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POSSIBLE FACTORS INFLUENCING THE TRANSMISSION AND CONTROL OF THE SOIL-BORNE WHEAT MOSAIC VIRUS IN KANSAS

by

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INTRODUCTION

The state of Kansas is primarily an agricultural state and the major share of the income of Kansans is derived from agriculture and industries which are directly dependent on agriculture (1). Wheat is one of the most important agricultural products in Kansas and the state leads all others in the United States in wheat production (5). This lead in wheat production has earned Kansas the title the "Wheat State" (5). In view of the important role wheat plays in the economy of Kansas, any threat to wheat production in the state, whether real or potential, becomes a direct threat to the state's economy.

Diseases are some of the threats to the production of wheat and one of these diseases is the soil-borne wheat mosaic. Since the disease was reported from Kansas in 1949 (41), three epiphytotics have been experienced in the state (55). The latest epiphytotic in Kansas occurred in the 1956-1957 growing season causing an estimated loss of some 2,082,000 bushels valued at approximately $3,950,000 (55).

Workers in Kansas and elsewhere in the United States of America and also abroad have been studying the disease since McKinney first observed it in Madison County Illinois in 1919 (32). These workers have studied various aspects of the soil-borne wheat mosaic such as the comparison of the characteristics of the disease in Kansas and elsewhere (55), soil factors influencing the incidence and symptom expression of the disease (38, 72, 73) and the genetics of resistance against the virus disease in wheat strains (47).

However, to date, the vector(s) involved in the transmission of the virus is (are) not known. From the fall of 1962 to the summer of 1964, attempts were made to find out at least some of the possible factors influencing the transmission of the virus in Kansas. This was the research problem taken and forms the theme of this paper.

LITERATURE REVIEW

History and Distribution

The soil-borne wheat mosaic was first reported from Madison County Illinois in 1919 (32). Humphrey and Johnson applied the name "take-all" to the disease shortly after its discovery (32, 19). The name "take-all" was applied to the disease because it was thought to be identical to "take-all" disease occurring in Australia and Europe (32, 45). Further investigations by McKinney, however, showed that the disease was not the same as the Australian "take-all" (32). The soil-borne wheat mosaic differs from take-all in symptomatology and host ranges. Also, Ophiobolus graminis Sacc. which causes take-all, has not been found in association with the wheat mosaic under discussion (45). McKinney therefore, gave the name "the rosette disease of wheat" to the disease (32). The name "rosette" was applied to the disease due to the abnormal appearance of the diseased plants of Harvest Queen, the variety in which the disease was first observed (25). In this wheat variety, Harvest Queen, the disease causes excessive proliferation and dwarfing, a condition known as rosette (25, 33).

McKinney showed that the infective agent was soil-borne (32). Further experimentation showed, by 1925 (33), that juice from diseased plants could be used to transmit the disease and that the same virus could produce rosette and leaf mottling. Various terms such as "wheat mosaic," "soil-borne wheat mosaic," and "Prairie wheat mosaic" have been used as common names for the disease since 1925, the term rosette having been retained as a name for the symptom produced only by some susceptible wheat varieties (25).

In the Great Plains, mosaic was first reported as such on wheat by Peltier in 1922 in experimental plots at Lincoln, Nebraska (33).

Though the first report of the soil-borne wheat mosaic in Kansas is given as 1949 (41, 55), the literature shows that as early as the spring of 1929

Fellows and Johnston had observed a yellow mosaic on wheat scattered on wheat plants in experimental plots at Manhattan, Kansas (36). In addition to Illinois and Kansas, the disease has been reported in Indiana (32), South Carolina (2), Iowa, Virginia, Maryland, North Carolina (41), Missouri (7), and Oklahoma (71).

Economic Importance

Roane et al, stated that the soil-borne wheat mosaic is a factor to be reckoned with in wheat production in Virginia (50). From Illinois, also, it is reported that but for the use of resistant varieties there would be no wheat grown in certain areas of the state (25). Kansas has experienced three epiphytotics of the soil-borne wheat mosaic disease, all in the eastern third of the state where the disease only occurs or has been found to occur so far. The first reported epiphytotic occurred in the 1951-1952 growing season. Average losses were estimated at 10% in the diseased fields checked, and valued at $1,500,000 (12, 57). In the 1953-1954 growing season there was another epiphytotic in Kansas. This one accounted for about 26% average reduction in yield with an estimated dollar loss of about $3,000,000 (57). The 1957 epiphytotic in Kansas cuased by the soil-borne wheat mosaic virus affected approximately 347,000 acres causing about 16% loss in yield and about $3,950,000 loss (58).

Losses due to this disease have been even higher elsewhere in the United States. In Illinois losses amounting to 40 per cent have been reported (45). Bretz, from a survey made in Missouri and Illinois reported losses up to 50% in some fields (7). The disease also causes late maturity and shrivelling of the grains of diseased plants (45).

Host Range

The soil-borne wheat mosaic virus has a narrow host range. Investigations have shown that several hundred varieties of winter wheat and two varieties of winter rye are susceptible to the virus (55). Barley, emmer, spelt, and a wild grass, Bromus commutatus Schrad., are also reported to be susceptible (25, 36). McKinney reports that "certain spring varieties develop mosaics when sown in the autumn in a region of mild winters (36). Sill, also reports that several varieties of spring wheat "developed possible but very mild symptoms in the field but never in the greenhouse under controlled environment (55).

Symptomatology

The symptoms that first attracted attention to the soil-borne wheat mosaic were field spotting, stunted diseased plants and the production of a large number of secondary tillers giving the plants a rosette appearance (32). This condition, found only in rosette-susceptible wheat varieties, such as Harvest Queen, has not been seen in Kansas in the field. However, the rosette symptoms have developed in control chambers in Kansas when rosette-susceptible varieties were seeded in virus-infested soil from Illinois (55). The rosetted plants appear a darker green than healthy ones (23).

Symptoms in Nonrosetting Wheat Varieties

Field Symptoms. Field symptoms, as a rule, are noticeable only in the spring when the wheat plants start their active growth. However, with a long period of cool growing weather in the fall occasional field symptoms have been observed (25, 36). In Kansas symptoms have not yet been observed in the fall (55)*. In the spring, the symptoms appear in the field as yellowish to light-green areas when compared to the adjacent healthy areas. Such areas vary in dimensions from a few inches to 50 or more feet across (25, 55). The yellowish to light-green color which distinguishes the diseased patches from the adjacent healthy areas may turn into yellow-bronze later. With the onset of hot weather, the yellowing begins to disappear, the disappearance becoming complete with persistent hot weather (55). Sometimes the diseased plants may completely recover in which case they can only be recognized by the slight stunting, the smaller and fewer heads, and the striking green spotting in the fields at the time of ripening since the diseased plants tend to be retarded in maturity. As regards topography this does not seem to be important in the initiation of the disease in the field. Poorly and well drained areas as well as low and high grounds have been observed to develop the disease (32, 55).

Individual Plant Symptoms. "Some other diseases, winter-killing, water damage, and lack of proper plant food may also show up in spots or patches and be mistaken for mosaic. The best way to tell mosaic patches from these others is to look for the mottling of the leaves" (25). Symptoms on individual infected winter wheat plants develop in the spring when the environmental conditions are favorable. These symptoms show as a mosaic and mottle condition consisting of yellowish areas intermingled with normal green tissues (23, 55). This mosaic and mottle condition is accompanied by some degree of purpling confined to the leaf edges but as the weather warms up considerably, these leaf symptoms may completely disappear (55). Under favorable conditions, however, the mottling may persist as long as the leaves are green, with some whitish streaks and blotches (25, 55). In addition to the mosaic and mottling condition, diseased wheat plants are also delayed in maturity, produce fewer and shorter heads with shrivelled grains. They are also characterized by moderate stunting (23, 24, 55).

