CROSS PROTECTION IN STONE FRUITS WITH THE RING SPOT VIRUS COMPLEX

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INTRODUCTION

Stone fruit trees are known to be infected with ring spot viruses in all areas where cherries and other Prunus species are grown. Cherry trees infected with a ring spot virus usually display symptoms only once, and then the virus remains in the tree in a latent condition. The severity of symptoms displayed by a tree infected with a ring spot virus will depend on the virus strain and the time of year of infection. Studies of stone fruit ring spot viruses have shown that strains exist, and that the symptoms they induce vary in intensity from severe to very mild according to the Prunus host inoculated with the virus. Mild virus strains have been shown to give protection against severe strains in herbaceous plants. If a mild strain of ring spot virus could be found then nursery trees might be infected and protected against the infection and shock reaction of more virulent strains of ring spot viruses under field conditions. Severe strains weaken the trees they infect and make them more susceptible to winter injury and intensify disease symptoms when the trees are infected with other stone fruit viruses.

The term ring spot virus is used in stone fruit literature to designate the latent necrotic ring spot and recurrent necrotic ring spot viruses. Ring spot virus strains are usually found as a virus complex, and host range studies produce disease symptoms of ring spot, sour cherry yellows, peach stunt, and prune dwarf. The relationship between these diseases has not been demonstrated, and they may be caused by strains of the same virus or are unrelated viruses. The term ringspot was interpreted as meaning a virus complex in this thesis, and will include all the related and unrelated viruses that may be found associated together in each ringspot source. The term ring spot virus will be used to denote a specific virus. The ringspot complexes have been given source tree numbers for the convenience of records.

The purpose of this study was (1) to determine through cross protection tests if interrelationships exist between ring spot viruses from different sources; (2) to find which Prunus plants would serve best to study these virus interrelationships; and (3) to discover, if possible, a mild reacting ring spot virus that would protect against severe strains.

LITERATURE REVIEW

The first indications of the phenomena of acquired immunity in plants was a report by McKinney in 1929 (11, p. 567), but it was not clearly demonstrated until 1931 by the work of Thung (18, pp. 450-463), who inoculated tobacco plants with a white mosaic strain of tobacco mosaic and then reinoculated with a common green strain of tobacco mosaic. The white mosaic strain completely protected against the green mosaic strain of tobacco mosaic. Likewise, if the green mosaic strain was introduced first it completely protected against the white strain. In 1933 Salaman (16, p. 468) inoculated tobacco plants with a very mild strain of potato virus X, and after five days reinoculated with a severe strain of the virus and no further reaction developed. When sub-cultures were made from doubly inoculated plants only the mild strain was recovered. This was the first demonstration of plant protection where the protective and challenging virus inoculations had little effect on the vigor of the plant.

The general subject of cross protection in plants has been reviewed by Bennett (3, pp. 39-67, and 4, pp. 295-308), Kunkel (10, pp. 251-273), and Price (13, pp. 338-361). In general their conclusions have been that no theory adequately explains the phenomena of cross protection. Many theories (4, p. 304, and 10, p. 252) have been advanced.

The one most generally accepted assumes that there is a limited amount of material essential for virus increase in the plant, and when this is exhausted by complete invasion by one virus the plant becomes immune from invasion by all viruses that require the same plant material for increase. The materials essential for virus increase apparently are certain protoplasmic constituents (14, p. 124), such as specific amino acids. Thung (13, pp. 124-125) suggested an antibody theory, but the presence of antibodies has not been demonstrated and any virus present in a plant usually remains active. Bawden and Kassanis (2, p. 56) advanced the theory that prior to multiplication virus particles attach themselves to certain cell constituents and there are only a limited number of such sites available in each cell. Unrelated viruses would combine at different specific sites, and related virus strains with the same site. If a site in a susceptible cell is already occupied by one strain then a second strain of that virus will be unable to attach itself and multiply. Another theory (4, p. 305) proposes that plants are immune from further infection by the same virus or its related strains because viruses exist in infected cells in the form of aggregates which have specific adsorptive properties. An additional virus introduced into the diseased plant does not increase since it is absorbed immediately by the primary aggregates. Immunity from related strains probably has its basis in the larger number of antigenic groups common to the various strains of a virus. This theory appears to have the advantage of providing for different degrees of interference between related strains since interference would be expected to depend, in part at least, on the number of antigenic groups available for adsorption. The above theories have been advanced to explain the phenomena involved in cross protection, but direct evidence to support any of these theories is lacking.

Several types of interactions occur between viruses and virus strains, but plants infected with one virus usually remain susceptible to infection by unrelated viruses (3, pp. 40-58). Many strains of viruses exist in nature, and strains of mosaics and ringspots have given a high degree of mutual protection. One type of interaction is that demonstrated by Thung (18, pp. 450-463), and Salaman (16, p. 468) where strains of the same virus mutually protected against each other, or a mild strain prevented invasion by a more virulent strain.

Antagonistic interactions may occur between unrelated viruses, and the concentration of one virus is markedly increased by the presence of a second unrelated virus. Ross (15, p. 24) has shown that the concentration of mild strains of potato virus X in _Nicotiana glutinosa_ Linn. may be increased as much as five-fold in the presence of potato virus Y. Unrelated viruses may cause a type of interaction where one virus reduces or completely suppresses the multiplication of the second virus. Bawden and Kassanis (2, pp. 52-57 demonstrated that severe etch virus prevented the multiplication of potato virus Y and Hyoscyamus virus 3, and was able to replace them even in plants in which they had become established.

Two unrelated viruses introduced into a plant may produce an interaction that results in an intensification of disease symptoms not characteristic of either virus alone (3, pp. 41-42). Tobacco mosaic, or potato virus X, introduced singly into tomato plants induces a mild type of disease, however, a tomato plant doubly infected with these viruses shows a marked increase in injury and expresses symptoms that are not typical of either virus.

The degree of interference offered by virus strains may vary between strains within a complex and will vary with different complexes (3, pp. 45-52). Related virus strains may give a high degree of mutual protection or no protection between its respective strains as in the case of curly-top virus.

Cross-protection as a criterion to determine virus relationships is now generally accepted (2, pp. 52-57, 3, pp. 39-67, 4, p. 306, 10, pp. 251-273, 13, p. 351, 16, p. 468, and 17, p. 66), but should be used in conjunction with other methods of determining relationships in the classification of viruses. Complete reliance should not be placed on immunity tests alone, but other criteria should be considered such as symptoms, host relationships, vector relationships, and physical or other characteristics.

Little information is available in the literature pertaining to cross protection in stone fruits. Cross immunological studies with the peach virus diseases, yellows, rosette, and little peach were reported by Kunkel in 1936 (8, pp. 201-219). The three diseases are alike in that they all cause stunting, abnormal production of secondary shoots, and yellowing of mature leaves. Cross immunity tests showed that rosette was not related to either yellows or little peach. Reciprocal inoculations with yellows and little peach showed the two viruses to be related.

Bodine, in Colorado, reported in 1942 (5, p. 1) that a mild strain of peach mosaic introduced into Elberta peach completely protected against a severe strain. Cochran in 1946 reported (6, p. 396) the existence of a large number of forms of the peach-mosaic virus capable of producing a symptom gradient on J. H. Hale peaches from severe strains to one so mild that diagnosis was difficult. Mild strains were obtained from sport-like branches, and trees developed from these branches were protected against the severe form from the mother tree. Results were conflicting when peach mosaic strains from widely separated areas were tested. A mild strain arising in Texas protected against the severe strains of that area, but not against the severe strain from Arizona. In other combinations, mild forms variously moderated the symptoms of severe forms as compared with checks.

Moore (12, pp. 470-471) inoculated Montmorency sour cherry trees showing recurrent necrotic ring spot and known to be infected with sour cherry yellows virus, with buds from four sour cherry and four peach sources known to contain latent necrotic ring spot, sour cherry yellows, and the prune dwarf viruses. The interaction effects produced by these inoculations fell into four groups: (a) shock symptoms on young leaves, then recovery; (b) shock symptoms produced one year later; (c) no shock symptoms, and inoculations back to Montmorency trees failed to incite recurrent necrotic ring spot; and (d) no shock symptoms, but recurrent necrotic ring spot was readily transmitted back to Montmorency trees.

Cochran (7, p. 512) studied interference between forms of the ring spot virus in peach trees. Seven mild forms were matched with three forms known to produce severe symptoms. No symptoms resulted from reinoculations on trees in which the original inoculations caused both leaf symptoms and bark necrosis. Three of the mild forms afforded protection against one of the severe forms, but were severely shocked by the other two. Symptoms were less severe than on trees which had not been infected with mild ringspots. This work indicated that ring spot virus forms afford varying protection against each other.

