Cultural and Inoculation Studies of Septoria nodorum, Cause of Glume Blotch of Wheat

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ABSTRACT

Septoria nodorum, cause of glume blotch of wheat, *Triticum aestivum,* exhibited cultural variability into the ninth single-spore transfer generation. Mycelial color and growth habit, number and formation of pycnidia and spores, and pathogenicity were prominent variable characters. Groups of cultures that produced abundant pycnidia and spores were selected in five transfer generations. From cultures that had many pycnidia and spores, isolates were selected in three transfer generations that produced no spores. When selection pressures were reversed, cultures were returned to original types with equal ease. Variants obtained from single-spore isolations were used to inoculate wheat lines to evaluate pathogenic variability. None of the isolates gave identical reactions on the test plants. Specific resistance to field populations of *S. nodorum* is not known among the *Triticum* spp. We have further documented the extreme variability in both cultural characters and pathogenicity of *S. nodorum,* and recommend not designating physiological races of this pathogen. Phytopathology 60:1480-1485.

Many reports of resistance to *Septoria nodorum* (Berk.) Berk. in wheat, *Triticum aestivum* L., have been published (1, 2, 3, 6, 10, 13, 14, 16, 17, 24). In subsequent tests, usually under altered environmental conditions, the reported resistances were not expressed (A. L. Scharen, *unpublished data* and A. L. Scharen, *personal communication*). But Bockmann (5) reported some cultivars of wheat to be distinctly less affected by the disease. Significant differences in reaction to *S. nodorum* were detected between some cultivars by Leijerstam (14). Differences in susceptibility among 25 winter and 16 spring wheats were detected by Zwatz (28). We have found differences in response to infection by *S. nodorum* among five wheat cultivars (21, 23).

The tolerance documented in the reports cited above appears to be equally effective against diverse collections of *Septoria;* i.e., nonspecific, as is the pathogenicity of the fungus. Brönnimann (8) studied the pathogenicity of 10 diverse cultures of *S. nodorum* on Hinal spring wheat in field experiments. Neither the intensity of the attack nor the components of yield gave any evidence of biotypes or races of the pathogen. But *S. nodorum* has fastidious requirements for favorable environmental conditions, with rainfall and high humidity being critical to epidemic development (7, 19, 20, 22, 25).

In the present study, we report on the cultural and pathogenic variability of *S. nodorum.* In addition, we have developed methods of rating seedlings for tolerance to *S. nodorum,* and have selected wheat lines with levels of tolerance to the pathogen that may be useful in breeding programs. A preliminary report has been published (22).

MATERIALS AND METHODS.—*Cultural studies.*—A single-spore (SS) culture of *S. nodorum* from a naturally infected field-grown plant was used to begin each study. The spores or the mycelia in the original slant-culture grown from a SS isolate were used to begin the selections. Additional SS isolates or mass transfers of mycelia (MT) were used as required.

Single-spore isolates were made as follows: (i) The selected culture was flooded with 5 ml of sterile distilled water and a spore suspension obtained; (ii) the suspension was decanted onto the surface of water agar in a petri dish, then incubated overnight at 20 C; and (iii) under a dissecting microscope, individual germinated spores were isolated and transferred to potato-dextrose agar, pH 5.5. Usually, 25 spores were isolated for each observation.

MT cultures were made by taking 25 to 30 bits of mycelia from the selected tube and placing them in new agar slants. All isolates were incubated at 20 C under constant fluorescent light (one 15-w cool-white tube), 2 weeks for SS isolates and 1 week for MT cultures. Observations were then made for growth characteristics and numbers of spores.

Spore counts were made by flooding slants with 5 ml sterile distilled water, shaking for 2 min, storing overnight at 20 C, then shaking for an additional 2 min. An aliquot of the spore suspension was then pipetted into a hemocytometer for counting.

