RUBUS VIRUSES IN BRITISH COLUMBIA AND THEIR RELATIONSHIP WITH THE APHID VECTOR *AMPHOROPHORA RUBI* KALTENBACH

by

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RUBUS VIRUSES IN BRITISH COLUMBIA AND THEIR RELATIONSHIP WITH THE APHID VECTOR *AMPHOROPHORA RUBI* KALTENBACH

INTRODUCTION

PURPOSE OF RESEARCH

In British Columbia, as in other parts of Canada and the United States where raspberries are produced commercially, growers and agriculturists have noted that many *Rubus* varieties tend to decline in productivity until they are no longer profitable. The most notable example in British Columbia is the Cuthbert variety of red raspberry which was, at one time, the most extensively planted commercial variety. To-day it has been almost completely replaced.

The factors that have contributed to the general decline of *Rubus* varieties include soil depletion, root rots, and virus diseases. This study was undertaken to investigate the relation of virus diseases to this decline with the hope that critical identification of the viruses would lead to an effective method of indexing *Rubus* varieties for viruses. Ultimately, the knowledge gained should assist in the establishment of a sound certification program.

PLAN OF RESEARCH

From a study of the literature, the need for three principle phases of research was apparent, namely :

(1) A survey of Rubus plantings in British Columbia and collection of virus-diseased material;

(2) Determination of a suitable indicator plant for Rubus viruses; and

(3) The separation of distinct virus entities and a study of their virus-vector relationships.

Cultivated Rubus plantings in the Fraser Valley region of British Columbia were surveyed to ascertain the extent of virus infection and to obtain sources of inoculum for experimental purposes. Clones of virus-diseased plants were established in a plot on the campus of the University of British Columbia. Most of the diseased material was obtained in the Fraser Valley, but some plants originated from other areas of the province.

Wild and escaped Rubus plants were examined also and a few virus-infected clones were established in the experimental plot.

In the second phase, experiments were undertaken to determine suitable indicator plants for Rubus viruses. It was considered essential to obtain an indicator plant having the following characteristics:-

(a) Ability to develop distinctive symptoms when infected with different viruses;

(b) Efficacy for inoculation by insect vectors; and

(c) Availability in large numbers.

Such an indicator plant was not available in abundance until 1953, consequently most of the virus-vector studies could not be undertaken until that year.

ETIOLOGY OF RUBUS VIRUS DISEASES

Research work on virus diseases of raspberry has been concentrated in three regions, namely : Great Britain, eastern North America and the west coast of North America. In each of these areas virus diseases have been observed and described usually by symptoms produced when a particular virus was inoculated into some commercial variety. As latent viruses are now known to be carried by a number of commercial varieties, the investigators often unwittingly described the symptom expression of a virus complex

rather than that of a single virus. Symptom expression also tends to vary on different varieties and under different environmental conditions. Since investigations proceeded independently in the three areas, there is a lack of uniformity in nomenclature and, generally speaking, analogous virus diseases have not been related.

In the following paragraphs Rubus virus diseases are divided arbitrarily into four groups based on the type of symptom they produce. Since some of these diseases have not been studied critically and comparative studies have not been made between the groups, the following classification, based largely on original descriptions, is tentative.

GROUP I - MOSAIC DISEASES : These diseases are characterized by a mottling or blotching of the foliage of various Rubus species. Some Rubus species or varieties are symptomless carriers of the viruses that cause the diseases in this group.

(a) Mild mosaic : Described by Dodge and Wilcox (20, p.3) in 1926 and by Bennett (2, pp.8-9) in 1927. Characterized by a mottling of the foliage of black raspberry.

(b) Red raspberry leaf mottle : Described in 1932 by Bennett (7, pp.8-9) as medium severe red-raspberry

mosaic and in 1951 by Cadman (8, pp.801-811) as leaf mottle. Characterized by a mild mottling of some varieties of red raspberry and necrosis of the terminals of black raspberry.

(c) Red raspberry leaf spot : Described in 1940 by Harris (22, pp.340-341) as Mosaic 2 and in 1952 by Cadman (12, pp.501-508) as leaf spot. Characterized by translucent, chlorotic, angular spots on the leaves of some varieties of red raspberry.

(d) Black raspberry streak : Described in 1922 by Wilcox (39, pp.1-12) as eastern blue-stem and in 1924 by Zeller (43, pp.9-11) as streak. Characterized by a stunting of the plant, a discoloration of the stems, and a curling and mottling of the leaves. This disease is known to occur only on black raspberry.

(e) Yellow blotch mosaic : Described on red raspberry in 1938 by Chamberlain (15, pp.118-124) as yellow blotch curl in Ontario. A similar disease was later called necrotic fern leaf mosaic by Chamberlain (16, pp.119-124). Described in 1943 by Zeller and Braun (45, pp.156-161) and in 1944 by Zeller and Schuh (47, pp.7-10) as decline in Oregon. Described in 1952 by Cadman (14, pp.212-214) and 11, pp.495-500) as

yellow blotch and severe yellows in Scotland. Described on wild blackberry in 1948 by Horn (23, pp.827-830) and on Loganberry in 1951 by Wilhelm, Thomas, and Koch (42, p.11) as yellow blotch mosaic. The foliage of infected plants shows yellow blotching or a ring spot mottling. Leaves may be slightly downcurled and the plant may be slightly stunted.

GROUP II - VEIN CHLOROSIS DISEASES : These diseases are characterized by the development of prominent chlorosis along the veins and the veinlets of the leaves. Beyond the tissue bordering the veins chlorosis is apparent as an irregular diffuse mottle.

(a) Yellow mosaic : Described by Bennett (2, p.9), 3, p.13) and 7, pp.9-10) as yellow mosaic. The tissue along the veins of the leaves of a diseased plant fades and the plant has a general yellow cast and is somewhat stunted. This is a disease of both black raspberries and red raspberries.

(b) Vein chlorosis : Described by Cadman (9, pp.61-68) as red raspberry vein chlorosis. Symptoms are analogous to yellow mosaic symptoms on red raspberry but the disease has not been described on black raspberry.

GROUP III - CURLY DWARF DISEASES : These diseases are characterized by a stunting of the plant and a down-curling of the foliage. Mottle and vein chlorosis are absent.

(a) Leaf curl: Distinguished from raspberry mosaic by Rankin and Hockey (33, pp.253-264) in 1922. Studied by Bennett (6, pp.787-802) in 1930. Characterized by a stunting of the plant and a tight curling of the foliage.

(b) Rubus stunt: Described in California by Wilhelm, Thomas, and Jensen (41, p.919) in 1948 as a dwarfing disease of bramble fruits. Later named Rubus stunt by Prentice (30, pp.35-42) who described the symptoms of the disease on several Rubus species in southern England. Characterized in all hosts by the production of very numerous weak canes.

(c) Curly dwarf: Described by Prentice and Harris (31, pp.122-127) in 1950 as "Curly Dwarf" of red raspberry. Characterized by the downward curling of the leaves and slight stunting of the plant.

GROUP IV - COMPLEX DISEASES : These diseases are characterized by Group I symptoms combined with symptoms of Group II or Group III. These diseases have been

described as being caused by a single virus. From a critical review of the literature on severe leaf curl and blackberry dwarf and from investigations of red raspberry mosaic, it is concluded that more than one virus is concerned in the etiology of each of these diseases.

(a) Red raspberry mosaic: First described by Stewart and Eustace (37, pp.362-364) in 1905 as raspberry yellows. This disease was later called raspberry mosaic by Rankin and Hockey (33, pp.253-264) in 1922, red raspberry mosaic by Bennett (2, pp.6-8) in 1927, red mosaic by Rankin (32, pp.19-20) in 1931, green mosaic by Cooley (19, pp.44-56) in 1936, Mosaic 1 by Harris (22, pp.340-341) in 1940, and veinbanding disease by Cadman (10, pp.69-77) in 1952. Characterized by the pronounced chlorosis of the tissue bordering the veins, a puckering of the interveinal tissue, and a downcurling and distortion of the leaf margins.

This disease probably results from the combined infection with the viruses that cause yellow mosaic disease and leaf mottle disease.

(b) Severe leaf curl: Described by Cadman and Harris (13, pp.201-211) in Scotland as raspberry Leaf Curl disease. A similar disease was also reported in British Columbia (18, p.96). Characterized by the production of

small tightly downcurled leaves that are blotched with conspicuous yellow or necrotic areas. The canes are stunted and the plants usually die during the winter following the appearance of this disease.

This disease probably results from the combined infection with the viruses that cause leaf curl disease (as described in America) and one or more of the mosaic diseases.

(c) Blackberry dwarf: Described in 1927 by Zeller (44, pp.629-648) as dwarf of Loganberry and Phenomenalberry in Oregon. The disease was later reported on Youngberry and Boysenberry (46, p.430). Characterized by the production of small, mottled and distorted leaves and a dwarfing of the plant.

This disease probably results from the combined infection with the viruses that cause Rubus stunt and one or more of the mosaic diseases.

This thesis is presented as a critical study of mild mosaic, red raspberry leaf mottle, yellow mosaic, and red raspberry mosaic, representing three of the four groups in this classification.

LEAF MOTTLE AND MILD MOSAIC

INTRODUCTION

Mild mosaic was described by Bennett (2, p.809) in 1927 on black raspberry and in the following year he noted that this mild mosaic also occurred on red raspberry varieties (3, p.12). Later Bennett (5, pp.49-51) and 7, pp.8-10) observed that mild mosaic of red raspberry produced a wide range of symptoms on black raspberry and he suggested that this range of severity might be produced by strains of one virus. Mild mosaic symptoms also occur on the purple raspberry and this source was used as inoculum in transmissions to black raspberry by Cooley (19, pp.44-56). The symptoms on black raspberry ranged from a mild mottling to a severe mottling accompanied by a necrosis of the stem tip and leaf petioles. Cooley concluded that one virus was involved and that the varying reactions obtained were due to raspberry variety differences and to the influence of environmental conditions.

