TECHNIQUES FOR STUDYING VARIETAL RESPONSE TO SEPTORIA LEAF BLOTCH OF WHEAT

By

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INTRODUCTION

Septoria leaf blotch of wheat, caused by *Septoria tritici* Rob., is known also by the common names speckled leaf blotch and nebular leaf spot. The straw colored lesions or blotches appear speckled due to numerous dark brown pycnidia which liberate thread like conidia in large numbers. As the blotches increase in number and size they cut off the conductive tissues supplying the leaf tips and a progressive dying back of the leaves results. In Oklahoma it usually first appears as small lesions on the seedling leaves during the later part of October. If rainfall is frequent or excessive during this period it continues to destroy the leaves almost as fast as the plant grows. Such infection materially reduces the amount of forage available for winter pasture, and at the same time, predisposes the plants to winter injury. The disease is usually quiescent during the cold and dry winter months, being carried over as pycnidia in old lesions. Spore production and infection resumes with the spring rains and continues into late May or June. The disease lesions cause a reduction in productive leaf surface; which in turn causes a reduction in yield and a lower test weight. However, the actual economic losses are extremely hard to measure.

Chester (3) reported an epidemic in Oklahoma in 1941 which resulted in 40 to 50 per cent of leaves destroyed by heading time. Again in 1952, the disease reached such a magnitude that Young and Wadsworth (21) found from 70 to 90 per cent of the flag leaves were destroyed by the time the kernels were in the milk stage of development. This disease, along with late winter temperatures, has been credited by Chester (4) for holding wheat leaf rust infection to a low level in 1943, because it was able to destroy rust infected leaves faster than the rust could produce spores.

In Arkansas, Rosen (15) reported that Septoria leaf blotch, leaf rust, and glume blotch (Septoria nodorum (Berk.) Berk.) have contributed to a steady decline in wheat acreage during the past decade. In the more humid parts of Oregon and Washington winter stands of wheat are almost universally covered with the salt and pepper lesions of S. tritici. This injury has a depressing effect on yield, even though the fungus often disappears entirely with the warm, drier weather in spring and summer, according to Sprague (19).

Several control measures have been suggested, among which are; crop rotation, sanitation, seed treatment with the organic mercury fungicides, and plowing as soon after harvest as soil conditions permit. However, the most satisfactory method of control would be the use of resistant varieties wherever they are available.

A primary prerequisite in finding or developing resistant varieties is the artificial production of disease epidemics. The purposes of these investigations were: (1) to determine methods of producing epidemics both in the field and in the greenhouse; and, (2) to determine by these methods the reaction of wheat varieties grown in Oklahoma, and other varieties which might be used as sources of resistance in a breeding program.

LITERATURE REVIEW

Temperature and moisture seem to be the most important environmental factors affecting spore germination and progress of the fungus within the host. Cardinal temperatures for germination of conidia have been established by several investigators (4, 5, 10, 20). Although there is some variation between these reports an average would place the minimum near 2 to 3° C., the optimum 24 to 26° C. and the maximum 30 to 32° C.

Agreement is universal that moisture is required during spore germination, both in greenhouse and field inoculations (1, 4, 10, 19). The method of maintaining high humidity or free moisture during the first part of the incubation period varied with each worker, but any practical method was satisfactory. The length of the wetting period required has been reported to be from 15 hours by Chester (4) to 120 hours by Luthra et al (10). In addition, Beach (1) indicated that a second wetting period is required before pycnidia will be produced in the lesions of infected leaves.

Spraying or otherwise spreading a spore suspension over the leaves has proved to be the best method of inoculation. Beach (1) notes, however, that leaves of plants grown in the greenhouse should be rubbed slightly to reduce surface tension before or as the spore suspension is applied. Inoculations made in this manner have given satisfactory infection when a sufficient wetting period at about the optimum temperature has been provided (1, 10, 11, 19, 20).

