COMPARISONS BETWEEN WHEAT AND BARLEY MOSAIC

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INTRODUCTION

In the spring of 1948, Dr. K. Starr Chester found a single barley plant in a row of Composite Hybrid Selection 242-9-4 that showed striking mosaic symptoms. Since this single plant was so characteristic of the symptoms in wheat mosaic, it aroused considerable interest, and it was turned over to the writer for investigation. As far as is known, this was the only barley plant in Oklahoma to show symptoms of mosaic, so it was considered worthy of further investigation. Therefore, many questions arose. Was it a virus? Could it be wheat mosaic in barley? If so, what was the vector, if any? So, it became the purpose of this thesis to attempt to identify the virus by comparative tests between the two viruses. Also, it was desired to find by what means the virus is transmitted, and to obtain information on the physical properties.

Though cereal mosaic in Oklahoma causes no appreciable loss at present, it is known to be quite severe in Illinois and Indiana (12). With this fact in mind, and due to the vast acreage of small grains in this state, it may be that this disease is potentially epiphytotic.

OCCURRENCE OF WHEAT AND BARLEY MOSAIC IN OKLAHOMA

A search of the literature reveals no information concerning wheat mosaic in Oklahoma. Furthermore, there are no data in existance about barley mosaic, because of its recent appearance in this state. Wheat mosaic has been observed for the past two years by the writer as it occurred under natural conditions in Pawnee wheat on experiment station plots at Stillwater. Numerous susceptible varieties of wheat are grown at the experiment station, but mosaic was noted only in Pawnee. Since mosaic is not yet a serious problem in Oklahoma, no data have been recorded dealing with losses from

these diseases.

Wheat mosaic occurs only on an occasional plant among many normal plants. Thus, it has been found lightly scattered throughout numerous plots. The fact that it does not seem to be confined to any particular part of the field, leads to the assumption that the causal agent is not necessarily associated with the soil. Nor, does it indicate that the disease is seed-borne, as is the case with certain legume viruses (3). It therefore, is something much more obscure, and at the present unsolved.

The same conditions may prevail for barley mosaic, but it is perhaps even less definite than for wheat mosaic. The reason lies in the fact that one, and only one diseased barley plant has been found in the field in Oklahoma, so far as the writer is aware. This one plant was found at the end of a short row on the alley way of the plot, and only a short distance from infected wheat. The location of the plant within the row, and its conspicuous accessibility, would lead one to suspect that an insect vector may be responsible for its infection.

LITERATURE REVIEW

According to McKinney *et al.* (13) a mosaic of winter wheat was first observed in the United States in April of 1919. At that time, the disease was centered around the Mississippi river bottoms near East St. Louis and Granite City, Illinois (18). However, it is possible that the disease was present for sometime prior to its recognition because of the uncertainty of its identity. At the time of its discovery, it was first suspected of being wheat take-all, a disease caused by abnormal winter conditions, unbalanced soil nutrition, nematodes or severe Hessian fly damage (13, 18). Later, (18) it was recognized as something different and called "rosette disease". Further investigations (9, 12, 13, 16) led to the conclusion that "rosette" was a severe expression or phase of mosaic. In the early observations by McKinney (10) during 1923 it was apparent that rosette of wheat in many cases was intimately associated with the *Helminthosporium* disease, and other wheat diseases which were also obscure at that time. McKinney (13) pointed out that nothing was known of the origin of rosette, but it is known that wheat mosaics occur in Japan as acknowledged by him (21). Furthermore, he notes (14) the occurrence of tropical mosaics mostly on the higher tribes of grasses as classified by Hitchcock (5). This information sheds little or no light on the origin, but it does give an idea of the possible distribution.

In 1937 McKinney (17) reiterated that mosaics constitute the largest known group of plant virus diseases. The group of mosaics includes many well known cereal diseases of ever increasing proportions as exemplified by the recent appearance of an oat mosaic as discussed by McKinney (20) in 1946, and barley mosaic described by Herbert and Middleton (4) in 1948. From the work of McKinney (17) with tobacco mosaic, it was thought that a virus might

be: (1) a microscopic organism, (2) an ultramicroscopic organism, (3) a connecting link between a strictly chemical system and living cells, (4) an enzyme, and (5) a protein. The latter view is supported by Stanley (23) in the statement: "that this unusual, high molecular weight protein is actually tobacco-mosaic virus." However, the results of research during the past few years tend to support the idea that the disease is caused by an ultramicroscopic organism (2). This is partially accounted for by the fact that viruses have a tendency to reproduce true to type (2), and on occasion give rise to mutations (17).

In 1937 McKinney (18) described symptoms of five different wheat viruses which he numbered from one to five. Each virus produced symptoms sufficiently different for visual identification. He pointed out that those plants developing rosette were especially susceptible to soil-borne parasites, specifically Helminthosporium sativum. He (8, 13) stated that Helminthosporium sativum causes a brown rot at the base of the tillers. So far as the rosette disease is concerned, McKinney (18) pointed out that it had not been isolated without mosaic. Though one cannot be isolated from the other, Webb (28) produced information to the effect that infection giving rise to mottling could manifest itself at later seedling stages, shorter time intervals, and over a wider temperature range than that for rosette. Webb brought out the fact that until a causal agent was found, the virus must be defined in terms of the host response. In further discussion he stated that the presence of symptoms always demonstrated the presence of the virus; but in the absence of symptoms, it could not be concluded that infection had not taken place. In the final stages of rosette, Johnson (6) reported that the plants tended to show considerable rotting of the roots and a brown rot where the tillers were underground. The symptoms of rosette and/or mosaic as

discussed by McKinney (18) are influenced by soil fertility, temperature, age of the plant and variety. He (17) also mentioned that the amount of light played an important part in the symptom-expression. Wada and Fukano (26) suggested that the infected varieties profoundly influenced the symptoms by their genetic constitution. McKinney (14) also suggested that varied expressions of symptoms were greatly influenced by genetic purity of the host, for continued selections in wheat varieties do not necessarily guarantee homozygosity for factors regulating susceptibility or resistance. In addition he (21) reported that nutritional factors modify symptoms; and with tobacco mosaic, nutrition is known to influence the amount of virus that the plant is able to produce. He further stated that mosaics tend to be very conspicuous when the nitrogen of the soil is rather low. In 1930 McKinney (15) announced that it is practically impossible to study the rosette phase of wheat mosaic under controlled conditions throughout the year. He pointed out that controlled conditions produce abnormally long sheaths and leaves, and tillering tends to be reduced. Also, some winter varieties fail to head satisfactorily. In speaking of rosette, McKinney (11) reported plants are sometimes found in which only part of the tillers are diseased while healthy tillers mature normally. Breed et al. (1) state that in wheat there is systemic chlorotic mottling.

