COMPARATIVE PHYSICAL PROPERTIES OF SEVERAL COLLECTIONS OF THE WHEAT STREAK MOSAIC VIRUS

by

JOHN M. KAINSKI

Dipl. Eng., University of Lwow, Poland, 1944

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Botany and Plant Pathology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

TABLE OF CONTENTS

INTRODUCTION

Different viruses of cereals and grasses induce chlorotic mottling, spotting, or streaking reactions of infected plants which are similar or almost identical. The symptom expression on the plants infected by one specific virus may be altered to some extent by the host or variety (Zashurilo and Sitnikova, 1940), temperature (Sill and Fellows, 1953), or other environmental conditions, and it would be unreasonable to differentiate the viruses according to symptoms alone.

The mode of spreading or mechanical transmission, the host range plants infected in nature or by manual methods of inoculation, the ability to withstand inactivation in liquid extract when subjected to different temperatures, the longevity of infectivity in plant juices or in desiccated tissue, the dilution end point and other physical and chemical treatments have greater diagnostic value in identification of these viruses.

The symptoms caused by soil-borne mosaic virus and western wheat streak mosaic virus cannot be distinguished easily when examining diseased wheat leaves even for the virus specialist (Sill, 1952) but behavior of viruses in extracted preparations, in dilution end point, in thermal inactivation point and longevity in dried tissues are dissimilar.

The brome grass mosaic virus induces symptoms on some grasses similar to those of the wheat streak mosaic virus. It spreads very easily by mechanical inoculations and has a wide host range among grasses and small grains. However, its thermal inactivation point is near 78° to 80° C and the dilution end point is 100,000 to 300,000 times, the highest among known viruses on grasses and cereals. The virus was found to be viable after 12 months in desiccated tissue preserved at room temperature and after 14 months in extracted juice stored at -17° C (McKinney, 1944).

The barley false stripe, a seed-borne virus disease of barley, is easily transmitted to wheat and some grasses by manual methods of inoculation. It has symptoms very similar to the other grass viruses mentioned such as chlorotic mottling, spotting and streaking. The virus is inactivated within 40 days in dried tissue at room temperature. Its dilution end point is slightly beyond 10,000 times when diluted in water, and the thermal death point is 68° C after a 10 minute exposure (U.S.D.A. Yearbook, 1953).

The symptoms of a new virus disease of cereals in California called barley yellow dwarf resemble wheat streak mosaic in some respects. The disease is destructive on barley, wheat, and oats (Oswald and Houston, 1951). No work has been reported on physical and chemical properties of the virus. However, it is differentiated specifically from the other viruses because it apparently cannot be transmitted by mechanical means and has been transmitted by only four different species of aphids.

No other literature has been found dealing with chemical and physical studies of grass viruses. More thorough investigations are needed on such properties as dilution end point, thermal inactivation point, and longevity in vitro, for these may be most useful in differentiating between the wheat virus diseases.

REVIEW OF LITERATURE

In spite of many attempts to introduce a system of classification to plant virus diseases there is still evidence of confusion in virus nomenclature. Nevertheless, it would be helpful to subdivide virus diseases of small grains into these groups:

(a) Soil-borne wheat mosaic.

(b) Plain's wheat mosaic or wheat streak mosaic.

(c) Wheat striate mosaic or Russian wheat mosaic.

(d) Barley yellow dwarf.

(e) Barley stripe mosaic (false stripe).

(f) Brome grass mosaic virus.

Soil-borne mosaic was reported in the United States in 1919 (McKinney, 1923) and later from Japan (Ikata and Kawai, 1939). It is a cool weather disease, the optimum symptom expression being near 15° C. The disease may be recognised in the early spring by the yellowish-brown areas of different size and irregular in shape which appear in the field. Affected plants are retarded in growth and leaves show yellowish to light green spots and some streaks. With the onset of warmer temperatures, the symptoms become less noticeable and later disappear.

A study of the physical properties of soil-borne mosaic showed that the virus was inactivated in 6 to 14 days in leaf tissue dried at room temperature. In extracted liquid the virus was inactivated at from 60° to 65° C after exposure for 10 minutes. The dilution end point was between 100 and 1000 times (U.S.D.A. Yearbook 1953).

Plain's wheat mosaic or wheat streak mosaic was first observed and reported in 1922 in Nebraska. It seems to have been increasing gradually since that time. In 1929 the disease appeared in Kansas (Melchers and Fellows, 1930) and caused a big epidemic in 1949 (Hansing et al, 1950). In 1949 wheat streak mosaic was reported from South Dakota (Slykhuis, 1952), in 1951 from California (Houston and Oswald, 1952) and by 1952 it was reported in Alberta, Canada (Slykhuis, 1953).

