AN INVESTIGATION OF CERTAIN MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF SEPTORIA TRITICI ROB. EX. DESM.

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INTRODUCTION

Septoria leaf blotch of wheat, also known as nebular leaf spot and speckled leaf blotch, caused by Septoria tritici Rob. ex. Desm., is world-wide in distribution. The infected leaf lesion is light tan in color and speckled with the dark brown pycnidia of the fungus.

Chester (5) reported an epiphytotic of Septoria leaf blotch in Oklahoma during 1941 that resulted in destruction of about half of the foilage by harvest. In 1943 he found the development of Septoria had played an important role in the epidemeology of wheat leaf rust. The rapidly advancing infections of Septoria destroyed the leaves before rust infections could develop, thus limiting what might have been a severe epidemic of leaf rust. These observations have been verified in many recent surveys and it should be noted that blotch and leaf rust cause a large portion of the leaf damage in the fall that many farmers later associate with "winter kill."

In 1952 Wadsworth and Young (26) reported complete destruction of leaves below the flag leaf by Septoria leaf blotch and from 70 to 90 percent of the flag leaf by the soft dough stage of maturity. State-wide surveys in Oklahoma over the past several years have shown the disease to be generally of rather minor importance in the western

wheat growing areas, while severe infections were often found in the eastern part of the state.

Caldwell (3) reported S. tritici to be one of the most destructive diseases in the soft red winter wheat area of the United States. He reported losses of 32 percent from natural infection, and as much as 50 percent in artificially inoculated plots in a statistically designed study involving several varieties with a wide range of maturity.

Chona and Munjal (8) in 1952 reported from India that S. tritici and Septoria nodorum caused damage of such proportions that less than five percent of the plants produced fertile heads; hence, most of the acreage could be used only for pasture.

Infections of S. tritici cause a reduction in forage for winter pasture and predispose the young plants to winter injury. Later, the reduced leaf surface, together with weakened sheaths and stems cause lodging and shriveled kernals. In total, these factors contribute to an average annual yield reduction of 5 to 15 percent (4) (7).

Control of this disease has been aimed at the reduction of the primary inoculum source through clean tillage and crop rotation. However, these practices have been only partially effective over large areas, and resistant varieties would provide the best control if they could be made available. The ultimate utility of resistant varieties depends upon the variability of the pathogen; therefore,

this study was undertaken to determine if variants in the pathogen could be recognized morphologically or pathologically.

LITERATURE REVIEW

S. tritici is pathogenic on the leaves and sheaths of wheat, with spore germination and penetration occurring within 24 to 30 hours under moist conditions. The germ tube penetrates the opened or closed stomata and hyphal spread is more or less confined by the vascular bundles. However, when mycelial growth reaches a certain level, one can observe hyphae crossing the vascular bundle directly under the epidermis. It takes from 7 to 16 days for water-soaked lesions to appear. Lesions develop more slowly at relatively low temperatures and in the intermediate and semiresistant varieties (11, 25). Hilu and Bever (11) found that pycnidia always developed in the substomatal chambers and were larger in the susceptible than the resistant varieties. The spores are exuded from pycnidia through the ostiole under wet conditions and are splashed to the leaves by rain where new infections are initiated (16, 25). A series of tests by Hilu and Bever proved that S. tritici oversummers in infected wheat plant residues lying on the surface of the soil and in infected volunteer wheat plants. They also showed that roots and crowns could not be infected by inoculated seed nor by artificially infested soil.

Beach (1) studied the characters used in differentiating species of Septoria and found the following to be most

useful: (1) spore shape, length, thickness, color and septation; (2) pycnidium shape, size, and color; (3) ostiole size. He also noted that the size, shape, margin, zonation, and color of the disease lesions, together with the location and distribution of lesions upon the host had been used for species descriptions. However, he found that specific descriptions in many cases were quite meager; the presence of a Septoria upon an unrecorded host, for instance, was the basis of the description of a new species. Nevertheless, the host range of Septoria species do tend to be limited to individual grass tribes which would suggest that the species have evolved through their association with a certain host. The effect of this association has evidently been more pronounced on the physiological than the morphological characters.

Sprague (22) in 1944, separated the genus Septoria from Stagonospora on the basis of spore length in relation to width. He suggested that all fungi in this group having spores predominantly less than 10 times as long as broad should be placed in the genus Stagonospora, while those with spores predominantly over 10 times as long as broad should be placed in the genus Septoria. In differentiating species and subdivisions within the genus Septoria, he used the ratio of length to width and the number of septations in the pycnospore. He noted that in most Septoria species the pycnospores are longer and contain more septations in the winter than in the summer on the same host. He found that septations fall into groups of 1, 3, and 7, depending on the number of nuclear divisions in the spore. The color of the

pycnidia also was used along with the length and shape of the pycnophores in species classification. Minor racial differences, including variations in pathogenicity, were noted, but were not given critical consideration due to difficulty of obtaining consistant infections. He stated that with improved inoculation techniques further racial differences could be expected.