Under controlled conditions when manual inoculation is desired, Rawlins and Tompkins (22) found infection could often result from use of carborundum. It was discovered that the sharp particles when sprinkled on leaves and rubbed with virus juice, allowed the virus to enter the cell.

In 1930 McKinney (14) indicated that so far as is known the host range of mosaic includes all the lower grasses in the Tribe Hordeae. Furthermore, the mosaics of the lower grasses show certain differences from those of the

higher grasses such as sugar cane, and Indian corn. He brought out the fact that certain varieties of winter barley became infected with mosaic when grown on infested wheat soil, and (18) that wheat virus number 2 was discovered on the grass Agropyron repens (L.) Beauv. where it persists in the underground rhizomes.

In 1923 and 1925 McKinney (8, 12) reported the causal agent of wheat mosaic to be soil-borne, attacking the underground portions during the seedling stage. Webb (28) stated that plants growing in infested soil for only one week would develop infection at favorable soil temperatures. He found that the soil temperature factor was more important than the length of exposure. He (29) also found the amount of oxygen and moisture in the infested soil to play an important part in the manifestation of the disease. Tests by Webb (loc. cit.) showed the infective agent to be in the silt fraction rather than in the filtrate. McKinney (13, 21) reported the virus to be more active in heavy silt and clay loam soils even though the disease could occur in all types of soil from poor sand to fertile gumbo. He (20) stated that the optimum temperature for soil-borne viruses is 60° to 65° F., and infection may require 35 to 60 days growth in infested soil.

Mosaic-rosette is spread by infested soil east of the Mississippi river (8) and another wheat mosaic is spread by rhizomes of Agropyron repens (L.) Beauv. There is no definite evidence that the western mosaics (west of the Mississippi river) are soil-borne. Investigation for insect vectors of eastern mosaics were negative, but there is reason to believe that an insect might be the vector in some of the western states. Breed et al. (1) reported the disease not to be seed-borne.

Cereal viruses are often identified by use of differential hosts along with various physical properties as described by McKinney (19).

In areas where the disease is soil-borne, the potential of mosaic is of great annual importance (18). The loss in heavily infested fields when uncontrolled may range from severe, where a field is worthless, to partial or slight. In severely infected plants an occasional plant may recover, but a recovered plant usually produces small imperfectly filled heads (11). Recovered plants mature at a later date, causing some difficulty in harvesting (13).

Control of the disease in the East is by three methods (18): soil disinfestation by formalin (8), very early or very late sowing (8, 28), and by resistant or tolerant varieties (8, 27). Little or no information is available for control of western mosaics (18). Though inactivation of infested soil is hardly a control method, Johnson (7) reported that infested soil could be inactivated when heated between 50° and 60° C. for 10 minutes. Also, plant juice can be inactivated near 55° C. when heated for 10 minutes; after 7 months in tissue frozen near -17° C.; and in dry tissue at room temperature after 34 to 40 days (19).

Webb et al. (27) stated in 1923 that at that time over 200 varieties and strains were tested for resistance to rosette and only 4 per cent were highly susceptible. Of these 200 varieties McKinney et al. (13) brought out the fact that a few varieties showed resistance to rosette, but all varieties were susceptible to mosaic in varying degrees. Webb et al. (loc. cit.) made it known that control of the virus is relatively simple where there are no complications from the use of resistant varieties. In some areas where flag smut is a complicating factor, Tisdale et al. (25) reported there were several varieties resistant to flag smut, but not so many as were resistant to rosette; however, the varieties resistant to both diseases were still fewer.

MOSAIC SYMPTOMS IN BARLEY

The symptoms of mosaic in barley have a wide range which is governed by the susceptibility of the variety, and the concentration of the inoculum. The conditions under which the inoculations were made, tend in many cases to influence the rapidity with which infection takes place, and the appearance of the first symptoms.

In barley the symptoms will begin to appear within 6 to 8 days, providing all conditions were favorable for normal infection. The first indication of infection is the pale-green color of the apical leaf. Frequently, this is in great contrast with the normal color of the remainder of the plant. This pale-green color may spread throughout the plant (Figs. 1, 5A). In the latter case the slight chlorosis persists until death of the plant. Within a very few days after the first discoloration, small chlorotic flecklings will appear. The flecks then elongate to form very definite stripes (Figs. 2, 3, 4). As the virus concentration increases within the plant, the chlorotic stripes may become enlarged, and the entire leaf or the major portion of the tip (Fig. 5C), then becomes necrotic. If the chlorotic to necrotic striping fails to appear, the symptoms may be more severe. This condition is often in the form of irregular chlorotic spots or blotches that harbor numerous tiny green islands. However, these small green islands are soon replaced by chlorosis.

While many severely infected plants as occur under artificial inoculation fail to mature, those that do reach maturity are often stunted, or fail to head, or produce imperfectly filled heads (Fig. 1).

In the field the symptoms are not so pronounced; since, infection must be from a source of low concentration, as would be expected if transmission

is by insect. The usual characteristics in the field are dwarfness, widespread growth habit, and a mosaic mottling in the upper leaves.

A B

Fig. 1 (Left). The healthy barley plants are about 3 weeks old, (A). The virus diseased barley plant which is approaching maturity is stunted, and has small, imperfectly filled heads, (B). The leaves show a pale-green mosaic pattern.

Fig. 2 (Right). Fifteen-day-old barley plants that have become chlorotically striped from barley mosaic. The darker plants do not show symptoms of infection.

A B

Fig. 3 (Left). Close up of barley plants diseased with barley mosaic.

Fig. 4 (Right). Healthy young barley plants (A), as compared to barley plants (B), which are infected with barley mosaic and are about 1 month from maturity.

MOSAIC SYMPTOMS IN WHEAT

The same governing factors for wheat mosaic are in effect, that were present in the control of symptoms in barley mosaic. Again, the variety is responsible for the segregation of symptoms, and the description will be limited to the varieties under observation. In Pawnee wheat the characteristic symptoms differ considerably from those in Michigan Amber wheat, even though inoculation techniques and conditions were identical. Here again, manual inoculation produces distinctly different characteristics from those seen under field conditions.

As a result of artificial inoculation, Pawnee wheat frequently assumes a stunted condition with rather prolific tillering. However, tillering is not as prolific as is ordinarily associated with the rosette disease, and many of the basal leaves retain a dark bluish-green color; while, the younger leaves show a mild mottling or mosaic (Figs. 5 F, G).

