al 3	2/24	(8.3)	5/15	(33.3)
Al 4	4/16	(25.0)	6/22	(27.3)
Up 1	3/27	(11.1)	4/20	(20.0)
Up 2	2/27	(7.4)	6/29	(20.7)
Up 3	4/31	(12.9)	7/25	(28.0)
Up 4	3/31	(9.7)	5/27	(18.5)

[a]Numerator = no. of plants infected; denominator = no. of trials; no. in parenthesis = % transmission.

[b]EL = East Lansing; WARF = Wisconsin Alumni Research Foundation; Ht = Holt; Ch = Cheboygan; Al = Alma; Up = Upper Peninsula.

Acquisition of pea enation mosaic virus by pea aphid Clones EL and WARF.--

The adults of two pea aphid strains, Clones EL and WARF, selected from the preliminary acquisition trials were compared in three experiments within a 2-month period to thoroughly characterize their efficiency of acquiring the California and New York PEMV isolates. Each trial consisted of acquisition-access times of 12, 24 and 48 hr and an inoculation-access period that was terminated 6 days after the 48-hr test insects were transferred to their test plants; the actual inoculation-access period was therefore 7.5 days for the 12-hr acquisition trial and 7 days for the 24-hr trial. Acquisition and inoculation took place at 75 F. Test aphid strains were reared on garden pea.

Clone WARF failed to transmit the California PEMV isolate after any of the acquisition periods whereas clone EL was 12.8, 21.8 and 34.6% efficient after the 12, 24 and 48-hr periods, respectively (Table 2). Both clones transmitted the New York isolate; however, Clone EL was significantly more efficient than Clone WARF--16.4, 28.6 and 47.4% versus 7.7, 7.2 and 15.6% efficiency after 12, 24 and 48-hr periods, respectively. Thus, Clone EL acquired the New York PEMV isolate more efficiently than the California isolate, and it was a more efficient vector of either virus isolate than Clone WARF.

The poor or nontransmission of California PEMV after the 24-hr acquisition-access period in the preceding experiment prompted a test with a long access period. In this test, adult aphids of each clone were picked from 10-week old colonies reared on garden pea and given

96-hr acquisition-access periods at 55 and 75 F on both the New York and California isolates; a 6-day inoculation-access period at 75 F followed.

Table 2.--Acquisition of the California and New York isolates of pea enation mosaic virus by pea aphid adults of Clones EL and WARF.

Virus isolate and test no.	Transmission[a] after specified acquisition-access periods (hr)					
	Clone EL			Clone WARF		
	12	24	48	12	24	48
California PEMV						
1	2/18	5/19	8/14	0/10	0/19	0/19
2	10/88	9/46	16/46	0/19	0/11	0/12
3	5/43	8/36	12/44	0/9	0/9	0/9
Total	17/149	22/101	36/104	0/38	0/39	0/40
% Transmission	(11.4)	(21.8)	(34.6)	(0.0)	(0.0)	(0.0)
New York PEMV						
1	7/22	6/20	11/24	2/20	2/15	7/44
2	2/45	8/31	17/32	2/18	0/10	2/10
3	10/49	12/40	18/41	0/14	1/17	1/10
Total	19/116	26/91	46/97	4/52	3/42	10/64
% Transmission	(16.4)	(28.6)	(47.4)	(7.7)	(7.1)	(15.6)

[a]Numerator = no. of infections; denominator = no. of trials.

Clone WARF was able to transmit the California isolate after a 96-hr acquisition-access period, but efficiency (5.0 and 9.1% at 75 and 55 F, respectively) was low (Table 3). As expected, both isolates were most efficiently transmitted by Clone EL. Transmission efficiency was greatest in all trials when acquisition occurred at 55 F; only the difference with the New York isolate and Clone WARF is large and it probably is reflected by the use of only a few trials. This indication

of temperature effects on acquisition led to experiments that will be described later.

Table 3.--Acquisition of pea enation mosaic virus by adults of pea aphid Clones EL and WARF during a long acquisition-access period.

Virus isolate	Transmission[a] after a 96-hr acquisition-access period at indicated temperature			
	Clone EL		Clone WARF	
	55 F	75 F	55 F	75 F
California PEMV	10/18 (55.6)	12/28 (42.9)	2/22 (9.1)	1/20 (5.0)
New York PEMV	17/21 (81.0)	15/20 (75.0)	3/14 (21.4)	2/21 (9.5)

[a]Numerator = no. of infections; denominator = no. of trials; no. in parenthesis = % transmission.