Cell Inclusions

Cellular inclusions have been found in wheat plants showing the green-mosaic and rosette condition but not in plants showing yellow mosaic (34). These cell inclusions have been found in the crown tissue, roots, leaf sheaths, and leaves of rosette or mottled plants. The shape is variable, ranging from round through irregular to plate-like. In size, they range from smaller to larger than the host nuclei and they usually occur singly in host cells either free from or in close contact with the nucleus (44).

In Japan these cell inclusions, also known as X-bodies, have been found associated with both the yellow and green mosaics of wheat (69). The Japanese workers reported two different kinds of X-bodies. The type A found in association with yellow mosaic is vacuolate, oval or elongated, smaller or larger than the host nucleus and occurs singly. The other type, type B., associated with the green mosaic is homogeneous, oval or irregular and smaller than the type A and occurring in groups of two to three or up to five in one cell. These workers also report finding an intermediate X-body which sometimes resembles type A and sometimes type B. This type has been found in connection with the composite type of mosaic which involves both yellow and green mosaics (69, 70).

Transmission

Various methods of transmission of the soil-borne wheat mosaic virus have been studied by various workers since it was shown that the infective agent was soil-borne (32).

Some success in transmission using abrasive inoculation has been reported (33, 34, 55). Sill failed to transmit the disease using seeds from diseased wheat plants (55). This inability to transmit the virus through seeds seems to be a general characteristic (25, 32, 45).

In addition to abrasive inoculation and seed transmission studies, other studies involving living organisms have been made. Among the living organisms which have been tried for transmission are leaf hoppers (23), species of aphid and an eriophyid mite (55). All these attempts have given only negative results. These negative results agree with McKinney's early work (32). Nematodes also have been tried. Aphelenchus avenae Bastian, 1865 and Panagrolaimus sp. Fuchs, 1930, failed to transmit the virus (43). Johnson also collected nematodes from infested soil and used them for transmission work. His results also were negative (23).

Work with infested soil has given rather consistent positive results (38, 42, 55).

Further work has pointed rather strongly to the existence of an "agent (vector) closely associated with the roots of mosaic diseased plants growing in naturally infested soil being involved in the natural overseasoning of, and inoculation with the soil-borne cereal viruses," so write McKinney et al. (46). Using autoclaved test-soils which had been inoculated several months prior to seeding with thoroughly washed roots from mosaic plants which had been naturally infected in the field, workers have obtained natural infection (46). Natural infection has failed to take place, on the other hand, when susceptible wheat varieties were seeded in autoclaved soil which had been inoculated with virus laden juice, or leaves, or roots from mosaic diseased plants that had been inoculated manually. Neither did infection develop in plants seeded in autoclaved soil to which had been added virus-laden leaves from mosaic diseased plants that had been infected naturally in the field (46).

Other workers have reported experiments and results which strongly suggest that a living factor besides the host and the virus is involved in the transmission of the disease (20,25, 73).

Factors Influencing Transmission. Soil moisture and soil temperatures are important factors which influence the transmission of the disease (38, 72). Symptoms develop best when temperatures are maintained around 60° F. with a photoperiod of about eight hours (35). Ikata et al. also reported the optimum soil temperature to be about 15° C. They reported that symptoms were fairly severe at 10° C., inconspicuous at 20° C., and imperceptible at 25° C. (20). Similar reports show that the optimum soil and air temperature for symptom development is around 60° F. (55). Ample rainfall during the growing season, and certainly from October to March, favors infection.(25, 38, 55).

Adequate sunlight and a photoperiod of 8-hours also favor infection (25, 42). With regards to the temperature and photoperiod, an exception is taken for a winter rye variety collected by Sill in Kansas. This winter rye develops symptoms in the greenhouse at temperatures of 70° F. or more and no attempt has been made to control daylength (53).

Using Currell wheat, Webb found that virus infection started before the 7th day after seeding and the greatest amount of mosaic occurred in plants that had been in the infested soil for 28 days before they were transplanted (72). It has also been reported that disease was more severe in fields where continuous wheat cropping without the application of fertilizers had been the practice. But where wheat had followed a legume the previous season or where adequate fertilizer had been applied, the disease was absent or less severe (71). Koehler et al. (25) reported that high nitrogen levels tend to obscure the symptoms of the disease and cause infected susceptible plants to look healthier.

Causal Agent

The soil-borne wheat mosaic is caused by a virus, Marmor tritici H. emend. McK. (37). There appear to be two strains at least, involved, namely the green or mosaic-rosette virus and the yellow-mosaic virus (38, 70). These strains were so named because plants artificially inoculated with purified strains of the virus and grown under controlled conditions showed either green mottling or yellow mottling depending on the virus strain used. Only the green variety, Marmor tritici var. typicum McK. causes both rosette and mottling (25). In nonrosetting wheat varieties the yellow mosaic, Marmor tritici var. fulvum McK., may cause the more severe injury (25). Wada etal. (70) on the other hand, reported that two "different viruses acting singly were believed to be responsible for the green and yellow mosaics...."

Control

As early as 1920 observations were made on the susceptibility of some wheat varieties to the rosette phase (32, 36, 72). In Kansas, from 1951 varietal test plots have been planted each year and among the varieties found to be resistant are Concho, Comanche, and Ottawa (55, 58, 59). Other workers elsewhere in the United States have also reported varietal resistance in their areas (4, 48, 50).

Workers have also tried the use of certain chemicals and also steam as means of controlling the disease. Among the chemicals which have proved effective against the virus are formaldehyde, chloropicrin, methyl bromide, carbon disulfide, calcium cyanide, naphthalene, and rotenone (22, 32, 41, 46). Ethylene dichloride gave no control (22), neither did Toluene (41).

McKinney also reports that late seeding to the extent that the seedlings to not emerge till the following spring also controls the disease (32). Sill however, reported that in Kansas the date of planting seemed to be of no consequence, rather the enviromental factors were the important ones.(55).

MATERIALS AND METHODS

Plants Used

The wheat varieties used in the experiments were Pawnee, Ponca and Ottawa. Pawnee is a wheat variety developed at the Kansas and Nebraska Agricultural Experiment Stations after the crosses had been made at Kansas State University. The cross was made between Kawvale and Tenmarq (6). The Pawnee variety is susceptible to the soil-borne wheat mosaic virus (59). Ponca, selected from a cross between selections of Kawvale x Marquillo and Kawvale x Tenmarq was also released by the Kansas Agricultural Experiment Station. It is susceptible to the soil-borne wheat mosaic virus. Ottawa on the other hand is resistant to the soil-borne wheat mosaic virus. It is one of the three resistant varieties recommended for the state of Kansas (5, 59). Ottawa was selected from a Mediterranean—Hope—Pawnee x Oro - Illinois No. 1 - Comanche cross (5).

A rye variety first tried by Sill (53) and found to develop mosaic symptoms at temperatures of 70° F. and above was also used in the studies.

Soil

One of the infested plots used is located approximately $\frac{1}{4}$ mile north-west of the Kansas State University campus at Manhattan. It is part of the Agronomy Farm and it is known to be infested with the soil-borne wheat mosaic virus. A similarly infested soil was selected at Powhattan, Kansas, where there are also University Experimental Farms.

Sand or soil to be autoclaved for greenhouse use was put in buckets measuring 12 inches across and 12.5 inches deep. The buckets were then left in the autoclave for two and a half hours at 15 lbs. pressure. They were then stored in the greenhouse and used later for the experiments. For the greenhouse experiments, 6 inch clay pots were used.

Temperature Control Chamber

The chamber was set to regulate the temperature at around 60° F. Both a thermograph and a thermometer were placed in the chamber. The thermometer was used to check the accuracy of the thermograph. The control chamber experiments were performed from early winter to late spring and no attempt was made to control day length. The plants were not placed in the temperature control chamber until the chamber had been found to maintain the desired temperature of about 60° F. The actual temperature oscillated between 55° F. and 65° F.