Beach (1) and Weber (25) both attribute the fluctuation in size of spores and pycnidia to environmental conditions. Beach (1) discovered that spores from leaves of wheat plants grown under freezing and thawing conditions were much longer than those from leaves grown under more favorable temperatures. Wheat seedlings inoculated with these longer spores and grown at normal temperatures always resulted in pycnidia of normal proportions and typically shorter spores. Weber also noted that spores from material collected during the winter were considerably larger and longer than spores from summer collections.

Reports vary greatly regarding media that best support fungal growth and sporulation. Sprague and Weber (21, 25) found potato-dextrose agar satisfactory, while Renfro (17) obtained better results with corn-meal agar, and Hilu and Beaver (11) obtained best results using Elliot-V8 agar.

The early cultural studies of Septoria tritici done by Weber (25) showed the cardinal temperatures for growth to be as follows: minimum 6°C., optimum 22° to 24°C., and maximum 32°C. The organism grew vigorously at a pH ranging from 3.8 to 8; however, different shades of pink

to white color developed depending on the hydrogen-ion concentration.

Bremer et. al. (2) reported pycnidia were produced on media similar to those found in nature; however, others have failed in many attempts to duplicate their results.

Similar methods of artificial inoculation have been used by most investigators. The most common technique consists of spraying or finger stripping the plants with a spore suspension and then keeping the plants moist for 24 to 72 hours after inoculation.

Resistance has been reported in many varieties of several species of Triticum. Resistance may be due to erratic and infrequent establishment of infection after penetration, to the slow growth of the fungus in certain hosts, or to specific physiological incompatibility (1, 11, 16). Hilu and Bever (11) stated that resistant varieties could be selected by measuring the size of the pycnidia produced with the smaller size occurring on the more resistant varieties or selections.

MATERIALS AND METHODS

Collections of Septoria tritici were obtained from four geographic areas in Oklahoma. These collections were obtained from the wheat, var. Comanche, grown in the State-wide Small Grain Yield Tests supervised by the Department of Agronomy, Oklahoma State University.

Collections of the fungus from eleven different varieties at a single location were made from the Wheat Field Plots of the Department of Agronomy, Oklahoma State University, at Stillwater, Oklahoma.

Spores were obtained for measurement by placing an infected wheat leaf in a Petri dish on moist filter paper at room temperature. After 6 to 12 hours, a portion of the spore mass oozing from a pycnidium was transferred to a microscope slide with a sterile glass needle. Semi-permanent mounts were made by ringing the cover slip over the water-spore suspension with petroleum jelly. Measurements were made with an ocular micrometer of all spores in each "high power" (45 x objective) field until the requisite number of spores were measured.

Samples were so obtained that the data could be subjected to statistical analysis.

The method of single spore isolation and transfer was a modification of the method of A. L. Hooker (12). A

dilute spore solution was prepared in sterile distilled water using the sterile glass needle transfer method previously described. This solution was dispersed over the surface of PDA in a Petri dish. These dishes were then slightly tipped so that excess water drained from the agar surface and they incubated in this position for 24 hours at room temperature (18° to 24°C). At first, single germinating spores observed under the microscope were removed with a sterile needle and transplanted to separate dishes. Later, single spore colonies observed with a binocular dissecting microscope were isolated. These isolates were maintained at a temperature of 10°C and transferred every 50 to 60 days depending on condition of the culture. Incubation at 10°C proved to be adequate for growth of the organism and, at that temperature, contaminants were not a problem when the Petri dishes were enclosed in paper or plastic bags.

Cultural growth was measured by the use of a bacterial colony counter in which the minimum gradations were units of 3 square millimeters. Each culture was placed in the counter and the number of units covered by the colony was counted. At the edges of the colony an estimation of the part of each unit covered by the colony was made and the figures rounded off to the nearest whole number. The first measurements were taken five days after transfer. Succeeding measurements were taken at five-day intervals, with the final measurement being made on the 20th day.

All cultures used for artificial inoculation were increased from single spore isolations and for any particular

test the same isolate was used throughout. Inoculum was obtained by gently scraping the agar surface with a scalpel and removing the yeasty mass of conidia with a minimum amount of agar. This conidial mass was placed in a small amount of water and stirred in a blender until a uniform suspension was obtained; the suspension was then diluted with distilled water to a spore count ranging from 200 to 300 spores in each "low power" microscopic field (10 X objective). After three or four drops of "Tween-20" were added to each liter of spore suspension, the plants were inoculated with a power paint sprayer that developed 20 pounds of pressure per square inch. In a later test, "Tween-20" was omitted and the inoculum was made up as a five percent gelatin solution. The wheat planted directly in the greenhouse was sprayed with inoculum, allowed to dry slightly, and then remoistened periodically for 72 hours. Potted plants were sprayed with inoculum, held in glass-covered, metal moist chambers for 12 hours, and then the plants were remoistened and held in the moist chambers for another 60 hours.

