CHERRY LATENT VIRUSES AS EXPRESSED ON CHERRY, PEACH, AND CUCUMBER

by

JULIUS LEO HEINIS

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APPROVED:

Redacted for Privacy

Professor of Botany and Plant Pathology

In Charge of Major

Redacted for Privacy

Chairman of Department of Botany

Redacted for Privacy

Chairman of School Graduate Committee

Redacted for Privacy

Dean of Graduate School

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Typed by Elsie L. Deep

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INTRODUCTION

Various papers have been presented which clearly demonstrate that stone fruit trees carry several viruses in a latent or more or less symptomless condition. The ring spot virus complex has received the most attention because it is widespread and can be detected on a number of different host plants. Some of the most used index plants are virus free peaches, Montmorency sour cherries, *Prunus tomentosa*, Thunb. (*Cerasus tomentosa*, Wall.) and oriental flowering cherries. The great variability of the symptoms produced by this ring spot virus complex, when different stonefruit trees were tested, has been noted by various workers. This suggests the complex is composed of a group of viruses or strains, or mixtures of strains of the same virus. A virus producing a definite pattern on cucumbers has been recovered by mechanical inoculation from this complex. The mechanical inoculation of stone fruit trees with virus cultures has not been possible, and the virus obtained in cucumbers has not been returned to any stonefruit plant. Therefore, the identity of this virus and its relationship to other members of the complex has not been established.

The purpose of this study was to attempt to compare the reactions of the ring spot virus and the virus obtained from cucumber on several host plants, and thereby determine their relationship.

The twenty-three stone fruit trees selected as sources of ring spot virus had already been indexed on Shiro-fugen and Kwanzan flowering cherries previous to this study. On these host plants there were different types of reactions, indicating there were different strains. Some of the sources had been indexed on peach, Montmorency and _Prunus tomentosa_, Thunb. The reaction of the sources on virus free Bing sweet cherry and J. H. Hale peach under greenhouse conditions was determined in this study. The major problem was to attempt to recover the ring spot virus from all sources on cucumber, and then study these viruses or this virus for strain differences noted on the other index hosts. If the virus recovered from stone fruits showed the same strain variance on cucumber as it did on the stone fruit index hosts, this would furnish strong evidence that the virus on cucumber was the ring spot virus and not some other latent contaminant.

LITERATURE REVIEW

The original description of ring spot virus on peach was accredited to Cochran and Hutchins (4, p.860) in 1941. They observed twig blight and severe dieback on J. H. Hale and Late Elberta. These symptoms disappeared after one year and the trees appeared normal. Nursery trees receiving graft-inoculum from diseased orchard trees showed severe twig blight, stem canker, ring spot, and chlorotic mottles on scattered leaves after two months. Most of the mottled leaves dropped and the new foliage was normal. Chamberlain, Willison, and Berkeley (3, pp. 63-64) in 1942 reported the necrotic ring spot (Canadian type) on sour and duke cherries. Berkeley (1, pp.2-3) described necrotic ring spot on sour cherry as being of the shock-type since the trees recover, while sour cherry yellows is of the chronic type because it persists year after year. Both are usually associated in the sour cherry yellows complex. If buds from diseased trees are inoculated on healthy peach trees the necrotic ring spot component produces shock reaction consisting in delayed foliage, chlorotic areas, and ring spots on the leaves. The chronic component, sour cherry yellows, causes rosetted shoots which persist. On Italian prune the chronic symptoms were similar to those of prune dwarf.

Berkeley concluded by suggesting that strains of both viruses were present because of the variation in degree of symptoms.

Tatter leaf was described by Willison, Berkeley, and Hildebrand (20, pp.141-146) on sweet cherry. Inoculations to Montmorency sour cherry first produced symptoms similar to necrotic ring spot, but later in the season they resembled those of sour cherry yellows. On peach, tatter leaf caused yellow green rings and chlorotic and necrotic spotting on the leaves first emerging after inoculation. The New York strain induced terminal dieback after which the tree recovered. An Ontario strain inoculated into Italian prune induced symptoms indistinguishable from those of prune dwarf. Willison _et al_. discussed the relationship of tatter leaf, necrotic ring spot, and prune dwarf.

In Oregon and Washington a similar disease was referred to as lace leaf condition by Zeller (23, pp.85-90). Peach trees inoculated with this virus indicated a severe shock reaction such as dieback, lace leaves, and sunken cankers around the inserted cherry bud.

To these forms or strains the rough bark disease, described by Milbrath and Zeller (8, pp.428-430) could be added. This virus on Kwanzan ornamental cherry has many characteristics which tie in with the ring spot virus complex.

When the stone fruit virus handbook (17) was written there was considerable disagreement among the interested workers as to what should be included under the peach ring spot virus. There was considerable evidence that peach ring spot, necrotic ring spot, and tatter or lace leaf were all caused by the same complex of viruses. However, since there was disagreement, the forms were discussed separately as different diseases.

Previous to 1944 Hildebrand (6) studied various fruit tree viruses, including parts of the ring spot complex, but he did not publish the results until 1953.

Milbrath and Zeller (9, pp.114-115) used the term latent viruses in stone fruits to cover this highly complex situation. In 1950 Milbrath (14, pp.374-375) reported growth reduction caused by latent ring spot virus of cherries on nursery trees, and the title indicates that he refers to ring spot as being a latent virus.

The possibility that cherry viruses of the ring spot type can be mechanically transmitted to herbaceous hosts was discovered by Moore, Boyle, and Keitt (15, pp. 623-624) in 1948. As source of inoculum they used young leaves of sour cherry trees carrying the ring spot virus. In a later paper Boyle, Moore, and Keitt (2, p.3) indicated the physical properties of the virus as expressed when transmitted to cucumbers. They obtained a thermal inactivation point of 52° C. for 10 minutes, a dilution end point of 1-20, and a longevity of 12 hours at room temperature. Varney and Moore (18, p.36) worked on the effect of temperature on symptom expression of the virus on cucumber. In 1951 Hobbs (7, pp.16-17) found 46 out of 47 cucumber varieties and 4 out of 5 pumpkin varieties susceptible to the virus from sour cherry. Squash and watermelons did not show positive results. His attempt to reinfect cherries with the virus from cucumbers by patch grafting and mechanical techniques failed.

In 1953 Willison and Weintraub (21, pp.175-177 and 22, pp.324-328) and Weintraub and Willison (19, pp.328-332) published the results of their studies on stone fruit viruses in cucurbit hosts. They described a standard technique for inoculating cucumber cotyledons and evaluating the infectivity of infectious juice. The results obtained when using buffers did not vary markedly from those obtained when using water. With higher dilution they found that infectivity decreased rapidly with most isolates. They also conducted experiments with longevity and found that one strain lost its infectivity after one hour when held at room temperature and after 12 hours at 0° C. Another strain lost its infectivity after 4 hours at room temperature, but remained infective for more than

24 hours at 0° C. Weintraub and Willison (19, pp.328-332) studied the inhibitors in cucumbers. When they added healthy cucumber leaf parts to the inoculum from cucumbers infected with a virus from sour cherries, they obtained an initial depression or delay of infectivity with some isolates. They found that if leaf tissue of a Hubbard squash was added to the inoculum, infectivity was totally lost. The inhibitory effect was considerably less with healthy cotyledons. They also found (21, p.175) that cucumber plants tend to become less susceptible as they grow older.

As a source of inoculum most workers have used the very young tip leaves of cherry or peach. Milbrath (12, p.479) also recovered the virus when the flower petals were used as a source of inoculum.

MATERIAL AND METHODS

Virus Sources

The virus sources used in these studies were selected from a group of trees growing on the Botany and Plant Pathology experimental farm. The 23 trees which were selected as the virus sources had previously been collected by J. A. Milbrath and designated as R.S. 2 to R.S. 23. The same letters and numbers have been adopted throughout these studies. These trees were chosen because when they were indexed on ornamental flowering cherry they indicated a range of virus response from no reaction to a very severe reaction, often killing the test tree. These sources are given below with a brief discussion of their previous index history.

R.S. 2. This is a Royal Ann (Napoleon) sweet cherry, found in a home yard at Hillsboro, Oregon. The tree used in these studies was established on mazzard roots at Oregon State College's experimental plots as tree 327. The tree has been tested several times on Shirofugen and Kwanzan and has never shown any indication of the ring spot virus. R.S. 2 has also been indexed on peach and Montmorency sour cherry without giving a ring spot reaction. On Montmorency an unreported tip canker virus reaction has been noted. On *Prunus tomentosa*, Thunb.

this source produced severe mottle symptoms. These records indicate that the source has not given any reaction suggestive of the ring spot virus.

R.S. 3. This Bing variety, from a tree in Cherry Park nursery, Fairview, Oregon, was established on mahaleb at the Oregon State College experimental plots as tree 310. Originally this tree tested non-necrotic on Shiro-fugen and had a very mild reaction on Kwanzan. The 1953-1954 tests on Shiro-fugen and Kwanzan demonstrated that this source tree has become contaminated with a more severe virus. There was a necrotic reaction on Shiro-fugen but the Kwanzan reaction was still mild.

R.S. 4. This Royal Ann tree from Walton's orchard in Eugene is now carried on mahaleb roots as tree 328. This source has been indexed on Shiro-fugen, peach, Montmorency, and English Morello sour cherry without a ring spot reaction. On Kwanzan there is a mild reaction.

R.S. 5. This is a Black Tartarian variety of sweet cherry from the Carl Dick home near Orenco. It has been established on mahaleb as tree 342. When tested on Shiro-fugen, a mild necrotic reaction occurred but there was no reaction on Kwanzan. The variety did not react on peach, but there was a necrotic ring spot reaction on Montmorency and English Morello.

R.S. 6. This Lambert is from Moller's nursery near

Fairview, Oregon, and is maintained on mahaleb tree 338. This tree gives a necrotic reaction on Shiro-fugen and a very mild reaction on Kwanzan. There is a medium retarding reaction on peach and a necrotic ring spot reaction on Montmorency.

R.S. 7. This is a Lambert tree from a home in Corvallis and is maintained on mahaleb as tree 341. This is a necrotic strain on Shiro-fugen and fairly severe reactor on Kwanzan. On peach the reaction is severe and necrotic ring spot is produced on Montmorency and English Morello.