Chemicals Used in the Field

The chemicals which were used for the study were vorlex, methyl bromide, 1,3-dichloroporopene-1, 2-dichloroproyane, (D-D) and nemagon. Nemagon is a soil fumigant put out by the Shell Chemical Corporation. It is a trade name for the compound 1,2-dibromo-3-chloropropane. It was applied directly to the wheat seeds as a wettable powder at the rate of 2 lbs. actual nemagon/75 lbs. of wheat which was then seeded at the rate of 1 gm/linear foot.

D-D, also produced by the Shell Chemical Corporation is the trade name for a volatile soil fumigant which is made up of 1,3-dichloropropene and 1,2-dichloropropane in about equal parts together with small amounts of other chlorinated compounds. It was applied by using a hand injector for soil calibrated to deliver the liquid at the rate of 40 gallons per acre. Small quantities of the D-D were injected into the soil to a depth of about eight inches at intervals of about a foot. The hole left by the injector was immediately covered with soil.

Methyl bromide is a volatile soil fumigant of bromomethane. It is a gas at ordinary temperatures with a boiling point at 40° F. It is therefore sold under pressure in 1 lb. containers under the trade name Dowfume MC-2. Dowfume MC-2 contains 98 per cent methyl bromide and 2 per cent chloropicrin. It is manufactured by the Dow Chemical Company. The methyl bromide was applied by evaporating it in shallow clay trays, placed on the surface of the area to be thus treated under a gas-tight plastic tarpaulin. The tarpaulin was supported a few inches above the surface of the soil by small mounds of soil, the edges of the tarpaulin being buried with soil to prevent escape of the methyl bromide when released. In the clay tray was placed a piece of board through which was sticking an iron nail with the sharp end up. The nail was then passed through the bottom of an empty can slightly larger than the methyl bromide container which was placed with the top end down in the empty can so that the methyl bromide can was balanced with its top end on the sharp point of the iron nail. When all was ready a pressure enough to make the nail puncture the methyl bromide can was applied with the hand. The tarpaulin was left in place for 48 hours after which time it was removed. The plot was seeded about a month after the treatments. It is necessary to allow two or three weeks' interval between treatment and planting in order to avoid injury to the plants. The methyl bromide was applied at the rate of 2 lbs. per 120 sq. ft.

Vorlex is made up of 20% methyl isothiocyanate and 80% chlorinated C_3 hydrocarbons. It was applied as the D-D at the rate of 40 gallons per acre.

Root Staining and Clearing Chemicals

For clearing roots for microscopic study, acid fuschin in lactophenol was used. The lactophenol was prepared by putting together the following:

Phenol crystals - 20 grams

Lactic acid - 20 grams

Glycerin - 40 grams

Distilled Water - 20 ml

The acid fuchsin solution was prepared by dissolving 1 gram of acid fuchsin in 100 ml water. 5 ml of this solution in 100 ml lactophenol gave the solution used in staining the roots. Clearing was done in clear lactophenol.

Field Studies

1962-1963 Growing Season. Field studies were initiated in the 1962-1963 growing season. A plot known to be infested with the soil-borne wheat mosaic virus was selected from the Agronomy Farm located on the north-west side of the Kansas State University campus. This plot has been consistently showing soil-borne wheat mosaic symptoms from year to year. The dimensions of the plot laid out was 40 feet long and 32 feet wide. After the plot had been disked, it was divided into two sections, lengthwise. An alley of two feet width was left between the two sections. Also a border four feet wide was left around the whole plot. The final measurements for each of the two sections of the plot, therefore were: length 32 feet; width 11 feet. As shown in Fig. 1, each section was then divided into eight sub-sections, each sub-section measuring 11 feet by four feet. Each sub-section was loosened to a depth of about six inches in preparation for the chemicals to be used. Loosening the soil is necessary to ensure deep penetration of the soil by the chemicals and also to facilitate aeration necessary for getting rid of excess chemicals from the soil. After loosening the soil each of the 16 sub-sections was divided up into four rows a foot apart. The first row of each sub-section was six inches away from the end of the sub-section and the fourth or last row was also six inches away from the other end. The last row of the first sub-section therefore, was a foot away from the first row of the second sub-section and so on.

Each sub-section was randomly selected for treatment with either vorlex, D-D, methyl bromide or to be left untreated. Each section of the plot had two treatments each for vorlex, D-D, and methyl bromide. There were also two controls in each section. In all, therefore, there were four treatments with vorlex, four with D-D, and four with methyl bromide. In addition, there were four untreated sub-sections which served as control.

The 1962-1963 field studies were made primarily for finding out whether the chemicals used had any effect on the yield. Planting of the plot was done with the help of a seed planter which planted seeds one row at a time. Each sub-section was seeded with four rows of Pawnee wheat. Only the middle two rows of each four-row unit were harvested. When the wheat was dry enough it was harvested by cutting with a sickle. The chopped wheat was tied in bundles according to treatment. Threshing was done in a threshing machine and the threshed wheat was later weighed.

1963-1964 Growing Season. The plot used in the 1962-1963 growing season was again used in the 1963-1964 growing season. In the latter year, however, the area was enlarged. The layout was similar to the 1962-1963 layout except that the 1963-1964 plot had 48 sub-sections each 16 feet long and three feet wide. Also, in addition to Pawnee wheat, Ottawa wheat was planted and the chemicals used were methyl bromide, D-D, and nemagon. Only three rows of either Pawnee wheat or Ottawa wheat were seeded in each of the 48 sub-sections. The distance between the rows was one foot.

Fig. 1. 1962-1963 Plot at Manhattan, Kansas

32'

11'	1 VORLEX	10 METHYL BROMIDE	7 CONTROL	9 VORLEX	15 D-D	11 CONTROL	3 D-D	2 METHYL BROMIDE
2'								
	14 METHYL BROMIDE	13 VORLEX	8 D-D	4 CONTROL	6 METHYL BROMIDE	12 D-D	5 VORLEX	16 CONTROL

In addition to the Manhattan Plot, in the 1963-1964 growing season, a plot known to be infested with the soil-borne wheat mosaic virus was selected at Powhattan, for similar studies. The plot was laid out as shown in Fig. 2. Only methyl bromide and D-D were used here, the rest of the plot being left as control. The D-D was applied at the rate of 40 gallons per acre and the methyl bromide at the rate of 2 bls./120 sq. ft. Harvesting was done with a sickle and the wheat was threshed with a threshing machine.

