EIGHT SHORT-TERM STUDIES ON PLANT VIRUS INFECTION

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I
SETTING UP A VIRUS-FREE COLONY

Setting up a virus-free colony (say, aphids) is important in the study of insect transmission of plant diseases. This is to assure the workers that the aphids to be used are not carrying the virus at the start of the experiment. Hence, transmission of virus can easily be determined and fully understood.

Virus-free colony of aphids can be set up by rearing the aphids on a host-plant (say, cowpea or bush sitao). The host plant will provide the aphids adequate amount of food to maintain its normal growth and development. If the host-plant will not show any characteristic symptoms of a virus disease, a virus-free colony of aphids is obtained. This can be done by transferring the aphids from one host-plant to another.

The main objective of this study is to establish a virus-free colony from field-collected aphids.

Materials and Methods

An aphid colony was collected in the field. It was identified as *Aphis craccivora* Koch. There were 5-10 adult aphids inoculated/transferred on virus-free healthy seedlings of bush sitao being grown from the previous experiment. Transfer of the adult aphids was done with the use of moist camel's hair brush. The two pots of virus-free healthy seedlings were then placed inside the cage for observation of virus infection. This was done to avoid

contamination.

The aphids on the bush sitao plants inside the cage were inspected everyday as the plants were being watered. When the adults from the first transfer produced nymphs, another 5-10 of these nymphs were transferred per plant to a new potted healthy bush sitao seedlings.

The two pots of bush sitao plants were taken out from the cage and the newly transferred nymphs on the new two pots of virus-free healthy seedlings of bush sitao were kept inside the cage for observation of virus infection.

In short, a series of transfer of aphids on the virus-free healthy bush sitao seedlings were done in the same manner as described above.

When characteristic symptoms of a virus disease were not shown by the bush sitao plants, another final transfer was done to be sure of obtaining a virus-free aphid colony.

Results and Discussions

The final transfer of aphids to a new set of virus-free healthy bush sitao seedlings resulted in the production of virus-free aphid colony as the bush sitao plants observed for two weeks did not show any characteristic symptom of virus infection.

The virus-free aphid colony was obtained after a series of transfer. This technique/method was also used by Smith (1931). He used *Myzus persicae* Sulz. on potato leafroll virus.

Simons (1954) mentioned that the extent of variation in the length of serial transfer is affected either by difference in species or within species. Similar observations were reported by Rochow (1960 a and b, 1961, 1963), Oswald and Housten (1953) and Slykhuis *et al.* (1959).

The two-week observation period on the bush sitao plants after the final transfer of aphids was more than enough; a virus-free aphid colony was obtained. Bernardo (1969) noted that nymphs of aphids reached reproductive periods of 6 days. In addi-

tion, characteristic symptom of virus infection can be shown, within two-weeks, by the plants especially leguminous plants including bush sitao.

Conclusion

A virus-free aphid colony reared on a host-plant, bush sitao, was obtained after a series of transfer of aphids in its nymphal stage. Production of virus-free colony is important in studying and understanding the role of insects as vectors in transmitting or spreading virus diseases. Moreover, it is a pre-requisite in a better understanding of virus-vector relationship (Smith, 1965) and the two broad types of virus transmission, namely, non-persistent (Carter, 1 962) and persistent type. Aphids as vectors of plant viruses was also studied by Kennedy, *et. al.* (1962).

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II
PRE-ACQUISITION STARVATION ON NON-PERSISTENT VIRUS TRANSMISSION

Introduction

Many workers reported that fasting before acquisition feeding greatly enhances the efficiency of non-persistent virus transmission. Day and Irzy Kiewics (1954) suggested that such enhancement of efficiency was brought about by changes in feeding behavior, stylet and ensheathment and formation of saliva. Smith and Brierly (1956) reported that the concept of a virus inactivator produced at one rate by vectors while feeding but at lower rate while fasting failed to furnish a satisfactory explanation for vector specificity and for non-transmission of highly infectious virus. Watson and Roberts (1939) reported that the efficiency of the vector was greatly increased if they were prevented from feeding for a time just prior to being placed on the disease inoculum. The transmitting efficiency increased with increased fasting time up to one hour. The efficiency of previously starved aphids decreased as the feeding time on the infected plants increased. If this time was extended to an hour there was no increase in efficiency over unstarved aphids. They explained that this was due to the inactivation of the virus by some substance such as enzyme produced by the aphids during feeding. This substance, they hypothesized, was