More recently, Cadman (8, pp.801-811) described a virus disease of raspberry in Scotland that he called leaf mottle. Some red raspberry varieties infected with this disease showed a mild mottling whereas other varieties

were symptomless carriers. When transmitted to black raspberry, by the aphid Amphorophora rubi Kaltenbach, the leaf mottle virus produced acute symptoms within eight days after inoculation. The symptoms included necrosing of the terminals and of the petioles, curling of the leaves and conspicuous yellow blotching of the foliage.

A mild form of mottling occurs on several varieties of raspberry in British Columbia. In the summer of 1950 several affected plants were collected and have since been under observation. Scions from these plants were used for graft transmissions and the virus-etiology of the mottling was established by grafting to healthy red raspberry plants (var. Washington). One symptomless variety, Seedling G, produced mild mottling when grafted on Washington. This mild mottle was occasionally transmitted to red raspberry by the aphid A. rubi but, even when large numbers of aphids were used, transmissions were not obtained consistently. In 1953, when black raspberry seedlings were available in quantity, more extensive aphid transmissions were made to determine the virus-vector relationships. The data obtained proved valuable in correlating this disease in British Columbia with raspberry virus diseases observed in other parts of North America and Europe.

EXPERIMENTAL

TRANSMISSION OF LEAF MOTTLE VIRUS : Black raspberry seedlings were used as indicator plants in transmission experiments. Seeds were stored in the refrigerator during the winter, planted in flats in the spring, and transplanted into pots when about two inches high. Plants were approximately four inches high and growing vigorously before being exposed to aphids.

In the first transmission tests, three sources of inoculum were used : (1) Seedling G variety of red raspberry that was a symptomless carrier; (2) Taylor variety of red raspberry that showed mild mottling; and (3) Munger variety of black raspberry that was severely stunted and mottled.

Aphids were given a twenty-four hour acquisition feeding on these sources and were then transferred to black raspberry seedlings. An aphid was placed on each of five plants from each of these three virus sources. Aphids were allowed to feed for fifteen minutes on the first plant of the series, after which they were transferred individually to other black raspberry seedlings and permitted to feed one hour, three hours and twenty-four hours on other plants in the series. After the last feeding, the aphids were removed and destroyed.

TABLE 1. Transmission* of leaf mottle virus to Rubus occidentalis by serial transfers of aphids from three virus sources

VIRUS SOURCE	DURATION OF TRANSFER FEEDING				
	15 min.	30 min.	1 hour	3 hours	1 day
Seedling G	2/5	3/5	3/5	0/5	0/5
Taylor	1/5	2/5	2/5	0/5	0/5
Munger	1/5	4/5	2/5	1/5	0/5

* Denominator indicates the number of plants inoculated. The numerator indicates the number of plants showing symptoms.

As shown in Table 1, transfer feedings of fifteen minutes were sufficient to transmit a virus from each of the sources, although more transmissions occurred following a longer feeding on the indicator plant. With one exception, aphids failed to transmit the virus to the fourth series of test plants. The aphid, therefore, is normally not capable of infecting the plant one hour and forty-five minutes after leaving the source of inoculum.

SYMPTOMS OF LEAF MOTTLE : The symptoms of infection on the black raspberry seedlings were the same for each of the three virus sources. In most instances, infection was evidenced within one week after inoculation by the downcurling of the tips of the young seedlings. Within a day of this initial symptom, small areas of necrosis

were seen on the young unfolding terminal leaflets (Fig. 1).

FIGURE 1. Leaf and stem necrosis of black raspberry seedling photographed ten days after inoculation with leaf mottle virus.

The stem terminals became markedly downcurled and brittle and then developed dark streaks which spread until the terminals died back two to three inches (Fig. 2).

FIGURE 2. Black raspberry seedling two weeks after inoculation with the leaf mottle virus.

Wilting and necrosing were restricted to the rapidly developing leaf and stem tissue at the terminals and to the buds in the axils of the more mature leaves. The necrotic areas generally occurred on the leaflets on one side of the petiole only (Fig. 3). A general yellow cast was observed on the leaves that were about three inches from the stem terminal, but the more mature basal leaves were unaffected.

FIGURE 3. Terminal leaves from black raspberry seedling one week after inoculation with the leaf mottle virus.

About a month after inoculation the axillary buds immediately below the necrotic area produced shoots, some of which were mottled.

In a later experiment, plants of varying sizes were inoculated with leaf mottle virus. In infected plants less than three inches high, the necrosis extended to the ground level, destroying all the photosynthetic area and axillary buds and most plants died.

SEPARATION OF LEAF MOTTLE AND MILD MOSAIC : In another experiment, the source of inoculum was a Cuthbert plant that showed a mild blotching on the leaves produced in the spring and fall (Fig. 4). Ten aphids were given an acquisition feeding of twenty-four hours on this plant. They were then transferred to black raspberry seedlings, each aphid being placed on a separate plant. After a

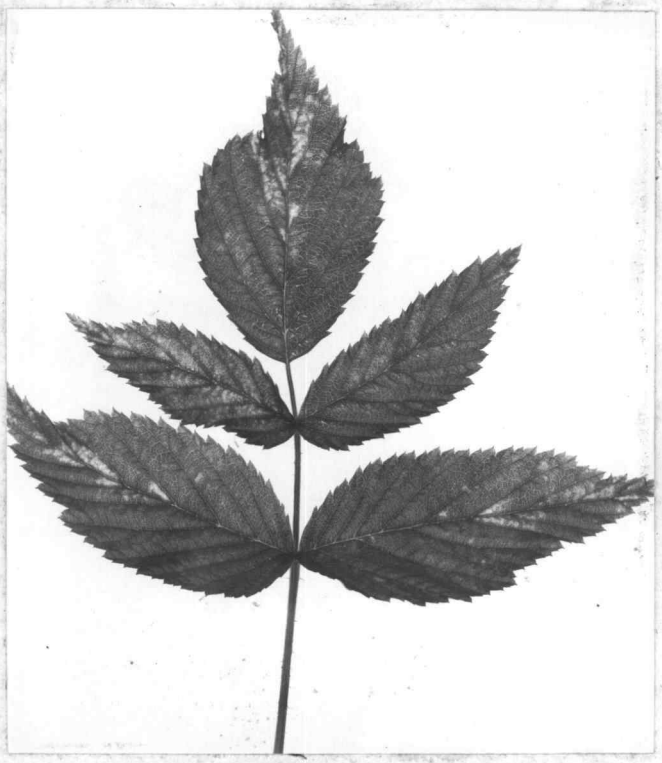

FIGURE 4. Leaf from Cuthbert red raspberry plant affected with complex of mild mosaic and leaf mottle - photographed in the fall.

transfer feeding of five minutes, each aphid was transferred to a second plant for ten minutes, then to a third for fifteen minutes, a fourth for thirty minutes, and to fifth, sixth and seventh plants for one hour each. The results, showing the transmission performance of each aphid, are given in Table 2.

TABLE 2. Incidence of leaf mottle (L) and mild mosaic (M) on Rubus occidentalis following serial transfer of ten single aphids from a Cuthbert raspberry plant infected with a virus complex

TEST APHID NUMBER	DURATION OF TRANSFER FEEDING						
	5 min.	10 min.	15 min.	30 min.	1 hr.	1 hr.	1 hr.
1	-	-	L	L	L	-	-
2	-	L	L	L	-	-	-
3	-	L	L	L	L	-	-
4	M	M	-	-	-	-	-
5	-	-	-	M	M	-	-
6	-	-	-	L	-	-	-
7	M	M	L	L	-	-	-
8	L	-	-	-	-	-	-
9	-	-	-	-	-	-	-
10	-	-	L	L	L	-	-

The plants indicated by "L" in Table 2 developed necrotic symptoms which were attributed to infection with leaf mottle virus. Those indicated by "M", however, did not have such a severe reaction. Their symptoms were attributed either to variation among the indicator plants or to a difference in the virus content. To investigate this, aphids were transferred from a plant with the mild symptoms to ten uniform leaf-bud cuttings taken from a single black raspberry plant (var. Munger). These cuttings developed mild symptoms whereas control cuttings, inoculated with the severe leaf mottle virus, developed the typical necrotic symptoms.

This proved that the symptom differences were not caused by variations amongst the indicator plants but by differences in the virus content. Either a separate virus entity was responsible or else the causal agent was a mild strain of the leaf mottle virus. For the purpose of this discussion, the causal agent of the mild reaction has been designated mild mosaic virus. Possible strain relationship is discussed later.

SYMPTOMS OF MILD MOSAIC : In the group infected with mild mosaic, the terminals wilted slightly about one week after inoculation, but there was no necrosis and the stems did not become brittle and downcurled. Within a

few days normal growth was resumed, except in the case of a few terminal leaves which remained stunted (Fig. 5).

FIGURE 5. Black raspberry seedling three weeks after inoculation with mild mosaic, showing downcurled, stunted leaves remaining after growth of stem had been resumed.

As the leaves on the new stem growth developed, they showed a slight mottling which became pronounced as the leaves matured. The mottling consisted of yellow-green patches which radiated from the main and secondary veins and encircled normal green islands of interveinal tissue. Growth was arrested in these yellowed areas, but the normal tissue continued to expand, with a resultant savoying of the leaf blade (Fig. 6).

When both viruses are simultaneously introduced into black raspberry seedlings, the leaf mottle virus predominates and completely masks the mild mosaic virus.