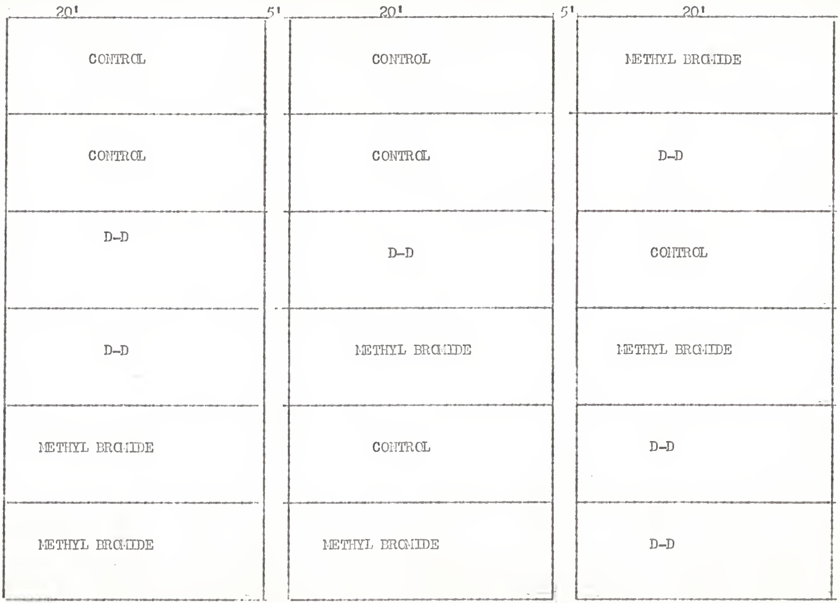
Laboratory Work

Nematode Studies. Soil samples were collected from the 1962-1963 plot from both treated and untreated areas to find out the types of nematodes present. The soil samples were taken with a shovel up to a depth of about six inches. These samples were put into plastic bags and taken to the laboratory for processing. The Cobb sifting and gravity method was used in processing the soil samples. Each sample was thoroughly mixed after which process about 2 lbs. of it was dished out into a large pail. The soil in the pail was then covered with water and the lumps were thoroughly broken up while working the mass into a thick mixture and picking out all plant debris. After allowing the thick mixture to settle for about 10 seconds it was poured through a 10-mesh screen to get rid of the coarse organic matter. The mixture was collected into a pan placed under the screen. A pint or two of water was added to the muddy mixture in the pan, thoroughly mixed and allowed to settle for about 10 seconds. It was then poured through a 25-mesh screen into another pan. The screen was then turned upside down and the bottom was washed with a spray of water into another dish. This washing was poured into a beaker. The muddy water which was poured through the 25-mesh screen was again poured through a 50-mesh screen into a pan. The 50-mesh screen was turned upside down over another pan and the bottom washed with a spray of water into the pan. This washing was added to that in the beaker. The process was repeated with a 100- and a 200- mesh screens. A Scotty tissue was placed over the mouth of the beaker and a piece of cheese cloth was placed over the Scotty tissue, and tied to the beaker with a rubber band. This done, the beaker was inverted into a Baermann funnel partly filled with water. Attached to the stem of the funnel was a piece of rubber tubing closed by a clamp. The setup was left for 48 hours after which time 20 ml portions were taken out into test tubes by releasing the clamp on the rubber tubing. The clear water drawn out was then poured into syracuse watch glasses and examined under a dissecting microscope for nematodes.

Root Staining and Clearing

To find out whether there were any organisms infecting the wheat roots, plants were carefully uprooted from a plot in Ashland near Manhattan, Kansas and also from treated as well as untreated areas of the 1963-1964 plots at Manhattan and Powhattan. The roots were thoroughly washed with water and then boiled in a .05% solution of acid fuschin in lactophenol. After boiling for two minutes the roots were taken out, thoroughly rinsed with water, blotted dry with paper towels and thinly spread in petri dishes containing clear lactophenol. They were left in the lactophenol until most of the red dye in the root cells was taken out. This took a few days. After this, with the help of a dissecting microscope, the roots showing some kind of cellular contents were picked out, placed on microscope slides and exmmined under the compound microscope after cover slips had been placed on them.

Fig. 2. 1963-1964 Plot at Powhattan, Kansas



Greenhouse Work

Nematode Studies. Soil samples were taken from the virus infested Manhattan plot of 1962-1963 as described elsewhere and put into clay pots. The pots were seeded with soybeans to increase the nematode population. The nematodes were later extracted from the soil by the Cobb sifting and gravity method. The nematodes thus extracted were divided into two sets. One set was disinfested with 1-1000 mercuric chloride for two minutes and washed with distilled water for another five minutes. The other set was not treated with mercuric chloride. Each set was then used separately to attempt to inoculate Pawnee wheat seedlings growing in autoclaved sand by pouring the nematodes on the roots as the seedlings were transplanted into fresh autoclaved sand. In all a total of 20 pots (10 for each set) with two Pawnee wheat seedlings in them and a few hundred nematodes added were used for the study. The pots were then left in a temperature control chamber with temperatures of 55° F. minimum and 65° F. maximum for a period of 60 days. The plants were examined periodically for soil-borne wheat mosaic symptoms.

Other Greenhouse Studies. Pawnee wheat and Ottawa wheat plants were collected from the control areas of the 1963-1964 plot at Manhattan. The plot was seeded on October 18 and the plants were collected on December 17. These plants were thoroughly washed with a spray of distilled water, then in a solution of Sun-flo cleanser for about five seconds, then again thoroughly rinsed with distilled water. Two each of the Pawnee wheat plants were transplanted into 10 pots of autoclaved sand. Two each of the Ottawa wheat plants were also transplanted into 10 separate pots containing autoclaved sand. Two each of Pawnee wheat and a rye variety (53) which had been growing on filter paper in petri dishes were transplanted into each of the 20 pots above. So that in all there were 10 pots each transplanted with two Pawnee wheat plants collected from the field, two Pawnee wheat seedlings transplanted from petri dishes and two rye seedlings transplanted from petri dishes. Also another 10 pots each transplanted with Ottawa wheat collected from the field, two petri dish-grown Pawnee wheat seedlings, and two petri dish-grown seedlings.

A similar setup was made, the only difference being that instead of the autoclaved sand used in the setup described above, infested soil collected from the field was put in each of the 20 pots.

Two more setups were made. One of these was set up in the same way as the first setup except that the wheat plants from the field were collected from a non-infested field. The last setup was also similar to the second setup except that the wheat plants came from a non-infested field and the soil in the pots was from a non-infested field. These pots were then put in the temperature control chamber with the temperature kept as described elsewhere in the paper. They were observed periodically for the development of the soil-borne wheat mosaic symptoms.

RESULTS

Field Work

The 1962-1963 field work was done to study the effects of the various treatments on yield. As described under "materials and methods" the chemicals used were vorlex, methyl bromide and D-D. There were two replications. Each replication had two areas with vorlex, two with D-D, and two control untreated areas. Only the two middle rows of each treatment unit were harvested. The wheat from each block or replication was threshed together treatment by treatment. The results are shown in Table 1. After weighing the wheat for the different treatments according to blocks, the weights for wheat from the same treatment were added together. The average yield for each treatment was then

Table 1. Yield data from soil-borne mosaic virus infested field plots of 1962-1963 at Manhattan, treated with vorlex, D-D, and methyl bromide and seeded with Pawnee wheat.

	Replications			
Treatment	I Weight in grams[a]	II Weight in grams[a]	Total Weight in grams	Average Weight in grams[b]
Vorlex	61.50	114.00	175.50	87.75
Control	141.00	159.00	300.00	150.00
D-D	176.50	230.00	406.50	203.25
Methyl bromide	225.50	241.50	467.00	233.50

[a] Average weight per two eight-foot rows.

[b] Average weight per two eight-foot rows for each treatment.

calculated. As shown by Table 1, the methyl bromide treatment gave the highest yield of 233.50 grams followed by the D-D treatment with 203.25 grams. The control followed next with an average yield of 150.00 grams for each two eight-foot rows. The average yield from the vorlex treatment was less than that from the control, the yield being 87.75 grams.

The 1963-1964 field work involved studying the effects of the various treatments on symptom expression as well as on yield. In the spring of 1964 the plots at Manhattan and Powhattan were examined for mottling and mosaic symptoms and also for stunting due to the soil-borne wheat mosaic. The observations are recorded in Tables 2, 3, 4, 5 and 6.

Table 2 shows the readings for mosaic symptom expression of the Manhattan Plot of 1963-1964 for Pawnee wheat. The Pawnee wheat plants on the control areas developed symptoms which involved from 90+% of the plants on one area to 99% of the plants in another. In all over 90% of the plants on each untreated control area developed soil-borne wheat mosaic symptoms. The disease also developed on over 90% of the plants on each of the areas seeded with nemagon-treated seeds. The situation was the same on the D-D treated areas; soil-borne mosaic symptoms were expressed by over 90% of the wheat plants growing on each D-D treated area. On the methyl bromide treated areas, on the other hand, the highest observed incidence of the disease involved only about two per cent of the Pawnee wheat plants. The rest of the methyl bromide treated areas showed symptoms involving less than one per cent of the plants.

