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THE EPIDEMIOLOGY OF *XANTHOMONAS TRANSLUCENS* (J. J. AND R.) DOWSON ON CEREALS AND GRASSES

A thesis

by

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THE EPIDEMIOLOGY OF *XANTHOMONAS TRANSLUCENS* (J. J. AND R.) DOWSON ON CEREALS AND GRASSES

INTRODUCTION

Xanthomonas streak[1] is a disease of wheat, barley, rye, brome grass (*Bromus inermis* Leyss) and quack grass (*Agropyron repens* (L.),) and is caused by several varieties of *Xanthomonas translucens* (J. J. and R.) Dowson (9, 21). The disease sometimes becomes epidemic and causes considerable damage to wheat, barley, and brome grass. There has been difficulty, however, in inducing epidemics artifically in the greenhouse and field. Investigations therefore were made to determine the factors that affect the natural spread of the pathogen and which may directly or indirectly play an important part in the epidemiology of the disease.

METHODS

The lack of a reliable standard technique for inoculating seedlings of cereals and grasses with *Xanthomonas translucens* is perhaps one reason why there are so many conflicting reports regarding many phases of

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[1] The common name of Xanthomonas streak is used throughout the paper for the disease of cereals and grasses caused by Xanthomonas translucens. Xanthomonas streak, bacterial blight, and black chaff are synonyms.

the disease. When conventional methods, such as spraying, inoculating with hypodermic needle, pricking or wounding, of inoculating plants with bacteria were found inadequate for inoculating a large number of seedlings quickly and effectively, the partial vacuum technique (2) was developed and used in most of the experiments reported in this paper.

A brief description of the procedure for the partial-vacuum technique for inoculating seedlings with bacteria is as follows. Over 1000 seedlings in the two-leaf stage are submerged in a water suspension of the bacteria in a pressure cooker. The suspension is prepared by mixing 10 ml. of a 48-hour-old bacterial culture, grown in potato-dextrose broth at about 27° C., with 4 liters of tap water. The pressure cooker containing the seedlings and the inoculum is evacuated to about 27 inches of mercury for 3 to 4 minutes. Such treatment causes water congestion of the leaves and withdraws the air surrounding the tissue, and the re-entrance of the air forces the bacterial suspension into the infection court. The inoculated seedlings are transplanted in non-sterilized soil or in vermiculite supplemented with a complete nutrient solution. Ordinarily, 100 percent of the inoculated seedlings become infacted when the plants are incubated on an open bench in a greenhouse at an average temperature of about 85°-90° F. or when the plants are transplanted in the field. The incubation period of Xanthomonas is 5 to 6 days at 85°- 90° F.

By using the partial-vacuum technique, infection

results when seedlings are inoculated with high dilutions of the bacteria; for example, infection resulted with inoculum containing about 20 to 35 bacterial cells per 1000 ml. of water.

Because of its efficiency, the vacuum technique of inoculation was adopted for use in detecting and isolating the pathogen from any material suspected of harboring the organism. By this means, the pathogenic bacteria were isolated from infested, sterilized soil that had been stored in the field during the winter. About 30 grams of the infested soil was placed in 3 liters of tap water and allowed to soak for approximately 10 hours. The water was then decanted and poured into the pressure cooker containing seedlings of wheat susceptible to the pathogen. The pressure cooker was evacuated as previously described and the inoculated seedlings were incubated in the greenhouse. About 5 days later disease symptoms appeared.

Xanthomonas can also be isolated relatively easily from diseased tissue by the same technique. To do so, however, about 100 grams of the suspected diseased tissue of the host is cut into pieces less than 0.5 cm. in length and soaked in water for about 24 hours. The small pieces of tissue are then removed with a fine mesh strainer, and the bacterial water suspension is used to inoculate the susceptible seedlings as described previously. In both instances, the host itself was used for selection isolation of the pathogen from other organisms which are also present

in the soil and in the diseased tissue. The number of infective bacteria per ml. of water prepared from soil, diseased tissue, or other suspected material is determined with moderate accuracy by comparing the average number of lesions on the first two leaves of 10 inoculated seedlings with the average number of lesions on the 10 seedlings inoculated with a known dilution of a 48-hour old culture of X. translucens.

Single-colony isolates of the pathogen are finally made from dilution plates filled with about 20 ml. of potato dextrose agar and one ml. of bacterial water suspension. The water suspension of the bacteria was prepared by macerating 5 to 7 translucent lesions of uniform size from the diseased leaves of the vacuum-inoculated seedling in 10 ml. of sterile, distilled water. A final dilution of 10^{-7} was used for making dilution plates. The isolates used in all experiments were derived from single-colony isolates which in turn were isolated from single colonies of 4 series of dilution plates.

It was noted that some of the bacterial isolates maintained on dextrose agar lost their viability, and all attempts to revive them failed. This was true of some of the isolates kept at 25° C. and of others stored in a refrigerator at 13° C. None of the isolates that lost their pathogenicity remained alive. To make sure that the bacterial isolates used in these studies retained their viability, a new method of storing the cultures was devised.

In this method, seedlings of a susceptible host are inoculated under vacuum with the desired bacterial culture. About 5 days after the incipient, water-soaked lesions appear on leaves of the infected plants, the leaves are cut, pressed and dried for about 48 hours, placed in glycine bags and stored in a refrigerator at 13° C. The bacterial isolates stored in this manner have remained viable for about 2 years. Whenever the bacterial isolates grown on dextrose-agar medium lost their viability, the culture was re-isolated from the infected leaves stored in the refrigerator.

MATERIALS

Sources of isolates.

During the investigation, from 1948 to 1951, many isolates of Xanthomonas translucens were obtained from diseased tissue of several hosts grown at various localities in Minnesota. The isolates used in the experiments are listed in table 1. Many of the isolates were obtained while Xanthomonas streak was epidemic on wheat, barley and Bromus inermis Leyss in the Red River Valley of Minnesota in 1949. The wheat isolates, W-1, W-2, W-3, were made, respectively, from water-soaked, translucent streaks on the leaf blades; from bacterial exudate on the surface of translucent streaks on the leaves, and from translucent streaks on the glumes. The barley isolates

Ba-1, Ba-2, and Ba-3, and the brome-grass isolates Br-1, Br-2, and Br-3 were obtained from similar material of the host as listed for the wheat isolates. The rye isolate R-1 was from translucent streaks on leaves of rye. Isolate Q-1 was from translucent streaks on the leaves of quack grass, Agropyron repens. Included with the isolates already mentioned was W-4, Xanthomonas translucens var. undulosa obtained from Dr. W. A. F. Hagborg in 1948. All the isolates listed were maintained on dextrose agar slants kept in a room at an average temperature of about 26° C.

Morphological and cultural characters of X. translucens.

The comparative morphologic and cultural characters of the varieties listed in table 2 were found to be similar to those reported by Hagborg (9, 10) and Wallin (21). Morphologically, the bacterial cells are cylindrical, with rounded ends, occurring solitary or in pairs. The average size of the rods was 0.4μ x 2.5μ. The bacterial rods were motile when observed in hanging drops and under dark-field illumination. All the varieties studied were gram negative, aerobic, non-spore formers and non-acid fast. On potato-dextrose agar and on dextrose agar, the varieties listed in table 2 formed yellow colonies which in turn formed concentric striations within.

Biochemical characters of isolates of X. translucens.

The methods used for the biochemical tests of the var. undulosa, hordei-avenae, and cerealis were those

Table 1. Source of isolates of Xanthomonas translucens used in the present study.

Isolate No.	Original Host	Source of Isolate	Year of Collection	Locality
W-1	Wheat	Translucent streaks on leaf blade	1949	Red River Valley, Minn.
W-2	do	Bacterial exudate drops on leaves	do	do
W-3	do	Translucent streaks on glumes	do	do
Ba-1	Barley	Like W-1	do	do
Ba-2	do	do W-2	do	do
Ba-3	do	do W-3	do	do
Br-1	Brome	do W-1	do	do
Br-2	do	do W-2	do	do
Br-3	do	do W-3	do	do
R-1	Rye	do W-1	1949	St. Paul, Minn.
Q-1	Quack Grass	do W-1	1948	Rosemount, Minn.
W-4	Wheat	Reisolate of Hagborg's X. trans. var. undulosa.	1946	Manitoba, Canada

recommended by the Committee on Bacteriological Technic of the Society of American Bacteriologists, reported in the Manual of Methods for Pure Culture Study of Bacteria (5). All isolates of the three var. of X. translucens (table 2) produced ammonia and hydrogen sulphide, liquified gelatin, reduced litmus milk, but not indol, did not hydrolyze starch, gave negative results in the methyl red and Voges-Proskauer tests. The isolates produced acid but not gas from glucose, d-fructose, sucrose, lactose, and xylose; none utilized maltose or salicin. X. translucens var. undulosa produced more acid than the other varieties, and variable results were obtained by this variety on glycerol and mannitol. All of the isolates of the 3 varieties of X. translucens were deficient in methionine, as determined by growth on a minimal medium.

Varieties of X. translucens based on pathogenicity.

Five varieties of wheat, two strains of Bromus inermis, 4 varieties of oats, 2 varieties of barley, one variety rye and soybean, and Agropyron repens were each inoculated with 6 isolates of Xanthomonas translucens and one isolate of Pseudomonas glycinea Coerper, the causal agent of bacterial blight of soybeans. P. glycinea, which is non-pathogenic to cereals and grasses, was used in the control treatment to determine whether or not the resistance of the host was broken down by the severity of the partial-vacuum technique. The criterion of infection for

Table 2. The effect of three varieties of Xanthomonas translucens on 14 cereal and grass hosts inoculated by the partial-vacuum technique in the greenhouse (40 plants of each inoculated).