R.S. 8. This Bing tree came from Carleton Nursery and is maintained on mazzard roots as tree 313. Shiro-fugen gives a necrotic reaction and Kwanzan indicates a severe strain. There was a strong reaction on peach and necrotic ring spot on Montmorency and English Morello.

R.S. 9. This is a Royal Ann tree purchased from Carleton Nursery and maintained as tree 329. There is a necrotic action on Shiro-fugen and a severe reaction on Kwanzan. A severe reaction on peach and necrotic ring spot on Montmorency and English Morello indicates that this tree has a severe strain of virus.

R.S. 10. This source tree is an Olivet sour cherry from Milton Nursery and has been numbered tree 432. This tree has given a severe reaction on all index plants. It is Shiro-fugen positive, very severe on Kwanzan and also on peach. R.S. 10 produces necrotic ring spot on Montmorency and English Morello.

R.S. 11. This Black Republican variety was found near Moller's Nursery at Fairview. This source was established on mahaleb as tree 330. This tree was indexed only on flowering cherry, giving a mild necrotic action on Shiro-fugen and no reaction on Kwanzan.

R.S. 12. This Bing does not react on Shiro-fugen locally and gives only a mild reaction on Kwanzan. The tree was found near a home east of Portland and has been established on mahaleb as tree 307.

R.S. 13. This is another Royal Ann, selected because it did not give a necrotic reaction on Shiro-fugen and only a mild one on Kwanzan, indicating a mild strain. However, when this tree was reindexed in 1953-1954 the Shiro-fugen gave a necrotic and the Kwanzan a mild reaction, demonstrating that this source now has a more severe strain. This tree came from the home of Carl Dick, Orenco, and is maintained on mahaleb as tree 325.

R.S. 14. This Bing tree was found near Gresham and is being maintained as tree 317 on mahaleb. Inoculations to Shiro-fugen gave a necrotic reaction, but the Kwanzan indicated a mild strain.

R.S. 15. This Black Tartarian is from a yard in Corvallis and was transferred to the experimental plots where it is maintained on mahaleb as tree 343. This gives a necrotic reaction on Shiro-fugen but no reaction on Kwanzan.

R.S. 16. This tree was obtained from a nursery at Fairview because it had developed a very rough bark condition which was described as the rough bark disease of Kwanzan (8, pp.428-430). When indexed on Shiro-fugen a necrotic reaction occurred and the effect on Kwanzan at first resembled ring spot. This could be considered a mild strain of ring spot.

R.S. 17. This Lambert tree was obtained from the Milton Nursery and is being maintained as tree 340. The reaction on Shiro-fugen is necrotic and on Kwanzan severe.

R.S. 18. This Black Republican came from N. E. Glisen Street, Portland, and is maintained on mahaleb as tree 336. This source does not produce a necrotic reaction on Shiro-fugen but gives a mild to severe reaction on Kwanzan.

R.S. 19. This English Morello is from Milton Nursery and maintained as tree 431. This is one of the very severe strains, being necrotic on Shiro-fugen and often killing Kwanzan.

R.S. 20. This is an Early Richmond sour cherry from Milton Nursery and has been given the tree number 433. There is a necrotic reaction on Shiro-fugen but no reaction was noted on Kwanzan. The 1953-1954 index on Kwanzan indicated some necrotic bud which might indicate a stronger reaction than shown by previous records.

R.S. 21. When this Bing tree, numbered 705, was inoculated with the Mora virus (11, pp.347-348) from Salem a severe lace leaf condition was noted indicating an invasion with a fairly severe ring spot virus. When indexed on Shiro-fugen in 1953-1954 a necrotic reaction developed, but on Kwanzan the response was fairly mild.

R.S. 22. This Lambert tree, number 896, was inoculated with rusty mottle from an orchard near Albany. When peach was inoculated with buds from this tree a severe green rosette condition resulted. The reaction on Shiro-fugen has been necrotic and on Kwanzan it is moderate in intensity.

R.S. 23 and R.S. 24. These are two Montmorency trees on mazzard roots which were inoculated with recurrent ring spot and sour cherry yellows. The inoculum was obtained from the University of Wisconsin through the courtesy of Dr. J. D. Moore. This source has been necrotic on Shiro-fugen but gave no reaction on Kwanzan. The ring spot symptom has not always been recurrent on these trees, especially on R.S. 24.

Host Plants

Cherry Trees

Bing, Lambert, Black Republican, and Royal Ann sweet cherry varieties were used in these studies. The Bing variety, propagated from a virus free tree known as "Oregon B 260," was used for most of these experiments. These trees were grown on mazzard roots at the Horticultural farm of Oregon State College. The other varieties, Royal Ann (Napoleon), Black Republican, and Lambert were obtained from a nursery. The Royal Ann was labeled "A 10" and was probably free from ring spot virus. The Black Republican and Lambert trees most likely were infected with a mild ring spot, since no virus free sources of bud material were available to the nurserymen. These trees had been propagated on mazzard roots.

One lot of 52 Bing cherry trees was planted in 18 inch pots and placed in the greenhouse. All the other trees were planted into the greenhouse soil in rows two feet apart and 1½ feet in the row. Fifty trees were also planted into the field.

In January, 1952, 23 virus sources were grafted on mazzard seedlings and planted into number 10 cans, in order to have a greenhouse source of inoculum for the experimental work on cucumbers during the winter.

Peach Trees

Sixty J. H. Hale peach trees, budded on Lovel seedlings, were planted in the greenhouse to be inoculated with the ring spot series. The trees were cut back to about three feet and inoculated when the new growth had started.

In order to have an inoculum for cucumber studies during the winter months, five Muir peach seedlings were inoculated in September, 1953 with buds from each of the 23 ring spot source trees. Three series were moved into the greenhouse, the first on January 5, the second on January 11, and the third on March 1, 1954. The trees were pruned back to 20 cm. above the cherry bud and the first two series potted in number 10 cans. The third series was planted in ground beds in the greenhouse. The second series was covered with a heavy paper cone to exclude the light and cause etiolated growth.

Cucumbers

National Pickling, which is a standard cucumber variety readily available in most seed stores, was chosen for this research. Hobbs (7, pp.16-17) reported that 46 out of 47 tested cucumber varieties were susceptible to necrotic ring spot. Since he found that most varieties of cucumber reacted to the ring spot virus, no further testing of varieties was necessary.

Transmission of Virus to Cherry and Peach

For the transmission of the virus to woody hosts the usual techniques of inoculation by T-buds and whip grafts were employed. When indexing for the ring spot virus, the methods outlined by Milbrath (13) were adopted. In the case of T-budding each two year old tree received two or three buds, varying in the different series. The buds were usually spaced 20-40 cm. apart. All T-budding was performed after the grwoth had started and the bark could easily be separated from the wood. When earlier inoculations were desired, whip grafts were made.

Transmission of Virus to Cucumber

Various methods of growing cucumbers were tried before a satisfactory method was found that would give uniform growth. When the work was first started in 1952 the cucumbers were germinated in vermiculite and transplanted to pots. Severe losses from damping-off made this method undesirable. The next method tried was to plant the seeds in clay pots and cover them with a mason sand to which Fermate was added. This prevented damping-off, but a severe virus-like mottle on the first foliage developed even on the check plants. In 1953 the cucumbers were grown in rows in flats of soil. The loss from damping-off was not too great, but uneven watering and an invasion of spider mites damaged the growth. The results of this preliminary work showed the importance of developing a satisfactory and uniform method of growing cucumbers.

In January, 1954, a standardized method of growing cucumbers which gave a uniform growth of plants, was developed and used throughout the remainder of these trials. Number 10 cans had been found satisfactory containers for other greenhouse work at Oregon State College and were selected as a standard unit for these experiments. Each can had six holes punched in the bottom for drainage. The cans were filled with a light soil which was mixed with a teaspoonful of a complete commercial fertilizer (10-18-18) and two tablespoonfuls of powdered sheep manure (1-1-1). Following this the soil was pressed to within one inch from the top and seven cucumber seeds were placed into each can. By covering the seeds with 3/4 inches of sand the danger of damping-off was reduced to almost zero in 1954. Twenty-five to fifty cans were so prepared once or twice every week.

The inculation of the cucumbers was done about 7-10 days after seeding when the cotyledons were expanded and green but not yet fully developed. First the cotyledons were dusted with carborundum (400 mesh), and the inoculum was prepared by grinding leaf tissue in a mortar. A drop or two of 0.2 molar dibasic sodium phosphate buffer of pH 8.3 was added. The leaf tissue used as inoculum consisted of the smallest possible leaves of cherry or peach. To transfer the virus from cucumber to cucumber only leaf parts which showed virus symptoms were used. Inoculation of the cucumbers was done by rubbing the juice over both cotyledons with the finger. Willison and Weintraub (22, p.324) gave one cotyledon 12 strokes. This was tried, but the plants showed severe mechanical injury, therefore the number of strokes was reduced to 6. Willison and Weintraub may have used plants with more fully developed cotyledons.

EXPERIMENTAL RESULTS

Inoculation on Cherry

Effect on Bing Cherry

In 1952 an experiment was set up to determine the reaction of the selected ring spot sources on two year old Bing trees grown in a greenhouse. Fifty trees were planted in pots and placed in rows two feet apart. By February 9, the buds had begun to swell and the trees were inoculated by budding three buds into the trunk of two trees for each of the ring spot sources.

These trees exhibited a variance in expression of symptoms. They varied from none to a slight mottling, necrotic rings, dieback, and even killing of the inoculated trees. On February 22, 1952, the top shoot of one tree started to wilt. The leaves showed a petiole and midrib necrosis, and a necrotic dieback developed on the new shoot growth. Successively other trees showed the same symptom. At first a bacterial infection was suspected and transfers of diseased tissue were made to agar plates, but no organism was isolated. The dieback was then considered to be a shock effect of the invading virus. Chlorotic mottles and ring spots started to develop as well as gum pockets between bark and wood, together with bud and stem necrosis. Two trees did not survive this shock, and they were dead before the growing season was over. The behavior of the individual sources is recorded in Table I.

At planting time the tips of each tree were cut off and indexed on Shiro-fugen to determine whether they were still virus free at the start of the experiment.

At mid-July the growth was practically terminated. Some trees even started to make secondary growth. For a numerical comparison of the 22 sources the three terminal shoots of each tree were measured, and the average length recorded in Table I.