A similar observation was made on the Powhattan Plot. This observation is recorded in Table 4. On the control areas the lowest incidence of the disease involved some 30% of the plants on one area and the highest incidence involved about 75% of the plants on one untreated control area. The development of the mosaic disease ranged from about 10% to about 20% on the D-D treated areas, whereas on the areas which were treated with methyl bromide it ranged from 0% to about 10%.

As shown in Table 3, from 0% to less than 1% of the Ottawa wheat plants developed the soil-borne wheat mosaic symptoms for any given treatment, whether the treatment was nemagon, D-D, methyl bromide or control. This was to be expected, however, as this variety is highly resistant to the virus.

Readings were also taken as regards the number of plants visibly stunted in the Ponca 1963-1964 Plot at Powhattan, Kansas. As shown in Table 4, the percentage of plants visibly stunted on the control plots was between 50% and 75%; on the D-D areas it was between 10% and 20%; and on the methyl bromide treated areas between 0% and 10% of the plants were observed to be stunted from one area to the other.

In the Manhattan Plot of 1963-1964, readings were taken for height of plants in each area. The average height of the Pawnee wheat plants on the methyl bromide treated areas was 33.25 inches; for the control it was 27.25 inches; with D-D 28.33 inches and nemagon 28.16 inches. For the Ottawa wheat plants the average heights were: methyl bromide, 33.50 inches, control, 33.13 inches, D-D 31.66 inches, and nemagon, 32 inches.

Tables 5 and 6 give the record of yields on the 1963-1964 Manhattan Plot and 1963-1964 Powhattan Plot respectively. On the Manhattan Plot the average yields for the Pawnee wheat variety were as follows:

Control:	170.87	grams
Methyl Bromide:	210.96	"
D-D:	153.61	"
Nemagon:	138.26	"

Table 2. Per cent symptom expression and average height of Pawnee wheat plants grown in soil-borne mosaic virus infested field plots at Manhattan, 1963-1964 and treated with D-D, methyl bromide and nemagon.

Wheat Variety	Treatment and Replications	% Plants showing symptoms	Height of Plants in inches
Pawnee	Nemagon 1[a]	90+	29
"	" 2	90+	26
"	" 3	90+	27
"	" 4	90+	26
"	" 5	90+	26
"	" 6	90+	35
	Ave. % symptom expression	90+	Average height 28.16
Pawnee	D-D 1	90+	35
"	" 2	90+	29
"	" 3	90+	25
"	" 4	90+	22
"	" 5	90+	28
"	" 6	90+	31
	Ave. % symptom expression	90+	Average height 28.33
Pawnee	Methyl bromide 1	Trace	36
"	" 2	2	31
"	" 3	Trace	34
"	" 4	Trace	32
	Average symptom expression	1	Average height 33.25
Pawnee	Control 1	99	26
"	" 2	90+	26

Table 2 (cont.)

Wheat Variety	Treatment and Replications	% Plants showing symptoms	Height of Plants in inches
Pawnee	Control 3	95+	25
"	" 4	90+	32
	Average % symptom expression	93.5+	Average height 27.25

a Seeds treated with Nemagon and then planted in plots.

Table 3. Average height of Ottawa wheat plants grown in soil-borne mosaic virus infested field plots at Manhattan, 1963-1964 and treated with D-D, methyl bromide, and nemagon.

Wheat Variety	Treatment and Replications	% of Plants Showing symptoms	Height of Plants in inches
Ottawa	Nemagon 1[a]	0	29
"	" 2	Trace	28
"	" 3	0	29
"	" 4	Trace	29
"	" 5	Trace	36
"	" 6	Trace	35
		Average Height	32
Ottawa	D-D 1	0	31
"	" 2	0	28
"	" 3	0	31
"	" 4	Trace	33
"	" 5	Trace	31
"	" 6	Trace	36
		Average Height	31.66
Ottawa	Methyl bromide 1	0	36
"	" 2	0	33
"	" 3	0	34
"	" 4	0	32
"	" 5	Trace	32
"	" 6	0	30

Table 3 (cont.)

Wheat Variety	Treatment and Replications	% of Plants Showing symptoms	Height of Plants in inches
Ottawa	Methyl bromide 7	Trace	36
"	" 8	0	35
		Average Height	33.50
Ottawa	Control 1	0	30
"	" 2	Trace	35
"	" 3	Trace	30
"	" 4	Trace	30
"	" 5	0	36
"	" 6	Trace	33
"	" 7	Trace	35
"	" 8	Trace	36
		Average Height	33.13

[a] Seeds treated with nemagon and then planted in plots.

Table 4. Per cent symptom expression and average height of Ponca wheat plants grown in soil-borne mosaic virus infested soil in field plots at Powhattan, 1963-1964, after the plots had been treated with D-D and methyl bromide.

Wheat Variety	Treatment and replications		% of Plants showing symptoms		% of Plants visibly stunted
Ponca	Methyl bromide	1	1		0
"	"	2	1		0
"	"	3	10		10
"	"	4	Trace		1
"	"	5	0		6
"	"	6	2		2
		Ave. % symptom expression	2.5	Average % stunting	3.16
Ponca	D-D	1	20		20
"	"	2	20		20
"	"	3	10		10
"	"	4	15		15
"	"	5	15		15
"	"	6	10		10
		Ave. % symptom expression	15	Average % stunting	15
Ponca	Control	1	65		65
"	"	2	75		75
"	"	3	65		65
"	"	4	50		65
"	"	5	30		50
"	"	6	50		50
		Average % symptom expression	54.16	Average % stunting	61.66

Table 5. Average yield of Pawnee and Ottawa wheat plants grown in soil-borne mosaic virus infested field plots at Manhattan, 1963-1964 and treated with D-D, methyl bromide, and nemagon.

Wheat Variety	Treatment	Average yield in grams[b]
Pawnee	Nemagon[a]	138.26
"	D-D	153.61
"	Methyl bromide	210.96
"	Control	170.87
Ottawa	Nemagon[a]	208.46
"	D-D	220.30
"	Methyl bromide	182.03
"	Control	238.53

[a] Seeds treated with nemagon and then planted in plots.

[b] Average yield per 16 foot row.

Table 6. Average yield of Ponca wheat plants grown on soil-borne mosaic virus infested soil at Powhattan, 1963-1964, after the plots had been treated with D-D and methyl bromide.

Wheat Variety	Treatment	Average Yield in grams[a]
Ponca	D-D	96.75
"	Methyl bromide	129.06
"	Control	65.28

[a] represents average yield per 16 foot row.

For the Ottawa wheat variety the results were:

Control:	238.53	grams
Methyl bromide:	182.03	"
D-D:	220.30	"
Nemagon:	208.46	"

On the Powhattan Pawnee Plot the average yield for each treatment was:

Control:	65.28	grams
D-D:	96.75	"
Methyl bromide:	129.06	"

Laboratory and Greenhouse Work

Nematode Studies. From the 1962-1963 Plot at Manhattan soil samples were taken and processed as described under "materials and methods." The nematodes extracted were examined under the dissecting microscope in order to find out the nature of the nematode population. The predominant nematodes were of four genera namely, *Pratylenchus* Filipjev, 1934, *Tylenchorynchus* Cobb, 1936, *Xiphinema* Cobb, 1931 a, and *Dorylaimus* Duj., 1945.