Host	Isolate, variety of X. translucens and degree of infection[1]						
	W-1 und.	W-4 und.	Ba-1 hor.-ave.	Br-1 cer.	R-1 und.	Q-1 cer.	S-1 Control[2]
Wheat							
Newthatch	2	2	0	2	0	2	0
Mida	2	2	0	2	0	3	0
Timstein	3	3	0	3	1	3	0
Pilot	3	3	0	3	1	2	0
Bromus inermis							
var. Fisher	2	0	0	3	0	2	0
var. Parkland	2	0	0	3	0	0	0
Oats							
Marion	0	0	0	1	0	0	0
Mindo	V	0	0	0	0	0	0
Bonda	V	V	1	0	V	2	0
Andrew	V	0	0	2	V	2	0
Barley							
Mars	3	3	3	3	2	3	0
Moore	3	3	3	2	2	2	0
Quackgrass	2	0	0	0	0	3	0
Imperial Rye	2	2	0	2	2	2	0
Habaro Soybeans	0	0	0	0	0	0	2

[1] 3 - severe infection 2 - moderate infection 1 - slight infection (susceptible reaction) 0 - no infection or a white, yellow or brown flecking V - variable infection (immune and resistant reactions).

[2] S-1 - Pseudomonas glycinea (control). See text for explanation.

these experiments and subsequent experiments was the development of water-congested, translucent, elongated lesions on the leaves of the inoculated plants. The rye isolates were less virulent on wheat and most virulent on rye as compared to the wheat isolates which were equally virulent on wheat and rye. Disease readings were made about 7 days after inoculation.

The six isolates of Xanthomonas translucens from cereals and grasses were differentiated on the basis of pathogenicity into 3 varieties, as shown in table 2. The variety undulosa and hordei-avenae are comparable to those in Hagborg's classification (9). The variety cerealis is similar to Wallin's (21) new description and change of rank of this variety, except that it is recommended that Wallin's (21) description should be amended to include Agropyron repens as a natural host. Hagborg's (9) variety hordei and secalis were not isolated from any of the diseased hosts listed in table 1. Some of the isolates of the 3 varieties of X. translucens listed in table 2 were used in subsequent experiments.

SYMPTOMOTOLOGY OF X. TRANSLUCENS ON WHEAT

Knowledge concerning variability in symptoms produced by a pathogen is a prerequisite to recognition of the disease and knowledge of its epidemiology. For this reason it seemed desirable to give special attention to the variable symptoms produced by Xanthomonas translucens on wheat.

The symptoms of Xanthomonas streak on wheat vary with the environment, the variety and, in all probability, with the severity of infection by root-rot and leaf-spot fungi. This variation is particularly noticeable with the symptoms of the head, which are usually associated with the disease. The symptoms on the heads are more frequently characterized by dark brown to black stripes on the glumes. Hagborg (10) recently reported, however, that "in varieties of durum wheat (Triticum durum Desf.), the glume discoloration is light brown and diffuse rather than dark brown and sharply delimited as in common wheats (T. vulgare = T. aestivum L.)."

A third symptom was noted on the heads of the variety Timstein and on some of its derivatives. Here, a diffused, translucent, water-soaked discoloration is produced on the glumes in contrast to a light brown or dark brown to black discoloration as described above. In some instances, only a portion of the head was infected; and in other instances, the entire head was involved. The diseased heads of wheat remain green much longer than the healthy heads; and, in general, matured 7-10 days later. The bacteria isolated from the diseased glumes were identified as Xanthomonas translucens var. cerealis (table 2, isolate W-3). The symptom described above was produced on the heads of the variety Timstein derived from seedlings that were inoculated with isolate W-3 of var. cerealis and transplanted in the field. The seeds from the diseased

heads of Timstein were severely shrivelled; and on the basis of kernel weight per 100 heads, they weighed about one-third as much as healthy seeds.

Since Timstein is being used as a parent stock in breeding for stem rust resistance, it is important to be able to recognize this new symptom of Xanthomonas streak in order select not only for rust resistance but also for resistance to Xanthomonas streak.

MODE OF OVERWINTERING OF XANTHOMONAS TRANSLUCENS

From his studies on Xanthomonas streak of wheat, Smith (15) concluded that the pathogen is carried from year to year on seed. Smith's conclusion was based on circumstantial evidence. No experiments were made to determine whether seeds from infected plants actually transmitted the disease to the emerging seedlings. Braun (3) presented more conclusive evidence that seed from diseased plants of wheat produced infected seedlings. Bamberg (1) was not able to get diseased plants from infected seeds, and therefore concluded that Xanthomonas translucens of wheat does not overwinter on the seed, but in the soil. His conclusion, however, was based on experiments in which sterilized soil was infested with a pure culture of the organism and exposed to freezing temperatures without any apparent injury to the pathogen. No experiments were made to determine whether the pathogenic bacteria can overwinter in non-sterilized field soil. More recently, Wallin (21) showed

that Xanthomonas streak of wheat overwinters on perennial hosts such as Bromus inermis and timothy. Jones et al (13) believe that infected seeds of barley are responsible for carrying over the organism from year to year.

Experiments were made to determine whether X. translucens can survive the winters of Minnesota in infected straw, on seed, in soil, and on perennial hosts. When any of the above habitats were found to harbor the organism through the winter, further studies were made to determine if the diseased material provided sufficient inoculum to cause the initial infection of the host in the spring.

Survival on wheat straw

Investigations were made in 1949 to determine whether Xanthomonas translucens can overwinter on the diseased wheat straw and whether the bacteria in the overwintered straw were infective on the susceptible host. Infested and non-infested straw was obtained in September from ten varieties of wheat grown at Crookston and at St. Paul, Minnesota. By the partial-vacuum technique, it was shown at this time that the infested straw contained X. translucens, while the straw from the clean plants was free from the pathogen. Xanthomonas streak developed on about 50 percent of the seedlings of Rival inoculated with the bacterial water suspension prepared from the infested straw. Most of the infected leaves had 3-4 water-soaked,

translucent lesions, but none of the leaves had more than five. The infective, bacterial population contained in the infested straw in October, 1949, was designated arbitrarily as "large" (L). Subsequent bacterial populations were designated as "medium" (M) and "small" (S) when 20 and 10 percent, respectively, of the inoculated seedlings succumbed to the disease. None of the seedlings of Rival treated with the suspension prepared from non-infected straw became infected.

In December, 1949, the infested and non-infested straw was cut into pieces less than 10 cm. long and placed in wooden flats 12" x 12" x 6". Four series of treatments with each series comprising two flats containing one pound of infected straw and two flats containing one pound of non-infested straw were treated as follows. The infested and non-infested straw in the two flats of series one was mixed thoroughly with non-sterilized field soil obtained from the wheat-disease nursery at the University Farm, St. Paul, Minnesota. The infested and non-infested straw in the other two flats of series one was mixed with the same type of soil as above except that it was sterilized for two consecutive days for 2 hours each day at 15 lbs. pressure. The straw in the flats of series 2 was treated like that in series one except that in this case the infested and non-infested straw was placed on top of the soil. The treatment for the straw in series 3 was the same as for series one ex-

cept that sand was used instead of field soil. The straw in series 4 was placed on top of the sterilized and non-sterilized sand. Flats in series 5 filled with field soil from the wheat-disease nursery served as the control. Two flats were filled with non-sterilized soil and the other two were filled with sterilized soil. The 5 series of treatments were replicated twice. The flats were covered with chicken wire with one replicate buried in a wheat field to the level of the screen covering, and the second replicate stored in a room whose average temperature was about 27° C.

In May, 1950, the flats stored in the field and at 27° C. were brought to the laboratory and isolations were made to determine whether or not the pathogenic bacteria in the infested straw survived through the winter. The presence of the infective bacteria in the straw was detected by the partial-vacuum technique as described under the section on methods. The results of this study show that Xanthomonas translucens overwinters in the infested straw of wheat kept in the field and in a room at about 27° C. The pathogen remained viable in the overwintering infested straw that was mixed with sterilized and non-sterilized field soil and sand. The greatest population of infective bacteria (M) was found in the infested straw which was placed on top of the above soils. There was no appreciable difference in the number of bacteria on the straw that was stored indoors or in the field. X. translucens was not recovered from the control.

Experiments were made under simulated field conditions to determine whether the surviving bacteria in the infested, overwintered straw can infect the emerging plants of wheat. Fifteen seeds of Rival wheat were planted in each 6-inch pot filled with the proper straw-infested soils listed in the previous experiment. The straw that was placed on top of the soil was chopped into small pieces and mixed with the soil in the pots. Each treatment was replicated 4 times. From planting time until the plants had formed their second leaves the pots were kept in the greenhouse during the day and in humidity chambers during the night. Disease readings were recorded each day until the plants reached the boot stage. As soon as symptoms of Xanthomonas streak appeared, the infected plants were removed from the pots so as to prevent the pathogen from spreading to adjoining plants.

The results of this study show that bacteria from the overwintered straw from diseased plants infected the emerging plants of wheat. Streak appeared on 5 to 7 percent of the plants resulting from seed planted in the sterilized and non-sterilized field soil and sand which was infested with straw from diseased plants that had overwintered on top of the soil. Only about 1 or 2 percent of the plants from seeds planted in the overwintered soil types mixed with the infested straw were infected with the pathogenic bacteria. No infection was detected on plants from seed

planted in soil infested with straw from clean plants. The infested straw apparently harbors viable inoculum from season to season that can cause initial infection in the field. The number of viable bacteria in the infested, overwintered straw was reduced when this straw was covered with soil.

Survival in the soil.

Investigations were made in the fall of 1949, to determine whether Xanthomonas translucens persists in the soil of fields cropped with wheat heavily infected with the pathogen. During September two collections of soil were obtained from infected fields of wheat at St. Paul, and from the Northwest Experiment Station at Crookston, Minnesota. Collections from the same area also were made from disease-free fields. Each of the four collections of soil weighed 200 grams and was taken from a composite of 100 samples collected at random from the top 3 inches of soil from five fields. Straw and chaff were removed from each collection by sifting the soil through a fine mesh screen. Seedlings of Rival wheat were inoculated by the partial-vacuum technique with a water suspension prepared from each of the soil samples and as a check with water suspension of sterilized soil to which X. translucens had been added a few hours prior to inoculation.

None of the seedlings inoculated with the water

suspension from the four collections of soil became infected, but about 90 percent of the plants inoculated with the suspension from the inoculated soil became infected. It is concluded from this study that the soil from wheat fields heavily infected with X. translucens and when freed of diseased plants parts does not harbor infective organisms.

Since the above collections of soil were made early in the fall, it was suggested that the bacteria did not get into the soil because the diseased straw had not decomposed sufficiently. Studies were made, therefore, to find out whether decomposing straw from diseased plants infests the soil with the pathogen.

