A STUDY OF FIVE MECHANICALLY TRANSMISSABLE CHERRY VIRUS ISOLATES WITH HERBACOUS HOSTS

by

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A B S T R A C T

Five virus isolates RS 2, RS 25, RS 26, RS 28 and RS 29, were transmitted by juice-inoculation technique from sour and sweet cherry trees to cucumber.

Four isolates were obtained from trees growing in the Kootenay cherry district of British Columbia. Another one was isolated from a tree growing in the coastal area of this province. Viruses known to occur in the source trees are Necrotic Ring Spot Virus, Sour Cherry Yellows Virus, Twisted Leaf Virus and Little Cherry Virus.

The relationship and the complexity of the virus isolates was studied with herbaceous hosts, using a mechanical transmission technique. Pincherry (Prunus pennsylvanica L.) was inoculated by the same technique as a means for provisional identification of the virus isolates.

The cucumber syndrome of isolate RS 25 was very mild, that of isolate RS 2 mild, that of isolate RS 29 was of medium severity and those of isolates RS 26 and RS 28 were very severe.

Inoculates RS 2 and RS 29 varied greatly in symptom expression on cucumber, whereas the symptom expression of the other isolates was less variable.

Isolate RS 29 was characterized by symptomless systemic infection of Nemesia sp., var. Triumph. Isolates RS 26 and RS 28 both infected

Petunia hybr., var. Blue Bee, without expressing symptoms, whereas the other isolates did not infect this species. Other host species too carried the isolates without expressing symptoms, whereas symptoms were produced on cucurbit hosts. Isolates RS 2, RS 26, RS 28 and RS 29 appeared to consist of more than one virus. Strains of a virus P occur in all isolates and isolate RS 25 itself is also a strain of this virus.

All five strains of virus P express similar very mild symptoms on cucumber, whereas a characteristic severe savoying type of symptom is produced on squash (var. Table Queen).

Species susceptible to virus P are cucumber, pincherry, squash, sweet pea, tobacco (under conditions of long day) and other species. Lathyrus odoratus L. and Lens culinaris Medic. are species useful in separating virus P from the other viruses occurring in isolates RS 2, RS 26, RS 28 and RS 29.

It is possible that virus P is related to cucumber-mosaic virus as suggested by symptoms on squash and tobacco. In previous work by other investigators a strain of cucumber-mosaic virus was also isolated from Prunus hosts.

On pincherry (P. pennsylvanica L.) isolate RS 28 caused acute symptoms of necrosis and shothole. The plants recovered but symptoms of mottling were systemic. Necrotic Ring Spot Virus caused similar symptoms on Prunus hosts and this virus and Sour Cherry Yellows Virus was present in the original source tree.

The other isolates in pincherry all caused similar symptoms of mottling on the young leaves. A few necrotic lesions were produced also.

On reisolation from pincherry virus P was obtained in case of isolates RS 2, RS 26 and RS 29. No virus was reisolated in the case of isolate RS 25. The complete parent isolate was reisolated in case of isolate RS 28.

The results with pincherry suggest that virus P is responsible for the mild symptoms whereas virus P in conjunction with an additional virus as in isolate RS 28 incites the severe shock symptoms. The identification of the viruses present in the isolates can be carried out by scion inoculation of a set of suitable Prunus indicator hosts.

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TABLE OF CONTENTS

Page

Introduction 1

Review of Literature 5
Relationship of Viruses of Stonefruits 6

Prunus Virus Isolates and Host Range Studies 11

Materials and Methods 19
The Isolates 19

Plant Growing and Environmental conditions 21

Inoculation Technique 22

General Course of Host Range Studies 22

Identification of the Cherry Virus Isolates 23

Preparation of Inoculum 24
Cherry Inoculum 24
Cucumber Inoculum 25
Testplant Inoculum 26
Photography 26
Observations and Results 27

Symptom Expression of the Isolates on Cucumber 27
Cucumber Syndrome of Isolate RS 2 27
Cucumber Syndrome of Isolate RS 25 . . . 28
Cucumber Syndrome of Isolate RS 26 and RS 28 28
Cucumber Syndrome of Isolate RS 29 . . . 30
Comparison of the Cucumber Syndromes . . 31

Inoculation trials with Herbaceous Hosts . . . 31
Virus Reisolated from Host Species 33
Symptom Expression of the Isolates on Pincherry 39
Virus Reisolated from Pincherry 40

Page

Discussion and Conclusion 42

Literature Cited 50

Appendix:

Figures 1 - 13 I - XIII

I N T R O D U C T I O N

General problems in research of virus diseases of plants.

Identification of the causal agent of a plant disease is of primary importance in plant pathological problems. Plant viruses are commonly identified by and their presence is observed from the symptoms they produce on their hosts. Particular problems however arise when symptomology is made the basis for identification.

Different viruses may produce similar symptoms on a single host or a single virus may produce dissimilar symptoms on different hosts. Furthermore, a single host may be infected simultaneously by more than one virus and the symptoms expressed may be the result of the combined effects or of the effect of only one, the others being latent. In addition there is the complication of the existence of forms or strains. Different strains of the same virus may cause widely different symptoms in one or more hosts.

In virus diseases affecting plants the causal agent may therefore be simple or complex. A single virus entity or two or more virus entities or virus strains or combinations thereof may be involved in the disease.

Different virus diseases on the same host may therefore have a virus factor in common. Consequently these diseases will be related

to each other.

In the Agriculture Handbook 10 (1) which deals with virus diseases of stonefruits only and published in 1951, forty-eight different diseases were described, another four are mentioned in the same publication and several more have been reported since. (6, 26, 27, 35).

Transmission experiments by budding or grafting techniques were the means by which stonefruit virus diseases were studied. Symptomology of a descriptive and comparative nature, host range studies and cross-protection experiments yielded data by which stonefruit viruses were distinguished. The transmission experiments were confined to the Rosaceae. Primarily Prunus species were used. Other species involved were apple (Malus sylvestris Mill.), Japanese Kerria (Kerria japonica (L.) DC.) and Rosa sp. (33).

Only when a virus is sap-transmissable can its properties be investigated in a convenient and thorough manner (2). Stonefruit viruses did not seem to be subject to juice-inoculation and in-vitro studies were virtually impossible.

In 1948 however Moore, Boyle and Keitt (28) transmitted a virus from sour cherry to cucumber using a mechanical transmission technique. The same group of workers (5) showed that the isolate was distinct from cucumber viruses. Other herbaceous hosts were thereafter reported, differentiating between isolates from Prunus hosts.

The scope of the study of stonefruit viruses was widened by these discoveries. Cucumber proved to be useful as a host in which a

number of stonefruit viruses could be maintained. Relationships between stonefruit viruses could be investigated on the basis of herbaceous host ranges.

In this investigation relationships between five isolates from five virus infected cherry trees were studies by herbaceous host range work. The isolates were transferred to and maintained in cucumber (Cucumis sativus L.). The trees were selected because of the range in severity of symptom expression of ring spot virus. The trees were also selected on the basis of regional occurrence. Four isolates were obtained from trees growing in the British Columbia Kootenay cherry district. Another one was isolated from a tree growing in the coastal area of British Columbia.

Some virus diseases occur in this province which are of economic importance. Little Cherry of sweet cherry is of importance because it reduces the value of the crop and its natural spread is extraordinarily rapid (9). Mottle Leaf of sweet cherry is important because growth of trees is seriously affected (22). Data on the economic importance of Necrotic Ringspot of sour cherry are not conclusive but the tree may be severely diseased in the first year when acute symptoms occur (3). Sour Cherry Yellows is economically the most important virus disease of sour cherry in the United States and Canada (20). The trees yielding the isolates were known to be infected with these viruses.

Part of the problem was concerned with determining if the virus entities transferred to cucumber consisted of only one component or whether a mixture or a complex of viruses was involved in the transfer.

The identity of the cherry virus isolates was probed by inoculation of pincherry (Prunus pennsylvanica L.). The use of this species for such a purpose and the method of transmission was suggested by the work of Fulton (10).

REVIEW OF LITERATURE

The use of herbaceous species in research on virus diseases of woody plants - and in particular virus diseases of stone fruits - has found wide application after the work of Kunkel and of Moore, Boyle & Keitt.

Kunkel (21) used dodder (Cuscuta campestris Yuncker) in transmitting a virus from X-diseased peach trees to carrot (Daucus carota L.), parsley (Petroselinum crispum Nym.), periwinkle (Vinca rosea L.) and to tomato (Lycopersicon esculentum Mill.).

Juice-inoculation technique was applied by Moore, Boyle & Keitt (28) to infect cucumber (Cucumis sativus L.) var. Ohio with a virus from sour cherry (P. cerasus L.). The trees were infected with Necrotic Ring Spot Virus alone, or in combination with Sour Cherry YellowsVirus. The virus was easily transmissable between cucumber plants.

The varietal susceptibility of cucumber was investigated by Boyle, Moore & Keitt (5) and by Hobbs (19). The first mentioned workers reported that all twenty varieties tested were susceptible to virus isolated from fifteen Prunus species. Hobbs reported that all except one out of forty-seven cucumber varieties tested could be infected with virus from sour cherry.

Using mechanical transmission technique cucumber has been used in many investigations to maintain virus isolated from a variety of Prunus species. In some studies the physical properties of stonefruit virus isolates were investigated. In other investigations the relationships between virus isolates was elucidated when herbaceous hosts other than cucumber were found, which differentiated between the isolates.

Some isolates also appeared to differ in symptom expression on cucumber and other herbaceous hosts. The source of the differences between the isolates in host range, symptomology and other properties can at least partly be explained on the basis of differences in virus contents of the source trees.