In the field the diseased plant is less conspicuous, but it can be recognized among the healthy plants. In most cases there is but one or two diseased plants among healthy ones in any given area, but its stunted, widespread growth habit makes it easily discernible. Further examination reveals mosaic mottling of the upper leaves.

The majority of plants showing infection in the field will sometimes head, though maturity may be somewhat later than ordinarily. The concentration of virus within the plant will, no doubt, determine whether the head is imperfectly filled or not.

In definite contrast, Michigan Amber wheat when infected by artificial means develops a yellowish mottling in the youngest apical leaf. With further development the entire plant takes on a yellowing and mottling, and is then enhanced by short chlorotic stripes, but rarely comparable to those of barley.

A B C D E F G

Fig. 5. Barley and wheat leaves: A. pale-green barley mosaic in barley. B. Barley mosaic infected barley leaf with chlorotic striping. C. Mosaic infected barley leaf with severe necrosis. D. Healthy barley leaf. E. Healthy wheat leaf. F, G. Wheat leaves infected with wheat mosaic.

Infected Michigan Amber wheat does not seem to tiller abnormally, but stunting is very characteristic. With concentrated inoculation, leaf distortion occurs as exemplified by twisting and rolling. This variety also produces a glossy, waxy appearance on the lower surface of infected leaves.

Since this variety is a soft wheat, it is not particularly adaptable to this region; and therefore, could not be observed under field conditions.

MATERIALS, METHODS, AND RESULTS

When these investigations were begun, 50 varieties of barley plants were available in the greenhouse as check plants for another problem. Since these plants were young and in good condition, they were manually inoculated with virus extract taken from the one diseased plant in the field. The inoculum was prepared from selected young leaves that showed the most obvious symptoms. The barley tissue was then thoroughly macerated by mortar and pestle. Since a very few leaves were available, the volume of virus extract was small and it was diluted with an equal volume of distilled water. The pulp was removed, but the juice was not filtered. The use of carborundum powder as suggested by Rawlins and Tompkins (22) (600 grain silicon carbide) was dusted on the oldest leaf of healthy plants, and the juice was applied to the surface of the dusted leaf by means of a swab. In most cases too much carborundum was used, and it resulted in the death of the inoculated leaf, but it did not hinder the entrance of the inoculum. In about a week, symptoms began to appear in 4 of the barley varieties. Thus, the first step of the problem was completed, and there was now no doubt that the condition of the plant in the field was the result of a virus infection.

At the time the barley leaves were selected in the field, several wheat plants with wheat mosaic were taken up in a ball of soil and transplanted in 6 inch pots for maintenance of fresh mosaic cultures.

Every day or so, other varieties showed virus symptoms until at the end of about 2 months, some 30 or more varieties had come down with the virus. The range of symptoms was often very inclusive. Though some varieties had but a single symptom, others seemed to have a combination of several which suggests a complex of viruses rather than a single virus as proposed by Wada and Fukano (24). Likewise, many symptoms were comparable to those previously

described by McKinney (18).

Due to the high summer temperatures, the infected varieties were repotted and placed outside the greenhouse for further observation. It was noted sometime later that certain varieties failed to reach maturity, and some varieties failed to head.

From the previous experiment, it was shown that 4 of the original 50 varieties were highly susceptible to the barley virus. Therefore, these 4 varieties designated as numbers 19, 28, 29, and 43 merely for convenience in writing, were selected for further study. These 4 varieties (4 pots each) were not seed treated, and were grown in standard greenhouse soil.[1] These conditions were maintained in all further tests. Seven or eight days after seeding, when the plants had developed 2 leaves, they were thinned to 4 plants per pot, and inoculated with extract from carefully selected, symptomatic leaf tissue from the original inoculation. The method of inoculation was repeated just as previously described, except that the juice was not diluted, and in addition, 1 pot of each variety was used as a carborundum check plant[2], and 1 pot of each variety was used as an absolute check.[3] Extreme care was used in handling the check plants to prevent possible transfer of the virus.

Juice left over from the inoculation was stored under refrigeration at 7° C. for later tests.

Five days after inoculation, symptoms were clearly visible in all 4 varieties, and possibly were distinguishable on the 4th day. With respect to

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[1] Standard is considered as two parts soil, one part sand, and one part barnyard manure.

[2] Carborundum check plant has the oldest leaf swabbed with carborundum, and no virus.

[3] Absolute check is without treatment of any kind.

the remainder of the test, there were no symptoms on either the carborundum checks or the plain checks. It was interesting to note that all 4 varieties of barley showed the same type of symptoms at the same time, and at least 75 per cent of all inoculated plants were infected.

Further observation of these 4 varieties substantiated the former statement, and allowed for the use of the varieties interchangeably with uniform results in later work. From this point on, no mention is made of the particular variety of barley used.

Of particular interest was the fact that possible virus symptoms had begun to show on a few of the carborundum and plain check plants. This was noted 10 days to 2 weeks later. Though the symptoms seemed to indicate infection, it required inoculation from expressed juice into healthy plants for positive conclusions. After inoculation from the check plants and incubation, the test plants came down with the virus. This fact proved that the check plants were somehow infected, as well as the manually inoculated ones. Whether this was due to negligent handling, or from an unknown source, was not known. As was mentioned earlier, care was exercised to prevent infection of the check plants; therefore, the possibility of an insect vector ventured into the realm of possibility.

Insect Vector Test

A simple insect transmission test was set up. Since it is practically impossible to keep aphids out of the greenhouse, there was an ample source on hand. Due to the fact that the aphids were common in the greenhouse, a supply was taken without any special attempt to identify the species. The green bugs were allowed to feed on diseased plants for 6 days, and at the end of that time, they were placed on young, healthy plants. The culturing

of green bugs on diseased plants, followed by the transfer to healthy plants, was not done in the greenhouse, but in a building some distance away. This was done, in order, to exercise as much control in the experiment as possible. One group of aphids was allowed to feed on healthy plants for 6 days before removal, and another group fed on healthy plants for a month. During the long feeding period, it was necessary to reduce the insect population for survival of the plants. After the aphids were removed from both groups of plants, they were under close scrutiny for 2 or 3 weeks. The results of both groups were negative. This rather insignificant vector experiment was not undertaken as a major problem of this thesis, but it was conducted in the hope that the discovery of a vector might be as simple as the experiment; or possibly, to discover the vector by sheer chance.