Varietal reaction to Septoria leaf blotch has received little attention in recent years. Information is particularly scarce on the more recently released hard red winter wheats. Fellows (6) found the most resistant varieties to be Red Chief, Ukraine, Wisconsin Pedigree No. 2, Brill, Mediterranean, Valley, Jenkin, Triplet, Yorkwin, and Minhardi; slightly less resistant were Sibley 81, Bald Rock, Denton, Nabob, Gladden, Prairie, Thorne, and Illinois No. 2.

Johnston (8) indicated that the soft red winter varieties were more seriously infected than the hard red winter varieties, and he found that Kawvale was particularly susceptible. Pawnee, like Kawvale, also was found to be more susceptible than most of the common varieties of Kansas by Reitz and Laude (14).

Josephson and Reid (9) found that Redhart and Purcam were susceptible in comparison to the varieties Thorne and Currells.

In earlier studies Beach (1) reported that Turkey Red, Minnesota Reliable, Red Cross, Red Hussar and Home Grown were about equally infected and among the more susceptible varieties. Pesterboden and Malakoff were infected to a lesser extent, and Hungarian was the most resistant. Marquillo was reported by Gardner and Mains (7) to be highly susceptible and Illinois No. 1 somewhat resistant.

Mackie (12) reported Septoria free plants could be produced by repeatedly crossing the most resistant selections of hybrid stocks. When he crossed these resistant plants with susceptible varieties a single factor ratio was obtained in the F_2 with susceptibility dominant.

Savulescu (17) found some differences in susceptibility of wheat varieties to S. tritici in Romania, and Roussakoff (16) in Russia reported that Kanred X Fulcaster 266287 was normally resistant to leaf blotch.

Sprague (18, 19) reported that the earlier maturing varieties appeared to be the most susceptible and that the variety Holland had less leaf blotching than Golden Hybrid 128, Albit or Rex.

Rosen (15), in breeding wheat for resistance to leaf rust, Septoria leaf blotch and Septoria glume blotch, found that a high type of resistance to leaf blotch and leaf rust was combined in the selection Red Rock - Hope X C. I. 12017.

Miller, et al (13) could find no differences between varieties in reaction to this disease.

MATERIALS AND METHODS

The isolates of *Septoria tritici* used in these experiments were obtained from lesions on several different wheat varieties grown at various locations in Oklahoma in the State-Wide Nursery Tests supervised by the Department of Agronomy, Oklahoma Agricultural and Mechanical College. Each isolate was derived from a single pycnidium obtained from these collections. Several different isolates were used in these tests; sometimes individually and sometimes in combinations of two or more. However, for any particular test the same isolate or combination of isolates was used throughout the test.

In the preparation of inoculum, cultures of the organism were grown on slightly acid corn meal agar in petri dishes for 3 to 4 weeks. The agar surface was then scraped in such a manner as to get as much of the fungus growth as possible with a minimum amount of media. Finally, the agar surface was rinsed with sterile distilled water to remove the remaining spores and mycelium. The resulting spore and mycelial suspension was then macerated in a blender. A dilution of approximately 75 mls. of water per petri dish was used in preparing the suspension. Spore counts of drops of the inoculum ranged from 150 to 250 spores per microscopic low power field (100 X).

The variety Westar, C. I. 12110, was selected for the greenhouse experiments because it appeared to be very susceptible in the field. Three replications were used in each test; each replication consisting of approximately 10 plants in a 4 inch pot. The plants were sprayed with water and placed in galvanized metal moist chambers for the wetting periods. After the wetting period in the moist chambers, the pots were set in open topped muslin isolation cages on the greenhouse bench. An average temperature of 68° F. was maintained in the greenhouse with temporary fluctuations from about 60° to 75° F. The plants were in the 3 to 4 leaf stage when inoculated, and second and third leaves were used to determine the extent of injury.

The field nursery, located on the west Agronomy farm, Oklahoma Agricultural and Mechanical College, included 17 varieties planted in single 3 foot rows. The design of the experiment was a randomized block with 3 replications.

These investigations dealt primarily with methods of producing artificial epidemics. Therefore, the methods of inoculation, periods of incubation used and so forth are discussed with each individual experiment.