GENERAL METHODS AND MATERIALS

The *Prunus* species used as host plants in these cross protection studies were: *Prunus avium* Linn. variety Bing (B 260); *Prunus cerasus* Linn. variety Montmorency (M 505); *Prunus serrulata* Lindl. variety Kwanzan; and *Prunus persica* (L.) Batsch. variety Muir. The cherry varieties Bing and Montmorency were propagated on seedlings of *Prunus mahaleb* Linn. The Kwanzan flowering cherry was propagated on mazzard seedlings of *P. avium*. The peach variety Muir was propagated on seedlings of *P. persica* variety Lovell. The propagating technique with all varieties was by the standard nursery practice of T-budding.

The ringspot virus source trees used in these experiments were part of a collection of stone fruit trees maintained by the Oregon Agricultural Experiment Station. These had been collected because of their complex of latent viruses. Included in this series were four source trees inoculated with ringspots originally obtained from Dr. J. D. Moore, University of Wisconsin, and were of special interest because all gave a positive reaction on *P. serrulata* variety Shiro-fugen, and a negative reaction on Kwanzan. The information pertaining to the virus content of each source tree was taken from the records of Dr. J. A. Milbrath.

Twenty-five stone fruit ringspot viruses were selected for the cross protection studies. The characteristics of each ringspot had been determined through indexing on the following hosts: Shiro-fugen flowering cherry; Kwanzan flowering cherry; Muir peach; Montmorency sour cherry; *Prunus domestica* Linn. variety Italian; *Prunus armeniaca* Linn. variety Moorpark; Bing sweet cherry; *Prunus tomentosa* Thumb.; and *Cucumis sativus* Linn. variety A and C.

The necrotic index reaction of Shiro-fugen to ring spot virus was a spreading necrosis in the cambium and phloem immediately underneath and adjacent to the inoculation buds. The Shiro-fugen systemic reaction consisted of small, scattered necrotic flecks in the phloem, beneath and next to the point of bud insertion, and a general stunting of the tree. Kwanzan characteristically reacted to ring spot virus with a systemic leaf epinasty and stunting, or severe dieback the year following insertion of the inoculation buds. Peach reacted to ring spot virus with a mild transient chlorotic and necrotic spotting in new leaves, or severe dieback with eventual recovery, or a persistent rosetting and stunting. The reaction of Montmorency to ring spot viruses varied from mild transient chlorotic rings or mottles to severe necrotic spots and rings. Certain ringspots caused the sour cherry yellows disease in Montmorency, while others induced terminal dieback. Prune dwarf was the only reaction observed on Italian prune when inoculated with certain of the ringspot virus collections. The *Prunus tomentosa* reaction varied from chlorotic mottles and line patterns to severe stunting with necrotic spotting. When Moorpark apricots were inoculated with buds from the ring-spot source trees the only reaction was a gumming in the immediate area of the inoculating buds from some of the test trees. The ring spot virus symptoms on the sweet cherry variety Bing varied from a mild chlorotic mottle to severe necrotic spotting and rings, terminal dieback, and stunting of the tree. Cucumbers reacted to juice inoculation with stone fruit virus ring spots with chlorotic spots on the inoculated cotyledons, severe stunting of the plant, mottling and deformity of leaves, death of the growing point, or death of the entire plant. The index reaction on cucumbers and Prunus hosts to stone fruit ring spot viruses varies in severity with the ringspot culture used. Consequently, the ringspots for the experiments were selected on the basis of the index reactions and were classified as either mild or severe. Mild ringspots gave a mild reaction on one or two index hosts, and no reaction on the other hosts. Severe ringspots gave very severe reactions on a majority of the index hosts.

Protecting viruses were primarily mild ringspots, and severe ringspots were used as the challenging viruses. Protecting or established viruses always refer to the first virus introduced in a <u>Prunus</u> host, and the challenging virus is the second virus introduced. The host reactions from the index tests indicated that most of the ringspots consisted of a complex mixture of virus strains or unrelated viruses. No method is known, however, whereby stone fruit ringspot cultures can be purified to a single virus entity, and this necessitated the use of the viruses as they existed in the source trees.

Peach was included in these studies in an attempt to determine through cross protection if a relationship existed between peach stunt, cherry ring spot virus, sour cherry yellows, and prune dwarf. The exact nature of the virus that causes peach stunt is not known. Peach inoculated with certain ringspots may show a stunt symptom as do other peach trees inoculated with sour cherry yellows or prune dwarf. Since sour cherry yellows and prune dwarf have never been found free of ring spot virus, either the ring spot virus is a common contaminant or these diseases may be caused by specific strains of ring spot virus. Cross protection studies were used to study this relationship.

The index reactions of the stone fruit ringspots used in these experiments are found in Table 1.

One ringspot culture of special interest was RS-10. The original index of this ringspot was a positive reaction on both Shiro-fugen and Kwanzan. The Kwanzan positive factor, apparently, was not present in all buds of a scion-wood source. In one Kwanzan index test of RS-10, the reaction was negative and this Kwanzan material was used to provide the protecting virus in a cross protection

Table 1. Host index reactions of the 25 ringspots employed in cross protection experiments.

Ringspot source	Index host plants: Shiro-fugen	Kwanzan	Muir	Montmorency	Italian prune	*Prunus tomentosa*	Cucumber	Apricot	Bing
RS-4	Sys	Sev	Neg	BS		Neg	Neg	Neg	Mild
RS-5	Nec	Neg	Neg	RS		Neg	Mild	Neg	Neg
RS-7	Nec	Sev	Mod	RS, Y		Mild	Sev	Neg	Mild
RS-8	Nec	Sev	St	RS, Y		Mild	Sev	Neg	Sev
RS-9	Nec	Sev	Sev	BS, RS		Mild	Sev	Neg	Sev
RS-10	Nec	Sev	St	RS, Y		Mild	Sev	SG	Sev
RS-11	Nec	Neg	St	Y		Neg	VM	SG	Mild
RS-12	Sys	Mod	Neg			Mild	VM	Neg	Neg
RS-13	Nec	Sev	St	RS	D	Sev	Sev	Neg	Mild
RS-14	Nec	Neg	Neg	RS		Mild	Sev	Neg	Mild
RS-15	Nec	Neg	Neg				VM	Neg	Neg
RS-17	Nec	Sev	Neg			Mild	Sev	Neg	Mild
RS-18	Sys	Mod	Neg	RS		Sev	Mild	Neg	Mild
RS-19	Nec	Sev	Sev	RS, Y		Sev	VS	SG	Sev
RS-20	Nec	Neg	Sev	RS, Y		Sev	VS	Neg	Sev
RS-21	Nec	Sev	Sev	RS			VS	MG	Sev
RS-22	Nec	Mod	St	RS, Y		Mild	Sev	SG	Sev
RS-23	Nec	Neg	St	RS, Y		Sev	Sev	SG	Sev
RS-26	Sys	Sev	Neg			Neg	Neg	Neg	
RS-27	Sys	Sev	Neg				Neg	Neg	
RS-28	Nec	Neg	Sev	RS, Y		Sev			
M 6-30	Nec	Neg		Rec, RS, Y					
S 5009	Nec	Neg		Rec, RS, Y					
B 3-22	Nec	Neg		RS	D				
G 2-1	Nec	Neg		Nec, RS					

Sys - Systemic, Nec - Necrotic, Sev - Severe, Neg - Negative, Mod - Moderate, St - Stunt, BS - Bark Split, RS - Ringspot, Y - Yellows, VM - Very mild, D - Dwarf VS - Very severe, SG - Severe gum, MG - Moderate gum experiment. The same segregating character of this ringspot culture was also noted by Dr. J. A. Milbrath in indexing tests on cucumber. The factors responsible for the instability of this ringspot is not known, but the phenomena could be caused by the interaction of two or more strains of the same virus. One of the virus components could dominate under one set of conditions, and the other dominate under conditions more favorable for its multiplication.

RS-15 was the mildest ringspot used in these experiments from the standpoint of host index reactions. This ringspot induced the systemic reaction in Shiro-fugen and none on additional Prunus hosts, but a very mild reaction in cucumber. RS-15 appears to be the least contaminated of any ringspot used in these cross protection experiments. The ringspot could be composed of two or more viruses, or the reaction on Shiro-fugen and cucumber could be the result of the same virus component.