First-instar virginiparae of Clones EL and WARF also were tested to determine if the interstrain difference in acquisition efficiency among adults also existed in the youngest aphid stage. Both clones were tested at acquisition and inoculation-access periods of 48-hr and 6 days, respectively; Clone EL was used in one experiment and Clone WARF in two.

Table 4 indicates that the interstrain differences in transmission efficiency that were demonstrated with adult pea aphids do exist among first-instars, but the difference is not great. Clone EL was totally efficient in the transmission of both virus isolates whereas the WARF Clone was 41.6 and 52.8% efficient with the California and New York isolates, respectively. Thus the nymph stage was more efficient than the adult (Table 3) in transmitting either PEMV isolate.

Table 4.--Acquisition of pea enation mosaic virus by first-instar pea aphids of Clones EL and WARF

Virus isolate and test no.	Transmission[a] after a 48-hr acquisition-access period			
	Clone EL		Clone WARF	
California PEMV				
1	---[b]		25/64	(39.1)
2	32/32	(100)	20/42	(47.6)
Total or mean	32/32	(100)	45/108	(41.7)
New York PEMV				
1	---[b]		22/40	(55.0)
2	38/38	(100)	24/47	(51.1)
Total or mean	38/38	(100)	46/87	(52.9)

[a]Numerator = no. of infections; denominator = no. of trials; no. in parenthesis = % transmission.

[b]Not tested.

A 48-hr acquisition-access period is usually exceedingly long for nymphal acquisition of PEMV (Bath and Chapman 1966). Generally near 100% transmission efficiency is expected with acquisition times of 12 to 24-hr; yet Clone WARF first-instars were still not more than 50% efficient after the 48-hr period on either virus isolate.

Acquisition of bean yellow mosaic virus by pea aphid Clones EL and WARF.--

The two pea aphid clones (EL and WARF) selected for efficiency of PEMV transmission were tested as vectors of a stylet-borne virus, bean yellow mosaic. At the same time, leaf (upper and lower surfaces), the stipule and stem of the virus source plant (garden pea) were tested as sites of virus acquisition by the aphid strains. Twenty aphids per clone were starved for 30 min, then one aphid at a time was transferred

onto one of the youngest pair of unfolded leaves of the virus source plant. Virus acquisition by the aphid was regarded as started from the time the tip of the aphid rostum touched the leaf surface; the acquisition probe was times in seconds. By the end of the 57th sec of an acquisition probe, the aphid was gently stroked on its abdomen with a wet sable hair brush to encourage it to terminate the probe. The aphid was discarded if it did not cease probing by the end of the 60th sec; thus, acquisition time was 57 to 60 sec in all cases. Test aphids were then transferred to a test plant and confined thereon with a cylindrical cage of the type used in the PEMV acquisition test. Groups of five aphids per clone were alternately tested until a total of 1 1/2 hr from the initiation of preacquisition starving had expired, then the remaining untested aphids were discarded, and another group of aphids were given the above treatment. The youngest pair of stipules and the young stem portion of the virus source plant were similarly tested as acquisition sites.

Aphids were allowed a 48-hr inoculation time at 75 F. Acquisition tests were replicated four times within a 2-month period. Temperature at the time of acquisition during all four acquisition tests ranged from 63 to 70 F.

Table 5 indicates a differential transmission of this virus by the test aphid strains and the site of virus acquisition as a factor influencing the efficiency of aphid strains to acquire and transmit the virus. Clone WARF, the less efficient strain in PEMV transmission, acquired and transmitted BYMV from all acquisition sites more efficiently than Clone EL, the more efficient PEMV vector strain. BYMV transmission by the two aphid strains was highest with acquisition from

onto one of the youngest pair of unfolded leaves of the virus source plant. Virus acquisition by the aphid was regarded as started from the time the tip of the aphid rostum touched the leaf surface; the acquisition probe was times in seconds. By the end of the 57th sec of an acquisition probe, the aphid was gently stroked on its abdomen with a wet sable hair brush to encourage it to terminate the probe. The aphid was discarded if it did not cease probing by the end of the 60th sec; thus, acquisition time was 57 to 60 sec in all cases. Test aphids were then transferred to a test plant and confined thereon with a cylindrical cage of the type used in the PEMV acquisition test. Groups of five aphids per clone were alternately tested until a total of 1 1/2 hr from the initiation of preacquisition starving had expired, then the remaining untested aphids were discarded, and another group of aphids were given the above treatment. The youngest pair of stipules and the young stem portion of the virus source plant were similarly tested as acquisition sites.