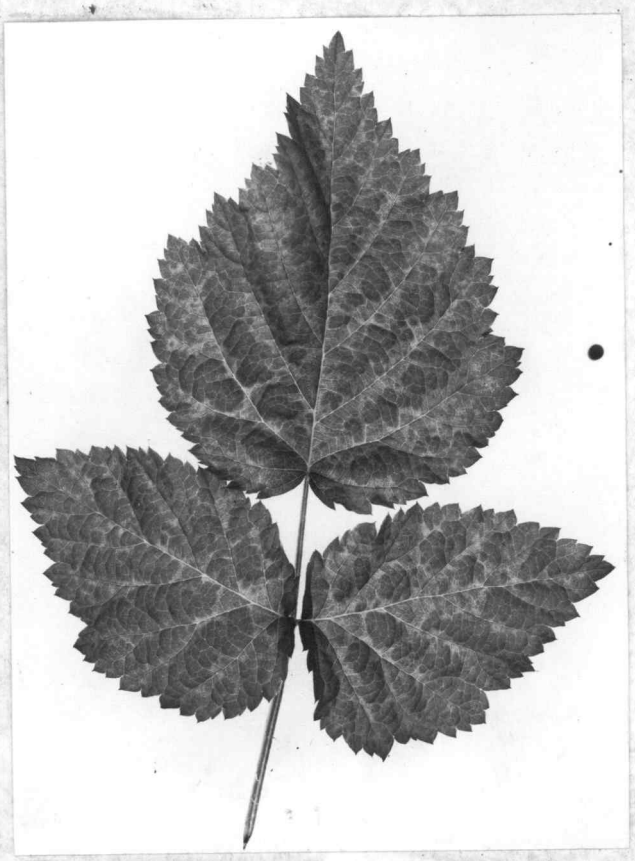

FIGURE 6. Leaf of black raspberry seedling, one month after inoculation with mild mosaic virus, showing yellowed areas encircling islands of normal green tissue with resultant savoying.

Undoubtedly a number of the test plants denoted in Table 2 as infected with the leaf mottle virus were infected with both viruses - the presence of the mild mosaic virus being masked by the more virulent leaf mottle virus.

CROSS PROTECTION TESTS : Aphids were fed for one day on a black raspberry seedling infected with the leaf mottle virus and were then transferred to fifteen plants infected with mild mosaic virus and to fifteen healthy plants as controls. Three aphids were transferred to each of the fifteen plants in each group. Within ten days, fourteen

of the fifteen control plants exhibited typical necrotic symptoms of leaf mottle infection, but none of the plants already infected with mild mosaic virus showed necrotic symptoms. However, within two weeks, some necrotic flecking was noted on a few of the plants in the mild mosaic group and, after a month, nine of the plants were showing definite necrotic symptoms. Apparently the presence of the mild mosaic did not completely protect the plant against infection with the leaf mottle virus but caused symptoms to develop more slowly.

PERSISTENCE OF LEAF MOTTLE AND MILD MOSAIC : As recorded in Tables 1 and 2, after a twenty-four hour acquisition feeding, both viruses persist in the body of the aphid from one to two hours. In later experiments, using acquisition feeding periods varying from five hours to forty-eight hours, the persistence of the virus in the aphid was shown to be unaffected by the length of the acquisition feeding. It was also shown that the aphids were seldom viruliferous for longer than two hours after leaving the source of inoculum. In only a few instances were the leaf mottle and mild mosaic viruses retained by an aphid for more than three hours.

MINIMUM TRANSFER FEEDING : The data in Table 2 show that some transmissions occurred on indicator plants upon

which the viruliferous aphid remained for only five minutes. The aphids in this transfer actually fed for less than five minutes, as they spent some time seeking suitable tissue for penetration before they inserted their stylets. The time lapse between placing the aphid on the test plant and the commencement of feeding - called the pre-penetration time - was taken into account in a later experiment.

Four aphids were first given an acquisition feeding of twenty-four hours on a leaf of a black raspberry plant infected with leaf mottle virus. Each was then placed individually on the lower surface of the leaf of the first of a series of five indicator plants, and observed with a binocular microscope. From the time that the stylet was inserted, each aphid was allowed a transfer feeding of two minutes' duration and was then transferred to the next plant in the series. The aphids were also permitted to feed individually on a check plant for one hour as this time had proved adequate, in earlier experiments, to determine whether or not an aphid was viruliferous.

Two of the series of plants, including the check plant, remained healthy. The two aphids that fed on the plants in these series apparently had not acquired the virus. The two other check plants developed symptoms of leaf mottle infection, as did some of the plants that were

exposed to these aphids for a two-minute transfer feeding. The results, given in Table 3, demonstrate that it is possible for the aphids to transmit leaf mottle virus with a transfer feeding as short as two minutes.

TABLE 3. Transmission of leaf mottle virus (L) to Rubus occidentalis by two-minute transfer feeding of single viruliferous aphids

TEST APHID	DURATION OF TRANSFER FEEDING				
	2 min.	2 min.	2 min.	2 min.	2 min.
1	L	L	-	-	L
2	-	-	L	-	-

ACQUISITION OF LEAF-MOTTLE VIRUS : In an experiment designed to determine the minimum time necessary for an aphid to feed on a virus source in order to acquire the leaf mottle virus, aphids were given varying acquisition feedings on diseased leaves and then transferred to healthy test plants. Three sources of inoculum were used, namely: (1) Himalaya blackberry, Rubus procerus; (2) Seedling G, red raspberry; and (3) Cuthbert red raspberry. The Seedling G and Himalaya blackberry exhibited no symptoms of virus infection and the Cuthbert showed mild mottling as previous described.

After an acquisition feeding of two hours, three

aphids from each source were transferred individually to indicator plants and given a transfer feeding of at least three hours. The aphids were then destroyed, previous experiments having shown that the aphids were non-viruliferous if they were unable to transmit the virus within three hours. The same number of transfers were made after acquisition feedings of three hours, six hours, twelve hours and one day. These experiments, summarized in Table 4, demonstrate that the efficiency of the aphid is not substantially increased by an acquisition feeding longer than two hours.

TABLE 4. Transmission* of leaf mottle virus to Rubus occidentalis from three virus sources by aphids allowed acquisition feedings of varying duration.

VIRUS SOURCE	DURATION OF ACQUISITION FEEDING				
	2 hr.	3 hr.	6 hr.	12 hr.	1 day
Himalaya	2/3	2/3	1/3	3/3	2/3
Seedling G	1/3	2/3	3/3	2/3	3/3
Cuthbert	2/3	2/3	3/3	3/3	3/3

* Denominator indicates number of plants inoculated. The numerator indicates the number of plants showing symptoms.

Since the above experiments showed that the leaf mottle virus was acquired by Amphorophora rubi within two hours, another experiment was conducted using shorter time

intervals to determine the minimum acquisition feeding required to render the aphid viruliferous (called, by some authors, the "acquisition threshold period" (34, p.29). Two aphid groups were used, one of which had fasted in a Petri plate for three hours prior to feeding on the virus source, which in this experiment was a leaf from a mottled Cuthbert plant.

After acquisition feedings of fifteen minutes, thirty minutes, forty-five minutes, one hour, one and a half hours, and two hours, respectively, aphids were transferred to healthy black raspberry seedlings. Five aphids were transferred from each group after each acquisition feeding and were fed singly on the indicator plants for at least three hours. Table 5 gives the results of this experiment.

TABLE 5. Transmission* of leaf mottle virus to Rubus occidentalis by non-fasted and fasted aphids allowed acquisition feedings of varying duration

APHIDS	DURATION OF ACQUISITION FEEDING					
	15 min.	30 min.	45 min.	1 hr.	1½ hr.	2 hr.
Non-fasted	0/5	0/5	0/5	0/5	2/5	4/5
Fasted	0/5	1/5	0/5	2/5	1/5	4/5

* Denominator indicates the number of plants inoculated. The numerator indicates the number of plants showing symptoms.

The minimum acquisition feed required by aphids that were not fasted, was one and a half hours, while one fasted aphid acquired sufficient virus in thirty minutes to transmit the disease. Two other fasted aphids became viruliferous after feeding on the virus source for one hour.

INCIDENCE OF LEAF MOTTLE : Graft inoculations had demonstrated that at least one variety of red raspberry (Seedling G) was a latent carrier of leaf mottle virus. By 1953, a technique had been devised for indexing Rubus plants for the presence of latent viruses. In place of grafting, the aphid Amphorophora rubi was used to transmit this latent virus to black raspberry seedlings, since they had proved to be excellent indicator plants. Rubus plants tested for virus infection included forty-one red raspberry plants and nine blackberry. The results are given in Table 6.

The variety Newburgh is the most widely planted red raspberry in the Fraser Valley region of British Columbia. Three of the four plants of this variety which were tested proved to be symptomless carriers of leaf mottle virus. Washington, the only other variety grown commercially, appears to be free from virus infection. No naturally infected plants of this variety have been found in British

Columbia, and the three indexed plants were free from leaf mottle.

TABLE 6. Incidence of leaf mottle virus in field plantings of red raspberry and blackberry demonstrated by aphid transmission to Rubus occidentalis

VARIETY	NUMBER TESTED	NUMBER DISEASED
RED RASPBERRY		
Cuthbert	4	3
Washington	3	0
Newburgh	4	3
Taylor	2	2
Latham	2	2
Indian Summer	1	1
Seedling G	2	2
Norfolk Giant	1	1
Malling Promise	1	1
Malling Enterprise	1	1
Malling Landmark	1	1
St. Walfried	1	1
Preussen	1	1
Agassiz Seedlings	17	6
BLACKBERRY		
Himalaya	2	2
Boysenberry	3	1
Nectarberry	1	0
Loganberry	3	0

Cuthbert was at one time the most common red raspberry variety in British Columbia, but it has now been largely replaced by Newburgh and Washington. The presence of leaf mottle virus in Cuthbert can be diagnosed without indexing since this variety shows visible symptoms of infection. Several small plantings of Cuthbert red raspberry have been examined in the past few years in British Columbia, and all

have been found to have a mild mottling of the foliage. Of the four plants indexed, all except one proved to be infected. This plant originated from Oregon, and was part of the raspberry certification stock at Oregon State College, having been selected by Dr. E. K. Vaughan[1] because of its vigour and absence of visible virus symptoms.

A few plants of the European varieties, Preussen, Norfolk Giant, Malling Promise, Malling Enterprise, Malling Landmark, and St. Walfried, maintained by the Department of Horticulture at the University of British Columbia, were indexed and all were found to be carrying leaf mottle. Only Norfolk Giant showed any definite symptoms of infection - small chlorotic spots, considered to be symptoms of leaf mottle, having been observed in the fall on the leaves of this variety.

Seventeen promising seedlings, developed from crosses made by the plant breeders of the Canada Department of Agriculture Experimental Farms Service at Agassiz, B.C., were indexed for the presence of latent viruses. Six of these were found to be infected with leaf mottle and, when examined in July, 1953, a very mild mottling was found on

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[1] Plant Pathologist, Oregon State College.

the basal leaves of some of the plants.