In this thesis symptomology and host ranges are criteria used in identification and differentiation of the isolates concerned.

The relationships between stonefruit virus isolates will reflect the relationships in virus contents of the source tree.

This review of literature will deal first with those viruses which are presumably mechanically trahsmissable to cucumber. After that discussion symptomological - and host range studies with Prunus virus isolates will be reviewed.

Relationships of Viruses of Stonefruits.

The study of stonefruit viruses was limited in scope prior to the discovery that they were mechanically transmissable to herbaceous hosts.

Valuable information however was obtained from transmission - and cross-protection experiments using scion-inoculation technique and from field observations. On the basis of symptomology on a variety of Prunus species certain diseases could be grouped together and relationships between causal viruses were recognized.

Generally recognized are the peach-yellows-little-peach group, the X-disease-little-cherry group, and the line-pattern group, the mottle-leaf-rugose-mosaic group and the cherry-yellows-necrotic ring-spot-group. (41). Viruses of the latter mentioned group and some other viruses are the subject matter in this thesis.

Willison et al. (41) consider that the following diseases - and by inference the causal viruses - belong to the cherry-yellows-necrotic-ring-spot group:

Sour Cherry Yellows (SCY)
Green Ring Mottle of sour cherry (GRM)
Necrotic Ring Spot of sour cherry (NRS)
Tatter Leaf of sweet cherry (TL)

Prune Dwarf (PD) was also included in this group by these workers because this virus gives shock symptoms on sour cherry (18). and because this virus was-presumably - transmitted to cucumber (38). The groups however are defined by their symptomology on Prunus hosts only and not by their reaction on cucumber. Cameron & Moore (7) report, that PD should be considered a virus distinct from the ones causing ringspot and (sour cherry) yellows.

In many cases NRS alone or in combination with SCY was reportedly present in the source trees, yielding isolates mechanically

transmissable to cucumber. From budding and grafting experiments it appears however that no tree with SCY has been found in which NRS did not occur also. (7, 20). On the other hand it has been shown that NRS can occur in trees without SCY being present (7). Both NRS and SCY are apparently different virus entities because they are able to incite different diseases in the same host.

It was suggested, that Sour Cherry Yellows is incited by a complex that includes NRS (20). Milbrath (25) suggested that SCY and Peach Ring Spot Virus are strains of each other. The latter virus and NRS are considered to be identical or closely related. (8).

Therefore in cases where reference is made to SCY, also implicated is NRS. The situation is more clearly expressed, when the term Sour Cherry Yellows Complex is used instead of Sour Cherry Yellows Virus.

Green Ring Mottle of sour cherry was also present in source trees from which an isolate was mechanically transmitted to cucumber. GRM is also a member of the SCY-NRS group because of its close relationship to SCY (30). A similar relationship exists between NRS and GRM as with NR^S and SCY. A more appropriate term to be used in cases where GRM is concerned would be the Green Ring Mottle Complex.

Another virus concerned in this discussion is Recurrent Necrotic Ring Spot Virus of sour Cherry (R-NRS). This virus and NRS are thought to be strains of each other (3). R-NRS therefore also can be included in the SCY-NRS group.

Tatter Leaf of sweet cherry (TL) also may be considered to be a strain of NRS (8) and was present in source trees yielding mechanically

transmissable isolates.

Investigations where members of the SCY-NRS group were reported to be present in the source trees are:

NRS alone or in combination with SCY (4, 5, 12, 15, 16, 19, 24, 28, 34, 36, 37, 38, 39.)

R-NRS alone or in combination with SCY (5, 12, 15, 28)

GRM (15, 34, 37, 38, 39) and

TL (36, 37, 38, 39).

In addition to members of the SCY-NRS group other viruses reportedly present in Prunus hosts from which viruses were mechanically transmitted to cucumber are Prune Dwarf (15, 24, 36, 38). Peach Stunt (24), Rough Bark of plum (44) and Line Pattern of plum (15).

A great variety of Prunus sp. contain virus mechanically transmissable to cucumber (5). Most of the studies however were concerned with virus isolates obtained from sour cherry (P. cerasus L.), sweet cherry (P. avium L.), peach (P. persica L.) and with plum (P. domestica L.).

These four Prunus species are all susceptible to two or more of the viruses mentioned above and some of these viruses can occur in a latent form. Prune Dwarf for instance can occur in a masked form in sour and sweet cherry and in some P. domestica varieties (18). Whether Prune Dwarf is present in the source tree can be ascertained only by scion-inoculation of an indicator host such as Italian Prune or Lombard Plum (18). This same virus is often found associated with Line Pattern Virus in P. domestica L. var. Italian Prune. (18). Also Line Pattern can be latent in sour cherry (29). Temperature conditions will determine symptom expression of SCY and hence the presence of this virus cannot

always be discerned. (20).

The complexity of the situation is well illustrated by symptomless sour or sweet cherry, which may be carrying NRS, SCY, PD, Peach Stunt and possibly other viruses not mentioned in this discussion.

Hence, the known virus content of a Prunus host as reported by an investigator is not necessarily identical with the actual virus "population" of the source tree. The reported virus contents can only be suggestive of the identity of the virus or viruses mechanically transmitted to cucumber.

Ideally studies concerned with mechanically transmissable isolates from Prunus hosts should start with inoculation of a set of suitable Prunus indicator hosts with scions from the trees yielding the isolates. The real virus contents of the source tree would be demonstrated hereby. However, it does not appear from the literature that such an indexing procedure has been followed. All the investigators concerned were therefore working with a subject virus which was defined only to the extent of the reported virus content.

Summarizing the above it is stated that the SCY-NRS group is comprised of NRS, R-NRS, SCY, GRM and TL. Cucumber appears susceptible to virus entities isolated from trees known to be infected with Prune Dwarf, Peach Stunt, Rough Bark of Plum, Line Pattern of Plum and members of the SCY-NRS group. The reported virus contents of the Prunus host is only suggestive of the identity of the isolate transmitted to cucumber because of the contamination with latent viruses.

Prunus Virus isolates and Host Range Studies.

Of interest in this literature review are the virus contents of the source tree, the symptom expression of the isolate in cucumber, differential hosts and the identity of the Prunus virus isolate. In some cases the identification procedure was determinate in nature as a result of backtransfer of the isolate to Prunus sp. In other cases the conditions of the investigation itself were only suggestive of identity. In some publications evidence was presented suggesting that the isolate included more than one distinct virus. A salient point as this one will be stressed also.

As stated before, Moore, Boyle & Keitt (28) were the first to transmit a virus from a Prunus host to cucumber by juice-inoculation technique. The eight sour cherry trees used in their investigation contained NRS alone or in combination with SCY. The syndromes incited in cucumber were all similar and were characterized by yellow rings, coalescence of yellow blotches on the cotyledons and bud proliferation after killing of the apical growing point. Backtransfer to indicator sour cherry trees was carried out by placing small pieces of cucumber leaf under the bark of the cherry trees. Symptoms indicating NRS were observed.

Boyle, Moore & Keitt (4) isolated virus from a sour cherry, infected with NRS. A similar syndrome as described above was obtained on cucumber. The isolate was studied in comparison with several cucumber viruses. Characteristic differences between the cherry virus and the

cucumber viruses were noted.

The same group of workers (5) transferred virus from sixty-six trees, comprising fifteen Prunus species. The trees were known to be infected with NRS, NRS and SCY or R-NRS and SCY. The syndromes on cucumber were all quite similar and are the same as described above. After backtransfer to Prunus hosts by bark transmission, one out of six sour cherry trees developed symptoms of NRS. Only NRS was also observed on two out of twenty-three pincherries inoculated. Because of the low proportion of successful backtransfers, the true identity of the viruses transmissable to cucumber was not determined. The results on sour cherry and on pincherry do show, that NRS belongs to those viruses which are mechanically transmissable to cucumber. This is also supported by the work of Moore et al. (28) already described above.

In an investigation by Heinis & Milbrath (17) twenty-three different stonefruit trees were indexed for ringspot virus on peach, Bing sweet cherry, and Kwanzan - and Shirofugen flowering cherry. All trees except one caused symptoms of ringspot in a varying degree of severity, ranging from very mild to very severe. The syndromes incited on cucumber also showed a range of severity. A close correlation was noted with severity of symptom expression on cucumber and on the indexing hosts. This correlation also suggests that Ring Spot Virus or NRS - is a virus mechanically transmissable to cucumber.

Extensive host range studies were also carried out by Boyle et al. (5). Fifty-seven species in twenty families were tested. Only

cucumber and squash (Cucurbita maxima Duchesne, var. Giant Summer Crookneck) were found to be susceptible.

Hobbs (19) made a comparative study of nine regional sour cherry isolates. All the trees showed symptoms of NRS. The isolates differed in infectivity and responded differently to different temperatures. All attempts to reinfect cherry with the isolates failed. Some herbaceous species were tested in the same investigation. Of watermelon, none out of seven varieties tested appeared to be susceptible. Of nine pumpkin varieties tested, four were found to be susceptible. None out of twelve squash varieties proved to be susceptible.

Milbrath (24) however was able to transfer twenty-five stone-fruit virus isolates to a number of squash varieties. The source trees were known to contain NRS, SCY, Peach Stunt and Prune Dwarf. Milbrath obtained his inoculum from peach trees which were bud-inoculated with scions from the source trees. On the variety Buttercup bright golden patterns developed when the inoculum was from source trees infected with SCY. Some strains developed local lesions on Hubbard squash. Other isolates all developed dissimilar symptoms.

