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Title APHID TRANSMISSION OF AN UNDESCRIBED VIRUS FROM SWEET CHERRY

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Information was sought on the mechanism of transmission of an undescribed latent virus by the green peach aphid, Myzus persicae (Sulzer). Feeding periods of various durations were used in the acquisition and test feeding experiments. Phaseolus vulgaris L. cv. Gill's Reliable bush bean was the best test plant for the aphid transmission experiments among the various species tried. Chenopodium amaranticolor Coste and Reyn, initially used as the indicator plant, was later replaced by P. vulgaris cv. Pencil Pod Black Wax bush bean, which proved to be more reliable. Although not conclusive, the data from the transmission experiments indicated that the virus is stylet-borne and semi-persistent in nature.

Transmission tests were conducted with three other aphid

species in addition to M. persicae. The pea aphid, Macrosiphum pisi Kaltenbach, transmitted the virus; however, attempts to transmit the virus by the black cherry aphid, Myzus cerasi (F.), and the plum leaf-curl aphid, Brachycaudus helichrysi Kaltenbach, were unsuccessful. Tests with these two species should be repeated when the transmission pattern of the virus is better known.

The virus was transmitted to Prunus mahaleb L. by Myzus persicae as indicated by recovery of virus to the indicator plant, Pencil Pod Black Wax bush bean.

APHID TRANSMISSION OF AN UNDESCRIBED VIRUS FROM SWEET CHERRY

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INTRODUCTION

In the Spring of 1962, an undescribed latent virus was isolated from a Bing cherry (Prunus avium L.) infected with rugose mosaic virus in an orchard near The Dalles, Oregon, by Dr. J. A. Milbrath, Botany Department, Oregon State University. The year before, during an attempt to recover and identify the Prunus ringspot virus from this tree, Dr. Milbrath noted that certain buds from Prunus mahaleb L. seedlings grafted to Shiro-fugen flowering cherry (Prunus serrulata Lindl.), the index plant, had not reacted in the usual fashion (20, p. 125). These plants were held over until the following spring when the discovery was made that the newly expanded leaves expressed vein-banding, a symptom typical of some virus diseases, but not of Prunus ringspot. The terminal leaves were macerated and Buttercup squash (C. maxima Duchesene) was mechanically inoculated with the expressed sap. A virus was recovered with symptoms entirely different than that ordinarily obtained with Prunus ringspot infections.

The virus was successfully inoculated mechanically into Vigna sinensis (L.) Endl. cv. Black-eye cowpea. The green peach

aphid, Myzus persicae (Sulzer), one of the more efficient vectors of plant viruses, was tested by Dr. K. G. Swenson and found to transmit this virus.

Phytopathogenic viruses may be divided into three classes based on the manner of transmission by insects: (1) nonpersistent viruses; (2) semi-persistent viruses; (3) persistent viruses. This paper reports the efforts made to determine the virus-vector relationships of this undescribed latent virus.

REVIEW OF LITERATURE

The Concept of Virus Transmission by Aphids

The concept of nonpersistent and persistent modes of virus transmission by insects was originally formulated by Watson and Roberts (30, p. 543-547). They found that certain viruses could be acquired and transmitted by aphids within seconds or minutes, and that this ability was soon lost unless the aphids had further access to a source of the virus. These viruses were termed non-persistent viruses. Other viruses were observed to require hours for acquisition and transmission, but the aphids were able to continue transmitting for many days after removal from the virus source. These they called persistent viruses. Recently, Kennedy et al. (16, p. 2) proposed that the terms "stylet-borne" and "circulative" be adopted as descriptive of the methods by which these viruses are carried by the insect.

Since the original definition of nonpersistence and persistence, a growing number of viruses have been discovered that have characteristics of both types of transmission. Sylvester (25, p. 800) suggested that these viruses be placed in an intermediate class called the semi-persistent viruses. All terms are now in common use.