RESULTS

Spore measurements. Collections of Septoria leaf blotch on the variety Comanche were made in March, 1961, from 37 of the State-wide Small Grain Yield Tests. Time did not permit the measurement of an adequate sample of spores from all of these collections so the state was divided into four major geographical areas (Fig. 1) and samples of spores were measured from two or three collections within each area. The sample from each area consisted of 1,000 spores. Somewhat over one hundred spores obtained from each of three pycnidia were measured from each collection within the area. In the southeast area, however, only two collections were available and the 1,000-spore sample was made up of 500 spores from each collection. The individual spore lengths were recorded and a mean was computed for each sample.

The mean of all spores measured was 62 μ with a range of 36 μ to 90 μ (Table I). These values compare favorably with those given by Sprague (22), although they are somewhat higher than he reports for either winter or summer collections of the fungus.

The data were analysed statistically and it was found that no significant differences existed between the mean spore lengths from the various geographical areas chosen. However, a trend was indicated; those spores collected in

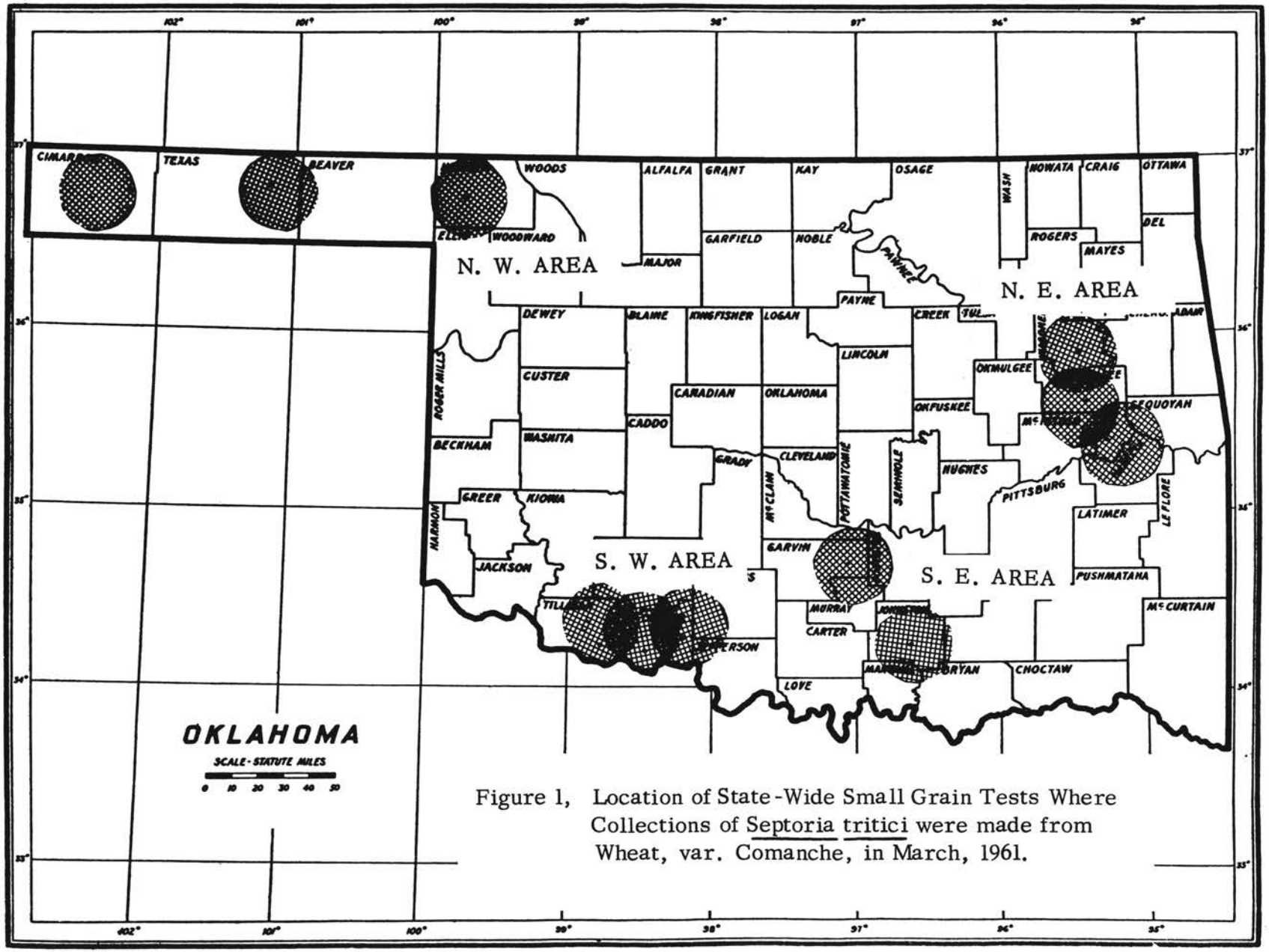

Figure 1, Location of State-Wide Small Grain Tests Where Collections of Septoria tritici were made from Wheat, var. Comanche, in March, 1961.

Table I - Spore Lengths of Septoria tritici collected on wheat, var. Comanche, from different geographical areas in Oklahoma, March, 1961.