RS-4, RS-12, RS-18, RS-26, and RS-27 were found to be Shiro-fugen systemic, Kwanzan positive ringspots. RS-13 was originally a Shiro-fugen systemic, Kwanzan positive ringspot, but became contaminated with the Shiro-fugen necrotic factor, apparently, through insect transmission of other ring spot virus components. RS-5, RS-11, RS-14, RS-20, RS-23, RS-28, M 6-30, S 5009, B 3-22, and G 2-1 were Shiro-fugen positive, Kwanzan negative ringspots. RS-7, RS-8, RS-9, RS-17, RS-19, RS-21, and RS-22 were Shiro-fugen positive, Kwanzan positive ringspots.

The first cross protection tests were designed to allow the protecting or established viruses to become systemic for a period of one year before inoculation with the challenging viruses. Virus-free Bing and Montmorency were propagated on seedling of P. mahaleb and the seedlings were inoculated with the protecting viruses at the time of budding. Scionwood for variety propagation was selected from virus-free trees to insure that no additional virus components would be unknowingly added to the protecting ringspot culture. Kwanzan trees for the first cross protection experiment were propagated with scionwood from trees systemically infected with the protecting ringspots. Inoculations with the challenging viruses were made when the Kwanzan trees were one year old. Later experiments with Kwanzan trees were designed so that buds which carried the protecting virus, and buds which carried the challenging virus were budded onto mazzard seedlings simultaneously. Scionwood, for the cross protection experiment with the peach variety Muir as the test host plant, was selected from peach trees systemically infected with the protecting ringspot viruses.

CROSS PROTECTION EXPERIMENTS

Field experiment with Bing Trees

The purpose of this study was to determine if certain strains of stone fruit ringspots would cross protect against each other when Bing sweet cherry is used as a host plant. Three mild reacting ringspots, RS-4, RS-7, and RS-15, and two severe ringspot strains, RS-9, and RS-10 were selected for this experiment, which was designed so that each ringspot was used in one series as a protecting virus, and in other series as a challenging virus against each of the other ringspots except RS-10, which was included only as a challenging virus.

One-hundred buds of virus-free Bing (B 260) were T-budded on P. mahaleb seedlings to provide trees for this experiment. Groups of twenty seedlings were bud-inoculated with one of the selected ringspots (RS-4, RS-7, RS-9, or RS-15), and twenty seedlings budded only with virus-free Bing were left for check inoculations. During the following growing season the developing Bing stems of each group were reinoculated with the same ringspot to insure the establishment of the culture. Inoculations with the challenging viruses were made just prior to bud-break at the beginning of the second growing season. Inoculation technique was by a method known as "patch budding", and three inoculation buds were placed on each tree. At least four trees from each group of twenty were cross inoculated with one of the four ringspots to be used as the challenging virus. Each challenging virus was also inoculated into the virus-free check plants to be used as controls.

The results of this experiment were determined during the following growing season. A series of disease classes were established based on the severity of virus reaction, and results were taken according to the numerical ratings noted below.

0 - Leaves normal. No ringspot symptoms.

1 - No necrotic spotting, but occasional mottles or chlorotic rings.

2 - A few leaves show necrotic spotting. New growth normal.

3 - Many leaves show necrotic spotting. Occasional tip blight or terminal dieback, and some terminal elongation.

4 - Most leaves show necrotic spots or rings. Terminal dieback or tip blight frequent. Trees stunted.

The results of this experiment are summarized in Table 2, where each figure represents an average of the disease rating for all replications in that set of inoculations.

RS-4 gave no protection against any of the challenging viruses. Where RS-7 was the challenging virus and RS-4 the established virus, the reaction was more severe than either RS-4 or RS-7 alone. When RS-7 was the protecting virus, however, and RS-4 the challenging virus, there was a very mild type of reaction considerably less than RS-7

Table 2. The relative effectiveness of four ringspots established in field grown Bing trees in protecting against four challenging ringspots.

Protecting virus	Average disease index when challenged with				
	RS-4	RS-7	RS-9	RS-10	RS-15
RS-4	-	3.0	3.0	4.0	1.2
RS-7	0.4	-	0.6	0.0	0.0
RS-9	0.7	0.6	-	0.8	1.0
RS-15	0.0	0.2	0.7	1.6	-
Check	0.0	1.7	3.5	4.0	1.2

alone. This would indicate an antagonistic effect where RS-4 is the established virus and is challenged by RS-7. Apparently an interaction caused one or both of the viruses to multiply at a greater rate when RS-4 was established first, and resulted in a more severe reaction than either ringspot alone. RS-7 is a more virulent strain of ringspot than RS-4 as indicated by a greater range of host reactions in index tests, and once RS-7 becomes established it apparently prevents the virus build-up of any additional introduced ringspots and thereby gives protection or prevents a shock reaction. RS-7 gave excellent protection against the two severe ringspot strains RS-9, and RS-10. RS-9 gave excellent protection against RS-10. RS-15 gave excellent protection against ringspot strains RS-7, RS-9, and RS-10. RS-15 is of special interest since it uniformly gave excellent protection against all the challenging ringspots. RS-4 is a Shiro-fugen systemic reacting ringspot, and did not give protection against RS-15 which has the Shiro-fugen necrotic factor. RS-7, RS-9, RS-10, and RS-15 all have the Shiro-fugen necrotic factor, and all gave mutual cross protection. These results indicated that the Shiro-fugen necrotic and the Shiro-fugen systemic factors are unrelated or distantly related viruses.

Numerical disease rating readings within the replications were quite uniform over the entire experiment, but there was some variation in certain protection sets. For example, the readings of the replications in the set RS-15 plus RS-10 were 1, 1, 1, 4, 1, and in the set RS-15 plus RS-9 the readings were 0,2,0,1. The lack of uniformity within replications could be due to the virus complexes that comprise the stone fruit ringspots.

The same trees were used in a second experiment to determine if trees conditioned with two ringspot selections would still give a ringspot shock reaction. RS-9 was selected as the challenging ringspot because it induces a severe shock reaction in virus-free Bing. Challenging inoculations were made by patch budding just prior to the start of spring growth. Two trees from each previous protection set were inoculated with RS-9, and two were left as controls. No ringspot or shock reaction symptoms were apparent during the growing season on any of the inoculated or uninoculated Bing trees. The combination of any two ringspot selections in this experiment with Bing as the host plant resulted in complete protection against RS-9. These results suggest that complete protection against ring spot virus shock reactions requires conditioning with a number of viruses, and that several strains of related and unrelated viruses comprise the stone fruit ringspot virus complex.

Field Experiment with Montmorency Trees

Montmorency sour cherry trees were used in this experiment because they commonly serve as index hosts in ring spot investigations. The purpose of this study was to determine if the selected strains of ring spot would cross protect against each other when Montmorency was used as the host plant. The same ring spot viruses were selected as in the Bing field experiment except RS-10 was not included as a challenging virus. The experiment was designed so that each ring spot was used in one series as a protecting virus, and in other series as a challenging virus against each of the other ring spots.

One-hundred buds of virus-free Montmorency (M 505) were T-budded on *P. mahaleb* seedlings to provide trees for this experiment. Simultaneously four groups of twenty seedlings each were bud-inoculated with one of the selected ring spots (RS-4, RS-7, RS-9, or RS-15), and twenty seedlings budded only with virus-free Montmorency were left for check inoculations. The trees were allowed to grow for one season to insure that the ring spots would become systemic in the Montmorency before inoculation with the challenging viruses. Inoculations with the challenging viruses were made at the beginning of the second growing season by the technique of patch budding. In the group where RS-4 was the established virus, eight trees survived out of the original twenty and as a result only RS-9 and RS-15 were used as challenging viruses. Four or five trees were available for inoculation with the challenging viruses in the three other groups.

Disease classes were established, based on the severity of virus symptoms, and the results were tabulated according to the following numerical classifications.

0 - Leaves normal. No ring spot symptoms.

1 - No necrotic spotting. Occasional leaf with chlorotic mottles or rings.

2 - A few leaves show necrotic spotting. New growth normal.

3 - Many leaves show necrotic spotting. Occasional tip blight or terminal dieback. Many leaves normal, and some terminal elongation.

4 - Most leaves show necrotic spots or rings. Terminal dieback or tip blight frequent. Trees stunted.

The results of this experiment are summarized in Table 3, and each figure represents an average of the disease rating of all the replications in that particular set of inoculations.

RS-4 gave only slight protection against RS-9. RS-7

Table 3. The relative effectiveness of four ringspots established in field grown Montmorency trees in protecting against three challenging ringspots.

