TRANSMISSION OF SOIL-BORNE WHEAT MOSAIC VIRUS

by

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B. S. A., University of the Philippines, 1958

A MASTER'S THESES

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Department of Botany and Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1966

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INTRODUCTION

Soil-borne wheat mosaic (SBWM) is one of the diseases which pose a serious threat to the wheat industry in Kansas. This disease was first observed in Illinois and Indiana in 1919 and was described by McKinney (25) as "The Rosette Disease of Wheat." During succeeding years SBWM was reported from various places in the United States. In 1925, it was found that the rosette disease of wheat was caused by a soil-borne virus (26).

The disease was believed to be present in the eastern part of Kansas as early as 1925 (10) and was first reported in Hodgeman and Pratt counties in 1949 (34). The significance and economic value of SBWM to the wheat industry in Kansas was not seriously felt until epiphytotics in 1952 (10) and 1954 which caused the wheat industry in Kansas an estimated loss of 3 million dollars (14). Again in 1956-1957, a severe epiphytotic of SBWM was reported in several counties of the eastern part of Kansas where about 347,000 acres were infested with an estimated yield loss of 2,080,000 bushels of grain worth about $3,950,000 (47).

Since the first reported occurrence of SBWM in 1919, plant disease investigators in this country have been studying the etiology of the disease. Various aspects on the nature of transmission such as natural transmission to winter wheat seedlings when planted in infested soil (32, 35, 46), transmission by insects (20, 25; 46), eriophyid mite (46), nematodes (1, 7, 20, 36), and mechanical transmission by expressed sap from infected leaves (20, 26, 27, 46, 54) have been explored. Recent studies have shown that symptoms of SBWM were induced on test plants by immer-

sing roots of germinated susceptible wheat seedlings in distilled water containing small pieces of SBWMV infected roots (7).

The organism(s) involved in the transmission of SBWMV is (are) not known at the present time. Various theories have been postulated and McKinney (36) in 1957 believed that a living vector of microscopic properties was involved, that the virus overseasoned in the vector and that natural inoculation of the plant was accomplished by this vector. Sill (46) also suggested that a very small living root vector which may also serve as an alternate host of the virus might be involved in its transmission. Brakke, et al. (7) also suggested that a microscopic organism was involved in the transmission of the virus. Addison (1) suggested that Olpidium brassicae (Wor.) Dang. and Polymyxa graminis Ledingham had good vector capabilities of SBWMV since he consistently found both organisms associated with naturally infected wheat roots.

The objectives of the present study conducted from November, 1964 to June, 1966 were to further elucidate factors involved in SBWMV transmission and to isolate the vector organism. The field experiments were to measure yield losses on susceptible and non-susceptible wheat varieties, to determine how quickly SBWMV moves back after partial soil sterilization and to further substantiate the timing and development of the disease.

REVIEW OF LITERATURE

History and Geographical Distribution. - Soil-borne wheat mosaic was first reported in the United States in Madison county, Illinois and in Indiana in the spring of 1919 (25). The disease

was first named "The Rosette Disease of Wheat" (25, 26). SBWM was later reported in the following states at the following times; Virginia, 1925 (11); Maryland, 1927 and North Carolina, 1929 (41); South Carolina, 1944 (2); Missouri, 1944 (9); Kansas, 1949 (34); Iowa, 1950 (41); Oklahoma, 1952 (53); and Nebraska, 1965 (7). A similar disease was also reported in Egypt (40), Japan (52) and Russia (56).

Economic Importance. - The first epiphytotic of SBWM in Kansas was in 1952 (10) when wheat fields in 36 eastern counties were severely infected. Yield reduction as high as 75% were claimed in some areas with average loses from 4.5 to 25%. These loses were estimated at $1,500,000 (45).

Another outbreak of SBWM occurred in Kansas in 1954 with an estimated loss of 3 million dollars (14). In the 1956-1957 growing season a severe epiphytotic was reported in 347,000 acres in eastern Kansas with an estimated yield loss of 2,080,000 bushels worth about $3,950,000 (47).

Losses of economic importance have been reported from Virginia (42), Oklahoma (53), and Illinois and Missouri (9).

Melchers (40), in Egypt, claimed that the yield loss due to wheat mosaic virus was 20 to 40% with 40 to 60% infection in many fields.

Host Range. - Soil-borne wheat mosaic virus host range is apparently confined to the tribe Hordeae. Many varieties of wheat and two varieties of rye have been found to be susceptible to the virus (45, 46). McKinney (27, 29) and Koehler et al. (22) induced SBWM symptoms on the following species of Hordeae: Triticum aestivum, T. compactum, T. turgidum, T. dicoccum, T. spelta,

Triticum monococcum, Hordeum sativum and Secale cereale.

In Kansas, the disease has been observed on a Bromus sp., several varieties of spring wheat and once on barley (46).

Symptomatology.-Rosette - Symptoms first noticed by McKinney (25) on wheat were field spotting, arrested spring development, excessive tillering of individual stools and dark blue-green foleage. In severe cases, dying of the outer leaves, browning of the base of affected plants and sometimes death was observed (21). On Harvest Queen, a rosette-susceptible wheat variety, the symptoms consisted of a mottling of light yellow areas intermingled with normal green of the leaves. These yellow patches may be varied from circular or nearly so to oblong or large chlorotic streaks parallel to the leaf veins may be formed (20). Though the rosette condition has never been observed in Kansas, Sill (46) was able to induce these symptoms under laboratory conditions when a rosette-susceptible wheat variety was seeded in infested soil from Illinois.

Nonrosette - Symptoms generally appeared in the spring when plants started vegetative growth and consisted of yellowish to light green patches when compared to the adjacent healthy wheat plants (22, 46). A little later they became yellow bronze, a color distinctly different from typical early spring yellowing due to nitrogen difficiency. Shortly after hot weather commenced, yellowing gradually disappeared. As the season progressed, diseased areas were recognized by a slight to moderate stunting, smaller heads and retarded maturity when compared with healthy areas (46). Individual infected wheat plants showed predominant yellow to bronze mosaic which was characterized by tiny green islands in a

lighter green to yellow background accompanied by a purpling condition of the leaf edges (10, 46).

Sometimes irregular streaks or blotches which varied from an inconspicuous pale green to a pronounced yellow were observed on infected leaves. These streaks or blotches sometimes involved the entire leaf area depending on the severity of the disease (22). Mottling symptoms of infected wheat plants often disappeared under hot weather conditions. However, under prolonged favorable weather conditions leaf symptoms persisted to the flag leaf stage of development as yellow-green or whitish streaks and blotches (46).

In addition to the mosaic condition, diseased wheat plants were also stunted, produced fewer and shorter heads and smaller and shrivelled grains (20, 21, 46). Mosaic symptoms may also occur in the fall with prolonged cool weather and adequate moisture (7, 22, 29). However, this condition has not been reported in Kansas (46).