The four series of soil and straw mixtures used in the previous experiment were also used here. In addition, the collections of soil made at St. Paul and Crookston were also infested with straw from diseased and clean plants and kept at 27° C. indoors and in the field from September, 1949, to May, 1950. As described in preceding experiment, isolations were made from the soils during the last week of May. None of the seedlings of Rival wheat became infected after inoculation with water suspension from the following soils infested with straw from diseased plants: sterilized and non-sterilized field soil from the wheat disease nursery; sterilized and non-sterilized sand; and sterilized and non-sterilized soil from fields of wheat heavily infected with X. translucens. The pathogen was not

isolated from the above soils that were infested with non-infested straw. It is concluded from this study that the decomposing, straw from diseased plants does not infest the soil with pathogenic bacteria of Xanthomonas streak; the pathogen apparently does not survive long in the soil.

Further studies were made to ascertain whether a mixture of 3 isolates of Xanthomonas translucens of wheat can survive in sterilized and non-sterilized soils stored in the field and at about 27° C. Isolates W-4 and R-1 of X. translucens variety undulosa and isolate Q-1 of X. translucens variety cerealis were grown separately in shake cultures of potato-dextrose broth for 48 hours. The 3 isolates were then mixed in equal parts of sterile, distilled water prior to inoculating the four types of soil listed in the previous experiment. Each collection of soil was divided into 16 lots, each weighing 15 lbs. and into 16 lots each weighing 250 grams. The 250-gram lots of soil were placed in a 250 ml. Erlenmeyer flask (Fig. 1,b) and the 15 lb. lots into wooden flats. Eight flasks and 8 flats from each soil collection were autoclaved for 2 hours at 15 lbs. pressure for 2 consecutive days (Fig. 1, c) and then infested, respectively, with 25 ml. and 1000 ml. of inoculum containing the 3 isolates of bacteria previously mentioned (Fig. 1, d). The control flasks and flats containing the sterile or non-sterile soil were treated, respectively, with 25 ml. and 1000 ml. of sterile

dextrose broth diluted with 100 parts of sterile, distilled water. Two flasks and 2 flats from each of the infested sterile and non-sterile soil and from the non-infested (control) sterile and non-sterile soil (Fig. 1, e) were stored in a room maintained at about 27° C. A replicate of the above treatment of soils was stored in the field. Individual Erlenmeyer flasks were wrapped with two layers of waxed paper and placed in sterilized tin containers, with each container holding 10 flasks. Asepsis was maintained in the flasks containing sterile soil and stored indoors and in the field. The flats were covered with a fine-mesh wire screen which was nailed to the sides of the flats. The flats stored in the field were spaced 6 feet apart and buried in the soil to the level of the screen; those stored indoors were piled in stacks of 10 with 4 inches between each flat for aeration. Asepsis was not maintained in the flats containing sterilized soil.

After the infested soils were stored in the field and indoors for seven months (September, 1949-March, 1950), isolations were made by the partial-vacuum method. Xanthomonas translucens was not recovered from any of the soils in the flats that were stored in the field and indoors. Comparable results were obtained from the infested soils in the Erlenmeyer flasks, except that the pathogenic bacteria were isolated rather easily from the sterile, infested soils of the four collections which were stored at 27° C. and in

the field. None of the control-treated soils contained the pathogenic bacteria. The results show that in Minnesota the three isolates of X. translucens were not able to survive in the four collections of soil kept at 27° C. and in the field unless the soil was sterile and asepsis was maintained. There probably are many organisms in the soil (7) that are antibiotic to X. translucens and this could account for its failure to survive.

Survival on seed of barley and wheat.

Another problem studied was whether or not Xanthomonas translucens can survive from year to year on the seeds of wheat and barley and, ultimately, produce infection on the emerging seedlings. An epidemic of Xanthomonas streak on barley and wheat in the Red River Valley in 1949 provided opportunity to study the problem. The seeds of Timstein and Rival wheat and Mars barley were used in these studies. One lot of seed of the above varieties of cereals was collected from severely diseased plants and the second lot was disease-free. The first isolation to determine if the pathogenic bacteria were on the seed was made after storage for one month in the laboratory at about 27° C. The bacterial inoculum used for isolating the pathogen from each variety of wheat and barley in the two lots, by the partial-vacuum technique, was prepared from 100 seeds. At this time it was shown that the pathogen was on the seeds collected from the

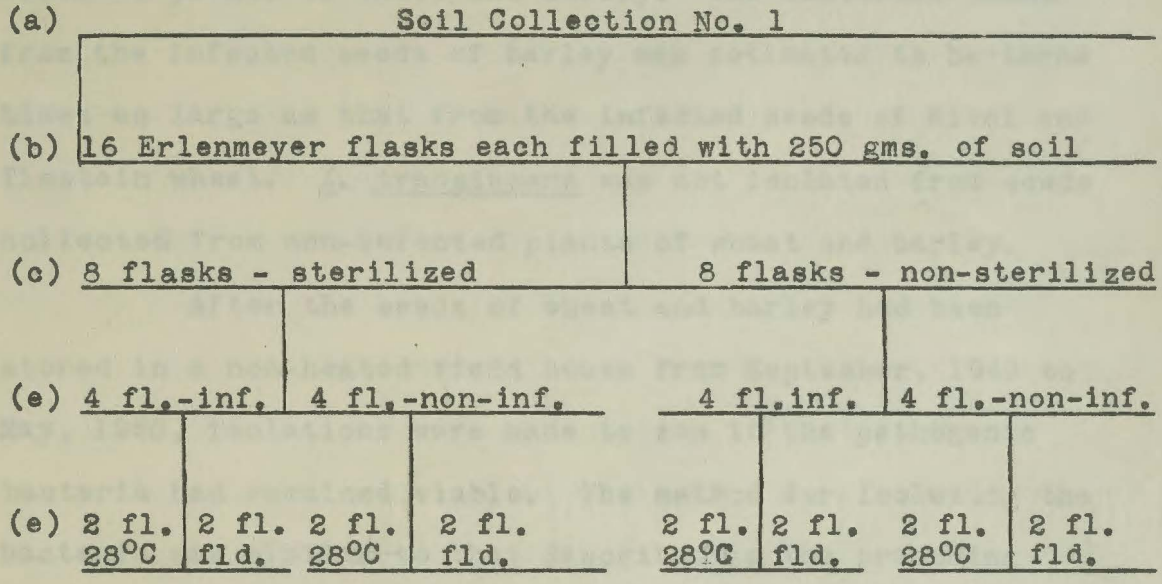

Fig. 1. Experimental design for determining whether 3 isolates of Xanthomonas translucens survive in sterilized and non-sterilized soil kept in 250 ml. Erlenmeyer flasks and stored in the field and at 27° C. The same design was used for three other collections of soil. A parallel experiment was run using wooden flats filled with 15 lbs. of soil.

/1 fl. = flasks.

infected plants of wheat and barley. The bacterial count from the infected seeds of barley was estimated to be three times as large as that from the infected seeds of Rival and Timstein wheat. X. translucens was not isolated from seeds collected from non-infected plants of wheat and barley.

After the seeds of wheat and barley had been stored in a non-heated field house from September, 1949 to May, 1950, isolations were made to see if the pathogenic bacteria had remained viable. The method for isolating the bacteria was similar to that described in the preceding paragraph. Many of the pathogenic bacteria remained viable on the infected seeds of Rival and Timstein wheats and on the seeds of Mars barley. The bacterial count, however, on seeds of wheat and barley was reduced about 70 and 33 percent, respectively, from that on the seeds of wheat and barley that were kept in the laboratory about 30 days after threshing. The number of bacteria on the seeds of barley was now about 4 times greater than on the infected seeds of wheat. The seeds of wheat and barley from the non-infected plants were free of the pathogen.

Experiments were made in the greenhouse and field to see if the bacteria on the seed could infect the emerging seedlings. The greenhouse experiment was made in sterilized and non-sterilized field soil from the wheat disease nursery at the University Farm. About 500 healthy seeds and 500 infected seeds of each variety of wheat and barley were planted separately in wooden flats filled with sterilized or

non-sterilized soil and germinated in a greenhouse kept at about 80° F. After the seedlings emerged, disease readings were made every 4 days until the plants reached the boot stage. The diseased plants were removed from each flat to prevent the dissemination of the pathogen to adjacent healthy plants. The barley plants from the diseased seeds planted in sterilized and non-sterilized field soil were the only ones infected with X. translucens. About 6 percent of the barley plants grown in the sterilized soil became infected, and only 3 percent of those grown in the non-sterilized soil became infected. The bacteria isolated from the diseased plants of barley were identified on the basis of pathogenicity as X. translucens variety hordei-avenae and variety undulosa.

Infected and non-infected seeds of Timstein and Rival wheats and Mars barley were planted in the field at the University Farm on June 10, 1950. The non-infected and infected seeds of each variety of wheat and barley were planted separately in randomized plots. Each plot included four 8-foot rows with an interval of one foot between rows. A distance of 3 feet separated each plot. About 200 seeds were planted in each row, making a total of 800 seeds in each plot. The procedure for taking disease readings in the greenhouse was also followed for the field readings. The results from this study were comparable with the results from the greenhouse experiments. The only plants infected with Xanthomonas translucens were those resulting from

diseased seeds of barley. About three percent of the total infected seeds of barley that germinated gave rise to plants with Xanthomonas streak. The pathogenic bacteria isolated from barley were identified as X. translucens variety hordei-avenae and variety undulosa. The pathogenic bacteria survived in great numbers on the seed of barley and caused infection on some of the emerging seedlings, while no infected plants resulted from seeds of wheat on which only a relatively small number of the bacteria remained viable through the winter.

The explanation for this may be found in the fact that the diseased glumes of barley remain attached to the seed. In our studies, the bacterial population of barley seed included the pathogen in the seed proper and in the glumes, whereas the number of bacteria on the seed of wheat included those of the seed proper only. It is expected then that each infected seed of barley contained a larger number of pathogenic bacteria than each seed of wheat. A higher percentage of the bacteria on diseased seed of barley may have remained viable because the glumes provided some protection to the pathogen against organisms antibiotic to it. (7)

Survival on perennial hosts.

