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Stone Fruit Virus Investigations

I. Inoculation Studies of the Ring Spot Virus Complex in Sweet Cherry

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D. F. MILLIKAN, Jr.*

INTRODUCTION

Virus diseases in stone fruits have caused great concern to nurseymen and fruit growers for several years. Two of these diseases, sour cherry yellows and ring spot, appear widespread, particularly in the cherry stocks of the Middle West.

Sour cherry yellows is a virus disease causing reductions of sour cherry yields in all of the major cherry growing areas. Since the source of the virus in the orchards has been traced to nursery stock, many states have initiated indexing procedures to eradicate the disease at this source. However, positive identification of yellows is complicated because of the critical temperature range (below 70° F.) required for optimum manifestation of symptoms. Other than Montmorency, no suitable host for detecting yellows has been found and the critical temperature conditions and long incubations necessary for the expression of optimum symptoms limit the usefulness of this host for any routine indexing.

The ring spot virus or virus complex infects most *Prunus* species. Unlike sour cherry yellows, the ring spot virus is apparently of minor importance because reduction in yields results only in the year of initial infection, Ring spot, however, causes a reduction in the vigor of cherry and other stone fruits and this has been associated with increased susceptibility to winter injury. Unlike sour cherry yellows, the ring spot virus symptoms are found under a wide range of temperatures, although the severity of symptoms is influenced by temperatures. Chlorotic rings or oak leaf patterns appear on the leaves of the infected plant at temperatures lower than 70° and at the higher temperatures such characteristic patterns tend to become necrotic and drop out. These leaf symptoms are usually limited to the initial phase of the disease. Since the disease is detected easily by the use of flowering cherry and Montmorency indexing hosts and appears as a contaminant in yellows as well as other stone fruit viruses, eradication of ring spot has been strongly urged and is practiced in most certification programs.

The early stages of this investigation involved hosts assumed to be specific indicators for sour cherry yellows. Both peach and Montmorency were thoroughly tested as indicator plants but due to the prevailing environmen-

*Part of a Ph. D. Thesis submitted to the Graduate School while the author was a member of the Botany Department.

tal conditions and long incubation period, neither host proved satisfactory. Consequently, it was thought that ring spot, symptoms of which are unaffected by temperatures and are expressed two to three weeks following bud break inoculations, would merit investigation. Detection of this disease would also detect yellows, for ring spot has appeared consistently as a contaminant or a component of every yellow strain found to date. Since little has appeared in the literature concerning this virus on sweet cherry, these investigations were set up to learn more concerning the symptomology and virulence of various isolates on a few representative varieties of sweet cherry.

REVIEW OF LITERATURE

The ring spot virus was first described on peach by Cochran and Hutchins (19) and more recently by Cochran, Hutchins, Milbrath, Stout and Zeller (5). However, it may have been recorded as early as 1932 by Valleau (17). In work with yellows and an unidentified plum disorder, Valleau observed symptoms on inoculated peach seedlings strikingly similar to those inoculated with ring spot. Thomas and Rawlings (16), working with the buckskin virus, described a disease that resembled ring spot and referred to it as mosaic of mazzard cherry.

Since the original reports, the same or similar disease has been observed on several hosts by many workers. Cation (2) in 1942 transmitted a disease resembling ring spot in many respects from sweet cherry to peach. However, the development of symptoms on the midseason leaves of trees affected with the disease indicated a difference from ring spot. Symptoms of ring spot in the infected trees ordinarily are restricted to leaves formed in the spring. Lace leaf of sweet cherry reported by Zeller (20) and Reeves (15), along with Tatter leaf described by Willison and Berkeley (18) and Willison, Berkeley and Hildebrand (19), are probably closely related viruses, if not the same virus that causes ring spot on sweet cherry as suggested by Hildebrand (7). In addition, it seems likely that the "latents" referred to by Milbrath and Zeller (10, 11) are less virulent strains of the ring spot virus and later were so considered by Milbrath (9). Necrotic ring spot, described independently by Hildebrand, Keitt, Moore and Miller (8) and Chamberlain, Willison and Berkeley (3) is probably the expression of ring spot on sour cherry. The exact relationship of these viruses has not been fully established but Parker and Cochran (14) were able to show similarities between these entities on host range composed of mazzard seedlings, sour cherry and peach.

MATERIALS AND METHODS

All of the trees used in the greenhouse studies were potted in 6 or 8 inch pots with ordinary compost soil. The trees inoculated during the 1950 season were transferred to the field for observation the next season. Trees from subsequent inoculation studies were merely repotted into larger pots

and set outside during the summer, as the outdoor sites available for growing cherry were unsatisfactory. Inoculations were made using the chip-bud method described by Moore (13). In this method a shield of bark which may or may not include a bud is removed from the stock, and a shield of bark of similar size and shape and always including a bud is removed from the scion wood and placed on the stock. The inserted bud is wrapped tightly with Sealtex, a latex type of material, but the bud itself is not covered. Bud take and, therefore, transmission, can be determined with ease by inspection of the bud.

The sources of trees used in the study were generally local nurseries affiliated with the certification program. Two clones, Napoleon A-114 and Lambert L-89, were purchased from commercial sources in Oregon. Sources of inocula and the virus content are listed in Table 1.