Area	Mean Length of Spores in Microns Pycnidia Number:			Collection Mean	Area Mean	Collection Range
Location	1	2	3			
N.E.					64	
Warner	62	65	60	62		39 - 83
Muskogee	70	62	45	59		36 - 90
Wagoner	68	74	70	71		45 - 90
S.E.					65	
Stratford	65	69	66	67		43 - 86
Tishmomingo	61	61	65	62		40 - 86
S.W.					62	
Randlett	59	65	64	63		40 - 83
Temple	57	62	63	61		40 - 83
Grandfield	65	66	56	62		40 - 83
N.W.					57	
Boise City	57	56	57	57		40 - 76
Buffalo	66	55	58	60		40 - 85
Hooker	57	55	53	55		36 - 76
			Population Mean		62	36 - 90

the eastern part of the state tended to be longer than those collected in the drier, colder areas of the west. There were no significant differences between the mean spore lengths of the various collections within an area, nor between the different pycnidia sampled within a collection. However, there was one pycnidium sampled from the collection made at Muskogee in which the spores were quite short and the range much less than other pycnidia within that collection (Fig. 2).

The foregoing study was made with collections of the pathogen from the same host variety at many locations. A similar study was then made with collections from many host varieties at a single location. For this purpose collections were made from all varieties in the Wheat Field Plots, Agronomy Research Station, Stillwater, Oklahoma, during April, 1961. From each of these collections one 100-spore sample was measured from each of three pycnidia.

These data were analysed statistically and it was found that the mean lengths of the spores from the collections from different varieties were not significantly different (Table II). This seemed rather unusual, since these means varied from 64 μ on the variety Concho to 94 μ on a selection of Triumph X Triticum species X Agropyron elongatum C.I. 13523[1] or almost one third of their total length. Such results would indicate that perhaps the sample of spores measured was too small, particularly when the range in spore

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[1] C.I. refers to the number assigned to this selection by the Cereal Crops Research Branch, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

COMANCHE

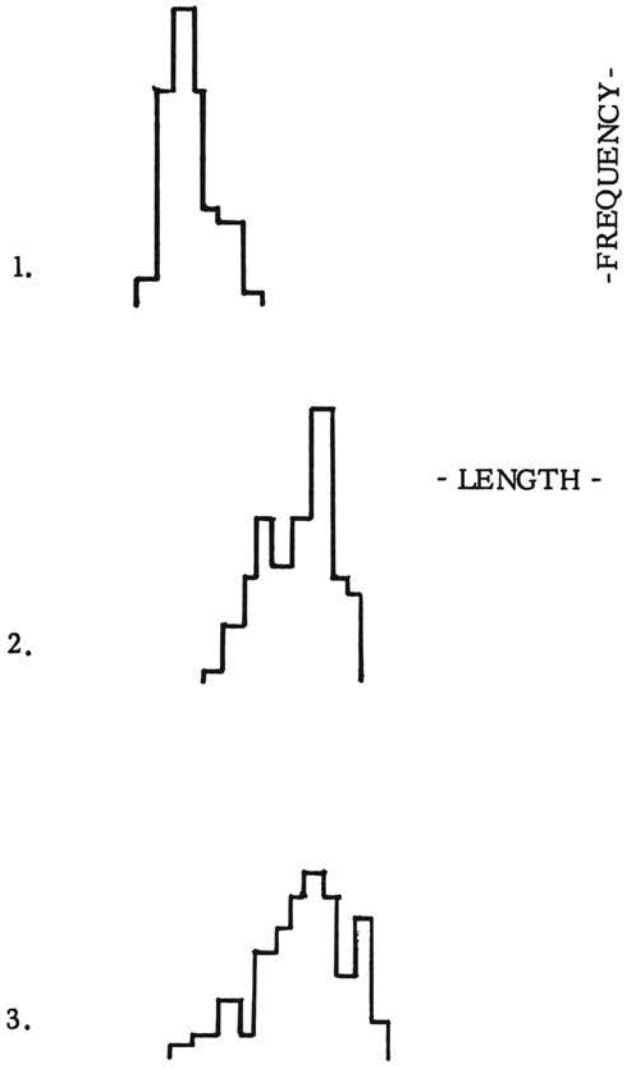

Figure 2. Variation in Length of Conidiospores of Septoria tritici from Three Pycnidia Collected at Muskogee, Oklahoma, March, 1961.

Table II - Spore Lengths of *Septoria tritici* Collected on Varieties in the Wheat Field Plots, Agronomy Research Station, Stillwater, Oklahoma, April, 1961.

Variety	CI No.	Mean Length of 100 Spores in Microns Pycnidia Number:			Collection	
		1	2	3	Mean	Range
Imp. Triumph	13667	75	69	78	74	40 - 119
F_3 X-comp	-	75	74	82	77	50 - 101
Kaw	12871	99	102	54	85	36 - 162
Concho	12517	58	57	78	64	36 - 104
Comanche	11673	50	84	68	67	29 - 144
Triumph	12132	69	75	72	72	43 - 108
Wichita	11952	60	102	83	82	36 - 148
Tascosa	13023	69	75	62	69	40 - 108
Ponca	12128	73	84	52	71	40 - 148
Crockett	12702	68	64	89	74	43 - 148
Tmp x *Tsp-Ae*	13523	100	98	84	94	50 - 126
				Population Mean	75	29 - 162

length (29 μ to 162 μ) was so wide.