In nature the vector is an eriophyid mite, Aceria tulipae (K.) (Slykhuis, 1953). It can be transmitted mechanically by many manual methods of inoculation (McKinney and Fellows, 1951). It is undoubtedly one of the most

dangerous wheat diseases of the Great Plains (Melchers, 1950).

Symptoms of wheat streak mosaic range from mild green to severe yellow mottling, spotting and streaking.

The previous study of physical properties of wheat streak mosaic indicated that the virus is inactivated near 55° C in plant juice when exposed for 10 minutes to heat. The dilution end point was reported as being 5000 times (McKinney, 1944).

In the leaf tissues dried and stored at room temperature wheat streak mosaic virus seems to be unstable; such longevities as 34 to 40 days (McKinney, 1944), 82 days (Slykhuis, 1952), and 21 to 30 days (Sill, 1953) have been reported. Infectivity was lost after five days when green infected leaves were preserved in moist condition at room temperature. With decreasing temperature viability of the virus increased, and it survived 350 days under most conditions at -23° C (Slykhuis, 1952). When desiccated in plant tissues and stored over $CaCl_2$ the virus retained its infectivity over one year at a wide range of temperatures (Slykhuis, 1952).

Wheat striate mosaic or Russian yellow wheat mosaic was described in the U.S.S.R. in 1939 (Zashurilo and Sitnikova, 1940) and in South Dakota in 1950 (Slykhuis, 1952). It is transmitted by leaf hoppers only. Wheat appears to be the major host of striate mosaic virus, but susceptibility of oats and barley has been reported. The disease may be distinguished by the fine chlorotic streaks and dashes which are first visible on the lower surface only and later on the upper leaf surface. Nothing appears to be known concerning its physical and chemical properties.

A relatively new virus disease of cereals, barley yellow dwarf, has been reported from California. This is transmitted by aphids only (Oswald and Houston, 1951). The symptoms of barley yellow dwarf resemble those caused

by excessive soil moisture, low temperature, or shortage of nitrogen. Leaves of infected plants show yellowing beginning at the tips and this rapidly progresses downward. On wheat chlorosis and stunting are the principal symptoms with occasional leaf reddening. Nothing has been reported concerning physical or chemical properties.

Barley stripe mosaic virus is seed-borne and was known for a long time as noninfectious barley false stripe. Its virus nature was recognized only in 1950 (McKinney, 1951). The leaves of infected young barley plants show a chlorotic mottling, spotting and streaking, often mild. On some infected leaves the usual mottling turns into broad irregular stripes, often brown in color, but usually yellow or gray-white. By manual methods of inoculation barley stripe mosaic virus can be transmitted to barley, wheat, rye, sweet corn, and some grasses.

The brome grass mosaic virus has been collected in nature on smooth brome plants only. The other viruses mentioned do not infect smooth brome grass. Although no insect or mite vectors are known, this virus can be easily transmitted by manual methods of inoculation to a wide range of grass species and several dicotyledonous plants (McKinney et al, 1942). The symptoms on the small grains induced by the brome mosaic virus can be confused with those produced by wheat streak mosaic virus.

MATERIALS AND METHODS

The object of this study was to compare certain physical properties of five collections of wheat streak mosaic virus, namely dilution end point, thermal inactivation point, and longevity in vitro. Five different collections of the virus, which varied somewhat in symptomatology, were used. The collections investigated were under code numbers SD-1, UK, 41, SC and MS.

SD-1 was collected in South Dakota in 1951 by Dr. J. Slykhuis, plant pathologist at the South Dakota Experiment Station. The collected sample was sent to Kansas State College in 1952.

UK was found near Ulysses, Kansas, in 1952 by A. Lowe, agronomist at the Garden City Branch Experiment Station, Kansas State College.

In 1932 H. H. McKinney, plant pathologist of the U.S.D.A. at the Plant Industry Station, Beltsville, Maryland, collected samples of wheat streak mosaic virus in Saline and Dickinson counties, Kansas. From these collections he isolated two viruses of wheat. First, the green wheat streak mosaic virus described by him as No. 6. It was used in this investigation under code number 41. Second, the yellow wheat streak mosaic virus under code number M3.

SC was collected in Siebert, Colorado in 1952 by Dr. H. Fellows, U.S.D.A. plant pathologist, Kansas State College.

Material collected in different fields and localities was desiccated by the collectors (unless otherwise stated) and sent to Kansas State College laboratory where all collections were stored in moisture-free conditions over $CaCl_2$ at about 1° C.