TABLE 1 -- INOCULA USED AND THEIR SYMPTOMS ON SWEET CHERRY WHEN GROWN IN THE GREENHOUSE AND FIELD, 1950-52

Virus Strain*	Virus Content**	Origin†
Goldman 20-5	Y & NRS	Wisconsin
Goldman 17-4	Y & NRS	Wisconsin
S-5009	Y & NRS	Wisconsin
S-16	Y & NRS	Missouri
S-80	Y & NRS	Missouri
B-557*	Y & NRS	Michigan
B-558*	Y & NRS	Michigan
S-1319	mild NRS	Wisconsin
Tatter leaf	RS	Ontario, Canada
E-253*	NRS	Michigan
C-297*	NRS	Michigan
Barnard 1-12	NRS	Wisconsin
MO-1	NRS	Missouri
S-8	NRS	Missouri
S-20	NRS	Missouri
V-2	L	Washington
L 6-9	NRS	Pennsylvania
L 3-9	NRS	Pennsylvania
S 2-5	NRS	Pennsylvania
C-34114	RS	California
Covert 1-7	RS	California
Prune dwarf	PD & NRS	Ontario, Canada
6.78w	L	Oregon
5.81k	L	Oregon
3.77z	L	Oregon
4.97aj	L	Oregon

* Virus strains B-557 and B-558 and strains E-253 and C-297 are considered identical by the original worker.

** Y - yellows, NRS - necrotic ring spot, RS - ring spot, L - latent and PD - prune dwarf

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INOCULATION STUDIES

Greenhouse, 1950

Ten strains of virus varying in virulence were used in the study. Five varieties of sweet cherry, selected because of availability and popularity, represented the variety host range. Results of the greenhouse inoculations on sweet cherry are listed in Table 2.

The symptoms of ring spot infection on Bing and Napoleon in the greenhouse were similar. Leaf symptoms of ring spot when present were usually limited to a basal leaf on the shoot of new growth and rarely became necrotic to form the shot hole phase. Some differences were noted in the exudate formed on the main stem or shoots. This exudate was formed heavily on Bing inoculated with S-16, S-20, S5009 and Barnard 1-12. Only with Mo-1 was there any indication of gumming in Napoleon. Neither variety showed symptoms when inoculated with prune dwarf or S-1319.

Black Tartarian seemed to react to virus inoculation similarly to Bing and Napoleon, except that the symptoms appeared to be less severe. Only inoculations with S-16 resulted in complete killing of tissues to the understock. Severe necrosis with killing of tissue exceeding 50 percent was observed in inoculations of S-80, S-5009 and Barnard 1-12. All of these were very severe on Bing and Napoleon. Tatter leaf infection caused a mild necrosis while both prune dwarf and Mo-1 were symptomless. Mild leaf patterns were expressed with inoculations of S-20 and Goldman 20-5 and a minor gumming of shoot tissue was the only indication of infection with the S-1319 isolate.

The necrosis characteristic on Bing and Napoleon was generally lacking in the inoculations of Gold and Hardy Giant. Only with virus strain Mo-1 was there any evidence of necrosis and this usually confined to a killing of the initial spurs. Chlorotic patterns, becoming necrotic, seemed to be a characteristic indication of virus infection on these varieties. This type of symptom was found on all of the test trees inoculated with all of the virus strains used except S-1319 and prune dwarf, both of which were symptomless on these two varieties.

Greenhouse and Field, 1951

In 1951, it was impossible to secure five strains of the virus used previously. Four additional but similar strains were substituted in the study. The Lambert variety replaced Black Tartarian since the source of the latter proved to be contaminated with ring spot. Following the greenhouse season, the trees were placed in larger pots and placed outside during the summer season. Observations were made during the subsequent two years in the greenhouse.

Field inoculations were made during the 1951 season. One-year-old nursery trees of Lambert and Hardy Giant trees, similar to those used in the

TABLE 2 -- REACTION OF FIVE VARIETIES OF SWEET CHERRY WHEN INOCULATED IN FEBRUARY, 1950, WITH TEN SOURCES OF THE RING SPOT COMPLEX

Variety	Inoculum	Symptoms*			
		NRS	CRS	Gum	Nec
Bing	Mo-1			xxx	VS
	S-80			xxx	VS
	S-16			xx	VS
	S-20			xx	none
	S-5009			xx	S
	Barnard 1-12			xx	VS
	Goldman 2-1				M
	S-1319				none
	Tatter leaf				none
	Unbudded				none
	Prune dwarf				S
Napoleon	Mo-1			xx	VM
	S-80				S
	S-16				VS
	S-20				none
	S-5009				VS
	Barnard 1-12				VS
	Goldman 2-15				none
	Prune dwarf				none
	Tatter leaf				VS
	S-1319				none
	Unbudded				none
Black Tartarian	Mo-1				none
	S-80				S
	S-16				VS
	S-20	x			none
	S-5009				VS
	Barnard 1-12				VS
	S-1319		x		none
	Goldman 20-5	xx			none
	Prune dwarf				none
	Tatter leaf	x			M
	Unbudded				none
Gold	Mo-1	xx	x		VM
	S-80				
	S-16	xx			
	S-20	x			
	S-5009	x			
	Barnard 1-12	xx			
	S-1319				
	Goldman 20-5	xx			
	Prune dwarf	x			
	Tatter leaf				
Hardy Giant	Mo-1	xx	x		VM
	S-80				
	S-16	xx			
	S-20	x			
	S-5009	x			
	Barnard 1-12	xx			
	S-1319				
	Goldman 20-5	xx			
	Tatter leaf	x			
	Prune dwarf				
	Unbudded				

*x indicates severity of symptoms
VM - very mild, necrosis 0-10%
M - mild, necrosis less than 25%
MS - moderately severe, necrosis less than 50%
S - severe, necrosis less than 90%
VS - very severe, necrosis 90-100%
CRS - chlorotic ring spot
Gum - gummosis
Nec - necrosis

greenhouse, were planted in rows and inoculated periodically with strains of ring spot. Observations were limited to the 1951 season since the trees were killed by a severe freeze during the late fall.