It was noteworthy that within three collections pycnidia were found which yielded spores quite a bit shorter than the other two pycnidia sampled (Fig. 3). The same phenomenon was observed in the collections made from a single variety at a number of locations.

An interesting observation from this study was the wide range in spore length and the excessive length of the population mean (75 μ). This mean exceeded the mean of the state-wide collections by 12 μ, or about 20 percent. Some spores were found which measured over 160 μ and exceeded any spores found in the state-wide collections by over 70 μ. These long spores were found to exceed by at least 60 μ anything reported by Sprague (22). It also was interesting that the total variation in spore length of samples from collections on different varieties (133 μ) so far exceeded the total variation found in samples from collections on a single variety at many locations (54 μ).

All of the morphological characters of these collections, except the exceedingly long length of the spore, fit the description of Septoria tritici given by Sprague (22). He does state, however, that discretion is necessary at all times in classifying the variable Fungi Imperfecti and that it should be emphasized that climatic factors may influence, to a considerable extent, the morphology of the pycnidia, pycnophores, and pycnospores. It was concluded, therefore, that the collections were Septoria tritici and the excessive length of the spores was due to environmental

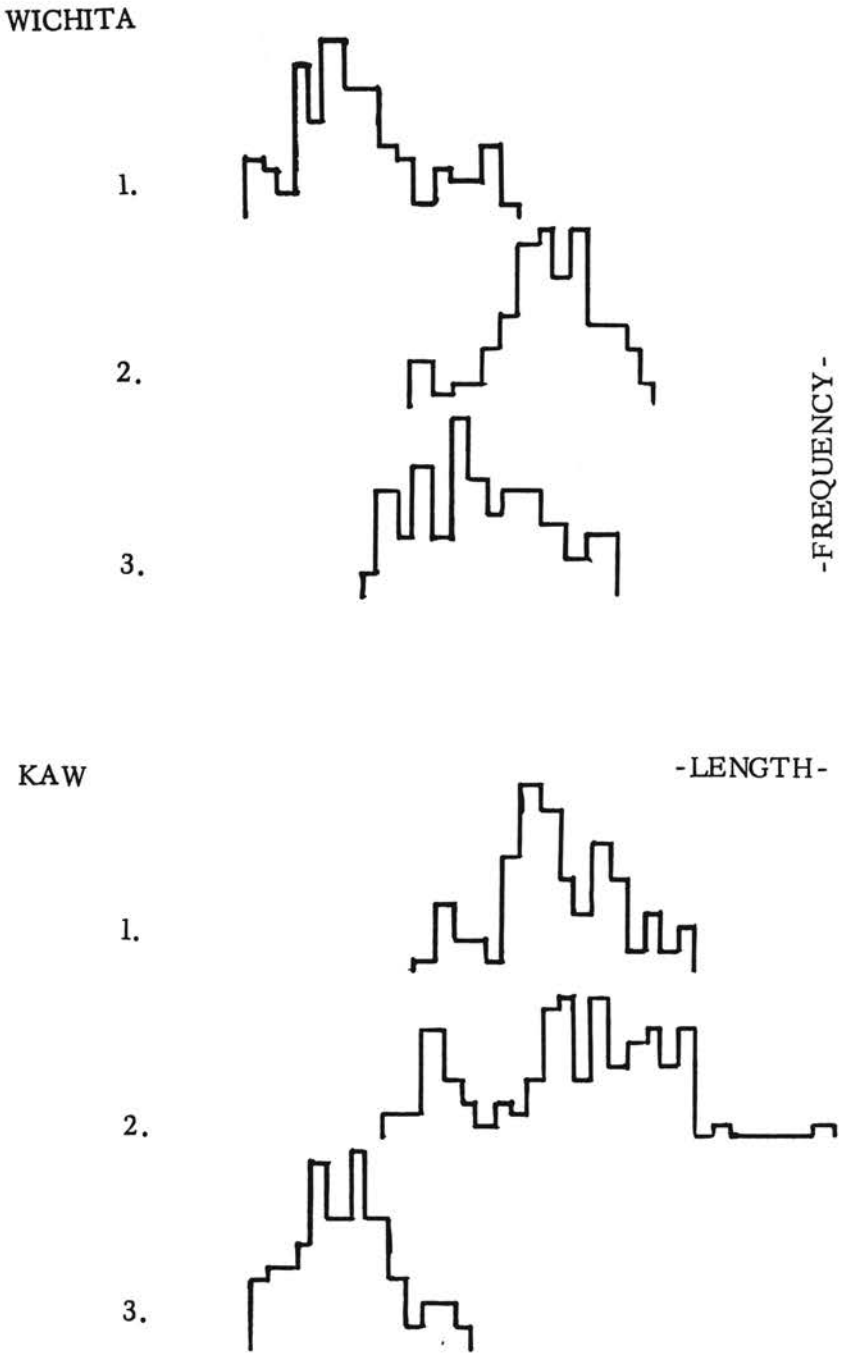

Figure 3. Variation in Length of Conidiospores of Septoria tritici from Three Pycnidia Collected from two Wheat varieties, Wichita and Kaw, at Stillwater, Oklahoma, April, 1961.

factors. Consequently, it was deemed advisable to make a test with somewhat more control of the environment and where a single known source of inoculum could be used.