Inoculation studies.—Wheats selected for inoculation studies were planted in 3-inch pots of soil in the greenhouse and allowed to grow to the three- to four-leaf stage. In screening for tolerance, 25 to 50 seedlings of each selection, five seedlings/pot, were inoculated with a standard culture. Seedlings were tested twice, and those that showed promise were inoculated twice more as seedlings and at least once as adult plants where possible. Wheat cultivars Asosan, C.I. 12665; Wisconsin Selection, C.I. 12632; Hadden, C.I. 13488; Little Club, C.I. 4066; and Nainari 60, C.I. 13747 were used to evaluate pathogenic variability in isolates of *S. nodorum.* Five to 10 seedlings of each were inoculated with each test culture. A spore-mycelial suspension was atomized onto the seedlings; the plants were kept in a high-humidity chamber at 20 C for 4 days, then placed on a greenhouse bench. Ten tests using 54 cultures were done throughout the year, except for the warmest periods of the summer. Greenhouse temp ranged from 22 to 44 C.

RESULTS.—*Cultural studies.*—Seventy-five MT were made from a SS culture of *S. nodorum,* N-38 (Fig. 1).

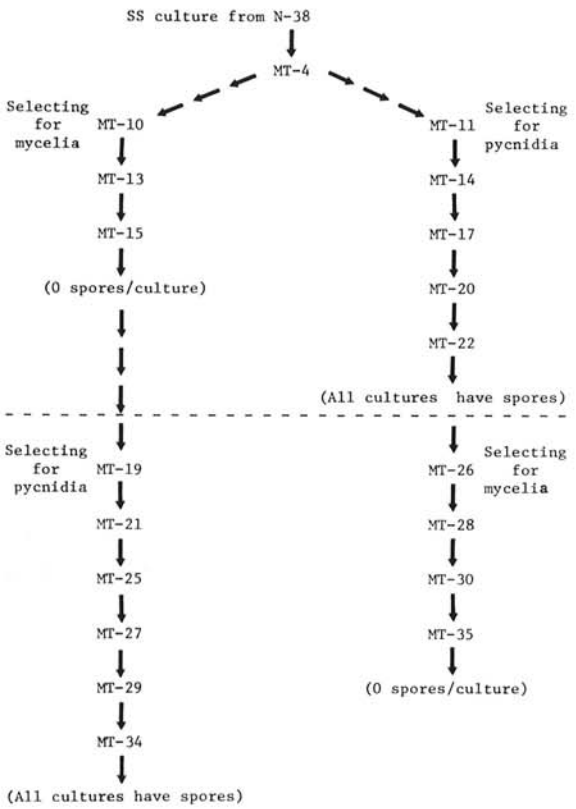

Fig. 1. Mass transfers in test-tube slant cultures from a single-spore culture (N-38, field collection) of *Septoria nodorum*. (Study V-2). None of the 30 cultures of MT-15 yielded spores, but a few pycnidia were present. Therefore, subsequent selection for pycnidia from MT-15 was possible.

Considerable variability was found among the group, designated generation MT-4 (Fig. 2-A). From MT-4, MT-4-51 was chosen because it had few pycnidia and a low spore count (1,100 pycnospores/ml). MT-4-7 was chosen because it contained numerous pycnidia and 17,700 spores/ml. Thirty transfers from each of these formed groups MT-10 and MT-11, respectively. Succeeding MT generations consisted of 30 test-tube slant cultures. From among these, one slant was chosen in each case to make 30 transfers for the next subsequent MT generation. After three groups of transfers, the 30 cultures of MT-15 yielded no spores, while most cultures from MT-17 had pycnidia and spores present (Fig. 2-B). Two additional transfer groups (MT-20 and MT-22) were grown before all 30 cultures yielded pycnidia and spores.

Selection pressure was then reversed from MT-15 through six transfer generations. Although none of the 30 cultures of MT-15 yielded spores, a few cultures had a small number of pycnidia. Selection for pycnidia from MT-15 therefore was possible. Group MT-34 consisted of 30 tube cultures of which all yielded pycnidia and spores. From MT-22, in which all cultures yielded spores, selections were made for increased mycelia and fewer spores. After four transfer generations, none of the 30 tube cultures yielded spores in MT-35 (Fig. 2-C).