Nonpersistent or Stylet-borne Viruses

The nature of the nonpersistent or stylet-borne viruses has been uncovered and reviewed by many investigators (8, p. 269) (12, p. 143) (14, p. 696) (23, p. 423) (27, p. 489) (28, p. 168-169) (30, p. 543-547). Several characteristics are readily apparent:

1. Stylet-borne viruses are of the common mosaic type, and affect mainly the epidermal tissue.
2. The viruses are easily sap transmissible.
3. Vector specificity is low.
4. Transmission efficiency is increased by a starvation period.
5. The viruses are acquired and transmitted in a short time.
6. The viruses are lost when the insect molts.

Bradley (4, p. 80) found that starved aphids do not settle down to feed immediately after transfer to a diseased plant, but probe the leaf repeatedly and become infective if they feed for only a few seconds in the epidermal cells. Roberts (21, p. 357) reported that at least one minute and usually several are required for the stylets of the aphid to reach the mesophyll, and 15 minutes or more are required to reach the phloem. Since starved aphids

become optimally infective in 20 to 30 seconds, this time is too short for the stylets to penetrate beyond the epidermis. Bawden et al. (2, p. 238) found that epidermal cells may contain 16 times as much nonpersistent virus as mesophyll cells.

The virus is transferred to other plants during the early stage of probing as the epidermis is penetrated. However, the vector may not reach the cells or tissue in which the virus can develop everytime it feeds.

The actual mechanism of transmission of stylet-borne viruses is very uncertain (28, p. 159), and several hypotheses have been advanced to explain the problem (4, p. 95-96) (8, p. 252-253) (24, p. 93) (26, p. 372) (31, p. 233). Generally, aphids transmit stylet-borne viruses by a mechanical process and virus contamination of the stylets occurs during saliva-free penetrations of the leaf epidermis. Vector efficiency and specificity are presumed due to interactions among the viruses, the saliva of the insect, the behavior of the species, and the reaction of the host cells being inoculated.

Persistent or Circulative Viruses

The workers who enumerated the characteristics of the stylet-borne viruses were, at the same time, able to record the characteristics of the persistent viruses.

1. Persistent viruses cause yellows, chlorotic streaks, necrosis of phloem, and tumors; they affect the conducting tissues.
2. The viruses are not, as a rule, sap transmissible.
3. Vector specificity is high and often only one or sometimes a few closely related species are found to transmit the same virus.
4. There is no starvation effect.
5. Both acquisition and transmission feedings require several hours or more for maximum efficiency.
6. The viruses are retained when the insect molts.
7. Persistent viruses are fewer in number than stylet-borne viruses (13, p. 52).

Circulative viruses are ingested by the aphids (17, p. 190) (18, p. 371). Several hours to several weeks are required for optimum infectivity due, in part, to the fact that some of the circulative viruses are located in the phloem that is not as readily accessible to the aphid (21, p. 357) (22, p. 472).

A latent period or delay in development of infective power in the aphid generally precedes the ability to transmit the virus to a healthy plant. The length varies with different viruses and may be a few hours or several weeks (1, p. 702) (5, p. 554). The

virus passes through the gut wall into the haemolymph. There is evidence that multiplication of the virus takes place to a limited extent, but it is not known with certainty at what locations within the body cavity the increases occur (7, p. 511). The virus then passes to the salivary glands from which it is injected back into the host via the saliva (17, p. 190) (18, p. 371).

Aphid-borne Viruses of Trees

In addition to the virus studied in this investigation, two other aphid-borne viruses of broadleaf trees are known : tristeza virus of citrus and plum pox virus. Aphid transmission of tristeza has been known for some time; aphid transmission of plum pox has only recently been reported.