Protecting virus	Average disease index when challenged with RS-4	RS-7	RS-9	RS-15
RS-4	-	-	3.0	0.5
RS-7	0.8	-	1.0	0.8
RS-9	1.2	1.0	-	1.0
RS-15	0.7	1.2	1.8	-
Check	0.2	3.0	4.0	0.0

gave excellent protection against RS-9. RS-7 plus RS-15, RS-9 plus RS-4, and RS-9 plus RS-15 resulted in a more severe reaction than either RS-4, or RS-15 alone. RS-9 gave good protection against RS-7. RS-15 gave good protection against RS-7, and RS-9. RS-7 and RS-9 gave reciprocal protection, but it was not complete in either case.

Disease index ratings were not always uniform in all replications of an inoculation set. For example, RS-4 plus RS-9 disease index readings were 4, 2, 3, and 2, and in the case of RS-15 plus RS-7 the readings were 1, 0, 2, 2, and 1. This lack of uniformity in disease index readings within replications could be due to the failure of the virus components of the protecting ringspots to become completely systemic in the test trees before the inoculations were made with the challenging viruses. Interactions between unrelated virus strains could be another reason for the variability in index readings.

Certain ringspot combinations gave virus symptoms unlike the check inoculations of either ringspot. At times the symptoms were modified to give a milder reaction. In other cases the virus symptoms were intensified over the control inoculations of the respective ringspot culture. The mild ringspots RS-4, and RS-15 are an example of the latter case, and both give essentially no reaction when inoculated to virus-free Montmorency, yet when they are combined, produce a very definite ringspot mottle.

The same trees were used the next year for a second experiment. The trees were reinoculated to see if Montmorency trees conditioned with two ringspot cultures would still display a ringspot shock reaction, or if they were completely protected. One challenging ringspot was used in this test, and RS-22 was chosen because it produces a very severe reaction when inoculated into virus-free Montmorency (Figure 1). One-half of the trees of a previous protection set were inoculated, and the other half left as check material. Nine virus-free Montmorency's were inoculated with RS-22 as controls. The trees, now two years old, were severely pruned before the challenging inoculation was made. Inoculations with the challenging virus were made just prior to spring growth by placing three buds on each tree by the patch-budding technique.

The results of this cross protection experiment are found in Table 4.

Table 4. Average disease index rating of Montmorency field trees conditioned with two ringspots then inoculated with RS-22 as a challenging virus.

Original protecting virus	Challenging virus	Original challenging virus RS-4	RS-7	RS-9	RS-15	Check
RS-4	None	-	-	0.0	0.0	0.0
RS-4	RS-22	-	-	2.0	1.0	4.0
RS-7	None	0.0	-	0.0	0.0	0.0
RS-7	RS-22	1.0	-	1.0	1.0	1.0
RS-9	None	1.0	0.0	-	0.0	0.0
RS-9	RS-22	1.7	0.5	-	1.0	2.0
RS-15	None	0.0	0.0	0.5	-	0.0
RS-15	RS-22	3.5	2.0	2.0	-	2.0
Check*	None					0.0
Check*	RS-22					4.0

* Virus-free Montmorency

This experiment demonstrates that even though Montmorency sour cherry trees are conditioned with two ringspot cultures, shock reactions may still be induced by inoculation with a third ringspot.

An interesting phenomenon is illustrated in the table; the combination of RS-4 plus RS-15 plus RS-22 resulted in a mild reaction or very good protection. The combination of RS-15 plus RS-4 plus RS-22, however, gave a reaction essentially the same as the check inoculation of virus-free Montmorency trees with RS-22.

The ringspot combinations of RS-15 plus RS-4, RS-15 plus RS-7, and RS-15 plus RS-9 gave no protection against RS-22. The ringspot combination of RS-9 plus RS-7 gave the most complete protection against RS-22.

The data in the table indicates that the RS-22 ringspot culture is composed of virus components not contained in the RS-4, RS-7, RS-9, and RS-15 ringspot complexes, and that Montmorency sour cherry is a more sensitive host plant than Bing to the ringspots used in these experiments.

This experiment strengthens the hypothesis that stone-fruit ringspot viruses are composed of a number of unrelated or distantly related viruses. Apparently, the viruses comprising the stone fruit ringspot complex are capable of inducing similar shock symptoms in Montmorency sour cherry.

Greenhouse Experiment with Bing Trees

The design and purpose of this experiment was to determine if "Kwanzan negative, Shiro-fugen positive", and "Kwanzan positive, Shiro-fugen negative" ringspots would protect against "Kwanzan negative, Shiro-fugen positive", and "Kwanzan positive, Shiro-fugen positive" ringspots. The protecting ringspots chosen for this study were RS-15, and RS-27, and the challenging ringspots selected were RS-8, RS-9, RS-10, RS-20, RS-23, and RS-28.

One-hundred and fifty seedlings of P. mahaleb were T-budded with virus-free Bing (B 260) to provide trees for this experiment. At the same time the Bing was budded, fifty seedlings each were bud-inoculated with RS-15, and RS-27, and fifty seedlings with virus-free Bing were left for the purpose of check inoculations. The trees were

Figure 1. The severe stunting effect of ringspot 22 on Montmorency sour cherry; healthy Montmorency on the right, and Montmorency inoculated with ringspot 22 on the left.

Figure 2. Greenhouse grown Bing trees graft inoculated with dormant scions from ringspot source trees. A severe type of dieback started at the point of graft inoculation and spread downward.

Figure 1

Figure 2

grown one year at the Plant Pathology farm, and the following winter the trees were dug and transplanted to greenhouse groundbeds. Inoculations with the challenging ringspots were made one week after planting by whip grafting a scion on the top of each tree. The Bing trees had been cut back to approximately thirty-one inches above the ground level.

The trees in this experiment had been numbered, and when the top was removed from each tree it was labeled, and placed in cold storage for subsequent indexing on Shiro-fugen. The indexing was thought necessary to determine if the one-year old Bing trees were systemically infected with the established viruses. All the virus-free Bing's and the trees inoculated with RS-27 conformed to expectations and were negative on Shiro-fugen. The indexing results with the trees inoculated with RS-15, however, were not as expected. Out of forty-eight trees indexed only thirteen were positive on Shiro-fugen and all should have given a positive reading. This could have resulted from faulty technique at the time of inoculation with the protecting virus, or the virus might not have been completely systemic in the source tree from which scions were taken. The virus might have failed to become entirely systemic in the one-year old Bing trees. The situation might have resulted from an unknown physiological relationship between the ringspot, the tree, and the environmental factors where the virus was kept at such a low titre that negative indexes resulted.

Growing the trees under greenhouse conditions modified symptom expression, and no sharp delineation could be made between the groups on the basis of leaf symptoms. Necrotic spots and rings were a common symptom, but definite disease classes could not be established. A dieback of the original trunk wood occurred in the trees, and was the most consistent and significant factor of symptom expression (Figure 2). Measurements were made in inches of the trunk dieback, and the readings of the seven replications were averaged to give a numerical rating for each cross protection set (Table 5).

Table 5. Average dieback of one-year old Bing trees in greenhouse cross-protection tests when inoculated with different ringspots.

Protecting virus	Average dieback in inches on seven trees inoculated with						
	RS-8	RS-9	RS-10	RS-20	RS-23	RS-28	Check
RS-15	8.1	15.1	22.2	7.5	11.9	7.8	0.0
RS-27	22.6	20.0	20.4	11.6	19.5	12.3	0.0
Check	12.1	21.4	19.0	5.7	10.5	15.2	0.0

The dieback that occurred in these trees is not a reaction usually associated with the symptoms resulting from ringspot inoculations to sweet cherry varieties. Winter or early spring inoculations with severe ringspots may result in tip blighting of current season growth, but not extensive dieback in the one-year old wood. There was not only extensive dieback in the test trees, but several trees were killed. The dieback in all cases started at the point of graft inoculation and progressed downward. This type of dieback is usually associated with some pathogenic organism, but none could be isolated from these trees. The organism most consistently isolated was a Pullularia sp. that is not considered to be pathogenic. The severe reactions which occurred from inoculations with the ringspots used in this experiment is difficult to explain. This type of reaction, however, emphasizes the role of environment and its effect on symptom expression of virus diseases.

The greatest amount of dieback occurred in the series where RS-27 was the established virus, and was very striking in contrast to the two other series. RS-27 is a more severe ringspot in comparison with RS-15, and the developing trees infected with only RS-27 were considerably dwarfed when compared to the other trees to be used in the experiment.