For this study a nursery consisting of fifteen varieties was planted in a polyethylene-covered greenhouse at Stillwater in the fall of 1961. At least two varieties, reported to possess a certain degree of resistance (Red Chief and Nabob), were included in the test together with seven of the varieties from which collections were made in the field, one highly susceptible variety (Westar), and five other varieties or selections. This study had a threefold purpose: (1) to further investigate the extreme variation in spore length encountered in collection from different varieties in the field in a test where environment and inoculum source could be better controlled; (2) to test various methods of handling the plants following inoculation in order to better induce epidemics; and (3) to measure varietal susceptibility.

A single spore isolate from a collection from the variety Comanche grown in one of the state-wide nurseries in which the spores were of medium length was used as a source of inoculum. Inoculations were made according to methods previously described. Many problems were encountered in maintaining moisture and temperatures suitable for spore germination and infection following inoculation. Following the first inoculations, the nursery was kept constantly wet for 72 hours, using a fine mist spray. This method allowed for too much water to accumulate on and in the soil for

adequate plant growth and, in addition, seemed to be washing the inoculum from the plants. Later, the inoculum was allowed to dry slightly following application, and then short rewetting periods were used to maintain a humidity level at or near 100 percent for approximately 72 hours. This method proved quite satisfactory and severe infection was produced in at least one section of the plot. The location of the nursery within the greenhouse proved to be the greatest limiting factor in the development of a uniform epidemic, however. It was planted in such a way that the headhouse shaded approximately one half the plot through most of the period this study was in progress, and it was found to be quite difficult to maintain adequate humidity in the unshaded portion of the plot. A sheet of polyethylene film was used as a wall surrounding that particular portion of the nursery to reduce air currents and evaporation but this measure was not too successful.

Plant disease susceptibility or resistance ratings could not be made on the various varieties because of the lack of uniformity in disease development. Some infections did occur on all varieties, however; and collections of mature pycnidia were made and spore measurements were obtained. The data from this nursery (Table III) indicate that there was no significant difference between varieties or between pycnidia within a collection. The mean of all spores measured in this test was 54.5 μ. This was 7.5 μ shorter than the mean of the population from a single variety in the state-wide nursery collections and 20.5 μ shorter

Table III - Spore Lengths of *Septoria tritici* Collected from Different Wheat Varieties Following Artificial Inoculation in the Greenhouse, Stillwater, Oklahoma, February, 1962.

Variety	PI or CI no.	Mean Length of 100 Spores in Microns Pycnidia Number: 1	2	3	Collections Mean	Range
Comanche	11673	44	51	53	49	32 - 72
Ponca	12128	47	45	42	45	32 - 68
Wichita	11952	59	72	56	63	40 - 94
Red Chief	12109	58	53	48	53	36 - 90
Nabob	8869	59	52	62	58	36 - 101
Triumph	12132	57	58	53	56	36 - 122
Westar	12110	60	48	47	52	36 - 86
Concho	12517	56	55	67	59	36 - 119
Tsp-Ae xPn	13020	67	44	51	54	32 - 119
Wanken	13659	57	56	57	57	32 - 76
Kaw	12871	50	58	52	53	36 - 76
Tmpx *Tsp-Ae*	13523	53	59	53	55	36 - 79
Pnx*Tsp-Ae*-PN	-	56	63	58	59	36 - 94
Aros	197656	51	46	55	51	36 - 79
Burpian	203084	55	54	52	54	36 - 79
				Population Mean	55	32 - 122

than the mean of the population collected from many varieties in the field at Stillwater. The total variation in spore length was 90 μ which was intermediate between the other two collections measured (Tables I and II).

One further test relative to spore length was made. During the spring of 1962, a single long spore from a pycnidia containing particularly long spores (150 to 160 microns) was isolated and increased. This culture was obtained from a collection made from the variety Kaw in the Stillwater Wheat Field Plots. Fifteen plants of each of the varieties Triumph, Kaw, and Ponca were inoculated with a 0.5 percent gelatin suspension of spores from this "long-spored" culture and placed in a metal moist chamber for 72 hours. The plants were then placed in a room with controlled temperatures ranging from 18° to 29°C. Although the plants were rewet by spraying with water within 12 hours after inoculation, infection occurred only on a single plant of Triumph. Pycnidia were produced on this plant and from three of these one hundred spores were measured. No significant difference was found between pycnidia (Table IV). The mean length was 51 μ with a range of 29 μ to 79 μ. No spores were found that even approached the length of the spore that was isolated to obtain the culture and the data indicate that spore length is quite strongly influenced by environment.