Other MT selections obtained incidentally during the course of the study included (i) a culture with pink mycelia and no spores; (ii) a pink selection from (i) that produced spores; (iii) a culture with white mycelia; (iv) a culture with yellow mycelia and no spores; and (v) a yellow selection from (iv) that produced spores.

In a representative study of SS isolates, we again started with *S. nodorum* N-38. Sixteen cultures from N-38, constituting SS-5, developed various numbers of pycnidia and spores (Fig. 2-D). From SS-5, 22 isolates were made and designated SS-12. From SS-12, a culture with prostrate mycelial growth and many pycnidia was chosen to make the 25 slant cultures in group SS-16; another culture with fluffy mycelia and few pycnidia was selected to make 25 cultures constituting SS-18. After three selection cycles, groups SS-32 and SS-33 (Fig. 2-E) were tested for uniformity by making two or three selections from each. Groups of cultures designated SS-39, SS-40, SS-42, and SS-51 were very uniform, all resembling SS-33 and having prostrate mycelia with many pycnidia and spores. There was continued variation in number of spores per ml. On the other branch of the selection scheme, SS-41 through SS-61 continued to exhibit considerable variability in mycelial type and number of spores.

During the SS selections for pycnidia, after the first group of selections only white mycelia were found. Mycelial growth was prostrate, and subsurface growth in agar occurred. Agar pigmentation became lighter during selections, until it could not be detected in SS-51. Pycnidia were numerous in all cultures after the first selections, but spores per ml varied from 14,300 to 1,861,200.

During the SS selections for mycelial growth, abundant yellow and white mycelial types were obtained, with pink, gray, and green appearing occasionally. Mycelia were mixed between prostrate, slightly fluffy, and aerial-fluffy growth. Selections for prostrate growth yielded only this type in subsequent transfer groups. Agar pigmentation became progressively lighter, but was still evident at the conclusion of the experiment. There was considerable variation in numbers of spores/ml (1,100 to 8,712,000) until the conclusion of the test.

Inoculation studies.—Typical results obtained with five cultural variants derived from a single-spore isolate of *S. nodorum* (MT-14, V-2) are shown in Table 1.

Since differences in disease reaction were not distinct, five categories of symptom expression were used: (i) lesion size; (ii) number of lesions; (iii) percentage chlorosis of leaf tissue; (iv) number of flecks, either chlorotic or necrotic; and (v) percentage necrosis of leaf tissue. Chlorosis and necrosis of the primary and second leaves were the measures showing the greatest differences between cultivars and cultures. Asosan and Nainari 60 had 0 to 60% chlorosis, depending on the inoculum culture, while Little Club inoculated with 14 to 23 had no more than 10% tip chlorosis. Flecks were not correlated with tolerance or resistance, as they are in some disease situations. Chlorosis varied