This was the type of the nematode population used in an attempt to inoculate Pawnee wheat seedlings. No soil-borne mosaic symptoms developed on any of the 40 Pawnee wheat seedlings on whose roots the nematodes were poured, as explained previously, and which were kept in the temperature control chamber at temperatures of 55° F. minimum and 65° F. maximum. The results of the 1963-1964 greenhouse work outlined under "Other Greenhouse Studies" is presented in Table 7. As shown by the table none of the Ottawa wheat plants developed soil-borne wheat mosaic symptoms; none of the rye and Pawnee wheat plants grown in the laboratory in petri dishes and transplanted together with plants from the field in autoclaved sand showed any symptoms either. Seven of the 40 laboratory-grown rye plants transplanted into infested soil developed soil-borne wheat mosaic symptoms; so did two of the 40 laboratory-grown Pawnee wheat plants transplanted into infested soil. Eight of the 20 Pawnee wheat plants transplanted from the infested field into autoclaved sand developed disease symptoms and nine of 20 similar plants transplanted into infested soil developed the symptoms of the disease.

Root Staining and Clearing Studies. Roots of wheat plants growing on various plots (See "Root clearing" under "materials and methods") were collected, washed, stained and cleared as described elsewhere in the paper. The roots were then examined on microscope slides under a compound microscope after the initial examination with the dissecting microscope.

Table 7. Number of plants developing mosaic symptoms in pots of soil-borne mosaic virus infested soil and autoclaved sand in the greenhouse.

	Pawnee[a]		Pawnee[b]		Rye[c]		Ottawa[d]	
	No. Planted	No. Infected	No. Planted	No. Infected	No. Planted	No. Infected	No. Planted	No. Infected
Autoclaved Sand	20	8	40	0	40	0	20	0
Infested Soil	20	9	40	2	40	7	20	0

[a] refers to Pawnee wheat transplants from soil-borne mosaic virus infested soil.

[b] refers to Pawnee wheat plants grown in petri dishes on filter paper and transplanted into pots together with Pawnee or Ottawa wheat transplants from soil-borne mosaic virus infested soil.

[c] refers to rye plants grown in petri dishes on filter paper and transplanted into pots together with Pawnee or Ottawa wheat transplants from soil-borne mosaic virus infested soil.

[d] refers to Ottawa wheat transplants from soil-borne mosaic virus infested soil.

Plates I and II are photographic records of the observations made on the roots after they had been cleared in lactophenol. Fig. 1, Plate I is a selective sample of the type of root cell inhabitants found in the roots from the wheat plants collected from Ashland Farm, a non-infested field. Figs. 2, 3, and 4 of Plate I and Figs. 5 and 6 of Plate II also represent selective samples of the infection-type found in the roots of wheat plants growing on the 1963-1964 Manhattan Plot and the 1963-1964 Powhattan Plots. The infection-type was the same from all areas. Figs. 1-3, Plate I show cysts of Olpidium Brassicae (Wor.) Dang. in the epidermal cells of the wheat roots. Fig. 4, Plate I, shows a zoosporangium of the same in the epidermal cell.(52). Figs. 5 and 6 show spore balls of Polymyxa graminis Ledingham in the root hairs of the wheat roots collected (28).

PLATE I

Fig. 1. shows cysts of Olpidium Brassicae (Wor.) Dang. in the root of plants collected from Ashland, a non-infested field.

Fig. 2 and 3 show cysts of Olpidium Brassicae (Wor.) Dang. in the epidermal cells of wheat roots collected from treated and untreated infested plots.

Fig. 4 shows a zoosporangium of Olpidium Brassicae (Wor.) Dang. in the epidermal cell of a wheat root.

Note that Figs. 2-4 are selective samples of the type of root cell inhabitants found in the roots of wheat plants growing on the 1963-1964 Manhattan plots and the 1963-1964 Powhattan plots.

PLATE I

Fig. 1

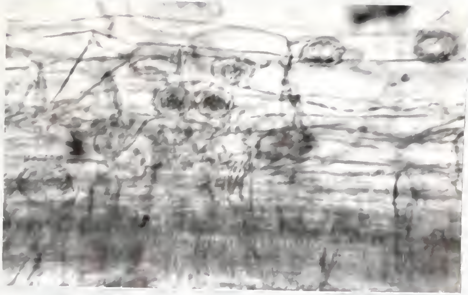


Fig. 2

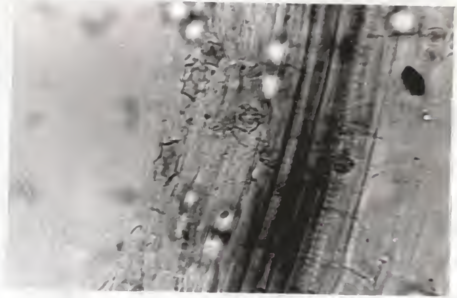


Fig. 3

Fig. 4

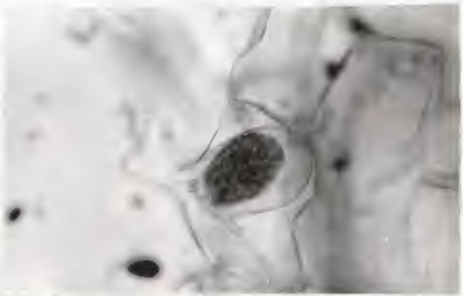


PLATE II

Figs. 5 and 6 are selective samples showing spore balls of *Polymyxa graminis* in the roots of wheat plants collected from 1963-1964 plots at Manhattan and Powhattan.

PLATE II

Fig. 5

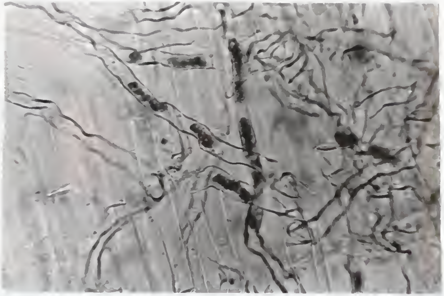


Fig. 6

DISCUSSION AND CONCLUSIONS

McKinney et al. (43) have presented evidence which strongly suggest that a microscopic living soil and/or root-inhabiting vector is involved in the transmission of the soil-borne wheat mosaic virus. In an attempt to find this possible living soil-or root-inhabiting vector, nematodes extracted from soil-borne mosaic virus infested soil were poured on roots of young Pawnee wheat plants grown in autoclaved sand. The nematodes failed to transmit the disease. Other workers have also reported failure to transmit the virus by means of nematodes (23, 43).

Twenty Pawnee wheat plants sowed in soil-borne mosaic virus infested field plots on October 18, 1963 were transplanted into autoclaved sand on December 17, 1963 and kept in a controlled chamber. Eight of these Pawnee wheat plants developed soil-borne mosaic symptoms. This indicates that by the time transplanting was done, some of the Pawnee wheat plants already had been infected. As reported under results and also shown in Table 7 none of the laboratory grown Pawnee wheat seedlings or the laboratory grown rye seedlings, transplanted into autoclaved sand developed soil-borne mosaic symptoms. In view of the fact that these laboratory grown plants were growing in the same pots with field-grown Pawnee wheat plants some of which developed soil-borne mosaic symptoms, it would seem that the virus did not move across from the field-grown plants to the laboratory grown plants through the autoclaved sand. A similar observation was made on the laboratory grown Pawnee wheat and rye plants transplanted into soil-borne mosaic infested soil. In all a total of nine such plants out of 80 transplants developed soil-borne mosaic symptoms. The low incidence of infection can only be explained in the light of Webb's report that transplanting susceptible wheat plants from non-infested to virus infested soil, the per cent infection decreased from 100% in swollen kernels to 0% in seedlings in the three-leaf stage (72). In other words, the level of development of the seedlings at the time of transplanting into virus infested soil appears to have a great influence on the transmission of the virus. The transplants from the laboratory were in the two-leaf stage.