<u>Effect on Other Varieties</u>

In 1953 thirty trees of three other cherry varieties were tested for a similar virus reaction. All trees came from a commercial nursery and were not indexed for the ring spot virus.

All trees were budded when the shoots were about 2 cm. long. The test was made with 12 Lambert, 12 Royal Ann, and 6 Black Republican trees. Half of each variety was budded with R.S. 10 and the other half with R.S. 24.

Lambert did not react to R.S. 10, but R.S. 24 gave chlorotic mottles on at least four of the 6 inoculated trees. These symptoms, however, were of a very slight nature.

On the three trees of Black Republican inoculated

TABLE I

The Effect of the Ring Spot Virus Sources on Bing

Source	Tree No.	Shiro reac- tion/1	Die- back	Other Symptoms/2	Length of Shoots/3
R.S. 3	1	S-	No	Ring and target spots	12.6 cm.
	2	S-	No	Ring and target spots	12.3 cm.
R.S. 4	3	S*	No	Mild RS, bud necrosis	9.3 cm.
	4	S*	No	Mild RS, bud necrosis	22.6 cm.
R.S. 5	5	S*	No	No symptoms	31.6 cm.
	6	S-	No	No symptoms	24.6 cm.
R.S. 6	7	S-	No	Mottles, target spots	14.3 cm.
	8	S*	No	Mottles, target spots	28.3 cm.
R.S. 7	9	S*	No	Green ring mottles	18.0 cm.
	10	S-	No	Green ring mottles	10.3 cm.
R.S. 8	11	S-	Yes	NRS, lace leaf	4.0 cm.
	12	S-	Yes	NRS, lace leaf	4.0 cm.
R.S. 9	13	S*	Yes	Chlorotic spots, gumming	14.3 cm.
	14	S*	No	No symptoms	18.6 cm.
R.S. 10	15	S*	Yes	Severe RS, gum pockets	5.3 cm.
	16	S*	No	No symptoms	8.3 cm.
R.S. 11	17	S-	No	Dying of 3 lateral leaves	14.0 cm.
	18	S*	No	Oak leaf pattern	10.0 cm.
R.S. 12	19	S*	No	No symptoms	13.6 cm.
	20	S-	No	No symptoms	21.0 cm.
R.S. 13	21	S-	No	Mottles, target spots	16.6 cm.
	22	S-	No	Intervenal mottles	11.3 cm.
R.S. 14	23	S-	Yes	Gum pockets	2.3 cm.
	24	S*	No	No symptoms	13.3 cm.

Table I. Continued

Source	Tree No.	Shiro reaction/1	Die-back	Other Symptoms/2	Length of Shoots/3
R.S. 15	25	S-	Yes	No symptoms	20.6 cm.
	26	S-	No	No symptoms	22.3 cm.
R.S. 16	27	S-	No	No symptoms	12.6 cm.
	28	S-	No	No symptoms	23.3 cm.
R.S. 17	29	S*	No	Green ring mottles	13.3 cm.
	30	S*	No	Green ring mottles	7.3 cm.
R.S. 18	31	S-	No	No symptoms	20.6 cm.
	32	S-	No	Chlorotic spots	22.3 cm.
R.S. 19	33	S-	Yes	Gumming, necrotic spots	1.0 cm.
	34	S*	No	Severe ring mottles	13.3 cm.
R.S. 20	35	S*	Yes	Ring mottles	8.6 cm.
	36	S-	Yes	Ring mottles	2.0 cm.
R.S. 21	37	S-	Yes	Killed by trunk necrosis	-
	38	S-	Yes	Killed by trunk necrosis	-
R.S. 22	39	S-	No	Mottles, gumming, bud necr.	5.6 cm.
	40	S-	Yes	Mottles, gumming, bud necr.	2.6 cm.
R.S. 23	41	S-	Yes	Gumming	2.6 cm.
	42	S*	Yes	NRS, golden RS	8.0 cm.
R.S. 24	43/4	S*	Yes	Gumming	-
	44	S-	No	Mild mottling, target spot	12.0 cm.
Checks	47	S-	No	No symptoms	28.6 cm.
	48	S-	No	No symptoms	32.3 cm.
	49	S*	No	No symptoms	32.3 cm.
	50	S-	No	No symptoms	18.0 cm.
	51	S*	No	No symptoms	28.3 cm.

/1 S* indicates a ring spot reaction on Shiro-fugen and S- indicates a negative reaction
/2 RS means ring spot and NRS means necrotic ring spot
/3 based on average length of 3 terminal shoots.
/4 This proved to be a mazzard seedling.

with R.S. 10 only one leaf was found with necrotic ring spot. With R.S. 24 one tree had some necrotic ring spot, a second tree chlorotic mottles, which later became target spots, and the third tree was symptomless.

Royal Ann (Napoleon) with three exceptions reacted positively to both sources. The affected trees had necrotic ring spot, and five trees developed the same type of dieback as shown on the Bing variety.

These results indicate some correlation between ring spot virus and the tip dieback symptom. From previous records both the Black Republican and Lambert variety were known to carry a ring spot virus, while Royal Ann was propagated from a source which does not carry this virus. Since both ring spot-free Bing and Royal Ann gave dieback when inoculated and the ring spot infected Lambert and Black Republican did not, there must have been some cross protection against the shock reaction. However, when examining the data in Table I exceptions to these results can be found. When the Bing trees were indexed on Shiro-fugen several of them gave a ring spot reaction. R.S. 9, R.S. 10, R.S. 20, R.S. 23, and R.S. 24 produced a shock reaction even though ring spot was present. Further studies are being conducted on this problem but are not included here.

<u>Effect of the Time of Inoculation</u>

All the previous budding was done at budbreak, i.e., as soon as the bark started slipping to permit T-budding. The susceptibility of the plant to a shock reaction seemed to be altered with the growth. To study this problem, an experiment was set up in 1952 and repeated in 1953. In principle, it consisted of inoculating cherry trees at intervals of around 15 days. The first inoculation was made as soon as the buds of the scions started breaking out of dormancy and the last when the young shoots were about 15 cm. long.

Forty-two Bing cherry trees were planted in ground beds in a greenhouse on March 13, 1952, and the first series was inoculated 9 days later. The next three series were inoculated 25, 40, and 64 days after planting. Two trees were inoculated with each source using two buds for each tree.

Since R.S. 14, 21, and 24 had previously shown the strongest tendency for tip dieback, they were selected for these studies. R.S. 10 was included in the last three series and R.S. 22 was used only in the second series.

The replicants for the most part behave very similarly, but in several cases more trees would have been desirable. The sources R.S. 10 and R.S. 23 gave the most significant results. The results of these inoculations can be summarized as follows:

R.S. 10. This source was used for the inoculations of April 7, 22, and May 16. Except for one tree they all developed stem necrosis, gummosis, and dieback. Chronologically these symptoms were observed as follows.

First series showed symptoms 44 days after inoculation.
Second series showed symptoms 39 days after inoculation.
Third series showed symptoms 36 days after inoculation.

R.S. 14. Inoculations were made on four different dates with this source, but only one tree of the series of April 7, had some symptoms of shot holes and rusty mottles by May 28.

R.S. 21. The two trees inoculated on March 22, showed dieback on one lateral shoot on April 18, 28 days after inoculation. One of the plants died on July 22, and the other one showed bud and stem necrosis on August 12. One tree, inoculated on April 7, had one leaf blighted and some dead buds on April 12, 5 days after inoculation. The other tree showed these symptoms on April 19, 13 days after inoculation. The third series, inoculated on April 22, showed no symptoms. Those budded on May 16 had one plant with a gum pocket and a slight necrosis near the middle bud on August 12.

R.S. 22. This source was budded into only two trees. On May 21 both trees showed a ring spot of the chlorotic type on an occasional leaf. On June 30, there were no new symptoms.

R.S. 23. With this source four inoculations were made, and the result was dieback, gummosis, ring spot, and lace leaves on one tree, dead buds and gum pockets on the other. In the last series one tree did not show symptoms at all, while the other had only a dead bud on August 12, 1952. The symptoms were first recorded at the following dates:

First series	20 days after inoculation.
Second series	12 and 44 days after inoculation.
Third series	90 days after inoculation.
Fourth series	44 days after inoculation.

R.S. 24. Of the four series of trees inoculated with R.S. 24, only the ones budded on March 22, showed symptoms. On April 19, leaves showed chlorotic spots and ring mottles, which became target spots by May 21. On June 30, no new symptoms could be seen.

Since these preliminary results indicated that the tip dieback reaction was influenced by the stage of growth of the tree at the time of inoculation, a larger experiment involving more trees and only two ring spot virus sources was planned and performed in 1953. R.S. 10 and R.S. 24 were selected for these studies. Sixty Bing trees were planted in the greenhouse beds on March 7, 1953, and the first inoculations were made March 15, as the buds were beginning to break. At this time seven trees were inoculated with R.S. 10 and with R.S. 24. The T- or slip-budding method was used, and seven trees were also inoculated by whip grafting a scion of the infected wood to the terminal bud of the test tree. The grafts were tried to determine whether this method might provide a faster and more virulent reaction. Fifteen days later seven more trees were inoculated with R.S. 10 and with R.S. 24 by the budding method only. By this time the new growth had developed about 5 centimeters long. The third series was budded 11 days after the second series, at which time the new growth was 10-15 centimeters long. Table II summarizes the results of these studies.