Since blackberries are not grown commercially in the Fraser Valley, only a few plants were tested. The tests, however, proved that leaf mottle occurs on the Himalaya blackberry and the Boysenberry.

DISCUSSION

EVIDENCE OF STRAIN RELATIONSHIP : When aphids are transferred from mottled Cuthbert plants to a series of black raspberry seedlings, two symptom types are recognized. One of these is characterized only by a mottling of the foliage: in the other, more severe type, a down-curling and death of the terminals is caused. When aphids are permitted to feed on indicator plants that are mottled but not necrotic, and are then transferred to healthy black raspberry seedlings, these seedlings develop only the milder symptoms. These tests demonstrate that the distinction between the mild and severe symptoms is not related to the influence of environment on the inoculated indicator plants, especially since the indicator plants were reasonably uniform and were incubated under controlled greenhouse conditions. It must be assumed that the mild mottling found on Cuthbert is caused by a complex of at least two viruses or virus strains, and

that this complex is separable by aphid transmissions.

Knight (27, pp.53-54) has summarized criteria which may be used somewhat arbitrarily to demonstrate strain relationship between two viruses as follows:-

(1) A similarity in host range.
(2) Coincidence of chemical and physical properties.
(3) Possession of common size and shape.
(4) Positive cross-interference tests.
(5) Positive serological cross reactions.
(6) Similar response to genetic change in host.
(7) Similar method of transmission.

He also points out that the argument for strain relationship is strengthened as an increasing number of the criteria are satisfied though, on the other hand, failure to satisfy any one of the criteria does not necessarily preclude strain relationship.

Because of the intangible nature of the Rubus viruses, several of the criteria (e.g. coincidence of chemical and physical properties; possession of common size and shape; and positive serological cross reactions) are inapplicable because no technique has yet been devised to purify or photograph these viruses.

For systemic viruses that are not sap-transmissible, one of the most valuable criteria is cross interference or cross protection. When the virus from a plant with necrotic symptoms is introduced into a plant with the mottle symptoms, necrotic symptoms eventually develop in the mottled plants but there is a considerable delay in the development of these necrotic symptoms. According to Bawden (1, p.272) plant protection tests are most easily made, and give most definite results, when one of the two strains involved produces local lesions. However, the method can be used reliably when both produce systemic symptoms provided these are sufficiently distinctive. Delay in development of symptoms characteristic of infection by the second strain introduced is usually sufficient to indicate interference with its progress. Consequently, the delay in symptom expression of the necrosis-producing virus indicates a strain relationship with the mottle-producing virus.

TERMINOLOGY : The symptoms of leaf mottle in Scotland, as described by Cadman (8, pp.801-811), are identical with the symptoms of leaf mottle in British Columbia. In both localities, some red raspberry varieties behave as symptomless carriers and other exhibit a mild mottling. When transmitted to black

raspberries, both produce necrosis of the terminals and leaf petioles and a mottling of the leaves.

The above evidence supports the hypothesis that leaf mottle in Scotland and in British Columbia are etiologically identical. Further evidence of this identity was obtained in the present study when a latent virus of several European varieties was found to be identical with the latent virus in American varieties in its symptom response on black raspberry seedlings. Also, the virus-vector relationships of leaf mottle in Scotland are in agreement with those reported here. Cadman (8, pp.807-809) reported that leaf mottle is occasionally acquired by the aphid in half an hour and rarely persists in the vector longer than four hours.

CONCLUSIONS

(1) Two Rubus viruses, termed leaf mottle and mild mosaic, were transmitted from red raspberry to black raspberry with the aphid Amphorophora rubi (Kalt.).

(2) For transmission of leaf mottle virus, the vector required an acquisition feeding of two hours, although fasted aphids acquired the virus with shorter feedings. The virus was transmitted in a two-minute

transfer feeding, and was seldom retained by the aphid longer than two hours.

(3) Mild mosaic virus was indistinguishable from leaf mottle virus in its vector relationships.

(4) Plants infected with mild mosaic virus were protected against infection with leaf mottle virus. This suggested the existence of a strain relationship between the two viruses.

(5) Leaf mottle virus was recovered from several red raspberry and blackberry varieties. The virus was latent in most of these varieties.

YELLOW MOSAIC

INTRODUCTION

LITERATURE : In 1927, Bennett (2, p.9) described yellow mosaic of black raspberry. According to his description, affected plants, soon after infection, begin to produce mottled shoots, the mottle varying from faint splotches to distinct yellow patches. In the following season, the entire infected plant shows symptoms of disease - fruiting shoots are weak and leaflets are flat or, at times, turned down at the edges. Affected plants are short-lived and usually die the second or third winter following infection.

In the following year, Bennett (3, p.13) noted that yellow mosaic occurred on red raspberry and dewberry. In red raspberry, leaflets of affected plants are yellow, due chiefly to fading of tissue along the veins and, also, in most cases the leaves are distinctly cupped. Bennett (7, p.10) elaborated further on the symptoms of yellow mosaic on red raspberry (var. Cuthbert and King), noting that symptoms are late in appearing in the spring and infected plants cannot usually be distinguished until many of the leaves are of full size. After this stage, and throughout the remainder of the season, infection is

usually obvious and symptoms are less masked by summer temperatures than those of some of the other Rubus mosaic diseases.

This virus, like leaf mottle, is transmitted by Amphorophora rubi, and Bennett (7, pp.29-30) demonstrated that it may be acquired by the aphid in two hours and transmitted to a healthy plant during the following forty-eight hour period.

The effect of yellow mosaic in red raspberry varieties was also discussed by Rankin in 1933 (36, pp.34-37) who noted that different varieties vary considerably in their symptom expression.

In British Columbia, no virus disease of raspberry with symptoms corresponding to Bennett's description of yellow mosaic has been observed. However, a virus obtained from Himalaya blackberry and transmitted to red raspberry and black raspberry produced symptoms in these hosts which agree with yellow mosaic as defined by Bennett.

EXPERIMENTAL

SYMPTOMS ON HIMALAYA BLACKBERRY : The mosaic diseased Himalaya blackberry plant was found in an abandoned field near Abbotsford, B.C. A portion of this plant was

transplanted to the laboratory plot in 1950 and has been under observation since. A vein chlorosis was evident when the leaves of this plant unfolded in the spring and, as the summer progressed, this chlorosis became more pronounced and extensive on the foliage of both fruiting canes and current year canes. The chlorosis spread outward, fanwise, from the veins towards the leaf margin, distinctly yellowing large areas of the affected leaves (Fig. 7). Eventually most of the leaves produced during

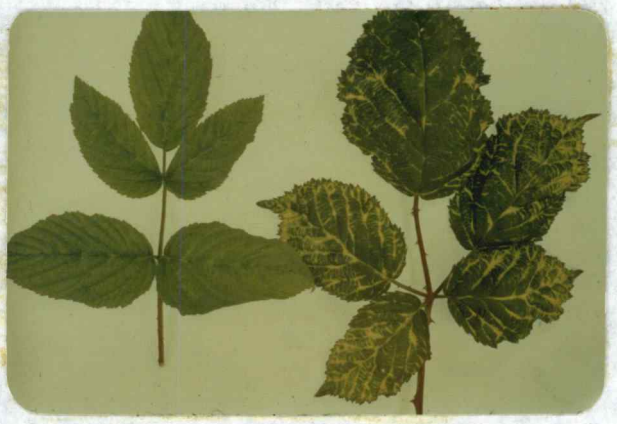

FIGURE 7. Symptoms of yellow mosaic virus on Himalaya blackberry (right) and on Washington red raspberry (left).

the summer were affected to some degree. Frequently symptoms were unilateral, all the chlorosis being restricted to the leaflets on one side of the petiole. By September, growth of the chlorotic areas was largely arrested, resulting in a cupping of the affected leaves but, at the same time, the greater part of the foliage then being produced was normal. This mosaic pattern has been repeated in succeeding years, but there is no apparent

dwarfing of the infected plant.

SYMPTOMS ON RED RASPBERRY : The virus nature of this disease of Himalaya blackberry was established by graft transmission to a red raspberry plant. A mature Washington plant in the field plot was grafted in the summer of 1951 and in the following spring the foliage of this plant was distinctly paler than the ungrafted controls. No distinct mottling was observed on the leaves of the grafted plant but the areas along the veins of the leaves were chlorotic. Most of the leaves produced during the summer were somewhat chlorotic and the plant as a whole remained pale green throughout the growing season. There was no distortion, downcurling, or stunting of the foliage and the plant was not noticeably stunted.

The symptoms on this grafted Washington plant are similar to Bennett's (7, pp.10-11) description of yellow mosaic on red raspberry varieties in Michigan. The virus causing the chlorosis of the Himalaya blackberry plant used as a source of inoculum is considered to be etiologically identical with yellow mosaic virus.

SYMPTOMS ON BLACK RASPBERRY : A colony of aphids (Amphorophora rubi) were permitted to feed for one day on a leaf of Himalaya blackberry infected with yellow mosaic

and then five aphids were transferred to each of ten black raspberry seedlings and given a transfer feeding of one day. Yellow mosaic virus was transmitted to two of the ten plants.

Symptoms of yellow mosaic on black raspberry seedlings can be first noticed three to four weeks after inoculation, when the fourth or fifth leaf beneath the tip of the inoculated plant develops diffuse chlorotic flecks along the veins. The chlorosis on such lower leaves is never extensive and remains confined to small patches (Fig. 8).