RESULTS

Greenhouse Experiments. The first experiment involving greenhouse techniques and proceedures was made in March, 1954. Four inoculation methods, 4 wetting periods, and 4 rewetting periods were used in all possible combinations. The methods of inoculation were: (1) Dipping the leaves into a beaker containing the inoculum; (2) spraying the inoculum on the leaves with a small hand sprayer; (3) stripping the leaves between the forefinger and the thumb with the inoculum; and (4) brushing the inoculum on the leaves with a small paint brush. The initial moist periods following inoculation were for 1, 2, 3 and 4 days. Ten days after inoculation the plants were subjected to a second wetting period or rewetting treatment. These treatments consisted of: (1) periodically rewetting the foliage with a hand sprayer 8 to 12 times over a 48 hour period while the plants remained on the greenhouse bench; (2) placing the plants back in the moist chambers for a 24 hour period; (3) placing the plants back in the moist chambers for a 48 hour period; and (4) the control which was given no rewetting treatment.

The data on the amount of infection were recorded 21 days after inoculation. The results were measured in two ways; by the per cent of inoculated leaves exhibiting fruiting lesions, and by the average number of fruiting lesions per leaf inoculated. The data obtained were subjected to statistical analysis. The computed "F" values for the different variables and interactions are given in Table 1.

Table 1. The "F" values and probability levels for the various inoculation methods and treatments derived from an analysis of the first greenhouse experiment.

Variation due to	Percent of leaves infected "F" value	Percent of leaves infected Probability level	Ave. lesions per leaf "F" value	Ave. lesions per leaf Probability level
Inoculation methods	30.58	0.0005	11.58	0.0005
Initial wetting periods	58.83	0.0005	25.74	0.0005
Rewetting treatments	48.08	0.0005	18.47	0.0005
Inoculation methods X wetting periods	2.20	0.05	1.24	0.30
Inoculation methods X rewetting treatments	2.42	0.025	1.77	0.10
Wetting Periods X rewetting treatments	4.98	0.0005	2.60	0.01
Triple Interaction	5.11	0.0005	2.15	0.005

A comparison of the different methods of inoculation is presented in Table 2. It was found that stripping the leaves between the fingers with the inoculum suspension was the most effective method. Using this method 46 per cent of the inoculated leaves were infected with an average of 0.73 lesions per leaf. Analysis indicated this method to be superior to the other 3 methods with a probability value of less than 0.0005. Spraying the plants with the inoculum proved to be the least effective method, with only 27 per cent of the inoculated leaves infected and only 0.31 lesions per inoculated leaf.

Table 2. A comparison of different methods of inoculation with *Septoria tritici* in the greenhouse.

Method	Percent of leaves infected*	Average lesions per leaf*
Dip	34	0.48
Spray	27	0.31
Strip	46	0.73
Brush	33	0.47
	6 LSD** - 5%	= 0.19
	7 LSD - 1%	= 0.23

* Expressed as a mean of 3 replications and including all initial wetting periods and all rewetting periods.

** Calculated according to Bennett and Franklin (2).

The data indicates that infection increased as the length of the initial wetting period increased (Table 3). When the inoculated plants were held in the moist chambers for 4 days, 48 per cent of the inoculated leaves were infected and there was an average of 0.84 lesions per leaf. This may be compared with only 23 per cent of the leaves infected and only 0.22 lesions per leaf when the initial wetting period was 24 hours.

Table 3. A comparison of different lengths of the initial wetting period after inoculation with <u>Septoria tritici</u> in the greenhouse.

Initial Wetting period (in days)	Percent of leaves infected*	Average lesions per leaf*
1	23	0.22
2	32	0.41
3	36	0.52
4	48	0.84
	6	LSD** - 5% = 0.19
	7	LSD - 1% = 0.23

* Expressed as a mean of 3 replications and including all methods of inoculation and all rewetting periods.