Soil Vector Test

To shed further light on a possible vector, several diseased pots of barley were selected. The choosing of these particular pots was based on the fact that the plants were obviously diseased, and had been growing in the same soil without repotting for well over a month. Therefore, 10 barley seeds were planted in a circle about the diseased plants approximately $\frac{1}{2}$ inch from the edge of the pot, and $\frac{1}{4}$ to $\frac{1}{2}$ inch deep. The young plants grew to maturity and showed no symptoms of mosaic irrespective of the fact that the original diseased plants died long before the experiment was concluded. The results of all plants in all pots were negative.

Here again, this test was not accepted as positive proof that the causal agent is not soil-borne.

In another test for a soil-borne vector, soil had been carefully saved from pots in which diseased barley plants had grown all summer. This soil

was diluted 50 per cent with soil which had not been exposed to diseased plants.

The mixed soil was placed in a large 8 inch crock, and about 20 barley seeds were equally spaced over the soil surface. They were then covered at a depth of approximately ½ inch. At maturity there was no symptom of the virus in any plant. The same experiment was duplicated with Pawnee wheat in soil from diseased barley plants with the same results.

Vector Test of Mosaic Infested Soil from out of State

Wheat mosaic infested soil from Clemson, South Carolina and Statesville, North Carolina (3½ qts. from each) was received from Dr. McKinney for soil-borne tests in Oklahoma. In addition 2 varieties of wheat, Michigan Amber and Red Winter spelt which are highly susceptible were also received. Each wheat variety was planted at the recommended date of planting for Oklahoma (Oct. 10-15) in four 6 inch pots with 10 seeds per pot sown in a circle. Each pot contained half Oklahoma soil and half mosaic infested soil with infested soil on top. In addition to Michigan Amber and Red Winter spelt, several pots of Pawnee wheat and barley were subjected to the same soils. Due to the fact that check plants occasionally became infected, it was necessary to provide insect proof cages outside the greenhouse for absolute control of pots with infested soil as well as those for checks. After seeding, the pots were placed in cages where seedlings were frequently protected by 10 per cent D. D. T., sulphur dust and nicotine sulphate spray.

An attempt was made to maintain the temperature in the greenhouse during the winter months between 60° and 65° F. Actually the temperature fluctuated considerably on both sides of this range. Shortly after bringing the plants into the greenhouse constant temperature recording ceased. In the greenhouse,

under warmer temperatures, the plants took on new growth. At the end of 3 weeks mosaic symptoms were showing on Michigan Amber and Red Winter spelt, but not on Pawnee wheat nor barley. At the time of this writing, some 3 months later, symptoms have still not appeared in Pawnee wheat or barley. If Pawnee wheat and barley fail to become infected, it would seem that under these conditions the soil-borne viruses from the Carolinas do not appear to be the same as those in Oklahoma.

According to McKinney (19) in 1944 certain varieties of wheat mosaic lose their infectivity with culture transfer. Likewise, certain varieties of wheat mosaic become inactivated from high summer temperatures. This fact is an important means of varietal separation. Thus, a running temperature log was kept in the greenhouse by thermograph during the summer and winter months. This data appears in Figure 6.

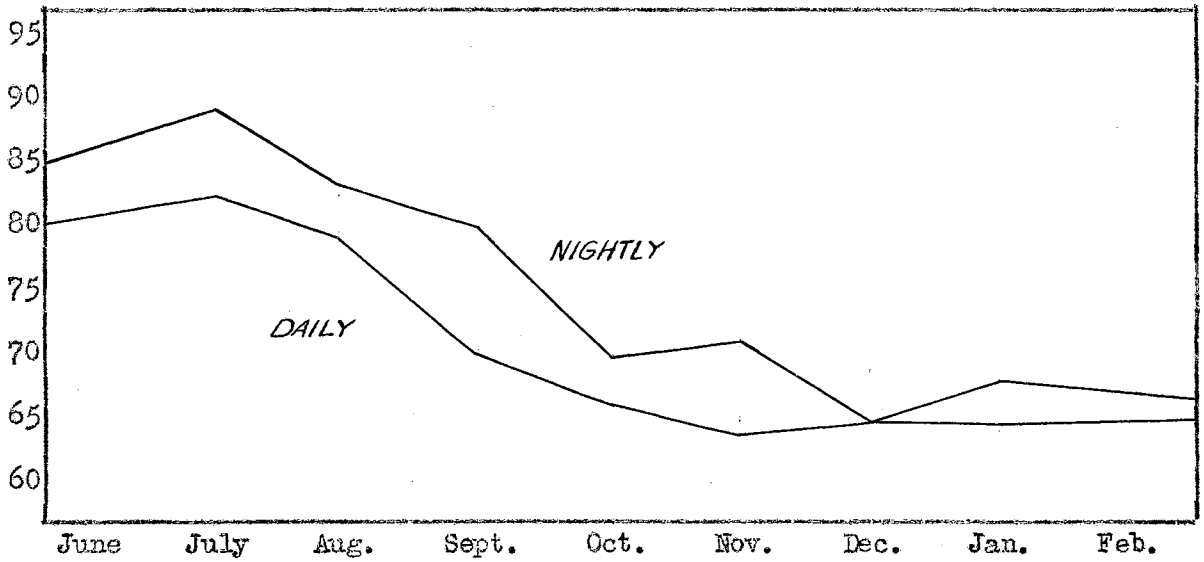

Fig. 6. Average greenhouse temperature in F. over a period of 9 months. These temperatures were recorded by thermograph. The daily average was from 6:00 a.m. to 6:00 p.m. and the nightly average was from 6:00 p.m. until 6:00 a.m.

In addition, with the approach of summer months when wheat can not be

grown, it was necessary to provide a method for carrying over diseased tissue. By personal correspondence with Dr. McKinney, it was suggested that infected leaf tissue could sometimes be kept viable by desiccation, and low temperature storage.

The desiccation process was carried out by again selecting obviously diseased leaf tissue, and cutting it into 1 inch lengths. The tissue was wrapped in gauze, and then placed in a regular desiccator containing calcium chloride. The tissue was under desiccation from 14 to 21 days at 7° C. At the end of this period it was removed from the desiccator, and placed in a small bottle which also contained calcium chloride. Then, the bottle was stored at 7° C. over the summer months. Diseased tissue from both wheat and barley was prepared in this manner.