Results of Greenhouse Inoculations

In general, the symptoms on sweet cherry hosts with the strains of virus used previously were similar. Those symptoms observed on Lambert appeared to be no different from those observed on Bing and Napoleon with any one strain of virus. The inoculations made in 1951 and the data recorded in 1951, 1952, and 1953 are listed in Table 3.

The observations during the 1951 season on Bing inoculated with Goldman 20-5, S-16, S-5009 and Barnard 1-12 were similar to those observed in 1950. Severe necrosis resulting in complete killing of all the scion tissue above the union occurred with inoculations of S-5009, S-16, B-557 and Barnard 1-12. Similar but somewhat less severe symptoms resulted from the inoculations with B-534 since the necrosis extended to the two or three basal spurs just above the understock union. The inoculations of Goldman 2-1, Goldman 20-5, and V-2 produced mild to very mild symptoms on Bing and symptoms were limited to single shoots on the V-2 infected trees. Only the unbudded controls and the trees inoculated with Goldman 2-1 and V-2 survived the winter. No symptoms were noted in 1952 or 1953 but the shoot growth of the uninoculated trees appeared to be more vigorous than that of the other surviving material.

The reactions of Lambert to virus infection appeared similar to, but milder than, those observed on Bing. Severe necrosis, approximately 90 percent of the scion tissue, occurred on trees inoculated with Goldman 2-1, Barnard 1-12, B-534 and B-557 while necrosis involving less than 50 percent of the varietal tissue was caused by inoculations of S-16 and E-253. Milder reactions appeared on the trees inoculated with S-5009 and Goldman 20-5 while inoculations of V-2 produced no symptoms on Lambert. These symptoms were limited to necrosis of the tip source and considerable gumming. All Lambert trees survived the winter and they did not show symptoms of ring spot during the subsequent seasons under observation.

Napoleon inoculated with S-16, Barnard 1-12, Goldman 20-5, and S-5009 showed the same type of symptoms that had been observed previously. There was very severe necrosis with the inoculations of S-16 and B-557 resulting in a killing of all spur and shoots completely to the understock. The basal spurs which recovered remained permanently dwarfed for the length of the growing season. Severe necrosis of less than 50 percent killing of varietal tissue was produced by the E-253, S-5009 and Barnard 1-12 strains. No symptoms appeared on the unbudded controls or the V-2 inoculations, while mild necrosis, or about 10 percent killing, was found on the Goldman

TABLE 3 -- REACTION OF FIVE VARIETIES OF SWEET CHERRY WHEN INOCULATED WITH NINE DIFFERENT STRAINS OF THE RING SPOT VIRUS IN THE WINTER, 1951

		Symptoms						
		1951				1952		1953
Variety	Inoculum	Nec	Gum	CRS	NRS	Vigor	NRS	NRS
Bing	Goldman 20-5	O				dead		
	S-5009	VS				dead		
	S-16	VS				dead		
	B-557	VS				dead		
	E-253	VS				dead		
	V-2	O-VM				VW		
	Goldman 2-1	M	xx			VW		
	Barnard 1-12	VS				dead		
	B-534	S				dead		
	Unbudded					MV		
Lambert	Goldman 20-5	VM	xx			MV		1
	S-5009	M				MV		
	S-16	MS				MV	2	
	Goldman 2-1	S				MV-V		
	E-253	MS				V	1	
	V-2	O				V-VV	1	
	B-534	S				V	1	
	B-557	S				V		
	Barnard 1-12	S				V		
	Unbudded					VV		
Napoleon	Goldman 20-5	M				MV		
	S-5009	S				MV		
	S-16	VS				W		
	B-557	M				V		
	E-253	S				V		
	B-534	S				V		
	V-2	O				MV-V		
	Goldman 2-1	M				W-MW		
	Barnard 1-12	VS				dead		
	Unbudded					V		
Hardy Giant	Goldman 20-5			x		V		1
	S-5009					V		
	S-16			x		V		
	B-557			x		V		
	E-253				xx	V		
	B-534			x		V	3	3
	V-2					VV		
	Goldman 2-1			x		V	2	2
	Barnard 1-12			x		V.V		
	Unbudded							
Gold	Goldman 20-5					VV	1	2
	S-16			xx		V	1	
	B-557	VM		xx		V		
	E-253	VM		x	x	V	1	
	V-2					V		
	Goldman 2-1	VM		x		VV	1	
	Barnard 1-12	M				MV	2	1
	B-534	VM		x	x	V	1	1
	S-5009			xx		V	1	1
	Unbudded					VV		

* Indicates the severity of symptoms, the numbers indicate the trees showing second or third season symptoms (inoculations were made in triplicate). Symbols under necrosis are the same as were used in Table 2. Under vigor the symbols are relative and represent: VW - very weak, growth of shoots spindly and short; W - weak, shoot growth about 8-10 inches; MV - moderately vigorous, growth about 10-18 inches; V - vigorous, growth about 18-24 inches; VV - very vigorous, shoot growth over 24 inches.

Reaction of four varieties of sweet cherry to the Barnard 1-12 strain of the ring spot virus complex. From left to right, mild reaction in Hardy Giant and Gold and severe reaction in Lambert and Napoleon.