Several readings were taken during the growing season. After 15 days, trees grafted with R.S. 10 started to develop dieback, which was moving from the tip in a downward direction (Figure 1). This dieback or tip blight was accompanied by necrotic ring spot, often causing a lace leaf condition on the leaves. The stem showed gummosis, expressed in gum flow and gum pockets, and sometimes stem canker. Trees inoculated with buds developed symptoms later than the trees inoculated by grafting, but the degree of severity was about the same (Figure 2). In the second series of trees inoculated 15 days later, only

TABLE II

Influence of Time of Inoculation on the Appearance of Dieback and Other Virus Symptoms

Source	Time of Inocula- tion /1	No. Trees Dieback /2	Days Neces- sary for Dieback	Other Symptoms /3
R.S. 10	8	4/7 *	28	6/7 NRS, gummosis
	8	7/7 **	17	7/7 NRS, gummosis
	23	4/7 *	21	6/7 NRS, lace leaf
	34	0/7 *	-	0/7 No symptoms
R.S. 24	8	5/7 *	26	7/7 RS, target spot, gummosis
	8	4/7 **	30	7/7 RS, target spot, gummosis
	23	0/7 *	-	7/7 chlorotic mot- tles, target spot
	34	0/7 *	-	0/7 No symptoms

/1 Number of days after planting, when inoculation was made
/2 * means bud inoculations; ** means graft inoculations
/3 NRS indicates necrotic ring spot; RS indicates ring spot

4 out of 7 trees produced tip blight symptoms (Figure 3), but all trees showed necrotic ring spot on the leaves (Figure 4). After the first shock most trees recovered and grew vigorously. There were no symptoms on all seven plants inoculated in the third series and the growth was very vigorous.

While with R.S. 10 a faster invasion of the trees by the virus was obtained with graft inoculation, the situation was reversed with R.S. 24 and the grafted trees developed dieback or tip blight four days after the budded ones. Dieback and tip blight were identical to that caused by R.S. 10, but the leaf symptoms were less severe with R.S. 24. Instead of laced or ring spotted leaves, R.S. 24 induced leaves with a chlorotic ring spot, which in later readings were diagnosed as target spots because the alternating yellow and green rings of 1-2 mm. in diameter resembled target discs used in the rifle and arrow shooting. Only the series of R.S. 24, inoculated at budbreak, induced dieback and tip blight combined with ring spot, target spot, and gummosis. The second series only gave chlorotic mottles and target spot on scattered leaves, and the trees, budded when the new shoots were 10-15 cm. long, grew vigorously and showed no virus symptoms. There was no difference in growth between the trees of the third series inoculated with the viruses R.S. 10 and R.S. 24 and the four check trees.

A similar experiment was performed in the field where two consecutive inoculations were made, the first one on April 20, 1953, and the second one two weeks later. Again seven trees were used for each source, and each inoculated tree received two virus-containing buds. Tip blight was observed only on two trees and these resulted from inoculations with R.S. 10 in April, 1953. The leaves developed necrotic ring spot and lace leaf, but these symptoms were located mainly on the base of the tree. In the second series the leaves showed chlorotic mottles, especially along the veins, but those were restricted to a few scattered leaves. The severe necrotic ring spot occurred only on leaves of three plants.

All seven trees inoculated in the field with R.S. 24 in the first series developed chlorotic mottles which later turned into target spots. In the second series the symptoms were slighter and reduced to some chlorotic mottling.

Another field experiment was started March 29, 1954, to determine whether earlier inoculation by whip grafting dormant trees would give a higher percentage of dieback. Five sources of ringspot which had indicated different strain reaction on various hosts were used in these trials. The trees were inoculated by whip grafting

3-5 branches on each of 4 trees with each virus source. Results have not been obtained in time to include them in this report.

These results clearly indicate that the time of inoculation has an influence on the appearance of virus symptoms. Trees inoculated immediately at budbreak were those which provided the shock reaction, consisting of dieback, tip blight, stem necrosis, gummosis, lace leaves, and ring spot. If the trees are inoculated when the current seasonal growth is about 5 cm. long a strong virus, like R.S. 10, may still induce tip blight and necrotic ring spot, but to a lesser degree, and also these trees have a better recovery than those inoculated earlier. When the new growth is over 10-15 cm. long the virus no longer produces any symptoms.

Inoculation on Peach

The aim of this experiment was to record the symptom expression which the 23 different sources cause on peach and use them for comparison with the other results. Sixty small J. H. Hale peach trees were budded on February 28, 1952, in the greenhouse. For each source two trees were budded with three cherry buds each. Symptoms were recorded as they developed and the final readings were made in August, 1952. Table III summarizes the reaction

TABLE III

The Effect of the Ring Spot
Virus Sources on J. H. Hale Peach

Source	Die-back	Other Symptoms
R.S. 3	None	Ring and target spot, Medium growth
R.S. 4	None	No symptoms, Vigorous growth
R.S. 5	None	No symptoms, Medium to strong growth
R.S. 6	None	No symptoms, Medium to strong growth
R.S. 7	None	Severe necrotic ring spot, Medium growth
R.S. 8	None	Ring spot on first leaves, Weak growth
R.S. 9	1 tree	Severe necrotic ring spot, Weak to medium growth
R.S. 10	2 trees	Very severe necrotic ring spot on first leaves, Medium to strong growth
R.S. 11	None	Chlorotic spots on some leaves, Weak to medium growth
R.S. 12	None	No symptoms, Strong growth
R.S. 13	None	Ring spot with leaf border necrosis, Growth very weak
R.S. 14	None	Some chlorotic spots on one tree, Vigorous growth
R.S. 15	None	No virus symptoms, Medium to strong growth
R.S. 16	None	Chlorotic rings on basis of one tree, Vigorous to medium growth
R.S. 17	None	Ring spot, Little to medium growth
R.S. 18	None	Chlorotic spots on basis of 1 tree, Medium to strong growth

Table III Continued

Source	Die-back	Other Symptoms
R.S. 19	None	Very severe ring spot with necrosis and lace leaves, Little growth
R.S. 20	None	Severe ring spot with necrosis Medium to weak growth
R.S. 21	2 trees	Severe ring spot, Weak to very weak growth
R.S. 22	2 trees	Severe ring spot, Necrosis, Rosetting, Weak growth
R.S. 23	None	Severe ring spot with necrosis and lace leaves, Medium growth
R.S. 24	None	Ring spot becoming target spot, Very little to medium growth

of the peach to the various ring spot sources.

According to the results presented in Table III the sources were assembled in groups.

Group 0: There was no die-back nor any other virus symptom. This group included the sources R.S. 4,5,6,12,15, and the check plants.

Group 1: There was some doubt as to whether the symptoms recorded for these sources were caused by the virus or by some other agent. R.S. 14, 16, and 18 were in this group. If the experiment were repeated on a larger scale, these sources would probably fall into group 0.

Group 2: There was no dieback; the ring spot was mild, and the new growth symptomless. R.S. 3, 8, 11, and 17 were in this group.

Group 3: Ring spot was very severe but no dieback occurred. This group included R.S. 7, 13, 19, 20, and 23.

Group 4: There was dieback or tip blight on the terminal growth and leaves, together with severe ring spot symptoms on the leaves. R.S. 9, 10,21, and 22 showed this reaction.

This data grouped the ring spot sources in definite units indicating virus strains, which varied in their response on peach. Some of them caused no effect, while others varied from a mild to a severe reaction.

Inoculation on Cucumber

The Transmission of the Ring Spot Virus Strains to Cucumber

The first objective of the studies on cucumber was to determine which of the ring spot sources were carrying the virus that was transmitted mechanically to cucumber. The second objective was to determine whether the variation of virus reaction on cucumber indicated a difference in virus strains. The third objective was to compare the cucumber reaction with the reaction obtained on other index hosts, thus indirectly determining whether the virus on cucumber could be considered the ring spot virus of peach.

The first attempts to recover virus from the ring spot sources were made in 1952 and 1953. Sweet and sour cherry stock from the selected source trees were grown in the greenhouse. Very young leaves were used as a source of inoculum. A virus was recovered quite easily from some of the sources, but for the most part, very low or no transfer was obtained from others. Similar results were obtained when flower petals and small fruits were used as a source of inoculum. The series of Muir seedlings inoculated in September, 1953, was used in the 1954 studies in order to get a uniform host plant inoculated with all sources. The peach proved to be a more reliable and satisfactory host plant from which the virus could be recovered. On January 5, the first series of Muir peach was taken into the greenhouse. As soon as the buds started breaking and the first leaves appeared, mechanical transfers to cucumber were made. One week later a second series was taken into the greenhouse. These trees were covered with a hood to produce an etiolated growth which might offer a better source of virus. The etiolated yellow leaves were ground up and the juice rubbed on cucumber cotyledons as in the preceding series. The third peach series was planted directly into the greenhouse ground.

In the Spring of 1954, inoculations from cherry flowers and cherry tip leaves were also repeated. In the case of the flowers 10 white petals were ground up with a few drops of buffer solution, and 15-20 plants were inoculated with each source. Fresh juice was prepared for each two cans, corresponding to 7-10 plants. The youngest leaf tips were used for a similar source of inoculum. Leaves and flowers were collected from the small trees in the greenhouse and from the original field trees.

The time required for symptoms to appear after inoculation varies greatly. Willison and Weintraub (21, pp. 175-177) took their readings after 10-14 days. Varney and Moore (18, p.36) got chlorotic circular lesions within

$1\frac{1}{2}$ to 2 days at 75° F. and above, 3 days at 70° F. and 4 days at 61° F. They found that at lower temperatures the virus tends to kill the cucumbers, while at 90-95° F. the virus caused mild symptoms in a few cases.

In these studies primary symptoms were seen as early as 3 days after inoculation with source R.S. 10, while other sources usually took 5-7 days or longer. Systemic symptoms started to appear after 8-10 days. Some virus strains were so strong that the plants died as early as 7 days after inoculation. Plants with virus symptoms were staked and recorded as soon as they appeared. Final readings could be taken after 10-14 days because by this time all infected plants showed symptoms.

For the purpose of definition symptoms were called primary if they appeared on the inoculated leaves, and secondary when the virus became systemic and moved into the rest of the plant.

The symptoms produced by the various ring spot sources were often difficult to classify into definite groups. There was an overlapping of strain differences and often a series of cucumbers inoculated with the same source would develop two or more symptoms. The first effect noted was chlorotic spotting or a tissue collapse and wilting on the inoculated cotyledon.

When a series of cucumber was inoculated with the

23 sources, five rather distinct reactions occurred. Many plants developed neither symptoms on the cotyledons nor on the secondary leaves, indicating no transmission of virus. Some of the cotyledons showed little symptoms while the first true leaf indicated a virus effect (Figure 5). In a third group the cotyledons developed scattered circular chlorotic lesions, 1-2 mm. in diameter (Figure 6A), which later faded into a general chlorotic mottle. Often the initial growing point was killed which resulted in the emergence of a short rosetted growth consisting of miniature leaves (Figure 7). The cotyledons remained turgid and the plant never became more than 2-3 inches tall. If the plants were inoculated when the cotyledons were larger and secondary leaves had formed, these leaves showed a strong mosaic pattern, but the growing point was killed, and the same rosetted growth developed later.