Xanthomonas translucens may overwinter in Minnesota on the infected leaves and leaf sheaths of winter wheat, winter rye, and quack grass. These findings are the

results from studies made on quack grass naturally infected with X. translucens and from studies on winter wheat, winter rye, and quack grass that were inoculated with the pathogen and transplanted in the field. The results from our studies also corroborate Wallin's (21) report stating that X. translucens overwinters on the infected leaves and stem of brome grass.

Investigations on the overwintering of X. translucens on naturally-infected quack grass were made in 1948-1949, at Rosemount Research Center, where severely infected quack grass was found. The symptoms of the disease were similar to those of brome grass (21). The bacteria isolated from the diseased leaves were identified as X. translucens variety cerealis. Many of the diseased plants were tagged and used for making isolations throughout the fall, winter and spring. Isolations were made from the leaves of diseased plants collected on October, 15, 1948, November 10, 1948, March 2, 1949, and April 10, 1949. Isolations by the partial-vacuum technique disclosed that the pathogenic bacteria were on the dead, diseased leaves collected on each of the above dates. During the investigation, it was observed that many of the dead, diseased leaves remained attached to the plants; and, in many instances, they were in contact with the new, growing shoots. Many of the newly-developed leaves that were in contact with the diseased leaves developed symptoms of Xanthomonas streak. The results indicate that X. translucens is capable of overwinter-

ing on naturally-infected leaves of quack grass and that, ultimately, the surviving bacteria can infect the newly formed shoots of the host.

X. translucens was shown to overwinter on several perennial and winter annual hosts that were inoculated with pure cultures of the pathogen. The winter annuals and perennial hosts used in this study were Black Hawk, winter wheat; Imperial winter rye; and quack grass. The seedlings of the above plants were inoculated with a mixture of isolates Br-1, and QR-1 of X. translucens var. cerealis, isolates W-1 and W-4 of X. translucens var. undulosa and isolate Ba-1 of X. translucens var. hordei-avenae. The inoculum was prepared by mixing 2 ml. of each of the 5 isolates, increased on potato-dextrose broth for 48 hours, with 5000 ml. of tap water. The non-inoculated seedlings (control) were evacuated in 5000 ml. of tap water containing 10 ml. of sterile potato-dextrose broth. The inoculated and non-inoculated seedlings were transplanted on September 2, 1949, in 2 adjacent blocks separated by a 3-foot alley. Each of the two blocks was divided into 4 plots and each plot into six 5-foot rows. The distance between the plots and between the rows was 3 feet and one foot, respectively. About 600 seedlings of each perennial and winter annual host were transplanted in each plot. To safeguard against the possibility of severe winter killing, 3 rows in each plot in the two blocks were covered on October 20, 1949, with straw from oats that were not infec-

ted with X. translucens. The straw was removed from the rows on May 20, 1950. The number of winter-killed plants in both the straw-covered and non-covered rows was negligible for the one perennial and 2 winter annual hosts. The disease developed in equal proportions on the straw-covered and non-covered inoculated plants of the perennial and winter annual hosts. Between June 5 and June 13 water-soaked, translucent lesions appeared on the lower leaves of the 3 hosts which had been inoculated the preceding fall. Symptoms of Xanthomonas streak were not noted on any of the hosts that were not inoculated with the pathogen. The pathogenic bacteria were isolated on June 7 from the diseased leaves of the one perennial and 2 winter annual hosts and identified as X. translucens var. cerealis and var. undulosa. X. translucens was not found on the leaves of the perennials that had not been previously inoculated with the pathogen.

On June 15 the upper leaves of approximately 5 percent of the inoculated rye and quack grass plants and about 2 percent of the inoculated plants of Black Hawk winter wheat were infected with X. translucens. None of the leaves of the non-inoculated plants of winter wheat, winter rye and quack grass were infected by the bacteria causing Xanthomonas streak.

The weather from June 15 to July 30 was relatively dry and unfavorable for the development of Xan-

thomonas streak; consequently, the development of the disease was arrested. The pathogenic bacteria, however, were isolated from the lower, diseased leaves of the 3 hosts on July 30. The pathogen was identified as X. translucens var. cerealis and var. undulosa.

The results of this study prove that X. translucens can overwinter on the inoculated leaves of Black Hawk winter wheat, Imperial winter rye and quack grass, and then infect the newly-formed leaves of the hosts. Under favorable environmental conditions, the diseased perennial and winter annual hosts could become the focal points from which an epidemic of Xanthomonas streak might develop.

In Minnesota, Xanthomonas translucens can survive from year to year on kernels of wheat and barley, on wheat straw, and on brome grass, winter wheat, winter rye, and quack grass. The above materials, except the diseased kernels of Timstein and Rival wheat, which harbor the pathogen through the winter, may also be the source which produces the initial inoculum. Since brome grass and quack grass are ubiquitous and are among the first plants to become green in the spring, there is an opportunity for X. translucens to spread from these weeds to cereals and other hosts.

TRANSMISSION OF XANTHOMONAS TRANSLUCENS

Jones et al (13) reported that the long range dissemination of Xanthomonas of barley was through infected seed, and that water is apparently the chief agent of local dissemination, although thrips and aphids may possibly play a considerable part. The writer observed that bacterial exudate from blighted tissue of barley was readily distributed by rain and dew, and to a lesser extent possibly by visiting insects (13). Smith's (17) investigations proved that X. translucens can be in and on the wheat grains. He (20) also stated that the Xanthomonas-streak bacteria of barley are seed borne. He suggested (17,20) that the wheat organism could be disseminated with infected straw, or by insects and birds. Bamberg (1) did not find infected wheat seedlings in the field or in the greenhouse from seed naturally infected with X. translucens.

Transmission by seed.

The preceding experiments show that Xanthomonas translucens in Minnesota remains alive and infectious from year to year on the infected seeds of barley. While the pathogen may remain alive on the diseased seeds of wheat, the resulting plants are free of Xanthomonas streak. Obviously, then, X. translucens may be carried on the seed of barley to new localities; and its long range dissemination is thus accounted for.

Transmission by straw.

The studies on the overwintering of Xanthomonas streak revealed that the pathogen remains alive and infectious on infested stubble and refuse of wheat and brome grass. This suggests that the pathogen may be disseminated in the infested straw or chaff which could be blown by the wind for long distances. Experiments therefore were made in 1950. The inoculum consisted of equal amounts of straw from diseased wheat and diseased brome grass. The wheat straw, which had been stored in the field during the winter, was collected from diseased plants grown the previous season. The straw from brome grass was obtained in the spring of 1950 from a low area in a severely infected field.

The mixture of straw from diseased wheat and brome plants was dried thoroughly and then ground by means of mortar and pestle to bits about 0.5 mm. in size. This dust-like inoculum was shaken over the seedlings of Timstein wheat and Fisher brome grass grown on folded strips of paper as described by Boosalis (2). Five treatments were used for each crop and about 200 seedlings in the second leaf were used in each treatment. The first treatment consisted of water congesting the seedlings by the partial-vacuum technique and dusting them with the infested straw inoculum. The inoculated seedlings were incubated in humidity chambers for 48 hours. The second treatment was similar to the first except that the seed-

lings were not water-congested. In the third treatment the seedlings of wheat and brome were inoculated with the straw from the diseased plants and placed in a greenhouse kept at about 85° F. for 48 hours. The fourth and fifth treatments were the controls in which the seedlings were dusted with non-infested straw and then placed, respectively, in the greenhouse and in humidity chambers for 48 hours. The treated seedlings of wheat and brome were then transplanted in the field. Each treatment was placed in separate plots spaced 3 feet apart. The transplanted seedlings were irrigated during the evening for seven consecutive days.

Xanthomonas translucens developed on the leaves of Timstein wheat and Fisher brome grass that had been water congested treated and inoculated with infested straw. The incipient symptoms appeared on leaves during the boot stage. About 6 percent of the plants of Fisher brome grass and about 4 percent of the Timstein plants succumbed to the disease and the bacteria isolated were identified as X. translucens. The corollary from these findings is that X. translucens can be disseminated in nature on infected straw of wheat and brome grass, which can be blown considerable distances by the wind. Conditions which permit water-congestion of tissue certainly favor infection by the bacteria in straw. The author saw spectacular water-congestion on wheat and barley in the Red River Valley in 1950. In one particular instance, about 80 percent of the

seedlings growing in a commercial field became water congested in less than 20 minutes when there was a sudden drop in the temperature, probably accompanied by a drop in the barometric pressure. Johnson (11) has made extensive studies on the frequency and importance of water-congestion in relation to bacterial diseases of plants.

Transmission by aphids.

In 1949 there was a heavy infestation of aphids on wheat and barley in the Red River Valley of Minnesota and at St. Paul. Many of the plants on which the insects were feeding were also severely infected with X. translucens. Aphids were seen feeding on areas of the leaf that had developed incipient symptoms of Xanthomonas streak. Diseased portions of the leaves that were dry or killed by the bacteria were relatively free from aphids, as they preferred to feed on the green, uninjured tissues. The insects frequently moved freely over the affected areas of the leaf that were studded with bacterial exudate. From these observations, it was postulated that aphids were possible vectors of X. translucens of wheat and barley and studies were made to find out.

Attempt was first made to determine whether aphids become contaminated with the pathogenic bacteria when visiting infected leaves of wheat and barley. About 1000 aphids were collected from infected plants of wheat and barley and placed in a 250 ml. Erlenmeyer flask containing 100 ml. of

tap water. The flask was then placed on a mechanical shaker for about 10 hours. Microscopic observations of drops of water from this flask revealed many types of bacteria, some of which were morphologically similar to X. translucens. The bacterial inoculum obtained from the aphids was then transferred to a pressure cooker filled with 2000 ml. of tap water. After the inoculum had been mixed thoroughly with the tap water, about 200 seedlings of Timstein wheat and 200 of Mars barley were placed in the pressure cooker and inoculated by the partial-vacuum technique. The control treatment consisted of evacuating the seedlings of wheat and barley in tap water.

The wheat and barley inoculated with water suspension from the aphids became lightly infected with X. translucens, with only 2 or 3 translucent, water-soaked lesions on the leaves of each diseased plant. Approximately 8 percent of the Mars plants and 5 percent of the Timstein plants became infected with the pathogen, while all control plants remained healthy. This indicates that aphids may become contaminated with X. translucens when visiting diseased plants of wheat and barley. The question now was whether contaminated aphids could effectively transmit the disease to healthy plants.