Aphids were allowed a 48-hr inoculation time at 75 F. Acquisition tests were replicated four times within a 2-month period. Temperature at the time of acquisition during all four acquisition tests ranged from 63 to 70 F.

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the leaf, more so from the upper than the lower leaf surface, less with acquisition from the stipules and least with acquisition from the stem.

Table 5.--Acquisition of bean yellow mosaic virus by agamic adult apterae of pea aphid Clones EL and WARF.

Aphid clone and test no.	Transmission[a] after a 57 to 60 sec probe on specified source-plant sites					
	Upper leaf	Lower leaf	Stipule	Stem	Total	
Clone EL						
1	3/56	1/47	1/39	0/27	5/169	(3.0)
2	4/24	5/24	2/23	1/24	12/95	(12.6)
3	3/20	5/23	1/24	1/24	10/91	(11.0)
4	7/24	4/23	4/24	2/24	17/95	(17.9)
Total or mean	17/124 (13.7)	15/117 (12.8)	8/110 (7.3)	4/99 (4.0)	44/450	(9.7)
Clone WARF						
1	12/51	7/49	5/41	2/24	26/165	(15.8)
2	14/23	12/24	7/24	4/25	37/96	(38.5)
3	11/24	9/23	4/24	5/24	29/95	(30.5)
4	15/25	10/24	3/20	4/23	32/92	(34.8)
Total or mean	52/123 (42.3)	38/120 (31.7)	19/109 (17.4)	15/96 (15.6)	124/448	(27.0)

[a]Numerator = no. of plants infected; denominator = no. of trials; no. in parenthesis = % transmission.

Influence of host plant and temperature on acquisition of pea enation mosaic virus by pea aphid Clones EL and WARF.--

Experiments were designed to indicate the influence of the aphid's host plant on subsequent virus transmission by that insect. Changes in diet could alter the physiology and biochemistry of the aphid and could result in transmission variations, as the circulative viruses are intimately related to the internal environment of the

vector. Likewise, it is possible that changes in temperature could affect the uptake of virus by insects and the survival of the virus in the insect body.

Both aphid clones (EL and WARF) were reared on three different host plant species--garden pea, Pisum sativum L.; common vetch, Vicia sativa L.; and broad bean, Vicia faba L.--and used in transmission trials 10 and 20 weeks after the colonies were initiated. Each test involved only those insects (of both clones) that were reared on one of the three host plant species and consisted of an acquisition-access period of 48-hr at 55 and 75 F on the California and New York isolates followed by a 6-day inoculation-access period at 55 and 75 F. Thus, the design was a 2 x 2 x 2 x 2 factorial for comparison of aphid clones, virus isolates, acquisition temperatures and inoculation temperatures.

Vector efficiency, regardless of temperature conditions and the virus isolate, was generally highest for aphids of both clones when common vetch was the aphid-host plant; garden pea reared aphids were the poorest vectors (Tables 6 and 7). While these data are not directly comparable due to differences in date of test, they do indicate definite areas for follow-up research.

The effects of temperature on transmission in general are more pronounced during virus acquisition than inoculation (Tables 6 and 7). Both aphid clones acquired California PEMV more efficiently at 55 than at 75 F, irrespective of aphid host. The garden pea colony of Clone WARF, as expected, failed to transmit the California isolate at 75 F, but it was barely capable at 55 F. Inoculation temperatures failed to

Table 6.--Effect of temperature on acquisition and inoculation phases of PEMV transmission by pea aphid Clones EL and WARF reared for 10 weeks on various host plant species.