The fourth reaction developed chlorotic lesions on the cotyledon leaves, but the center of these areas became necrotic within 2-3 days (Figure 6B). The cotyledons became chlorotic; the growing point was killed, the plants wilted, and many died after a few days. Some plants remained alive for several weeks without any growth development, but they eventually died.

The most severe reaction showed up first as a wilted area on the inoculated cotyledon. This wilting gradually increased until the entire cotyledon wilted and collapsed. When the plants were inoculated while they were young, the wilting developed very rapidly and both cotyledons and the growing point would be dead within seven days (Figure 8C). When the larger plants were inoculated when secondary leaves were present, the virus first caused wilting of the tip. The wilting gradually spread downward and the plants died.

Table IV summarizes the data obtained when cucumber was inoculated with the 23 sources of ring spot by taking the inoculum from the different hosts.

Some of the ring spot strains, such as R.S. 19 and R.S. 20 were more easily recovered from all sources. Some were isolated more easily from peach than from cherry, while others were recovered only from cherry. R.S. 6 was difficult to transmit and was only obtained once from the small leaves of the mahaleb rootstock. R.S. 16 was transmitted only from the flower petals. Some of the strains like R.S. 6, 12, 14, and 15 were isolated on only a few cucumber plants, and R.S. 2, 4, and 18 failed to infect cucumber from any of the sources.

Once the virus was isolated in cucumber most strains were fairly easy to transfer and maintain in this host. Some had to be transferred quite frequently or they would be lost and would have to be recovered from the original

TABLE IV

The Recovery of the Ring Spot Virus on
Cucumbers by Mechanical Inoculation

Virus	Source of Inoculum	Plants Inoculated	Number Positive	Percentage of Transmission
R.S. 2	Cherry leaves	15	0	0.0
	flowers	17	0	0.0
	Peach normal	40	0	0.0
	etiolated	9	0	0.0
R.S. 3	Cherry leaves	13	0	0.0
	flowers	37	1	2.7
	Mahaleb leaves	6	5	83.5
	Peach normal	14	13	93.0
	etiolated	5	5	100.0
	Cucumber	60	9	15.0
R.S. 4	Cherry leaves	12	0	0.0
	flowers	17	0	0.0
	Mahaleb leaves	5	0	0.0
	Peach normal	39	0	0.0
	etiolated	8	0	0.0
R.S. 5	Cherry leaves	9	6	66.5
	flowers	14	0	0.0
	Mahaleb leaves	9	0	0.0
	Peach normal	42	0	0.0
	etiolated	5	1	14.3
R.S. 6	Cherry leaves	19	0	0.0
	flowers	11	0	0.0
	Mahaleb leaves	23	15	65.2
	flowers	14	0	0.0
	Peach normal	36	0	0.0
	etiolated	5	0	0.0
R.S. 7	Cherry leaves	20	9	45.0
	flowers	18	0	0.0
	Peach normal	14	4	28.5
	etiolated	5	4	80.0
	Cucumber	81	26	32.1

Table IV Continued

Virus	Source of Inoculum	Plants Inoculated	Number Positive	Percentage of Transmission
R.S. 8	Cherry leaves	24	6	25.0
	flowers	39	7	17.9
	Peach normal	15	6	40.0
	etiolated	5	2	40.0
	Cucumber	52	18	34.6
R.S. 9	Cherry leaves	16	0	0.0
	flowers	20	0	0.0
	Peach normal	16	5	31.2
	etiolated	5	1	20.0
	Cucumber	52	4	7.7
R.S. 10	Cherry leaves	19	2	10.5
	flowers	25	0	0.0
	Peach normal	15	8	53.3
	etiolated	3	1	33.3
	Cucumber	144	85	59.0
R.S. 11	Cherry leaves	8	6	75.0
	flowers	14	0	0.0
	Mahaleb leaves	13	4	30.8
	flowers	5	0	0.0
	Peach normal	19	12	62.2
	etiolated	8	4	50.0
	Cucumber	82	27	33.9
R.S. 12	Cherry leaves	12	3	25.0
	flowers	14	1	7.1
	Mahaleb leaves	8	0	0.0
	flowers	7	0	0.0
	Peach normal	19	0	0.0
	etiolated	9	0	0.0
	Cucumber	31	5	16.1
R.S. 13	Cherry leaves	11	1	9.0
	flowers	42	0	0.0
	Peach normal	15	9	60.0
	etiolated	6	5	83.5
	Cucumber	110	50	45.4

Table IV Continued

Virus	Inoculum	Plants Inoculated	Number Positive	Percentage of Transmission
R.S. 14	Cherry leaves	8	0	0.0
	flowers	16	0	0.0
	Mahaleb leaves	12	8	66.7
	flowers	5	2	40.0
	Peach normal	18	1	5.5
	etiolated	9	3	33.3
	Cucumber	39	9	23.1
R.S. 15	Cherry leaves	12	2	16.6
	flowers	20	0	0.0
	Peach normal	17	2	11.7
	etiolated	11	0	0.0
	Cucumber	36	8	22.2
R.S. 16.	Cherry leaves	12	0	0.0
	flowers	29	6	20.6
	Peach normal	17	0	0.0
	etiolated	10	0	0.0
	Cucumber	34	22	64.8
R.S. 17	Cherry leaves	25	5	4.0
	flowers	26	0	0.0
	Peach normal	17	5	29.4
	etiolated	12	1	8.3
	Cucumber	72	16	22.2
R.S. 18	Cherry leaves	21	0	0.0
	flowers	20	0	0.0
	Peach normal	42	0	0.0
	etiolated	11	0	0.0
R.S. 19	Cherry leaves	21	1	4.7
	flowers	20	2	10.0
	Peach normal	14	12	85.7
	etiolated	6	3	50.0
	Cucumber	70	36	51.4
R.S. 20	Cherry leaves	4	1	25.0
	flowers	6	1	16.6
	Peach normal	12	10	83.2
	etiolated	6	1	16.4
	Cucumber	46	12	26.1

Table IV Continued

Virus	Source of Inoculum	Plants Inoculated	Number Positive	Percentage of Transmission
R.S. 21	Cherry leaves	17	0	0.0
	flowers	15	0	0.0
	Peach normal	11	4	36.4
	etiolated	6	2	33.3
	Cucumber	9	2	22.2
R.S. 22	Cherry leaves	14	5	35.6
	flowers	16	3	18.7
	Peach normal	12	10	83.2
	etiolated	5	2	40.0
	Cucumber	50	8	16.0
R.S. 23	Cherry leaves	18	3	16.6
	flowers	15	0	0.0
	Peach normal	15	6	12.0
	etiolated	3	0	0.0
	Cucumber	46	10	21.7
R.S. 24	Cherry leaves	9	0	0.0
	flowers	20	1	5.0
	Peach normal	10	8	80.0
	etiolated	5	0	0.0
	Cucumber	101	77	76.2

tree. Some of the severe strains could be maintained longer on larger plants.

Occasionally some strain might infect all of the plants inoculated but such results were not consistent for any of the selections. The milder strains usually gave a lower percentage of transmission than the more severe strains. In one experiment 25-30 cucumber plants were inoculated with the sources R.S. 7, 10, 11, 13, 17, and 24. The strong viruses R.S. 10 and 24 gave symptoms on nearly all inoculated plants, and R.S. 11 and 13 also had a high transfer, although lower than the first two. R.S. 7 and 17 had only a few plants with symptoms and these occurred among the first plants inoculated, indicating the inoculum lost its infectivity rather rapidly. This was taken into account in later inoculations and fresh inoculum was prepared after inoculating each can of plants.

In order to compare the viruses recovered from the various R.S. sources on a series of cucumber plants of the same age and growing under similar climatic conditions the following experiment was performed on March 28, 1954. Seventeen of the ring spot sources were available in cucumber as uniform source of inoculum. Five cans with 5-7 one-week old cucumbers were placed in rows on a bench in the same greenhouse and inoculated with each of the 17 virus sources. These plants were examined at daily intervals and records were made starting with the first cotyledon symptoms and the experiment was concluded five weeks later. This experiment gave valuable data on strain differences which will be discussed in the next section.

In addition to inoculating cucumber plants in the small stage, larger plants with one or more secondary leaves were also inoculated. These larger plants withstood the shock reaction better and offered a larger source of inoculum over a longer period of time. The plants were inoculated on the enlarged cotyledons or on the secondary leaves, or both, depending on the nature of the experiment.

In one experiment 3-5 cucumber plants, having 3 or more true leaves, were inoculated with several of the R.S. sources to help in strain differentiation. Also earlier experiments had shown that older plants are difficult to infect with some of these strains, therefore this series was planned to obtain additional information.

The sources R.S. 3, 8, 10, 13, 15, 19, 22, and 24 were transmitted to larger plants, but R.S. 7, 9, 12, 14, 16, 17, 20, and 23 were not transferred in this experiment. The symptoms generally were the same as those of cotyledon inoculation, except that fewer plants died. R.S. 10 and R.S. 19 often caused wilting of the tip, then death from the tip downward. Other sources, like R.S. 13 caused mosaic and stunting. R.S. 24 killed the growing point, and later axillary buds developed into a bushy plant. These results indicated that the virus moves through the inoculated leaf into the tip of the plant, infecting all new leaves and sometimes moves downward causing a wilting and finally the death of the plant. The more severe strains, such as R.S. 10, developed large golden chlorotic lesions on the inoculated leaf and the older leaves above it. With these strains the tip started wilting after a few days, the wilting progressed downward, killing the plant.

In order to determine how long infection could be obtained by inoculating the cotyledon leaves an experiment was conducted using R.S. 10 and 24. Cucumbers were planted in the usual method and the inoculations were made on the cotyledons when the plants were 9, 13, 16, 21, and 28 days old. Table V gives the results of these inoculations.