Transmission of Barley Mosaic into Wheat

Thus far, the experimental work has been centered about virus transmission into barley, but several tests were conducted whereby the barley virus was inoculated into wheat. In personal correspondence Dr. McKinney pointed out that both Michigan Amber and Red Winter spelt wheat are highly susceptible to wheat mosaic. Pawnee wheat from this section is also susceptible. Therefore, since these varieties have already been proved to be susceptible to wheat mosaic, they were tested to ascertain their susceptibility to barley mosaic. This inoculation was begun when plants had developed two leaves (about 8 days old) as previously described, but with this modification: carborundum was thoroughly mixed with the virus extract rather than being applied directly to the leaves. Inoculation was then accomplished by stripping the oldest leaves between the fingers. In earlier inoculations it was found unnecessary to use both carborundum and plain checks; so control plants

were limited to healthy plants without the use of carborundum. After 10 days, symptoms were showing on all 3 varieties, and displaying somewhat different characteristics. For instance, severe chlorosis was observed on the apical leaves of spelt. This led to early death of that portion of the plant as compared to somewhat less severe effect on the other 2 varieties. The virulence of barley mosaic in spelt as compared to wheat mosaic in spelt was the first indication that the two viruses are possibly not the same. Since the check plants remained free from infection, a great deal of significance could be attached to this experiment.

Since an occasional control plant becomes infected, a few seedlings of the wheat, oat, and barley varieties were grown in insect proof cages. No infection developed on any of these seedlings.

Since barley mosaic extract was left over from the wheat varietal inoculations, it was used to inoculate several 6-day-old barley plants and several 11-day-old plants. On the 6th day the youngest leaf was just beginning to protrude while 11-day-old plants had 2 or more well developed leaves. Inoculation was done by clipping off the oldest leaves $\frac{1}{2}$ inch from the tip and merely inserting the cut edge of the leaf into the virus juice for 3 to 5 seconds. Infection developed in both age groups 7 days later. This test illustrates the ease and variability of plants in which infection can occur.

Miscellaneous Inoculations

After barley mosaic extract had been under refrigeration for $3\frac{1}{2}$ months at 7° C., it was diluted with an equal amount of distilled water to increase its volume. Immediately following dilution 16 pots of barley were inoculated by the extract-carborundum stripping method. Following an ample incubation

period, infection failed to appear, indicating inactivation of the virus from either length of storage or the fact that the virus was in liquid suspension.

Not being able to keep wheat mosaic in culture during the hot summer months necessitated the preservation by desiccation of diseased wheat leaf tissue, and diseased barley leaf tissue for comparison. After storage for 3 months at 7° C. and the beginning of cooler weather, inoculations on wheat were resumed. Inoculum from both Pawnee wheat and barley was prepared by grinding the leaf tissue in mortar and pestle with 10 cc. of phosphate buffer solution (23). Immediately after preparation, 16 pots of 11-day-old Pawnee wheat were inoculated with wheat mosaic extract, and 16 pots of 11-day-old barley were inoculated with barley mosaic extract by the carborundum-stripping method. In this case no check plants were set aside, because there were numerous healthy plants of all age-groups available.

The results were negative with the barley desiccate, and only one wheat plant ever developed infection. Since the purpose of this experiment was to oversummer the wheat mosaic virus, its recovery in but a single plant was insufficient as a source of inoculum. Therefore, a live culture had to be obtained from Dr. McKinney at Beltsville, Maryland.

In the meantime, Michigan Amber wheat was planted and was ready for inoculation upon arrival of the culture. This inoculation proved effective, but only about 50 per cent of the plants were infected. Inoculum was immediately obtained and increased on more Michigan Amber, but again the infection percentage in wheat from wheat mosaic was not as high as for barley inoculated with barley mosaic; thus indicating once again the possible difference between the two viruses.

Differential Host Tests

In 1944 McKinney (19) reported that wheat mosaic[4] infected many hosts among which were Zea mays, variety Golden Giant sugar, Triticum aestivum, varieties Harvest Queen and Turkey, and Avena sativa, variety Victoria. Seed of these cereals were obtained for differential testings.

Four 6 inch pots with 6 to 8 fifteen-day-old Golden Giant sugar corn plants were inoculated with desiccated wheat mosaic by the carborundum-stripping method with negative results. Check plants were also negative.

Thirty 4 inch pots with 2 to 4 Golden Giant sugar corn plants per pot that were 4 inches to 1 foot high were inoculated with fresh barley mosaic extract by the pin point method.[5] Several plants appeared to be infected, but positive results could not be ascertained. The experiment was repeated with equally indeterminate results.

Victoria oats were inoculated by the carborundum method with fresh barley virus juice on two occasions with negative results. Also, diluted extract was poured on the soil of several pots of Victoria oats with negative results.

Turkey and Harvest Queen wheat were inoculated by carborundum method with weak to mild susceptibility and results. Also diluted extract was poured on the soil of several pots with negative results.

The value of these differential tests points out that barley mosaic does not appear to give clearly recognizable infection in Zea mays as does

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[4] McKinney stated that the wheat mosaic sent to him from Stillwater probably was Marmor virgatum.

[5] Consists of placing a drop of virus juice from a pipette at the ligule of the oldest leaf and making 15 - 20 punctures through the drop with a fine point, preferably an insect mounting pin.

wheat mosaic, nor does barley mosaic give comparable results in Harvest Queen and Turkey wheats.

Effect of Temperature and Dilution with Barley Mosaic

Two experiments were set up for barley inoculation with fresh unfiltered barley virus, one at 70° F., and the other at 93° F. in combination with extract dilutions. Each test consisted of 20 pots of young barley plants with 4 to 6 plants per pot. In both cases inoculation was by the carborundum-stripping method where one group of plants was inoculated at 93° F., and held at that temperature for 24 hours. Each group of 20 pots was divided into 4 sections of 5 pots per section for dilutions. A large volume of extract was expressed from diseased tissue in 2 cc. of distilled water, and this procedure was repeated until 8 cc. of extract was obtained.[6] The virus juice was then divided into 4 parts of 2 cc. each for the 2 experiments. Inoculation proceeded with 2, 12, 22, and 32 cc. dilutions of virus by distilled water respectively, and at the end of 24 hours the plants were taken to the greenhouse.[7] Results are shown in Table 1 and 2. The higher temperature at 22 cc. dilution obviously gives a higher percentage of infection while the concentrated virus at either temperature gives poor results.

This information was utilized in preparation and inoculation of further tests. Though 22 cc. dilution gave the greatest percentage of infection, 12 cc. dilution was preferred from an ease of working standpoint.

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[6] Extraction of virus juice is aided by a small quantity of distilled water.

[7] These dilutions were not end point determinations, but for information concerning the best temperature and dilution for inoculation.

TABLE 1.--Infectivity of barley mosaic in barley at 70° F. with dilutions as shown below.

Virus extract dilution	Infected plants	Healthy plants
2 cc. (concentrated virus)	1	27
Do + 10 cc. of distilled water	3	20
Do + 20 do	7	16
Do + 30 do	3	19

TABLE 2.--Infectivity of barley mosaic in barley at 93° F. with dilutions as shown below.