Virus isolate and host plant	Temperature during 6-day IAP[a]	Transmission[b] after a 48-hr acquisition-access period at specified temperatures			
		Clone EL		Clone WARF	
		55 F	75 F	55 F	75 F
California					
Broad bean	55 F	7/15 (46.7)	4/13 (30.8)	2/19 (10.5)	1/27 (3.7)
	75 F	11/14 (78.6)	5/14 (35.7)	3/18 (16.7)	1/26 (3.8)
Common vetch	55 F	7/13 (53.8)	4/11 (36.4)	10/25 (40)	4/16 (25)
	75 F	5/13 (38.5)	4/11 (36.4)	3/12 (25)	7/20 (35)
Garden pea	55 F	4/11 (36.4)	3/11 (27.3)	1/12 (8.3)	0/12 (0)
	75 F	1/8 (12.5)	2/12 (16.7)	1/10 (10)	0/15 (0)
New York					
Broad bean	55 F	4/18 (22.2)	9/17 (52.9)	0/12 (0)	6/29 (20.7)
	75 F	6/17 (35.3)	11/18 (61.1)	0/12 (0)	7/27 (25.9)
Common vetch	55 F	2/8 (25)	7/13 (53.8)	11/23 (47.8)	7/16 (43.8)
	75 F	2/7 (28.6)	5/12 (41.7)	4/11 (36.4)	6/18 (33.3)
Garden pea	55 F	2/12 (16.7)	5/11 (45.5)	2/11 (18.2)	1/12 (8.3)
	75 F	3/11 (27.3)	5/12 (41.7)	3/12 (25)	2/14 (14.3)

[a]Inoculation-access period.

[b]Numerator = no. of infections; denominator = no. of trials; no. in parenthesis = % transmission.

Table 7.--Effect of temperature on acquisition and inoculation phases of PEMV transmission by pea aphid Clones EL and WARF reared for 20 weeks on various host plant species.

Virus isolate and host plant	Temperature during 6-day IAP[a]	Transmission[b] after a 48-hr acquisition-access period at specified temperatures			
		Clone EL		Clone WARF	
		55 F	75 F	55 F	75 F
California					
Broad bean	55 F	6/20 (30)	3/20 (15)	2/20 (10)	1/25 (4)
	75 F	10/20 (50)	3/23 (13)	2/24 (8.3)	0.25 (0)
Common vetch	55 F	8/20 (40)	5/25 (20)	5/24 (20.8)	3/25 (12)
	75 F	5/20 (25)	4/25 (16)	4/24 (16.7)	2/20 (10)
Garden pea	55 F	3/20 (15)	3/24 (12.5)	2/25 (8)	0/20 (0)
	75 F	2/20 (10)	3/25 (12)	1/25 (4)	0.25 (0)
New York					
Broad bean	55 F	5/20 (25)	7/16 (43.7)	3/14 (21.4)	3/19 (15.8)
	75 F	8/20 (40)	8/17 (47.1)	5/20 (25)	4/18 (22.2)
Common vetch	55 F	6/23 (26.1)	10/20 (50)	6/14 (42.9)	8/19 (42.1)
	75 F	5/16 (31.3)	6/18 (33.3)	9/20 (45)	5/18 (27.8)
Garden pea	55 F	3/20 (15)	8/20 (40)	2/18 (11.1)	1/15 (6.7)
	75 F	4/20 (20)	6/20 (30)	3/16 (18.7)	2/20 (10)

[a]Inoculation-access period.

[b]Numerator = no. of infections; denominator = no. of trials; no. in parenthesis = % transmission.

cause significant effects on transmission efficiency with this virus isolate.

The New York isolate was best acquired by Clone EL at 75 rather than at 55 F whereas Clone WARF was most effective vice-versa (Tables 6 and 7). Again no major influences of temperature on inoculation were detected.

These tests supported the evidence presented earlier that Clone EL was more efficient in acquisition than Clone WARF. Additionally, the New York isolate was transmitted with greater efficiency than the California isolate.

While the number of individuals tested per factorial level was low (generally 10 to 20) and host plant effects were not comparable, these tests indicated variables that influence vector-efficiency. Further studies of temperature effects on, perhaps, common vetch and broad bean, seem well warranted.

Inhibition of pea enation mosaic virus by homogenates of pea aphid Clones EL and WARF.--

Mixtures of homogenates of the aphid strains and PEMV isolates were mechanically inoculated to healthy garden pea plants which were subjected to a pre- and post-inoculation temperatures of 55 and 75 F. This was to determine the extent to which these homogenates inhibit PEMV infectivity and the influence of temperature treatments of the inoculated plants on the inhibitive property of the homogenates.