not produced in sufficient quantity to inactivate the virus being ingested, resulting to increase efficiency of virus transmission after fasting and short feeding periods.

The main objective of this study is to demonstrate the effect of pre-acquisition starvation period on the transmission of non-persistent virus.

Materials and Methods

About 300 nymphs of aphids (*Aphis craccivora* Koch) were starved in petri dishes for the following periods :0, 30 minutes; 60 minutes, and 120 minutes. After each starvation period, these aphids were allowed to feed (acquisition feeding) on virus-infected bush sitao for 5 minutes (acquisition feeding period).

After acquisition feeding period, the aphids were transferred to virus-free healthy seedlings of bush sitao (test plants) for inoculation feeding period of 5-10 minutes.

There were three aphids allowed for inoculation feeding period pre-test plant. Observation for virus infection was terminated after two weeks.

Results and Discussions

The virus-infected bush sitao is show in Table 1.

Table 1. Virus-infected bush sitao as a result of different pre-qualification starvation periods of *Aphis craccivora*.

Pre-acquisition starvation	Total Infection	Percent Infection
0	1/3	33.33
30 minutes	3/3	100.00
60 minutes	2/3	66.66
120 minutes	2/3	66.66

The results proved a marked influence of pre-acquisition starvation (fasting) when compared to control (0 starvation). The highest percentage of infection (100) was obtained when the aphids was starved for 30 minutes before acquisition feeding of virus-infected source plant. This could be attributed to suggested hypotheses advanced by Day and Irzy Kiewics (1954), Smith and Brierly (1956), Watson and Roberts (1939), Sylvester (1950), Watson (1938 and 1946) mentioned earlier.

Conclusion

A marked influence of pre-starvation period was evident when the test plants exhibited higher percentages of virus infection when compared to control. A 30-minute fasting period proved highly efficient in enhancing transmission of non-persistent virus.

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III
TRANSMISSION OF NON-PERSISTENT APHID-BORNE VIRUSES

The terms "persistent" and "non-persistent" refer to the length of time for which the viruses remain active in the vectors and that, in general, the persistent viruses are retained longer in the vectors than are the non-persistent. These two classic concepts were developed first by Watson and Roberts (1940).

Sylvester (1954) listed the acquisition threshold, inoculation threshold, and transmission threshold periods for a number of non-persistent viruses. The acquisition and inoculation threshold are defined as the periods necessary for successful acquisition and inoculation, respectively; the transmission threshold as the sum of the first two.

Watson and Roberts (1940) hypothesized the mechanism of transmission of the non-persistent virus as the following :(1) that the transmission thresholds is too short to have permitted the virus to have entered the insect's body and passed into the plant via the blood and salivary secretion; (2) that non-persistent viruses are only transmitted by aphids to a single plant or, rarely, a second, after a single acquisition feeding, the assumption being that the feeding process cleans the stylets, the loss of infectivity therefore being also a mechanical process; and (3) retention during fasting

was longer than during feeding, indicating that the stylets were not being decontaminated as rapidly during fasting, but that virus was being lost at rates comparable with those of inactivation *in vitro*. They concluded that the loss of infectivity was not due to mechanical cleansing of stylets but rather to the activity of a substance produced by the aphid while feeding but not while fasting. They further added increasing the efficiency of non-persistent virus vectors was ascribed to the absence of this inactivating substances in the fasted insects.

It is the main objective of this study to define characteristics of non-persistent virus by transmission.