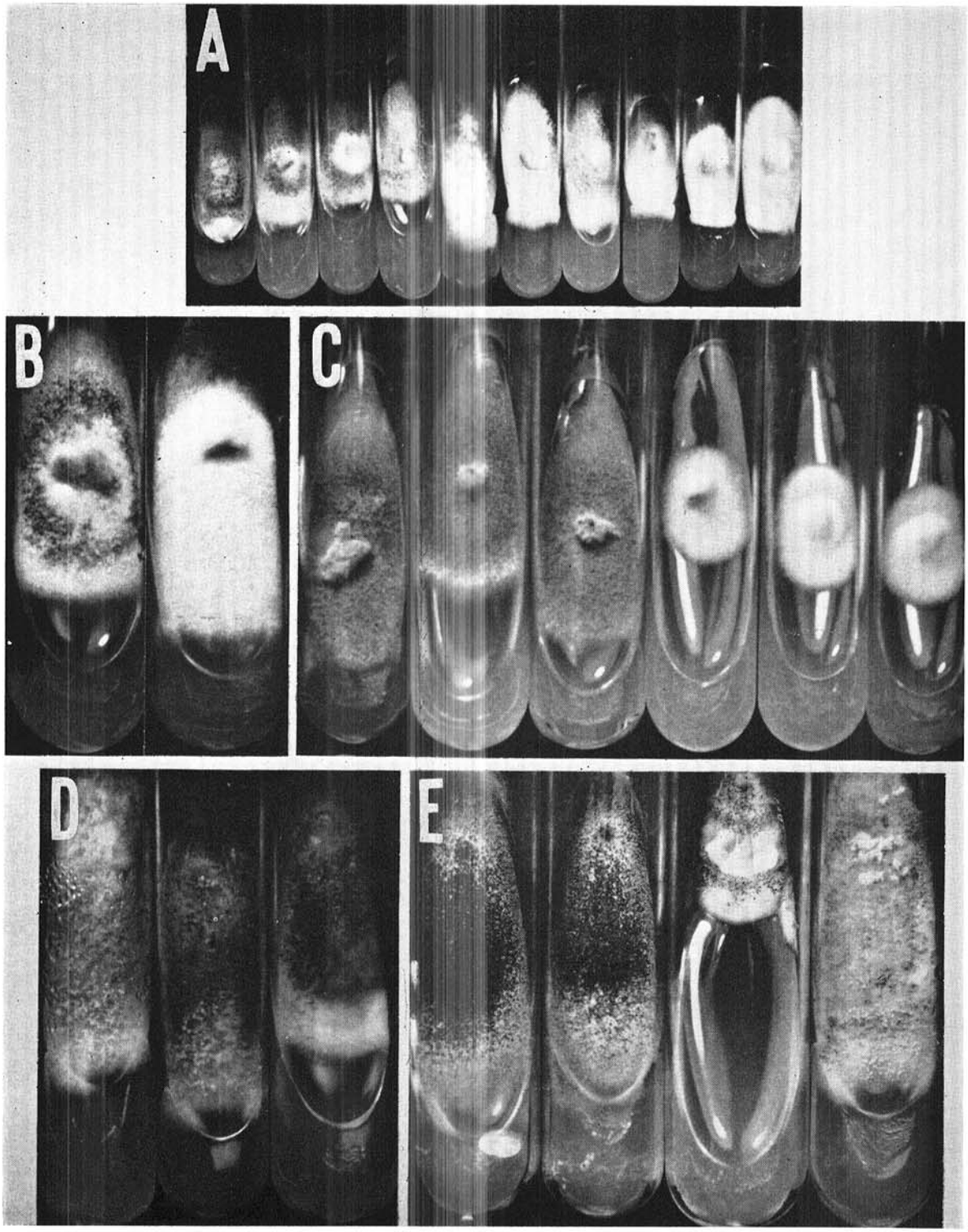

Fig. 2. Cultures of *Septoria nodorum*. **A)** Ten variant cultural types among 75 mass mycelial transfers (MT-4), all derived from a single-spore culture. **B)** Left, a pycnidia-producing culture (MT-17); right, a mycelial culture without pycnidia (MT-15), each selected in three transfers from MT-4. **C)** Left, three tubes, pycnidia-producing cultures (MT-34) selected in 6 transfers from MT-15; right, three tubes, mycelial nonsporulating cultures (MT-35) selected in four transfers from MT-22. **D)** SS-5 (V-3) representative variants of single-spore isolates from N-38. **E)** Left, two tubes, SS-33 obtained after three transfers selecting for pycnidia; right, two tubes, SS-32 obtained after three transfers selecting for mycelia.