Transmission. - Since McKinney (26) demonstrated that the infective agent of the rosette disease of wheat was soil-borne, various methods of transmission have been studied. Transmission attempts which involved living arthropods included the leafhoppers Laevocephalus (Deltocephalus) striatus, Agalia sanguinolenta, A. constricta, Delphacodes campestris, Toxicoptera gramineum (20), three species of aphids and an eriophyid mite (Aceria tulipae, Keifer) (46). All these attempts failed to transmit the virus. Earlier studies on the transmission of the virus involving insects also yielded negative results (25). The possibility of nematodes as potential vectors of soil-borne mosaic virus was also explored in numerous cases. Aphelenchus avenae and Panagrolaimus

sp. failed to transmit the virus (36). Similar studies by Brakke, et al. (7), using species of Helicotylenchus, Dorylaimus, Tylenchus, Tylenchorhynchus, Psilenchus, and Pratylenchus, likewise gave negative results. Johnson (20) collected nematodes from infested soil and used them for transmission work but his results were negative. Addison (1) screened nematodes from infested soil and found species of Pratylenchus, Tylenchorhynchus, Xiphinema and Dorylaimus to be predominant. He was unable to transmit SBWMV with these nematodes.

Sill (46) failed to transmit soil-borne mosaic using seeds from diseased wheat plants. This seemed to agree with earlier findings regarding the general characteristics of the virus (22, 27, 38).

Natural infection from infested soil gave consistent positive results (32, 35, 46). Likewise success was reported by abrasive inoculation of healthy susceptible wheat leaves with expressed sap from infected plants although the percentage of infection was generally low (26, 27, 38, 39, 46).

Additional work on transmission of SBWMV strongly suggested the involvement of a microscopic living organism closely associated with the roots of plants and which also served as an alternate host for the over-seasoning virus (7, 16, 22, 39, 46, 55). McKinney and associates (39) induced SBWM symptoms on wheat planted in autoclaved soil mixed with washed roots of diseased plants. On the other hand, no symptoms developed in plants seeded in autoclaved SBWMV-infested soil or wheat plants seeded in autoclaved soil in which was added virus laden juice from leaves or roots from diseased plants that had been mechanically inoculated (39).

Positive transmission of SBWMV was reported by Brakke, et al. (7) when the roots of healthy wheat seedlings aseptically germinated were emmersed in distilled water containing small pieces of washed, naturally infected roots.

The constant association of Polymyxa graminis (1, 39) and Olpidium brassicae (1) with the roots of diseased plants, strongly suggested the vector capabilities of these organism.

Factors Influencing Infection and Symptom Development. - Webb (54) reported that virus infection started before the 7th day after seeding and a maximum infection was obtained if the plants were grown for 28 days in infested soil before being transplanted.

In recent studies by Brakke, et al. (7), electron microscopy of a root-dip or leaf-dip preparations of wheat seedlings grown in infested soil showed that 2 to 3 weeks after planting the virus was localized in the roots in most of the symptomless plants. Adequate moisture during the growing season was necessary for infection. (7, 22, 31).

Symptoms of SBWM developed best when the temperature was maintained around 60°F. with adequate sunlight and a photoperiod of about 8 hours (22, 28, 29, 35). In a study of symptom expression of yellow mosaic disease of wheat, Ikata and Kawai (16) observed that symptoms were severe at 10°, fairly inconspicuous at 20°, and almost none at 25°C. The optimum soil temperature was 15°C.

In Kansas, Sill (44) induced symptoms of SBWM on winter rye which served as an excellent bait plant in the greenhouse at temperatures of 70°F. or slightly more and no control of photoperiod.

Wadsworth (53) reported that SBWM incidence was greater and severity more intense in fields which were continuously cropped with unfertilized wheat or in inadequately prepared seedbeds. The disease was less severe or nearly absent in fields where wheat followed a legume or where adequate fertilizers were applied (33).

Causal Agent. - The soil-borne wheat mosaic disease is caused by a virus. Holmes (15) designated the latin binomial as Marmor tritici H. McKinney (30) ammended the name to Marmor tritici var. typicum Mck. in 1944. Wada and Hukano (51) and McKinney (32) separately reported that there were two strains of SBWMV; the green or mosaic-rosette inducing virus and the yellow mosaic inducing virus. The green strain, Marmor tritici var. typicum Mck., caused rosetting and mottling and the yellow mosaic strain, Marmor tritici var. fulvum Mck., caused more injury than the former (25).

Electron micrographs of the virus from leaf extract of wheat rosette mosaic and the wheat yellow mosaic infected plants showed no distinquishable differences in the shape of the two virus strains (12). All were rod-shaped with a modal length in a class value between 120 and 135 mu with a mid-point of 128 mμ. Width was 24.6 to 26.1 mμ (12). Brakke, et al. (7) showed by electron microscopy of root-dip and leaf-dip preparations of SBWMV infected wheat plants that the virus particle was also rod-shaped and its length usually ranged from 160 to 300 mμ. The Japanese wheat mosaic virus particles were also rod-shape and their size ranged from 150 to 170 mμ long (43).

Intracellular Bodies. - Plants showing the green-mosaic and rosette symptoms contained cellular inclusions but none were observed in plants with yellows symptoms (37). Crown tissue, roots

and leafsheath of rosetted or mottled plants invariably contained intracellular bodies (37). The shape varied from roundish to almost irregular or plate-like. These bodies were smaller or larger than the host nuclei and usually occurred either free from or in close contact with the host nucleus (25). Johnson (20) observed that wheat infectd with SBWMV in the eastern part of the Mississippi river valley had vacuolated intracellular bodies while mosaic-infected wheat in the western part of the valley contained no such bodies.

Cell inclusions of the Japanese green and yellow mosaic of wheat was described as X-bodies (51). These X-bodies were of two types. Type A was vacuolated, oval or elongated, smaller to almost larger than the host nucleus, occurred singly and was always associated with yellow mosaic. Type B was homogeneous, oval or irregular, smaller than type A occurred in groups of 2 to 5 per cell and associated with the green-mosaic (51). In addition, wheat plants infected with both the yellow and the green mosaic types were found to have intermediate X-bodies which resembled either type A or type B strains (51, 52).

Control. - Early workers on the SBWMV observed that a number of commercial wheat varieties were susceptible to the disease (21, 25, 29, 54). It was suggested that the best way to control the disease was to develop resistant varieties that also possessed all other characteristics necessary for satisfactory yield of high quality grain (21, 22, 25, 26, 41, 42). In Kansas, experiments conducted in 1951 to screen wheat varieties showed that Concho, Comanche, and Ottawa were resistant to SBWMV(46, 47, 48).

Wheat varieties resistant to SBWM have been reported elsewhere in the United States (4, 41, 42).

Chemical soil fumigants and steam effectively prevented the disease. Such chemicals were formaldehyde, chloropicrin, methyl bromide, carbon disulfide, calcium cyanide, rotenone, napthalene, D-D (dichloropropene, dichloropropane), and ethyl bromide (1, 18, 25, 34, 36). Ethylene dichloride (19) and toluene (34) had no effect in reducing SBWM incidence.

Cultural practices such as late seeding so that seedlings emmerge the following spring also prevented the disease (25). However, Sill (46) found that the time of planting has no actual bearing on the occurrence of the disease and that environmental factors were of prime importance in forecasting the disease.

MATERIALS AND METHODS

Host. - Winter wheat varieties used in field experiments were Pawnee and Ottawa. Pawnee resulted from a cross between Kawvale and Tenmark and was developed jointly by the Kansas State University and the University of Nebraska Agricultural Experiment Stations (6). This winter wheat variety was susceptible to SBWM (48). Ottawa, a winter wheat variety, was selected from a cross between Mediterranean-Hope-Pawnee and Oro-Illinois No. 1-Comanche (5). This winter wheat variety was resistant to SBWM (46, 47, 48).