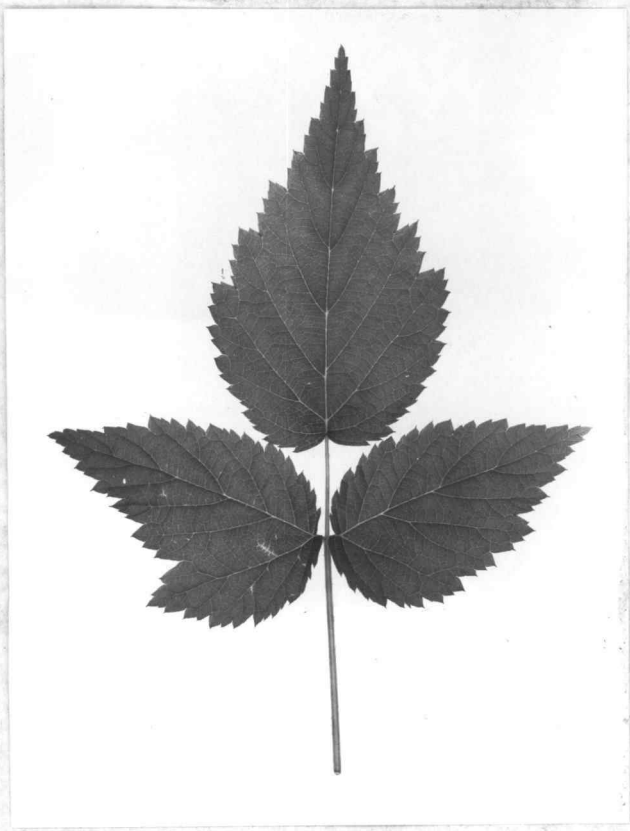

FIGURE 8. First symptom observed on black raspberry plant three weeks after inoculation with yellow mosaic. Photographs shows chlorotic flecks along veins of fifth leaf below terminal.

Within two or three days after the first symptom, vein chlorosis can be observed on the next youngest leaf. Here it is more extensive, presenting a diffuse, net-like appearance, and usually it is unilateral, involving one of the basal leaflets and the lower edge of the terminal leaflet (Fig. 9).

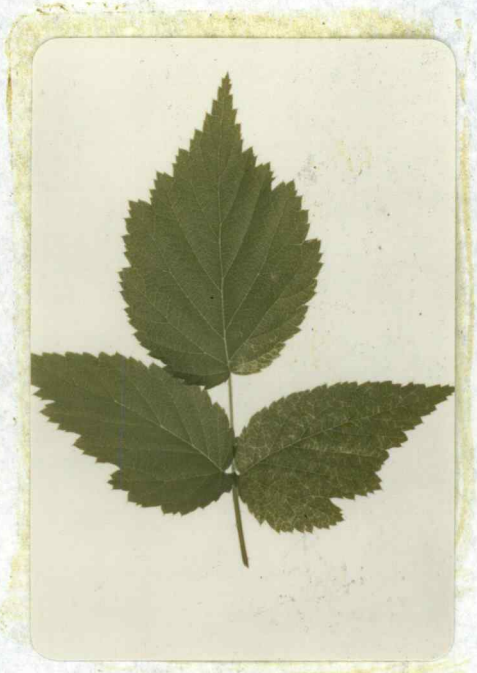

FIGURE 9. Fourth leaf below terminal of affected black raspberry plant three days after onset of symptoms shown in Figure 8.

Symptoms appear on the remaining two or three leaves in rapid succession, the affected areas becoming chlorotic and severely stunted. Since the chlorosis develops unilaterally, one of the basal leaflets is much smaller than the other, and the midrib of the terminal leaflet is bent towards the stunted side (Fig. 10).

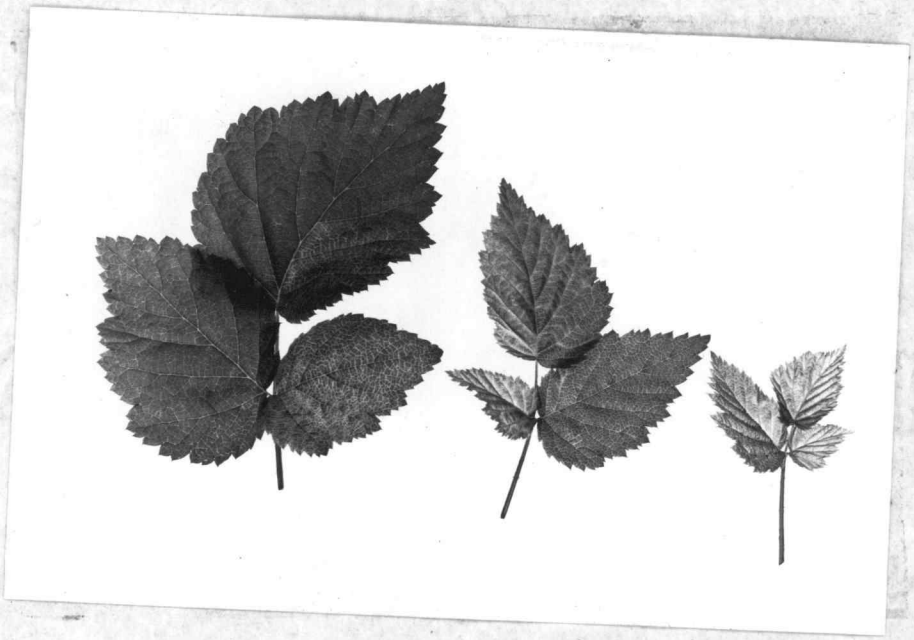

FIGURE 10. Symptoms of yellow mosaic on the terminal leaves of black raspberry one month after inoculation.

The initial symptoms, therefore, involve the four or five terminal leaves, the leaves below the first affected leaf remaining normal. The tip of the plant continues to grow and produces yellow-green leaves that are distorted to varying degrees. The vein chlorosis involves most of the leaf blade (Fig. 11). The affected plant is somewhat stunted.

On black raspberry seedlings, yellow mosaic can be differentiated from leaf mottle virus in three distinct ways, namely - the length of the incubation period in the inoculated plant, the location of the first symptoms, and

FIGURE 11. Chronic symptoms of yellow mosaic on black raspberry

the expression of the chronic symptoms. In leaf mottle infection, symptoms can usually be seen within one week after inoculation whereas, in yellow mosaic, the first symptoms do not appear until three or four weeks after viruliferous aphids have fed on the plant. First symptoms of leaf mottle appear in the terminals and consist of wilting or necrosis, but yellow mosaic is first evident as diffuse flecks of vein chlorosis on a leaf several inches below the tip of the plant. Leaf mottling is the chronic symptom of leaf mottle disease but in

yellow mosaic the chronic symptom consists of varying degrees of vein chlorosis.

PERSISTENCE OF YELLOW MOSAIC : After an acquisition feeding of one day on a leaf from an infected black raspberry seedling plant, nine aphids were placed individually on other black raspberry seedlings and transferred through a series of plants. Each aphid was permitted to feed for fifteen minutes, thirty minutes, one hour, two hours, four hours and sixteen hours, respectively, on the plants in the series. Table 7 gives the results of these transfers.

One or more transmissions occurred in eight of the nine series. Six of the aphids were able to transmit the virus to the first plant in the series, that is, after a transfer feeding of fifteen minutes. In two instances yellow mosaic was transmitted to the fourth plant in the series, but no transmissions occurred in the fifth and sixth plants. In other words, yellow mosaic persists in the body of the aphid for longer than one hour and forty-five minutes but for less than three hours and forty-five minutes.

TABLE 7. Incidence of yellow mosaic (Y) on Rubus occidentalis following serial transfer of single aphids from an infected black raspberry plant

TEST APHID NUMBER	DURATION OF TRANSFER FEEDING					
	15 min.	30 min.	1 hr.	2 hr.	4 hr.	16 hr.
1	Y	Y	Y	-	-	-
2	Y	-	-	-	-	-
3	Y	Y	-	Y	-	-
4	-	Y	Y	Y	-	-
5	Y	-	Y	-	-	-
6	-	-	-	-	-	-
7	Y	-	-	-	-	-
8	-	-	Y	-	-	-
9	Y	Y	-	-	-	-

ACQUISITION FEEDING PERIOD : The feeding time necessary to render aphids viruliferous was then investigated. A colony of aphids was placed on a leaf from an infected black raspberry plant and, at varying intervals, aphids were transferred to other black raspberry seedlings. An aphid was placed on each of five plants after one hour, three hours, four hours, twenty-four hours and forty-eight hours, respectively, and then permitted to feed on the indicator plant for at least three hours. The results, given in Table 8, demonstrate that yellow mosaic can be

acquired by the aphid in a three-hour acquisition feeding and the virus is transmitted as efficiently after a four-hour acquisition feeding as after a longer feed.

TABLE 8. Transmission* of yellow mosaic virus to Rubus occidentalis by aphids allowed acquisition feedings of varying duration

DURATION OF ACQUISITION FEEDING				
1 hour	3 hours	4 hours	24 hours	48 hours
0/5	1/5	5/5	3/5	4/5

* Denominator indicates the number of plants inoculated. The numerator indicates the number of plants showing symptoms.

DISCUSSION

The virus disease which was found in British Columbia on Himalaya blackberry can, on the basis of its symptom production on red raspberry and black raspberry, be identified with yellow mosaic as described by Bennett. However, it does not appear to correspond to any virus disease described in Europe, with the possible exception of moderate vein chlorosis as described by Cadman (9, pp.62-65) on European varieties of red raspberry. Cadman's virus, although producing a vein chlorosis, is rarely, if at all, transmitted by Amphorophora rubi, and

for this reason it cannot be identified with yellow mosaic.

CONCLUSIONS

(1) Yellow mosaic virus disease was found naturally on Himalaya blackberry in British Columbia.

(2) Yellow mosaic symptoms on Washington variety of red raspberry and black raspberry seedlings is described.

(3) Yellow mosaic virus is transmitted by the aphid Amphorophora rubi. It may be acquired in three hours, and persists at least one hour and forty-five minutes in the vector.

RED RASPBERRY MOSAIC

INTRODUCTION

LITERATURE IN NORTH AMERICA : In 1905, Stewart and Eustace (37, pp.362-364) described a disease of red raspberry in New York that they designated "yellows" because of the similarity of this disease to peach yellows. Affected plants had yellow, mottled, and curled foliage and were generally unproductive. During the next twenty years, a similar disease was recognized in other states and in eastern Canada. Diseases of this type in other crops were termed "mosaics", therefore this raspberry disease became known as "raspberry mosaic" and the causal agent was termed "raspberry mosaic virus".