** Calculated according to Bennett and Franklin (2).

All of the rewetting treatments were superior to the control in producing infection (Table 4). A 24 hour period in the moist chamber was significantly better than the other treatments. The plants handled in this manner had 46 per cent of the inoculated leaves infected with an average of 0.77 lesions per leaf. When the rewetting treatment was extended to 48 hours in the moist chamber many of the older leaves turned yellow and died. No fruiting bodies of <u>S. tritici</u> could be found on these leaves and their death was attributed to other causes. Consequently, the per cent of infection with this treatment was reduced.

Table 4. A comparison of the effect of rewetting treatments during the latter part of the incubation period on infection by <u>Septoria tritici</u> in the greenhouse.

Rewetting Treatment	Percent of leaves infected*	Average lesions per leaf*
None	22	0.23
Periodical spray	37	0.54
1 day (moist chamber)	46	0.77
2 days (moist chamber)	35	0.45
LSD** - 5% =	6	0.19
LSD - 1% =	7	0.23

* Expressed as a mean of 3 replications including all methods of inoculation and all initial wetting periods.

** Calculated according to Bennett and Franklin (2).

The interaction between initial wetting periods and the rewetting treatments was significant, and simply indicated that a combination of an initial wetting period of 4 days and a 24 hour rewetting treatment gave better infection than any other possible combination of these two factors (Tables 5 and 6).

The best method of inoculation, stripping the leaves with the inoculum, in combination with a 4 day period of initial wetting and a 24 hour rewetting treatment gave 100 per cent infection of the inoculated leaves and an average of 1.81 lesions per leaf. No other combination of these variables gave such good results, and when the plants were sprayed with inoculum, given only 24 hours initial wetting and no rewetting treatment only 8 per cent of the inoculated leaves were infected and there were only 0.08 lesions per leaf.

The results indicate that all three of the factors tested play an important part in developing infection with <u>Septoria tritici</u>, and that when the best combination of these variables is used very satisfactory infection can be obtained.

Table 5. The interaction between initial wetting periods and rewetting treatments as indicated by the per cent of inoculated leaves infected.*

Rewetting treatments	Initial Wetting period (in days)			
	1	2	3	4
Control	17	22	19	33
Periodic Spray	27	35	44	42
24 Hours (moist chamber)	25	46	49	64
48 Hours (moist chamber)	23	26	34	55

LSD** - 5% = 14.
LSD - 1% = 16.

* Expressed as the mean of 3 replications and including 4 different methods of inoculation.

** Calculated according to Bennett and Franklin (2).

Table 6. The interaction between initial wetting periods and rewetting treatments as indicated by the average number of lesions per leaf.*

Rewetting treatments	Initial wetting period (in days)			
	1	2	3	4
Control	0.16	0.20	0.18	0.38
Periodic Spray	0.28	0.51	0.57	0.78
24 Hours (moist chamber)	0.22	0.63	0.87	1.32
48 Hours (moist chamber)	0.22	0.28	0.44	0.86

LSD** - 5% = 0.50
LSD - 1% = 0.57

* Expressed as the mean of 3 replications and including 4 different methods of inoculation.

** Calculated according to Bennett and Franklin (2).

In October, 1954, portions of this experiment were repeated to obtain a more critical determination of the initial wetting period and of the rewetting treatment. The plants were inoculated by stripping the leaves between the fingers with inoculum. The remaining inoculum was sprayed on the plants after they were placed in the moist chambers for the initial wetting period.

The duration of the initial wetting period was for 2, 3, 4 and 6 days. The plants were then subjected to rewetting treatments in the moist chambers 8 days following inoculation for periods of 12, 24 and 36 hours. The 12 possible combinations of these 2 variables were made and 3 pots of approximately 10 plants each were used for each combination. Infection was determined in this experiment by estimating the per cent of leaf area destroyed and by recording the per cent of leaves inoculated which became infected. The data are presented in Tables 7 and 8.

Statistical analysis of these data indicated that the small differences observed between the various treatments were not significant. Excellent infection was obtained regardless of the method in which the plants were handled.