Materials and Methods

About a hunred aphids (*Aphis craccivora* Koch) were starved for 30 minutes. Then the starved aphids were allowed to feed (acquisition feeding) on the virus-infected cowpea. Acquisition feeding period was five to 10 minutes.

Serial transfer of aphids after acquisition feeding period was done on the cowpea test plants. Inoculation feeding period for each set of virus-free cowpea test plants was 15 minutes, 15-30, 30-45, and 45-overnight.

Observation was done everyday for 10 days as the plants were watered every day.

Results and Discussions

Table 1 below shown the results of the experiment.

Table 1. Serial transmission of mosaic virus in cowpea by serial transfer/inoculation feeding of aphids (*Aphis craccivora*)

Serial Inoculation Feeding Period	Observation of No. Plants Infected	Percent Infections

	7th day	8th day	9th day	10th day	
15-minutes	3/9	4/9	4/9	5/9	55.5
15-30 minutes	2/7	2/7	3/7	3/7	42.8
30-45 minutes	2/6	2/6	2/6	2/6	33.3
45-overnight	0/8	0/8	0/8	0/8	0

From the data presented at Table 1, highest percent infection was obtained in 15 minutes inoculation feeding period and followed by 15-30 minutes, 30-45 minutes in that order of decreasing percent infection. Inoculation feeding period of 45-overnight yielded 0% infection.

The inverse relationship of infectivity and time of transmission of non-persistent virus can be attributed to decrease concentration of virus titer in the mouth-part of aphids and that the virus did not multiply in the vector's body.

The same observations were obtained by many workers on the transmission of non-persistent virus. Maramorosch (1963) reported that non-persistent viruses can be acquired within a matter of second or minutes, but the vectors (aphids) soon lose the ability to transmit the virus unless they have access to another virus source. This shows the lack of multiplication of non-persistent virus in the vector's body because they are only "stylet-borne" (Kennedy, et. al, 1962; Finsley, 1973).

Summary and Conclusion

The non-persistent virus is one in which the transmission time is short because there is no virus multiplication in the vector's body.

Jansen (1946) characterized non-persistent viruses, namely : (1) They usually require no incubation period in the vector; (2) they are not long retained by the vectors after the latter leaves a diseased plant; (3) they are usually transmissible by mechanical

means; and (4) there is usually a relatively low degree of specificity between the virus and its vector, particularly with reference to the aphids.

Day and Venables (1961) have set up rather precise definitions of the term "non-persistent" virus as follows : (1) the transmission time is short; (2) the virus is not recoverable from the haemolymph; (3) the vector is not capable of transmitting following a moult; and (4) the vector does not become infective when purified virus is inoculated into the haemocoele.

There is an inverse relationship between inoculation feeding time and infectivity of virus transmission.

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IV
INOCULATION FEEDING PERIOD IN NON-PERSISTENT VIRUS TRANSMISSION

The Federation of British Plant Pathologists (1973) defined inoculation threshold as the period necessary for successful inoculation of the virus. Hence, inoculation feeding time is the time that a vector is allowed to spend on a test plant in transmission experiment; this is also called as inoculation access time or inoculation feeding period.

Sylvester (1954) noted a number of inoculation thresholds in some non-persistent virus. This proves the difference in inoculation thresholds among different non-persistent aphid-borne viruses.

The main objective of this study is to compare various lengths of inoculation feeding periods in non-persistent transmission following short acquisition feeding period.

Materials and Methods

Appreciable number of nymphs of *Aphis craccivora* were starved in petri dish for one hour. After fasting, the aphids were allowed to acquire the virus for five to minutes in bean mosaic virus infected bush sitao plants.

After acquisition of the virus, aphids were transferred with the use of damp camel's hair brush into virus-free healthy cowpea seedlings and allowed to feed (inoculation feeding) for one minute, 15 minutes, 30 minutes, and 60 minutes (acquisition feeding period). There were five aphids per test plant used in the experiment.