The host used in greenhouse experiments was an unnamed winter rye variety (Secale cereale L.) which was reported by Sill (44) to be a good bait plant for SBWM and which always developed excellent symptoms at temperatures of 70°F.

Soil and Field Plot Site. - Infested plots were located on the Agronomy Farm 1½ miles northwest of the Kansas State University campus at Manhattan. This area was formerly used by Addison (1) in his field studies. Infested soil used in greenhouse experiments were taken from the infested field plot.

Noninfested soil was obtained from the Kansas State University Ashland Experimental Farm about 6 miles southwest of the Kansas State University.

Soil on which the control plants were grown in greenhouse experiments was placed in 5 in. clay pots and autoclaved for 3 hours at 15 psi one day prior to seeding or transplanting.

Temperature Control. - Temperature was controlled by air conditioning units in a 137 cubic ft. chamber placed in a greenhouse (Fig. 1). Temperature was set for 60°F. but actually oscillated between 55 and 65°F. No attempt was made to control daylength. Experiments conducted inside the chamber were performed from November, 1964 to late April, 1965 and from August, 1965 to early June, 1966.

Transmission Experiments. - Stored and dried SBWMV infested soil. - To determined if infested soil stored for a long time was still a carrier of SBWMV, infested soil stored in 1953, 1954, 1955, and 1961 was tested for infectivity. The 1953, 1954, and 1955 infested soils were stored in an unsealed container inside a greenhouse. All these soils were powder dry. The 1961 infested soil was tightly sealed and stored inside an office. Infested soil was mixed with sterilized soil 1 to 1 to increase the volume and placed in 5 in. pots. Ten pots were used for each designated year. Two rye seedlings were transplanted in each pot and placed at

Figure 1. The temperature controlled chamber.

60 ± 5°F. In addition 5 lb. of fresh infested soil was spread thinly over an aluminum sheet and air-dried for about 28 days. After drying, it was placed in 1 ft. X 1 ft. X 3 in. wooden flats and seeded with rye. Non-air-dried infested soil was seeded with rye to serve as a control. Plants were placed in the temperature controlled chamber and observed for symptoms for 90 days.

Serial transmission. - Plants infected with SBWMV and growing in the greenhouse were carefully removed from pots. Roots were thoroughly washed with running tap water for 5 minutes and immediately immersed in a beaker containing distilled water. After 3 to 5 hours, the roots were removed and discarded. The water-root leachate was then passed through cheese cloth to removed remaining root and soil debris. Rye seedlings on filter paper were carefully lifted and their roots placed in the water-root leachate for 12 to 18 hours. Roots were then removed and washed in running tap water for 1 minute. For check plants, rye roots were soaked in distilled water for the same length of time. All the transplants were potted in sterilized soil. Plants were placed in the greenhouse at 60 ± 5°F. and observed for 70 days.

Rye plants which developed SBWM were used again for a second transmission. The same procedures for obtaining water-root leachate, germination of rye test plants, soaking time, and soil were followed.

Soil where SBWM symptoms developed in the serial transmission test was repotted in sets of 10 and germinated rye seedlings transplanted into it. Controls consisted of pots containing sterilized soil transplanted with rye. Observation for symptoms was carried out for 40 days after transplanting.

Transmission from mechanically inoculated plants. - Inoculum was prepared by cutting infected rye leaves into small pieces and grinding with a mortar and pestle in distilled water. The expressed leaf sap was passed through a clean cheese cloth and then centrifuged at 8000 rpm for 30 minutes. After centrifugation, the supernatant was collected in a beaker and a fine grade (800-mesh) of carborundum added. Test plants were rye about 4 in. tall with 4 to 5 leaves. Inoculation of healthy rye leaves was done by dipping "Q-Tip" into inoculum-carborundum mixture and rubbing the upper leaf surface (17). Five separate trials were made and the 1st, 2nd, and 3rd trials were inoculated twice to get maximum infection. Observations were made for 45 days.

To determine whether SBWMV from roots of mechanically inoculated plants grown in sterilized soil can be transmitted, infected plants were removed from containers and their roots washed in running tap water for 5 minutes. Water-root leachate was obtained and inoculations were made by the same procedures previously mentioned. Observations for symptoms were made for 75 days.

The same procedures described above were followed using noninfested soil.

Transmission with noninfested water-root leachate plus expressed sap from SBWMV infected leaves. - To further establish evidence of an organism(s) that may act as a vector of the virus associated with either wheat or rye roots, water-root leachate of healthy rye roots grown in noninfested soil was mixed with an equal volume of partially purified extract from infected leaves and allowed to stand for 3 hours at room temperature. Roots of germinated rye seedlings were placed in the mixture for 12 to 18

hours and then washed in running tap water for 1 minute before planting in potted sterilized soil. Roots of germinated rye in the control sets were soaked either in water-root leachate or leaf extract from SBWMV infected leaves alone. Plants were transferred to the temperature controlled chamber and observed for symptoms for 60 days.

Vector Isolation and Transmission Procedures. - Media used for isolations were the following; nutrient broth, nutrient broth with isoleucine, nutrient broth with wheat root decoction, and nutrient agar.

Nutrient broth contained 8 g dehydrated nutrient broth in 1000 ml distilled water.

Root decoction was prepared by macerating washed healthy roots in a Waring blendor for 10 minutes and then passing through a cheese cloth. This solution was mixed with an equal amount of nutrient broth. Nutrient agar was prepared by adding 20 g of Bacto-agar to 1 l of prepared nutrient broth. Media were autoclaved for 1 hour at 15 psi.

Roots of infected plants were thoroughly washed with tap water and blotted with paper towels. The roots were then carefully examined with the aid of a magnifier and only those roots showing dark brown discoloration were picked out. The selected roots were further examined under the dissecting scope or under the compound microscope for the presence of fruiting bodies within the root cells. The selected roots were surface sterilized in undiluted commerial bleaching solution (Chlorox-6% sodium hypochlorite) for 5 minutes and transferred to a nutrient medium and incubated at room temperature.

Mixtures of equal volume of partially purified infected leaf extract and 40 day old isolates (see discussion) were allowed to stand for about 3 hours. Roots of germinated rye were soaked in this mixture for 12 to 18 hours. The seedlings were washed in running tap water for about 5 minutes and planted in autoclaved soil. Roots of rye, for controls, were soaked either in plain isolates or in extract from infected leaves alone. Symptoms appearance on test plants was observed for 60 days.

Staining Procedures. - Roots from wheat plants infected with SBWMV and healthy wheat from Ashland Farm near Manhattan, Kansas were stained to check for possible microorganisms. Roots were thoroughly washed with tap water and blotted dry with paper towels. Clean, dry roots were boiled in a 0.05% solution of cotton blue in lactophenol for 2 to 3 minutes. After boiling, the roots were blotted with clean paper towels and transferred to a 150 ml beaker containing warm lactophenol. Two to 3 changes of warm lactophenol were made to remove excess dye. Stained roots were transferred to another beaker of clear lactophenol and left until most of the dye in the root cells was remove. This took 7 days or more. Roots were examined under a dissecting microscope and those showing abnormal conditions were mounted in lactophenol on glass slides for observations under a compound microscope. Roots of rye plants infected with SBWMV in the serial transmission experiment were also stained, following the same techniques.

Control Experiments. - Methyl bromide was applied by releasing 1 lb of the fumigant from a sealed container as illustrated in Figure 2 under a 4 mil plastic cover. It was applied at the rate of 1 lb/48 sq. ft. The cover was removed after 48 hours.