It was noted that for a period of several days following the inoculations the weather remained cloudy. This condition caused an increase in the relative humidity, particularly among the rather closely spaced pots on the greenhouse bench. Plants removed from the moist chambers at the end of the designated 2 and 3 day initial wetting periods remained wet for up to 12 or 15 hours after being placed on the bench. This, of course, extended the initial wetting period and gave rise to increased infection for those treatments.

Plants which were held in the moist chamber for an initial wetting period of 3 and 4 days during the cloudy weather were severely etiolated and water soaked. Many of these plants later died (Table 9). Leaves which were only partially killed had leaf blotch lesions toward the proximal end, but it was difficult and often impossible to determine how much of the leaf blade had been destroyed by the Septoria parasite. The plants left in the moist chamber for an initial wetting period of 6 days were almost completely destroyed, so that it was impossible to make an estimate of the injury caused by _Septoria tritici_ itself.

Table 7. The average per cent of leaves infected with _Septoria tritici_ following various wetting periods and rewetting treatments.

Rewetting Treatment (Hours in moist chamber)	Wetting periods (in days) 2	3	4	Mean
12	95	100	89	95
24	95	95	100	97
36	97	80	90	89
Mean	96	92	93	

Table 8. The average per cent of leaf area destroyed by _Septoria tritici_ following various wetting periods and rewetting treatments.

Rewetting Treatment (Hours in moist chamber)	Wetting periods (in days) 2	3	4	Mean
12	40	37	29	35
24	40	21	38	33
36	43	18	44	35
Mean	41	25	37	

Table 9. The number of plants killed following various initial wetting and rewetting treatments.

Rewetting treatment (Hours in moist chamber)	Wetting periods (in days) 2	3	4	Total
12	0	3	12	15
24	0	1	4	5
36	0	1	7	8
Total	0	5	23	

Field experiments. An experiment was designed during the winter of 1954 to determine methods which might be used to produce artificial epidemics in the field. The same experiment was used to test the reaction of many of the hard red winter wheat varieties commercially grown in Oklahoma, together with some selections and strains which might be used as sources of resistance in the breeding program. In the beginning, two methods of inoculation were used; spraying the inoculum on the plants with a hand sprayer, and brushing it on with a 2 inch paint brush. Later, a power paint sprayer developing 30 to 40 pounds pressure was substituted for the hand sprayer.

A total of 7 inoculations were made on a block consisting of single row plots of 17 varieties in 3 replications. The first inoculation was made on January 6, 1954, and subsequent inoculations were made on February 12, March 20, March 24, April 11, April 22 and May 1. When the first two inoculations were made the ground was thoroughly soaked with water and the plants were covered with corregated cardboard for a period of 4 days to maintain a high humidity. Later inoculations were timed to coincide with periods of rainy or foggy weather in order to dispense with the laborious task of spreading and removing these covers. Brushing the inoculum on the plants had to be abandoned after the fourth inoculation because the plants were too large to make this method practical.

The first lesions of S. tritici were observed in this block on April 28. Infection was rather general throughout the spray inoculated plots by May 1. Very few lesions ever developed in the plots inoculated by brushing and the control plots also remained free of infection. Since, in the greenhouse, the brushing method of inoculation was generally more effective than spraying it is reasonable to assume that none of the first four inoculations in the field produced infection. Also, the average incubation period in the greenhouse tests was 14 to 16 days. If it is assumed that a similar incubation period prevails in the field at a like temperature, then the general infection observed about May 1, must have developed from the inoculation made on April 11. A summary of certain climatic conditions prevailing during a four day period subsequent to each inoculation date is presented in Table 10. These weather factors following the inoculation on April 11 were generally favorable for infection. During this time there was over one-half inch of rainfall and a period of 35 hours when there was free moisture on the leaf surfaces. In addition, the average temperature was 60° F. and the minimum temperature during the 4 day period was only 53° F. It appears from these data that free moisture or rainfall and a period of low temperatures were more critical than the average temperature during the initial phase of the incubation period.