Table IV - Spore Lengths of *Septoria tritici* Collected from Wheat, var. Triumph, Plants Following Artificial Inoculation and Grown Under Controlled Conditions in the Greenhouse, Stillwater, Oklahoma, February, 1962.

Variety	CI No.	Mean Length of 100 Spores in Microns			Collections	
		Pycnidia Number			Mean	Range
		1	2	3		
Triumph	12132	51	52	50	51	29-79

Cultural Studies. The original objective in this portion of the study was to determine if consistant differences between isolates could be detected by growing cultures of the fungus on different substrates. However, S. tritici ordinarily grows quite slowly in culture and a substrate which would provide adequate growth needed to be found.

The following test was designed to find suitable media and growing conditions to obtain abundant growth. Seven different media (Table V) at four temperatures with and without light were used. Growth measurements were made at five-day intervals for 20 days as indicated previously. Transfers from a single-spore isolate were used throughout the study. The data are given in Table VI and indicate no differences in colony size when eight hours of light were added; however, a slight mounding of the colony could be observed under light.

Growth of the cultures increased with temperature. Many of the colonies produced at 21°C were larger than those at a lower temperature; but, due to contamination, growth could not be accurately measured. The lowest temperature

Table V - Media Formulations Used for Testing the Growth of *Septoria tritici* in Culture.

Media[1/]	Amounts
Potato Dextrose Agar (PDA)[2/]	
Potatoes	200 gm.
Dextrose	10 gm.
Agar	20 gm.
Potato Glactose Agar (PGA)	
Potatoes	200 gm.
Glactose	10 gm.
Agar	20 gm.
Potato Peptone Glactose Agar (SPGA)	
Potatoes	200 gm.
Peptone	10 gm.
Glactose	20 gm.
Agar	20 gm.
Campbells V-8 Juice Agar (V-8A)	
Campbells V-8 Juice	200 ml.
$CaCo_3$	3 gm.
Agar	20 gm.
Prune Agar (PA)	
Bacto prepared prune agar. Ref. No. 284866	
Corn Meal Agar + Dextrose (CMDA)[3/]	
Corn Meal	20 gm.
Dextrose	20 gm.
Agar	20 gm.
(slightly acidified)	
Corn Meal Agar + Glactose (CMGA)[3/]	
Corn Meal	20 gm.
Glactose	20 gm.
Agar	20 gm.
(slightly acidified)	

1/ Each medium was made up to 1000 ml. with distilled water.

2/ Abbreviations used in text.

3/ Prepared according to Rikers (19) Manual.

Table VI - The Cultural Growth of Septoria tritici on Seven Different Agar Medias over a 20-day Period.

Average amount of growth in units[1] for 20 days

Media	No Light					8 Hrs. Light Daily					Media
	Temperature in degrees C.					Temperature in degrees C.					
	4°	10°	15°	21°[2]	$\bar{X}$	4°	10°	15°	21°[2]	$\bar{X}$	$\bar{X}$
PDA	2.7	10.6	12.0	4.4	7.4	3.5	10.9	16.6	3.9	8.7	8.1
PGA	2.4	11.0	9.0	4.3	6.7	2.4	11.2	13.4	5.4	8.1	7.2
V-8A	4.7	10.7	10.7	3.1	7.3	2.3	10.8	15.6	2.8	7.9	7.6
P.A.	1.7	4.0	4.3	2.8	3.2	1.6	3.9	8.4	2.2	4.0	3.6
SPGA	3.4	14.2	12.3	5.3	8.8	3.9	12.8	5.4	4.5	6.7	7.7
CMDA	1.3	3.0	5.0	1.9	2.8	.7	1.1	3.7	1.9	1.9	2.4
CMGA	1.0	3.3	3.8	1.4	2.4	.6	1.2	4.4	1.4	1.9	2.1
TEMPERATURE $\bar{X}$	2.5	8.1	8.2	3.3		2.1	7.4	9.6	3.2		
$\bar{X}$ of 8 hrs. Light vs No Light					5.5					5.6	

1/ Average of 10 colonies expressed in number of 3 square millimeter units.

2/ Contamination was a problem at this temperature, limiting growth throughout the study and finally becoming so severe that readings were not made after 15 days.

(4°C) proved to be too low for adequate growth; however, contaminants were quite adequately controlled at this temperature. For this reason cultures were later stored at 4°C.

The temperatures ranging from 10° to 15°C were found to be optimum for production of spore colonies under conditions of this test. The selected isolate of S. tritici grew well on four of the seven media used (PDA, PGA, V-8A, and SPGA). The prune and corn meal agars proved unsatisfactory as a substrate for this organism.