The development of the mosaic symptoms on the Ottawa wheat plants of the 1963-1964 plots at Manhattan varied from 0% to a trace. These results were expected as the Ottawa wheat variety is highly resistant to the soil-borne mosaic virus.(59).

On the Pawnee wheat seeded plots, however, the incidence of the disease seemed to have been affected by the type of treatment. On the control plots of the 1963-1964 field experiment at Manhattan the average per cent of Pawnee wheat plants developing symptoms was 93.5+, on the DD plots it was 90+, and on the nemagon plots it was 90+ also. However, on the methyl bromide treated plots the average percentage of Pawnee wheat plants developing soil-borne mosaic was less than one per cent. This would suggest that methyl bromide almost completely controlled the disease in the field. The Powhattan plots of 1963-1964 produced similar results. On the methyl bromide treated plots the average per cent of Ponca wheat plants showing symptoms was 2.5, on the D-D treated plots 15, and 54.16 on the control. Here again the plants on the methyl bromide treated plots had much the lowest incidence of infection. D-D also reduced considerably the incidence of infection on the plots of 1963-1964. Except for the possibility of a higher virus infestation on the 1963-1964 Manhattan plots than on the 1963-1964 Powhattan plots as suggested by the higher per cent of plants showing symptoms on the control plots of the former than on the latter, the high incidence of the disease on the D-D treated plots of the 1963-1964 Manhattan plots cannot be explained. Working in the greenhouse, other workers have reported the control of the disease by the use of methyl bromide and D-D, among other chemicals (22, 42).

The degree of stunting was also measured on the Pawhattan plots of 1963-1964. On the average, the per cent of Ponca wheat plants which were visibly stunted in the methyl bromide treated plots was 3.16. In the D-D treated plots an average of 15% of the plants were visibly stunted whereas in the control plots it was 61.66%. Methyl bromide therefore again lead in effectiveness in controlling stunting caused by the soil-borne wheat mosaic virus.

As shown by Table 6, the average yield of Ponca wheat per 16 foot row was affected by the type of treatment on the 1963-1964 plots at Powhattan. On the control plots the average yield per 16 foot row was 65.28 grams. The average yield from the D-D treated plots was 96.75 grams per 16 foot row, almost one and half times higher than that from the control plots. From the methyl bromide treated plots the average yield was 129.06 grams which is only a fraction less than twice the average yield from the control plots. Therefore considering these results for yield, it would seem that D-D raised the yield but methyl bromide raised the yield much more. On the 1962-1963 plot at Manhattan, D-D, vorlex, and methyl bromide were used. The average yield per two eight-foot rows for the vorlex-treated plots was 87.75 grams. From the D-D-treated plots the average yield was 203.25 grams, with the methyl bromide treated plots giving an average yield of 233.50 grams and the control plots 150.00 grams. The trend follows the other results discussed, in that methyl bromide gave the highest yield, followed by D-D, and control in that order. The vorlex treated plots fell below the control plots in yield. This might have resulted from the phytotoxic effect of vorlex on Pawnee wheat plants. Also, McKeen, working on "the destruction of viruses by soil fumigants", reported that vorlex was less viricidal than methyl bromide (Dowfume MC-2) (30).

On the 1963-1964 Manhattan plots the average yield of the plots seeded with nemagon treated Pawnee wheat seeds was 138.26 grams; on the D-D-treated plots it was 153.61 grams, whereas on the methyl bromide treated and control plots the average yields were 210.96 grams and 170.87 grams respectively. The methyl bromide treated plots yielded higher than the other plots. The higher average yield of the control plots over that from D-D treated plots cannot be explained beyond the observation that the incidence of the disease for each of the last two mentioned treatments involved over 90% of the plants on the plots. Nemagon is phytotoxic to Pawnee wheat and this may explain at least in part the higher yield of the control as compared to the nemagon plots.

The yield records of the Ottawa wheat variety on the Manhattan plot of 1963-1964 also defies explanation based upon the effects of the soil-borne mosaic virus. This wheat variety is highly resistant to the virus and therefore the type of treatment should have made no difference as regards infection with the soil-borne mosaic virus. As shown by Table 3, this was the case, the percent of Ottawa wheat plants developing soil-borne mosaic ranging from 0 to a trace. Should this have been reflected directly in the yield, the yields from all the treatments would have been approximately the same. But as shown by Table 5, the average yields were 208.46 grams for nemagon treatment, 220.30 grams for D-D treatment, 182.03 grams for methyl bromide treatment, and 238.53 grams for the control. The suggestion is that some unknown factor seems to be involved here. It is probably that the erratic Ottawa yields were due to the prolonged spring drouth as Ottawa yields were extremely erratic in the Manhattan area farmer fields.

The root staining and clearing studies showed that the roots of the Pawnee wheat and Ponca wheat plants whether obtained from infested or non-infested soils were inhabited by Olpidium Brassicae (Wor.) Dang., and Polymyxa graminis Ledingham. These fungi have been reported previously to inhabit wheat roots (28, 29, 52). In view of reports that Olpidium Brassicae (Wor.) Dang. transmits virus diseases such as tobacco necrosis (62), and the big vein disease of lettuce (9), coupled with the circumstantial evidence that a living soil- or root-inhabiting vector is probably involved with the transmission of the soil-borne wheat mosaic virus, further work should be done using the above mentioned fungi as possible vectors.

There are other soil inhabitants which could be considered as possible vectors. The evidences point to an organism or organisms which are capable of persisting in dry soil for several years (55) and which have close association with wheat plant roots (43). Spore forming bacteria and fungi besides Olpidium Brassicae (Wor.) Dang. and Polymyxa graminis Ledingham should also be tried in attempts to transmit the virus.

SUMMARY

Among the threats to wheat production in Kansas are diseases and one of these diseases is the soil-borne wheat mosaic viruses. From the fall of 1962 to the summer of 1964 attempts were made to find out at least some of the possible factors influencing the transmission of the soil-borne wheat mosaic virus in Kansas.

McKinney first reported the disease in 1919 from Illinois. The disease was at first confused with the Australian "take-all" but further investigations showed that it was different from "take-all." The wheat variety in which the disease was first observed was Harvest Queen. In this variety, the disease causes excessive proliferation and dwarfing, a condition known as rosette. Hence McKinney gave the name "the rosette disease of wheat" to the disease. He showed that the infective agent was soil borne and that juice from diseased plants could be used to transmit the disease.

The first report of the soil-borne wheat mosaic in Kansas was in 1949. Other states from which reports about the occurrence of the soil-borne wheat mosaic have been made are Indiana, South Carolina, Iowa, Virginia, Maryland, North Carolina, Missouri, and Oklahoma.

In Kansas, the soil-borne wheat mosaic virus is apparently confined to the eastern third of the state. In the 1951-'52 growing season an epiphytotic causing average losses estimated at 10% in the diseased field checked was reported. The dollar value was estimated at $1,500,000. In the 1953-'54 growing season another epiphytotic accounting for about 26% average reduction in yield and an estimated loss of about $3,000,000 was recorded. Another epiphytotic in 1957 reduced yields by about 16% which accounted for about $3,950,000 loss. Even higher losses have been reported from other states. The virus is reported to infect several hundred varieties of winter wheat, two varieties of winter rye,

barley, emmer, spelt, and a wild grass, Bromus commutatus Schrad.

As a rule, field symptoms appear only in the spring. Diseased areas appear yellowish to light-green as compared with the adjacent healthy areas. The yellowing disappears with persistent hot weather. On individual plants symptoms show as a mosaic and mottle condition consisting of yellowish areas intermingled with normal green tissues. The leaf symptoms may also disappear in persistent hot weather. Delayed maturity, fewer and shorter heads, shrivelled grains, and stunting are other characteristics of the disease.

Cellular inclusions have been found in wheat plants showing the green-mosaic and rosette condition but not in plants showing the yellow mosaic.