During the 1920's, other raspberry mosaic diseases were recognized and Bennett (4, p.89) adopted the terms yellow, mild, and red raspberry mosaic to indicate groups of related symptoms. Yellow mosaic was recognized as a distinct virus entity, but there was no clear line of separation between the more severe forms of mild mosaic and the milder forms of red raspberry mosaic. As to the number of viruses involved in the production of red raspberry mosaics, Bennett (7, p.7) stated his opinion as follows :-

> "Since there is a graduated range of severity of symptoms from very mild to very severe, and since so little is actually known regarding the properties of the causal agent, it seems questionable whether the present state of our knowledge justifies the use of virus or viruses as a basis for classification in this group of symptoms. It seems best for the present to consider all of the temperature-limited, necrosis-producing forms of mosaic as belonging to one group and perhaps caused by one virus, or by several strains of virus all of which have had a common origin."

Rankin (36, p.37) also favoured the theory that one virus was responsible for the wide range of symptoms of red raspberry mosaic (shortened by him to red mosaic), but he differed from Bennett in his interpretation. Bennett suggested that several strains of a single virus were responsible for the variation in symptom expression, whereas Rankin considered the wide range of symptoms to be a function of varietal susceptibility and rate of growth.

Cooley (19, pp.44-56) conducted experiments which he interpreted as supporting the Rankin theory that there are only two viruses involved in mosaics of raspberry in eastern North America, namely - red (raspberry) mosaic and yellow mosaic. Using black raspberry plants as indicators and a mild type of mosaic of Columbian hybrid raspberry as the source of inoculum, Cooley found that

the inoculated plants developed all degrees of other-than-yellow mosaic symptoms, from extremely mild to extremely severe phases. Cooley thought it improbable that more than one virus or virus strain could have been present in the mottled plants used as sources of inoculum, and he attributed the varying reactions of the plants to varietal differences and to the influence of environmental conditions as they affected the growth of each individual inoculated black raspberry plant.

Cooley suggested a change in nomenclature, recommending green mottle mosaic as a substitute for the terms red raspberry mosaic and red mosaic. He characterized green mottle mosaic as follows: "It is meant to include the entire range of symptom expression from very mild to very severe cases; in fact, all types of mosaic of raspberry other than the distinct cases known to be due to the yellow-mosaic virus." The terminology proposed by Cooley is now in fairly general usage in extension literature on raspberry diseases (17, pp.4-5), 35, pp.26-34), 24, pp.775-783) and 25, pp.770-775).

LITERATURE IN EUROPE : From 1921 to 1932, Harris (21, pp.237-255) made observations on mosaic symptoms on raspberries in Great Britain and found a range of leaf symptoms varying from faint and evenly distributed mottle

to a sharply defined chlorotic speckle with pronounced laminal distortion. He classified the range of symptoms into three categories - naming them, types a, b, and c. Type b symptom corresponds to the severe red raspberry mosaic in North America and was described by Harris as follows :-

> "Chlorotic patches ill defined, tending to group themselves towards the leaf margins and between the main veins, slightly sunken and accompanied by a symmetrical, downcurling of the laminae about the midrib. The degree of mottling and downcurling both vary on the individual leaflets of a single leaf. This type of mosaic is masked by high summer temperature conditions."

This symptom type was later called Mosaic 1 by Harris (22, pp.318-343) and he states that it is probably of a single virus origin.

More recently, in 1952, Cadman (10, pp.69-77) substituted the term veinbanding disease for Mosaic 1, since Mosaic 1 denotes a disease category rather than a specific disease. He transmitted veinbanding virus to some North American red and black raspberry varieties and noted that the symptoms resembled those of severe red raspberry mosaic described and illustrated in the United States and Canada. Cadman considered the veinbanding disease to be caused by a single distinct virus and named it "veinbanding virus".

RED RASPBERRY MOSAIC IN BRITISH COLUMBIA : A severe virus disease, characterized by yellowing of the tissue adjacent to the veins, by puckering of the interveinal tissue and downcurling of the leaf blade, is commonly found in plantings of Newburgh, Cuthbert and Latham varieties of red raspberry in British Columbia (Fig. 12).

FIGURE 12. Red raspberry mosaic symptoms on Cuthbert var. of red raspberry.

This disease has not been observed on the Washington variety of red raspberry, although plantings of this variety are frequently located near infected plants of other varieties. This virus disease appears to be identical with the severe red raspberry mosaic disease described and illustrated in other parts of North America

by Bennett (3, pp.6-8), Jones and Baur (26, pp.6-7) and Zeller and Schuh (47, pp.12-13). It also appears to be identical with veinbanding disease described and illustrated by Cadman (10, pp.69-77).

In this discussion, the severe virus disease on red raspberry varieties is referred to as red raspberry mosaic. The name corresponds to severe red raspberry mosaic as used by Bennett (7, p.9) and to veinbanding disease as used by Cadman (10, pp.69-77).

EXPERIMENTAL

TRANSMISSION BY GRAFTING : A Cuthbert plant showing typical symptoms of severe red raspberry mosaic was established in the laboratory plot in 1950, and has been used since then as a source of inoculum in grafting experiments. Scions from this plant were bottle-grafted to five healthy Washington plants and to five mild mosaic-infected Cuthbert plants in the fall of 1951. By the following spring, three of the Washington plants and all the grafted Cuthbert plants were showing severe mosaic symptoms (Fig. 13) indistinguishable from those on the Cuthbert plant from which the scions were obtained. Five ungrafted control plants in each group remained free from severe mosaic.

FIGURE 13. Severe red raspberry mosaic symptoms on Washington raspberry that had been grafted to an infected Cuthbert plant.

TRANSMISSION BY APHIDS : Severe red raspberry mosaic on the varieties Newburgh, Cuthbert and Seedling G, were used as sources of inoculum in transmission experiments with the aphid Amphorophora rubi and black raspberry seedlings were used as indicator plants After a twenty-four hour acquisition feeding, five aphids were transferred to each of three black raspberry seedlings from each virus source and permitted to feed for six hours. The aphids were then transferred to other groups of plants and permitted to feed for one day, two days, and three days, respectively. As shown in Table 9, a virus was transmitted to each plant in the first transfer but to none of the plants in later transfers.

TABLE 9. Transmission* of red raspberry mosaic to Rubus occidentalis by serial transfer of aphids from three virus sources

VIRUS SOURCE	DURATION OF TRANSFER FEEDING			
	6 hours	1 day	2 days	3 days
Seedling G	3/3	0/3	0/3	0/3
Newburgh	3/3	0/3	0/3	0/3
Cuthbert	3/3	0/3	0/3	0/3

* Denominator indicates the number of plants inoculated. The numerator indicates the number of plants showing symptoms.

Symptoms of virus infection were evident within a week of inoculation when the stem terminals of the inoculated plants became downcurled and brittle and the terminal leaves wilted and died. The tip died back two to three inches. Thus the initial symptoms were indistinguishable from the symptoms of leaf mottle virus on black raspberry seedlings described in a previous section. Within three weeks of inoculation, axillary buds immediately below the necrotic terminals developed and the shoots from these buds were mottled, distorted, and showed slight veinbanding (Fig. 14). The symptoms on the black raspberry seedlings suggested that red raspberry mosaic is caused by a virus complex, one of the components being leaf mottle virus and another being

yellow mosaic virus.

FIGURE 14. Tip of black raspberry seedling one month after inoculation with red raspberry mosaic showing terminal necrosis and distortion of foliage on axillary shoot.

SEPARATION OF RED RASPBERRY MOSAIC VIRUS COMPLEX: A further experiment was conducted when aphids were given shorter transfer feeds than in the previous experiment. Aphids were allowed a five-hour acquisition feeding on diseased Cuthbert leaves and then, as shown in Table 10, sixteen individual aphids were transferred through a series of seven black raspberry seedlings. Each aphid was given

a transfer feeding of five minutes on the first plant of the series, ten minutes on the second, fifteen minutes on the third, thirty minutes on the fourth, and one hour each on the fifth, sixth, and seventh plants in the series.

TABLE 10. Incidence of leaf mottle (L), mild mosaic (M), and yellow mosaic (Y) on Rubus occidentalis following serial transfer of sixteen single aphids from a Cuthbert raspberry plant infected with red raspberry mosaic

TEST APHID NUMBER	DURATION OF TRANSFER FEEDING						
	5 min.	10 min.	15 min.	30 min.	1 hr.	1 hr.	1 hr.
1	L	-	L	-	-	-	-
2	-	L	L	-	-	-	-
3	L	L	L	-	-	-	-
4	-	-	L	L	M	-	-
5	L	-	-	L	-	-	-
6	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-
8	-	L	-	-	-	-	-
9	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
11	M	Y	L	M	Y	-	-
12	-	L	L	-	-	-	-
13	-	L	L	L	L	M	-
14	-	Y	L	L	-	-	-
15	M	-	-	-	-	-	-
16	-	-	-	L	L	-	-

Some of the inoculated plants began to exhibit symptoms of virus infection within a week of inoculation. These plants, denoted by "L" in Table 10, were indistinguishable from plants infected with leaf mottle virus. Other plants, denoted by "M" in Table 10, wilted at the terminals but did not become necrotic and developed symptoms typical of the mild mosaic virus infection described in a previous section.

Four weeks after inoculation, two of the plants in the second transfer group (ten minutes transfer feeding) and one plant in the fifth transfer group (one hour transfer feeding) showed flecks of vein chlorosis on one of the leaves beneath the terminal of the plant. Within a few days a more pronounced vein chlorosis was observed on the younger leaves above the first infected leaf, and subsequent symptoms were typical of symptoms produced by yellow mosaic virus. These three plants are denoted by "Y" in Table 10. On the basis of the length of the incubation period and the symptom expression on black raspberry, this component of red raspberry mosaic was identified as yellow mosaic virus. Apparently a short transfer feeding may introduce one virus into the test plant, whereas a longer transfer feeding is more likely to introduce a complex of viruses into the susceptible cells

of the test plant.