20-5, Goldman 2-1 and B-534 inoculated Napoleon trees. Only the trees inoculated with B-557 failed to survive the winter.

The individual inoculations on Hardy Giant of Goldman 20-5, S-16, and Barnard 1-12 were of the same order of severity as noted in 1950 but there were no symptoms on the test trees inoculated with S-5009. While these symptoms were quite similar they did tend to be less virulent, with the ring spots remaining chlorotic rather than becoming necrotic and falling out. V-2 was symptomless, while the Michigan strains, E-253, B-557 and B-534 were positive and exhibited striking patterns on the leaves of the inoculated host. Test trees inoculated with B-534 and Goldman 2-1 showed symptoms in both 1952 and 1953 and a single tree inoculated with Goldman

20-5 showed ring spot symptoms in 1953 but not in 1952. These symptoms always were localized on a few leaves of single spur.

The leaf patterns on the inoculated Gold trees likewise tended to remain chlorotic rather than becoming necrotic. Goldman 20-5 in 1951 was symptomless on Gold which contrasted with the mild ring spotting noted the previous year. Only in the inoculations with B-534 did the chlorotic patterns become necrotic, while similar chlorotic patterns which failed to become necrotic developed on the trees inoculated with B-557, E-253, S-16 and Goldman 2-1. With the exception of Goldman 2-1, these chlorotic patterns were accompanied with some spur and tip necrosis. With the Barnard 1-12 inoculations, this necrosis was so severe as to kill one of the trees, and the leaf symptoms, absent in 1951, were conspicuous in 1952. Ring spot symptoms were expressed on at least one tree inoculated with each strain except B-557 and V-2 in 1952. In 1953 symptoms were observed on two trees that had been inoculated with Goldman 20-5 and single trees infected with Barnard 1-12, B-534 and S-5009.

Results of Field Inoculations

Ample numbers of certified sweet cherry varieties, Lambert and Hardy Giant, were available for field inoculations in 1951. In this study, interest was centered on whether the necrosis observed in the greenhouse would occur in the field and if any symptoms would occur if the inoculations were made at periods other than bud break. Results are listed in Table 4.

TABLE 4 -- REACTION OF TWO VARIETIES OF SWEET CHERRY WHEN INOCULATED IN THE FIELD WITH THREE SOURCES OF RING SPOT IN THE SPRING, 1951

Variety	Inoculum	Time of Inoculation after bud break*	Symptoms		
			Nec	Gum	NRS
Lambert	Barnard 1-12	0	S		
	E-253	0	MS		
	Goldman 2-1	0	M		x
	Barnard 1-12	3 weeks	M		
	C-297	6 weeks			
	Goldman 2-1	6 weeks			
	Non-budded				
Hardy Giant	C-297	0			xx
	Goldman 2-1	0			xx
	Barnard 1-12	3 weeks			
	C-297	6 weeks			
	Non-budded				

* Bud-break (April 19, 1951)

The results of the field observations made in 1951 indicated that the results obtained under greenhouse conditions would be duplicated in the field. But the evidence suggested that these symptoms would be expressed

Reaction of Napoleon sweet cherry to Goldman 20-5 (left) and to S-16 (right) strains of the ring spot virus complex.

only if the inoculations were made at bud break. Due to winter injury resulting in death to the experimental trees, observations could not be made during the subsequent seasons.

In the Lambert variety inoculated with the ring spot strains used in the study, there were symptoms very similar to those observed in the greenhouse. Although the bud break inoculations of E-253 and Barnard 1-12 did not kill the trees completely to the understock, necrosis varying from slightly less than 50 percent up to 90 percent, was observed in the field as in the greenhouse. Evidence of ring spot infection in the Goldman 2-1 inoculations was revealed by mild necrosis accompanied by ring spotting. Inoculations on Lambert with Barnard 1-12 on May 4, about four weeks after bud break, caused a mild necrosis of a few shoots. All inoculations thereafter failed to produce symptoms.

Hardy Giant inoculated in the field did not show symptoms of necrosis. Inoculations with C-297 and Goldman 2-1 made at bud break resulted in severe ring spots on the leaves of this variety, but all inoculations made on later dates were symptomless. Whether the trees that were inoculated

but symptomless in 1951 would have expressed any indication of ring spot infection in 1952 could not be determined since the test trees winter killed.

Greenhouse, 1952

In 1952, it was thought that virus strains from other regions would merit investigation. Work completed in 1951 indicated that some strains presumed to be ring spot were symptomless on sweet cherry. Furthermore, most of the research in the various states has been limited to strains of virus indigenous to those areas despite the fact that movement of nursery stock has likely permitted a wide dissemination of the strains. Accordingly, cultures of the ring spot virus were obtained from several areas and were inoculated into sweet cherry. The virus strains added to the study in 1952 were tested on every variety except Bing, which was not available in adequate quantities. Due to limitations of greenhouse space, some of the strains used previously were not inoculated into all of the hosts. Results of inoculations on sweet cherry are listed in Table 5.

The greenhouse work in 1952, with the virus strains used previously, gave about the same results. The moderately severe necrosis on Lambert inoculated with Goldman 2-1 was somewhat more extensive than noted in the earlier inoculations but still considerably less than that found with the more severe strains. None of the Oregon latents expressed any symptoms following inoculation, nor did C-34114, L 3-9, or Goldman 20-5. A severe necrosis of varietal tissue similar to that observed in Barnard 1-12 infected trees was noted on the trees inoculated with S-2-5 and Goldman 17-4. Heavy gummosis was observed only on the trees inoculated with Covert 1-7 and Barnard 1-12. In 1953, in all Lambert trees inoculated previously with Goldman 20-5, Goldman 17-4, or B-558 there was a very mild necrosis. This mild necrosis was accompanied with some necrotic ring spotting on the trees inoculated with Goldman 20-5. Second season symptoms were absent on Lambert inoculated with the other virus strains.