Virus extract dilution	Infected plants	Healthy plants
2 cc. (concentrated virus)	3	23
Do + 10 cc. of distilled water	14	10
Do + 20 do	19	7
Do + 30 do	8	18

Dilution End Points for Wheat and Barley Mosaic

Dilution of virus juice for maximum infection sheds very little or no light on the dilution end points. Thus, the reason for the following two experiments. Twenty-four pots of Michigan Amber in 4 groups of 6 pots each were inoculated at 93° C. at the following dilutions: 2 cc. of concentrated extract, 1 cc. of concentrate + 9 cc. of distilled water (designated as "A"), 1 cc. of "A" + 9 cc. of distilled water - "B", 1 cc. of "B" + 9 cc. of distilled water. At these concentrations high percentage infection appeared at all dilutions, the maximum of which was 1: 1000, indicating of course that the dilution end point is beyond 1 part virus infected juice to 1000 parts

of distilled water. Actually, there is little need to carry dilutions beyond this point because end points vary according to the original concentration of virus. Determinations of the original concentration can be done by Kjeldahl nitrogen measurements. This calls for elaborate equipment not available in this department. Results are summarized in Table 3.

The same method was employed with barley mosaic except that the dilution was carried out 1 step farther in hopes of a difference in end points. Infection appeared at a dilution of 1: 100, but not at 1: 1000 or above. Analysis of these differences under the conditions of this experiment suggests either a physical property difference or a difference in virus concentration of the original solution or both. Data of this test are shown in Table 4.

TABLE 3.--Dilution end point of wheat mosaic extract.

Dilution by distilled water	Infection	Non-infection
Full strength	positive	---
1: 10	Do	---
1: 100	Do	---
1: 1000	Do	---

TABLE 4.--Dilution end point of barley mosaic extract.

Dilution by distilled water	Infection	Non-infection
Full strength	positive	---
1: 10	Do	---
1: 100	Do	---
1: 1000	---	---

Thermal Inactivation of Wheat and Barley Virus Extract

No data are available on the thermal inactivation of infected barley juice. However, McKinney (19) in 1944 reported inactivation of wheat mosaic juice to be near 55° C. after 10 minute subjection. Therefore, a test was designed in 2 parts for comparison with McKinney's data. To obtain these results 9 pots of barley averaging 10 plants per pot were inoculated, 3 at 50° C., 60° C., and 70° C., by carborundum-stripping. While the plants for inoculation were held at 93° C., the juice was prepared in the following manner: diseased barley tissue was ground in 2 cc. of distilled water, and diluted with 10 cc. of distilled water. The solution was not filtered; however, the pulp was removed. Next, the solution was placed in a small beaker and suspended in a water bath that was being held at 50° C. (within 1° + or -) for 10 minutes.

Since a constant temperature bath was not available, a bath was improvised. This was done by using a large pan of water[8] which was heated by a bunsen burner. A centigrade thermometer was suspended in the water near the beaker for the temperature readings. The same method was employed for all 10 degree temperature changes. Following each treatment, the inoculation was performed as previously mentioned. With completion of the above test the same sequence was repeated for wheat mosaic, and the results of both experiments are presented in Tables 5 and 6.

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[8] Larger the volume, the easier the temperature control.

TABLE 5.--Thermal inactivation of barley virus juice.

Temperature	Degree of infection	Non-infection
50° C.	Severe	---
60° C.	Mild	---
70° C.	---	None

TABLE 6.--Thermal inactivation of wheat virus juice.

Temperature	Degree of infection	Non-infection
50° C.	Severe	---
60° C.	Do	---
70° C.	Do	---

In the case of severe infection of wheat mosaic at 70° C. in Table 6, it should be explained that only one plant out of thirty developed symptoms. Due to the fact that space was inadequate for keeping all experiments in insect proof chambers, the one diseased plant may be the result of an unknown vector. But the fact, that the infection in barley was mild at 60° C. and severe in wheat at that temperature seems to indicate once again the more probable difference between the two viruses.

pH Range of Infectivity for Wheat and Barley Mosaic

The final experiment for comparison was for the range of pH infectivity. In this case titration was done with .1 N sodium hydroxide and 10% acetic acid over a pH range of 1.85 to 11.85. Since this type of experiment is time-consuming, it was not possible to run tests for both viruses in the

same day. This meant that the Beckman pH meter had to be calibrated for room temperature on two separate occasions. Before using the pH meter the barley virus extract was expressed in 2 cc. of distilled water and diluted with 10 cc. of distilled water. This extraction gave a pH reading of 5.85, which was approximately the same as the wheat virus. Each successive extraction was varied one unit from the previous reading until the indicated range was covered. The barley virus at each pH change was transmitted into 2 pots of barley and 2 pots of wheat averaging 8-10 plants per pot. The plants were then held at 93° C. for 24 hours before being taken to the greenhouse. The same technique was followed for the transfer of wheat mosaic into barley and wheat. Summarization appears in tabular form in Tables 7 and 8.

TABLE 7.--Range of infectivity in wheat and barley seedlings by pH changes in expressed juice from mosaic infected barley leaves.

	Barley mosaic			
	Wheat		Barley	
pH	No. infected	Healthy	No. infected	Healthy
1.85	0	16	0	14
2.85	0	18	1	13
3.85	2	16	6	10
4.85	19	1	17	0
5.85	18	0	15	0
6.85	17	0	18	0
7.85	18	0	14	4
8.90	17	1	16	1
9.85	7	10	13	7
10.85	0	18	0	18
11.85	0	19	3	13

Realizing that symptoms alone are often misleading the 3 infected barley plants at a pH of 11.85 may be in error. Likewise, one infected barley plant at a pH of 2.85 is doubtful, but there is a significant difference in suscep-

tibility at 3.85 and 9.85. Also, it was interesting to note that symptoms from barley mosaic appeared in barley before symptoms were observed in wheat at 4.85 and 5.85.

TABLE 8.--Range of infectivity in wheat and barley seedlings by pH changes in expressed juice from mosaic infected wheat leaves.

	Wheat mosaic			
	Wheat		Barley	
pH	No. infected	Healthy	No. infected	Healthy
1.85	0	21	0	13
2.85	0	19	0	15
3.85	2	18	0	15
4.85	17	0	3	15
5.85	18	0	5	9
6.85	21	1	15	4
7.85	16	0	7	10
8.85	13	4	5	13
9.85	16	2	1	18
10.85	0	17	0	17
11.85	0	21	0	17

These data show a consistant trend for less susceptibility in barley than in wheat when infected with wheat mosaic. Also, the symptoms appeared first in wheat and later in barley at a pH of 5.85 and 6.85.