The test plants were then caged. Observation of viral symptom expressions was done every day, for two weeks, as the test

plant were watered every day.

Results and Discussions

Results are tabulated below.

Table 1. Total Virus-infected cowpea and percentage infection as influenced by different inoculation feeding times of *Aphis craccivora*.

Inoculation Feeding Period	Total Plants Infected	Percent Infection
1 minute	4/6	66.66
15 minutes	3/6	50.00
30 minutes	2/6	33.33
60 minutes	4/6	66.66

Highest percent of infection was obtained at one minute and 60 minutes inoculation feeding period, with both exhibiting 66.66% . This proved that inoculation feeding period affected percentage infection of virus diseases.

The highest percent infection observed with one minute inoculation feeding period was even longer than what was reported by Sylvester (1956), Bradley (1952) and Simons (1956). They observed that inoculation occured within as little as five seconds after an aphid affixes its labium to a plant.

The effect of the duration of a probe on inoculation has been studied both by allowing the probe to be completed and by interrupting it at a pre-determined time by dislodging the aphid. When viruleferous aphids are allowed to complete their probes on the test plants, the probability of infection resulting is 100%, high for probes that last from 15 to 30 seconds as for those that last longer (Bradley, 1952 and Bradley and Rideout, 1953). And when aphids are interrupted at various intervals during their first probe on a test plants, the probability of infection resulting reached its

maximum by about 15 seconds and sometimes earlier (Sylvester, 1949 and 1950b and McLean (1959).

In the experiment, aphids might have been interrupted during their first probe that accounted higher percentage virus infection in one minute and 60 minutes as have been noted from the previous reported study on aphid inoculation.

Conclusion

Inoculation feeding period of non-persistent virus using aphids had a marked influence on the percentage of virus infection. Highest percent of virus infection was both obtained in one minute and 60 minute inoculation feeding periods.

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V
SEED TRANSMISSION OF VIRUS DISEASES

Earlier works in seed transmission of plant viruses have been conducted. Some workers presented suggestive rather than conclusive pieces of evidence while others presented uncontestable evidence of seed transmission of virus diseases. It was known from these early works that virus transmission through seed occured commonly among legumes and relatively uncommon among non-leguminous plants.

In 1919 Reddick and Stewart were probably the first who demonstrated the seedborne nature of the bean viruses and noted considerable variability in the percentage of virus transmission in the seed obtained from different mosaicked plants. In 1929, Pierce and Hungerford found that seed from infected plants transmitted the virus to about 33% of the seedlings and about 48% of primary-infected seed transmitted the disease. In 1932, Nelson showed that about 50% of the seed of primary-infected plants was infected.

An experiment was conducted to determine the percentage infection of common bean mosaic virus disease in Los Banos Bush Sitao seeds. Bush sitao was used in this study because it is one of the most important legumes in the country. This crop is grown as cash crops and is very popular in the Filipino diet. Improvement of this crops would be beneficial to the bush sitao-eating people.

Materials and Methods

Weevil - or hole-free Los Banos Bush Sitao seeds were chosen from the pool of bush sitao seeds treated with Sevin. Immediately after selection, the seeds were sown in four earthen pots (8-inch in diameter) filled with soil at ten seeds per pot. The

plants were grown in Entomology greenhouse for observation of the appearance of the common bean mosaic virus disease symptoms.

After a week from planting, the plants were fertilized once with ammonium sulfate and complete fertilizer to ensure normal and vigorous growth. Watering the plants was done occasionally to insure enough moisture for normal growth and development.

When the plants had developed the first compound leaves, they were carefully examined for mosaic symptoms. After two weeks, mosaic symptoms appeared on the leaves and the reading of the percentage infection was made by differentiating the healthy and diseased plants.

Results and Discussion

The identification of the common bean mosaic virus disease during the experiment was based mainly on the appearance or the external symptom expressions. Fajardo (1930) and Pierce (1934, 1935) were probably the first workers who attempted to identify viruses on symptom expressions, host range and physical properties.