Gilmer (15) reported that an apparent latent virus in cucumber developed distinct veinbanding symptoms in squash Cocozelle. Such a syndrome was only observed when the isolate was derived from source trees containing Line Pattern Virus. Gilmer suggested that this or another virus was responsible for the veinbanding symptoms on Cocozelle. Apparently the particular isolates comprised at least two distinct viruses. Willison & Weintraub (39) were reportedly also dealing with isolates which were complex

in nature. An isolate designated G. 1 was obtained from a source tree infected with Green Ring Mottle. Another isolate T. 2 was obtained from a Prunus host infected with Tatter Leaf. Both these two isolates could be separated into two distinct components, designated G. 1.A and G. 1.B. and T.2.A and T.2.B. respectively. Components B infected cucumber, tobacco and other herbaceous hosts, whereas components A infected cucurbit hosts only. From later work by the same investigators (40) it appeared that G.1.B. and T.2.B. were strains of the same virus.

This virus named CMVP infected bean, cowpea, cucumber, Datura stramonium, petunia, tobacco, spinach, sugar beet, Swiss chard and zinnia. Because of host range, symptomology and physical properties of CMVP, it was considered to be an atypical strain of cucumber mosaic virus. Willison & Weintraub were of the opinion that this virus was latent in Prunus hosts and was not implicated in the etiology of cherry yellows and related stonefruit diseases. Besides source trees containing Tatter Leaf and Green Ring Mottle, other Prunus hosts with different virus contents were involved in the studies of Willison & Weintraub. Present in these Prunus hosts were NRS, SCY, NRS and PD. Because of inoculation to tobacco it was suggested that CMVP was also present in these isolates.

Tobacco and zinnia were differential hosts also reported by Varney & Moore (33). Virus was mechanically transmitted from leaves of some Prunus hosts to tobacco, zinnia and to cucumber. All the isolates that transmitted to tobacco or zinnia also transmitted to cucumber, but some sources were only transmittable to cucumber and did not infect

tobacco or zinnia. Also certain sources infected tobacco or zinnia but not both. Isolates were obtained from sour cherry, mahaleb, peach and Italian Prune.

Cowpea (Vigna sinensis Endl.) gave a local lesion reaction to isolates of Thornberry (32) and of Milbrath (23). The source tree in Thornberry's study was a sour cherry. Thornberry suggested cherry ring-spot virus as the incitant of the local lesion reaction. Milbrath used two types of inoculum: one type was prepared from flower petals, the other type was made from leaves. The flower inoculum gave numerous local necrotic lesions on the primary leaves of cowpea. Cowpea did not react with the leaf inoculum. Both the two inocula incited symptoms on cucumber. The syndromes however differed from each other. The sour cherry trees contained latent viruses only. Milbrath suggested that different viruses or strains were transmitted.

Cowpea in investigation of Willison & Weintraub (40) already discussed above did not react with local lesions to CMVP.

In an investigation by Yarwood (42) Peach Yellow Bud Mosaic Virus was transmitted from peach to cowpea also. The plant reacted with local lesions on inoculated leaves and with systemic shock symptoms. Also susceptible were bean, cucumber, guar, sunflower and tobacco. Bean also reacted with a local lesion symptom. The virus was backtransferred by mechanical inoculation to peach, aided by heat treatment.

The same worker (43) reported that bean also reacted to a strain of peach ring spot obtained from apricot. It appeared that

this strain could be transmitted from bean to bean by contact rubbing. Other strains of peach ring spot could not be transmitted in this manner.

Most extensive host range studies were carried out by Fulton (12). Four isolates were investigated by this worker. Sour cherry was the source tree for all the isolates used. In case of isolate A, the source tree was affected by R-NRS and by SCY. Isolate B was obtained from Varney & Moore (33), and had been maintained in tobacco (Nicotiana tabacum L.). This isolate originated in a tree, affected only by SCY. The source tree in case of isolate E was also affected by SCY. Virus E was separated from another virus thought to be a strain of isolate B. The sour cherry tree yielding isolate G did not show symptoms, but was known to be carrying necrotic ringspot virus. Numerous differences in hostrange between the isolates were found. Investigations involving the same isolates and carried out by the same worker (13) at a later date showed, that the isolates also differed in physical properties. Incidentally, the four isolates all caused similar symptoms in cucumber. Fulton however did not describe the symptoms produced in this species.

When backtransferred to sour cherry, the symptoms produced by the four isolates were also different and reflected the differences in virus content of the source trees. Fulton (14) reported, that isolate A causes recurrent Necrotic Ringspot, isolate G causes ordinary Necrotic Ringspot, isolate E causes necrotic spotting similar to ringspot in sour cherry except that enations appear on the lower surface of the leaves. Isolate B produces a chlorotic spotting in sour cherry accompanied by some rings. This isolate does not produce symptoms in the second year. Isolate B, according to Fulton is widely distributed in infected stone-

fruits, including sweet cherries on the west coast. In Fulton's opinion the symptoms produced by isolate B in cherry are distinct from previously described virus diseases in stonefruits. Isolate B therefore would represent a new disease.

Useful in assay of virus infectivity, Fulton (12) also reported hosts giving a local lesion reaction. Local lesion hosts were guar (Cyamopsis tetragonoloba (L.) Taub.), Momordica balsamina L., Sesbania spp., Crotalaria Spectabilis Roth, and C. capensis Jacq.

Certain plant families seem to provide more species susceptible to Prunus isolates than others. Computation of Fulton's data gives the following information. The number behind the family name gives the number of species susceptible to one or more of Fulton's isolates.

Apocynaceae	2
Compositae	9
Cucurbitaceae	24
Labiatae	2
Leguminosae	23
Scrophulariaceae	2
Solanaceae	26
Eleven other families each	1

Of the twenty-six solanaceous species, twenty are of the genus Nicotiana. The importance of the Cucurbitaceae, Leguminosae, and Solanaceae in host range work with stonefruit virus isolates is obvious.

The virus CMVP of Willison & Weintraub (40) already discussed above infected a .o. some members of the Chaenopodiaceae (spinach, sugar beet, Swiss chard). This family might be useful also in differentiation of isolates from Prunus hosts.

Some species found susceptible to Prunus isolates by workers other than Fulton (12) are bean, cowpea, spinach, sugar beet and Swiss chard, (23, 32, 40, 42, 43). Apparently these species were not susceptibile to any of Fulton's isolates. Fulton does not state however, whether they were tested or not.

Other hosts reported by those workers are also susceptible to one or more of Fulton's isolates.

MATERIALS AND METHODS

The Isolates

Twelve cherry trees were selected in the experimental orchard of the Science Service, Plant Pathology Laboratory, Summerland, British Columbia, substation Creston, B.C. The range in severity in symptom expression of Necrotic Ring Spot Virus and related virus was the basis for their selection. Some of the trees were without virus symptoms. Others showed a severe chlorotic mottling, a shothole or a laceration effect.

The first isolation was made on June 2, 1957, about three weeks after petal fall. After repeated attempts lasting throughout the months of June, July and August five of the source trees yielded the isolates RS 2, RS 24, RS 26, RS 28 and RS 29. No virus could be isolated from the other seven cherry trees. The isolate RS 24 was lost because of a severe outbreak of powdery mildew on cucumber in the greenhouse. The otherfour isolates were used in this investigation. Also used was isolate RS 25, which was received from the Science Service, Plant Pathology Laboratory, Vancouver, B.C. It was isolated from a cherry tree on the campus of the University of British Columbia at Vancouver, B.C. Nomenclature of these isolates is based on systems in use with these laboratories.

The isolates were transferred to and maintained in cucumber (Cucumis sativus L.), var. National Pickling. The same variety of

cucumber was also used in making isolations from inoculated test plants.

The identity of the cherry virus isolates was probed by inoculation of pincherry (Prunus pennsylvanica L.). The use of this species for such a purpose was suggested by the work of Fulton (10).

Virus Contents of the Source Trees.

No formal diagnostic studies were made to determine the virus contents of the source trees. Certain definite data are provided however from symptoms present on the trees and from scion-inoculation experiments of previous years in the case of the Creston trees. The tree yielding isolate RS 25 did not show virus symptoms in the spring of 1958. Data are given in Table 1.

TABLE 1

Virus Contents of the Source Trees *

Isolate	Source Tree	Virus Contents	Remarks
RS 2	Sweet Cherry Oregon Lambert	NRS Tw. Lf. LC	NRS symptoms present only on uppermost leaves
RS 25	Sweet Cherry	Symptomless	Observed only in spring 1958
RS 26	Mazzard Seedling	NRS Mottle Leaf Virus LC	Very strong chlorotic mottle. Symptoms confined to basal portion of the current season's growth and to the spurs. Also laceleaf appearance.
RS 28	Montmorency Sour Cherry	SCY NRS	Depending on weather conditions symptoms of NRS and SCY are apparent every year. No symptoms in 1957.
RS 29	Mazzard Seedling	R-NRS	Symptoms confined to the basal portions of the current season's growth. Laceleaf appearance.

* LC - Little Cherry Virus
NRS - Necrotic Ring Spot Virus
R-NRS - Recurrent NRS
SCY - Sour Cherry Yellow Virus
Tw.Lf. - Twisted Leaf Virus

Plant Growing and Environmental conditions.

Actual isolation work was carried out at Creston, B.C. during the summer of 1957. All other work was done at Vancouver, B.C. in the period October 1957 - June 1958.

At Creston the cucumbers were grown in flats, incubated in a greenhouse where little or no control of temperature could be exercised. Temperatures of 90 -100 F were common and often prevailed for the greater part of the day. Such high temperatures are thought to be a factor affecting the isolation of the virus.

At Vancouver the cucumbers were grown from seed, sown directly into benches containing a 6" deep layer of fertile greenhouse soil. The distance between plants was three inches. Seedlings of pincherry were grown in 4" flower pots. Other plant species were grown as transplants or they were seeded directly into the soil benches and thinned out when necessary.