Originally, tristeza was thought to be due to sweet orange scion and sour orange rootstock incompatibility, but the disease was later shown to be a virus. In 1946, Meneglini (19, p. 285-287) demonstrated the existence of a virus-vector relationship between tristeza and the Oriental brown citrus aphid, Aphis citricidus Kirkaldy. He utilized large numbers of aphids in his acquisition and transmission tests to determine transmissibility. Bennett and Costa (3, p. 218), using similar mass inoculation procedures, found that a starvation period had no effect and that the aphids required a

60-minute acquisition feeding period to become infective. Further tests by Costa and Grant (6, p. 106-111) indicated that A. citricidus became optimally infective after 24 hours on the source. Maximum transmission was obtained after four hours on the test plants, but the aphids became noninfective after 24 hours without further access to the virus source.

Dickson et al. (9, p. 175) (10, p. 5) reported that tristeza is carried by the melon aphid, Aphis gossypii Glover, in the United States. Recently, he showed that the virus is stylet-borne (11, p. 206). Aphids were starved for two hours or more, placed on infected plants (20-25 per plant) for five minutes, then transferred to test plants (duration of time on the test plants not given). Infection was obtained in three out of 95 test plants. In a concurrent series, aphids were grown on infected plants, transferred to the healthy test plants for two hours, then transferred two more times for periods of 48 hours and one week. Three infections were obtained, all during the two-hour feeding period. Dickson concluded that the virus was not circulative, but that a short acquisition feeding period was sufficient for transmission.

After a six-year investigation concluded in 1961, Jordović (15, p. 167-169) reported that plum pox virus spread most in orchards where the activity of certain aphids was high. Concurrent

laboratory tests showed both Brachycaudus helichrysi Kaltenbach and Phorodon humuli Schrank to be vectors.

MATERIALS AND METHODS

General

This investigation was conducted in the Oregon State University greenhouses, Corvallis, Oregon, from October 1962 to November 1963. The plants used in the experiments were raised in a greenhouse maintained at approximately 75 degrees by day and night. This system was, however, subject to change throughout the 24-hour period due to the daily fluctuation of external climatic conditions.

The inoculations for the experiments were made in the headhouse under day-to-day conditions of temperature, humidity, and light; no attempt was made to record these variables. The temperature of the headhouse was difficult to control for any length of time; therefore, during experiments with extended acquisition and test feeding periods, the test plants with their aphids were placed on a bench in the greenhouses to obtain more uniform conditions.

Definition of Terms

Acquisition feeding - the feeding period of aphids on the diseased plant.

Colony - a number of green peach aphids infesting the same individual plant, referred to as the colony plant.

Indicator plant - an alternative host used to confirm the presence of virus in the test plants by the formation of local lesions.

Mechanical inoculation - rubbing of a plant with sap, diluted with a buffer, from a diseased plant. To facilitate infection, the plant was dusted with carborundum.

Preliminary starvation - the period of starvation before the acquisition feeding.

Source plant - the diseased plant used for aphid acquisition feeding and for preparation of sap for mechanical inoculation.

Test feeding - the period of feeding of the aphid on a healthy plant once the acquisition feeding was accomplished.

Test plant - the healthy plant used for the test feeding of the aphid.

Materials

The soil used was a one to three mixture of peat moss and riverbottom loam. In addition, one measuring cup each of ammonium nitrate and 6-10-4, and one-half cup of lime per 22-25 gallons of soil was added to increase fertility. The fertilizers were thoroughly mixed with the soil to insure good dispersal.

Vigna sinensis cv. Early Ramshorn cowpea and later Phaseolus vulgaris L. cv. Gill's Reliable bush bean were the main test plants used throughout the investigation.

Chenopodium amaranticolor Coste and Reyn and Phaseolus vulgaris cv. Pencil Pod Black Wax were used as indicator plants. Chenopodium responded best when used as young plants eight to ten inches in height; only the fully expanded leaves, excluding the top four to five purple leaves, were inoculated. Pencil Pod Black Wax was most susceptible in the primary leaf stage.

Clay pots were used for all plants. Seeds were treated with Phygon, a fungicide, at the time of sowing.