The first discernible symptoms observed was characterized by prominent wein-clearing on the trifoliate and later, turning into mild or marked mottling or coarse mosaic pattern associated with leaf curling. Systematically infected leaves developed a light and dark green mosaic pattern along the major veins. Noticeable rugosity and downward cupping of the leaf margin accompanied the mottle on affected leaves. Some symptoms consisted of only very mild mottling, the dark green patches seen only when viewed against the light. These external symptom expressions observed were more or less similar to those reported by earliest workers (McLean,1941, Snyder, 1942; Shepherd and Fulton, 1962.)

The range of percentage infection of the common bean mosaic virus disease in Los Banos Bush Sitao plants was 2.7% to 40% as shown in Table 1 below.

Table I. Percentage Determination of Seed-borne Virus Disease (Common bean mosaic) in Los Banos Bush Sitao.

Replicates	No. of Plants Infected / No. of Plants Planted	%Infection
I	1/36	2.7%
II	16/40	40.0%
III	2/33	6.0%

The variability of the percentage infection can be attributed to the time of data collection. The data of Rep I and II were taken after two weeks from planting, while that of Rep III were taken more than two weeks. This showed that the percentage of seed infection can be correlated with certain stages in the growth of the plants. Moreover, the number of seeds planted and germinated vary from Replicate I to III that resulted to the variability in percentage seed transmission of the common bean mosaic virus disease.

Nelson (1932) reported the variability of virus transmission in the seed might be explained by certain characteristics in the vascular anatomy of the bean pod, assuming that virus might be present only in certain elements of the vascular bundles and seeds having a direct connection with the infected tissues would be infected.

Fajardo (1930 b) proved and later confirmed by Harrison (1935) that the percentage of seed infection is correlated with certain stages in the growth of the plants. If infection occurs before the plant blooms, the seed might carry the virus. However, if they become infected after the blossom set, no seed infection results.

Smith and Hewitt (1938) noted that, in general, varieties most infected produced higher percentage of infected seed than

those less affected.

Crowley (1957) reported seed transmission of plant virus diseases and found pieces of evidence that transmission through seeds was accomplished only when the virus was able to infect the mega or micro-spore of infected mother plant. Likewise, Esau (1956) presented additional evidence supporting the work of Crowley.

Summary and Conclusion

The common bean mosaic virus disease was readily transmitted by Los Banos Bush Sitao seeds. The difference in percentage infection of seed-borne virus disease transmission in Los Banos Bush Sitao seeds could be attributed to the time of data collection in which percentage virus infection can be correlated with certain stages in the growth of the plants.

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VI
VECTOR EFFICIENCY (SPECIFICITY) IN VIRUS TRANSMISSION

Fortunately not all species of aphid vectors transmit all the known non-persistent and persistent viruses. If they do, our very existence will be threatened for lack of food.

As a rule several species have been shown to transmit each virus but some appear unable to do so, experimentally at least (Kennedy, et. al., 1962). The most interesting cases are when one species fails to transmit a virus under conditions where another species of the very same genus is an efficient vector (Doncaster and Kassanis, 1946 and Kassanis, 1947).

When those species that transmit a given virus are compared as vectors of it, usually one causes a different level of transmission. A species that transmits one virus efficiently will often do so with others, but there are notable exceptions to this. For example,

Sylvester (1952) found that *M. persicae* was a more efficient vector of beet mosaic virus than *M. circumflexus* (Buckt), but it was quite the reverse when these species were compared as vectors of celery mosaic virus (Simons and Sylvester, 1953).

Carter (1962) named many kinds of specificity such as species specificity, area specificity, group specificity, regional specificity and vector nonspecificity. He attributed these kinds of specificities to ecological factors, feeding behavior, ingestion, blood, salivary glands, and immunity of insects.