Simultaneously with storage of the viruses in the desiccated tissues, the cultures of all above-mentioned viruses were maintained through sub-inoculation on wheat plants in the mosaic greenhouse at Kansas State College.

Under greenhouse conditions the five collections varied somewhat in outward appearance of infected plants, and they are described below according to symptoms. The plants used were varieties Pawnee and Marquillo-Oro X Pawnee Sel. No. 462666. The host plants were inoculated by the carborundum-rub method, described later, and were grown under greenhouse conditions at about 22° C. The symptoms were read six weeks after inoculation.

SD-1. Faint green streaks and dashes on young leaves turn to white long bands and streaks on older leaves. On the oldest leaves the streaks and dashes become prominent and numerous. Some coalesce and form chlorotic areas and stripes white-yellow in color and irregular in shape. Infected leaves become typically elongated when compared with other collections. There is no marked stunting and no proliferation or rolling of the leaves under greenhouse conditions.

UK. Infected leaves are remarkably narrow and are covered with faint white-greenish streaks and dashes, which in older plants become white and yellow-white. There is a pronounced stunting effect, excessive proliferation and longitudinal rolling of infected leaves.

41. The virus stunts the plants and stimulates proliferation of leaves, which remain short and broad. Blades of the infected leaves seldom roll or wrinkle except at very high temperatures. Light-green and yellow-green streaks and bands run along the leaves parallel to the axis. In some leaves narrow stripes or bands run along the entire leaf length. Mosaic mottling and crinkling of the leaves may be observed two to three weeks after inoculation. They later tend to disappear.

SC. The symptoms are very similar to those produced by UK. Leaves are usually rolled longitudinally and have streaks which are broadened and form white necrotic or yellowish spots. Stunting is evident and severe. Some plants may die. Proliferation is marked but not quite so prominent as in plants infected with UK virus.

MS. Four to five days after inoculation distortion of the leaves commences, after which crinkling and mottling symptoms develop. The first leaves which emerged after inoculation are rolled, with desiccated tips. The tips usually wither, leaves developing later are distinctly shorter.

Plants inoculated when young often die and when inoculated at the 5 to 6 leaf stage diseased leaves are shorter, erect and tend to be broader than normal. Small spots, dashes, and streaks of yellow color develop on the infected leaves. These gradually increase in size and number. On older leaves they coalesce and form distinct chlorotic to yellow spots. The spots increase in size and sometimes all leaves become wholly yellow and then may die. These symptoms were typical only of the desiccated source of the MS virus. The greenhouse source was similar to 41 only slightly more severe.

From the time of collection until this investigation the different viruses used were preserved in two ways. First, in cultures maintained through subinoculation in the greenhouse, and second, in desiccated leaf tissues prepared according to the method described by McKinney (1947) and stored over $CaCl_2$ at 1° C.

The virus preparations were obtained from cultures in the greenhouse in all preliminary trials. For final experiments the virus preparations were obtained from cultures prepared from the desiccated virus source.

Pawnee wheat was planted in 8-inch pots. To avoid any contamination with other viruses by leaf contact and to prevent to some extent possible mite contamination, these pots were placed in cages with surgical gauze walls. Twice weekly the section was fumigated with Plantfume 103 (tetraethyl dithiopyrophosphate) to prohibit insect population. At the four leaf stage the plants were inoculated with the proper virus extracted from desiccated tissue. Before inoculation plants to be inoculated were carefully examined and only healthy symptomless plants were used. No symptoms were ever seen on these plants before inoculation. After inoculation plants were placed in the cages, each virus collection in a separate section, and thereafter were

always handled with extreme care to prevent possible contamination.

In all experiments the virus extract was obtained by macerating infected leaves of wheat plants in a mortar and pestle with added quartz sand. The extract was prepared only from the juice present in the leaves without any added water. The pulp of ground leaves was collected in double sterile surgical gauze and the extract was squeezed through by hand. The collected liquid was immediately diluted with distilled water to a ratio of 1 to 10. Virus extracts prepared in this way from each of the virus collections were used separately to determine the dilution end point, thermal inactivation point, and longevity in vitro.

For the dilution end point experiments a series of sterilized test tubes containing 9 cc of distilled water and 1 cc pipettes were involved. From the prepared inoculum in ratio 1 to 10, 1 cc of diluted extract was placed in 9 cc of water, diluting it 1 to 100. Again 1 cc of diluted 1 to 100 extract was transferred to 9 cc of water, making the dilution 1 to 1000. The same procedure was repeated with dilution 1 to 10,000, and 1 to 100,000.