The virus has been transmitted through abrasive inoculation but not by seeds. Neither have workers using nematodes, aphids, and mites reported any success. Further work has pointed strongly to the existence of a vector organism in close association with the roots of mosaic diseased plants growing in naturally infested soil as being involved with the natural overseasoning of, and the transmission of the soil-borne mosaic virus.

Soil moisture, soil temperature, and photoperiod are some of the factors which influence the development of the soil-borne wheat mosaic. Symptoms develop best when temperatures are maintained around 60° F. with a photoperiod of about eight hours and an adequate soil moisture during the growing season.

Workers have reported wheat varieties resistant to the virus and also chemicals such as methyl bromide, D-D, chloropicrin, carbon disulfide, and calcium cyanide have been reported to control the disease in the greenhouse under controlled conditions. Steam sterilization has also controlled the disease.

The studies done at Kansas State University from fall 1962 to summer 1964 involved field work, greenhouse work, and laboratory work. For the field work the effects of methyl bromide, D-D, vorlex, and nemagon on yield, symptom expression, and stunting of Pawnee, Ponca, and Ottawa wheat varieties due to the soil-borne mosaic virus were studied. Plots known to be infested with the soil-borne mosaic virus were laid out at Manhattan and Powhattan, Kansas. In the 1962-'63 growing season when the field studies were initiated, the Manhattan plots were treated with either methyl bromide, D-D, vorlex, or left untreated to serve as a control. Pawnee wheat was seeded on the plots. In the 1963-'64 growing season the Manhattan plots of the previous growing season were used. This time the chemicals used were methyl bromide, D-D, and nemagon, and Pawnee and Ottawa wheat varieties were seeded on the plots.

At Powhattan, a similar plot was laid out in the 1963-'64 growing season. Methyl bromide and D-D were the chemicals used and the wheat variety sowed was Ponca.

From the field experiments it was observed that methyl bromide almost completely controlled the disease. As regards yield, methyl bromide gave the highest yield for the susceptible wheat varieties Pawnee and Ponca. It was the leading chemical in reducing stunting and mosaic symptoms. The effects of D-D, however, were not consistent on the susceptible wheat varieties Pawnee and Ponca. On Ponca wheat, the yield from D-D treated plots was higher than that from the control. On the Pawnee wheat variety, however, the yield from D-D treated plots was higher than that from the control plots in the 1962-'63 experiment, but the reverse was true in the 1963-'64 experiment at Manhattan. D-D reduced incidence of the disease considerably on Ponca wheat as compared to the control. It did not reduce the symptom expression on Pawnee wheat to any appreciable extent.

The yield resulting from the nemagon treatment was also below that from the control plots, neither was the incidence of the disease reduced to any appreciable extent. Vorlex treatment also resulted in a yield less than that from the control plots. Nemagon and Vorlex also were phytotoxic to Pawnee wheat.

One of the greenhouse studies involved the use of nematodes in an attempt to transmit the virus. The nematodes were extracted from samples of infested soil by the Cobb sifting and gravity method. The nematodes thus obtained were poured on the roots of Pawnee wheat seedlings which were being transplanted into fresh pots of autoclaved sand. The pots were then placed in a chamber whose temperature was maintained between 55° F. and 65° F. The nematodes used in the experiment failed to transmit the virus. Also Pawnee and Ottawa wheat plants which had been sown on infested plots on October 18, 1963 were collected on December 17, 1963 and after the roots had been thoroughly washed, were transplanted into either autoclaved sand or infested soil in clay pots. Into each of these pots were transplanted two each of Pawnee wheat plants and a rye variety both of which had been growing on filter paper in petri dishes. The above experiment was repeated using wheat plants from a non-infested field instead of wheat plants from an infested field and also replacing the infested soil in the pots with soil from a non-infested field. These pots were placed in a temperature controlled chamber maintained at 55° F. to 65° F.

Some of the Pawnee wheat plants collected from the infested field proved to have been infected with the virus before they were transplanted as evidenced by eight of the 20 transplanted into autoclaved sand developing mosaic symptoms. The virus failed to move from the old plants to the young seedlings through autoclaved sand; none of the young seedlings transplanted into autoclaved sand from petri dishes developed mosaic symptoms. However, a total of nine out of 80 of the Pawnee wheat plants and the rye variety both of which had been transplanted from petri dishes into infested soil, developed soil-borne mosaic symptoms. None of the Ottawa wheat plants developed mosaic symptoms. Ottawa wheat is highly resistant to the soil-borne mosaic virus.

The laboratory work involved staining wheat roots collected from infested and non-infested plots in acid fuschin and lactophenol and then clearing them in lactophenol. The cleared roots were then examined under the microscope for root inhabitants. The examination showed that the roots of the Pawnee and Ponca wheat plants, whether obtained from infested or non-infested soils were inhabited by Olpidium Brassicae (Wor.) Dang., and Polymyxa graminis Ledingham.

The evidence suggests that these fungi and other soil-inhabiting fungi and bacteria with very persistent spores or similar structures should be studied as possible vectors of the soil-borne wheat mosaic virus.

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POSSIBLE FACTORS INFLUENCING THE TRANSMISSION AND CONTROL OF THE SOIL-BORNE WHEAT MOSAIC VIRUS IN KANSAS

by

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Among the diseases of wheat plants in Kansas is the soil-borne wheat mosaic. Since the disease was reported from Kansas in 1949, three epiphytotics have been experienced in the state. In view of the above-mentioned epiphytotics in Kansas, the soil-borne wheat mosaic virus can be considered at least a potential danger to wheat production in Kansas. From the fall of 1962 to the summer of 1964, therefore, attempts were made to find out at least some of the possible factors influencing the transmission of the virus in Kansas. The work done forms the theme of this paper.

McKinney reported the disease from Illinois in 1919. Among the names which have been applied to the disease since that time are "take-all", "the rosette disease of wheat," "wheat mosaic" and prairie wheat mosaic." In addition to Illinois and Kansas, the disease is known in Indiana, South Carolina, Iowa, Virginia, Maryland, North Carolina, Missouri and Oklahoma.

The studies reported in this paper involved greenhouse, laboratory and field work. In the 1962-1963 growing season a field at Manhattan known to be infested with the soil-borne mosaic virus was treated with methyl bromide, dichloropropene-dichloropropane (D-D), and vorlex to study their effects on the yields of Pawnee wheat, a variety susceptible to the virus.

In the 1963-1964 growing season also the above Manhattan field and a similarly infested field at Powhattan, Kansas, were used to study the effects of methyl bromide, D-D, and nemagon on Pawnee, Ponca and Ottawa wheats as regards yield, symptom expression, and stunting caused by the soil-borne mosaic virus.

Pawnee and Ponca wheat roots from soil-borne mosaic virus infested and non-infested plots were also stained in acid fuschin in lactophenol and cleared in lactophenol to study the type of root inhabitants. In addition, Pawnee and Ottawa wheat plants were collected from infested plots and transplanted into pots of either autoclaved or infested soil, together with Pawnee wheat and rye seedlings grown on filter paper in petri dishes.

Methyl bromide was the most consistent chemical in reducing symptom expression, stunting, and also increasing the yield of Pawnee and Ponca wheat plants in the field. The greenhouse studies showed that some of the Pawnee wheat plants were already infected with the virus before they were transplanted into pots and also that the virus failed to move from Pawnee wheat plants transplanted from the field to Pawnee wheat and rye seedlings grown in petri dishes through autoclaved sand.

The wheat root inhabitants found through root staining and clearing studies were Olpidium brassicae (Wor.) Dang. and Polymyxa graminis Ledingham. The evidences suggest that these fungi and other soil inhabiting fungi and also bacteria with persistent structures should be studied as possible vectors of the soil-borne wheat mosaic virus.