Many workers studied vector efficiency (specificity). Radam (1958) found that a strain of the stylet-borne cucumber mosaic virus was readily transmitted by *Myzus persicae* at first, but it lost this property during transfers in the greenhouse. The isolate remained transmissible by *M. ascolonicus* and *Aphis gosypii,* but not by *M. persicae.* The latter remained an efficient vector of other virus. This indicate that the basis of this type of vector specificity involved a change in the virus and not in the vector. Similar loss of adaptation to affinity to insect vectors has been discovered in leafhopper-borne virus.

Paine and Legg (1953) obtained transmission of sheep mosaic virus by spring winged form of *Phorodon humili,* but not by the wingless summer form.

Orlob and Aray (1960) found that only oviporate of *Rhophalusiphum fitchii* was capable of transmitting the circulatory bowley yellow dwarf virus, while other forms of the same aphid species failed to transmit the virus.

Orlob (1962) further found that oviparous females of *Macrosiphum euphorbias* and *Brevicoryne sp.* transmitted both potato virus Y and cabbage virus B, as did apterous viviparous females.

Anderson (1951) noted the difference among developmental stage of *Macrosiphum geganicola* in the transmission of circulatory virus of filaru read leaf. The young nymphs seemed to acquire the virus more readily than did adults. The minimum latent period was shorter in nymphs than in adults.

Stubbs (1955) demonstrated the variation between different culture of *Myzus persicae* in their ability to transmit a circulatory

virus from spinach.

Williams and Ross (1957) reported variability among cloves. of *M. Persicae* in the transmission of potato leaf roll virus.

Maramorosch (1963) recognized the kinds of variation within an applied species. The first kind concerns variations in virus transmissions among different cloves in strains. The second deals with variation among various developmental stage. The third type distinguished variation among forms of one species.

Bawden and Kassanis (1947) suggested that only occasionally individuals of *Myzus persicae* might be vector of potato virus C in contrast to most individuals of this species which are unable to transmit the virus.

The main objective of this study is to compare the efficiency (specificity) of three species of aphids as vector of bean mosaic virus disease.

Materials and Methods

Three species of aphids, *Aphis craccivora, Myzus persicae,* and *Aphis gosypii* were used in this experiment. About a hundred of each species were starved for 30 minutes in petri dishes.

After starvation, all the starved aphids were allowed to feed on the bean mosaic from bush sitao for 30 minutes (acquisition feeding period).

After acquisition feeding period, the viruleferous aphids were transferred to the virus-free healthy test plants, mungbean and bush sitao for / hour (inoculation feeding period). There were 10 aphids used per species per test plant.

Results and Discussions

Results are shown below.

Table 1. Vector efficiency (specificity) on virus transmission of bean mosaic.

Vector Species	Test Plants Mungbean	Bush Sitao	Percent Infection (M)	(BS)
A. craccivora	3/3	2/3	100	66.66
A. gosypii	1/3	1/3	33.33	33.33
M. persicae	1/3	1/3	33.33	33.33

These results evidently show that some degree of efficiency (specificity) was exhibited by the vector species as well as the test plants species. The *Aphis craccivora* seemed to be the most efficient compared to *Aphis gosypii* and *Myzus persicae.* This could be attributed to the rearing of aphids/vectors to different hosts (Simons, 1955).

Aphis craccivora seemed to be an efficient vector of mosaic virus on mungbean rather than on bush sitao. Similar observation were obtained by Sylvester and Simons (1951) when they did an experiment using *M. persicae* and *Rhopalosiphum pseudobrassicae* (Davis) as vector of a turnip mosaic virus. They reported that when mustard seedlings were used as test plants, *M. persicae* was the more efficient vector, but it was just the reverse when chinese cabbage was used.

Conclusion

Aphis craccivora was the most efficient vector of bean mosaic virus, when compared to *Aphis gosypii* and *Myzus persicae,* using mungbean and bush sitao as test plants. Moreover, *A. craccivora* had increased and decreased levels of transmission of infection on mungbean and bush sitao, respectively.