After each dilution the contents of each test tube were well shaken before subsequent dilution.

Thermal inactivation was accomplished with the virus extract in the ratio 1 part of plant sap and 10 parts of distilled water. The extract was placed in pipettes approximately 25 cm in length and 4 mm in outer diameter. The capillary ends of the pipettes were sealed in a gas flame. To avoid heating the extract in the pipettes, the pipettes containing the virus extract were kept in a horizontal position while sealing the ends in the side of the flame. The capillary end of each prepared pipette was inserted point first through a cork until the surface of the virus extract was about 1 cm below the bottom of the cork. During the heat treatment these

prepared pipettes were floated in a water bath with a thermostatic temperature regulator. The surface of the virus extract within the pipettes when immersed in the bath was approximately 4 cm below the water surface. Each virus preparation was heated for 10 minutes in the water bath. The pipettes were then immediately removed and immersed in cool water held at about 7° C. The thermal inactivation tests were made at 45, 50, 55, 60, 65, 70, 75, 80, and 85° C. These temperatures were measured by a thermometer placed in the water bath. Fluctuations in water temperature were present during thermal treatment, and increased with an increase in temperature. At 60° C these were about ± 0.5° C, increasing to ± 1° C at 85° C.

After the heat treatment and before inoculation to plants the sealed capillary ends were broken to release the heat treated virus extract.

For the aging in vitro tests the virus extracts from each collection also were tested at a 1 to 10 ratio. They were placed in separate flasks with cotton corks, and stored at room temperature, about 22° C.

The carborundum-rubbing method was used in all inoculations (McKinney, 1930). In this inoculation method the thumb and index finger are dipped into the inoculum to which 400 mesh carborundum powder has been added. The leaves are then rubbed gently, but with enough pressure to make slight injury to the epidermal cells. The wheat plants were inoculated at the 3 to 5-leaf stage. The inoculations for the dilution end point started with the highest dilution (10-5) and proceeded to the lowest (10-1). As a similar precautionary measure in the thermal inactivation tests the first inoculation was done with inoculum subjected to the highest temperature and the last with inoculum treated at the lowest temperature. The last inoculation was always made with the unheated infected control extract. All inoculations were done within one day after preparing the inocula. In the longevity in vitro tests

the first inoculation was done the same day in which the virus extract was prepared and subsequently every second day. Before each inoculation the inocula source flasks were well shaken. All inoculated plants were maintained at about 22° C in the greenhouse. This temperature should be considered as the lowest limit. Fluctuations in temperature upward were observed during warmer days.

Systemic symptoms on individual inoculated wheat plants were the criteria of infection. Symptomless plants were considered to be healthy since the wheat variety used is very susceptible to the virus and shows clear symptoms at these temperatures. Final readings were never made earlier than 3 weeks after inoculation in order that all plants might have adequate time to develop symptoms.

During the experimental work all utensils coming in contact with the virus were sterilized in the autoclave for 30 minutes at 15 pounds pressure or in a steam steriliser for one hour without pressure. Prior to all inoculations hands and fingers were washed with soap and water, dried, washed in 95 percent ethyl alcohol, and then washed again in water to remove the alcohol.

PRELIMINARY TRIALS

The purpose of the preliminary trials was to find (a) a host plant for quantitative assay, (b) a well adapted method of mechanical inoculation, and (c) an approximate estimation of dilution end point, thermal inactivation point, and longevity in vitro of the virus collections studied.

Golden Giant sweet corn plants and Agropyron x Triticum, No. 6605, plants were inoculated with SD-1, UK, 41, SC, and MS virus collections in dilution 10-1, 10-2, 10-3, 10-4, and 10-5. Simultaneously with the corn and

Agrotricum plants, Marquillo-Oro x Pawnee Sel. No. 462666 wheat plants were inoculated with the same inocula. After two trials to find the approximate dilution end point, it was found that systemic infection in the wheat plants gave more reliable data than the erratic local lesions produced on the corn and Agrotricum plants.

Experiments were conducted to find the effect of the amount of finger pressure during inoculation upon infectivity. Wheat plants were inoculated with three different finger pressures by the carborundum-rubbing method with the SD and 41 virus. The inocula were diluted 1 to 10 with distilled water. It was found that gentle rubbing of inoculum over the leaf surface induced as high a percentage of infection as heavier pressures. Results are presented below and these indicated equally good infection may be obtained with light or heavy pressures. (Table 1).

Three trials with the dilution end point indicated that all collections tested were inactivated somewhere near 10,000 times when diluted with distilled water.