Artificial light was provided during the short day season. An area of approximately 300 square feet was used during the investigation and facilities of two greenhouses A and B were necessary. Environmental conditions in these greenhouses differed considerably.

From October 1957 to April 1958 when the greater part of the host range studies was carried out, the temperature in greenhouse A was at a constant 75 F. During the same period however, the temperature in greenhouse B fluctuated between 55 and 65 F. After April 1958 tempera-

ture conditions in the two greenhouses were comparable. Fluorescent light tubes in greenhouse A gave 10 hours extra light of a high intensity. In greenhouse B light intensity was lower and light provisions were such, that often many plants had to be grown under conditions of natural light only.

In general plants grew better in greenhouse A and therefore stock cultures of the isolates were maintained in this greenhouse. However cucumbers used in backtransfers had often to be grown in greenhouse B. Pincherry was cultured in greenhouse A.

Inoculation Technique.

All inoculations were made by mechanical transmission technique. Expressed crude plant juice was rubbed onto the upper surface of leaves that had previously been dusted with carborandum 400 mesh. Using the forefinger three to five strokes per leaf were applied in an inoculation. Previous work has indicated that such a technique is satisfactory. (12, 16.)

General Course of Host Range Studies.

In general inoculations were made, when the plants were in a state of most rapid growth and presumably most susceptible to virus infection. Cucumber was inoculated in the cotyledon stage, before the young bud had started to unfold. In this stage it is most susceptible to infection by cherry virus (5). It seemed logical to inoculate other members of the Cucurbitaceae in the cotyledon stage as well and therefore

this procedure was followed. For other plant species inoculations were made when 2-8 leaves had developed. In testing a species three to seven plants were used for each isolate. As a check upon the infectivity of the inoculum three to five cucumber plants were inoculated at the same time with the same inoculum. Three to five plants of the species tested were kept as an additional control.

Backtransfers to cucumber to determine whether infection had taken place were made 12-18 days after inoculation. Three to five cucumber plants were used for each backtransfer. Young leaves were taken from all plants inoculated with the same isolate and a representative sample was used in preparation of inoculum, regardless of whether the young growth showed symptoms or not. Inoculum prepared in this method would demonstrate systemic infection.

Local infection was only investigated when inoculated leaves showed symptoms. In this case inoculum was prepared from such symptom bearing leaves.

A backtransfer sometimes yielded a syndrome clearly differing from that connected with the parent isolate. Such a backtransfer was cultured in cucumber for a better comparison with the parent isolate and with other similar re-isolates.

Identification of the Cherry Virus Isolates.

It was thought that the identity of the cherry virus isolates could be determined at least provisionally by inoculation of pincherry (P. pennsylvanica L.). The use of this species for such a purpose and the method of inoculation was suggested by the work of Fulton (10)

Ultimate identification however must take place by inoculation of a set of suitable Prunus indicator hosts, using scions of P. pennsylvanica. This latter phase of the identification procedure was not carried out in this investigation.

Pincherry seedlings in the 6-leaf stage were inoculated on March 9, 1958. Three seedlings numbered 1, 2 and 3 were used for each isolate. Three young succulent leaves of a seedling were rubbed with cucumber-inoculum.

Backtransfers to cucumber were carried out after the period of incubation. The inoculum used in re-isolation was prepared from each seedling separately. For some seedlings, backtransfers were repeated several times.

Preparation of Inoculum.

Inoculum was prepared by macerating rubbed and mature leaves or leaves of the young growth in a small amount of 0.03 M K_2HPO_4- KH_2PO_4 buffer. The pH level used was eight. The particular pH level was suggested by the work of Fulton (11, 12, 13) and of Heinis (16). The work of Fulton also suggested the particular molar concentration of the buffer. In some cases tapwater was used as the diluent.

Cherry Inoculum.

Principles of inoculation as outlined by Boyle, Moore & Keitt (5) were applied here. Young succulent leaves, preferably those showing

initial ringspot symptoms were selected as the source of inoculum. Tapwater was used in making isolations from the cherry trees, because no buffer was available at that time. In later work however when making re-isolations from pincherry the phosphate buffer formed the suspending medium.

Cucumber Inoculum.

Cucumber inoculum was used in routine transfers to maintain the stock cultures and with inoculations of test plants. Regular transfers of isolates to maintain the stock cultures were made every 14 - 17 days. Inoculum prepared from the stock cultures showed a high degree of infectivity upon cucumber.

In the case of isolates RS 2, RS 25 and RS 29, the inoculum was prepared from systemically infected leaves. Plants inoculated with isolates RS 26 and RS 28 showed little or no growth beyond the cotyledon stage and locally infected cotyledons had to be used.

Phosphate buffer was used as a diluent with isolates RS 25, RS 26, RS 28 and RS 29. The infectivity of RS 2 dropped considerably with the beginning of the short day season when the phosphate buffer was used. However when tapwater was substituted, high infectivity was restored and this procedure was followed in subsequent inoculations.

Previous work has shown that cherry viruses in cucumber extracts have only a short lifetime in-vitro (11,38). In this investigation cucumber inoculum applied to any plant was in no case older than

five minutes.

Testplant inoculum.

Testplant inoculum was used to demonstrate virus infection. It was prepared as explained above. (p.23).

Photography.

Photopictures of figures 1-10 were taken with a 35 mm. Contraflex camera (f =4.5) on Kodachrome film. Those of figures 11-13 were taken with a 35 mm. Practica camera (f =2.8) on Anscochrome film. The photopictures presented are enlarged copies of the original ones.

OBSERVATIONS AND RESULTS

Symptom Expression of the Isolates on Cucumber.

In general the symptom expression of the isolates varied with length of daytime, temperature and season of the year. Some isolates were more subject to variation than others. Characteristic differences were noted in degree of stunting, occurrence of necrosis, chlorotic lesions and bud proliferation. Minor differences were noted in color of the syndrome, and type and degree of mottling. (Fig. 1 - 9 in Appendix). Isolate RS 2 was characterized by a different requirement of pH of buffer as explained under Materials and Methods.

Cucumber Syndrome of Isolate RS 2 (Fig. 2).

Bud proliferation did not occur with this isolate and chlorotic lesions on the cotyledons did not develop. Except during a prolonged hot spell necrosis did not take place. The plants were only slightly stunted and mottling was mild.

The initial symptom was a chlorosis of the first true leaf, starting at the margin and becoming interveinal later on. The leaf then became mottled. As the plant aged the mottling of the leaves became less pronounced. In general symptom expression was more severe during the long day season. During the short day season symptoms produced were very mild and the syndrome was very similar to that of

isolate RS 25.

Cucumber Syndrome of Isolate RS 25 (Fig. 3,4,5)

The first symptom was observed on the first true leaf. Pale green chlorotic areas appeared which followed the outline of the bigger veins. These chlorotic areas were bordered by dark green bands and they enclosed islands of dark green tissue. When the second true leaf had developed the dark green dissue of the first leaf had become chlorotic also. By this time the first leaf often showed a chessboard effect where the lighter colored bigger veins formed the outline of the blocks. The youngest growth showed a few chlorotic areas of an irregular shape and often concentrated along the main vein. The chessboard effect on the first leaf was persistent, whereas no symptoms remained on the other older leaves.

Under short day conditions the syndrome was dark green, whereas under long day conditions it was light green colored. Lesions on the cotyledons were observed only until the start of the short day season in 1957. These lesions were pale yellow and had a rather definite outline. Development of symptoms was slow, stunting was not observed and the syndrome was very mild.

Cucumber Syndrome of Isolates RS 26 and RS 28 (Fig. 6,7,8)

Bud proliferation, severe mottling, severe stunting and development of chlorotic lesions on the cotyledons were characteristics of both these isolates. The lesions were of two types.

In the one case the lesions were diffuse pale green, chlorotic, circular 2-3 mm. in diameter and appearing 3-6 days after inoculation. These spots coalesced readily resulting in chlorotic areas, which often became yellow as the plant matured. In the other case the lesions were yellow, circular, measuring 1-2 mm. in diameter and appeared during or after coalescence of the other lesions. This type however had a distinct margin, remained mostly separate but sometimes two or seldom three adjacent lesions coalesced also. These lesions were often surrounded by a dark green - complete or partial - ring. One to ten lesions of this type were present on a cotyledon. The greater part of the cotyledons was taken in by the first mentioned chlorotic areas.

Both the two isolates showed necrosis of the first true leaf. The cucumber plants were of a general chlorotic light green appearance, but with plant maturity the cotyledons became nearly completely yellow.

The isolates were distinguished by the degree of severity of the syndrome. Killing of the primary bud and the degree of bud proliferation were differentiating features.

The primary bud was killed mostly before the first true leaf had fully developed. The first leaf became necrotic, and bud proliferation was immediate and conspicuous. The buds were severely mottled. Sometimes under conditions of hot weather the first true leaf was not killed so soon and bud proliferation was delayed. This was the more severe syndrome. Isolate RS 28 behaved in this manner during the summer and fall of 1957 until the start of the short day season.

When the primary bud was not killed, bud proliferation was less pronounced. The internodes were very short measuring $\frac{1}{4}$ - $\frac{1}{2}$ inch. The plants slowly increased in length, though the leaves were severely stunted. Isolate RS 28 behaved in this manner from the start of the short day season in 1957 until July 1958. During this period isolate RS 26 was more severe than isolate RS 28.

Both the two isolates, however, were very similar in symptom expression. Also, variability in expression of symptoms was minor when compared with isolates RS 2 and RS 29.