According to these results cotyledons remained susceptible to virus infection for at least 3-4 weeks. Symptoms were more severe when nine day old seedlings were inoculated, but when older cotyledons were inoculated they developed much slower. The virus must take longer to move through the cotyledon to the tip. The virus seems to build up in the tip of the plant, often to an amount able to kill the growing point, and in the more severe forms the killing action precedes in a descending direction

TABLE V

Effect of the Age of Cotyledons at Time of Inoculation on Virus Susceptibility

Source	Age of Plant/1	Plants with Symptoms	Kind of Symptoms
R.S. 10	9	6/7	Wilting and dying
	13	2/4	Wilting and dying
	16	5/5	Two died, Three with tip necrosis
	21	5/5	Golden mosaic (Figure 9)
	28	1/4	Tip with golden mosaic
R.S. 24	9	5/7	Yellow cotyledons, 1 plant rosetted
	13	5/5	Died
	16	5/5	Cotyledons died, First internode and first true leaf survived
	21	5/5	Growing point killed, 1-3 leaves survived (Figure 10)
	28	4/4	Tip with golden mosaic, Growing point killed.

/1 Days from time of planting

over the whole plant.

<u>A Comparison of the Various Ring Spot Virus Strains Recovered on Cucumber</u>

When cucumber plants were inoculated with the viruses from the ring spot sources some of them developed definite symptom patterns indicating degrees of severity. With others there was an overlapping of strain variations without sharp lines of demarcation. Those sources, from which at least two distinct strains could be isolated, were the most difficult to classify. In order to show the variation of symptom expressed by the different strains, each ring spot source will be discussed separately.

<u>R.S. 2</u>. From a total of 81 inoculated cucumbers using peach, cherry flowers, and tip leaves, not a single transmission was obtained.

<u>R.S. 3</u>. The virus in this source was easy to transmit from peach and from the mahaleb rootstock of the original tree. From 37 cucumbers inoculated with juice from macerated flower petals one plant showed severe symptoms, but the 13 inoculations from tip leaves failed to produce positive reactions. In January inoculum from peach produced a target spot type of local lesion (Figure 6C) on the cotyledon. A few days later the small secondary leaf wilted and died, and eventually the seedling wilted and died. This indicated that a rather severe strain of virus

was present. After several transfers through cucumber a much milder reaction occurred. Some plants became quite large, but nevertheless showed a severe mosaic pattern and the internodes were shortened in comparison to normal plants. This variability strongly suggests a mixture of two or more strains in this source tree. This would agree with the flowering cherry index history in which the tree was originally selected because it did not react on Shiro-fugen. Later re-indexing gave a necrotic reaction on Shiro-fugen, indicating that the source had become infected with a more severe strain.

R.S. 4. No virus was recovered in cucumber from this source. The flowering cherry history indicates that ring spot was present, but 81 attempts to transfer it to cucumber from peach, cherry leaves, and flower petals failed to give an infected plant.

R.S. 5. At first the ring spot virus seemed to be difficult to recover from this source. Forty-seven cucumbers were inoculated, using young peach leaves, but only one plant showed suspicious symptoms. Cherry flowers also gave no positive results, but when 9 cucumbers were inoculated with juice from cherry tip leaves 6 of them developed chlorotic lesions on the cotyledons and a mosaic pattern on the secondary leaves.

R.S. 6. From the 85 transfers to cucumber with peach and cherry inoculum no transmission was obtained. However, the virus could be recovered from the leaves of the mahaleb rootstock. All 14 plants inoculated in 1953 with this inoculum developed local lesions, mosaic, and they finally died. In 1954 one out of 9 similarly inoculated cucumbers showed local lesions on the cotyledons and severe mosaic on the first true leaf.

R.S. 7. A fairly high percentage of transmission was obtained from peach and from cherry material. Chlorotic lesions formed on the cotyledons and severe mosaic on the true leaves. Several plants wilted and died. The percentage of transmission from cucumber to cucumber was rather high. From the series inoculated on March 29, 1954, 17 out of 27 plants showed severe symptoms. R.S. 7 caused the wilting and dying of the tip, yellowing and border necrosis of the cotyledons. The growth was almost completely suppressed and the few leaves were very small and showed evidence of mosaic and chlorosis.

R.S. 8. Good transmission resulted from peach and cherry flowers and tip leaves. The infected plants showed chlorotic lesions in the cotyledons. This was occasionally followed by wilting and dying of the plant. Secondary symptoms, on the plants which survived the shock, were a dark type of mosaic and also severe stunting of the plant. The fact that some virus infected plants attained considerable size while others were killed by the virus shock is another indication of a mixture of strong and mild strains.

R.S. 9. This source gave fair transmission from peach and only occasionally from cherry. The plants on which virus symptoms were observed showed chlorotic and necrotic lesions on the cotyledons and mosaic on the secondary leaves. The diseased plants usually died, thereby inactivating the virus before it could be transmitted. This explains the low percentage of cucumber to cucumber transmissions.

R.S. 10. This was one of the severest strains in the series. Transmissions from peach were very successful in winter, causing the death of the inoculated plants after very few days (Figure 8C). In March and April, when cherry material was used as a source of inoculum, transmission decreased. Possibly the warmer climatic condition was a cause of this change of reaction. When R.S. 10 was transmitted from cucumber to cucumber the plants wilted and died in the first transfers the same as when peach to cucumber inoculations were made. However, after several transfers to cucumber the symptoms seemed to be less severe since they were restricted to severe stunting and mosaic. This indicated a separation in that the weaker strain had been transferred from cucumber to cucumber while the severe one had been lost in the quick-wilted plants. If large plants were inoculated the tip leaves showed a golden type of mosaic (Figure 9), often the growing point was killed, and the wilting and dying spread downward, killing the plant.

R.S. 11. A high percentage of transmission was obtained both from peach and cherry inoculum. The symptoms were consistently different from those obtained from other sources. Usually the cotyledons did not show local lesions, and only when the first true leaf appeared could virus presence be determined. The true leaves usually were darker than normal, the veins showed clearing and were close together, and the intervenal areas often were somewhat raised giving the appearance of a mild crinkle or rugosity (Figure 8A). The growth was markedly reduced. With this virus the growing point was never killed but the plant remained small and the leaves very small. The internodes were shortened but not to the degree of those producing rosetting. These diseased plants produced a profusion of normal sized flowers.

R.S. 12. This virus was not transmitted when peach was used as the source of inoculum. With juice from young cherry leaves the cucumbers had chlorotic lesions on the cotyledons. The secondary symptoms were similar to those caused by R.S. 11, and consisted of a dark type of a coarse mosaic combined with reduced growth. Cucumber to cucumber transfers were fairly successful.

R.S. 13. This strain was readily transmissible from peach leaves. Subtransfers from cucumber to cucumber were successful at a high percentage. The first reaction of the diseased plant was local lesions on the cotyledons, often in the form of target spot (Figure 6C). In many plants the growing point was killed and sometimes the whole plant died. The plants surviving the shock had a very stunted, rosetted growth, and mosaic developed on the leaves. Larger plants, when inoculated, showed a very coarse mosaic and often a dead growing point. Frequently the plant wilted in a downward direction.

R.S. 14. Peach inoculum gave a rather low amount of transmission. The virus produced chlorotic lesions on the cotyledons and reduced the growth. Inoculum from the mahaleb rootstock caused a severe mosaic. In the cucumber to cucumber inoculations the responding plants wilted and died rapidly.

R.S. 15. The percentage of transmissions of virus from this source was low. If established on cucumber it could be transmitted to healthy cucumber, but the transmission was relatively low. The symptoms consisted of local lesions on cotyledons and mosaic on true leaves. Several large inoculated plants first grew normally, but after about 2 weeks the tip leaves showed mosaic. Later the terminal growth started to wilt and die, and the necrosis spread downward, eventually killing the plant.

R.S. 16. This roughbark virus was not transmitted to cucumber from peach, but could be obtained from flowers of a diseased Kwanzan cherry tree in the greenhouse. Eleven cucumbers were inoculated with flowers from this tree and 6 of them collapsed shortly after showing numerous local lesions of the cotyledons. If this first isolate was transmitted to cucumber the reaction was less severe. The cotyledons showed local lesions and the secondary leaves mosaic. Killing of the growing point caused a very dwarfed growth which developed from axillary buds.

R.S. 17. Peach proved to be a good source of inoculum, while cherry gave only transmission in one of 25 cases. Transmissions from cucumber to cucumber were successful with 57 per cent of the attempts. The symptoms were local lesions on the cotyledons, mosaic on the secondary leaves, reduced growth of the plants, and occasionally the growing point was killed. R.S. 17 was finally classified as a mild strain.

R.S. 18. A total of 94 attempts to transfer a virus from source to cucumber using cherry leaves and flowers, normal and etiolated peach leaves, failed to yield a single case of virus transmission.

R.S. 19. The virus transmission was very successful from peach and to a lesser degree from cherry when this source was used as inoculum. Transmission from diseased cucumber to cucumber also gave a very severe reaction. From the series of March 29, only 7 of the 32 inoculated plants remained normal. After 4 days the rest showed local lesions on the cotyledon leaves. Within a few days these plants wilted and died. When larger plants were inoculated mosaic symptoms developed on the youngest leaves and then the growing point died. The wilting progressed downward eventually killing the plant.

R.S. 20. Peach and cherry leaves gave good transmission. Cucumber to cucumber transfers also were successful. The inoculated plants had local lesions, some of them of a necrotic type (Figure 6B) and the true leaf usually started wilting shortly afterwards. Most diseased plants died quickly while others survived for several weeks with only chlorotic cotyledons or at most one mottled leaf.

R.S. 21. During January and February, 1954, this virus was established on one-third of the inoculated cucumber plants when peach leaves were used as the source of inoculum. The symptoms were necrotic lesions on the cotyledons (Figure 6B), mosaic on the first true leaf, and completely repressed growth with most plants dying. In the attempt to recover the virus from peach in March no transmission was recorded.

R.S. 22. Most of the cucumbers inoculated from infected peach showed virus symptoms. From cherry the virus could be transmitted to cucumber in a high percentage of the cases. Cucumber to cucumber transfers were fairly successful. The virus caused local chlorotic or sometimes necrotic lesions (Figure 6B) on the cotyledons, mosaic on true leaves, and a killing of the growing point. Often the plant died. The surviving plants were small and rosetted or ocassionally became larger and showed an asteroid type of mosaic.

R.S. 23. The percentage of transmission was not very high with this source, and the following symptoms were noted: chlorotic and necrotic lesions on the cotyledons, mosaic on the first true leaf (if this leaf developed at all), and killing of the growing point. On the younger tip leaves of large inoculated cucumber plants the virus caused yellow veins which became necrotic, and a golden type of mosaic developed on some leaves.