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VII
TRANSMISSION OF FIELD COLLECTED VIRUS

Fulton (1964) noted that the property of transmissibility is a fundamental characteristic of viruses as it is of other biological agents that cause disease. He further added that the transmission of a virus provided the only experimental evidence of its existence as an independent entity.

Kinds/types of transmission of plant viruses are many, namely: by grafting, dodder, seed, mechanical, soil, and arthropod. Many workers have worked on these types of transmission in their attempts to control or prevent spread of plant virus diseases. Smith and Brierly (1956) reported that many of our most devasta-

ting plant (pathogens) diseases are attributable to virus transmitted by insects. This statement was supported by Carter (1962), who reported that the largest group of insect vectors from the standpoint of both in number of viruses as well as species of insects involved are the aphids. This was evident in the works of Doolittle (1920), who used *M. persicae* to transmit cucumber mosaic; Brandes (1920), who used *A. maidis* to transmit sugar cane mosaic wilt; Jagger (1921), who used *M. persicae* to transmit lettuce mosaic; and Doolittle and Jones (1925), who used *Macro-siphum pisi* to transmit pea mosaic.

The main objective of this study is to determine the transmissibility of unknown viruses collected in the field based on external symptoms.

Materials and Methods

Healthy seeds of mungbean, bush sitao and cowpea were sown in earthen pots (eight inches in diameter) filled with soil, at five seeds per pot. Thinning to three seedlings per pot was done after the plants developed the first compound leaves. These served as test plants for aphid virus transmission.

The virus-infected bush sitao plants from previous experiment were saved purposely for this experiment as inoculum source.

About a hundred nymphs *of Aphis craccivora* were allowed to feed (acquisition feeding) on virus-infected bush sitao for 15 minutes (acquisition feeding period).

After acquisition feeding period, the viruleferous aphids were removed from the inoculum source and 10 aphids per plants were allowed to feed (inoculation feeding) on the test plants. They were placed in the cage of observation of the transmissibility of the virus based on external expression.

As the plants were watered every day, the compound leaves were carefully examined for viral symptoms. After two weeks, mosaic symptoms appeared on the leaves and the reading of percentage infection was made by differentiating the healthy and diseased plants.

Results and Discussions

The identification of the virus disease was based mainly on the appearance of the external symptom expressions. The viral symptoms, which was characterized by mild or marked mottling or coarse mosaic pattern associated with leaf curling, appeared after two weeks of observation on each leguminous test plants. These external symptoms observed were more or less similar to those reported by Fajardo (1930), Pierce (1934, 1935), McLean (1941), Snyder (1942), Shepherd and Fulton (1962).

It was observed that all the test plants were infected with mosaic virus disease. However, the rate of transmission differed on each test plants. Bush sitao exhibited the viral symptoms earlier followed by cowpea and mungbean in that order. This can be attributed to the incubation period of the virus in each leguminous species as test plants.

Conclusion

The experiment confirmed the transmissibility of virus using aphids as vectors of the disease as shown by the test plants, which were 100% infected.

The rate of virus infectivity among test plants varied. Bush sitao showed the first viral symptoms followed by cowpea and mungbean, in that descending order.

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VIII
ARTHROPOD TRANSMISSION OF TWO PLANT VIRUSES

Transmission of virus disease by the aphids has long been studied. Kennedy, et al. (1962), reported 242 species of aphids as vectors of virus diseases. Watson and Plumb (1972) reported aphid as the vector that transmits a great number and variety of viruses and attributed it to its specialization to utilize food, reproduce through parthenogenesis and mutate to adopt to the changing

conditions. Maramorosch (1963) noted two types of aphid transmission of plant viruses, the non-persistent and persistent. He also noted that leafhoppers ranked second to aphids as transmitters of virus diseases.

Leafhopper was linked with the spread of plant diseases as early as 1902, when Japanese entomologists discovered that *Nephotetix apicalis* was responsible for the transmission of rice stunt.