The first preliminary test concerning thermal inactivation of the 41 and SC viruses showed that these were inactivated near 60° C in 10 minutes exposure to heat. In each group 18 wheat plants were inoculated. (Table 2).

In the next thermal inactivation test all five virus collections were used. In each group 24 wheat plants were inoculated. Again each virus was inactivated at approximately 60° C. (Table 3).

The preliminary test for longevity of the viruses in vitro at room temperature in extracted liquid indicated that the infectivity in all collections of wheat streak mosaic decreases after 10 days, and ceases by 22 days. In each trial 20 wheat plants were inoculated. (Table 4).

Table 1. The effect of finger pressure during inoculation upon infectivity.

Virus Collection used for inoculum	Different degrees of pressure during rubbing.			Number of Plants			Remarks
	Light Pressure	Medium Pressure	Heavy Pressure	Inoculated	Devel. Symp.	Healthy	
A1	x			24	24	0	
A1		x		24	23	1	
A1			x	24	24	0	
SD-1	x			24	24	0	
SD-1		x		24	24	0	
SD-1			x	24	23	0	1 plant died after inoculation.

Table 2. The effect of thermal treatment upon infectivity of two collections of wheat streak mosaic virus.

Temperature °C	No. of plants infected by	
	Virus 41	Virus SC
90	0	0
80	0	0
70	0	0
60	3	3
50	15	18
not treated	17	18

Table 3. The effect of heat upon the infectivity of five collections of wheat streak mosaic virus.

Temperature °C	Collections used and number of plants infected				
	SD-1	UK	41	SC	MS
75	0	0	0	0	0
70	0	0	0	0	0
65	0	0	0	0	0
60	0	3	2	6	2
55	13	24	14	24	6
50	22	24	24	24	24
45	23	24	24	24	24
not treated	24	24	24	24	24

Table 4. Longevity in vitro of 5 collections of wheat streak mosaic virus.

Days after inoculation	Collections used and number of plants infected				
	SD-1	UK	41	SC	MS
0	20	19	20	20	20
2	20	20	20	18	20
4	19	18	20	20	20
6	20	20	20	19	20
8	20	20	20	20	20
10	18	20	20	20	13
12	10	17	15	9	4
14	4	15	9	10	1
16	1	3	4	2	2
18	0	2	1	3	0
20	1	0	2	2	1
22	0	0	1	0	0
24	0	0	0	0	0
26	0	0	0	0	0
28	0	0	0	0	0

FINAL EXPERIMENTAL RESULTS

Three final tests of dilution end point, thermal inactivation point, and longevity in vitro of the five collections were conducted. Desiccated virus sources were used.

Table 5 presents data showing dilution end point for collections SD-1, UK, 41 and SC to be near 10,000 times and for collection MS near 100,000 times, when diluted with distilled water.

Table 6 gives the final results for thermal inactivation. Collections SD-1, UK, 41, and SC were inactivated near 60° C and collection MS near 80° C after 10 minute exposures to heat.

Table 7 presents data concerning the longevity in vitro test. It was found that infectivity of collections SD-1, UK, 41 and SC decreased after 10 days and ceased completely after 20 days. Collection MS was inactivated in 30 days. Two additional longevity in vitro tests are under investigation at the time of writing this thesis.

In preliminary trials 272 plants of Golden Giant sweet corn, 450 plants of Agrotricum #6605, and 2820 wheat plants were used. Final tests involved 1500 wheat plants in the dilution end point studies, 2160 plants for thermal death point determinations, and 5400 in longevity in vitro investigations. In addition to these several hundred wheat plants were used as healthy checks and for inocula source material.

Table 5. Dilution end points of five collections of wheat streak-mosaic virus.

Dilution	SD-1 1/			UK			41			SC			MS		
	I 2/	II	III	I	II	III	I	II	III	I	II	III	I	II	III
10-1	20 3/	20	20	19	20	20	19	20	20	19	20	20	20	20	20
10-2	20	17	20	20	20	20	20	20	18	20	20	20	20	20	20
10-3	8	16	7	9	20	13	9	19	14	8	20	18	17	20	20
10-4	2	13	0	3	5	1	2	4	1	5	5	6	7	8	11
10-5	0	1	0	0	1	0	0	0	0	0	1	0	1	2	5

1/ Collections tested: (SD-1) South Dakota, (UK) Ulysses, Kansas, (41) Salina intermediate, (SC) Siebert, Colorado, and (MS) Salina severe.

2/ (I) First trial, (II) Second trial, and (III) Third trial.

3/ 20 plants inoculated in each trial at each dilution. Numbers indicate number of diseased plants.