Table 10. A summary of certain climatic factors during a 4 day period following field inoculations with <u>Septoria tritici</u>.

Inoculation Date	Ave. Temp. in degrees F.	Minimum Temp. in degrees F.	Total No. of Hours below 50° F.	Total Rainfall in inches	Hours when free water was present
Jan. 6	49	28	57	0	No data
Feb. 12	58	44	20	0	No data
Mar. 20	56	37	34	0	42
Mar. 24	56	39	30	0.16	16
Apr. 11	60	53	0	0.60	35
Apr. 22	69	54	0	0	12
May 1*	50	35	49	1.79	13

* Infection generally present throughout spray-inoculated plots on this date.

An evaluation of the varietal reaction was made on May 15, 1954. At that time 15 leaves were selected at random from each variety in each plot. The first leaf below the flag leaf was used in all cases. An estimate was made of the per cent of the leaf area destroyed and an average of the 15 leaves constituted the reading for each plot. These data are presented in Table 11. Practically no infection occurred in the plots which were brush-inoculated nor did any appreciable number of lesions develop in the control plots. The plots which were sprayed, however, developed very satisfactory infection and varietal reactions could be determined. The varieties in this test can be placed in 3 main groupings, although the distinction between the latter two groups is not as striking as it is between the resistant and intermediate groups. Red Chief and Nabob constitute one group which is significantly more resistant than the other varieties. An intermediate group was composed of 11 varieties; Concho, Cheyenne, Ponca, Clarkan, Blue Jacket, Comanche, Tenmarq, Harvest Queen, Blackhull, Kawvale and Pawnee. The remaining 4 varieties, Wichita, Early Blackhull, Triumph and Westar were the most susceptible and composed the last group.

Table 11. The comparative response of 17 winter wheat varieties to Septoria Leaf Blotch following inoculation in the field.

Rank	Variety	C. I. No.	Sprayed	Brushed	Control
1.	Red Chief	12109	9*	Tr	0
2.	Nabob	8869	9	0	0
3.	Concho	12517	30	1	0
4.	Cheyenne	8885	30	2	Tr
5.	Ponca	12128	33	0.5	Tr
6.	Clarkan	8858	33	Tr	Tr
7.	Blue Jacket	12502	34	0.5	0
8.	Comanche	11673	35	3.5	Tr
9.	Tenmarq	6936	36	Tr	Tr
10.	Harvest Queen	(Ros. Res.)	41	Tr	Tr
11.	Blackhull	6251	41	Tr	Tr
12.	Kawvale	8180	43	0.5	1.0
13.	Pawnee	11669	48	Tr	1.0
14.	Wichita	11952	59	1.5	Tr
15.	Early Blackhull	8856	62	Tr	Tr
16.	Triumph	12132	68	1.5	Tr
17.	Westar	12110	71	2.5	Tr
		LSD**-5% =	25		
		LSD - 1% =	30		

* An average of the per cent of leaf area destroyed.
** Calculated according to Bennett and Franklin (2).

An attempt was made in the greenhouse during the winter of 1954-1955 to screen 4 to 5 thousand F_2 individuals from crosses of the variety Nabob with several desirable hard red winter wheats. These plants were grown in individual plant bands in flats and were inoculated by stripping the second and third leaves between the fingers with inoculum. Each flat was given an initial wetting of 2 days and 8 days after inoculation was given a 24 hour rewetting treatment in the moist chamber.

Not one lesion developed from all of these inoculations. Since this inoculation method and handling proceedure had previously produced satisfactory infection, spores of the culture were examined and found to be viable. Therefore, another inoculation was made on the variety Westar following the same proceedures. Again, no infection occurred. From these tests it was concluded that the pathogenicity of this culture had been lost. Such a phenomenon is not uncommon among many fungi and it is not surprising that the pathogenicity of cultures of *Septoria tritici* could be lost in cultural transfer. It is a significant factor to be reckoned with in producing artificial epidemics either in the field or the greenhouse.