Cucumber Syndrome of Isolate RS 29 (Fig. 9)

Under long day conditions the syndrome was quite similar to that of isolate RS 2. The RS 29 syndrome however showed a more severe type of mottling, more stunting and chlorotic lesions were produced on the cotyledons. In the summer of 1957 these lesions were 1-2 mm. in diameter, were of a pale yellow color and had a definite margin. During the spring of 1958 lesions were not produced.

Under short day conditions the syndrome was entirely different and was very similar to that of isolate RS 28. The RS 29 syndrome however was dark green in color in contrast to that of isolate RS 28 which was light green in appearance. Stunting was very pronounced, internodes were short and bud proliferation occurred. On the cotyledons circular chlorotic lesions developed 2-3 mm. in diameter and had a definite margin. The true leaves were severely mottled, the cotyledons became dark green.

Comparison of the Cucumber Syndromes.

Table 2 gives features distinguishing and differentiating the syndromes of the respective isolates. (p.31-A).

Inoculation Trials with Herbaceous Hosts.

Seventy-five species in twenty-three plant families were tested. On backtransfer virus was reisolated from twenty-three species and varieties belonging to nine families (Table 3, p.32 B-H). In general only the occurrence of systemic infection was checked.

The species which were not susceptible to systemic virus infection are listed on Table 4 (p. 32 I-K). It is possible that some of these species were locally infected.

Virus symptoms were shown by a few species. Most of the experiments were carried out during the short day season when growth of plants was slow. Symptoms usually occur on actively growing leaves (2) and one can expect that virus infection is also favored by an active state of growth.

Symptoms on host plants were shown by members of the Cucurbitaceae, by Nicotiana tabacum L., var. Haranova, by Lens culinaris Medic., by Crotalaria spectabilis Roth and by Cassia marylandica L.

The last named species showed a local lesion reaction on the terminal leaflets when inoculated with isolate RS 25. These lesions had a necrotic centre surrounded by a yellow chlorotic ring. Only a few lesions were produced on a plant and only a low proportion of inoculated

plants reacted with this symptom. Hence only little inoculum was available for backtransfer to cucumber and as a result the causal virus could not be reisolated.

The inoculation trials with Cassia were conducted three times and the same typical symptom was reproduced on every occasion. For this reason it is believed that isolate RS 25 is the incitant of the local lesion reaction.

The results with some species were inconsistent with time. On some occasions virus was recovered showing that systemic infection had taken place whereas in repeat trials virus could not be re-isolated. Frequently only one out of four or five cucumber plants used in a backtransfer showed virus infection. Results as these might be due to host materials which inactivate or inhibit virus. Another cause would be the low susceptibility of the host to virus infection.

In some cases only a limited quanitity of seed was available or only a low proportion of the seeds germinated. As a consequence repeat trials could not be carried out or not all isolates could be tested in an inoculation trial.

The experimental value will be limited of those inoculation trials which were performed only once and where only a low proportion of the cucumbers used in re-isolation became infected.

The results with other experiments are however of more value and these will be dealt with below.

OBSERVATION AND RESULTS

TABLE 2

Comparison of the syndromes on cucumber of the cherry virus -isolates.*

Isolate	Syndrome in general	Bud Killing	Bud Proliferation	Necrosis	Lesions		Stunting	Color	Variation in symptom expression
					Diffuse Margin	Definite Margin			
RS 2	Mild	No	No	Sometimes (H.T.)	-	-	Some	LG(LD) DG(SD)	Considerable
RS 25	Very mild	No	No	No	-	Yes (LD)	No	DG (SD)	Negligible
RS 26	Severe	Yes (SD)	Yes	Yes	Yes	Yes	Severe	LG	Some
RS 28	Severe	Yes (LD)	Yes	Yes	Yes	Yes	Severe	LG	Some
RS 29	Medium	No	Yes	Yes (HT)	No	Yes	Severe (SD) Medium (LD)	DG(SD) LG (LD)	Considerable

* LG - Light green; DG - Dark green; LD - under day conditions; SD - under short day conditions; HT - under high temperature conditions.

OBSERVATIONS AND RESULTS

TABLE 3

Host Range Studies of Five Cherry Virus Isolates with Herbaceous Species

Family & Species	Date of Inoculation	Tested	Symptom on Test Species	Backtransfer to Cucumber		
				Ratio infected/ inoculated	Syndrome RS-0	Parent Isolate
AMARANTHACEAE						
Gomphrena globosa L.	Jan. 12'58	RS 2	-	2/4	-	+
	Mar. 8'58	All isolates	-	0/4		
	" 9'58	" "	-	0/4		
BORAGINACEAE						
Brunnera macrophylla Johnston	Ma. 25'58	RS 2	-	0/4		
		-25	-	0/4		
		-26	-	1/4	+	-
		-28	-	2/4	+	-
		-29	-	1/4	+	-
CARYOPHYLLACEAE						
Silene armeria L.	Mar. 9'58	-2	-	0/4		
		-25	-	0/4		
		-26	-	0/4		
		-28	-	1/4	-	+
		-29	-	0/4		
CHAENOPODIACEAE						
Chaenopodium ambrosi-doides L.	Apr. 12'58	RS 2	-	0/4		
		-25	-	1/4	-	+
		-26	-	0/4		
		-28	-	0/4		
		-29	-	0/4		

TABLE 3 Cont'd.

COMPOSITAE						
Dahlia sp., var. Ideal	Apr. 4'58	RS 2	-	1/4	+	-
Bedding		-25	-	0/4		
		-26	-	0/4		
		-28	-	0/4		
		-29	-	0/4		
Helianthus annuus L.	Feb. 2'58	RS 2	-	0/4		
		-25	-	0/4		
		-26	-	0/4		
		-28	-	0/4		
		-29	-	1/4	+	-
CUCURBITACEAE						
Cucurbita moschata Duchesne	Nov. 26'57	RS 2	?	0/4		
var. Buttercup		-25	chlorosis	5/5	-	+
		-29	?	0/4		
var. Cocozelle	Nov. 26'57	RS 2	mottle	3/4	-	+
		-25	"	5/5	-	+
		-26	"	4/5	-	+
		-29	?	3/4	-	+
var. Table Queen	Nov. 26'57	RS 2	mottle	3/4	+	-
		-25	"	3/4	-	+
		-26	"	4/4	-	+
		-28	"	4/4	-	+
		-29	"	3/4	-	+
	May 22'58	RS 2	savoying	5/5	+	-

TABLE 3 Cont'd.

var. Table Queen Cont'd.	June 19'58	RS 2	savoying	4/4	+	-
		-25	"	4/4	-	+
		-26	"	2/4	+	-
		-28	"	4/4	+	-
		-29	"	5/5	+	-
Cyclanthera sp.	Mar. 18'58	RS 2	mottle	2/4	+	-
		-25	"	3/4	-	+
		-26	-	0/4		
		-28	-	0/4		
		-29	mottle	2/4	+	-
Momordica balsamina L.	Feb. 22'58	RS 26	-	0/4		
		-28	mottle	0/4		
		-29	"	2/4	+	-
Momordica sp.	Mar. 13'58	RS 2	mottle	2/4	+	-
		-25	"	3/4	-	+
		-26	"	0/4		
LEGUMINOSAE						
Cassia marylandica L.	Dec. 11'57	RS 25	necr.lesion	0/4		
		-28	-	0/4		
		-29	-	0/4		
	Mar. 8'58	RS 2	-	0/4		
		-25	necr. lesion	0/4		
		-26	-	0/4		
		-28	-	0/4		
		-29	-	0/4		

TABLE 3 Cont'd.

LEGUMINOSAE Cont'd.						
Cassia marylandica L.	Mar. 25'58	RS 2	-	0/4		
		-25	necr. lesion	0/4		
		-26	-	0/4		
		-28	-	0/4		
		-29	-	0/4		
Crotalaria spectabilis Roth	Jan. 26'58	All isol.	-	0/5		
	Jan 26. & Feb. 16'58 *	RS 2	?	0/4		
		-25	chlorosis	4/4	-	+
		-26	?	0/4		
		-28	-	0/4		
		-29	-	1/4	+	-
	Feb. 16 & Mar. 8'58 *	RS 2	chlorosis & necrosis	3/4	+	-
		-25	chlorosis	5/5	-	+
		-26	necr/chlor	0/4		
		-28	" "	1/4	+	-
		-29	" "	1/4	+	-
	Mar. 9'58	-26	chlorosis	2/4	+	-
Lathyrus odoratus L.	Jan. 11'58	-26	-	3/3	+	-
		-28	-	1/3	+	-
	Feb. 15'58	RS 2	-	0/4		
		-25	-	3/4	-	+
		-26	-	0/4		
		-28	-	0/4		
		-29	-	3/4	+	-

* Same plants reinoculated.

TABLE 3 Cont'd.

LEGUMINOSAE Cont'd.	Feb. 22'58	RS	2	-	3/4	+	-
Lathyrus odoratus L.			25	-	4/4	-	+
			26	-	3/4	+	-
			28	-	2/3	+	-
			29	-	3/4	+	-
	May 22'58	RS	2	-	2/3	+	-
			25	-	3/3	-	+
			26	-	2/3	+	-
			28	-	2/3	+	-
			29	-	3/3	+	-
Lens culinaris Medic.	Feb 2'58	RS	2	-	1/4	+	-
			25	-	3/3	-	+
			26	-	2/4	+	-
			28	-	?		
			29	-	?		
	May 22'58	RS	2	chlorisis	2/4	+	-
			25	"	3/3	-	+
			26	"	2/3	+	-
			28	"	?		
			29	"	1/3	+	-
Medicago sativa L.	Mar. 9'58	RS	2	-	0/4		
var. Rhizome			25	-	1/4	-	+
			26	-	0/4		
			28	-	0/4		
			29	-	0/4		
SCROPHULARIACEAE							
Nemesia sp., var. Triumph	Dec.30'57	RS	2	-	0/4		
			25	-	0/4		
			26	-	0/4		
			28	-	0/4		
			29	-	2/4	-	+

TABLE 3 Cont'd.