Aphids collected from infected plants of wheat and barley and transferred to healthy ones in cages did not induce the disease. This was true whether the infes-

ted plants were kept in the greenhouse or in the field. Attempt was then made to find out whether the aphids could transmit the disease to water-congested plants. Seedlings of wheat and barley were water-congested by the partial-vacuum technique and transplanted to pots filled with sterilized soil and then placed in insect cages. In each cage, there were 50 seedlings of wheat and 50 seedlings of barley. The seedlings in one cage were infested with about 100 aphids collected from diseased plants of wheat and barley. About 100 aphids collected from healthy plants of wheat and barley grown in the greenhouse were transferred to the water-congested seedlings in a second cage. The same number of contaminated aphids collected from wheat and barley were transferred to the third cage which contained seedlings that were not water-congested. The fourth cage contained only the water-congested seedlings of wheat and barley. The four cages were then placed in a low area in the field that had been irrigated. This was done in the evening to prevent the water-congested tissue from drying out. The plants were kept in the cages for 10 days and were then removed and transplanted in the field.

Xanthomonas streak developed on the leaves of wheat and barley that had been water-congested and infested with contaminated aphids. The symptoms of the disease were apparent when the plants were in the boot stage. The infection was very light with a total of 2-3 translucent

lesions developing on the lower leaves of the diseased plants. About 5 percent of the barley plants and 3 percent of the wheat plants became infected. Variable results were obtained when the experiment was replicated 4 times. In 2 experiments none of the wheat and barley plants became infected regardless of treatment; in another experiment about 10 percent of the barley and less than one percent of the wheat plants that had been water-congested and infested with contaminated aphids succumbed to the disease. The results indicate that under favorable environmental conditions aphids can transmit X. translucens to wheat and barley and that they may aid in long distance dissemination.

Transmission by rain and winds.

During periods of high humidity abundant exudate is frequently produced on wheat, barley and Bromis inermis in the field. In one instance over 20 ml. of bacterial exudate was collected in less than 10 minutes from severely infected plants of brome grass growing on the banks of a steep ditch. Field observations indicated that the exudate can be readily distributed by beating rains and wind; therefore experiments were made to determine whether infection could result on adjacent non-infected plants.

Copious amounts of bacterial exudate was produced on the leaves of wheat, barley and brome grass by placing vacuum-inoculated plants in a humidity chamber for 24

hours. Plants whose leaves were studded with bacterial exudate were then placed on a platform that extended one foot above the surface of the bench. Water-congested seedlings of Timstein wheat, Mars barley, and Bromus inermis, were placed on the same bench directly in line with the infected plants on the platform. The distance between the two sets of plants was about four feet. A driving rain blown by strong winds was simulated in the greenhouse by a fine spray of water under about 50 pounds of pressure. The spray jet was directed into the infected plants at such an angle that many of the droplets of water deflected from the leaves fell on the leaves of the non-infected, water-congested plants. When the leaves of the non-infected plants were thoroughly covered with droplets of water, the plants were placed in a moist chamber for 48 hours. The control plants were sprayed with tap water and similarly treated. Immediately after the plants were removed from the humidity chambers, they were transplanted in the field.

The water-congested plants of wheat, barley and brome grass inoculated with bacterial exudate carried by driving spray of water became infected with X. translucens. The disease was first observed when the plants were in the boot stage. About 15 percent of the Mars plants, 10 percent of the brome grass plants, and 8 percent of the Timstein plants were diseased, but no symptoms were ob-

served on any of the control plants. It appears therefore that bacterial exudate of X. translucens may be disseminated effectively in the field by driving rains and wind. The likelihood of infection is probably increased by environmental conditions which produce water-congested tissue, such as are likely to occur during rainy periods in the summer.

Transmission by contact.

Studies were made to determine if healthy plants could become infected from contact with diseased ones. Seedlings of Timstein wheat, Mars barley, and Bromus inermis were inoculated with the variety cerealis and placed in a greenhouse kept at about 85° F. When the incipient symptoms appeared on the infected leaves, healthy, water-congested seedlings of the above varieties of cereals and brome grass were placed adjacent to the infected plants in such a way that many of the diseased and healthy leaves were in contact. Precautions were taken to eliminate the possibility of disseminating the pathogen by wind, splashing water or insects.

About 14 days after the experiment was started, the first symptom of Xanthomonas streak appeared on many of the leaves that had been in contact with the diseased leaves: the percentage of infected leaves was about 20 percent for Mars barley, 10 percent for brome grass, and 5 percent for Timstein. Contact between infected and healthy

leaves probably occurs frequently in nature and can result in spread of the disease.

ROOT ROT AND LEAF SPOT FUNGI IN RELATION TO X. TRANSLUCENS OF CEREALS

During the 1949 epidemic of Xanthomonas streak in the Red River Valley of Minnesota, a high percentage of the wheat and barley plants infected with root rots were also infected with Xanthomonas translucens and many of the wheat and barley plants free of root rots were relatively free from Xanthomonas streak. These observations suggested that root rots might predispose to Xanthomonas streak. It was also postulated that the initial infection of X. translucens on the plants of wheat and barley was caused by the pathogenic bacteria in association with the host invading fungi. Experiments were made, therefore, to determine whether or not root rot fungi play a part in Xanthomonas streak of wheat and barley and rye.

Laboratory and greenhouse experiments for studying root rot and leaf spot fungi in relation to Xanthomonas streak of cereals.

Two isolates of Helminthosporium and 3 isolates of Xanthomonas translucens were used in these studies. The isolates of Helminthosporium designated as H-1 and H-2 were obtained from diseased roots of barley and wheat, respectively. Isolate H-1 was identified as Helminthosporium sativum but for isolate H-2 no species

identification was made. Pathogenicity tests made in sterilized field soil in the greenhouse in October, 1949, showed that isolates H-1 and H-2 were moderately pathogenic on barley and wheat. Isolate H-1 caused pre-emergence and post-emergence damping-off and spot blotch of Mars and Moore barley. Pre-emergence and post-emergence damping-off of Rival, Newthatch, Timstein and Mida wheat was produced by isolate H-2. The three isolates of X. translucens selected for this investigation were W-1, Ba-1 and R-1 (table 2).

Each of the 2 isolates of Helminthosporium was grown with each of the 3 isolates of X. translucens on potato-dextrose agar, beef-peptone agar, and in shake cultures of potato-dextrose broth. The paired isolates of fungi and bacteria grew normally with no indication of antagonism. The rate of growth of each of the paired isolates of the 3 media was comparable to that for each of the isolates grown alone on the 3 media. The fungus isolates grew over the entire surface of the potato-dextrose agar and beef-peptone agar media in Petri plates, while the 3 isolates of bacteria grew slightly beyond the area over the bacterial colonies. Microscopic observations of the mycelium growing over the bacterial colonies showed that the hyphae were in intimate contact with many bacterial cells. The bacteria, however, did not penetrate any of the hyphae.

After each of the paired isolates of Helmintho-

sporium and Xanthomonas had grown in shake cultures of potato-dextrose broth for about 96 hours, the mycelial mat was filtered off and dilution plates were made from the broth. The number of bacteria in one ml. of the filtered broth from the culture which contained both the bacterial and fungus isolates was about 20 percent less than that in one ml. of broth in which only the bacterial isolate was grown. The number of bacteria removed from the broth with the mycelium was about 15 percent of the total number in a 96 hour old bacterial culture grown in a shake culture of potato-dextrose broth. The mycelium of each isolate of Helminthosporium grown with each isolate of X. translucens produced new growth when transferred to fresh potato-dextrose agar. Xanthomonas streak was produced on the proper host when inoculations were made with the bacteria from each of the 3 isolates of X. translucens grown with each isolate of Helminthosporium. Evidently the 2 isolates of Helminthosporium, H-1 and H-2, and the 3 isolates of X. translucens, W-1, Ba-1 and R-1, are not antagonistic or antibiotic to each other when grown together on potato-dextrose agar, beef-peptone agar or potato-dextrose broth.

When seeds of wheat, barley and rye were inoculated with both a root rot fungus and X. translucens grown on grain medium, a relatively high percentage of the resulting plants were infected with the pathogenic

bacteria. Experiments were made in the greenhouse in the winter of 1949 in sterilized and non-sterilized field soil. Seeds of Rival wheat, Imperial winter rye and Mars barley were inoculated with 4 types of inoculum listed in table 3. The isolate of X. translucens used for each cereal is listed in table 3 under two types of inoculum because it was grown on a grain medium and in potato-dextrose broth. The other two types of inoculum were increased on a grain medium. Non-infested grain medium and non-infested potato-dextrose broth were used for the control.

The grain medium consisted of oats, wheat, barley and corn. These components were mixed and soaked in tap water 8 hours before they were autoclaved in quart jars. Each jar was half-filled with the grain medium and autoclaved at 15 lbs. pressure for 3 hours on each of 2 consecutive days. Five-day-old cultures of Helminthosporium and 2-day-old cultures of X. translucens grown in test tubes, each of which contained 10 ml. of potato-dextrose broth, were used for inoculating the sterilized grain medium. Two test tubes of the fungus inoculum and 6 of the bacterial inoculum were transferred to each of the prescribed jars of grain medium. Five tubes of the sterilized potato-dextrose broth were transferred to each jar reserved for the control treatment. Five replicates of each type of inoculum listed for each cereal in table 3 were increased on the predetermined medium.

The following procedure was used in increasing the types of inoculum listed in table 3. The grain medium was inoculated with an isolate of Helminthosporium 8 days before the bacterial isolate was added. The fungus and bacterial inoculum was then allowed to grow for 14 days before it was used to inoculate the seeds. The inoculum containing only an isolate of Helminthosporium was grown on the grain medium for 22 days; and the inoculum containing an isolate of X. translucens was grown on the grain medium only 4 days. The 3 bacterial isolates were also increased in sterilized, 250 ml. Erlenmeyer flasks, each filled with 100 ml. of potato-dextrose broth. The broth in each flask was inoculated with 100 ml. of potato-dextrose broth. The broth in each flask was inoculated with a loop (4 mm. in diameter) of a 24-hour-old culture of X. translucens grown on potato-dextrose broth. One loop of sterile potato-dextrose broth was transferred to the flasks of broth used for the control treatment. The bacterial isolates were grown in the potato-dextrose broth for 48 hours. The partial-vacuum technique for inoculating seedlings with bacteria revealed that the 3 isolates of X. translucens, increased either alone or with an isolate of Helminthosporium, were pathogenic at the time the seeds were inoculated.