DISCUSSION

Methods for producing epidemics of Septoria leaf blotch in the greenhouse were found. The inoculum used was a water suspension of spores and mycelium diluted to a concentration of approximately 150 to 250 spores per low power field (100 X). Stripping this inoculum on the leaves with the fingers was the most satisfactory method of inoculation. Beach (1) mentioned that stripping the leaves prior to or during inoculation was desirable. The purpose of this operation is to reduce surface tension so that the inoculum will be adequately distributed. Any method of applying the inoculum would probably be satisfactory if the leaves are first stripped or rubbed. Such a proceedure would require two operations which can successfully be combined by stripping the leaves with the inoculum itself. An initial wetting period of 3 to 4 days was optimum which falls within the range found by most other workers. Infection was found to be enhanced by a rewetting treatment of 24 hours in the moist chamber about 8 days following inoculation or when the first symptoms were observed. Beach (1) also noted that a rewetting treatment was desirable for fruiting of the organism, but did not indicate that this type of treatment would enhance the amount of infection. In these tests the plants which were not given a rewetting treatment had fruiting lesions, indicating that such a treatment is not essential for fruiting. Stripping the inoculum on the leaves, followed by an initial wetting period of 3 to 4 days and a rewetting treatment of 24 hours in the moist chamber produced infection on 100 per cent of the inoculated leaves. Epidemics of this magnitude would be satisfactory for the testing of early generations of hybrid material, as well as for testing established varieties and selections.

A test of several F_2 hybrid populations was attempted using the methods described above. Unfortunately the isolate of Septoria tritici used for this purpose had lost its pathogenicity. This was the only occasion in all of the tests made in this study that such a phenomenon occurred. However, it is a factor which should be recognized in tests involving this pathogen. Wherever possible fresh isolates from field material should be used.

A field epidemic also was successfully created. The methods used were somewhat different than in the greenhouse tests. Stripping the leaves with the inoculum, which proved most effective in the greenhouse, was not practical in the field. Instead, it was found that spraying the inoculum on the plants would produce satisfactory infection in the field. Certain climatic conditions immediately following inoculation were found to be essential. Of primary importance was the presence of free moisture for a period of 36 to 72 hours. During this same period an average temperature of about 60° F. was satisfactory, but if the minimum temperature fell to 50° F. or below for any length of time infection was inhibited. The reaction of 17 winter wheat varieties was determined in a field nursery inoculated under these conditions. It was found that the varieties Red Chief and Nabob were the most resistant. Fellows (6) also found that Red Chief and Nabob were among the more resistant varieties he tested. The varieties Kawvale and Pawnee were found to be susceptible as was reported by Johnston (8) and Reitz and Laude (14). However, in this test there were other varieties more susceptible; notably Westar, Triumph, Early Blackhull and Wichita.

SUMMARY

1. Stripping the plants between the fingers with the inoculum was superior to spraying or brushing the inoculum on the plants for inoculations made in the greenhouse.

2. An initial wetting period of 3 to 4 days was required to produce satisfactory infection in greenhouse tests. Longer wetting periods were unsatisfactory due to etiolation.

3. A rewetting treatment of 24 hours in the moist chamber about 8 to 10 days after inoculation enhanced infection significantly.

4. A combination of these factors produced epidemics of a magnitude sufficient for the testing of early generation hybrid material.

5. The pathogenicity of isolates of *Septoria tritici* is likely to be lost in cultural transfer.

6. Inoculation in the field can be satisfactorily made by spraying the inoculum on the plants.

7. Post-inoculation weather requirements in the field are essentially similar to those found to be satisfactory in the greenhouse. A field epidemic was produced when, during the 4 day period following inoculation, the average temperature was 60° F., the minimum temperature was 53° F., and free moisture was present for a period of 35 hours. No infection was obtained when there was no free moisture present or when the minimum temperatures ranged between 40 to 50° F. or below during this post-inoculation period.

8. The reaction of 17 winter wheat varieties was determined. Red Chief and Nabob were found to be quite resistant, while Triumph and Westar were the most susceptible.

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