Nemesia sp. var. Triumph cont'd	Mar.8'58	RS 2	-	0/4		
		25	-	0/4		
		26	-	0/4		
		28	-	0/4		
		29	-	3/4	-	+
	Mar.9'58	RS 2	-	0/4		
		25	-	0/4		
		26	-	0/4		
		28	-	0/4		
		29	-	3/4	-	+
SOLANACEAE						
Nicotiana tabacum L.	Jan. 11'58	RS 2	-	2/4	-	+
var. Havanna 38		25	-	0/4		
and also		26	-	0/4		
var. Turkish Tobacco		28	-	0/4		
		29	-	0/4		
var. Turkish Tobacco	Apr. 1'58	All isolates		0/4		
var. Haranova	June 19'58	RS 2	Ringspot & mottle	3/6	+	-
		25	"	1/4	-	+
		26	"	3/4	+	-
		28	"	1/5	+	-
		29	"	3/6	+	-
Petunia Hybr.var. Blue Bee	Dec. 30'57	RS 2	-	0/4		
		25	-	0/4		
		26	-	2/4	-	+
		28	-	2/4	-	+
		29	-	0/4		

TABLE 3 Cont'd.

Petunia Hybr. var. Blue Bee Cont'd.	Apr. 1'58	RS	2	-	0/4		
			25	-	0/4		
			26	-	4/4	-	+
			28	-	4/4	-	+
			29	-	0/4		

TABLE 4

LIST OF SPECIES NOT SUSCEPTIBLE TO SYSTEMIC INFECTION BY THE ISOLATES

	Isolates not Tested.
APOCYNACEAE	
Apocynum androsaemifolium L.	
ASCLEPIADACEAE	
Asclepias curassavica L.	28
BORAGINACEAE	
Anchusa azurea Mill. var. Dropmore	2, 26, 28
A. capensis Thumb.	2, 25, 29
Cynoglossum montanum L.	25
CAMPANULACEAE	
Adenophora farreri L.	
Campanula medium L.	
CARYOPHYLLACEAE	
Dianthus serotinus L.	
CHAENOPODIACEAE	
Beta vulgaris L.; var Detroit Dark Red	
Chaenopodium album L.	
Ch. amaranticolor Coste & Rein.	
Spinacia oleracea L.	
COMPOSITAE	
Ageratum Houstoninum Mill., var. Blue Cap	
Aster sp.; var. Giant of California	
Calendula officinalis L.	
Centaurea moschata L.	
C. imperialis Hort.	
Cosmos bipinnatus Cav.; var. Early Sensation	
Lactuca scariola L.	
Tagetes erecta L.	
Tharaxacum officinale Weber	
Zinnia elegans Jacq.	
CRUCIFERAE	
Cheiranthus cheiry L. var. Golden Wonder	
EUPHORBIACEAE	
Euphorbia Lathyrus L.	

TABLE 4 Cont'd.

	Isolates not Tested
LABIATAE	
Salvia liliaefoliae L.	
LEGUMINOSAE	
Baptisia australis R. Br.	
Crotalaria capensis Jacq.	25, 26, 28
Cyamopsis tetragonoloba L. Taub.	
Dolichos Lablab L. (D. soudanensis Hort).	
Glycine Max Merr. (G. hispida Maxim.)	
Lupinus polyphyllus Lindl.	
Phaeseolus coccineus L. (P. multiflorus Lam.)	
P. vulgaris, L.; var. Golden Wax	
P. vulgaris L. var Golden Wax Dwarf	
Pisum sativum L. var. Little Marvel	
P. - - var. Onward	
P. - - var. Perfection	
Vicia faba L.	
V. Sativa L.	
Vigna sinensis Savi. var. California Black Eye	
MALVACEAE	
Althea rosea Cav.	26, 28
NYCTAGINACEAE	
Mirabilis jalapa L.	25
ONAGRACEAE	
Clarkia elegans Dougl.	
Epilobium angustifolium L.	
Godetia amoena Don	
PAPAVERACEAE	
Papaver nudicaule L.	
PLANTAGINACEAE	
Plantago lanceolata L.	
RANUNCULACEAE	
Delphinium sp.	25, 28, 29
SCROPHULARIACEAE	
Antirrhinum majus L. var Early Sensation	
Digitalis purpurea L. var. gloxiniaeflora Vilm.	

TABLE 4 Cont'd.

	Isolates not Tested
SOLANACEAE	
Atropa belladonna L.	
Capsicum frutescens L.	
Datura Stramonium L.	2, 26, 28
D. innoxia L.	2, 26, 28
Salpiglossis sinuata, Ruiz & Pav., var. Emperor	
TROPAEOLACEAE	
Tropaeolum majus L.	
VIOLACEAE	
Viola tricolor L. var. Red Giant	

Virus Re-isolated from Host Species.

In many cases virus obtained in re-isolation was maintained in cucumber for a better comparison with the parent isolates and for a better comparison of all re-isolates together. In some cases the cucumber syndrome of the re-isolated virus was the same as that caused by the original parent isolate. In many cases however it was different.

Many re-isolates originating from sources RS 2, RS 26, RS 28 and RS 29 all produced the same cucumber syndrome irrespective of the parent isolate. The parent isolates however differred in greater or lesser detail as already described. For example, virus re-isolated from sweet pea, Lathyrus adoratus L., inoculated with these dissimilar isolates all produced similar symptoms on cucumber. This particular syndrome will be referred to henceforth as SO. In all cases syndrome SO was reproduced constantly in repeated transfers between cucumbers and in no case did the syndrome of the original parent isolate manifest itself.

It appeared that SO was indistinguishable from the cucumber syndrome of isolate RS 25. This does not mean however, that the causal viruses are identical, because dissimilar viruses can produce similar syndromes in the same plant species. The observations suggest only that the four isolates concerned are not simple but complex in virus composition.

For convenience however, the general symbol RS-O will be used to denote a virus factor found in isolates RS 2, RS 26, RS 28 and

RS 29, which produces the same syndrome SO in cucumber.

Some species notably Lathyrus odoratus L. and Lens culinaris Medic. appeared to be useful in separation of RS-O from the parent isolates. Isolate RS 28 however did not yield RS-O in infectivity trials with Lens culinaris. Trials were repeated three times with sweet pea and two times with Lens culinaris. Control plants of these species did not yield RS-O. Symptoms were not observed on Lathyrus whereas in one experiment with Lens conducted in May 1958, the young leaflets showed a distinct chlorosis. The other experiment with this species was carried out in February 1958.

Other species useful in separation of RS-O from the parent isolates are tobacco (var. Haranova) and squash (var. Table Queen). The results with these species however are inconsistent and need explanation.

In one experiment carried out in greenhouse A in January 1958 the tobacco varieties Havanna 38 and Turkish tobacco were readily infected with isolate RS 2. On reisolation all five cucumber plants used in each backtransfer clearly showed the syndrome distinctive of isolate RS 2. Symptoms on tobacco were not observed. No other isolate infected either of these two tobacco varieties. Another experiment carried out in the same greenhouse and taking place in April 1958, using Turkish tobacco only was unsuccessful, however.

Questionable results were also obtained with squash (var. Table Queen). In an experiment conducted in November 1957, taking place in greenhouse A, all five isolates proved to be infective. Isolates RS 2

and RS 29 caused a similar slight systemic mottle, isolate RS 25 caused a mottle accompanied by a light green chlorosis, isolates RS 26 and RS 28 caused severe mottling and stunting. On reisolation into cucumber isolates RS 25, 26, 28 and 29 yielded a syndrome identical to that of the parent isolates. Isolate RS 2 yielded a mild syndrome, which might have been due to RS-0.

In a later experiment conducted in May 1958 and taking place in greenhouse B where many of the seeds failed to germinate, isolate RS 2 only was used. After ten days the young growth showed a light green chlorosis. Nine days later the symptoms were very severe and conspicuous to the extreme. Stunting was very pronounced and the plant had a savoyed appearance. On the younger growth dark green blisters with the opening pointing downwards were conspicuous amidst the pale green chlorotic areas. The blisters were variable in size and were often coalescing. The veins protruded beyond the leaf edge giving it a fringe-like appearance. Often the leaf lamina was almost entirely lacking and the leaf was reduced to fringes of tissue only (Fig. 10). Spatula shaped leaves and other variations in leaf malformations were noted also. On the leaf petioles, especially of the older leaves, enations were produced appearing initially as pin-point areas and later forming bands of tissue raised above the surface of the petiole. These bands were all running parallel to each other. When backtransfered to cucumber, RS-0 was produced.

Smith (31) described a symptom on squash caused by cucumber mosaic virus which is reminiscent of the squash symptom described above. The same writer related that cucumber mosaic virus produces

characteristic symptoms on Nicotiana tabacum and on N. glutinosa. Another experiment, the third, with tobacco Haranova was therefore conducted. At the same time a more extensive trial with squash (var. Table Queen) was carried out.

Squash-inoculum of isolate RS 2 was used in both these experiments, along with cucumber-inoculum of RS-0 as the sweet pea filtrate of isolate RS 28 and finally all the regular stock isolates. Symptoms on squash were the same for all virus sources. Also the symptom was similar to the one already described above. In all backtransfers RS-0 was reisolated.