About 200 seeds of each cereal were inoculated with one of the types of inoculum listed in table 3, and

planted in 10-6 inch pots which were filled with sterilized field soil within 4 inches from the top. About 20 seeds were placed on top of the soil in each of the 10 replicated pots and covered with about 40 grams of the proper inoculum grown on the grain medium or with 30 ml. of the bacterial inoculum grown in potato-dextrose broth. The depth of planting after the seeds were covered with inoculum and sterilized soil was about 3.0 inches. The pots for each of the cereals were randomized in a greenhouse maintained at about 85° F, and watered daily with tap water.

A high percentage of the plants of wheat, barley and rye resulting from seeds inoculated with both Helminthosporium and X. translucens were infected with X. translucens. Spot blotch also developed on a high percentage of the plants of Mars barley. The first symptom of Xanthomonas streak usually developed on the first leaves of the diseased wheat, barley, and rye. In a few instances, however, the symptoms of the disease developed simultaneously on the first and second leaves. It was evident on the leaves anywhere from 5 to 20 days after the seedlings emerged. The first symptoms often occur on the tips of the leaves but not infrequently other areas of the leaves were infected first. The initial lesions often coalesced and continued to develop until the entire leaf area was involved. As soon as the sym-

ptoms of the disease appeared most of the infected plants were extirpated to prevent the pathogen from spreading to adjacent non-infected plants.

Free-hand sections and isolations were made from the leaves of the 3 cereals derived from seed that was treated with the types of inoculum shown in table 3. Motile, rod bacteria were seen oozing from within the infected tissue of the barley, wheat and rye plants resulting from seed inoculated with Helminthosporium and X. translucens. Fungus hyphae were also evident on about 5 percent of the sections from the three cereals. X. translucens and Helminthosporium were isolated from many leaves of barley, wheat and rye infected with Xanthomonas streak. Fungus hyphae occurred on the free-hand sections cut from spot-blotch infected areas of the leaves of barley derived from seed inoculated only with Helminthosporium sativum, and a few unidentified saprophytic bacteria were isolated also. A relatively small number of bacteria and a few hyphae were seen in the free-hand sections of non-infected leaves of barley, wheat and rye derived from seed inoculated with only X. translucens, while several species of saprophytic bacteria and Alternaria were also isolated.

Table 3 shows that 30 percent of the plants of Mars barley, 20 percent of the plants of Imperial rye and 15 percent of the plants of Rival wheat derived from seed

inoculated with both Helminthosporium and Xanthomonas developed symptoms of Xanthomonas streak. About 3 percent of the Moore barley and 2 percent of the Imperial rye and Rival wheat resulting from seed inoculated with X. translucens increased on the grain medium or on potato-dextrose broth were infected with the pathogenic bacteria. The seeds of the 3 cereals inoculated with Helminthosporium sp. did not give rise to plants infected with X. translucens. Plants from the control treated seed of the 3 cereals were free of symptoms caused by X. translucens.

The above experiment was replicated in non-sterilized field soil in October, 1949. The results were comparable to those of the preceding experiment. The greenhouse studies show that root rot and leaf spot fungi associated with X. translucens increased the incidence of Xanthomonas streak on barley, wheat, and rye planted in sterilized or non-sterilized field soil.

Field experiments on the relation of root rots to Xanthomonas streak

The 1950 field experiments proved that seeds of wheat, barley and rye inoculated with Helminthosporium and X. translucens grown together on a grain medium produced a high percentage of plants infected with Xanthomonas streak bacteria. It was also noted that the root rot isolate H-2 of Helminthosporium predisposed wheat to Xanthomonas streak.

Three varieties of wheat, Timstein, Rival and Lee, two varieties of barley, Moore and Mars, and one

variety of winter rye, Imperial, were used for the field experiments. The seeds of each variety of the 3 cereals were inoculated with 4 types of inoculum (table 4). The inoculum was increased on a grain medium or on potato-dextrose broth as described in the preceding experiment. All varieties of the 3 cereals treated with a comparable type of inoculum were planted in the same block. Each variety, however, was planted in separate, randomized plots within the block. Each plot consisted of four 5-foot rows spaced one foot apart. The blocks were randomized and each treatment was replicated twice.

About 100 seeds were planted in each row, making a total of 400 seeds in each plot. One quart of the inoculum grown on the grain medium was mixed with one quart of field soil and spread on top of the seeds sown in one row. Approximately 500 ml. of the bacterial inoculum grown on potato-dextrose broth for 48 hours was diluted with 2000 ml. of tap water and sprinkled on the seeds planted in one row. Sterilized grain and sterilized potato-dextrose broth were used at the same rate as above for control. The depth of planting after the seeds were covered with field soil was 3 to 4 inches. After the seedlings emerged, disease readings were recorded every 3 days until the kernels were in the milk stage. The diseased plants were not rogued as was done in the prededing greenhouse experiment. The plots were irrigated 3 times during

Table 3. The percentage of plants of three cereals infected with Xanthomonas translucens resulting from seed inoculated with Helminthosporium sp.; X. translucens and with both Helminthosporium sp. and X. translucens grown on a grain medium, when seeds were planted in sterilized field soil in a greenhouse kept at about 80° F.

Host	Organism and percentage of plants infected with X. translucens/1		
	Helmintho-sporium sp.	X. translucens/2	Helm. sp. & X. translucens
Rival-wheat	0	2	15
Mars-barley	0	3	30
Imperial-rye	0	2	20

/1 Rival wheat was inoculated with isolates H-2: Helminthosporium sp., W-1: X. translucens var. cerealis; Mars barley with isolates H-1: Helminthosporium sativum, Ba-1 X. translucens var. hordei-avenae; Imperial rye with isolates H-1: Helminthosporium sativum, R-1: X. translucens var. undulosa. Perfect emergence would be 200. Percentage of diseased plants was based on total number of plants emerging, ranging from 150 to 190 plants.

/2 Similar results were obtained when the seeds of the three cereals were inoculated with the bacterial pathogen grown on potato-dextrose broth.

the course of the experiment.

Initial infection of Xanthomonas translucens usually occurred on the first leaf when the plants had developed their third leaves, and symptoms also appeared on the second and third leaves of about 80 percent of the diseased plants. The only plants infected in the third leaf stage were those resulting from seeds of wheat, barley and rye inoculated with isolates of Helminthosporium sp. and X. translucens grown together on the grain medium. The highest incidence of Xanthomonas streak was on Mars barley, which had 40 percent of its plants, in the third leaf stage of development, infected with the pathogen. The percentage of diseased plants for the other varieties of cereals listed in table 4 was as follows: Moore barley-37, Imperial rye-30, Timstein wheat-28, Rival wheat-25, and Lee wheat-10.

The symptoms of Xanthomonas streak did not develop on the leaves of the diseased plants formed after the third leaf, until flowering time when the kernels were in the milk stage. New centers of infection appeared on the non-infected leaves of most of the diseased plants at this time. The infection was also evident on the leaves of many adjacent plants which were previously free of the disease. The percentage of diseased plants for each variety of cereal at the milk stage of the kernel development was more than twice the percentage of diseased

plants counted at the third-leaf stage of development (table 4). For example, 28 percent of the plants of Timstein wheat were infected during the third leaf stage of development, but it was 85 percent when the kernels of the plants had reached the milk stage. A comparable increase in the percentage of diseased plants was also noted for the other varieties of wheat, barley, and rye listed in table 4. The plants infected with X. translucens were also severely infected with root rot.

When the kernels were in the milk stage, it was observed that a substantial number of plants of Timstein, Rival, and Lee resulting from seeds inoculated with isolate H-2 of Helminthosporium sp. were infected with X. translucens. Table 4 shows that 40 percent of the Timstein plants, 25 percent of the Rival plants and 10 percent of the Lee plants were infected with the bacterial pathogen. The symptoms of the disease occurred primarily on the leaves, although translucent lesions developed on the glumes of many plants. The roots of the plants infected with X. translucens were also severely infected with root rot fungi. About 60 percent of the fungi isolated from the diseased roots of wheat were identified as Helminthosporium sp. whose cultural characteristics were similar to those of isolate H-2. A relatively low percentage of the plants of all the varieties of wheat, barley and rye derived from seeds inoculated with X. translucens grown on a grain medium or on potato-dextrose broth were found

to be infected with the bacteria. In some instances a few of the plants from the control treated seeds were infected also.

The greenhouse and field experiments show that a high incidence of Xanthomonas streak occurs on wheat, barley and rye derived from seed inoculated with a pathogenic root rot isolate of Helminthosporium grown together with an isolate of X. translucens on a grain medium. The mechanism by which the root rot isolate grown with the bacterial isolate causes the initial infection on the plants was not investigated in this study. The field experiments also showed that wheat severely infected with root rot fungi was also infected with X. translucens. The source and the mode of dissemination of the bacterial inoculum causing Xanthomonas streak on the plants of wheat from seed inoculated with a root rot fungus was not determined. Although the above experiments were made only once, the results indicate that root rot fungi may be an important factor in the epidemiology of Xanthomonas streak of wheat, barley and rye and possibly grasses.

VARIETAL SUSCEPTIBILITY OF WHEAT TO X. TRANSLUCENS

Goulden and Neatby (8) reported that all varieties and strains of wheat grown in the nursery at Winnipeg, Canada, in 1928 were infected to some degree by Xanthomonas

Table 4. Percentage of plants of wheat, barley and rye infected with X. translucens when grown from seeds planted in the field and inoculated with Helminthosporium sp.; X. translucens; and with both grown together on grain medium.

Host	Organism and percentage of plants infected with X. translucens/1					
	Helminthosporium sp.		X. translucens/2		Helm. sp. & X. translucens	
	3rd leaf stage	milk stage of grain	3rd leaf stage	milk stage of grain	3rd leaf stage	milk stage of grain
Wheat:						
Rival	0	25	0	7	25	60
Timstein	0	40	0	5	28	85
Lee	0	10	0	3	10	25
Barley:						
Mars	0	6	0	0	40	80
Moore	0	4	0	2	37	78
Rye:/3						
Imperial	0	-	0	-	30	-

/1 Wheat was inoculated with isolates H-2: Helminthosporium sp., W-1: X. translucens var. cerealis; barley with isolates H-1: Helminthosporium sativum, Ba-1: X. translucens var. hordei-avenae; rye with isolates H-1: Helminthosporium sativum, R-1: X. translucens var. undulosa. Perfect emergence would be 200. Percentage of diseased plants was based on total number emerging, ranging from 285 to 390.