Inoculum sap of both PEMV isolates (California and New York) was prepared by grinding the terminal portions of young diseased plants that showed severe symptoms and expressing the inoculum sap through

cheesecloth. A 1:5 dilution of each virus isolate in distilled water was prepared.

The aphid homogenates were prepared by separating active adults of each clone (EL and WARF) into groups of 5, 10, 15, 30, 60 and 90 individuals, placing them in 6-dram vials and macerating them with a glass spatula. Five or six replicates were conducted per group.

One ml of each virus isolate was added to each insect-homogenate, mixed and inoculated to 20 pea seedlings. One half of the test plants were kept at 55 F for 2 days before and for 6 days after inoculation; the other half received a 75 F condition. All plants were exposed to a 12-hr photoperiod. Five to 10 plants were inoculated with a sap extract of each virus isolate, as a control on the homogenate treatments.

Pea enation mosaic virus infectivity was strongly inhibited by the homogenates of the two aphid clones (Table 8). At 75 F Clone EL inhibited the New York isolate less than the California isolate whereas the WARF Clone was totally inhibitory to both virus isolates. Both isolates were totally inhibited at 55 F by Clone EL, but Clone WARF inhibitor-factor of Clone EL is more active at 55 than 75 F and vice-versa for Clone WARF.

No attempt was made to determine whether inhibition was on the virus or the test plant and only differences between aphid clones were sought. Such differences would indicate biochemical variations between the clones. Further work needs to be conducted on this aspect with the use of a local lesion host and accurately measured homogenate-virus concentrations.

Table 8.--Inhibition of PEMV infection of pea plants held at different preinoculation and postinoculation temperatures by homogenates of pea aphid Clones EL and Warf.

Virus isolate and number of aphids homogenized	Transmission[a] from the mechanical inoculation of pea plants with mixtures of aphid homogenate and virus and the degree of inhibition of virus infection							
	Clone EL				Clone WARF			
	75 F		55 F		75 F		55 F	
California								
0[b]	5/5	(0)	5/5	(0)	5/5	(0)	5/5	(0)
5	1/10	(90)	0/10	(100)	1/10	(90)	1/10	(90)
10	0/10	(100)	0/10	(100)	0/10	(100)	0/10	(100)
15	0/10	(100)	0/10	(100)	0/10	(100)	0/10	(100)
30	0/10	(100)	-		0/10	(100)	-	
60	0/10	(100)	-		0/10	(100)	-	
90	0/10	(100)	-		0/10	(100)	-	
New York								
0[b]	5/5	(0)	5/5	(0)	5/5	(0)	5/5	(0)
5	1/9	(88.9)	0/10	(100)	0/10	(100)	0/10	(100)
10	1/10	(90)	0/10	(100)	0/10	(100)	0/10	(100)
15	0/10	(100)	0/10	(100)	1/10	(100)	0/10	(100)
30	0/10	(100)	-		0/10	(100)	-	
60	0/10	(100)	-		0/10	(100)	-	
90	0/10	(100)	-		0/10	(100)	-	

[a]Numerator = no. of plants infected; denominator = no. of plants inoculated; no. in parenthesis indicates % inhibition of virus infectivity.

[b]Fresh virus sap without aphid homogenate.

Characteristics of pea aphid Clones EL and WARF.--

1. Mean body size.

It was observed during transmission tests that adult apterae of Clone WARF nearly always appeared larger in size than those of Clone EL; thus, a number of the adult apterae, third- and first-instar virginiparae of these clones were measured to determine accurately their mean body

sizes. Twenty active adult apterae of each aphid strain were randomly picked from the broad bean aphid colony and killed directly in 80% ethyl alcohol. Measurement was begun 1 hr after aphids had been in alcohol. Groups of five aphids per clone were alternately measured, one aphid at a time; a total of 10 aphids per stage per clone were measured. The width of the mid-abdominal region and the length from the anterior to the posterior end were measured in millimeters. Similarly first- and third-instar virginiparae also were measured.

An interclonal difference in mean body size was evident for the adult apterae and the third-instar virginiparae; in both cases Clone WARF was larger (Table 9). There was no significant interclonal difference in the mean body sizes of the first-instar virginiparae of these aphid clones.

Table 9.--Body sizes of adult, third-instar and first-instar pea aphids of Clones EL and WARF.