While it was quite evident that growth of S. tritici adequate to measure differences in colony size and characterization could be obtained on any of the four media mentioned above, it was also clear that at temperatures above 15°C contamination presented a very real problem even though extreme care was exercised. It was observed that the Petri dishes which were not handled following seeding seldom became contaminated. Consequently, means of reducing the entrance of air into the dishes following seeding were investigated. Placing the plates in Kraft paper bags during storage helped to reduce contamination, but the best method tested was to tape the dish closed with cellulose tape. When this was done, colony growth at 21°C could be adequately tested.

DISCUSSION

The variation reported for morphological characters, such as spore length, in Septoria tritici (22, 24) is quite large, but even greater variability was found among cultures collected within the confines of the State of Oklahoma on a single variety of wheat. That ecotypes might develop with such wide limits seems plausible. Indeed, if the present study had ended after the first investigations, such a conclusion might have been drawn. It was found that spores collected in the western area tended to be shorter than those collected in eastern area. But it was also found in subsequent studies that at a single location even greater variability was noted when collections were made from different varieties. Such findings might also tend to indicate that host or substrate exerted considerable influence on total variation. However, when a single-spore isolate was cultured on susceptible and resistant hosts in the greenhouse, total variation as well as total spore length was reduced. These studies certainly would indicate that the variability in spore length in S. tritici is a function of the environmental, particularly temperature, variation.

A brief look at the hereditability of the spore length character was had in an experiment with a single long-spored isolate. While it is undoubtedly true that spore length is

an hereditable character in this fungus, it must also be greatly influenced by environment. At any rate, growing such a culture under controlled conditions in the greenhouse failed completely to reproduce either the spore length or variability in length that was experienced with this culture as it was isolated from the field. Unfortunately, a short-spored isolate was not cultured simultaneously. It is entirely possible that such a culture might have been proportionately reduced in size and variability under the conditions of this experiment.

In spite of the wide range in spore length that was encountered in these investigations, there is little evidence from the study of this character that ecotypes exist, at least not within the geographical confines of these tests. It most certainly would be interesting to widen the scope of such a study, as was done with Diplodia maydis (27).

S. tritici makes a rather slow growth on common media in culture (25). Consequently, before any study of cultural characteristics in relation to the possible existence of ecotypes could be made, a study of media and cultural conditions suitable for more rapid growth was required. Such a study was made with seven media, four of which supported what might be termed adequate to good growth. With these media colonies approximately 7 to 10 mm. in diameter were obtained in 20 days at 10° to 15°C. Such a quantity of growth would be adequate for increasing inoculum, perhaps, but for a study of cultural characteristics it was minimal. It seemed evident that the use of higher temperatures, at

least to 21°C , would have given somewhat better growth; but contamination then became a real problem, particularly when the dishes were handled during the process of measurement. Sealing the dishes with cellulose tape after seeding was quite beneficial in reducing the degree of contamination, but the entire problem of cultural growth needs further study before adequate measures of cultural characteristics can be made.

SUMMARY

1. Spore measurements of Septoria tritici collections made from the various State-wide Small Grain Yield Tests showed no significant difference in mean spore length.

2. The spores collected in the eastern part of the state tended to be longer than those collected in the drier, colder areas of the west.

3. The spores of one pycnidium sampled from the Muskogee collection were shorter and the range in length was much less than spores from the other pycnidia within that collection.

4. The mean lengths of the spores measured from eleven varieties grown in the Stillwater Field Plots showed no statistically significant difference, although spore length varied from 64 μ on the variety Concho to 94 μ on a selection of Triumph X Triticum species X Agropyron elongatum C.I. 13523, or almost one third of their total length. The range of individual spore lengths for this collection ranged from 29 μ to 162 μ and had a population mean of 75 μ.

5. The spores of a single pycnidium in three collections from the Stillwater Field Plots were found to be shorter than spores from the other two pycnidia sampled in each collection. This same phenomenon was observed in the Muskogee collection made from a single variety.

6. Long spores were found in the Stillwater Field

Plots collection which measured 162 μ and exceeded any spores found in the state-wide collections by over 70 μ. These long spores were found to exceed by at least 60 μ anything reported by Sprague (22).

7. No significant difference could be found between the mean spore lengths of collections made from 15 varieties grown in an artificially infected greenhouse nursery.

8. The mean spore length from the greenhouse nursery was 7.5 μ shorter than the mean of the population from the state-wide nurseries and 20.5 μ shorter than the mean of the population collected from many varieties in the field at Stillwater. The total variation in spore length was 90 μ, which was intermediate between the other two tests.

9. Severe infection was produced with artificial inoculation, but varietal susceptibility or resistance ratings could not be made because of the lack of uniformity in disease development.

10. Triumph wheat plants were infected with inoculum increased from a single long-spored isolate; however, none of the spores produced approached the spore length of the parent isolate.

11. Four substrates (Potato Dextrose Agar, Potato Glactose Agar, Campbells V-8 Juice Agar, and Potato Peptone Glactose Agar) were found to support adequate growth of Septoria tritici for cultural studies.

12. In general, growth in culture increased with temperature above 10°C. At approximately 21°C, however, contamination presented a real problem to studies of cultural growth.

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VITA

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