Some mild necrosis, consisting of killing of a few scattered tip shoots, was observed again in 1952 on the Gold variety. The necrosis noted on the test trees inoculated with E-253, Barnard 1-12, and Goldman 2-1 appeared earlier than previously but the Goldman 2-1 infection was noticeably milder than the other two. Mild necrosis similar to that caused by Goldman 2-1 appeared with the inoculations of S-8 and Covert 1-7 while a very mild necrosis, generally limited to a few individual spurs, was found on Gold inoculated with S-2-5, The only chlorotic patterns on this variety were found on the test trees inoculated with L 3-9 and Goldman 2-1, while necrotic ring spot symptoms were found when inoculated with Covert 1-7, L 6-9, S 2-5, Barnard 1-12, and S-5009. The Oregon latents (6.78w, 5.81k, 3.77z and 4.79aj) and the weak peach ring spot, C-34114, were symptomless. In 1953, trees inoculated with Barnard 1-12, S-8, and S-5009 showed second season symptoms of chlorotic ring spot.

TABLE 5 -- REACTION OF FIVE VARIETIES OF SWEET CHERRY WHEN INOCULATED WITH 14 REGIONAL STRAINS OF RING SPOT IN THE SPRING, 1951

Variety	Inoculum	Symptoms						
		1952				1953		
		Nec	CRS	NRS	Gum	Nec	CRS	NRS
Napoleon	6.79w							
	5.81k							
	4.79aj							
	3.77z			xx				
	C-34114			x				
	Covert 1-7			xx				
	S 2-5		x	xx				
	L 6-9			x				
	L 3-9		x					
	V-2							
	B-558	VM		xx				
	E-534	M		xx				
	Goldman 17-4	VM		x				
	Goldman 20-5			x				
	S-16			xx				
	S-8			x				
	Unbudded							
	C-34114							
	L 3-9		x					
	L 6-9			x				
	S 2-5	VM		x				
	E-253	VM						
	Barnard 1-12	M		x				
	Goldman 2-1	M	x					
	S-8				x			x
	S-5009			xx				x
	Unbudded							
Hardy Giant	6.78w							
	5.81k							
	3.77z			xx				
	4.79aj							
	Covert 1-7			xx				
	C-34114							
	V-2							
	S-8			x				
	Goldman 2-1			x				xx
	E-253			x				x
	Goldman 17-4			xx				x
	S-16			x				
	B-534			xx				
	Goldman 20-5			x	x			
	Unbudded							
Bing	6.78w							
	5.81k	S						
	4.79aj							
	3.77z	S						
	Covert 1-7	S						
	C-34114							
	Barnard 1-12	VS						
	V-2							
	Unbudded							

X indicates severity of symptoms, symbols under necrosis are the same as those used in Table 2.

Variety	Inoculum	Symptoms 1952 Nec	CRS	NRS	Gum	1953 Nec	CRS	NRS
Napoleon	6.79w							
	5.81k							
	4.79aj							
	3.77z			xx				
	C-34114			x				
	Covert 1-7			xx				
	S 2-5		x	xx				
	L 6-9			x				
	L 3-9		x					
	V-2							
	B-558	VM		xx				
	E-534	M		xx				
	Goldman 17-4	VM		x				
	Goldman 20-5			x				
	S-16			xx				
	S-8			x				
	Unbudded							

X indicates severity of symptoms, symbols under necrosis are the same as those used in Table 2.

The only difference noted between the inoculations made on Hardy Giant in 1952 and those made previously was the tendency of the chlorotic ring spot symptoms noted earlier to become necrotic. Positive ring spotting was found for all of the strains used in the study except 6.78w, 5.81k, 4.79aj, C-34114, and V-2 with the greatest ring spotting occurring with the 3.77z, Covert 1-7, Goldman 17-4 and B-534 strains. Test trees infected with Goldman 2-1, E-253, and Goldman 17-4 in the second season all showed symptoms of the ring spot, generally localized to a single spur and usually limited to the basal leaves of that spur.

Insufficient material of Bing was available to make a very extensive study of this variety. However, with the limited amount of work that was done, it appeared that 5.81 k was capable of causing a mild necrosis. In fact, the only symptoms observed on Bing were of the dieback type with severe to very severe necrosis occurring on all of the test trees except those inoculated with 6.78w, 4.79aj, C-34114, and V-2 which were symptomless. None of these trees were available for observation during the 1953 season.

Necrosis varying from moderately severe to very severe occurred on Napoleon inoculated with all the strains used in 1952 except the Oregon latents, C-34114, L 3-9, and V-2. Very severe necrosis, generally terminating in death to the tree, resulted with inoculations of Covert 1-7, Barnard 1-12, and S-8 while necrosis of over 30 percent was observed on the E-253 and Goldman 2-1 inoculations. Chlorotic ring spot patterns were found only on the trees inoculated with L 3-9 and S-5009 while symptoms of the necrotic type characterized the inoculation of 3.77z and L 6-9. Only in the inocula-

tions with E-253 and S-5009 were there any symptoms in 1953 and these consisted of very mild necrotic rings limited to two or three basal leaves of a single shoot.