Figure 2. Parts of fumigating device (A).

B - The fumigating device ready for methyl bromide release.

plots were seeded 7 days after treatment.

Nellite M-2466 is a water soluble powder containing 90% technical nellite (phenyl N, N'-dimethylphosphorodiamideate). In the present study, the chemical was used as seed treatment applied at the rate of 2 lb/A.

Field experiments were started in the Fall of 1964. The plot area was the one previously used by Addison (1). Field plot design was as illustrated in Figure 3.

Soil was prepared with a cultivator run parallel to the length of the plots to avoid mixing soil from different treatments. Treatments used were methyl bromide applied the Fall of 1963, methyl bromide applied the Fall of 1964, Nellite seed treatment applied the Fall of 1964 and a control. Methyl bromide-1963 labelled replications were treated by Addison (1) in his 1963 field studies. Methyl bromide-1964 treatment was previously treated with Nemagon. Seed treated with Nellite was seeded in plots previously fumigated with a dichloropropene-dichloropropane mixture.

Two winter wheat varieties, Ottawa and Pawnee, were seeded separately for each treatment and replicated 6 times. Each replication had 3 rows of plants, 1 ft. apart. Only the center row of each plot was harvested for data.

The same plots were used for field experiments conducted in the Fall of 1965. Prior to treatment and seeding, the soil was prepared the same way as mentioned above. Replications previously seeded with Nellite treated seeds were fumigated with methyl bromide. The rest of the plot was not given further treatment.

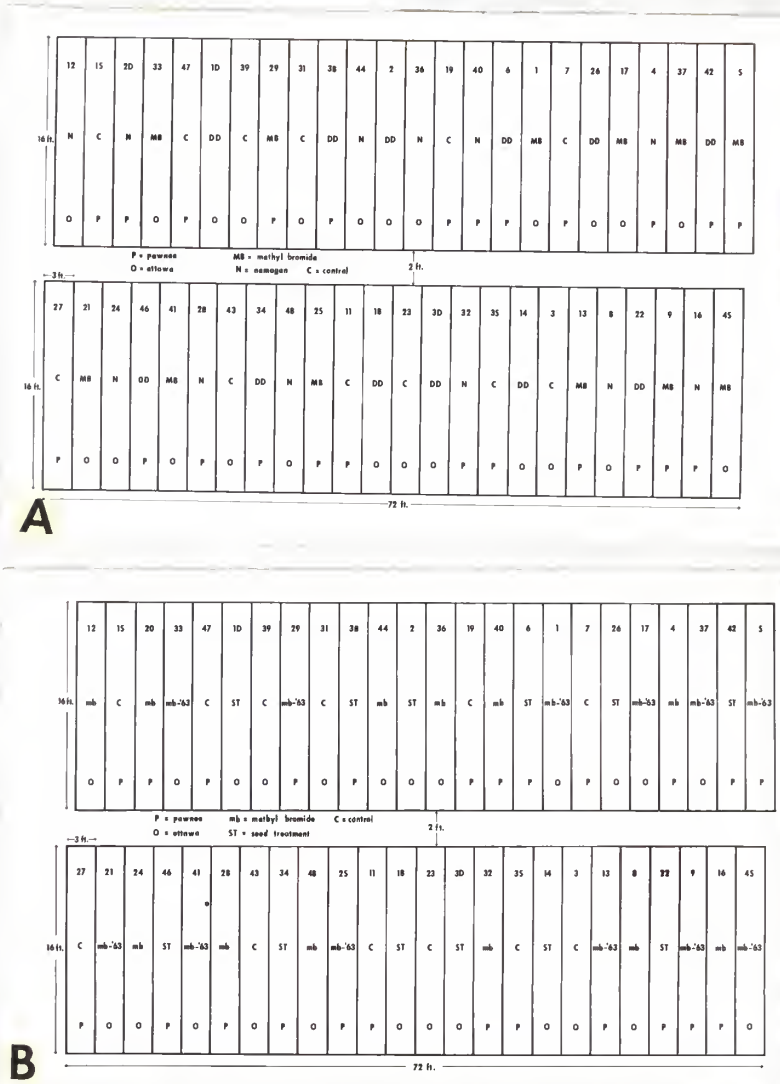

Figure 3. A - Layout of experimental field plots used by Addison during the 1963-1964 growing season at the Agronomy Farm, KSU.

B - The 1964-1965 field plots. In the Fall, 1965, plots planted with Nellite treated seeds were fumigated with methyl bromide.

RESULTS

Transmission Experiments.- No SBWMV symptoms developed on test plants that had been transplanted into infested soil stored in 1953, 1954, 1955 and 1961. Symptoms developed on 21% of the test plants in infested soil dried for 28 days and 91% in fresh infested soil (Table 1).

All susceptible winter wheat and rye seedlings transplanted into infested soil normally developed symptoms of SBWM within 35 to 75 days.

Table 1. Transmission of SBWMV from air-dried infested soil

Trials	Air-dried infested soil		Fresh infested soil[a/]	
	No. of plants Inf./planted	% Infection	No. of plants Inf./planted	% Infection
1	2/10	20	8/10	80
2	3/10	30	9/10	90
3	3/15	20	15/15	100
4	2/15	13.3	14/15	93.3
	Averages	20.8		90.8

a/ = Incubation period ranged from 40 to 57 days

b/ = Incubation period ranged from 30 to 57 days

An average of 28.7% of rye seedlings soaked in a water-root leachate from naturally infected plants developed symptoms in sterilized soil. These infectedsplants were used as another source of water-root leachate. An average of 32.5% of rye seedlings soaked in this leachate developed symptoms (Table 2).

Table 2. Serial transmission of SBWMV from naturally infected plant roots to healthy rye seedlings via water-root leachate

Trials	First transmission: No. of plants Inf./Inoc.	Incubation (days)	% Infection	Control
1	10/20	37 - 66	50	0/20
2	6/20	25 - 49	30	0/20
3	2/20	31	10	0/20
4	5/20	35 - 43	25	0/20
Average			28.7	
	Second transmission			
1	6/20	22 - 32	30	0/20
2	10/20	25 - 33	50	0/20
3	4/20	28 - 33	20	0/20
4	6/20	30 - 39	30	0/20
Average			32.5	

An average of 26.6% of uninoculated rye seedlings transplanted into soil used in the preceeding experiments developed symptoms within 30 days (Table 3). No symptoms developed on rye seedlings soaked in distilled water and planted in sterilized soil.

Fifty three percent of rye seedlings mechanically inoculated and grown in sterilized soil developed SBWM symptoms (Table 4).

No symptoms developed when roots of rye seedlings soaked in a water-root leachate obtained from infected plants that had been mechanically inoculated. Likewise, no symptoms developed on rye

seedlings grown in sterilized soil that had contained mechanically inoculated plants.

Table 3. SBWMV infestation of sterilized soil that had contained rye inoculated with water-root leachate from infected rye.