The tabulated results of the dieback showed a surprising trend, and agreed to some extent with the results obtained in the Bing field experiment. RS-15 gave excellent protection against RS-4, RS-7, RS-9, and RS-10 in the Bing field experiment. In the Bing greenhouse experiment where RS-15 was the established virus, and RS-8, RS-9, and RS-28 were the challenging viruses, the amount of dieback was considerably reduced when compared with the check inoculations. RS-27 gave no protection against any of the challenging ringspots, and in the case of the challenging viruses RS-8, RS-20, and RS-23 the amount of dieback was greater than that of the check inoculations. This same situation occurred in the Bing field experiment where RS-4 was the established virus. RS-4 gave no protection against any of the challenging ringspots, and where RS-7 was the challenging virus the severity of reaction was increased over both the RS-4, and RS-7 check inoculations. Both RS-4 and RS-27 react with the Shiro-fugen systemic reaction when indexed on Shiro-fugen. The Shiro-fugen systemic factor could be an unrelated virus and, when combined with virus components of certain ringspots, is capable of producing a synergistic effect that results in a more virulent reaction than either virus is capable of producing alone. There were no ringspot or dieback symptoms in any of the uninoculated RS-15, RS-27, and virus-free Bing check trees.

Greenhouse Experiment with Montmorency Trees

This experiment was performed to test the suitability of Montmorency trees for cross protection studies under greenhouse conditions, and to study the relationship of Kwanzan positive, and Shiro-fugen positive ringspots. The experiment was designed to determine if ringspots that indexed positive on only one flowering cherry host would cross protect against ringspots that indexed either positive on one, or positive on both Shiro-fugen and Kwanzan. If Kwanzan positive and Shiro-fugen positive ringspots do not protect against each other then the assumption may be made that they are unrelated viruses.

One-hundred and fifty seedlings of P. mahaleb were T-budded with virus-free Montmorency (M 505) buds to provide trees for use in this experiment. To introduce the protecting test viruses, fifty seedlings each were bud-inoculated with RS-15, and RS-27. Fifty seedlings with virus-free buds of Montmorency were provided for check inoculations with the challenging viruses. The trees were grown at the Plant Pathology farm one year to provide test plants of a suitable size, and to allow the established viruses to become systemic. The trees were dug in late winter and transplanted to greenhouse groundbeds. Inoculations with the challenging viruses were started immediately after the trees were planted, and the inoculation technique was either by patch budding or the standard nursery T-budding practice. Inoculations with the challenging ringspots were replicated seven times within each series.

Disease classes were established (Figures 3 and 4) based on the severity of the virus symptom, and the results were tabulated (Table 6) according to the following numerical classifications.

0 - Leaves normal. No ringspot symptoms.

1 - Occasional rough leaf, but no necrosis or leaf spotting.

2 - Some leaves show necrotic spotting, but new growth normal.

3 - Most leaves show necrotic spotting and necrosis, but a few normal leaves. Some terminal elongation.

4 - Nearly every leaf with severe leaf spotting, with large necrotic areas or necrotic rings. Frequent tip blighting, and little or no terminal elongation.

Each figure in Table 6 is an average of the disease index rating of the seven replications in each inoculation set.

Table 6. The relative effectiveness of two ringspots established in greenhouse Montmorency trees in protecting against six challenging ringspots.

Protecting virus	Average disease index of seven trees challenged with						
	RS-8	RS-9	RS-10	RS-20	RS-23	RS-28	Check
RS-15	2.0	2.4	2.2	1.3	1.7	1.4	0.0
RS-27	2.8	2.0	3.3	1.4	2.6	2.3	0.0
Check*	2.0	2.0	3.4	1.1	3.6	2.3	0.0

*Virus-free Montmorency

RS-15 gave good protection against RS-10, RS-23, and RS-28, but the protection was partial and not complete. RS-27 gave some protection against RS-23, but the protection was incomplete. An interaction occurred with the combination of RS-27 plus RS-8 that gave a more severe disease index than RS-8 alone. All challenging ringspots

Figure 3. Greenhouse grown Montmorency illustrating three of the milder disease classes established for degree of cross protection. Tree on left is class 0 and illustrates normal tree or no reaction; center tree is class 1 with a mild reaction of chlorotic mottling; trees on right show a more severe reaction with some necrotic spotting.

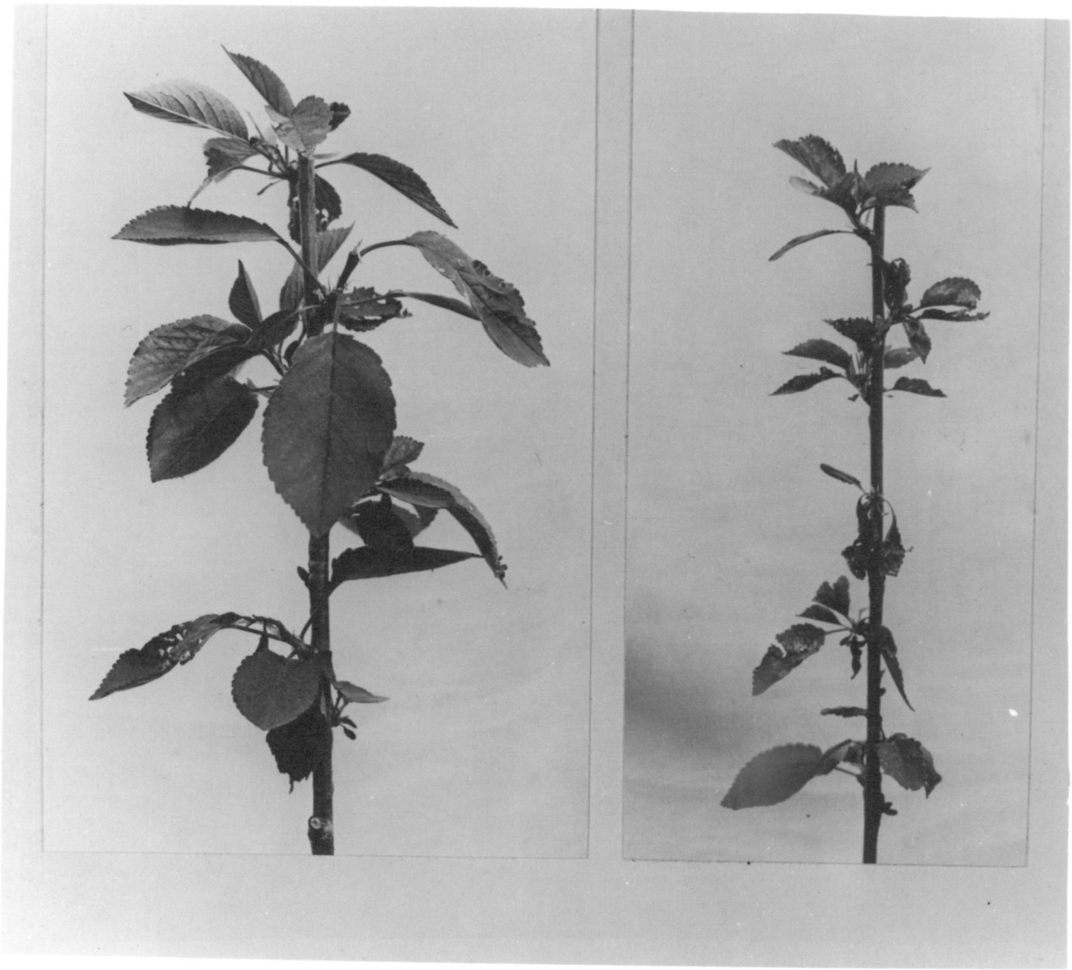

Figure 4. Greenhouse grown Montmorency illustrating two of the more severe disease classes established for degree of cross protection. Tree on the left is class 3 and shows many leaves with necrotic spotting; tree on the right is class 4 with most leaves showing necrotic spotting and an occasional tip blight.

contain the Shiro-fugen positive factor, and the protecting virus RS-27 is a "Shiro-fugen systemic, Kwanzan positive" ringspot. RS-27 gave no protection against any of the challenging ringspots. Apparently the Shiro-fugen systemic, and the Shiro-fugen necrotic factors are unrelated virus components.

Field Experiments with Kwanzan Trees

Kwanzan trees inoculated in late summer with certain stone fruit ringspot viruses reacted the following spring with a systemic leaf epinasty symptom. The symptoms varied in severity with the ringspot culture, and ranged from a slight downward cupping of the leaves to severe leaf distortion and rosetting, or killing of the trees. Other ringspots do not induce the leaf epinasty symptom. This experiment was designed to test the hypothesis that Kwanzan negative ringspots might protect against Kwanzan positive ringspots. The experiment also served the purpose of determining the suitability of Kwanzan as a host for cross protection studies with stone fruit ringspot viruses.