	Mean size (mm) of 10 individuals per specified clone			
	Clone EL		Clone WARF	
Stage	Length	Width	Length	Width
Adult	3.51	1.33	4.12	1.52
Third-instar	1.93	0.75	2.10	0.81
First-instar	1.31	0.50	1.33	0.49

2. Chromoreaction to ethanol.

An observation that more adult apterae of Clone WARF than Clone EL lost their normal green color within unit time in 95% ethyl alcohol

necessitated further investigation. Aphids that lost their green color turned yellow on the abdomen while the head, thoracic and terminal abdominal regions turned dark. A number of adult apterae of both aphid clones were therefore tested in several concentrations of ethyl alcohol with the objective of determining accurately the differences in clonal color changes (chromoreaction).

Ten adult apterae of each aphid clone (reared on broad bean) were put into 4 dram shell vials with 5 ml of 95, 90, 85, 80 and 70% ethyl alcohol; the vials were then corked. The number of aphids which underwent a color change was noted 5 and 10 minutes after the initial observation of mild paling of the normal green color, which occurred 30 minutes after aphids were killed in the alcohol.

Table 10 indicates that both aphid strains responded with color change in 95% and 90% but not in lower alcohol concentrations. Within the units of time, interclonal difference in color response was significantly greater in 95 than in 90% alcohol.

Table 10.--Chromoreaction of Clones EL and WARF to ethyl alcohol used as a preservative.

% Ethyl alcohol concentration	No. of individuals out of 10 per specified clone to change color after indicated time lapse			
	Clone EL		Clone WARF	
	35 min	40 min	35 min	40 min
95	2	3	8	10
90	0	3	1	2
85	0	0	0	0
80	0	0	0	0
70	0	0	0	0

Within 35 min color change by both aphid strains was greater in 95% than in 90% alcohol with more individuals of Clone WARF responding. In 90% alcohol Clone EL showed no response and only one individual of Clone WARF responded. After 40 min all of the Clone WARF aphids changed color whereas only three of 10 Clone EL aphids changed color. Thus, the two test clones differed in their chronoreaction to alcohol.

3. Fecundity.

Two experiments were conducted to establish the fecundity of pea aphid Clones EL and WARF. In one experiment the two clones were reared on each of five host plant species and evaluated on the basis of the number of nymphs produced during a 24-hr period on each of the five plant species. In the other experiment, both clones were reared on broad bean and evaluated on each of four garden pea varieties.

Garden pea variety Perfected Wales, common vetch, alfalfa variety Vernal, broad bean and bur clover (Melilotus hispida Gaertn.) were used as host and test (larvipositional) plants in the initial experiment. Each colony was started with many first-instar nymphs; reproductive evaluations were made when the insects were 13 days old. Six adults from each colony were caged singly on young seedlings of each test species for a 24-hr larvipositional period. This experiment was conducted in the rearing room.

In every trial but two (the vetch-colonies tested on bur clover and the broad bean-colonies on vetch), the EL Clone produced more nymphs in 24 hr than Clone WARF (Table 11). Colonies reared on pea and broad bean were generally most prolific regardless of the plant species on which tested. It was interesting that the larviposition-plant had considerable affect on fecundity, even though the insects spent only

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1-day on them. Bur clover was the poorest larviposition-species for all colonies except those reared on bur clover; alfalfa was only slightly more suitable.

Table 11.--Reproduction by variously-reared Clones EL and WARF on several host plants.

Aphids reared on:	Clone[a]	Mean no. of nymphs produced by 13-day old adults during a 24-hr period on specified host plants				
		Pea	Common vetch	Alfalfa	Bur clover	Broad bean
Pea	EL	14.0	12.8	6.0	4.2	13.3
	WARF	11.4	10.3	4.5	3.0	12.6
Common vetch	EL	9.2	10.3	7.3	6.2	10.0
	WARF	5.0	9.4	5.6	6.5	9.1
Alfalfa	EL	7.0	8.5	7.1	5.7	7.4
	WARF	5.9	4.7	5.0	5.2	6.0
Bur clover	EL	6.1	4.8	5.5	9.5	10.7
	WARF	5.7	4.3	3.2	7.0	8.0
Broad bean	EL	14.6	12.6	12.0	10.8	16.7
	WARF	13.5	13.2	10.1	8.2	14.0

[a]EL = East Lansing; WARF = Wisconsin Alumni Research Foundation.