Aside from aphids and leafhoppers as arthropod vectors of plant virus diseases, Carter (1962) listed whiteflies (*Aleurodidae*), bugs (*Miridae*), mealybugs (*Pseudococcididae*), thrips (*Thripidae*), planthoppers (*Delphacidae*) and some other species belonging to the order *Orthoptera, Coleoptera* and *Acarina.*

The main objective of this study is to demonstrate that viruses can be transmitted by arthropods such as aphids and leafhoppers.

Materials and Methods

Part I – Aphid Transmission

After one hour starvation period, the aphids (*Myzus persicae* Sulz.) were allowed to feed (acquisition feeding) on the inoculum source (virus-infected pechay) for 15 minutes (acquisition feeding period).

The ready germinated pechay seedlings were transplanted in plastic bags filled with moistened soil. There were seven virus-free seedlings transplanted per plastic bag. Fertilization was done after transplanting.

After acquisition feeding period, 10 aphids were transferred per test plant (pechay) with the use of damp camels hair brush. Inoculation feeding period was four hours after which the aphids were killed and the test plants were caged together with the control.

Observation was done everyday for 11 days as the plants were watered.

Plant II – Leafhopper Transmission

Taichung Native 1 (TN1) rice seedlings were used as test plants for the transmission of rice tungro virus by green leafhopper (*Nephotettix virescens* Distant).

The adult non-viruleferous green leafhoppers were allowed to feed (acquisition feeding) on the virus-infected TN1 for one hour and 24 hours with the following inoculation feeding periods for each: 12 hours, 24 hours, 36 hours and 48 hours.

Inoculation feeding period was done inside the test tubes with mouths covered with cheese cloths to prevent leafhoppers from escaping. Leafhoppers were killed after inoculation was done. Twenty days later, reading was done for the number of infections and symptoms expression of the disease.

Results and Discussions

Aphids transmission of mosiac virus disease was observed on the 8th, 9th and 11th day after inoculation feeding period. There was a total of four pechay plants infected with the virus disease out of the seven pechay transplanted in the plastic bags. Hence, the percentage virus infection (4/7) was 57.1%. This proved that pechay mosaic virus was readily transmitted by aphids.

Leafhopper transmission of rice tungro virus disease was evident when its characteristic symptoms were observed in one hour acquisition feeding period with 24 hours inoculation feeding period and in 24 hours acquisition feeding period with 36 hours inoculation feeding period. Table 1 presented the results of leafhopper transmission of rice tungro virus disease.

Table 1. Leafhopper transmission of rice tungro virus disease.

Acquisition Fedding Period	Inoculation Feeding Period	Virus Infected Plants	Percent Infection
1 hour	12 hrs	0	0

1 hour	24 hrs	3/3	100
1 hour	36 hrs	0	0
1 hour	48 hrs	0	0
24 hrs	12 hrs	0	0
24 hrs	24 hrs	0	0
24 hrs	36 hrs	2/3	66.7%
24 hrs	48 hrs	0	0

A closer look at the table will reveal that 100% infection was obtained in one hour acquisition feeding period with 24 hrs inoculation feeding period. On the other hand, 66.7% infection was observed in 24 hrs acquisition feeding period with 36 hrs. inoculation feeding period. This proved that acquisition feeding periods and inoculation feeding periods affected the percentage of virus infection. Similar observations were reported by Rivera, et al. (1965). They further observed that acquisition feeding period of three to five days provided the insect with maximum amount of virus. Maramorosch (1963) reported that successful acquisition and transmission of the virus by leafhoppers are affected by age of plant leaves, feeding time, temperature and increased food uptake.

Ling (1968) reported shortest inoculation feeding period of seven minutes for leafhoppers to readily transmit the virus disease. He further added that a single probing of an infective green leafhopper can cause rice seedling to become infected.

Conclusion

Plant virus diseases such as mosaic virus disease of pechay and tungro virus of rice can be readily transmitted by black bean aphids (*Aphis craccivora* Koch) and green leafhopper (*Nephotettix virescens* Distant), respectively.

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