The Kwanzan negative ringspots chosen for this study were the Wisconsin ringspots S 5009, B 3-22, M 6-30, and G 2-1. The challenging Kwanzan positive ringspots used were RS-7, RS-8, RS-9, and RS-10. The scionwood for propagation was taken from trees previously inoculated and systemically infected with the Kwanzan negative viruses. Twenty-five Kwanzan trees with each protecting ringspot and twenty-five trees of healthy Kwanzan were propagated by T-budding on mazzard seedlings.

The Kwanzan trees carrying the established viruses, and the healthy Kwanzan checks were inoculated with the challenging ringspots the first growing season. Three buds carrying the challenging virus were T-budded to each tree, and challenging inoculations were replicated four times in each series.

The results of this experiment were taken the second growing season. Check trees for this experiment consisted of Kwanzan trees inoculated with the protecting virus, healthy Kwanzan inoculated with the challenging ringspots, and uninoculated healthy Kwanzan. A series of disease classes were established based on the severity of virus reaction, and the results were tabulated (Table 7) according to the numerical ratings noted below.

0 - Trees normal.

1 - Leaves normal except slight arching and cupping of some leaves.

2 - All leaves small, rosetted, twisted, and arched. All leaves affected, but no killing.

3 - Leaves very small, and in tight rosettes. Very little growth.

4 - Top buds and wood killed, with some leaves alive at base of trunk which are not normal.

5 - Kwanzan killed.

The tabulations in the table represent an average figure of the disease readings of the four replications in each inoculation set.

Table 7. The relative effectiveness of four ringspots established in field grown Kwanzan trees in protecting against four challenging ringspots.

Protecting virus	Average disease index of four trees when challenged with			
	RS-7	RS-8	RS-9	RS-10
M 6-30	2.0	1.0	2.0	0.0
B 3-22	2.0	1.0	2.0	0.0
S 5009	3.0	1.0	3.0	0.0
G 2-1	2.0	1.0	2.0	0.0
Check	2.0	5.0	3.5	4.0

All the Kwanzan negative ringspots gave excellent protection against RS-8 and RS-10 which are both very severe on healthy Kwanzan. All established viruses gave a measure of protection against RS-9, except S 5009. No established virus gave protection against RS-7, and in the case where S 5009 was the established virus there was an interaction of the ringspots and the reaction was more severe than RS-7 alone. The protection by the established viruses was not complete against RS-8, but the results were very striking because RS-8 kills healthy Kwanzan (Figure 5).

Kwanzan was found to be a very excellent host to study interrelationships of stone fruit ringspot viruses.

Figure 5. Kwanzan trees used in cross protection experiments. A to E illustrates type of reaction when healthy Kwanzan is inoculated with: A, RS-10; B, RS-7; C, RS-9; D, RS-8; and E healthy check. F to J shows Kwanzan trees protected with ringspot G 2-1 and challenged with: F, RS-10; G, RS-7; H, RS-9; I, RS-8; and F healthy check.

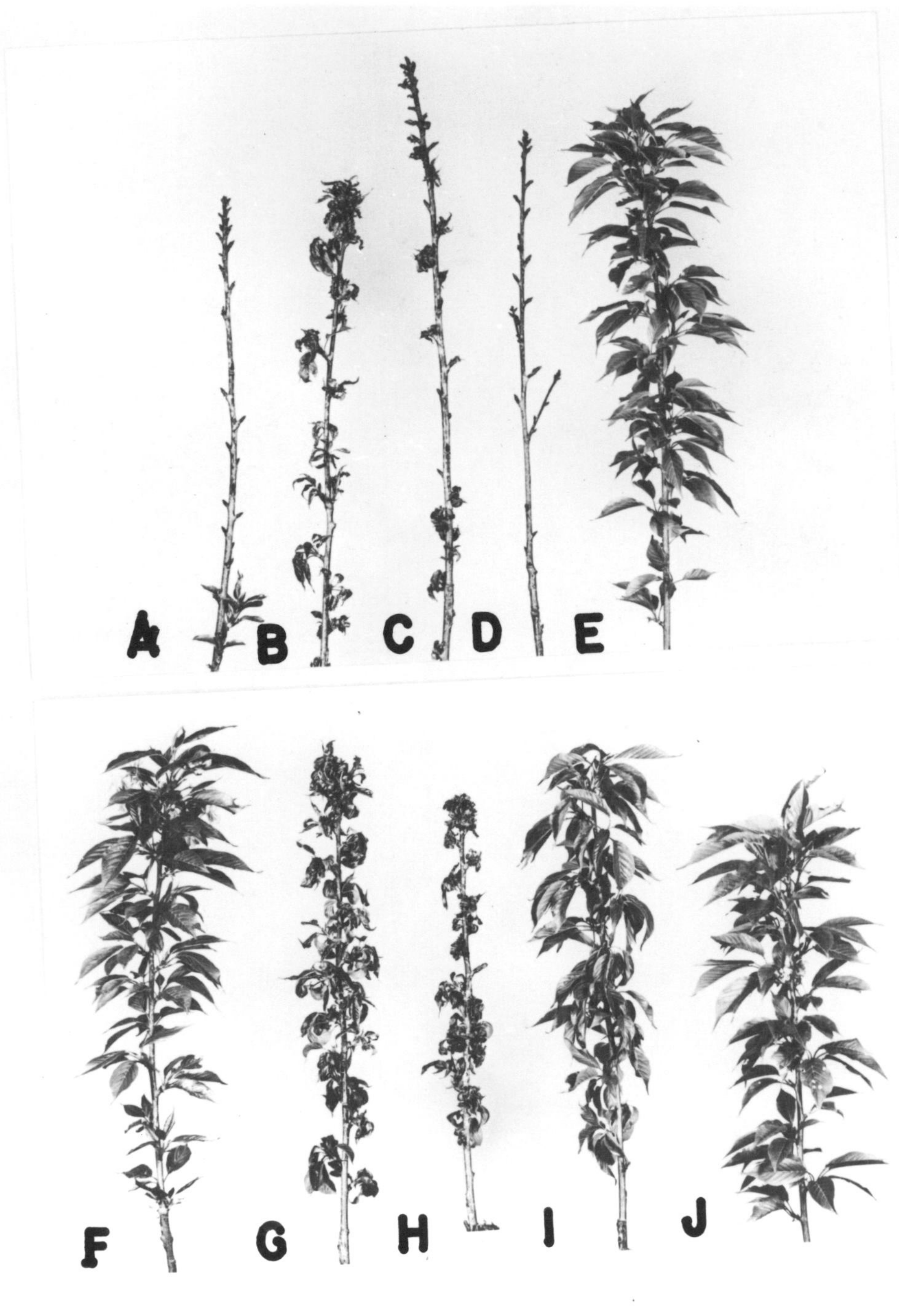

Figure 5

The process of conducting any experiment employing stone fruit trees requires a considerable amount of time. Seed or seedlings are planted in the early spring, and may be budded with the desired variety late in the summer of that year. The following summer is required for growing the trees into a suitable size for use. Inoculations can be made the second year to these one-year old trees, with the results observed the following year. This process requires a period of three growing seasons. The present experiment was designed to circumvent this time consuming process and to determine if seedlings could be topworked with buds carrying the protecting viruses, and at the same time bud-inoculate with the challenging viruses and get reliable results. If the above technique proved to be feasible, seedlings could be budded one summer and results taken the following spring and thereby save many months of valuable research time.

The Kwanzan negative protecting ringspots chosen for this experiment were RS-5, RS-10, RS-11, RS-12, RS-15, and RS-23. The challenging Kwanzan positive ringspots selected were RS-8, RS-9, RS-10, and RS-19. RS-10 and RS-12 have been considered Kwanzan positive ringspots. The cultures of RS-10, and RS-12 protecting ringspots used in this experiment, however, were obtained from test indexes of several ringspots where RS-10 and RS-12 had segregated and gave a Kwanzan negative index. These Kwanzan index trees served as the source material of the RS-10, and RS-12 cultures used in this experiment.

One-year old mazzard seedlings were used for topworking. Sixteen seedlings each were topworked by T-budding with the six protecting ringspots (Figure 6). Inoculations with the four challenging ringspots were made simultaneously and replicated four times in each protection series. This experiment was exploratory in nature as only a limited number of mazzard seedlings were available, and no healthy Kwanzan could be provided for inoculations with the challenging ringspots.

A series of disease classes was established based on the severity of virus reaction, and results were tabulated according to the numerical ratings noted below.