The results on tobacco however were in contrast to those obtained in earlier experiments. Ten days after inoculation ringspot symptoms were observed only in the case of the stock isolates. The ringspots were present on locally infected leaves and the symptom did not become systemic. These spots were 3-4 mm. in diameter and appeared as dark green water-soaked areas surrounded by a ring of light green tissue.

On the day following this observation and with careful examination no such ringspots could be discerned. The number of ringspots produced were counted:

Virus	No. of Ringspots		
	Plant 1	Plant 2	
RS 2	2	0	
- 25	6	0	
- 26	14	0	(mottle)
- 28	6	1	
- 29	2	0	
- 2 (in squash)	0	0	
RS-0 Sweet Pea filtrate of RS 28	0	-	
Control	0	0	

On plant 2 of isolate RS 26 another systemic symptom was produced. It consisted of an initial dark green veinbanding effect, which later changed into a dark green mottle. It was very reminiscent of the syndrome of a cucumber-mosaic virus. Except for this plant, a doubtful inconspicuous mottle was noticeable on all the other plants. On backtransfer and irrespective of the virus source, RS-0 was obtained only. Plant 2 of isolate RS 26 from which separate inoculum was prepared also yielded this syndrome. No virus was isolated from the control tobacco plants. All inoculum was prepared from systemically infected young leaves. No attempt was made to isolate virus from the locally infected leaves.

The experiments reported above were all concerned with reisolated virus which produced cucumber syndromes dissimilar to those caused by isolates RS 2, RS 26, RS 28 and RS 29.

In other trials the reisolated virus consistently produced a cucumber syndrome which was always similar to that caused by the parent isolate. This was the case with Nemesia sp., var. Triumph which was susceptible only to isolate RS 29. It was also apparent with Petunia hybr., var. Blue Bee, from which only isolates RS 26 and RS 28 could be reisolated. These experiments were repeated several times and the same results were reproduced on every occasion.

When virus reisolated from Nemesia and Petunia was used to infect sweet pea, syndrome SO only was obtained in backtransfer to cucumber. Evidently passage through Nemesia and Petunia had not deprived isolates

RS 26, RS 28 and RS 29 from virus factor RS-O responsible for syndrome SO.

Symptom Expression of the Isolates on Pincherry.

The first virus symptoms were observed nine days after inoculation. Plants inoculated with isolate RS 28 were characterized by acute symptoms of necrosis and curling of the young leaves. (Fig. 13). Another six days later these plants showed a severe shothole effect. Plants inoculated with the other isolates all showed similar symptoms of mottling on the young succulent leaves. (Fig. 12). In some cases a few necrotic lesions developed shortly after the initial symptoms of mottling.

Subsequent symptoms observed on inoculated plants are of doubtful experimental value because of fungus contamination. The occurrence of the fungus was not noted until near the end of the investigation when a few necrotic lesions-pf a similar type as found on virus inoculated plants - were observed on one control plant. Some of these lesions on the control plant were accompanied by a white superficial mycelial growth occurring at the underside of the leaf at the site of the lesion. Similar mycelial growth also occurred on leaves of inoculated plants. Microscopic examination on one occasion showed the presence of one celled hyaline globular catanulate conidia. The conidia of Coccomyces hiemalis Higgins and Coryneum beijrinckii Oud., both incitants of leaf spot on cherry, do not correspond to the description of the condia referred to above. No time was available to pursue a more

thorough investigation of the fungus contaminant and its association with the necrotic lesions.

On all virus inoculated plants similar symptoms of necrotic lesions, shothole effect, shredding and defoliation were noted, whereas on one out of three control plants a few necrotic lesions developed. Conspicuous differences in disease severity between control and virus inoculated plants are therefore present. A combined virus-fungus effect could explain the more severe symptoms on the inoculated plants.

The inoculated plants however do retain their value as a stock of scions to be grafted or budded onto suitable Prunus indicator hosts. Identification of the virus isolates can be carried out by such a procedure.

Virus Reisolated from Pincherry.

The cucumbers used in backtransfers from pincherry inoculated with isolates RS 2, RS 26 and RS 29 all yielded the syndrome SO. In other words only virus RS-O was reisolated in these cases.

No virus was reisolated from plants inoculated with isolate RS 25. Attempts to reisolate virus were made on three different occasions. Plants inoculated with isolate RS 28 yielded on backtransfer a cucumber syndrome similar to that of the parent isolate. Inoculation of sweet pea with this reisolated virus and subsequent backtransfer to cucumber yielded syndrome SO. Evidently virus RS-O still formed part of the complex reisolated from pincherry inoculated with isolate RS 28.

The inoculum used in backtransfer was always prepared from the seedlings with the most pronounced symptoms. In case of isolate RS 28 all three seedlings showed similar acute symptoms. Table 5 gives the results of backtransfer in a tabulated form

TABLE 5

Results of Backtransfer to Cucumber from Pincherry Inoculated with the Virus Isolates

Isolate	No. of Seedling	Initial Symptom on Seedling	Date of Reisolation in 1958	Ration infected/ inoculated plants	Backtransfer to Cucumber Syndrome RS-0	Parent isolate
RS 2	1	mottle	July 6	1/4	+	-
	2	"	Apr. 9	1/4	+	-
			May 26	3/3	+	-
	3	"	"	3/3	+	-
RS 25	3	"	Apr. 9	0/4		
			" 28	0/4		
			May 5	0/4		
RS 26	2	"	May 26	3/3	+	-
	3		Apr. 9	1/4	+	-
RS 28	3	acute necrosis shothole	Apr. 9	5/5	-	+
RS 29	3	mottle	Apr. 9	0/4		
			" 28	0/4		
			May 5	0/4		
			" 26	1/4	+	-

D I S C U S I O N AND C O N C L U S I O N

It is evident from the experiments that several species of herbaceous plants can be symptomless carriers of viruses isolated from cherry, whereas symptoms are produced in cucurbit species. This confirms the work of Fulton (12) and of Willison and Weintraub (40). Species as Lathyrus odoratus L., Lens culinaris Medic. and Nemesia sp. differentiate between viruses isolated from cherry and have not been reported by other workers. Another differentiating host is Petunia hybr. var. Blue Bee also reported by Fulton (12).

From the inoculation experiments it is evident that isolates RS 2, RS 26, RS 28 and RS 29 are complex in nature, i.e. they consist of more than one virus. Isolates complex in nature were also involved in work done by Gilmer (15), Milbrath (23) and Willison and Weintraub (40).

Lathyrus odoratus and Lens culinaris are useful in separating the virus RS-O present in isolates RS 2, RS 26, RS 28 and RS 29. Virus RS-O causes a mild syndrome SO in cucumber which was very similar to that of isolate RS 25. The same virus in squash (var. Table Queen) causes a severe savoying type of symptom characterized by fringe-like outgrowths at the leaf edge. Tobacco (var. Haranova) was systemically infected by virus RS-O under conditions of long day.

On pincherry (P. pennsylvanica L.) isolate RS 28 causes acute symptoms of necrosis and shothole. The plants recovered but symptoms of mottling were systemic. Necrotic Ring Spot Virus caused similar symptoms on Prunus hosts and this virus and Sour Cherry Yellows Virus was present in the original source tree.

The other isolates in pincherry all cause similar symptoms of mottling on the young leaves. A few necrotic lesions were produced also. On reisolation from pincherry virus RS-0 was obtained in the case of isolates RS 2, RS 26 and RS 29. No virus was reisolated in the case of isolate RS 25.

The results with pincherry suggest that virus RS-0 is responsible for the mild symptoms whereas virus RS-0 in conjunction with an additional virus as in isolate RS 28 incites the severe shock symptoms. The identification of the viruses present in the isolates can be carried out by scion inoculation of a set of suitable Prunus indicator hosts.

Some species used in extensive host range work by Fulton (12) proved to be useful in differentiation of Prunus virus isolates. Cassia marylandica L. gave a systemic symptomless reaction to virus E of Fulton, whereas in this investigation it reacted with a necrotic local lesion symptom to isolate RS 25. Crotalaria spectabilis Roth reacted with symptoms of necrosis and chlorosis to all isolates, whereas in Fulton's work it gave a local lesion reaction to virus B. Cyamopsis tetragonoloba (L.) Taub. reacted with symptoms to viruses A, E and G of Fulton, whereas this species was not susceptible to any of the five isolates tested in this study. Gomphrena globosa L. became systemically infected by virus A of Fulton. Isolate RS 2 infected this species on one

occasion but the results could not be reproduced. Zinnia elegans Jacq. reacted with a mottle to virus B of Fulton whereas it was not susceptible to any of the isolates used in this investigation. Other cases similar to Zinnia can be cited.

It would appear that there is little relationship between the virus isolates studied here and those studied by Fulton. Necrotic Ring Spot Virus and Sour Cherry Yellows Virus however occur in the source trees concerned in this investigation as well as in Fulton's study. Different viruses or different strains are evidently involved.

Isolates RS 26 and RS 28 are distinguished from the other isolates by systemic symptomless infection of Petunia hybr., var Blue Bee. Isolate RS 28 in one experiment with Silene armeria L. infected this species whereas isolate RS 26 failed to do so. The value of this experiment is not great because on reisolation only one out of four cucumber plants became infected. Also on the basis of very similar symptom expression on cucumber isolates RS 26 and RS 28 appear to be closely related to each other.

Isolate RS 29 is characterized by symptomless systemic infection of Nemesia sp., var. Triumph.

Isolate RS 25 differs from the other isolates by local infection resulting in necrotic lesions on Cassia marylandica L. Under the conditions of the experiment it does not appear that this species is useful for assaying the infectivity of this isolate because only a few lesions per plant were produced and because some plants escaped infection.