/2 Similar results were obtained when seeds of the three cereals were inoculated with the bacterial pathogen grown on potato-dextrose broth.

/3 The winter variety of rye, Imperial, did not head.

translucens. Burton (4) found that all varieties of wheat tested at the Njaro Plant Breeding Station, Kenya, in 1931, were susceptible. Although Bamberg (1) stated that Marquis, Mindum and Kubanka were resistant under field conditions, he concluded from his experiments that resistance of wheat is only relative.

Thirty-three varieties and hybrids of wheat were tested to Xanthomonas translucens in the field in 1950. About 100 seedlings of each variety and hybrid were inoculated by the partial-vacuum technique with a mixture of isolates W-1, W-4, R-1, and Br-1 of var. undulosa and var. cerealis (table 2). The inoculum was prepared by mixing 2 ml. of a 24-hour-old shake culture grown on potato-dextrose broth from each of the isolates. The control treatment consisted of water congesting the leaves of the seedlings. The inoculated and control seedlings were transplanted in separate adjoining blocks. Each variety was planted in separate plots which contained two 6-foot rows. The distance between plots and between blocks was 3 feet. All of the plots were irrigated when conditions became relatively dry.

Disease readings were made when the kernels were in the soft dough. All the varieties and hybrids listed in table 5 were infected to some degree. The relative susceptibility of each variety and hybrid was designated as moderately resistant (MR); moderately susceptible (MS), and

susceptible (S) (see footnote2, table 5). It was found (table 5) that Steward, Carleton, and 8 hybrid lines are moderately resistant, whereas the other 23 varieties and hybrids are either moderately susceptible or susceptible. The control plants were not infected. These results show that some varieties and hybrids of wheat are less subject to the disease than others. It is believed that the partial-vacuum technique for inoculating seedlings with bacteria was not unduly severe for testing varieties for resistance. It is unlikely that morphologic or functional resistance of wheat were broken down by this method of inoculation; many of the varieties and hybrid lines shown to be susceptible by this method were also found to be susceptible as a result of natural infection in the field.

Effect of X. translucens on kernel weight of wheat.

Smith et al (17) reported reduction in yield of several varieties of wheat ranging from 50 to 80 percent. The effect of X. translucens on the kernel weight was determined for 6 varieties of wheat (table 6). Five hundred heads of wheat from each of the 6 varieties were collected at random from each of the blocks containing the infected and non-infected plants. The heads were allowed to dry before the grain was threshed and weighed. The kernels from the diseased heads were severely shrivelled and those from

the non-infected heads were plump. The percentage reduction in weight of kernels due to the disease in Mida, Haynes Bluestem, Rival, Timstein, Ceres and Newthatch was 57, 44, 40, 38, 36 and 20, respectively.

Effect of X. translucens on seed germination of wheat.

The effect of Xanthomonas translucens on seed germination was determined for the 6 varieties of wheat listed in table 6. About 100 diseased seeds and 100 non-infected seeds from each variety were planted in non-sterilized field soil and germinated in a greenhouse kept at about 85° F. Counts of seedling emergence were taken about 3 weeks after the seeds were planted. The results in table 6 show that the percentage of diseased seeds that germinated ranged from 50 to 72 percent for the six varieties while that for the healthy seeds was from 78 to 95 percent. The greatest reduction in seed germination, 50 percent, was with the variety Timstein (table 8). X. translucens not only reduced the weight of the kernels of the six varieties of wheat but also reduced the percentage of seed germination.

Table 5. Susceptibility of 33 varieties and hybrid lines of wheat to 4 isolates (W-1, W-4, R-1, Br-1) of X. translucens var. undulosa, and var. cerealis.

Variety or Hybrid Line	Minn. No. or Nursery Stock No.	Degree of Susceptibility/1	Variety or Hybrid Line	Minn. No. or Nursery Stock No.	Degree of Susceptibility
Steward	2708	MR	Thatcher	2303	MS
Carleton	2707	MR	Marquis	1239	MS
1552 x Mida	2083	MR	Kenya 117A	-	MS
Timstein x Newthatch	II-42-30	MR	Ceres	2223	MS
Pilot x Merit	1996	MR	Lee	2776	MS
1764 x Henry	2211	MR	Newthatch	2752	MS
Timstein x Mida	II-42-27	MR	Henry	2753	MS
1750 x 1753	2092	MR	AM^{10}x Newthatch	Ns3684	MS
Ns2744 x2809	Ns3274	MR	Hope x Timstein	2789	MS
Mida x Kenya 117A	II-44-2	MR	H.R.R. x Mercury	SD1691	MS
Hope	2297	MS	Timstein x Newthatch	II-42-41	MS
Mindum	470	MS	Haynes Bluestem	169	S
Timstein x Newthatch	II-42-10	MS	Timstein	5990	S
Redman	2777	MS	Rival	2670	S
Progress	2225	MS	K58 x Newthatch	II-44-3	S
Pilot	2687	MS	Regent x1315	1950	S
Mida	2689	MS			

/1 Relative susceptibility classes are designated by MR, MS, and S. MR, 10-20 percent of leaf area infected; MS, 20-40 percent of the leaf area infected; S, 40-80 percent of the leaf area infected.

Table 6. The effect of X. translucens on the kernel weight and seed germination of 6 varieties of wheat.

Variety	Weight of grain from 500 heads of wheat.		Percent difference in weight	Percentage of seed germination. [1]	
	Diseased	Control		Diseased	Control
Mida	150 grams	350 grams	57	63	85
Rival	170	305	44	55	78
Timstein	150	250	40	50	89
Ceres	200	310	38	72	95
Haynes Bluestein	100	160	36	65	90
Newthatch	170	210	20	58	88

[1] One hundred diseased and healthy seeds of each variety were planted in non-sterilized field soil and germinated in a greenhouse maintained at about 85° F.

DISCUSSION

Although Xanthomonas translucens causes a common and destructive disease of cereals and grasses, previous investigations have been confined primarily to the classification and natural host range of the pathogen (1,6,9,21). The present investigations have shown how the pathogen overwinters in Minnesota, and some of the ways in which it is disseminated. It is also shown that root-rot fungi may predispose cereals to Xanthomonas streak. This information should help in the prevention of the disease by cultural methods and in understanding the occurrence of epidemics.

The common names given to the diseases caused by Xanthomonas translucens do not adequately describe the symptoms. For example, although the name "black-chaff" of wheat is commonly used, it is not entirely descriptive. Although common names need not necessarily be descriptive, this name has given the impression that all black discoloration of the head is caused by X. translucens. It is believed that "Xanthomonas streak" proposed by Wallin (21) is a more appropriate common name.

It is difficult to make an accurate diagnosis of Xanthomonas streak in the field by studying only the symptoms on the head, because other microorganisms, including fungi and bacteria, and environmental factors may produce black discolorations similar to those produced by X.

translucens (10). The symptoms on the head may also vary with the variety. The most frequent symptom reported on the heads is dark brown to black stripes on the glumes. On durum wheats, however, the glume discoloration is light brown; and on Timstein and some of the Timstein derivatives, there is no light brown, dark brown to black discoloration but only a diffused, translucent, water-soaked discoloration of the glumes. Xanthomonas streak can be diagnosed fairly accurately, however, if bacterial exudate in the form of transparent scales or yellow beads is present on some infected portion of the tissue. Microscopic observations and cultural studies are necessary, however, to be certain of the causal agent.

It was shown that Xanthomonas streak, under conditions favorable to the parasite, may seriously reduce the quality of the grain. The kernel weight of six varieties of wheat severely infected with Xanthomonas translucens was reduced 20 to 50 percent and the germination of the seed was 20 to 30 percent lower than that of the healthy seeds. X. translucens and root rot and leaf spot fungi undoubtedly were the pathogens primarily responsible for the reduction in yield and seed germination of many varieties of wheat and barley grown in the Red River Valley of Minnesota in 1949.

One of the most important reasons for the paucity of information regarding many phases of the epidemiology of

Xanthomonas streak is undoubtedly the lack of a technique for detecting the causal agent when present in relatively small numbers. The author, therefore, devised and adopted the partial-vacuum technique for inoculating seedlings with bacteria especially for this purpose. Because of its efficiency, the partial vacuum technique was used for inoculating simultaneously a large number of seedlings with the same batch of inoculum, for detecting naturally surviving bacteria in small numbers on overwintered material and on possible agents of dissemination, and for inoculating uniformly and heavily many varieties and hybrid lines of wheat to be tested for resistance in the field.

This new technique was also used for water-congesting the host tissue. Such water-congestion apparently is requisite for infection of plants when inoculated by other methods. Water congestion occurs commonly in nature (11) and, in all probability, is important in the effective transmission of X. translucens in the field. Although this new technique of inoculation may be somewhat drastic as compared to natural means of inoculation, it served its purpose in elucidating many phases of the epidemiology of X. translucens.

Xanthomonas translucens can persist from one season to the next on winter wheat, winter rye and quack grass; on the straw of wheat and brome grass; and on seeds of barley and wheat. The winter hosts afforded the bac-

teria the best means for overwintering, and much of the initial inoculum in spring probably comes from these sources. The number of viable bacteria in straw was considerably reduced when the infested straw was mixed with soil. It may be that B. subtilis (7), which is antibiotic on artificial media, and other soil-inhabiting microorganisms are deleterious to X. translucens in soil and tend to eliminate it through antibiotic action. This could also explain why the pathogenic bacteria do not survive in the soil for any appreciable time. Only a small number of bacteria remain viable on severely infected seed of wheat; consequently, the resulting plants have been found to be free from Xanthomonas streak. Bamberg (1), who also made experiments at Minnesota, reported that plants from diseased seed of wheat were not infected with the pathogen.

The mode of overwintering of X. translucens suggests three possible sanitary measures for controlling the disease -- using clean seed, plowing under crop residue, and destroying perennial weeds susceptible to the pathogen.