The green peach aphid, Myzus persicae (Sulzer), was the insect used in experiments on the mechanism of transmission. Brassica pekinensis Rupr., Chinese cabbage, was used as the colony plant for the green peach aphid. New colonies of the insects were started by single parthenogenic individuals, a culture maintained by Dr. K. G. Swenson for several years. This was done to keep genetic variation in the aphids to a minimum. The colony plants were individually caged and kept in a greenhouse separate from the test plants to prevent contamination.

Methods

Aphid Inoculation. For each experiment, apterous aphids, other than very young nymphs, were obtained from thriving colonies, and, when possible, from the same mature colony plant leaf. The

aphids were placed in Erlenmeyer flasks for a preliminary starvation period of 15 minutes or longer.

At the end of the preliminary starvation period, the aphids were removed from the flasks and placed on the desired source plant leaf with a moistened camel-hair brush. The source plants were clipped of all but the most viruliferous leaves (generally the younger leaves identified by the usual expression of virus symptoms). For each experiment, the aphids were allowed to feed on only one leaf so as to make the replications as uniform as possible. The aphids were observed through a 10X hand lens and the 10- to 60-second feeding periods were measured with a stopwatch. The periods were measured from the time the tip of the proboscis contacted the leaf surface until it was removed. Those aphids which fed for less than ten seconds or more than 60 seconds were discarded.

The aphids were placed on test plants in the early primary leaf stage. The plants were enclosed with eight-inch glass chimneys to restrict the aphids to the test plants.

Once the 10- to 60-second tests were completed a small cage, consisting of a four-inch plastic glass with the bottom cut out, was placed on the source plant such that the desired source leaf was entirely within the cage. A paper back was fashioned around the stem and attached to the glass by rubber bands. Starved aphids,

that were to feed for one hour or more, were placed inside and the open end was closed with gauze. The aphids, restricted to the chosen leaf, could be left in this manner for 24 hours or longer.

At the end of the period, those aphids found feeding on the leaf were utilized in the tests, while those not in a feeding position or found crawling on the glass were discarded. When the appropriate test feeding periods elapsed, the plants and aphids were fumigated, checked for living aphids, and put back into the greenhouse.

Mechanical Inoculation. The following technique was used for mechanical inoculation. Leaf tissue was ground in one percent potassium dibasic phosphate buffer to improve transmission by prevention of inactivation of the virus in the expressed sap. Prior to applying the inoculum to the test leaf, the upper surface was dusted with carborundum (400-mesh silicon carbide). The test leaf was supported from below by the left hand while the inoculum was applied by a finger of the opposite hand. The finger was dipped into the inoculum at the end of each one or two strokes until the entire surface of the leaf was covered. Finger pressure was moderated according to the "feel" of the leaf being inoculated; in most cases only moderate pressure was necessary. The mortars and pestles were washed with tap water and detergent at the end of each

transfer if other sources of inoculum were used. A possibility of contamination was thus avoided.

Chenopodium amaranticolor was heavily relied upon as the local-lesion indicator plant in the first experiments. Later, Pencil Pod Black Wax bush bean was found to be a more reliable indicator.

RESULTS

Virus Transmission Tests Employing Myzus persicae (Sulzer)

The first four experiments involved two acquisition feeding periods, 10 to 60 seconds and 1 to 4 hours, and two test feeding periods, 15 minutes and 12 to 18 hours on the test plants. This gave the following four treatment combinations:

Acquisition Feeding Period	Test Feeding Period
10 to 60 seconds	15 minutes
10 to 60 seconds	12 to 18 hours
1 to 4 hours	15 minutes
1 to 4 hours	12 to 18 hours

The source of the virus for the first two experiments was a mechanically inoculated Black-eye cowpea. The source for the third experiment was an aphid-inoculated Early Ramshorn cowpea retained from the second experiment. Mechanically inoculated Early Ramshorn cowpea and Gill's Reliable bush bean, and an aphid-inoculated Early Ramshorn cowpea retained from Experiment 3 were used for the fourth experiment. Black-eye and Early Ramshorn cowpeas were used as test plants for the first three experiments, and Gill's Reliable bush bean was used as the test plant in Experiment 4.