Effect of Time of Inoculation Upon the Expression of Ring Spot Symptoms on Sweet Cherry, 1952-53

Results of early work suggested that the expression of symptoms might be limited to inoculations made only at bud break or shortly thereafter. This seemed of interest for, in the case of Montmorency, inoculations as late as nine weeks following bud break generally produce typical ring spot symptoms. Consequently, it was thought that a time-of-inoculation study in relation to the expression of symptoms would merit investigation.

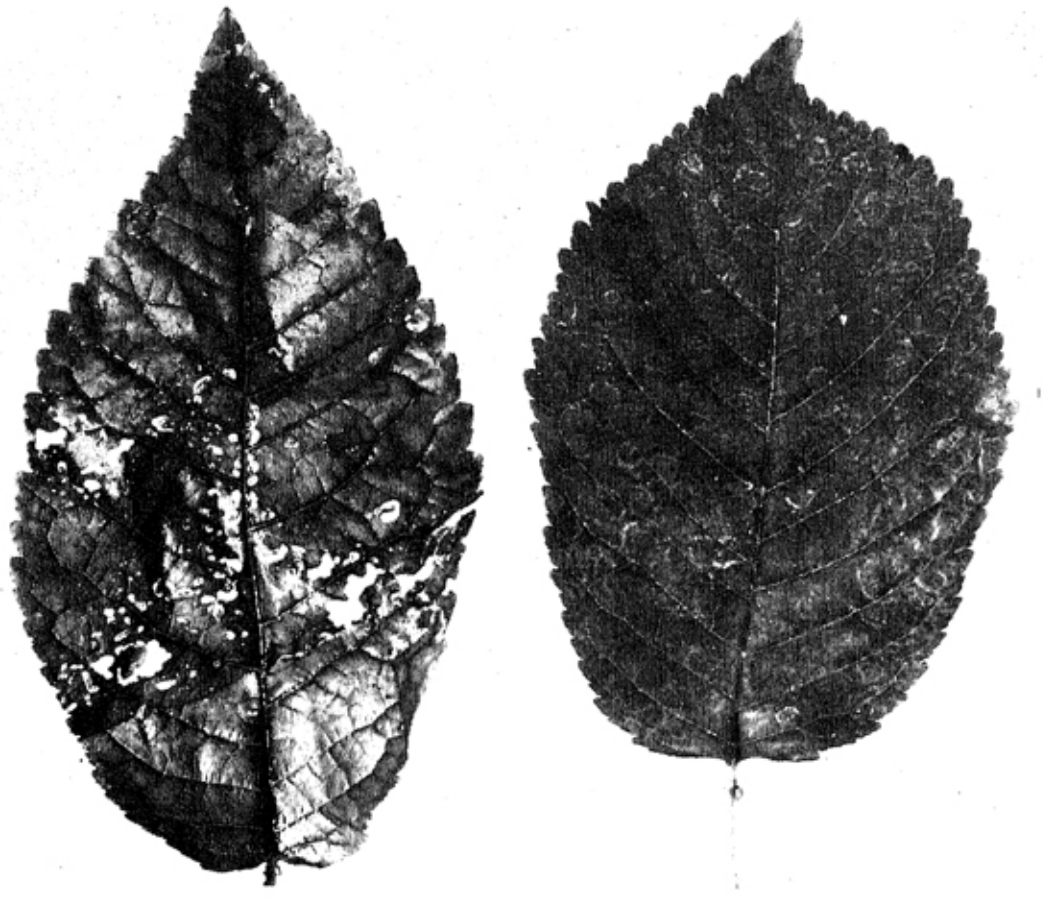
To simulate natural conditions as closely as possible, the trees were potted and grown outside for one season. Inoculations were made using a virulent and mild ring spot strain in early October just prior to the start of the dormancy period, at bud break (February 17), and periods of two to three weeks following bud break. These were made in the greenhouse and the results are listed in Table 6.

TABLE 6 -- REACTION OF TWO VARIETIES OF SWEET CHERRY WHEN INOCULATED IN EARLY DORMANT STAGE, AT BUD BREAK AND INTERVALS FOLLOWING BUD BREAK, 1952-53

Variety	Inoculum	Date of Inoculation	Symptoms* 1952 Date	1952 NRS	1952 Nec	1953 Date	1953 NRS	1953 Nec
Hardy Giant	Goldman 2-1	9- 2-51						
	Barnard 1-12	9- 2-51						
	Goldman 2-1	2- 7-52	3- 3	x		1-15	x	
	Barnard 1-12	2- 7-52	2-22	xx		1-14	x	
	Barnard 1-12	2-22-52				1-16	x	
	Barnard 1-12	3-21-52				1-14	xx	
	Unbudded							
Lambert	Goldman 2-1	9- 2-51						
	Barnard 1-12	9- 2-51	2-22	x				
	Goldman 2-1	2- 7-52	2-18	x	M			
	Barnard 1-12	2- 7-52	2-18	xx	MS			
	Goldman 2-1	2-22-52				1-30	x	VM
	Barnard 1-12	2-22-52				1-15	x	VM
	Barnard 1-12	3-12-52				1-20	x	VM
	Unbudded							

* Trees were brought into the greenhouse January 20, 1952 and January 2, 1953. Bud break inoculations were those made on February 7, 1952. Symptoms under necrosis are the same as those used in Table 2.

The results substantiate some of the observations noted in the field inoculations made during the spring of 1951. The necrosis characteristic of virulent ring spot infection on the Lambert-Napoleon group seemed to occur only with inoculation at bud break (February 7). Otherwise, the symptoms, if expressed, were the usual chlorotic patterns which may or may not become necrotic.