DISCUSSION

It is unusual that a single virus diseased barley plant could give rise to such extensive investigation, not only for its own physical properties, but for comparisons between two hosts. On this basis it was earnestly endeavered to distinguish between wheat mosaic and barley mosaic, or to prove then one and the same. It was a natural, so to speak, in the way of a research problem for these reasons: (1) the virus host was barley which can be grown during the high summer temperatures, (2) the virus was not inactivated by high summer temperatures, (3) it could be readily transmitted by infected juice, (4) it can be cultured indefinitely without loss of infectivity, (5) masking of symptoms is not pronounced at high temperatures on the more susceptible varieties, and (6) fifty varieties of barley were at a desirable age for infection when these researches were begun.

Since only one barley plant was known to be diseased, there has been considerable speculation about whether it will reappear in the field or whether it was just a freak of nature. In either event it seems logical that the virus was transmitted as wheat mosaic or a mutation, thus becoming a distinctly separate virus. Both conditions are plausible, due to the fact, that either virus will infect the two hosts, wheat and barley. From the data of the experiments the writer leans toward the supposition of different viruses. Though the viruses are undoubtedly closely related as a result of common origin, barley mosaic seems to be somewhat better adapted to barley than is wheat mosaic. If barley mosaic continues to appear in the years to come, its evolutionary change may result in a greater separation between the two viruses.

At present in Oklahoma, it is not known how mosaic manages to oversummer. Yet, it is known that there are native grasses in this area which

harbor virus. Whether this is a source for reinfection, is likewise unknown. Still, alternate grass hosts seem to be a good possibility, especially since much experimentation discourages all but the insect vector.

In viewing some 35 virus infected barley varieties, one can not avoid noticing similar symptoms in various combinations and varieties. This fact could easily infer that a single virus was not isolated, but rather, a composite of two or more. A determination of this type lies beyond the scope of this paper, and is presented only for consideration. When many varieties are infected by a virus, the resulting reactions can be accounted for by varietal differences; but if there is a composite of viruses, it seems logical to surmise that segregation will occur in certain varieties; thus, accounting for different incubation periods, virus combinations, and symptoms.

With regard to high summer temperatures, masking of virus symptoms frequently presents a serious handicap. For instance, a variety may be obviously infected, but with a steady rise in temperature, as manifested in summer, the symptoms gradually disappear. In this condition the plant is actually a carrier, that reiterates virus symptoms with the approach of lower over-all temperatures. Up to a given point, the lower the temperature, the more severe the symptoms. At its maximum the virulence is ultimately limited by varietal susceptibility. Consequently, many diseased plants escape detection through masking.

The insect transmission trial produced negative results, but certainly did not prove that an insect is not a vector. The fact that check plants occasionally develop infection, intimates that the causal agent may be insect-borne. At any rate, this preliminary experiment has led to a much more thorough approach in the way of a separate problem and an extensive investigation is currently in progress.

Why infection takes place at one time and not at another, when conditions appear to be identical, is a most perplexing problem. Equally baffling, is the difference in per cent of infection from time to time under similar circumstances. In culturing barley mosaic 100 per cent infection was not uncommon, but infection in the culturing of wheat mosaic was rarely, if ever, 100 per cent. No doubt, the answer can be summed up by physiological differences within the plant, but that sheds no light on the cause. This difference makes it difficult to evaluate differential inoculations. Hence, an inoculation may have to be repeated many times before infection occurs. The big question is where to stop before assuming that a plant is not susceptible. Therefore, the fact that Zea mays L. and Victoria oats were not infected by barley mosaic, in contrast to wheat mosaic, is not conclusive proof that infection could not take place. Consequently, it only tends to indicate a difference between the two viruses.

For some time virus research has been on a cooperative basis, with free exchange of materials. Samples of wheat mosaic sent to Dr. McKinney were tentatively identified by him as Marmor virgatum, and on the basis of properties as observed at Stillwater this identification was verified. Also, he stated that barley mosaic had certain properties in common with Marmor virgatum; yet, it possibly was a different virus. As a result of tests at Stillwater, that possibility is substantiated.

Mosaic infested soil from east of the Mississippi river produced infection in wheat under Oklahoma environmental conditions. However, soil west of the river is not known to produce infection. This means that the mechanism for infection is something within the soil proper, rather than environmental. The factor within the soil which allows for transmission may be the result of eastern environmental conditions, but there seems to be little information

regarding this phenomenon.

In the beginning, culture of the barley virus was aided materially by natural conditions. Since a higher percentage of infection was obtained at temperatures near 93° F. (Table 2), the hot summer weather played an important and favorable part. Likewise, the fact that extraction of pure virus juice yields such a small volume, it was necessary to dilute it slightly for sufficient solution with which to work. Diluting 2 to 3 cc. of extract with 10 to 20 cc. of distilled water gave maximum infection as shown in Table 2. Thus, high summer temperatures and required dilution were factors of necessity, that later experimentation proved most satisfactory.

SUMMARY

In the spring of 1948 a single barley plant was found near Stillwater, Oklahoma, that appeared to be infected with a virus disease. Juice was expressed from this one barley plant and manually inoculated into 50 varieties of barley. Over half of these varieties developed symptoms that resembled to some degree, those seen on the original host plant.

The history of wheat and barley mosaic is reviewed as it occurs in or about Stillwater, together with descriptions of symptoms of wheat mosaic in wheat, and barley mosaic in barley. Though, it is possible to infect either host with either virus, no attempt has been made to distinguish between viruses on the basis of minute symptom differences.

Because of irregularity in occurrence and the scarcity of infected plants, wheat mosaic accounts for no appreciable loss in Oklahoma. As a result, no control measures are in effect. In event of an increase in prevalence and severity, control would not be a major problem, because there are many varieties of wheat that are resistant or partly resistant to mosaic.

A description is given of the technique employed for manual inoculation with carborundum and virus extract. Also it was found that infection could be aided by dilution of virus extract (1 part virus juice to 5 parts distilled water) and inoculation at temperatures near 93° F. Inoculated plants remained at that temperature about 24 hours.

Diseased leaf tissue from barley and wheat plants was desiccated by calcium chloride and held under refrigeration about 3 months. The barley desiccated tissue failed to produce infection when inoculated into barley, and the wheat desiccated material produced very mild infection when inoculated into wheat.

No infection occurred in barley from barley virus juice that had been

under refrigeration for 3½ months.