Perfected Wales, Little Marvel, Dwarf Alderman and Laxton's Progress varieties of garden pea were used as host plants to test the fecundity of the two clones in the second experiment (Table 12). Fourth-instar virginiparae from colonies that were reared on broad bean were placed singly on each of three young test plant seedlings

per the above-named varieties. The number of nymphs produced by each insect was recorded daily until the insects were age 17 days.

Table 12.--Reproduction by broad bean-reared pea aphids on four varieties of garden pea.

Age (days) of test insect	Mean no. of nymphs produced daily on specified pea varieties							
	Perfected Wales		Little Marvel		Dwarf Alderman		Laxton's Progress	
	EL	WARF	EL	WARF	EL	WARF	EL	WARF
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	6.0	5.3	5.0	2.6	5.0	4.3	5.6	4.6
9	7.0	6.6	6.3	5.6	6.3	5.0	9.0	8.0
10	10.6	8.6	7.6	6.6	9.3	7.3	10.6	9.6
11	12.3	10.0	8.6	7.3	8.0	8.3	10.0	7.0
12	10.3	11.3	6.6	6.3	8.6	6.6	7.0	7.6
13	10.0	9.0	5.6	4.0	8.0	7.0	6.0	5.6
14	8.3	8.0	6.0	4.3	6.3	5.0	4.3	4.6
15	9.3	7.6	5.3	4.0	5.6	4.6	5.0	3.3
16	8.0	6.0	4.0	3.0	6.0	4.5	4.6	4.3
17	7.0	5.5	5.0	4.0	4.5	5.0	5.3	4.0
Mean[a]	8.9	7.8	6.0	4.8	6.8	5.8	6.7	5.9

[a]Mean of means.

Interclone variations in fecundity also occurred on the four pea varieties (Table 12). The mean of the daily mean productivity was 1.0 to 1.2 nymphs greater for the EL Clone than the WARF Clone on

each variety. Reproduction varied with host plant; with Perfected Wales the most suited larvipositional variety and Little Marvel the least; the other two varieties were almost alike and intermediate to the other two.

DISCUSSION

The choice of a 48-hr acquisition-access time for PEMV acquisition tests is based on the knowledge that a long acquisition would not only decrease and sometimes eliminate the latent period of the virus in the aphid vector (Simon 1954; Ehrhardt and Schmutterer 1965) but also would ensure greater intake of virus through an increased ingestion of the virus-contaminated phloem food of the aphid.

An increased virus concentration in the aphid vector has a dual significance; first there is a correlation between transmission (acquisition) efficiency and the length of the latent period of a virus in the vector and second there is an association of the length of the latent period with the concentration of a virus in the vector since it has been suggested that the latent period is dosage sensitive in persistent aphid-borne viruses (Day 1955; Duffus 1963; Sylvester 1965). In either case, an increased virus concentration in a prospective aphid vector would increase the probability of transmission and in this case it would appear therefore that PEMV has a shorter LP_{50} in Clone EL than in Clone WARF adults. This would hold true if backed up with evidence of differences in adult aphid body size (Table 9) since the more efficient Clone EL compared with the less efficient Clone WARF has a smaller mean body size that automatically would make for a higher virus concentration per unit body volume for the same amount of virus ingested by each of the two clones within a unit

acquisition access-period. Such a correlation would inevitably be in agreement with the explanation for differences in transmission (acquisition) efficiency between adult and nymph vectors as also has been recorded by Simons (1954) and Sylvester (1965).

Even a doubling of the acquisition-access time (Table 3) was not enough to offset any interclonal differences in transmission efficiency that the aphid body size could have introduced. Furthermore the difference in transmission efficiency between the first-instar virginiparae of both Clones EL and WARF is evidently not related to their body sizes which show no significant differences (Table 9). Also interclonal differences in body size cannot be used as a sufficient, if acceptable, explanation for the differences in transmission (acquisition) efficiency between Clones EL and WARF in the stylet-borne transmission of the nonpersistent BYMV (Table 5).