Trials	No. of plants Inf./Inoc.	Incubation (days)	% Infection	Control
1	3/10	25	30	0/10
2	2/10	31	20	0/10
3	3/10	26 - 30	30	0/10
	Average		26.6	

Table 4. Mechanical inoculation of SBWMV on healthy rye planted in autoclaved soil

Trials	No. of plants Inf./Inoc.	Incubation (days)	% Infection
1	14/20[a]	20-- 27	70
2	8/10[a]	21 - 31	80
3	14/20[a]	22 - 38	70
4	14/60	28 - 32	23.3
5	13/60	28 - 35	21.9
	Average		52.9

a = Test plants were inoculated twice with SBWMV infected leaf extract to get maximum infection

An average of 15 % of rye seedlings transplanted into non-infested soil and mechanically inoculated developed SBWM symptoms (Table 5). No symptoms developed on uninoculated plants. When water-root leachate was obtained from these infected plants and

healthy rye seedlings soaked therein, 10% developed SBWM symptoms (Table 6, Figs. 4 & 6). An average of 8.3% of rye seedlings transplanted into noninfested soil that had contained infected plants that had been mechanically inoculated developed SBWM symptoms (Table 7).

Table 5. Mechanical inoculation of SBWMV on healthy rye planted in noninfested soil

Trials	No. of plants Inf./Inoc.	Incubation (days)	% Infection
1	10/70	45 - 60	14.2
2	22/90	50 - 55	24.4
3	4/30	45 - 55	13.3
4	4/35	43 - 50	11.4
5	3/25	45 - 57	12.0
	Average		15.06

Table 6. Transmission of SBWMV via water-root leachate from mechanically inoculated rye grown in noninfested soil

Trials	No. of plants Inf./Inoc.	Incubation (days)	% Infection	Control
1	3/20	39 - 54	15	0/20
2	1/20	33	5	0/20
3	2/20	34 - 52	10	0/20
	Average		10	

Figure 4. Rye plants showing SBWM symptoms (arrow). These plants were inoculated via water-root leachate from diseased mechanically inoculated rye grown in noninfested soil.

Roots of rye seedlings were soaked in a mixture of partially purified leaf extract from SBWMV infected leaves and water-root leachate from roots of rye grown in noninfested soil. These seedlings were transplanted into sterilized soil where 10% developed symptoms (Figs. 5 & 6). No symptoms were observed when roots were either soaked in leaf extract or water-root leachate alone (Table 8).

Table 7. SBWMV infestation of noninfested soil planted with mechanically inoculated rye

Trials	No. of plants Inf./Inoc.	Incubation (days)	% Infection	Control
1	1/20	50	5	0/20
2	2/20	52	10	0/20
3	2/20	34 - 54	10	0/20
	Average		8.3	

Table 8. Transmission of SBWMV via water-root leachate from healthy rye grown in noninfested soil mixed with leaf extract from infected plants

Trials	Root leachate + leaf extract	Incubation (days)	% Infection	Root leachate only	Leaf extract only
1	2/20	39 - 46	10	0/20	0/20
2	1/20	42	5	0/20	0/20
3	3/20	32 - 49	10	0/20	0/20
	Average		10		

Figure 5. Rye plant showing SBWM symptoms (arrow). The roots of this plant were soaked in a mixture of water-root from rye plants grown in noninfested soil and leaf extract from SBWMV infected plants.

Figure 6. Selected leaves of rye test plants showing SBWM symptoms. Note the tiny dark green islands in the lighter green background of the leaves.

Vector Isolation and Transmission. - The primary purpose of isolation procedures at the beginning was to culture Olpidium and Polymyxa species. None were obtained. However, 2 and probably 3 species of bacteria were consistently found in the isolations. These bacteria were not identified. It was postulated that perhaps one or more of these isolates could be a factor in SBWMV transmission. When roots of rye seedlings were soaked in a mixture of 40 day old isolates in nutrient broth and leaf extract from SBWMV infected plants, an average of 12.5% of the seedlings developed typical symptoms. No symptoms developed when roots of seedlings were soaked either in the nutrient broth culture or the leaf extract alone (Table 9).

Table 9. Transmission of SBWMV by soaking rye seedlings in a mixture of culturable isolates and leaf extract from SBWMV infected plants

Isolate No.	Isolate + Leaf extract	Incubation (days)	% Infection	Isolate only	Leaf extract only
NBI-1	2/20	44 - 52	10	0/20	0/20
NB-1	3/20	44 - 54	15	0/20	0/20
	Average		12.5		

Root Staining. - Roots of wheat plants growing in infested and noninfested soil were stained. The organism consistently noted were the fungi Olpidium sp.(Fig. 7) and Polymyxa graminis (23) (Fig. 8).

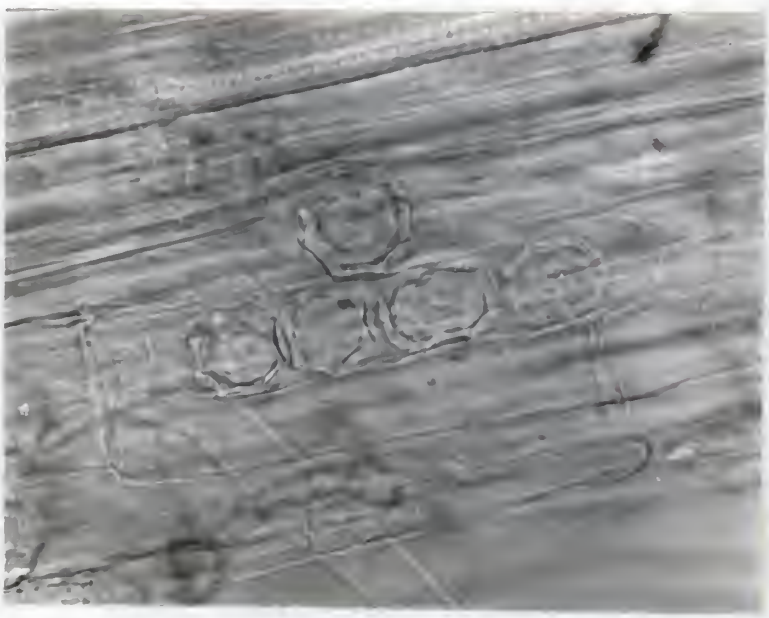

Figure 7. Cysts of *Olpidium* sp. in a SBWMV infected wheat root collected at the Agronomy Farm, KSU. Stained root samples collected from a noninfested field also had the same type of cysts.

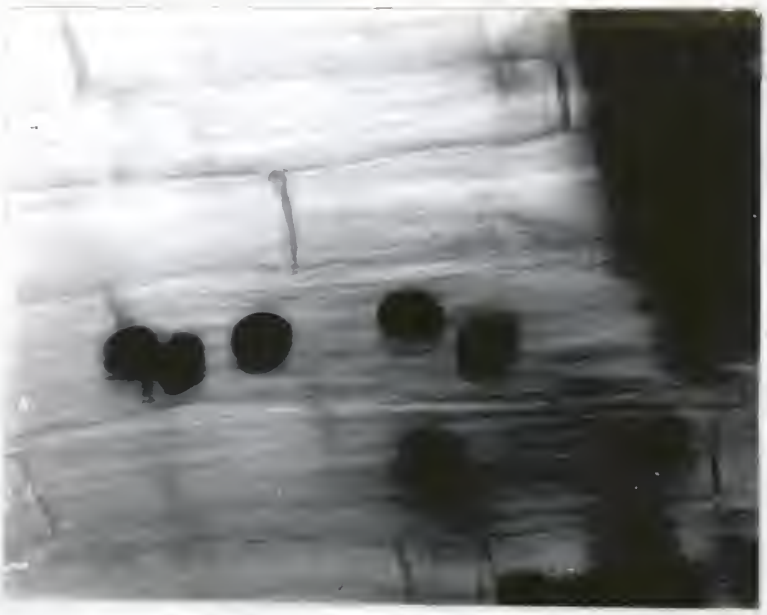

Figure 8. Spore balls of Polymyxa graminis in stained wheat roots collected from SBWMV infested and noninfested fields.