Necrotic and chlorotic symptoms on sweet cherry caused by two strains of the ring spot virus complex. Note the shot holing caused by the falling out of the necrotic areas.

On Hardy Giant, the only indication of infection in 1952 resulted from the inoculations with Barnard 1-12 and Goldman 2-1 made on February 7. The Goldman 2-1 infection was characterized by mild chlorotic patterns later becoming necrotic to give a lace leaf effect. These symptoms appeared about three weeks after inoculation. The Barnard 1-12 inoculations made February 7 showed symptoms of ring spot infection two weeks later. These symptoms were initially faint leaf patterns that gradually became necrotic. No symptoms of any sort appeared in 1952 or 1953 on Hardy Giant inoculated with either isolate October 1951. However, second season symptoms were observed in 1953 on the other inoculations.

No symptoms of ring spot infection appeared in 1952 on Lambert inoculated after February 7. With the inoculations made on October 2, 1951, only the trees inoculated with Barnard 1-12 showed any indication of infection in 1952 while those inoculated with Goldman 2-1 failed to express any symptoms in either 1952 or 1953. The inoculations with Goldman 2-1 and

Barnard 1-12 made at bud break on February 7 showed initial spur and shoot necrosis. This necrosis generally was limited to two or three terminal spurs with the Goldman 2-1 inoculations, but as much as 30 percent of the host tissue was killed with the Barnard 1-12 inoculations. Both isolates caused leaf patterns. These patterns were mild and localized in the Goldman 2-1 inoculations but were more extensive and necrotic on the test trees inoculated with Barnard 1-12. Second season symptoms were observed on all of the inoculated test trees except those inoculated at bud break and in October 1951.

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1953

The possibility of inoculating clones of cherry with a mild strain of ring spot to confer protection against the more virulent strains has often been considered. Some experimental work in this regard has been done within the necrotic ring spot group but relatively little work has been done with strains falling within the latent group. These strains are so mild that they produce no recognizable symptoms on cherry and should be desirable ones to use for such a purpose. Therefore, Napoleon and Lambert clones known to be infected with a flowering cherry positive latent were inoculated at bud break in triplicate with strains of necrotic ring spot. Comparable inoculations were made on certified Napoleon and Lambert to serve as controls. The results are listed in Table 7.

TABLE 7 -- REACTION OF TWO SWEET CHERRY CLONES CARRYING LATENT VIRUSES COMPARED WITH TWO CERTIFIED CLONES WHEN INOCULATED AT BUD BREAK WITH NECROTIC RING SPOT

Variety	Inoculum	Symptoms		
		Nec	CRS	NRS
Lambert L-89	Barnard 1-12			
	Goldman 2-1			
	S-16	VM*		
	Goldman 20-5			
	Unbudded			
Lambert L-1 (certified)	Barnard 1-12	S		
	Goldman 2-1	M		
	S-16	VS		
	Goldman 20-5	M		
	Unbudded			
Napoleon A-114	Barnard 1-12	VS		
	Goldman 2-1	M		
	S-16	VS		
	Goldman 20-5	M		
	Unbudded			
Napoleon A-23	Barnard 1-12	VS		
	Goldman 2-1	M		
	S-16	VS		
	Goldman 20-5	VS		
	Unbudded			

* One of the three trees inoculated showed a very mild browning on the tips of two terminal shoots.

The comparison between Lambert L-89 and the certified clone inoculated with the same virus showed a striking contrast. Only on a single tree inoculated with S-16 was there any indication of infection on Lambert L-89. This appeared as a mild necrosis on two of the tip most shoots about three weeks after inoculation. On the other hand, inoculations with the same strain inoculated on Lambert L-1 produced the symptoms observed previously of severe to very severe necrosis in the case of Barnard 1-12 and S-16 and a mild necrosis generally restricted to a few initial spurs in the trees inoculated with the milder strains, Goldman 20-5 and Goldman 2-1.

The inoculations of the Napoleon clones with the same isolates produced symptoms quite similar to each other and to those observed previously on this variety. Inoculations with Barnard 1-12 and S-16 were similar on both clones. First there was a browning of the terminal shoot tips and within two weeks the top one-half of the inoculated tree had been entirely killed. Six weeks following inoculation, necrosis extended to the understock, resulting in death to all of the varietal tissue in both clones. The necrosis noted from the Goldman 20-5 and Goldman 2-1 inoculations was much milder and showed less than 30 percent necrosis of shoot and spur growth. Initial symptoms were expressed about three weeks following inoculation. Usually, this necrosis proceeded downward about 6 inches from the tip, killing all of the side and terminal spurs in the affected area.

Results of Indexing L-89 and A-114 Clones on Montmorency

In the original work at the Oregon Station, the Napoleon A-114 clone was considered to be Shirofugen negative and Kwanzan mild while the L-89 clone was found to be Shirofugen positive and Kwanzan mild. This implied that both hosts were infected with a virus which was positive on Kwanzan. On the other hand, the early work at the Missouri Station indicated that both certified clones were negative on all three hosts. In order to determine if necrotic ring spot was involved in either or both of the western clones, buds from sticks removed L-89 and A-114 prior to inoculation were placed into Montmorency. Every tree of the Lambert L-89 clone was found to be infected with a ring spot while the A-114 clone, if infected, was carrying a very mild form of necrotic ring spot that is symptomless on Montmorency.