Table 6. Effect of thermal inactivation upon five collections of wheat streak mosaic virus.

°C Tempr.	SD-1 1/			UK			41			SC			MS		
	I	II	III 2/	I	II	III	I	II	III	I	II	III	I	II	III
85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
80	- 3/	-	-	-	-	-	-	-	-	-	-	-	-	-	0
75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20
70	0	0	0	0	0	0	0	0	0	0	0	0	20	20	20
65	0	0	0	0	0	0	0	0	0	0	0	0	20	20	20
60															
55	0	0	0	0	0	0	1	0	0	0	1	0	20	20	20
50	10	11	4	9	6	9	5	8	13	7	6	17	20	20	20
45	19	20	16	15	12	20	10	19	17	14	11	20	20	20	20
not treated	19	20	19	19	17	20	20	20	20	20	18	19	20	20	20
	20	20	20	19	20	20	19	20	20	19	20	20	20	20	20

1/ SD, UK, 41, SC, and MS Collections used in thermal inactivation.

2/ I, II, III, - First, Second and Third trial.

3/ 20 plants inoculated with each virus in each trial at each temperature. Numbers indicate number of diseased plants.

Table 7. Longevity of five collections of wheat streak mosaic virus in extracted plant juices.

Days after inoculation	Collections used in investigation				
	SD-1	UK	Al	SC	MS
	1/				
0	20	20	20	20	20
2	20	20	20	20	20
4	20	20	20	20	20
6	20	20	20	20	20
8	20	20	20	20	20
10	18	20	20	17	20
12	9	18	16	9	20
14	3	15	8	2	20
16	0	4	2	3	20
18	1	2	0	2	20
20	0	2	0	1	20
22	0	0	0	0	20
24	0	0	0	0	20
26	0	0	0	0	20
28	0	0	0	0	6
30	0	0	0	0	1
32	0	0	0	0	0
34	0	0	0	0	0

1/ 20 plants inoculated for each virus collection and on each day inoculated. Numbers indicate number of diseased plants.

DISCUSSION

McKinney (1937, 1944) made valuable suggestions concerning the classification and differentiation of several grass viruses, including wheat streak mosaic virus. Since that time little effort has been made to rearrange these viruses using criteria other than outward symptoms. Since wheat streak mosaic virus collections have been readily transmitted by manual methods of inoculation, and because they occur largely on one host plant in which symptoms may be confusing, such characteristics as dilution end point, thermal inactivation point and longevity in vitro may have considerable value in separating other distinct grass viruses from various collections of streak mosaic. Such studies are and must be done with extreme care to be accurate.

During this type of study precautionary measures must be utilized consistently to avoid contamination of the different virus cultures in the greenhouse either by handling or by insect or mite vectors. Another difficulty where wheat streak mosaic is concerned is the fact that no plants outside of the grasses can be infected and no local lesion semiquantitative hosts suitable for virus assay have been found as yet. For this reason large numbers of systemically infected wheat plants must be used in assay work.

McKinney experimented with crude wheat streak mosaic extracts from infected wheat plants and found the dilution end point near 5000 times and thermal inactivation near 55° C. The data were the same for all four virus collections isolated by him from infected wheat plants. He did not notice any difference among those viruses in dilution end point and thermal inactivation point. Unfortunately, there is no information available concerning the methods used by McKinney so that no exact comparisons of technique can

be made. As far as known work on longevity in vitro of wheat streak mosaic has never been reported.

The object of this study was to compare physical properties of different collections of the virus which had been obtained in the field in widely separated areas and which differed somewhat in symptomatology on wheat.

Several preliminary trials with dilution end point, thermal inactivation point, and longevity in vitro using crude extract from cultures maintained through manual subinoculations in the greenhouse did not reveal any remarkable differences among collections studied, and results in general agreed with those by McKinney (1944).

As stressed by McKinney (1944) some wheat streak mosaic virus collections may lose the property of infectivity and other characteristics when cultured through manual subinoculation for a long period of time. This phenomenon has not been observed in any cultures maintained at Kansas State College, but it did indicate that more information might be obtained about original stocks of a virus by preparing new cultures from desiccated material collected in the fields.

All final tests with dilution end point, thermal inactivation point, and longevity in vitro were done with inocula sources from desiccated cultures. The final results represented in Tables 5, 6, and 7 indicated that only four collections SD-1, UK, A1, and SC behave alike in dilution end point, near 10,000 times. Thermal inactivation point for these collections was near 60° C, and longevity in vitro, 20 days. By contrast collection MS had a higher dilution end point, near 100,000 times. It was inactivated by heat near 80° C and its longevity in vitro was about 30 days.