Since the pathogenic bacteria overwinter on straw and seed, they may be disseminated long distances on these materials by man or wind. Local dissemination of Xanthomonas translucens is by wind-blown rain, contact, and aphids. In view of the fact that barley refuse may harbor X. translucens as well as such leaf spot fungi as Helmonthosporium teres and Helminthosporium sativum, it is

also possible that hyphae of these fungi may subsequently carry the bacteria to the emerging seedlings of cereals. Further studies should be made to investigate the possibility that another disseminating agent of the bacterial pathogen may be the spores of the leaf spot fungi which are on plants infected with X. translucens.

Experiments have disclosed the existence of a biotic relationship between root rots such as Helminthosporium sativum and Helminthosporium sp. and Xanthomonas translucens which appears to have a profound effect on the epidemiology of Xanthomonas streak of cereals. This relationship may help to explain the conflicting reports regarding many phases of the disease and the difficulty encountered in causing epidemics artifically.

Wheat infected with root fot fungi (Helminthosporium spp.) apparently is predisposed to the attack of Xanthomonas translucens. In controlled experiments a greater incidence of natural bacterial infection occurred on plants infected with root rot fungi than on the plants free from root rot; and there was a positive association between the incidence of the two diseases in commercial fields of wheat.

Root rot associated with Xanthomonas translucens may have a strong influence on the incidence of Xanthomonas streak on the seedlings of barley, wheat and rye. Seeds of the three cereals inoculated with X. trans-

lucens alone resulted in a few infected seedlings, whereas 25 to 40 percent of the seedlings from seeds inoculated both with a root rot fungus (Helminthosporium sp.) and X. translucens succumbed to Xanthomonas streak.

The mechanism by which root rot fungi increase the incidence of Xanthomonas streak was not extensively studied. It is possible, however, that plants infected with root rot fungi may be more subject to water-congestion, which is an important requisite for the abundant infection of the host by bacteria. It is also possible that bacteria associated with root-rot fungi are carried to the infection court of the emerging seedlings by the host-invading hyphae.

Although thirty-three varieties and hybrid lines of wheat tested in the field were susceptible to Xanthomonas translucens, it was evident that there was a difference in the degree of susceptibility of some varieties and hybrid lines. For example, Stewart, Carleton and 8 hybrid lines listed in table 5 were moderately resistant and the remainder of the varieties and hybrid lines were either moderately susceptible or susceptible. It may be possible to use some of the moderately resistant lines of wheat to develop new varieties highly resistant to the disease. Investigations should also be made to determine if there is a correlation between root rot resistance and resistance to X. translucens.

SUMMARY

1. Xanthomonas streak of cereals and grasses caused by several varieties of Xanthomonas translucens occurs commonly in Minnesota.

2. The partial-vacuum technique was devised for use in this study, for inoculating seedlings with bacteria, for isolating the pathogen from materials suspected of harboring the pathogen, and for water-congesting the host tissue. By this means it was possible to determine the mode of overwintering of Xanthomonas translucens and possible agents of dissemination.

3. The isolates of X. translucens from cereals and grasses were identified on the basis of pathogenicity on wheat, barley, rye, oats, brome grass and quack grass as belonging to the varieties undulosa, hordei-avenae, and cerealis.

4. X. translucens maintained on the host tissue stored at about 13° C. remained viable for over two years.

5. A new symptom of Xanthomonas streak was noted on the heads of Timstein and some Timstein derivatives of wheat, on which a glistening, translucent, diffused, water-soaked appearance of the glumes is characteristic, with absence of any appreciable light brown, dark brown to black discoloration.

6. In Minnesota the pathogenic bacteria may persist from one season to the next on seed of barley and wheat, on

straw of barley and brome grass, and on winter hosts such as winter wheat, winter rye, quack grass, and brome grass.

7. The above material, with the exception of wheat seed, which harbors the pathogen through the winter, may also provide the initial inoculum which ultimately results in producing the initial infection in the spring.

8. X. translucens does not survive in non-sterile soil for an appreciable length of time unless it is in or on plant parts.

9. X. translucens may be transmitted by aphids, straw, wind and rain, by diseased plants coming in contact with healthy plants, and, probably, by root rot and leaf spot fungi.

10. Effective transmission by aphids, straw, and wind and rain and contact was produced only when the host tissue was water-congested.

11. Experimental evidence indicates that root-rot and leaf-spot fungi predispose wheat to attack by X. translucens.

12. Thirty-three varieties and hybrid lines tested in the field became infected to some degree by Xanthomonas streak. Differences in susceptibility, however, was evident in some of the varieties and hybrid lines. Stewart, Carleton, and 8 hybrid lines listed in table 5 were moderately resistant, whereas the remaining varieties and hybrid lines were either moderately sus-

ceptible or susceptible.

13. The reduction in kernel weight of six varieties of wheat infected with X. translucens ranged from 20 to 50 percent, and the germination of the seed was 20 to 30 percent lower than that of healthy seed.

LITERATURE CITED

1. Bamberg, R. H. Black chaff of wheat. Jour. Agr. Res. 52:397-417. 1936.

2. Boosalis, M. G. A partial-vacuum technique for inoculating seedlings with bacteria and fungi (Abstr.) Phytopath. 40:2-3. 1950.

3. Braun, H. Presoak method of seed treatment: a means of preventing seed injury due to chemical disinfectants and of increasing germicidal efficiency. Jour. Agr. Res. 19: 363-392. 1920.

4. Burton, G. J. L. Annual report of the senior plant breeder, 1930, 1931. Kenya Dept. Agr. Ann. Rept. 1930: 228-243, 1931; 1931: 176-201, 1932. (Abstr. in Rev. Appl. Mycol. 11:164, 1932; 12:13, 1933).

5. Committee of Bacteriological technic. Society of American Bacteriologists. Manual of methods for pure culture study of bacteria. Geneva, N. Y. 1946.

6. Fang, C. T., O. N. Allen, A. J. Riker, and J. G. Dickson. The pathogenic, physiological and serological reactions of the form species of Xanthomonas translucens. Phytopath. 40:44-64. 1950.

7. Goodman, J. J. and A. W. Henry. Action of subtilin in reducing infection by a seed-borne pathogen. Sci. 105:320-321. 1947.

8. Goulden, C. H. and K. W. Neatby. A study of disease resistance and other varietal characters of wheat-application of the analysis of variance, and correlation. Sci. Agr. 9:575-586. 1929.

9. Hagborg, W. A. F. Classification revision in Xanthomonas translucens. Canad. Journ. Res. (C) 20: 312-326. 1942.

10. _____ The diagnosis of bacterial black chaff of wheat. Sci. Agr. 26:140-146. 1946.

11. Johnson, J. Water-congestion in plants in relation to disease. Wisc. Research Bull. 160. 1947.

12. Jones, L. R., A. G. Johnson, and C. S. Reddy. Bacterial blights of barley and certain other cereals. Sci. n. s 44: 432-433. 1916.

13. _____ A bacterial disease of barley. Jour. Agr. Res. 11:625-644. 1917.

14. Melchers, L. E. Plant diseases attacking the wheat crop in Kansas. Kansas State Board of Agriculture 39. 220-248. 1920.

15. Reddy, C. S., J. Godkin, and A. G. Johnson. Bacterial blight of rye. Jour. Agr. Res. 28: 1039-1040. 1924.

16. Smith, E. F. A new disease of wheat. Jour. Agr. Res. 10:51-54. 1917.

17. _____ U. S. Dept. Agr. Bur. Plant Ind. Plant Disease Survey Bull. 1:40. 1917.

18. _____ Black chaff of wheat. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Survey Bull. 2: 98-99. 1918. Mimeographed.

19. _____, L. R. Jones and E. S. Reddy. Black chaff caused by Bact. translucens var. undulosa. U. S. Bureau of Plant Industry. Plant Disease Bull. Supplement 4:135. 1918.

20. _____ An Introduction to Bacterial Diseases of Plants. 1st ed., 688 pp. W. B. Saunders Co. 1920.

21. Wallin, J. R. Parasitism of Xanthomonas translucens (J. J. and R.) Dawson on grasses and cereals. Iowa State College Jour. of Sci. 20: 171-193. 1946.

Biography of Michael G. Boosalis

Michael G. Boosalis was born September 20, 1917, in Faribault, Minnesota. He attended elementary school through the fourth grade in Niata, Greece, and completed elementary and high school in Faribault. In 1941 he was granted the degree of Bachelor of Science by the University of Minnesota, with a major in plant pathology and a minor in botany. Each summer from 1937 to 1941 he was the assistant corn breeder for the Minnesota Seed Company at Faribault.

Mr. Boosalis entered the United States Army in 1941 as a private. In 1942 he transferred into the United States Army Air Corps and received his commission as 2nd Lieutenant in 1944. From 1944 to 1945, he was with the Fifteenth Air Force, stationed in Italy. After completing fifty combat missions, he returned to the United States and served as instructor for bombardiers and navigators until he was released in 1945 with the rank of 1st Lieutenant. At present he is a 1st Lieutenant in the Air Corps Reserve.

In August, 1945, he entered the Graduate School of the University of Minnesota where he became research assistant in the Division of Plant Pathology. He received his Master of Science Degree in 1948. In the same year he was appointed teaching assistant in elemen-

tary plant pathology and forest pathology. During the spring and summers of 1948 and 1949, he was responsible for the spring wheat testing for the United States Department of Agriculture in the wheat disease garden, University Farm, St. Paul, Minnesota. In 1949 he was appointed Research Fellow and assigned to investigate the effects of radioactive materials on bacteria under a grant from the Atomic Energy Commission.

Mr. Boosalis is an associate member of the Society of the Sigma Xi, a member of Gamma Alpha and of the American Phytopathological Society.

He has published the following papers:

Necrosis of soybean stem and rot caused by Rhizoctonia solani Kuhn. (Abstr.) Phytopath. 37:3. 1947.

Fungi that poison soybeans (with M.F. Kernkamp) Minnesota Farm and Home Sci. 4. November, 1948.

A partial-vacuum technique for inoculating seedlings with bacteria and fungi. (Abstr.) Phytopath. 40:2-3. 1950.

Studies on the parasitism of Rhizoctonia solani Kuhn on soybeans. Phytopath. 40: 820-831. 1950.