Field Experiments. - No SBWM symptoms were observed on Pawnee or Ottawa in field plots during the Fall of 1964. In the Fall of 1965, about 1% of Pawnee wheat plants had SBWM symptoms (Fig. 9). but none were found on Ottawa. Temperature at this time was about the same as that of 1964, but the moisture was more favorable for SBWM symptom development (Tables 10, 11).

In the 1964-1965 growing season, the incidence of SBWM from the 4 treatments used varied from 4.3 to 6.8% on Ottawa. On Pawnee, the averages of SBWM incidence were between 64 to 100%. Yields in terms of computed bushels/acre for Ottawa varied from 29.3 to 38.4 while Pawnee varied from 20 to 35 bushels/acre.

Based on computed LSD of 12.38 (Table 12), the mean incidence of SBWM for Pawnee in methyl bromide-1964 treatment decreased significantly when compared to the rest of the treatments. On Ottawa, the mean incidence of SBWM between treatments was non-significant but was significantly lower than that of Pawnee. Yield difference between methyl bromide-1964 and methyl bromide-1963 treatments for Ottawa was significant. Likewise, yield difference between methyl bromide-1964 over Nellite seed treatment or control on Pawnee was significant. The mean yields of Ottawa obtained from the treatments used were significantly higher than of the Pawnee control (Table 12).

In the 1965-1966 growing season, averages of SBWM incidence on Ottawa varied from 2.3 to 6.0% and on Pawnee from 50 to 97.5%. Average yields of Ottawa at various treatments varied from 22.2 to 24.4 bu./A. On Pawnee, the yield averages were from 15.7 to 33.4 bu./A.

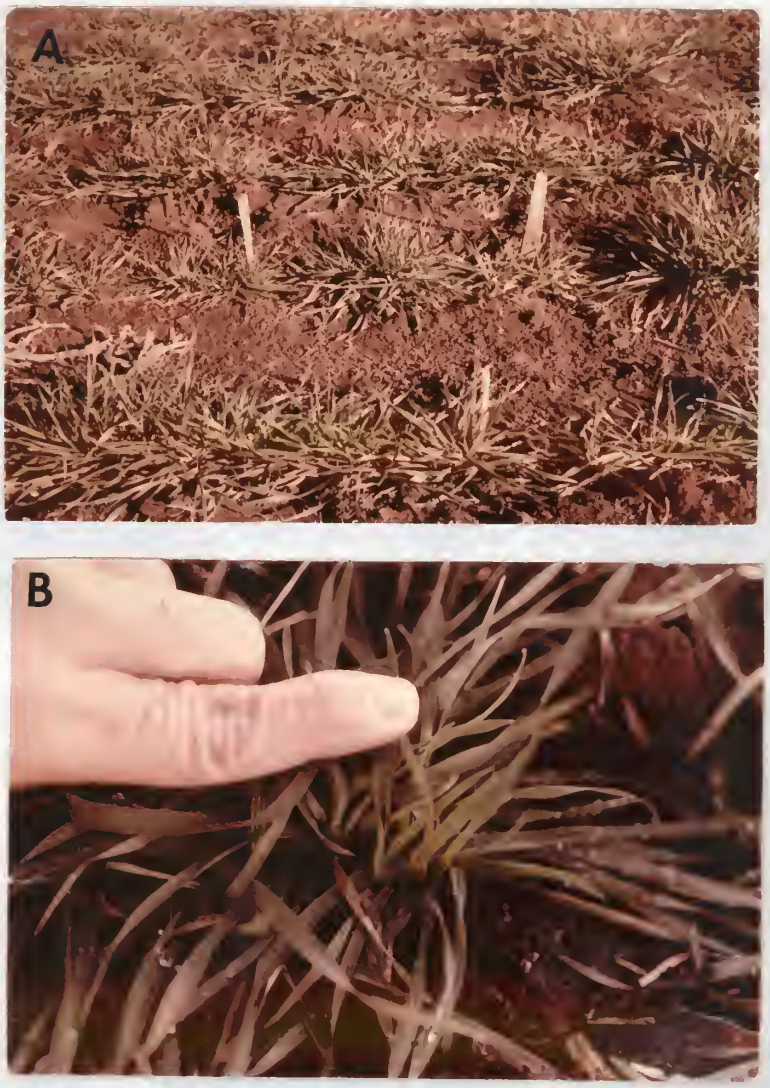

Figure 9. A-SBWM symptoms on Pawnee wheat (staked) in infested plots on the Agronomy Farm, KSU in the Fall of 1965.

B-A close-up of a Pawnee plant showing SBWM symptoms.

Table 10. Weekly average temperature and precipitation at the KSU Agronomy Farm from August 30 to November 28, 1964

Date	Temperature (°F)			Precip. (in.)
	Maximum	Minimum	Average	
Aug. 30 - Sept. 5	86	65	75	1.17
Sept. 6 - Sept. 12	85	56	75	0
Sept. 13 - Sept. 19	76	57	67	0.12
Sept. 20 - Sept. 26	78	54	66	0.03
Sept. 27 - Oct. 3	75	43	59	0
Monthly average	80	57.4	68.8	Total 2.03
Oct. 4 - Oct. 10	66	34	50	0
Oct. 11 - Oct. 17	73	40	57	0.25
Oct. 18 - Oct. 24	70	34	52	0
Oct. 25 - Oct. 31	70	44	57	0.01
Monthly average	69.75	38	54	Total 0.26
Nov. 1 - Nov. 7	54	50	58	0.24
Nov. 8 - Nov. 14	71	43	58	0
Nov. 15 - Nov. 21	41	32	36	0.45
Nov. 22 - Nov. 28	48	24	36	0
Monthly average	53.5	39.75	47	Total 0.69

Table 11. Weekly average temperature and precipitation at the KSU Agronomy Farm from August 30 to November 28, 1965

Date	Temperature (°F)			Precip. (in.)
	Maximum	Minimum	Average	
Aug. 30 - Sept. 5	81	58	70	1.84
Sept. 6 - Sept. 12	85	61	73	1.52
Sept. 13 - Sept. 19	76	54	65	1.31
Sept. 20 - Sept. 26	64	46	55	3.61
Sept. 27 - Oct. 3	74	48	61	0.36
Monthly average	78	54	65	Total 8.64
Oct. 4 - Oct. 10	78	45	61	0
Oct. 11 - Oct. 17	76	48	62	0.68
Oct. 18 - Oct. 24	69	44	56	0.43
Oct. 25 - Oct. 31	70	38	54	0
Monthly average	73.25	43.25	58.25	Total 1.11
Nov. 1 - Nov. 7	69	41	55	0
Nov. 8 - Nov. 14	55	34	45	0.28
Nov. 15 - Nov. 21	58	30	44	0
Nov. 22 - Nov. 28	57	31	44	0
Monthly average	59.75	34	47	Total 0.28

Note: Wettest September since records began in 1858

Table 12. The effect of soil fumigation and seed treatment on SBWM incidence and wheat yields