SUMMARY AND CONCLUSIONS

Five collections of supposed wheat streak mosaic were studied. Such physical properties as dilution end point, thermal inactivation point and aging in vitro were compared in plant sap-distilled water extracts obtained from artificially inoculated diseased wheat plants.

The data presented show a dilution end point for collections SD-1, UK, 41 and SC near 10,000 times, and for MS near 100,000 times.

The thermal inactivation point for collections SD, UK, 41 and SC was near 60° C, for MS near 80° C after 10 minutes exposure to heat.

Aging in vitro for collections SD-1, UK, 41, and SC was 20 days, for MS 30 days, when virus preparations were held at room temperatures of about 22° C.

Four collections of wheat streak mosaic virus SD-1, UK, 41, and SC resemble each other in such properties as dilution end point, thermal death point, and longevity in vitro. Collection MS from the desiccated virus source is similar in thermal inactivation and dilution end point to brome grass mosaic virus. Since the wheat streak mosaic virus has never been described in such virulent form there is a probability that with this collection we are dealing either with the brome grass mosaic virus, a mixture of wheat streak mosaic and brome grass mosaic virus occurring either in the field or greenhouse, or possibly with a new virus disease of wheat in Kansas. If it is the brome grass mosaic virus, this would be the only recorded time when it has been collected from wheat plants. Hitherto it has been found only in smooth brome grass.

It should be stressed that the desiccated collection MS used in the final tests showed distinct differences in dilution end point, thermal inactivation

point and aging in vitro, from collection MS maintained in plants in the greenhouse and used in the preliminary trials, although both supposedly were from the same original source.

The desiccated MS culture behaved more constantly in its infectivity than all other collections studied. The disease is very easily transmitted by manual inoculation with almost one hundred percent of infection. This is not consistently the case in other investigated collections.

The symptoms induced by the desiccated MS virus also resemble those on plants infected with brome grass mosaic virus. The possibility of this collection being brome mosaic virus or a mixture including it will be the subject of further investigation.

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. W. H. Gill, Jr., for direction and encouragement during the course of this investigation and the presentation of this thesis. Also, the author is fondly indebted to Prof. L. E. Melchers for inspiration and valuable suggestions during the period of study.

LITERATURE CITED

1. Hansing, E. D., L. E. Melchers, H. Fellows, and C. O. Johnston. Kansas phytopathological notes, 1949. Trans. Kans. Acad. Sci. 53: 344-354, 1950.

2. Houston, B. R., and J. W. Oswald. A mosaic disease of wheat, barley, and oats new to California. Phytopath. 42: 12. 1952.

3. Ikata, S., and I.Kawai. Some experiments concerning the development of yellow mosaic disease (white streak) of wheat. Relation between the development of yellow mosaic disease of wheat and soil temperature. Jour. Plant Prot. 24: 491-501, 847-854. 1937. In Japanese. Abstracted in Rev. Appl. Mycol. 18: 98. 1939.

4. McKinney, H. H. Investigations of the rosette disease of wheat and its control. Jour. Agr. Research 23: 771-800. 1923.

5. _____. A mosaic of wheat transmissible to all cereal species in the tribe Hordeae. Jour. Agr. Res. 40: 547-556. 1930.

6. _____. Mosaic diseases of wheat and related cereals. U.S.D.A. Circ. 442. 1937.

7. _____. Descriptions and revisions of several species of viruses in the genera Marmor, Fractilinea, and Galla. Wash. Acad. Sci. 34: 322-329. 1944.

8. _____. Stability of labile viruses in desiccated tissue. Phytopath. 37: 139-142. 1947.

9. _____. A seed-borne virus causing false-stripe symptoms in barley. Plt. Dis. Reptr. 35: 48. 1951.

10. McKinney, H. H., and H. Fellows. A method for inoculating varietal test nurseries with wheat streak-mosaic virus. Plt. Dis. Reptr. 35: 264-266. 1951.

11. McKinney, H. H., H. Fellows, and C. O. Johnston. Mosaic of Bromus inermis. Phytopath. 32: 331. 1942.

12. Melchers, L. E., and H. Fellows. Wheat mosaic in Kansas. Plt. Dis. Reptr. 14: 158. 1930.

13. _____.
The 1949 outbreak of wheat mosaic. Dien. Rept. Kans. St. Bd. Agr. 42: 37-40. 1950.

14. Oswald, J. W., and B. R. Houston.
A new virus disease of cereals transmissible by aphids. Plt. Dis. Reptr. 35: 471-475. 1951.