In the first two experiments, one aphid per plant was used,

but the number was increased to five per plant in Experiment 3 and ten per plant in Experiment 4 as the low rate of transmission became obvious.

After aphid inoculation, the test plants in the first three experiments were maintained for three to four weeks to allow enough time for proper virus symptom development. At the end of the period, plants with virus-like symptoms were retained and the sap from a leaf of each was mechanically inoculated into Chenopodium for recovery of the virus.

Transmission was low (Table 1). The best combination of feeding periods (10 to 60 seconds, 12 to 18 hours) produced only 10 infected plants out of 150 test plants.

Due to the poor results from the first three experiments using cowpeas as test plants, Gill's Reliable bush bean was used as the test plant in the fourth experiment. Gill's Reliable was found to be a better test plant; expression of virus symptoms was much improved, particularly after aphid inoculation. As before, test plants were held for a three-to four-week period after aphid inoculation. At the end of the period, plants with virus-like symptoms were retained and the sap from a leaf of each was mechanically inoculated into Chenopodium.

The increase in transmission for each combination of feeding

periods was quite pronounced (Table 1). The best combination of feeding periods was 10 to 60 seconds, 12 to 18 hours; eight of nine test plants were infected. Thus far, this feeding period combination showed the highest rate of virus transmission in the four experiments.

Because of the improved rate of virus transmission in Experiment 4, the feeding conditions for the fifth experiment were revised to include feeding periods of 10 to 60 seconds and 4 to 24 hours on the source, with a 24-hour feeding period on the test plant. A third combination consisted of 4 to 24 hours on the source and 24 hours on the test plant, with a four-day intermediate feeding period on Chinese cabbage included to establish a latent period, if present.

Acquisition Feeding Period	Intermediate Feeding Period	Test Feeding Period
10 to 60 seconds	none	24 hours
4 to 24 hours	none	24 hours
4 to 24 hours	4 days on Chinese cabbage	24 hours

The source plant was an aphid-inoculated Gill's Reliable bush bean from the fourth experiment; the test plants were also Gill's Reliable. One aphid per plant was used.

Once again, test plants were held for three to four weeks

after aphid inoculation and sap from the suspected plants was inoculated into Chenopodium. As predicted from a count of the diseased plants before virus recovery, transmission was poor (Table 2). Virus was transmitted to only 2 of 50 test plants (10 to 60 seconds, 24 hours). Although some plants of the second combination of feeding periods appeared to be diseased, no virus was recovered to Chenopodium. No virus was recovered to Chenopodium from plants of the third feeding combination and there was no indication of transmission.

The following feeding combinations were used in the sixth experiment:

Acquisition Feeding Period	Test Feeding Period
10 to 60 seconds	24 hours
1 hour	24 hours
2 hours	24 hours
4 hours	24 hours
8 hours	24 hours
23 hours	24 hours

A mechanically inoculated Bachicha bean and an aphid-inoculated Gill's Reliable bush bean were used as source plants. The test plant was Gill's Reliable bush bean. One aphid was used per test plant. Chenopodium was discarded as the indicator plant in favor of Pencil Pod Black Wax bush bean. Evidence, based on prior experience, indicated that many test plants with virus-like

symptoms, subsequently shown to be infected, failed to produce lesions when virus recovery was attempted to Chenopodium. Pencil Pod Black Wax bush bean was found to be susceptible when inoculated with virus from these same plants.

After three to four weeks, virus recovery was accomplished following the procedures already established. The presence of the virus was indicated by an epinastic reaction in which the petioles of the primary leaves formed an obtuse angle of approximately 135 degrees with the stem of the indicator plant.