R.S. 24. This virus source and R.S. 10 gave the most consistent transmission. The virus was obtained from peach, cherry tip leaves, flowers, and even young unripe fruits. Cucumber to cucumber transfers also had a high percentage of take, and even larger plants showed symptoms when inoculated. At first local lesions appeared on the inoculated cotyledons, and if the first true leaf developed, it showed mosaic. In nearly all cases the growing point was killed and the rest of the plant developed into a rosette, while the cotyledons persisted with a very dark green color for several weeks (Figure 7).

DISCUSSION

Virus free Bing cherry trees had not been tested for reaction to 23 selected sources of ring spot virus. Therefore an experiment was set up to determine what symptoms those viruses cause on this host, and to what extent cherry can be used in ring spot strain differentiation.

Tip blight and dieback were the most severe symptoms which were characteristic with some sources. The time of inoculation proved to be important, since an experiment repeated for two years showed that trees with new growth over 12-15 cm. long would not be affected by a virus shock. More of the severe strains of the ring spot virus caused dieback symptoms than did the mild ones. Most conspicuous and consistent results were obtained with R.S. 10 and 24 when they were budded or grafted at budbreak or before the shoots were over 8 cm. long. This indicated that all inoculations should be done at this time, if shock reactions are desired, since virus containing budsticks do not cause an infection of trees with well developed new growth. The results are similar to those that Cochran and Reeve's (5, pp.714-721) found when peach was inoculated.

Some strain difference could be shown on Bing cherry and J. H. Hale peach. In Table VI the symptoms were recorded according to their severity, no symptoms meaning the mildest form, mild reaction to indicate that the virus caused some leaf symptoms of a mild form like mottles and ring spot, while the classification severe was applied to sources causing severe necrotic ring spot and particularly dieback and tip blight. There were some irregularities, for instance with R.S. 11, one tree only showed oak leaf pattern which later disappeared, but the other tree had slight tip blight which might indicate this virus to be either mild or severe. However, since all other hosts indicated that this is a mild strain, it was classified as mild for cherry. With R.S. 14 one tree showed severe dieback, but since eight subsequently inoculated trees did not show this symptom the strain was classified as a mild one.

The data in the third column of Table VI was taken from the records of J. A. Milbrath on the reaction of flowering cherry to these various source trees. This classification was based on the combined response of Kwanzan and Shiro-fugen to each source. If neither host reacted to the inoculation the source is listed as none. If one host reacted and the other did not, the virus is listed as mild. Also listed as mild are those where Shiro-fugen reacted, but the Kwanzan reaction was very mild. A

TABLE VI

Virus Symptoms on Various Hosts

Virus	Bing Cherry	J.H. Hale Peach	Flowering Cherry	Cucumber
R.S. 2	-	-	None	None
R.S. 3	Mild	Mild	Severe	Severe
R.S. 4	Mild	None	Very mild	None
R.S. 5	None	None	Very mild	Mild
R.S. 6	Mild	None	Mild	Severe
R.S. 7	Mild	Severe	Severe	Severe
R.S. 8	Severe	Mild	Severe	Severe
R.S. 9	Severe	Severe	Severe	Severe
R.S. 10	Severe	Severe	Very severe	Very severe
R.S. 11	Mild	Mild	Very Mild	Very mild
R.S. 12	None	None	Very mild	Very mild
R.S. 13	Mild	Mild	Severe	Severe
R.S. 14	Mild	Mild	Very mild	Very severe
R.S. 15	None	None	Mild	Very mild
R.S. 16	None	None	Mild	Severe
R.S. 17	Mild	Mild	Severe	Mild
R.S. 18	Mild	Mild	Very mild	None
R.S. 19	Severe	Severe	Very severe	Very severe
R.S. 20	Severe	Severe	Severe	Very severe

Table VI Continued

Virus	Bing Cherry	J.H. Hale Peach	Flowering Cherry	Cucumber
R.S. 21	Severe	Severe	Severe	Very severe
R.S. 22	Severe	Severe	Severe	Severe
R.S. 23	Severe	Severe	Mild	Severe
R.S. 24	Severe	Mild	Mild	Severe

severe virus is one that gave a necrotic reaction on Shirofugen and a severe reaction on Kwanzan and a very severe reaction indicated that the Kwanzan was killed. This grouping is based on general observations, but sufficient work has not been done on all of these reactions to be sure that there are not exceptions. For instance a Shirofugen reaction and a Kwanzan negative reaction may not always indicate a mild strain of virus. These readings were available at the beginning of the experiments in 1952, and a new reading was taken in 1954. These readings confirmed most of the previous records, but for R.S. 3 and 13 the mild reaction became a severe one, which could be explained by a possible contamination with a new and stronger virus.

The last column in Table VI summarizes the cucumber reaction in the same type of grouping as those used for the cherry, peach, and flowering cherry. The sources called very severe were those where some or most mechanically inoculated cucumber plants wilted and died as a result of the virus shock. The severe group was the cucumber where the cotyledons remained living and turgid for several days and often for three or more weeks. The original growing point was killed and only short, dwarfed, rosetted plants develop if any secondary growth appeared at all. Mild indicated those where the virus was not of a killing type but still produced definite virus symptoms, such as local lesions on the cotyledons, mosaic on the secondary leaves, dwarfing or rosetting of the plants. They often became several inches tall and remained alive for a long time. The term none was used when no reaction was ever obtained on cucumber.

Whenever several cucumber plants were inoculated with the same source at any one time, often some of the plants wilted and died and would have been classified as a very severe strain. However, other plants in the same pot would survive and produce secondary growth which was mottled and rosetted and would be considered a mild strain. This would suggest that two or more strains were present, and which ever one was transmitted or developed first would determine the predominating symptom. This separation occurred in plants inoculated with R.S. 3 and 13 where flowering cherry reactions had indicated that two strains of ring spot were present. R.S. 10 also was found to have two strains present. One of these strains caused a severe wilting and early dying, while the other one killed the growing point, but the cotyledons persisted and a dwarfed rosetted growth developed.

When the reactions on cucumber were compared with those that are accepted as a ring spot reaction on Bing, peach, and flowering cherry, a significant correlation can be seen. There are a few exceptions, but as more work is done with this virus complex, perhaps there will be a logical explanation even for these.

From R.S. 2 no virus could be transmitted to cucumber, but since this source had never given any visible reaction on either Shiro-fugen or Kwanzan ornamental cherry no reaction on cucumber should be expected.

R.S. 5 and R.S. 13 reacted severely on both flowering cherry and cucumber but mildly on cherry and peach. Cherry and peach were tested in 1952 when flowering cherry still gave a negative reading, and therefore were expected to give a mild reaction on the two hosts. In 1954, flowering cherry gave a positive reading. If cherry and peach were tested in the same year, they should give a severe reaction if there is a positive correlation between all hosts.

R.S. 4 and R.S. 18 were never isolated on cucumber, however, the flowering cherry reaction does indicate a mild ring spot virus is present in both. Since there has not been any satisfactory method for purifying stone fruit virus cultures or for isolating and maintaining single virus entities the true significance of the flowering cherry reaction is not known. Some of them could be caused by entities other than the ring spot virus.

From 47 attempts to transfer R.S. 5 to cucumber, using peach inoculum, only one plant showed symptoms, but with young cherry leaf tips 6 out of 9 cucumbers contacted the virus.

R.S. 6 was never recovered from young peach or sweet cherry leaves, but it was recovered from leaves of the mahaleb rootstock of the original tree both in 1953 and 1954 transfers.

R.S. 16 was obtained only from Kwanzan flower petals of a tree grafted on mazzard seedling and kept in the greenhouse. When flowers from the original field tree were used, no virus transmission was obtained. Perhaps the greenhouse tree became infected from the mazzard rootstock and hence a different virus was recovered on cucumber than the one present in the field tree.

R.S. 17 gave a severe reaction on flowering cherry and a mild one on cherry, peach, and cucumber.

R.S. 23 had a lower percentage of virus transmission than R.S. 24, although the two were supposedly identical. The two sources reacted positive on Shirofugen but negative on Kwanzan, which classed them into a mild group. The symptoms on cucumber, however, were severe. This seems to support the theory that a negative reaction on Kwanzan does not always indicate a mild strain of ring spot virus. The reaction of this source on Montmorency, which is a recurrent type of ring spot rather than the shock type indicates this virus to be of a different nature. This might explain why this source did not follow the expected pattern of a mild reaction on cucumber.

The source of the inoculum had a marked influence on the recovery of the virus (Table VII). Several sources could be recovered by using flower petals but at a much lower percentage than with other methods. Young leaves of peach trees inoculated with the virus proved to be a good source of inoculum, but to put the trees in darkness to induce etiolated growth did not increase virus transmission. In several cases where the virus could not be recovered from either cherry or peach leaves, this could be done by using young leaves of the mahaleb rootstock. With some strains the virus was not recovered until several different sources had been used as inoculum. The possibility remains that R.S. 2, 4, and 18 could be recovered if other intermediate hosts were selected to furnish inoculum.

The data assembled in these studies strongly supports the theory that the virus which has been recovered on cucumber from stone fruit sources is the ring spot virus. There was a close relationship, with a few exceptions, between the strain behaviour in the host plants studied. What had been accepted as ring spot reaction in

TABLE VII

Summary of All the Mechanical Inoculations to Cucumber

Virus Source	Plants Inoculated	Plants Positive	Percentage of Transmission
Cherry leaves	308	44	14.3
Cherry flowers	457	22	4.8
Mahaleb leaves	67	23	34.3
Mahaleb flowers	26	2	7.7
Peach normal	454	115	25.3
Peach etiolated	157	35	22.9
Cucumber	1105	382	34.6
Total	2574	623	24.2

stone fruits also gave the same strain reaction in cucumber. Likewise, when a strain mixture was indicated in stone fruits, it was also detected in cucumber. The correlation shown by as many cases as found in these studies is too close not to be considered significant.

SUMMARY

1. Cherry latent viruses can be in trees without showing symptoms. They appear in an active condition if healthy index hosts are inoculated with diseased budwood. Shirofugen and Kwanzan ornamental cherries have been previously reported to be good index hosts.