Treatments	Mean SBWM incidence		Mean yields in bu./A	
	Ottawa	Pawnee	Ottawa	Pawnee
Methyl bromide-1963	4.66	98.3	29.3	31.0*
Methyl bromide-1964	4.33	64.0*	38.4*	35.0*
Nellite seed treatment	5.00	99.6	31.0	23.0
Control	6.80	100	32.0	20.0

* = Significant

LSD for SBWM mean incidence at 5% level = 12.38

LSD for mean yield at 5% level = 8.09

There was no significant differences of SBWM incidence and yields within Ottawa among treatments. However on Pawnee, SBWM incidence in the methyl bromide-1965 treatment was significantly lower when compared to the rest of the treatments. Between methyl bromide-1964 and methyl bromide-1963, SBWM incidence on Pawnee was likewise significant. Yield of Pawnee in methyl bromide-1965 was significantly higher when compared to the rest of the treatments. The yield of Pawnee control was significant over yield in methyl bromide-1963 treatment. Between the two wheat varieties, average yield of Ottawa in methyl bromide-1963 was significantly higher than on Pawnee for the same treatment. In methyl bromide-1965 treatment, yield of Pawnee was significantly higher than Ottawa. The value of significance was based on computed LSD's of 6.03 for SBWM mean incidence and 6.77 for mean yield (Table 13).

Table 13. The effect of methyl bromide fumigation on SBWM incidence and wheat yields in 1965-1966 growing season

Treatments	Mean SBWM incidence		Mean yields in bu./A	
	Ottawa	Pawnee	Ottawa	Pawnee
Methyl bromide-1963	3.00	97.50	25.33 *	15.79
Methyl bromide-1964	4.83	90.83*	22.28	19.44
Methyl bromide-1965	2.33	50.00*	26.44 *	33.47*
Control	6.00	94.16	23.77	23.88*

* = Significant

LSD for SBWM mean incidence at 5% level = 6.03

LSD for mean yield at 5% level = 6.76

DISCUSSION AND CONCLUSIONS

The susceptible wheat varieties, Red Winter Spelt, Harvest Queen susceptible, McKing, and a variety of winter Rye seeded in SBWMV infested soil normally developed symptoms in the greenhouse. These findings seemed to essentially agree with earlier studies that natural infection of susceptible wheat varieties with SBWMV from infested soil under controlled temperature was efficiently successful (32, 35, 46).

The role of nematodes as possible vectors of the virus was disregarded in this study since earlier reports proved that many of these organisms failed to transmit SBWMV (1, 7, 20, 36).

Recovery of the virus from infested soil stored in the greenhouse from 3 to 11 years was negative. When fresh infested soil was air-dried for 28 days before seeding with bait plants, SBWMV infection decreased to almost 21%. Infection of plants seeded in nonair-dried infested soil was about 91%. The prolonged desiccation of infested soil probably inhibited or killed the vector or the virus or both normally present in the soil or within the infested root refuse. Sill (46) observed that fresh infested soil was more infectious than soil from the same source stored in the greenhouse for 1 year of more. However, McKinney (31) reported that infested soil remained infectious for about 9 years. In this case, the soil used was not devoid of moisture.

Serial transmission of the virus via water-root leachate obtained from roots of naturally infected plants yielded positive results. None of the control plants showed SBWM symptoms. Furthermore, 26.6% mosaic incidence on rye plants was observed when seeded in the sterilized soil used in the preceeding experiments. Similar results were reported by Brakke, et al. (7) when roots of healthy wheat seedlings were soaked in distilled water containing small pieces of roots from naturally infected plants. Apparently, when roots of plants naturally infected or roots of plants from a serial transmission were soaked in distilled water, certain root-inhabiting organism(s) carrying the virus moved into the distilled water. When they came in contact with roots of healthy seedlings transmission was effected.

The positive identification of SBWM from serial transmission studies via water-root leachate did not rule out the possibility

that virus particles may exude from infected roots and become dispersed in distilled water. Checking this possible lopehole, rye plants grown in sterilized soil were mechanically inoculated with SBWMV. Recovery of the virus via water-root leachate from roots of mechanically inoculated plants was negative. Similar results were obtained on rye plants seeded in sterilized soil that had contained mechanically inoculated plants. These results indicate that subterranean transmission of SBWMV from naturally infected plants to healthy ones occur only in the presence of certain soil and/or root-vector organism(s).

Studies were made to determine if root-inhabiting organism(s) capable of transmitting SBWMV were in noninfested soil. Water-root leachate from the roots of mechanically inoculated rye plants grown in noninfested soil was obtained. As shown in Table 6, an average of 10% infection on test plants was observed with an incubation period between 33 to 54 days. Similar results were obtained when noninfested soil that had contained mechanically inoculated plants was transplanted with healthy rye seedlings. Additional transmission studies using equal volumes of water-root leachate from roots of healthy rye plants grown in noninfested soil and leaf extract from SBWMV infected plants yielded positive results. On the other hand, when water-root leachate or infected leaf extract was used alone, test plants did not developed symptoms (Table 8). The low incidence of mosaic obtained from mechanical inoculations and from mixtures of water-root leachate and leaf extract from SBWMV infected plants can be explained if the titer of the virus in the expressed sap was low. Johnson (20); McKinney (26, 27), and Sill (46) reported that infection rate from mechan-

ical inoculations was low. If the virus titer was low, then it would follow that not all of the vector organisms would become viruliferous.

Evidence seems to clearly indicate that soil and/or root-inhabiting organism(s) which act as a vector for the virus are not restricted to infested soil as originally postulated (36, 46) but are also present in noninfested soil. The fact that transmission of the disease was only obtained from the mixture of water-root leachate and leaf extract from SBWMV infected plants and none from either alone would indicate that the organism(s) present in noninfested soil are nonviruliferous.

Reports by earlier investigators on the transmission of SBWMV contained enough evidence to suggest that either a microscopic soil or root-inhabiting organism(s) is (are) involved in the natural infection of SBWMV. Polymyxa graminis (23) and Olpidium brassicae, consistently observed within the roots of SBWMV infected wheat, had been suggested as possible vectors of the disease (1, 39).

Results of the root staining and clearing studies as shown in Figures 7 & 8 demonstrated roots to be inhabited by Polymyxa graminis (23) and Olpidium spp. These observation were in agreement with studies conducted by Addison (1) and Lindford and McKinney (24).

While roots of SBWMV infected wheat invariably showed resting spores of Olpidium, no zoospores were observed in nutrient broth culture when isolation was made from such infected roots. In contrast, Barr (3) reported having isolated Olpidium zoospores to the second tube transfer from roots of lettuce showing big-vein symptoms. Olpidium brassicae has been demonstrated as the

vector of big-vein virus of lettuce (BVV) (8, 13).and tobacco necrosis virus (TNV) (49). Only motile zoospores transmitted these viruses and were found to remain as such for just 1 hour when suspended in distilled water (49). Olpidium zoospores suspended in dilute solution of L-isoleucine were motile for 52 hours (50). Even with the addition of L-isoleucine to the culture media, Olpidium zoospores were not observed by microscopic examination of the media. However, certain bacteria were constantly isolated. It was decided that these cultures should be checked as possible vectors. Since these cultures were quite old it was assumed that Olpidium zoospores would be inactive. Dilution plates of the cultures indicated that 2 and probably 3 species of bacteria were present. No fungi were recovered.