No herbaceous host was found which gives consistent and reproduceable results in differentiating isolate RS 2 from the other isolates. The results with Gomphrena globosa L. and with Nicotiana tabacum L. were obtained on one occasion only and could not be reproduced.

It appeared that isolates RS 2, RS 26, RS 28 and RS 29 consist of more than one virus. This was apparent by inoculation of Lathyrus odoratus L. and of other species. The reisolated virus of these four isolates all yielded a cucumber syndrome SO which was dissimilar to that of the parent isolates. At the same time syndrome SO could not be distinguished from the cucumber syndrome of isolate RS 25.

The question arises whether syndrome SO is caused by the same virus, by different viruses or by different strains of the same virus, because similar symptoms on the same host can be caused by viruses which are dissimilar (I) or similar (II).

For convenience the symbol RS-O is replaced by P. In case I isolate RS 25 e.g. comprises virus P-25, isolate RS 2 comprises virus P-2, etc. In case II the same virus e.g. P-25 is present in all isolates. Virus P may consist of only one virus or may comprise more than one virus.

Because the parent isolates do differ in symptom expression on cucumber and because it is assumed that virus P alone always gives the same cucumber syndrome SO, an additional virus must be present in all isolates except RS 25. Virus P-25 alone distinguishes this isolate already from isolates RS 2, RS 26, RS 28 and RS 29. The additional factor present in the last named isolates may be the same for all (1) or it may be different (2). In case 1 the letter A will denote the same factor, in case 2,

letters A, B, C and D will denote the different factors. The situation is as follows:

Isolate	Virus Composition			
	I-1	I-2	II-1	II-2
RS 2	A P-2	A P-2	A P-25	A P-25
RS 25	P-25	P-25	P-25	P-25
RS 26	A P-26	B P-26	A P-25	B P-25
RS 28	A P-28	C P-28	A P-25	C P-25
RS 29	A P-29	D P-29	A P-25	D P-25

Case II-1 can be dismissed because then the parent isolates RS 2, RS 26, RS 28 and RS 29 would give the same cucumber syndrome. This was not the case.

In case II-2 one would expect that in an experiment as with (e.g.) Brunnera macrophylla which appears susceptible to isolates RS 26, RS 28 and RS 29, also isolate RS 25, would infect this species. The concentration of virus P-25 in the respective cucumber inocula used for inoculation of Brunnera should be higher for isolate RS 25 than for the other isolates where additional factors were present. Isolate RS 25 does not infect Brunnera.

A similar way of reasoning applies in the case of Dahlia (only isolate RS 2 infects) and in the case of Helianthus (only isolate RS 29 infects).

One would also expect that in case of Nemesia (susceptible to isolate RS 29) and of Petunia (susceptible to isolates RS 26 and RS 28) isolate RS 25 also would infect this species, because these isolates RS 26 and RS 28 still contain the virus factor responsible for syndrome SO. Both these species could not be infected with isolate RS 25 in spite of repeated attempts.

Case II-2 therefore is rejected and it is considered that Case I-1 and I-2 represent pictures closer to reality.

Case I-1 seems possible only if virus A alone is latent in cucumber. Gilmer (15) reported a Prunus virus latent in cucumber which caused a veinbanding symptom in the squash variety Cocozelle.

In the experiment with Nemesia the original cucumber syndrome of isolate RS 29 was obtained in backtransfer. One may assume that this syndrome is due to the interaction of both viruses A and P-29, because P-29 alone yields the dissimilar cucumber syndrome SO. In this case Nemesia is apparently susceptible to virus A. Virus A present in the other isolates can only be discerned if symptoms are produced. The cucumber used in backtransfer for the other isolates did not show symptoms however. This can only be accounted for if virus A is latent in cucumber.

Case I-2 accounts for the results obtained with Nemesia and with Petunia on the basis of the differences between viruses A, B, C and D.

The results with Brunnera, Dahlia and Helianthus can be explained on the basis of the dissimilarity between the strains. Brunnera e.g. would be susceptible only to P-26, P-28 and P-29 and not to P-2 and P-25.

Similarly Dahlia would only be susceptible to strain P-2 and Helianthus only to strain P-29.

The results with Nemesia and Petunia can be explained on the same basis. For example, Nemesia is susceptible to both viruses D and P-29 present in the parent isolate RS-29.

The varying results with tobacco can be explained on the basis of differential susceptibility to virus P under long - and short day conditions. Virus P does not infect tobacco under short day conditions whereas conditions of long day are favorable to infection.

Viruses P-2, P-25, P-26, P-28 and P-29 may be considered to be different strains of the same virus because of common host ranges. Cucumber, pincherry, squash, sweet pea and tobacco are all susceptible to each of the five strains of virus P. All strains also give the same or similar syndrome on cucumber, pincherry, squash and tobacco and all strains react without symptoms in sweet pea.

The differences between the cucumber syndromes of the parent isolates can be explained on the basis of the differences in reaction of the strains of virus P with the same or different viruses.

The results with Nemesia and Petunia and other species are suggestive also of a relationship between the strain of virus P and the other viruses present in the isolates.

Obviously strain P-29 and virus E have common host ranges. Both viruses are isolated from cherry, and both infect cucumber and Nemesia. Strain P-26 and virus B and strain P-28 and virus C similarly have host ranges in common.

The identity of virus P has not been determined because of lack of time. It is apparently widespread in nature because it occurs in isolates of both the Kootenay district (RS 2, RS 26, RS 28, RS 29) as well as in isolate RS 25 originating from the coastal area. From experiments not described here it appears that virus P is resistant to aging in-vivo. The symptoms on squash are reminiscent of a strain of cucumber-mosaic virus (31). One of two tobacco plants infected with isolate RS 26 showed symptoms suggestive of a strain of cucumber-mosaic virus also. Species of a number of different families, other than Cucurbitaceae are apparently susceptible, especially members of the Leguminosae.

Willison and Weintraub (40) isolated a virus CMVP from Prunus hosts. Its particle size and immunological reactions suggested that it was a strain of cucumber-mosaic virus. A more complete discussion of the work of these investigators is found in the review of literature.

Virus P and virus CMVP of Willison and Weintraub may well be related to each other.

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Fig. 1
Healthy cucumber plant, of same
age as plants in figures 3, 6, 7,8.

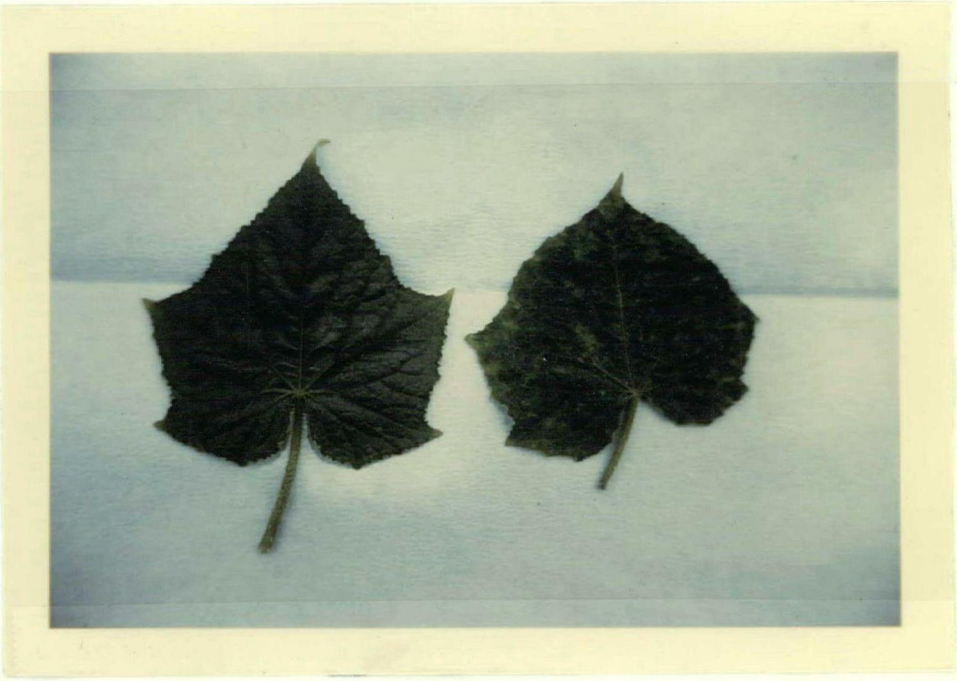

Fig. 2
Young cucumber leaf showing typical mottle of isolate RS 2 (right)
Leaf of healthy plant at left.

Fig. 3.
Isolate RS 25 in cucumber, 11 days after inoculation.

Fig. 4.
First true leaf of cucumber infected with isolate RS 25 showing the chessboard appearance (right). Leaf of healthy plant at left.

Fig. 5.
Cucumber leaves showing typical mottle of
isolate RS 25.

Fig. 6
Isolate RS 26 in cucumber, 11 days after inoculation. Apical bud has been killed.

Fig. 7
Isolate RS 28 in cucumber, 11 days after inoculation. Proliferation of buds has started, youngest leaf shows necrosis at tip.

Fig. 8
Isolates RS 26 (left) and RS 28 (right)
in cucumber, 27 days after inoculation.

Fig. 9
Isolate RS 29 in cucumber, 11 days
after inoculation.

Fig. 10
Typical syndrome of virus RS-0 in squash
(var. Table Queen),
showing dark green blisters on chlorotic
leaf lamina and the fringe-like leaf edges.

Fig. 11
Healthy seedling of Pincherry.

Fig. 12
Isolate RS 2 in pincherry, 32 days after inoculation. The leaf in front of the picture is mottled.

Fig. 13
Isolate RS 28 in pincherry, 32 days after inoculation. Leaf with shock symptoms at right and mottled leaf in front of the picture.