0 - Trees normal.

1 - Slight cupping and arching of some leaves.

2 - Leaves small, rosetted, twisted, and arched.

3 - Leaves very small in tight rosettes. Growth badly stunted.

4 - Growth started and then was killed.

The results of this experiment have been summarized in Table 8, and the figures represent an average of the four replications of each inoculation set.

All Kwanzan negative ringspots gave excellent protection against RS-10 and RS-19 (Figure 6). Both RS-10 and RS-19 killed healthy Kwanzan when originally indexed, and

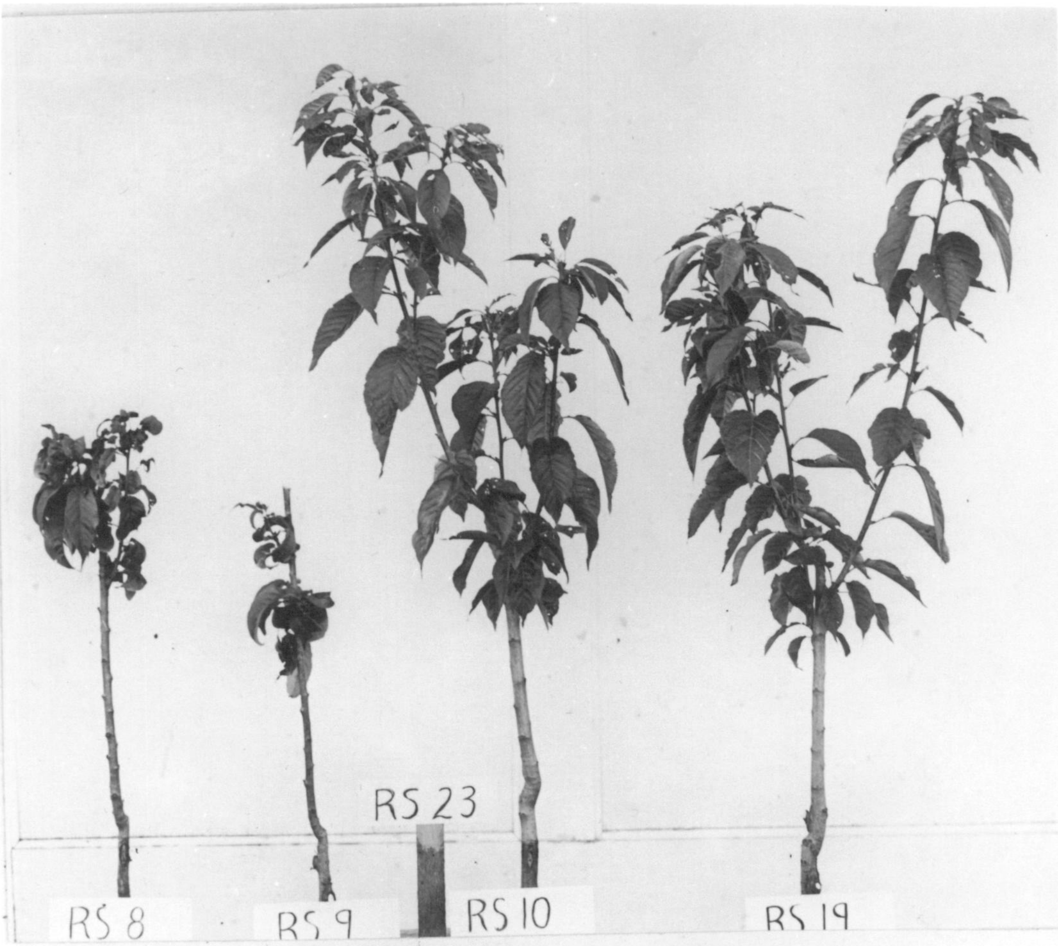

Figure 6. Mazzard seedlings topworked with Kwanzan systemically infected with ringspot 23 and simultaneously each seedling was inoculated with a challenging ringspot. The two trees on left illustrates no cross protection to RS-8 and RS-9, and the two trees on the right show complete protection to RS-10 and RS-19.

Table 8. The relative effectiveness of six ringspots established in highworked Kwanzan field trees in protecting against four challenging ringspots.

Protecting virus	Average disease index of four trees when challenged with			
	RS-7	RS-8	RS-9	RS-10
RS-5	2.0	2.0	0.0	0.0
RS-10	3.0	3.0	0.0	0.0
RS-11	2.0	3.0	0.0	0.0
RS-12	2.0	3.0	0.0	0.0
RS-15	2.0	2.0	0.0	0.0
RS-23	2.0	2.0	0.0	0.0

gave severe ringspot reactions on most of the index hosts.

The results of this preliminary experiment indicated that the technique employed can be used as a tool to study interrelationships of stone fruit ringspot viruses.

Greenhouse Experiment with Kwanzan Trees

The preceding study was exploratory in nature, so the present experiment was designed to test the technique adequately and provide a suitable number of check inoculations for comparison purposes. Since time was a limiting factor the mazzard seedlings were topworked and inoculated during the summer, and were dug and transplanted to number ten cans the following winter. Forcing the trees under greenhouse conditions permitted obtaining results several months ahead of normal field procedures.

The Kwanzan buds were examined when the mazzard seedlings were dug, and many of the buds appeared to have died. These buds could have been killed by the action of the challenging viruses, or they might have died as a result of a premature cold period which occurred during the month of November. Kwanzan scionwood carrying the protecting viruses was available, so each protection series was topworked with dormant scions by the technique of whip-grafting as the test trees were brought into the greenhouse. The topworking procedure served to test an additional hypothesis that the dormant scions infected with the protecting virus might be induced to display the leaf epinasty symptom when topworked to ringspot infected mazzard seedlings. Usually nine or ten months must elapse following Kwanzan index inoculations before results can be obtained under ordinary field conditions. Symptoms have not been observed in Kwanzan trees the same summer they were inoculated with stone fruit ringspots.

Seven Kwanzan negative protecting viruses, RS-5, RS-11, RS-12, RS-15, RS-20, RS-23 and RS-28, and eleven Kwanzan positive challenging viruses, RS-4, RS-7, RS-8, RS-9, RS-10, RS-13, RS-17, RS-18, RS-19, RS-21, and RS-26 were chosen for interrelationship study in this experiment. Each protection group consisted of sixty trees; five trees were provided for each challenging inoculation, leaving five trees carrying only the protecting virus. Sixty seedlings were budded high with healthy Kwanzan buds to provide material for check inoculations with the challenging ringspots. Each seedling was budded high with three buds carrying the protecting virus, and simultaneously inoculated with two buds carrying the challenging virus. The inoculating buds were placed below the Kwanzan buds on the mazzard seedling trunk.

The disease classes for this experiment were the same as those used in the previous experiment (page 40).

The results of this experiment have been summarized in Table 9, and the figures represent an average of the five replications of each inoculation set.

The data in Table 9 show that no single ringspot will protect against all stone fruit ringspots. All established viruses except RS-12 gave complete protection against RS-10, and RS-19. RS-5, and RS-12 gave some protection against RS-4, but it was incomplete. RS-5, RS-11, RS-12, RS-20, and RS-23 gave some protection against RS-7. All established viruses gave some protection against RS-8, but RS-23 and RS-28 gave the best protection. Only RS-5 gave notable protection against RS-9. RS-12, and RS-15 gave the best protection against RS-13. All established ringspots gave fair protection against RS-17. RS-5 gave the only significant protection against RS-18 and RS-21. All established viruses

Table 9. The relative effectiveness of seven ringspot viruses established in high-worked Kwanzan trees in protecting against eleven challenging ringspots.

Protecting virus	Average disease index of five trees challenged with											
	RS-4	RS-7	RS-8	RS-9	RS-10	RS-13	RS-17	RS-18	RS-19	RS-21	RS-26	Check
RS-5	1.4	0.8	2.8	1.3	0.0	2.3	1.0	1.2	0.0	1.8	1.0	0.0
RS-11	2.3	1.8	3.9	2.7	0.0	2.5	1.8	2.2	0.0	3.0	1.9	0.0
RS-12	1.1	1.0	3.7	2.0	3.7	1.1	1.7	2.0	1.5	2.2	1.2	0.4
RS-15	3.0	3.0	2.3	3.2	0.0	1.8	1.6	2.7	0.0	2.3	2.3	0.0
RS-20	2.7	1.6	2.0	2.0	0.0	2.7	1.8	2.7	0.0	2.7	1.2	0.0
RS-23	3.0	1.7	1.7	2.6	0.0	2.3	1.1	2.2	0.0	3.0	1.5	0.0
RS-28	3.0	2.3	1.4	2.5	0.0	2.9	2.2	2.8	0.0	3.0	1.8	0.0
Check*	2.7	2.8	4.0	3.0	4.0	2.8	3.0	2.5	3.0	3.4	2.3	0.0

* Healthy Kwanzan

except RS-15 gave some protection against RS-26.