The ring spot infecting the Lambert L-89 clone is somewhat different from those encountered in routine indexing or generally used in our investigations. This particular virus strain is peculiar in that it does not appear on the first leaves to unfold but appears on the second and third leaves. The first symptoms appear as mottlings which gradually assume the shape of ring patterns resulting in a roughened appearance of the leaf. Usually, these rings remain as intensified chlorotic patterns but may become necrotic and drop out. Subsequent foliage remained symptomless, at least until they were destroyed at the end of the greenhouse season.

DISCUSSION

Sweet cherry varieties appear to fall into two groups regarding host response to ring spot infection. Varieties such as Bing, Napoleon, and Lambert when inoculated with ring spot show severe necrosis of host tissue that may extend completely to the understock. These symptoms differ considerably from the milder ones of leaf patterns observed on Gold and Hardy Giant. Thus, there appears to be a difference in the sensitivity of the host tissue between varieties belonging to the sweet cherry group. Possibly this sensitivity may be reflected in the susceptibility of cells in the host. For example, the cells of the more sensitive varieties may be readily invaded and once invaded, the virus particles are able to multiply at a rapid rate. Conversely, the cells of the less sensitive varieties would then be less readily invaded and the multiplication of the virus particles would proceed at a less rapid rate. In this event, an increase of temperature increases the sensitivity of the host tissue to virus infection and multiplication. Therefore, the increased necrosis observed under higher temperatures would not be inconsistent with this theory.

Bennett (1) cites a precursor exhaustion theory to explain susceptibility of hosts to virus invasion. In this theory, the sensitive varieties under normal temperatures would have a greater supply of virus precursor metabolites than would the less sensitive varieties. Increased temperatures could then increase enzymatic action which would in turn supply more precursor. In any event, the observed necrosis is thought to associate quantitatively with the virus. Unfortunately, we do not have a suitable assay method to follow ring spot multiplication and therefore cannot confirm this theory.

The production of severe symptoms apparently is limited to inoculations made at bud break or shortly thereafter. Under normal greenhouse conditions, no symptoms appeared when the inoculations were made later than two weeks after bud break, and mild or no symptoms occurred with the inoculations made prior to the beginning of the dormancy period of the previous autumn. However, mild symptoms of ring spot appeared in 1953 on most of the trees inoculated after bud break in 1952. Field inoculations made after June 6 or about six weeks after bud break, failed to produce symptoms on the host while the necrosis caused by the bud break inoculations was replaced by leaf symptoms diminishing in severity. This apparent relationship between time of infection and the expression of symptoms was noted as early as 1942 by Hildebrand (6) and was considered to be an association between virus and food movement. Additional work on the fundamental aspects of virus infection is needed to clarify this point.

No apparent differentiation is discernable between strains of virus containing ring spot alone or those in which ring spot is part of a complex such as yellows. This would imply that ring spot, itself, is the causative factor for the adverse affect on the inoculated host. Although growth studies pre-

viously reported by the author (12) indicated that ring spot alone does significantly reduce the growth of the Lambert and Hardy Giant varieties, absolute confirmation must await until direct comparisons can be made with the same clone propagated from a source infected with yellows.

The leaf patterns associated with ring spot infection on Gold and Hardy Giant are similar to those associated with ring spot infection on any of the sweet cherry varieties used in the study. Temperature, however, plays an important role in the severity of the symptoms of ring spot on Gold and Hardy Giant for the chlorotic spotting becomes necrotic whenever the temperature is above 80° F. Under the higher temperatures some slight necrosis occurs but this is limited generally to those strains causing extensive necrosis on the varieties belonging to the sensitive group.

The latent viruses still remain a very perplexing problem in regard to their identity and their use in economic control problems. In the past, the relationship of viruses has been considered positive if one virus when inoculated into a susceptible host would protect this host against another virus inoculated subsequently. If this is true, then the latents used in this study are not closely related to the viruses of the necrotic ring spot group. Until a satisfactory method of virus purification can be worked out and serological studies then made, the exact relationship of this group and necrotic ring spot can not be determined satisfactorily.

SUMMARY

A study of the ring spot complex on sweet cherry indicates that strains of virus thought to be the same on the basis of symptoms expressed on sour cherry are, in fact, different entities. That they are closely related is attested by the similarity of symptoms on one or more hosts. However, the extensiveness of necrosis on sweet cherry suggests a variance in virulence that seems to be due to the ring spot component, whether the entity appears alone or as a contaminant in the virus culture.

Regional isolates of ring spot or yellows differ from each other no more than do isolates collected from the same region. Furthermore, ring spot strains collected from different hosts when inoculated into the same host produce symptoms so similar that there seems little reason for considering them as belonging to different groups.

The variance in virulence found in the strains used in the study indicates that the role of ring spot may be more serious than it has been considered previously. While no difference between virus strains of ring spot alone or in combination with yellows occurred with regard to overall symptoms, the same extensiveness in the observed symptoms of necrosis strongly suggests that the ring component is the causative entity.

Host response to ring spot inoculations indicated that sweet cherry varieties fall into two groups. Certain strains of ring spot alone or in com-

bination with yellows were found to cause a severe necrosis, killing from 90 to 100 percent of the varietal tissue while others cause a necrosis limited to a few tip spurs. These symptoms may or may not be accompanied with foliar symptoms of necrotic ring spot. Plants of the second group react to inoculations of ring spot with foliar symptoms similar to those on Montmorency. Virulence of isolate on this latter group is usually restricted to the degree of ring spotting or shotholing with a very mild necrosis occurring only under conditions of high temperatures.

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