Average transmission of the virus by aphid inoculation from the Gill's Reliable and Bachicha source plants increased from 8 of 30 test plants (10 to 60 seconds) to 16 of 30 test plants (23 hours) (Table 3), indicating that transmission increased with increasing time on the diseased plant.

Virus Transmission Tests with Three Aphid Species

Two aphid species, Brachycaudus helichrysi Kaltenbach, the plum leaf-curl aphid, and Myzus cerasi (F.), the black cherry aphid, were tested concurrently for the ability to transmit the virus. The source plant was an aphid-inoculated Early Ramshorn cowpea; the test plants were also Early Ramshorn cowpeas. The feeding periods were:

Acquisition Feeding Period	Test Feeding Period
10 to 60 seconds	15 minutes
10 to 60 seconds	12 to 18 hours
1 to 4 hours	15 minutes
1 to 4 hours	12 to 18 hours

There were eight test plants, four per test, with one plant for each feeding combination. Ten aphids per plant were used. The indicator plant was Chenopodium. Four weeks after aphid inoculation, virus recovery to Chenopodium was attempted. Lack of recovery to Chenopodium indicated no transmission in either test.

The pea aphid, Macrosiphum pisi Kaltenbach, was the third aphid species tested. Pea aphids were placed on the source, an aphid-inoculated Gill's Reliable bush bean, for four hours; then on four Gill's Reliable test plants for 24 hours, ten aphids per plant. After three weeks, definitive symptoms of the virus appeared in four out of four test plants. No attempt was made to recover the virus to Chenopodium.

Susceptibility of Prunus mahaleb

Several attempts were made to transfer the virus from P. mahaleb to test and indicator plants via aphid and mechanical inoculation. Aphid transmission by Myzus persicae to Black-eye cowpea was attempted from a P. mahaleb seedling budded from a

viruliferous peach by Dr. J. A. Milbrath. The feeding period on the source was 23 hours. The aphids were transferred to four cowpeas, 25 aphids per plant, for a test feeding period of 96 hours. No transmission of the virus to the cowpeas was evident after six weeks.

Sap from young, viruliferous leaves of the same P. mahaleb budded by Dr. Milbrath were macerated and mechanically inoculated into the leaves of six Chenopodium plants. Every fourth leaf of each plant was left as a check. No lesions appeared after 23 days.

An attempt was made to transmit the virus from P. mahaleb to Chenopodium by aphid. The virus source was ten leaves of an infected P. mahaleb growing on the Oregon State University Plant Pathology Farm, Corvallis, Oregon. The leaves were divided and placed into five petri dishes. Aphids were placed on the leaves and allowed to feed for four hours. They were then transferred to four Chenopodium plants, 25 aphids per plant, for a test feeding period of 20 hours. No lesions were produced after two weeks.

An aphid-inoculated Gill's Reliable bush bean was the virus source in an attempt to inoculate four P. mahaleb seedlings by aphids. The aphids were allowed to feed on the source for three hours, then were transferred to the test plants, ten aphids per plant. The aphids were allowed to remain on the test plants for

24 hours. After six weeks, leaf patterns developed indicating possible virus. Sap from terminal leaves of each P. mahaleb test plant was mechanically inoculated to four Gill's Reliable bush beans. Virus-like symptoms developed in some of the beans after seven weeks. Sap from the leaves of the plants suspected to be infected in each groups was mechanically inoculated to three Pencil Pod Black Wax bush beans. An epinastic reaction appeared in the indicator plants that had been inoculated with sap from plants of three of the four groups of Gill's Reliable bush beans. However, the symptoms were weak.

Plants Tested as Possible Indicators

Numerous plant varieties, selected on the basis of availability, were tested for use as possible indicator plants by mechanical inoculation (Table 4). The virus source was Black-eye cowpea. Plants found to react positively were: Phaseolus vulgaris cvs. Bachicha, Dwarf Horticultural, Gill's Reliable, Michelite Pea, Pencil Pod Black Wax, and Pure Gold Wax; Vigna sinensis cv. Early Ramshorn cowpea.