These transmissions suggest that red raspberry mosaic is not caused by one virus but is the result of infection with leaf mottle virus or mild mosaic virus combined with yellow mosaic virus, and that the combined viruses are more severe in their effect than either component alone. There is, of course, a possibility that other unidentified viruses were present in these transmissions. Final analysis of red raspberry mosaic will have to await a synthesis of leaf mottle, mild mosaic, and yellow mosaic in virus free red raspberry and a comparison of the resultant symptoms with those of red raspberry mosaic.

PERSISTENCE OF VIRUSES IN FASTING APHIDS : Three Rubus viruses - mild mosaic, leaf mottle and yellow mosaic - have been shown to persist in the body of infective aphids for relatively short periods, usually less than two hours. The periods were determined by transferring aphids directly from the source of the inoculum to a series of test plants (Table 10).

In a later experiment, the persistence of these viruses in fasting aphids was investigated by giving aphids an acquisition feeding and then fasting them for

varying periods prior to the transfer feeding. In this experiment the source of inoculum was a shoot from a Cuthbert red raspberry plant known to be infected with a combination of the three viruses mentioned above. Aphids were permitted to feed on this shoot for one day. One group of aphids was then transferred directly from the virus source to the indicator plants while other groups were fasted in moist Petri plates held at 66°-72°F for periods of six hours, twelve hours, one day, and two days, respectively, before being transferred to the indicator plants. Individual aphids were given a transfer feeding of at least three hours on each test plant.

Some of the plants began to show symptoms of infection with leaf mottle virus and mild mosaic virus within one week after inoculation. Other plants showed symptoms of yellow mosaic infection after three weeks. As noted earlier, infection with leaf mottle virus produces severe symptoms and masks the presence of yellow mosaic and mild mosaic. Only the initial symptom type was recorded, hence some plants diagnosed as infected with leaf mottle may also have been infected with yellow mosaic and mild mosaic. The results of this experiment are summarized in Table 11.

TABLE 11. Incidence of symptoms on Rubus occidentalis of the component viruses of red raspberry mosaic complex transmitted by aphids fasted at 66°-72°F

FAST PERIOD	TRANS-MISSION RATIO*	PERCENT. TRANS-MISSION	SYMPTOM EXPRESSION		
			Leaf Mottle	Mild Mosaic	Yellow Mosaic
none	15/20	75	13	2	0
6 hrs.	6/20	30	3	1	2
12 "	8/50	16	3	0	5
1 day	3/25	12	2	0	1
2 "	0/15	0	0	0	0

* Denominator indicates the number of plants inoculated. The numerator indicates the number of plants showing symptoms.

As the fasting period was lengthened, there was a gradual decrease in the number of transmissions. None of the aphids fasted for two days transmitted any of the three viruses. Some fasting aphids retained the capacity to transmit mild mosaic virus for at least six hours after leaving the source of inoculum and others transmitted leaf mottle virus and yellow mosaic virus one day after removal from the virus source. The fasting aphids, therefore, retained the capacity to transmit each of the three component viruses considerably longer than aphids transferred directly from the source of the inoculum and fed continuously on healthy plants.

In a parallel experiment to determine the effect of temperature on persistence of viruses, aphids were given a one-day acquisition feeding on the same virus source. One group of aphids was then transferred directly from the source of inoculum to the indicator plants: other groups were held in Petri plates at 32°-34°F and fasted for periods of six hours, twelve hours, one day, two days, four days, and eight days. Aphids were then placed individually on the test plants and permitted to feed for at least three hours. The results of this experiment are given in Table 12.

TABLE 12. Incidence of symptoms on Rubus occidentalis of the component viruses of red raspberry mosaic complex transmitted by aphids fasted at 32°-34°F

FAST PERIOD	TRANS-MISSION RATIO*	PERCENT. TRANS-MISSION	SYMPTOM EXPRESSION		
			Leaf Mottle	Mild Mosaic	Yellow Mosaic
none	6/10	60	6	0	0
6 hrs.	5/10	50	4	1	0
12 "	8/15	53	6	2	0
1 day	8/20	40	5	2	1
2 "	12/37	32	4	1	7
4 "	11/40	27	4	3	4
8 "	0/25	0	0	0	0

* Denominator indicates the number of plants inoculated. The numerator indicates the number of plants showing symptoms.

As in the previous experiment, there was a gradual decrease in the number of transmissions as the fasting period was lengthened. None of the aphids fasted for eight days transmitted a virus. However, each of the three component viruses was transmitted by some of the aphids that had fasted for four days. The capacity to transmit the viruses was retained longer when aphids were fasted at low temperatures. Temperature, therefore, affects the persistence of these Rubus viruses in the fasting aphids.

DISCUSSION

INSECT TRANSMISSION : The susceptibility of black raspberry to red raspberry mosaic was first demonstrated in 1924 by Wilcox and Smith (40, p.55) who used the aphid Amphorophora rubi to transmit the virus. Three years later this was confirmed by Bennett (2, pp.15-16). In 1952, Cadman (10, pp.69-77), in Scotland, also used the aphid A. rubi to transmit an analogous disease to black raspberry plants, and obtained the same results as the North American workers, noting that the initial symptoms on black raspberry were not clearly distinguishable from those caused in Scotland by leaf mottle virus.

In all previous transmissions, viruliferous aphids

were permitted to feed for at least an hour on the indicator plants and several aphids were used in each transfer. In the experiments reported here (see Table 9), when aphids were given transfer feeds of six hours, the necrotic symptoms of leaf mottle virus developed on all of the plants inoculated, and yellow mosaic was not separated from leaf mottle or mild mosaic. It has been demonstrated previously that yellow mosaic is readily transmitted by A. rubi (see Table 7) in as short a transfer feed as fifteen minutes. Hence a large number of plants diagnosed as infected with the leaf mottle virus were probably infected with both viruses, the leaf mottle masking the presence of the yellow mosaic virus in the initial symptoms.

Cadman used a source of inoculum which, in addition to veinbanding, was carrying at least two other viruses, both of which produce necrosis on black raspberry. It is, therefore, evident that the necrosis-producing viruses were transmitted in mass transfers involving several aphids and long transfer feeds. The initial symptoms of infection were necrosis, but the chronic symptoms were veinbanding, and Cadman concludes that veinbanding and leaf mottle had been separated. Cadman also states : "It is concluded that the veinbanding disease

identified on Norfolk Giant and other varieties in Scotland is identical with the mosaic 1 disease described by Harris (1940) and the data agree with the author's supposition that the symptoms are caused by one virus". If veinbanding disease in Scotland is the same as severe red raspberry mosaic in America, as it appears to be, it is difficult to see how Cadman was able to conclude that veinbanding was caused by a single virus.

TERMINOLOGY : At the present time the terminology of this mosaic is not standardized in the literature. While "green mosaic" is generally used in American literature, it is objectionable because such usage implies acceptance of Cooley's theory on the identity of raspberry mosaics - namely, that all mosaics, other than yellow, are caused by a single virus and the different symptoms are essentially the result of environmental influences and varietal susceptibility.

It is considered preferable to retain the term "red raspberry mosaic" for this disease since the name was originally chosen by Bennett because it seemed to be by far the most common and destructive virus disease on red raspberry. As he uses it, it is a general term, including the severe disease discussed here and, in addition, the milder forms of mosaic.

Bennett was aware of the limitations of his observations and, after discussing the symptoms of the severe form of red raspberry mosaic, he made this statement concerning the mild form - "Whether this less severe form of mosaic is caused by the same virus as the above operating under different conditions or whether it is a modified product of the severe form or is distinct altogether, is not known."

EFFECT OF FASTING : Consideration of the persistence of viruses in infective aphids has led several workers to investigate the effect of fasting the vector between the acquisition feeding and the transfer feeding. Watson (38, p.201) notes that the nonpersistent viruses, Hyoscyamus virus 3, potato virus Y, cucumber virus 1, tobacco etch virus, all persist in their aphid vectors for about one hour when aphids are feeding continuously, but are retained for longer periods when aphids are fasted. On the other hand, the persistence of some viruses is not appreciably affected by fasting the infective aphids. For example, Mellor and Fitzpatrick (28, p.416), and Miller (29, pp.311-312), have shown that the nonpersistent component of strawberry yellows does not persist as long as six hours in either the feeding or the fasting aphid. Miller (29, pp.311-312) further investigated the effect

of temperature on the persistence of viruses and noted that the nonpersistent component of strawberry yellows survived considerably longer than six hours when the aphids were fasted at a cool temperature.

As shown in Table 11, fasting the aphid has a definite effect. The viruses were retained approximately ten times longer in the fasting aphids than in aphids fed continuously. Fasting itself is rendered more effective if the fasting aphids are held at low temperatures. Table 12 shows that when aphids were fasted at 32°-34°F the viruses were retained considerably longer than when they were fasted at 66°-72°F.

Since the component viruses of the red raspberry mosaic complex are indistinguishable in their vector relationships, separation of the complex is difficult, although it has been achieved by transferring individual aphids through a series of test plants. However, when aphids were fasted after the acquisition feeding, separation of the component viruses was effected in several instances (Tables 11 and 12). The results indicate that some of the viruses were inactivated after two to four days at 32°-34°F while others survived, thereby resolving the complex into the various components. This suggests that a Rubus virus complex may be more efficiently

resolved by fasting the aphids after the acquisition feeding than by transferring them immediately to test plants. Apparently the viruses were gradually inactivated in the body of the fasting aphid with none surviving a prolonged fast of two days at 66°-72°F or eight days at 32°-34°F.

Knowledge of the persistence of Rubus viruses in fasting aphids is important for understanding the natural transmission of the viruses in the field. Both apterous and winged forms of Amphorophora rubi are capable of transmitting the viruses when they move from a diseased plant to a healthy plant. When wingless aphids are disturbed by cultural operations, or by rains or wind, and fall to the ground, they are very active and able to crawl considerable distances. If the viruses are retained for two hours or less, as has been demonstrated with aphids fed continuously after leaving the source of inoculum, the winged forms can be assumed to account for most of the field transmissions. However, in view of the fact that viruses can persist in fasting aphids for at least one day after an acquisition feeding on a source of inoculum, the apterous form is also important as a potential agent for natural dissemination of the viruses.