2. Twenty-three virus sources were tested on cherry, peach, and cucumber. The previous history of these sources suggested that 22 of them are infected with a virus considered to belong to the ring spot complex.

3. On sweet cherry the sources were tested on the varieties Bing, Lambert, Royal Ann (Napoleon), and Black Republican. Bing and Royal Ann gave a necrotic shock reaction when inoculated with some of these source trees, and Bing was henceforth used in most experiments.

4. Two year old Bing trees, propagated from the virus free "Oregon B 260," were budded in the greenhouse and in the field. Symptoms appeared within two or three weeks. They consisted of dieback, ring spot, and chlorotic mottles.

5. An experiment was set up in 1952 and repeated in 1953 to determine whether the time of inoculation influences symptom appearance. The cherry trees were budded at intervals of about 15 days. Those budded at budbreak and when the shoots were up to 8 cm. long showed severe virus

symptoms, while those budded when the new shoots were over 12-15 cm. long remained symptomless.

6. All 23 sources were budded on J. H. Hale peach, and according to the symptoms recorded five groups were established. Group 0 included sources which remained symptomless, group 1, 2, and 3 were intermediate and group 4 contained the most severe sources.

7. The virus was recovered on cucumber from 20 of the 23 sources selected for these studies. Different sources of virus inoculum were used. These included peach leaves in a normal or etiolated condition, cherry leaves and flowers, and leaves of the mahaleb rootstock. The percentage of transmission varied, depending on virus strain, source of inoculum, and climatic conditions.

8. Symptoms on the cucumbers appeared within 4-7 days. They consisted of primary symptoms such as local lesions in the inoculated leaves, wilting and dying of the cotyledon, and eventually dying of the whole plant. Secondary symptoms appeared in the form of mosaic, killing of the growing point, and stunting and rosetting of the diseased plant.

9. For the most part the strains of virus recovered on cucumber followed a definite pattern which could be classified as mild, severe, or very severe. However, some sources gave a variance of reactions which were difficult to classify in any one group.

10. In several cases a cucumber plant reacted differently if inoculated from cherry or peach or if the inoculum had gone through cucumber several times. This was explained with virus separation and selection of the less potent virus in the subsequent transfers. The more severe component killed the plant and so the virus would be lost.

11. The data presented show that there is a close correlation between the reaction of flowering cherry and that of cucumber when inoculated with the same source of virus. The accumulated data strongly supports the theory that the virus recovered on cucumber from stone fruit sources is the ring spot virus.

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APPENDIX

Explanation of Figures

Figure 1. Two Bing trees inoculated at bud break by whip grafts. Both trees show dieback that occurred on the young terminal growth.

Figure 2. A Bing tree inoculated at the same time as those shown in Figure 1. T-buds were used instead of grafts. The type and severity of tip dieback are similar to those inoculated by grafting.

Figure 1

Figure 2

Explanation of Figures

Figure 3. Tip blight symptoms which developed when a Bing tree was inoculated with a severe strain of ring spot when the growth was 5-8 cm. long.

Figure 4. The two Bing cherry leaves on the left show necrotic ring spot and a lace leaf condition, and the one on the right target spot symptoms which developed when some strains of ring spot virus first invaded the tree.

Figure 3

Figure 4

Explanation of Figures

Figure 5. The two young cucumber plants on the left were inoculated with the virus strain R.S. 10 and the plant on the right with R.S. 24. The cotyledons show primary symptoms as local lesions and wilting, and the first leaves show secondary symptoms of mosaic.

Figure 6. The cotyledon leaves from three cucumber plants inoculated with different strains of ring spot.

A. Typical chlorotic lesions frequently produced by several strains.

B. Larger chlorotic lesions which soon developed a necrotic center.

C. The target spot type of lesions produced by some strains.

Figure 5

Figure 6

Explanation of Figures

Figure 7. The effect of R.S. 24 on subsequent growth of a cucumber plant. The inoculated plant on the right shows the severe dwarfing and rosetting of the new growth as compared with the normal growth of the check plant on the left.

Figure 8. Virus symptoms on cucumbers two weeks after mechanical inoculation with 3 different strains of the ring spot virus.

A. Plants showing mosaic and rugosity on first true leaf when inoculated with the virus strain, R.S. 11.

B. Local lesions on cotyledons and killing of the growing point, when inoculated with R.S. 24.

C. Dead cucumber plants which resulted from virus shock, when inoculated with R.S. 10.

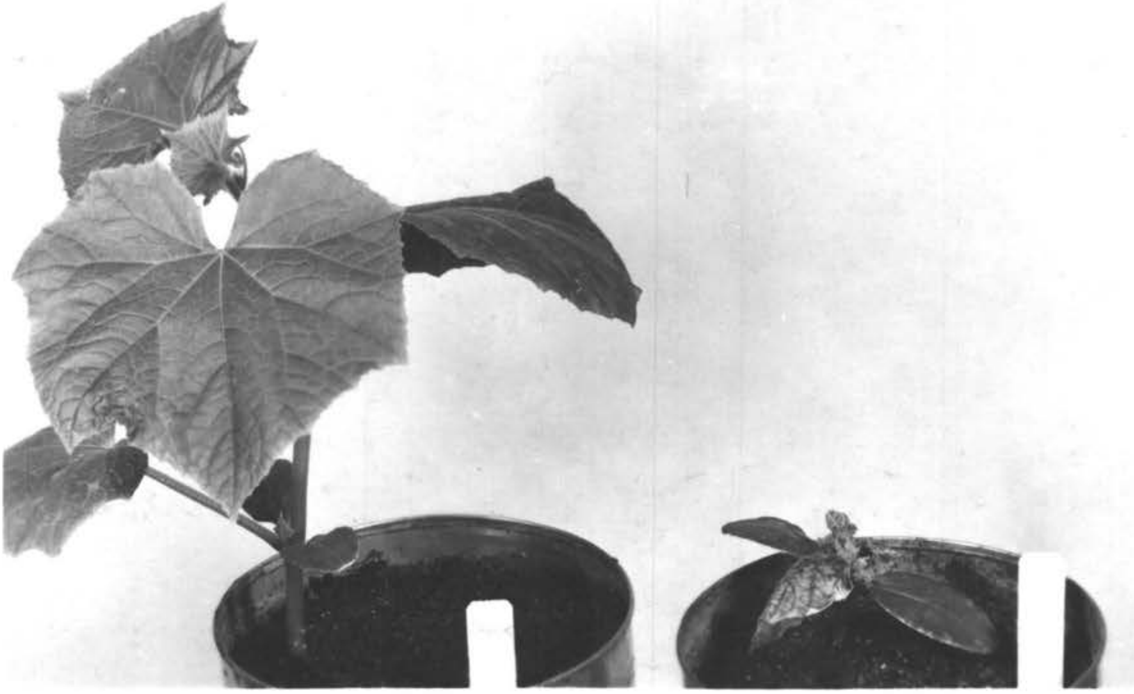

Figure 7

Figure 8

Explanation of Figures

Figure 9. Virus symptoms on large cucumber plants caused by the virus strain R.S. 10 when inoculated 21 days after planting. The plants show a golden type of mosaic, particularly on the tips.

Figure 10. Large cucumber plants similar to those of Figure 9 but inoculated with strain R.S. 24. The virus killed the growing point and then a dwarfed, rosetted growth developed from the axillary buds. Some of the larger leaves developed mosaic symptoms.

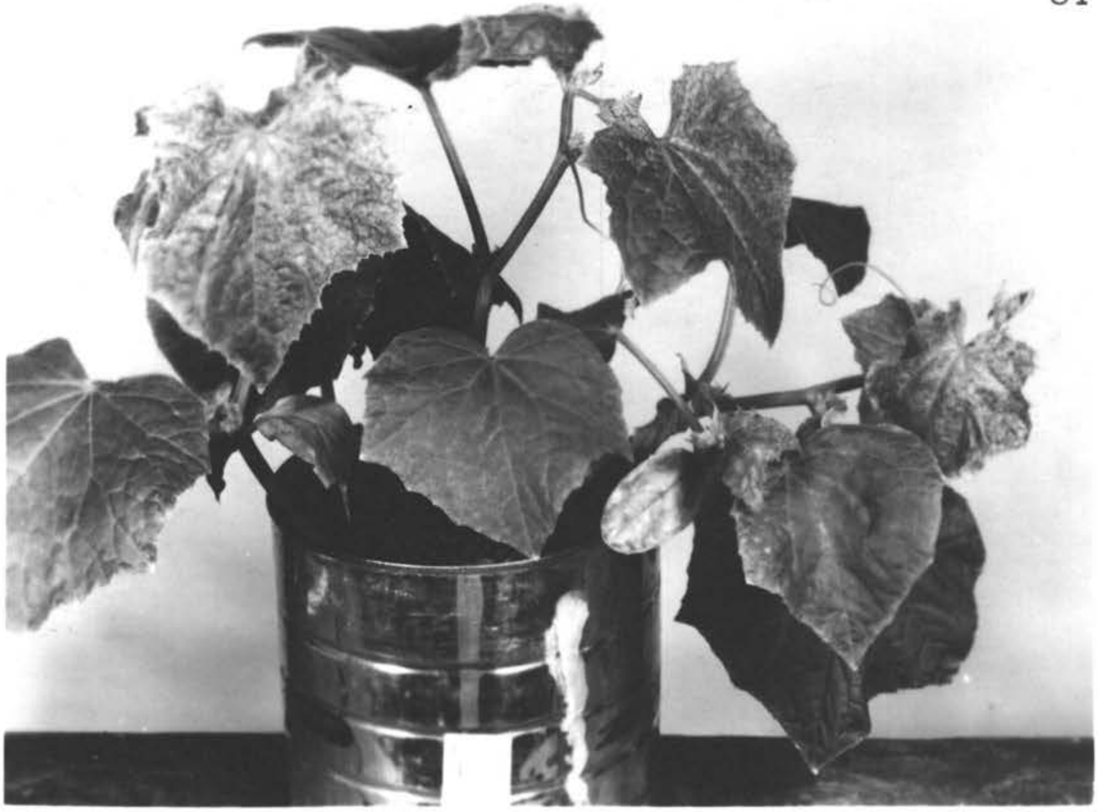

Figure 9

Figure 10