Transmission of SBWMV by the isolates in nutrient broth from the roots of infected wheat plants was positive. When roots of test plants were soaked in a mixture of NBI-1 or NB-1 isolates and leaf extract from SBWMV infected plants, 2 of 20 and 3 of 20 plants developed symptoms of mosaic, respectively. No symptoms developed on plants soaked in either nutrient broth containing the isolates or leaf extract alone. Initial results obtained in this particular experiment seems to indicate that the isolates contain a vector of SBWMV. Until more data are available, however, it would be premature to consider that any one organism is the vector of the virus.

About 1% of the Pawnee plants in the field plots developed SBWM symptoms during the Fall of 1965 but none was reported in previous years. Factors attributed to symptom development in the field are prolonged cool weather and sufficient moisture (7, 22,

29, 46). Apparently this conditions were satisfied in the Fall of 1965.

Results obtained from 1964-1965 field experiments showed that methyl bromide-1964 fumigation reduced SBWM incidence on Pawnee with a correspondingly significant increase in yield. On Ottawa, a similar increase in yield was noted. The increase in yield however, could not be correlated with the decrease of SBWM incidence, since mosaic infection obtained in different treatments was about equal. This increase may be due to other factors. These unknown factors were possibly fungi, bacteria, nematodes or a combination of such organisms. Similar conditions were noted in the 1965-1966 growing season. Methyl bromide-1965 fumigation reduced SBWM incidence on Pawnee and correspondingly increased yield. However, on Ottawa, no significant increase in yield was observed in methyl bromide-1965 treatment. Yields of Ottawa among treatments were almost equal, as was SBWM incidence.

It was evident that methyl bromide fumigation decreased SBWM incidence on Pawnee. However, this reduction lasted for only 1 growing season. The use of methyl bromide in a large scale basis to control SBWMV infestation is not advisable. Development of symptoms in the field can be expected when the right environmental conditions are present. Similar condition have been observed by other workers on the disease (7, 22, 29).

SUMMARY

Natural infection of SBWMV on rye plants seeded in infested soil stored for 3 to 11 years in the greenhouse was negative. Infested soil air-dried for 28 days prior to seeding with test

plants decreased in infectivity.

Serial transmission of SBWMV via water-root leachate from naturally infected plants was successful. In addition, healthy rye seeded in sterilized soil that had contained infected plants from serial transmission developed symptoms. These results indicated that some soil or root-inhabiting organism(s) is (are) involved as a vector of the virus.

Recovery of the virus via water-root leachate from mechanically inoculated plants grown in sterilized soil was negative. Likewise, no transmission of SBWMV was noted when this sterilized soil was replanted with healthy bait plants. These results indicated that the virus requires a vector for its transmission.

Further studies showed that the possible vector organism was not restricted to infested soil but was also present in noninfested soil. Transmission of SBWMV via water-root leachate from roots of mechanically inoculated plants grown in noninfested soil was positive. Noninfested soil became infested with the virus when seeded with mechanically inoculated plants.

Positive transmission of the disease in the preceeding experiment from noninfested soil showed strong evidence that a soil or root-inhabiting organism(s) is (are) involved in the transmission of the virus. This condition was further substantiated when positive transmission of SBWMV was obtained from rye plants soaked in a mixture of water-root leachate from healthy plants grown in noninfested soil and leaf extract from SBWMV infected plants. No transmission of the virus was observed on test plants when either of the inoculum sources were used alone.

Test plants showed positive symptoms of SBWM when soaked in a mixture of isolated organisms in broth cultures and leaf extract from SBWMV infected plants. No symptoms of SBWM were noted on plants soaked in either of the inoculum-sources alone.

Root staining studies of wheat roots grown in infested and noninfested soil revealed resting spores of Olpidium sp. and Polymyxa graminis.

Field fumigation with methyl bromide in Fall of 1964 significantly reduced the incidence of SBWM on Pawnee and correspondingly increased its yield. However, an increased in yield of Ottawa in methyl bromide-1964 treated plots could not be correlated to the low mosaic incidence. The decrease in yield on Pawnee was correlated with the high rate of SBWM incidence.

SBWM incidence was reduced and yields correspondingly increased in methyl bromide-1965 fumigation trials. On Ottawa, SBWM incidence remained almost the same in both treated and untreated plots. No significant differences were found on Ottawa yield within treatments.

Symptoms of SBWM in the field were noted in the Fall of 1965. This condition was due primarily to favorable environmental conditions.

ACKNOWLEDGEMENT

I wish to thank the Rockefeller Foundation for sponsoring my graduate studies at Kansas State University and Dr. Webster H. Sill, Jr. for helping me come to this country.

I wish to express my gratitude to Drs. W. H. Sill, Jr. and O. J. Dickerson for their unselfish guidance, patience and encouragement while acting as my advisors and to other members of the Department of Botany and Plant Pathology for their advice and materials.

A special note of thanks is due to Dr. Dickerson for helping in the preparation of this manuscript.

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TRANSMISSION OF SOIL-BORNE WHEAT MOSAIC VIRUS

by

Rodulfo P. Pacumbaba
B. S. A., University of the Philippines, 1958

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Department of Botany and Plant Pathology

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Manhattan, Kansas

1966

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Among the diseases of wheat of economic importance in Kansas is soil-borne wheat mosaic (SBWM). The first reported occurrence of SBWM in Kansas was in 1949 and since, 3 severe epiphytotics have been reported.

Studies conducted from the Fall, 1964 to June, 1966 were to determine the mechanism of transmission of the virus, to isolate the vector organism, to determine the effect of soil fumigation on the disease incidence and yields on susceptible and nonsusceptible wheat varieties, and to develop a way of forecasting SBWM outbreaks.

Transmission of SBWMV from infested soil stored from 3 to 11 years was negative. Infectivity of fresh infested soil was greatly reduced when air-dried for 28 days. Serial transmission of the virus via water-root leachate from naturally infected wheat plants was positive. Sterilized soil became infective when planted with rye that had been inoculated with water-root leachate from infected plants.

Negative transmission of SBWMV was noted on test plants soaked in water-root leachate from diseased mechanically inoculated plants grown in sterilized soil or from rye seedlings replanted in this soil.

Transmission of SBWMV was recorded when water-root leachate was obtained from mechanically inoculated plants grown in non-SBWMV-infested soil. Under controlled conditions, noninfested soil containing diseased mechanically inoculated rye seedlings became infested with SBWMV. A mixture of water-root leachate from healthy rye plants grown in noninfested soil and leaf ex-

tract from SBWMV infected plants yielded positive transmission.

Stained SBWMV infested and healthy roots revealed resting spores of Olpidium sp. and Polymyxa graminis. Attempts to isolate these organism in nutrient broth were not successful. However, 2 or possibly 3 species of bacteria were consistently observed. A small percentage of rye seedlings soaked for 12 to 18 hours in a mixture of these bacteria and leaf extract from SBWMV infested plants developed SBWM symptoms. Seedlings soaked in either the bacterial mixture or leaf extract from infected plants alone did not became infected. These experiments have not been repeated.

SBWMV was noted in the field plots in the Fall of 1965. This is the first report in Kansas of Fall-symptom expression.

Methyl bromide treatment reduced incidence of SBWM on Pawnee wheat resulting in an increase yield as compared to the rest of Pawnee under treatment. Partial soil sterilization by methyl bromide was effective for just 1 growing season. On Ottawa, mosaic incidence was unaffected by the fumigant. The yield increase of Ottawa in methyl bromide-1964 treated plots could not be attributed to the low incidence of SBWM.