CONCLUSIONS

(1) In British Columbia red raspberry mosaic is common on Cuthbert, Newburgh and Latham varieties of raspberry. It has not been found on the Washington variety, although this variety exhibits typical symptoms when graft-infected.

(2) Red raspberry mosaic is not caused by one virus but is the result of infection with a virus complex which includes yellow mosaic virus, leaf mottle virus and mild mosaic virus.

(3) The components of red raspberry mosaic can be separated by giving infected aphids short transfer feedings on indicator plants.

(4) The component viruses of red raspberry mosaic are retained longer in fasting aphids than in aphids fed continuously. This persistence is further prolonged by lowering the temperature at which the fasting aphids are held.

BIBLIOGRAPHY

1. Bawden, F. C. Plant viruses and virus diseases. 3d ed. Waltham, Chronica Botanica, 1950. 336p.

2. Bennett, C. W. Virus diseases of raspberries. East Lansing, Michigan state college, 1927. 38p. (Michigan. Agricultural experiment station. Technical bulletin no. 80)

3. Bennett, C. W. Michigan raspberry diseases. East Lansing, Michigan state college, 1928. 52p. (Michigan. Agricultural experiment station. Special bulletin no. 178)

4. Bennett, C. W. Some raspberry mosaic symptoms. Phytopathology 19:89. 1929. (Abstract of paper presented at the twentieth annual meeting of the American phytopathological society, New York, N.Y.)

5. Bennett, C. W. Diseases of raspberries and blackberries. In J. S. Shoemaker, C. W. Bennett and J. S. Houser's Raspberries and blackberries in Ohio. Wooster, Ohio agricultural experiment station, 1930. pp.43-78. (Ohio. Agricultural experiment station. Bulletin no. 454)

6. Bennett, C. W. Further observations and experiments on the curl disease of raspberries. Phytopathology 20:787-802. 1930.

7. Bennett, C. W. Further observations and experiments with mosaic diseases of raspberries, blackberries, and dewberries. East Lansing, Michigan state college, 1932. 32p. (Michigan. Agricultural experiment station. Technical bulletin no. 125)

8. Cadman, C. H. Studies in Rubus virus diseases I. A latent virus of Norfolk Giant raspberry. Annals of applied biology 38:801-811. 1951.

9. Cadman, C. H. Studies in Rubus virus diseases II. Three types of vein chlorosis of raspberries. Annals of applied biology 39:61-68. 1952.

10. Cadman, C. H. Studies in Rubus virus diseases III. A veinbanding disease of raspberries. Annals of applied biology 39:69-77. 1952.

11. Cadman, C. H. Studies in Rubus virus diseases IV. Yellows diseases of raspberries. Annals of applied biology 39:495-500. 1952.

12. Cadman, C. H. Studies in Rubus virus diseases V. Experiments in the analysis of Lloyd George decline. Annals of applied biology 39:501-508. 1952.

13. Cadman, C. H. and R. V. Harris. Leaf curl, a virus disease of raspberries in Scotland. Journal of horticultural science 27:201-211. 1952.

14. Cadman, C. H. and R. V. Harris. A lethal virus disease of Lloyd George raspberry in Scotland. Journal of horticultural science 27:212-214. 1952.

15. Chamberlain, G. C. Yellow blotch-curl: a new virus disease of the red raspberry in Ontario. Canadian journal of research C,16:118-124. 1938.

16. Chamberlain, G. C. A necrotic "fern-leaf" mosaic of raspberry. Scientific agriculture 22:119-124. 1941.

17. Chamberlain, G. C. and W. L. Putman. Diseases and insect pests of the raspberry in Canada. Ottawa, Canada Dept. of agriculture, 1952. 32p. (Canada Dept. of agriculture. Science service publication 880)

18. Conners, I. L. and D. B. O. Savile. Diseases of fruit crops. In Canada Dept. of agriculture plant disease survey 32d. annual report, 1952. Ottawa, Canada Dept. of agriculture, 1953. pp.81-100.

19. Cooley, L. M. The identity of raspberry mosaics. Phytopathology 26:44-56. 1936.

20. Dodge, B. O. and R. B. Wilcox. Diseases of raspberries and blackberries. Washington, U.S. Government printing office, 1926. 33p. (U.S. Dept. of agriculture. Farmers' bulletin no. 1488)

21. Harris, R. V. Mosaic disease of the raspberry in Great Britain. I. Symptoms and varietal susceptibility. Journal of pomology and horticultural science 11:237-255. 1933.

22. Harris, R. V. Mosaic disease of the raspberry in Great Britain. II. Experiments in transmission and symptom analysis. Journal of pomology and horticultural science 17:318-343. 1940.

23. Horn, Norman L. A new virus disease of blackberry. Phytopathology 38:827-830. 1948.

24. Jeffers, W. F. Diseases of berries in the east. In U.S. Dept. of agriculture yearbook, 1953. Washington, U.S. Government printing office, 1953. pp.775-783.

25. Johnson, Folke. Diseases of berries in the west. In U.S. Dept. of agriculture yearbook, 1953. Washington, U.S. Government printing office, 1953. pp.770-775.

26. Jones, Leon K. and Karl E. Baur. Mosaic and related diseases of raspberries in Washington. Pullman, Washington state college, 1936. 19p. (Washington. Agricultural experiment station. Bulletin no. 324)

27. Knight, C. A. Some salient points concerning plant viruses. In M. Delbruck's (ed.) Viruses 1950. Pasadena, California institute of technology, 1950. pp.52-54.

28. Mellor, Frances C. and Randal E. Fitzpatrick. Studies of virus diseases of strawberries in British Columbia II. The separation of the component viruses of yellows. Canadian journal of botany 29:411-420. 1951.

29. Miller, P. W. Relation of temperature to persistence of strawberry yellows virus complex in strawberry aphid. Plant disease reporter 36:311-312. 1952.

30. Prentice, I. W. Rubus stunt: a virus disease. Journal of horticultural science 26:35-42. 1950.

31. Prentice, I. W. and R. V. Harris. Mosaic disease of the raspberry in Great Britain. III. Further experiments in symptom analysis. Journal of horticultural science 25:122-127. 1950.

32. Rankin, W. Howard. Virus diseases of black raspberries. Geneva, New York state agricultural experiment station, 1931. 24p. (New York. Agricultural experiment station. Technical bulletin no. 175)

33. Rankin, W. Howard and J. F. Hockey. Mosaic and leaf curl (yellows) of the cultivated red raspberry. Phytopathology 12:253-264. 1922.

34. Simons, John N. and Edward S. Sylvester. Acquisition threshold period of western celery mosaic virus for four species of aphids. Phytopathology 43:29-31. 1953.

35. Slate, George L., A. J. Braun, and F. G. Mundinger. Raspberry growing. Geneva, New York state agricultural experiment station, 1947. 68p. (New York. Agricultural experiment station. Cornell extension bulletin 719)

36. Slate, George L. and W. Howard Rankin. Raspberry growing in New York state: cultural practises and disease control. Geneva, New York state agricultural experiment station, 1933. 62p. (New York. Agricultural experiment station. Station bulletin no. 625)

37. Stewart, F. C. and H. J. Eustace. Raspberry cane blight and raspberry yellows. Geneva, New York state agricultural experiment station, 1902. 36p. (New York. Agricultural experiment station. Station bulletin no. 226)

38. Watson, M. A. The transmission of beet mosaic and beet yellows viruses by aphides; a comparative study of a non-persistent and a persistent virus having host plants and vectors in common. Proceedings of the royal society of London B, 133:200-219. 1946.

39. Wilcox, R. B. Eastern blue-stem of the black raspberry. Washington, U.S. Government printing office, 1922. 12p. (U.S. Dept. of agriculture. Department circular 227)

40. Wilcox, R. B. and Floyd F. Smith. Transfer of mosaic disease from red to black raspberries. Phytopathology 14:55. 1924. (Abstract of paper presented at the fifteenth annual meeting of the American phytopathological society, Cincinnati, Ohio.)

41. Wilhelm, Stephen, H. Earl Thomas and D. D. Jensen. A dwarfing virus disease of bramble fruits. Phytopathology 38:919. 1948. (Abstract of paper presented at the thirtieth annual meeting of the Pacific division of the American phytopathological society, Berkeley, California.)

42. Wilhelm, Stephen, H. Earl Thomas and Edward C. Koch. Diseases of the Loganberry. California agriculture 5:11, 14. January, 1951.

43. Zeller, S. M. Mosaic and other systemic diseases of brambles in Oregon. Corvallis, Oregon agricultural college, 1923. 15p. (Oregon. Agricultural college experiment station. Station circular no. 49)

44. Zeller, S. M. Dwarf of blackberries. Phytopathology 17:629-648. 1927.

45. Zeller, S. M. and A. J. Braun. Decline diseases of raspberry. Phytopathology 33:156-161. 1943.

46. Zeller, S. M. and J. A. Milbrath. Blackberry dwarf in boysenberries and youngberries. Plant disease reporter 24:430. 1940.

47. Zeller, S. M. and Joe Schuh. Diseases and insect pests of cane fruits in Oregon. Corvallis, Oregon state college, 1944. 58p. (Oregon. Agricultural experiment station. Station bulletin no. 418)

APPENDIX

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In the experiments reported in this thesis, all the aphids (Amphorophora rubi Kaltenbach) used, originated from one viviparous adult obtained in Vancouver, B. C., from a virus-free Washington red raspberry plant. Progeny of this adult were transferred at birth to healthy black raspberry seedlings: thus the original stock culture was non-viruliferous. Stock cultures were reared in the greenhouse on seedling black raspberry plants.

Transfers of aphids were made by the standard technique of using a camel-hair brush. In all transmission experiments, immature aphids (about three to four days old) were selected. These aphids were gently disturbed and permitted to withdraw their stylets before being transferred, on the moistened brush, to the source of inoculum. They were then placed on the lower surface of the youngest, fully opened leaf on the indicator plant, and the entire plant was then caged in a glass cylinder five inches in diameter (a Coleman lamp shade) covered with muslin.

All experiments were conducted in the greenhouse which was fumigated at approximately two-week intervals with Plantfume 103 (tetra ethyl pyrophosphate) to eliminate mites and as a precaution against stray aphids.