15. Sill, W. H., Jr.
Progress in knowledge concerning wheat mosaic. Minutes Branch Station Conference (appendix). Kansas State College. 1952.

16. _____.
Some characteristics of the wheat streak-mosaic virus and disease. Trans. Kans. Acad. Sci. 56: 418-424. 1953.

17. Sill, W. H., Jr., and H. Fellows.
Symptom expression of the wheat streak-mosaic virus disease as affected by temperature. Plt. Dis. Reptr. 37: 30-33. 1953.

18. Slykhuis, J. T.
Virus diseases of cereal crops in South Dakota. S. Dak. Agr. Exp. Sta. Tech. Bull. 11. 1952.

19. _____.
Wheat streak-mosaic in Alberta and factors related to its spread. Canad. Jour. Agr. Sci. 33: 195-197. 1953.

20. United States Department of Agriculture.
Plant Diseases the yearbook of agriculture 1953: 350-360. 1953.

21. Zashurilo, V. K., and G. M. Sitnikova.
Mosaic of winter wheat. C. R. Acad. Sci. U. R. S. S. 25: 798-801. 1939. Abstract Rev. Appl. Mycol. 19: 268. 1940.

COMPARATIVE PHYSICAL PROPERTIES OF SEVERAL COLLECTIONS OF THE WHEAT STREAK MOSAIC VIRUS

by

JOHN M. KAINSKI

Dipl. Eng., University of Lwow, Poland, 1944

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Botany and Plant Pathology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

Plant viruses, particularly dicotyledonous viruses, usually give characteristic or diagnostic symptoms which are unique on one or more hosts. Such does not seem to be the case among grass viruses. Instead, symptoms of different viruses may be very similar, often essentially identical, on the same host. Hence, it has been necessary to seek other criteria for identifying these viruses. Among these are the so-called physical properties, namely, dilution end point, thermal inactivation point, and aging in vitro.

Because of the above difficulties a physical property study of five collections of wheat streak mosaic virus was started to see whether these collections, differing somewhat in symptoms on wheat, were actually strains of the same virus or possibly were distinctly different where physical properties were concerned.

The five virus collections used were kept under code numbers SD-1, UK, 41, SC, and MS. These were collected in different localities on naturally infected wheat plants; SD-1 in South Dakota, UK near Ulysses, Kansas, 41 near Salina, Kansas, SC near Siebert, Colorado, and MS near Salina, Kansas.

All collections were stored in the desiccated tissues of infected wheat plants under moisture free conditions at about 1° C. Simultaneously all viruses also were maintained through subinoculations on wheat plants in the greenhouse.

Inoculations from each of the virus sources were made to Pawnee wheat plants maintained before and after inoculation in the greenhouse at about 22° C. Final readings were never made earlier than three weeks after inoculation.

In preliminary trials, dilution end point, thermal inactivation point, and longevity in vitro were compared, using virus preparations obtained from cultures maintained in the greenhouse. Final trials were conducted, using inocula prepared from desiccated virus sources.

The preliminary trials did not reveal any remarkable differences among collections studied. All collections tested were inactivated near 10,000 times when diluted with distilled water, the thermal inactivation point was near 60° C, and longevity in vitro was 22 days when the extracts were stored at about 22° C.

The final results showed a dilution end point for viruses SD-1, UK, 41, and SC near 10,000 times and for MS near 100,000 times when diluted with distilled water.

The data showed a thermal inactivation point for collections SD-1, UK, 41, and SC near 60° C, but for MS near 80° C after 10 minutes exposure to heat.

Longevity in vitro for collections SD-1, UK, 41, and SC was 20 days, but for MS it was 30 days. The infectivity in viruses SD-1, UK, 41, and SC decreased after 10 days and ceased by 20 days. In virus MS infectivity decreased after 26 days and ceased by 30 days, when the virus extract was diluted 1 to 10 and held at about 22° C.

From these studies it appears that five collections, SD-1, UK, 41, SC, and MS from subinoculations in the greenhouse were similar in dilution end point, thermal inactivation point, and aging in vitro. The MS virus from the desiccated source used in the final tests showed distinct differences in properties studied from collection MS source maintained through subinoculations in plants and from all other collections used in the preliminary and final experiments. The desiccated collection MS was more infectious and more easily transmitted by manual inoculation than all other collections. Therefore, assuming that physical properties are adequate to differentiate strains from other distinctly different viruses, it would seem to be either an entirely different virus such as brome grass mosaic virus or a mixture of brome grass mosaic virus and wheat streak mosaic virus.