All protecting viruses were originally negative when indexed on Kwanzan. Two Kwanzan inoculated with RS-12, however, gave a slight leaf epinasty symptom which could have resulted from contaminated mazzard seedlings.

This experiment re-emphasizes the complex mixture of viruses comprising the stone fruit ringspots. Several virus entities are involved, but this study could not attempt to show how many are involved.

The Kwanzan scions whip-grafted to the top of the test trees displayed the leaf epinasty symptom. The technique should be tested further to determine if mazzard seedlings could be whip-grafted with healthy Kwanzan scions, and simultaneously bud-inoculated with ringspots and still induce the leaf epinasty symptom. If the above procedure were successful, the indexing of stone fruit trees for latent ringspot viruses could be done in a much shorter time, and with considerable less expense.

Greenhouse Experiment with Peach Trees

Prunus host range studies have shown that certain cherry ringspot viruses induce no reaction on Muir peach, while others cause a very severe reaction. This experiment was designed to determine if mild non-reacting ringspots would protect against severe-reacting ringspots when

Muir peach was used as the host plant. The mild ringspots selected were RS-7, RS-12, RS-14, and RS-15. The severe ringspots chosen were RS-8, RS-9, RS-10, RS-13, and RS-20. All the challenging ringspots had previously given a very severe reaction on Muir, and RS-8, RS-10, and RS-13 carry the peach stunt factor. RS-7, RS-8, RS-10, and RS-20 are the ringspots in this group that carry sour cherry yellows. RS-13 also has the factor that causes prune dwarf in Italian plum. None of the mild protecting viruses carry peach stunt or prune dwarf. The relationship that exists between these disease components is not known, and they have never been found free of ring spot virus. This experiment was conducted to explore the possibilities of using cross protection to determine if any relationships exist between peach stunt, sour cherry yellows, prune dwarf, and cherry ringspots.

Scionwood for the propagation of test trees was obtained from Muir peach trees systemically infected with the protecting viruses. Four groups of twenty-five peach seedlings were each T-budded with buds from one of the four mild ringspot source trees. An additional group of twenty-five seedlings were budded with virus-free Muir. The seedlings were dug in the fall and transplanted to number ten cans. The following summer each protection series and the healthy controls were inoculated with the challenging ring spots.

The trees remained outside until the middle of the second winter when they were brought into the greenhouse.

The data from this experiment was inconclusive, and will not be included here. The control inoculations did not induce the symptoms which were displayed by infected Muir peach trees under field conditions, and no satisfactory comparisons could be made. Cochran (7, p. 512) has stated that the severity of symptoms caused by the ring spot virus in peach trees apparently depends on certain environmental factors such as temperature, vigor of host plant, time of year of infection, and others. The failure of the control trees to respond properly could have been due directly to the greenhouse environment.

DISCUSSION

These cross protection studies have produced results that show various trends, and for the most part no consistent protection was achieved with any mild ringspot. The interaction effects produced in these studies, in general, fell into four groups (1) complete protection by the established virus against the challenging virus; (2) incomplete protection, but a milder type of reaction than control inoculations; (3) no protection, with symptoms of test and control inoculations of the same severity; and (4) a synergistic reaction resulting in symptoms more severe than those produced by either the established or challenging ringspots. The incomplete protection could be due either to distantly related ring spot viruses, or to virus mixtures of related and unrelated viruses. The virus combinations that result in more severe reactions are probably due to unrelated viruses.

Two types of reaction result from indexing ringspots on Kwanzan. One type is the leaf epinasty reaction, and the other is that induced by ringspot inoculations of RS-10 and RS-19. The latter reaction is a severe dieback of the Kwanzan tree which may or may not result in death of the tree. If death of the tree does not result, the new growth will be stunted and the leaves somewhat off color, but there will be no leaf epinasty. The severe dieback was considered the reaction of a severe ringspot. The leaf epinasty symptom and the dieback could be due to different virus components, and they are either distantly related or are unrelated viruses. If the two reactions are the result of unrelated viruses the symptom caused by the ring spot virus is not known.

Two types of reaction result from indexing ringspots on Shiro-fugen trees, one systemic and the other necrotic. All established viruses except RS-12 gave complete protection against RS-10, and RS-19 in Kwanzan plants. RS-12 is one of the ringspots that indexed Shiro-fugen systemic, and this virus gave no protection against any challenging virus in either Bing trees or Montmorency trees. RS-12 gave some protection against RS-4 in Kwanzan host trees, and the two ringspots appear to be related. Most challenging ringspots used in Kwanzan experiments indexed Shiro-fugen necrotic and Kwanzan positive. The type of interaction that occurred where RS-4 and RS-12 did not protect against Shiro-fugen necrotic type ringspots indicates that the systemic and necrotic components are probably unrelated viruses. Interactions, as a general rule, between ringspots in Kwanzan experiments resulted in complete protection, no protection, or symptoms milder than those usually induced by the challenging ringspot in control plants.

No one <u>Prunus</u> host can be designated as the best plant for cross protection tests in the study of ring spot virus interrelationships. Many factors are involved in the interactions of ringspot combinations, and results will vary in many cases according to host plant and environment. For example, under field conditions RS-15 gave protection against RS-9 in Bing and Montmorency trees, but not in Kwanzan trees. Also, RS-5 protected against RS-8 in Kwanzan trees, and RS-15 protected against RS-9 in Montmorency trees under field conditions but not in the greenhouse.

The cross protection experiments did not reveal any relationships between the virus diseases, ring spot, sour cherry yellows, peach stunt, and prune dwarf.

The results of these experiments, in general, indicate that the ringspot complex is composed of several entities some of which appear to be related, while others do not. Results are difficult to interpret since it is not known what components are present in any two competing ringspots, or which are interacting.

SUMMARY

Cross protection studies were conducted to determine interrelationships of stone fruit ring spot viruses from several sources. The host plants used were Bing sweet cherry, Montmorency sour cherry, Kwanzan flowering cherry, and Muir peach.

All established viruses except RS-12 gave complete protection against the challenging viruses RS-10 and RS-19. The combination of any two ringspots in a host usually resulted in incomplete protection and a milder reaction than that produced by the challenging ringspot in control plants. A syngeristic reaction resulted in certain ringspot combinations and the symptoms produced were more severe than control inoculations. The interaction resulting from the ringspot combination of RS-4 plus RS-7 is an example of this type of reaction.

Bing trees conditioned with two ringspots gave complete protection on reinoculation with a third ringspot. These results suggested that conditioning against ringspot shock reactions in Bing trees requires a number of viruses or virus strains.

Montmorency sour cherry trees conditioned with two ringspots were induced to display shock reaction when infected with a third ringspot.

Symptoms in Bing trees grown under greenhouse

conditions were modified, and severe dieback resulted which was not a typical virus reaction.

The time interval in indexing stone fruit varieties on Kwanzan was shortened by simultaneously bud-propagating with Kwanzan, and ringspot bud-inoculating mazzard seedlings.

The cross protection experiments indicated that the two types of Shiro-fugen index reactions, the systemic and the necrotic, were caused by unrelated viruses. Two types of index reaction occurred on Kwanzan, one the leaf epinasty symptom and the second a severe dieback without leaf epinasty. The dieback symptom could be caused by a very severe strain of the same virus or an unrelated virus.

Certain ringspot combinations may result in protection in one host, but not in another, and under field conditions but not in the greenhouse.

No single _Prunus_ host was found to be better suited for cross protection studies with stone fruit ring spot viruses. Host reaction to ring spot virus inoculations vary between _Prunus_ hosts and time and method of inoculation.

The interaction effects produced in these studies, in general, fell into four groups (1) complete protection by the established virus against the challenging virus; (2) incomplete protection, but a milder type reaction than control inoculations; (3) no protection, with test and control inoculations of the same severity; and (4) a synergistic reaction resulting in symptoms more severe than those produced by either the established or the challenging ringspots. These interaction effects may be explained if the assumption is made that variation in degree of protection obtained is due to variation in degree of relationship between the established and challenging ringspot viruses. Closely related viruses were assumed to have given complete protection, and incomplete protection was due to viruses with a distant relationship. No protection and the synergistic reaction was assumed to be due to unrelated viruses.

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