Table 1

Transmission by aphids (Myzus persicae) having acquisition feedings and test feedings of various durations

Experiment	Aphids per plant	Feeding Combinations: Acquisition Feeding Period	Feeding Combinations: Test Feeding Period	Results
1	1	10 to 60 seconds	15 minutes	*0/50
		10 to 60 seconds	12 to 18 hours	9/50
		1 to 4 hours	15 minutes	5/50
		1 to 4 hours	12 to 18 hours	3/50
2	1	10 to 60 seconds	15 minutes	1/50
		10 to 60 seconds	12 to 18 hours	0/50
		1 to 4 hours	15 minutes	1/50
		1 to 4 hours	12 to 18 hours	1/50
3	5	10 to 60 seconds	15 minutes	0/50
		10 to 60 seconds	12 to 18 hours	1/50
		1 to 4 hours	15 minutes	0/50
		1 to 4 hours	12 to 18 hours	0/50
4	10	10 to 60 seconds	15 minutes	7/9
		10 to 60 seconds	12 to 18 hours	8/9
		1 to 4 hours	15 minutes	5/9
		1 to 4 hours	12 to 18 hours	5/9

* Number of plants infected/total number of plants in series

Table 2

Transmission by aphids (Myzus persicae) having acquisition feedings and test feedings of various durations

Experiment	Aphids per plant	Feeding Combinations: Acquisition Feeding Period	Intermediate Period	Test Feeding Period	Results
5	1	10 to 60 seconds	none	24 hours	*2/150
		4 to 24 hours	none	24 hours	0/150
		4 to 24 hours	4 days on Chinese cabbage	24 hours	0/150

* Number of plants infected/total number of plants in series

Table 3

Transmission by aphids (Myzus persicae) having acquisition feedings and test feedings of various durations

Experiment	Aphids per plant	Feeding Combinations: Acquisition Feeding Period	Feeding Combinations: Test Feeding Period	Results
6	[a] 1	10 to 60 seconds	24 hours	* 3/15
		1 hour	24 hours	6/15
		2 hours	24 hours	3/15
		4 hours	24 hours	6/15
		8 hours	24 hours	4/15
		23 hours	24 hours	7/15
	[b] 1	10 to 60 seconds	24 hours	5/15
		1 hour	24 hours	1/15
		2 hours	24 hours	6/15
		4 hours	24 hours	6/15
		8 hours	24 hours	6/15
		23 hours	24 hours	9/15

* Number of plants infected/total number of plants in series

[a] - Gill's Reliable bush bean used as the source plant

[b] - Bachicha bush bean used as the source plant

Table 4

Plants tested as possible indicators

Latin Name	Cultivar	Result
Asclepsia tuberosa L.	Butterfly Milkweed	* 0/10
Brassica oleracea L.	Copenhagen Market	0/5
Brassica Rapa L.	Purple Top White Globe	0/10
Callistephus chinensis Ness.	Crego Pink	0/10
Cucumis sativus L.	Early Fortune	0/5
Ipomoea L.	Candy Pink Giants	0/10
	Flying Saucers	0/10
	Scarlet O'Hara Giants	0/10
	Summer Skies	0/10
Lycopersicum esculentum Mill.	Bonny Best	0/5
Petroselinum hortense Hoffm.	Triple Moss Curled	0/5
Phaseolus vulgaris L.	Bachicha	5/5
	Blue Lake	0/5
	Dwarf Horticultural	4/5
	Gill's Reliable	5/5
	Improved Supergreen	0/5
	Michelite Pea	5/5
	Pearlgreen	0/5
	Pencil Pod Black Wax	5/5
	Pure Gold Wax	5/5
	Top Crop	0/5
	Wadex	0/5
Pisum sativum L.	Lincoln	0/5
Vicia faba L.	Windsor	0/5