Barley virus juice failed to produce infection when poured on soil growing oat and wheat seedlings.

When eastern wheats were grown at Stillwater in wheat mosaic infested soil from North and South Carolina, they became infected, whereas barley and Pawnee wheat from this area failed to produce symptoms. Unfortunately, the same experiment was not duplicated in North and South Carolina. As a result it would seem that environmental conditions in Oklahoma are not a limiting factor for soil-borne infection.

It is known that wheat mosaic is not seed-borne, either east of the Mississippi river or west of it. While, it is soil-borne east of the Mississippi river; it does not appear to be soil-borne in Oklahoma. Such tests as were and are being carried out at Stillwater with insects, have not shed any light on the vector problem. However, the ever accusing finger persistantly points towards an insect vector.

On the basis of, and under the prevailing conditions of experiments as conducted at Stillwater, wheat mosaic and barley mosaic do not appear to be the same virus. In spite of the fact that the two viruses infect either host and are difficult or impossible to distinguish on the basis of symptoms, each virus tends to be better adapted to its respective host. For instance, barley mosaic in spelt was much more virulent than wheat mosaic in spelt, also percentage of infection in barley from inoculation with the barley virus is, for the most part, consistantly higher than wheat mosaic in wheat. In addition when both hosts are inoculated with the same virus, symptoms regularly appear somewhat sooner on the host to which the virus is more adapted. Furthermore, differential inoculations with barley mosaic failed to infect corn, oats and produced poor results in Turkey and Harvest Queen wheats. The previously

mentioned facts are in opposition to wheat mosaic.

The difference in end point dilutions between the two viruses may indicate a difference of the viruses, but there are too many variables in dilutions for this to be accepted as reliable datum.

Thermal inactivation indicated that barley mosaic extract is somewhat less tolerant of high temperatures than is wheat mosaic.

When extracted juice from barley mosaic tissue is subjected to changes in pH, there is little difference in susceptibility of wheat and barley. However, there is a perceptible difference when wheat mosaic extract undergoes pH changes. In the latter case infection in wheat was considerably higher.

Despite the obvious differences between the two mosaics, they are undoubtedly very closely related; therefore, experimental results show similarities as well as differences.

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LITERATURE CITED

1. Breed, R. S., E. G. D. Murray, and A. P. Hitchens. Bergey's Manual of Determinative Bacteriology. 1529 pp. The Williams and Wilkins Co., Baltimore, Md. 1948.

2. Burnet, F. G. Virus as Organism. 134 pp. Harvard University Press, Cambridge, Mass. 1946.

3. Chester, K. Starr. Nature and Prevention of Plant Diseases. 525 pp. The Blakiston Co., Philadelphia, Pa. 1947.

4. Herbert, T. T., and G. K. Middleton. Mosaic of barley in North Carolina. U. S. Dept. Agr., Pl. Dis. Reptr. 32: 435-436. 1948.

5. Hitchcock, A. S. The genera of grasses in the U. S. with special reference to the economic species. U. S. Dept. Agr. Bul. 722. 1920.

6. Johnson, A. G., H. H. McKinney, R. W. Webb, and C. E. Leighty. The rosette disease of wheat and its control. U. S. Dept. Agr. Farmers' Bul. 1414: 1-10. 1924.

7. Johnson, Folke. Heat inactivation of wheat mosaic virus in soils. Science 95: 610. 1942.

8. McKinney, H. H. Investigations of the rosette disease of wheat and its control. Jour. Agr. Res. (U. S.) 23: 771-800. 1923.

9. _____, S. H. Eckerson, and R. W. Webb. The intracellular bodies associated with the rosette disease and a mosaic-like leaf mottling of wheat. Jour. Agr. Res. (U. S.) 26: 605-608. 1923.

10. _____. Influence of soil temperature and moisture on infection of wheat seedlings by Helminthosporium sativum. Jour. Agr. Res. (U. S.) 26: 195-218. 1923.

11. _____, and W. H. Larrimer. Symptoms of wheat rosette compared with those produced by certain insects. U. S. Dept. Agr. Bul. 1137: 1-8. 1923.

12. _____. A mosaic disease of winter wheat and winter rye. U. S. Dept. Agr. Bul. 1361: 1-11. 1925.

13. _____, R. W. Webb, and G. H. Dungan. Wheat rosette and its control. Ill. Agr. Exp. Sta. Bul. 264: 275-296. 1925.

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

15. _____, and W. J. Sando. The behavior of winter wheat in artificial environments. Science 71: 668-670. 1930.

16. _____. Differentiation of viruses causing green and yellow mosaics of wheat. Science 73: 750-651. 1931.

17. _____. Virus mutation and the gene concept. Jour. Heredity 28: 51-57. 1937.

18. _____. Mosaic diseases of wheats and related cereals. U. S. Dept. Agr. Cir. 442. 1937.

19. _____. Descriptions of viruses. Jour. Wash. Acad. Sci. 34: 322-329. 1944.

20. _____. Mosaics of winter oats induced by soil-borne viruses. Phytopath. 36: 359-369. 1946.

21. _____. Soil factors in relation to incidence and symptom-expression of virus diseases. Soil Sci. 61: 93-100. 1946.

22. Rawlins, T. E., and C. M. Tompkins. The use of carborundum as an abrasive in plant-virus inoculations. (Abstr.) Phytopath. 24: 1147. 1934.

23. Stanley, W. M. Crystalline tobacco-mosaic virus protein. Amer. Jour. Bot. 24: 59-68. 1937.

24. Thornberry, H. H. Effect of phosphate buffers on infectivity of tobacco-mosaic virus. Phytopath. 25: 618-627. 1935.

25. Tisdale, W. H., G. H. Dungan, and C. E. Leighty. Flag smut of wheat, with special reference to varietal resistance. Ill. Agr. Exp. Sta. Bul. 242: 511-538. 1923.

26. Wada, E., and H. Fukano. On the difference and discrimination of wheat mosaics in Japan. Jour. Imp. Agr. Sta. Tokyo 3: 124-128. 1937. (In Japanese, English summary.)

27. Webb, R. W., C. E. Leighty, G. H. Dungan, and J. B. Kendrick. Varietal resistance in winter wheat to the rosette disease. Jour. Agr. Res. (U. S.) 26: 261-270. 1923.

28. _____. Soil factors influencing the development of the mosaic disease of winter wheat. Jour. Agr. Res. (U. S.) 35: 587-614. 1927.

29. _____. Further studies on the soil relationships of the mosaic disease of winter wheat. Jour. Agr. Res. (U. S.) 36: 53-75. 1928.

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