Short of morphological differences, a factor that could influence transmission efficiency (Rochow 1960), and vector behavior (Volk 1961), both of which are absent in Clones EL and WARF, one other vectoral factor that can influence transmission is the aphid saliva. An "Inactivator-behaviour" factor (Watson and Roberts 1939; Day and Irzykiewicz 1954) and an "Inactivator-Host plant cell" compatibility factor (Sylvester 1954) have been hypothesized in explanation of intraspecific variation in transmission efficiency for the stylet-borne viruses.

It is possible that a form of salivary inactivation or inhibition of the same or different kind as that of the stylet-borne virus inactivation is involved in the interclonal differences in vector efficiency in the circulative transmission of the persistent PEMV

by Clones EL and WARF since the saliva inevitably comes in contact with the virus in the virus source plant during the process of ingestion of phloem food and with the healthy young test plant during inoculation, also involving the ingestive mechanism of the aphid.

Although salivary inactivation is unrelated to differences in physical behaviour between the PEMV isolates, but rather to something intrinsic in the virus isolates (Ruppel and Hagedorn 1963), such differences alone are insufficient to explain the complete inability of some individuals of both aphid clones to transmit the persistent PEMV.

It has been shown that the inability to transmit a virus by some individuals of a given vector species is genetic and persistent in the progeny of the specific nonvector individuals (Storey 1932; Bjorling and Ossiannilsson 1958).

Since the number of individuals of a given vector species that become active vectors varies with the environmental air temperature condition (Sylvester 1965; Ehrhardt and Schmutterer 1965), it follows that within a population of a given vector species there exists some sort of gradient of a genetically regulated mechanism subject in some way to modification by temperature and as such accountable for the ability of some individuals to transmit only under certain temperature condition and for the total inability to transmit in certain individuals under all temperature conditions. Therefore, interclonal difference in transmission efficiency may represent only a difference in the intensity of this genetically oriented, thermo-regulated mechanism which is more susceptible to variation in the more efficient Clone EL than in the less efficient Clone WARF. This pattern of transmission by Clones EL and WARF can be negatively correlated with the percentage of

individuals failing to show color change in 95% ethanol for the 35-min test period.

The aphid color change in alcohol indicates a possible difference in the concentration of some alcohol-sensitive chemical component of the aphids of both clones. If this were the case then a difference in the rate, or perhaps the entire nature, of some physiological mechanism involved directly or indirectly with the virus-transmission mechanism in the aphid Clones EL and WARF can be postulated. Evidence of physiological variation between the aphid clones is furnished by the significant interclonal difference in fecundity (Tables 11 and 12).

Differences in clonal transmission efficiencies in different host plant cultures of the aphid clones (Tables 6 and 7) indicates two possibilities: (1) that the vectoral factor responsible for interclonal difference in transmission efficiency, perhaps related to the interclonal difference in chromoreaction and possibly fecundity differences, is subject in some way to modification by the aphid host plant as indicated by the differential transmission of both PEMV isolates; (2) that the same vectoral factor is capable of modification to various degrees by different air temperature conditions at which either the aphids are acquiring the virus or the virus is undergoing latent period in the aphid and/or the aphid inoculating the healthy plants.

Interclonal differences in transmission efficiency at the various combinations of acquisition and inoculation-feeding temperatures may possibly reflect in part the effect on the aphids themselves of temperature since 75, not 55 F, makes for the uptimum metabolic activities of pea aphids (Sylvester 1965) and perhaps of temperature on the

virus within the aphid body or on the vector-virus-host plant interactions.

Strong inhibition of the infectivities of both isolates of PEMV by homogenates of aphid Clones EL and WARF is evident from mechanical inoculation of young garden pea plants with mixtures of aphid homogenates and the virus (Table 8). It has been suggested that the ability of live insects to transmit plant viruses is independent of the inhibitors within such insects and that such inhibitors are active only in insect macerates (Bawden 1954); however, this hypothesis is not backed with exhaustive experimental evidence. From the foregoing discussion and from the evidence of differential inhibition of the PEMV isolates at 75 F and 55 F by homogenates of aphid Clones EL and WARF, a possible negative correlation between this inhibition pattern and the differential transmission of these virus isolates at the corresponding temperatures by live aphids of both clones is inescapable. If the same vectoral factor were responsible for the largely oppositely variable transmission of PEMV and BYMV in terms of efficiency by aphid Clone EL and WARF, then the aphid saliva would be directly incriminated as the source of variation in the inter-virus, inter-isolate and intra-isolate transmission.

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