* Number infected/number inoculated

Table 4 (Cont'd)

Plants tested as possible indicators

Latin Name	Cultivar	Result
Vigna sinensis (L.) Endl.	Black cowpea	0/5
	Cream Crowder cowpea	0/5
	Early Ramshorn cowpea	5/5
Zea mays L	Golden Cross Bantam	0/10
Zinnia elegans Jacq.	California Giant Double	0/10
	Cherry Sun Pac	0/10
	Dahia Flowered	0/10
	Floradale Scarlet	0/10
	Giant Fantasy	0/10
	Peppermint Stick	0/10

DISCUSSION

When cowpea was used as the test plant, difficulty was experienced in separating the infected plants from the uninfected after aphid inoculation. Some infected plants showed an indefinite thickening of some leaves, a degree of leaf curling, and some evidence of local lesion development in the plants more severely infected, whereas other plants were essentially symptomless. The appearance of similar conditions of leaf thickening and curling in uninoculated plants further complicated separation of the test plants. When attempting to recover virus from those plants with virus-like symptoms to Chenopodium, the results were often negative. Therefore, a more satisfactory test plant was sought.

The bush beans expressed symptoms similar to those that appeared in cowpea; however, they were more pronounced. In addition, shortly after aphid inoculation, the primary leaves often showed a yellow, necrotic area that expanded into an area often an inch or more in diameter where the aphid apparently had fed. The leaves often expressed vein-banding. Those plants that had not been infected by aphid inoculation had an obviously healthy appearance. Many plants expressed virus-like symptoms, but virus recovery to Chenopodium, as with cowpea, was often unsuccessful.

However, these and similar plants were subsequently shown to be infected by recovery of virus to Pencil Pod Black Wax bush bean.

The unreliability of Chenopodium was suspected, but not demonstrated until Pencil Pod Black Wax was shown to have indicator plant capabilities. Individual Chenopodium plants often did not produce local lesions when inoculated from plants known to be infected. This was apparently due to the variation among individual Chenopodium plants and to factors such as age of the plant, age of the inoculated leaf, time of day inoculated, and perhaps light and growing conditions affected by the time of year.

Despite generally poor results, the transmission tests produced enough data to show that the virus seems to be stylet-borne, and may be semi-persistent in nature. The reasons for this conclusion are the following: (1) the virus was easily sap transmissible; (2) there was no conspicuous difference in transmission between the short (10 to 60 seconds) and the long (23 hours) acquisition feeding periods, which is indicative of a semi-persistent virus; and (3) persistent viruses are virtually never transmitted with a 60-second acquisition feeding period.

SUMMARY

On the basis of this investigation, several statements may be made. Bush beans were better test plants than cowpea. Virus symptoms such as leaf curling and local lesions were similar to those symptoms in cowpea, but expression was more pronounced. In addition, development of a yellow, necrotic area on the primary leaves after aphid inoculation and vein-banding made possible early recognition of infected test plants.

Chenopodium was found to be unreliable as an indicator plant. Often local lesions did not develop when inoculated with sap from plants known to be infected. Pencil Pod Black Wax proved to be an excellent indicator plant.

Transmission of the virus by the green peach aphid, Myzus persicae, and the pea aphid, Macrosiphum pisi, has been demonstrated. Tests with the black cherry aphid, Myzus cerasi, and the plum leaf-curl aphid, Brachycaudus helichrysi, gave negative results but should be repeated when the transmission pattern of the virus is better known. While not conclusive, the virus seems to be stylet-borne and may be semi-persistent in nature.

Virus transmission to P. mahaleb by aphid inoculation from Gill's Reliable bush bean was successful. Sap from the P. mahaleb

seedlings suspected of being infected was mechanically inoculated to other Gill's Reliable bush beans and virus was recovered to Pencil Pod Black Wax bush beans. However, the epinastic reaction was weak.

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