TABLE 1. Reactions of five wheat cultivars to five cultural variants from a single-spore isolate of *Septoria nodorum*

	14-8(2200)[a]					14-10(4425)					14-12(2200)					14-23(2200)					14-24(2200)				
Culture MT-14	U[b]	V	W	X	AF	U	V	W	X	AF	U	V	W	X	AF	U	V	W	X	AF	U	V	W	X	AF
SYMPTOMS																									
Primary Leaf:																									
Lesion size[c]	*	*	*	*	*	*	M	*	*	M	S	S	*	*	*	*	*	*	*	M	*	*	*	*	*
No. lesions[d]	*	*	*	*	*	*	M	*	*	F	F	M	*	*	*	*	*	*	*	F	*	*	*	*	*
% Chlorosis	20	10	0	0	0	50	20	20	0	50	30	20	0	0	0	10	30	20	10	0	25	60	0	0	20
% Necrosis	10	40	0	0	5	50	10	10	0	50	20	10	0	0	0	20	10	40	0	10	10	40	0	0	20
No. flecks	0	0	F	F	M	0	0	F	0	0	0	0	F	F	F	M	0	F	F	M	M	0	M	F	M
Second Leaf:																									
Lesion size	*	*	*	*	*	*	S	*	*	M	M	S	*	*	*	*	*	*	*	M	*	*	*	*	*
No. lesions	*	*	*	*	*	*	F	*	*	M	M	F	*	*	*	*	*	*	*	F	*	*	*	*	*
% Chlorosis	5	20	40	0	40	40	20	20	0	50	0	20	0	0	0	0	30	0	0	0	20	10	0	0	40
% Necrosis	0	10	10	0	10	10	10	0	0	20	0	10	0	0	10	10	20	10	0	10	0	0	10	0	20
No. flecks	0	0	M	F	0	0	0	0	0	0	0	0	M	0	M	M	M	M	0	M	M	M	F	0	M

[a] Number of conidia per ml of inoculum.
[b] Cultivars: U, Asosan; V, Wisc. Sel.; W, Hadden; X, Little Club; AF, Nainari 60.
[c] Size: S = small; M = medium; L = large; asterisk (*) indicates no discrete lesions were present.
[d] Number: F = few; M = moderate.

from 0 to 50% between cultivars inoculated with a single culture.

A total of 54 wheat selections were tested in a series of inoculation experiments using a single field collection of *S. nodorum*. The five cultivars mentioned above were included in these tests to evaluate the inoculation techniques and conditions of incubation. On the basis of results similar to those described above and in Table 1, 9 lines were determined to have a useful degree of tolerance to *S. nodorum*. Those were (i) *Triticum aestivum* (Fn × Th II), Pelotas, Brazil; (ii) *T. fungicidum*, Castelar, Argentina; (iii) *T. durum* (Kubanka) × *T. monococcum* (Oued Allal), Julio de Castilhos, Brazil; (iv) *T. aestivum* (Carazinho), Pelotas, Brazil; (5, 6, 7, 8, 9) selections from the cross, (*Aegilops ventricosa* × *T. turgidum*) × *T. aestivum* (Chancellor), Beltsville, Md.

DISCUSSION.—*Variation.*—Our studies show that a SS isolate of *S. nodorum* does not necessarily give a stable culture of the fungus. Generally there were great variations among cultures at the beginning of each study. Similar variability was reported in *Septoria avenae* Frank (9, 11).

In the V-2 study (see Fig. 1), there was sufficient variability among the initial SS isolates so that cultures having a large amount of fluffy mycelia and few or no pycnidia, and cultures that produced sparse mycelia with large numbers of pycnidia could be selected easily. When selection pressure was reversed, pycnidial cultures were selected from those producing only mycelia, and vice-versa. Other variants already described were also found.

Substantial variability was also noted among SS cultures. Cultures of *S. nodorum* having many pycnidia, and prostrate white mycelia or prostrate yellow mycelia were found. In the selection for fluffy mycelia, much variability appeared within and between groups and cultural generations.

Stability.—Even though great variability was the norm, a stable culture was obtained from the SS series. With the exception of number of spores produced, a "stable culture" was one in which all SS slant-cultures for several transfer generations had identical cultural characteristics as far as we could determine them. The stable culture in this series had a prostrate form of mycelial growth and many pycnidia. Hooker (11) obtained stable cultures of *S. avenae* in about the same way.

In contrast, no cultural stability was obtained by single-sporing for fluffy mycelia in study V-3. Subcultures SS-32 and SS-38 exhibited great variability, indicating that selections for abundant fluffy mycelia would probably yield unstable, variable cultures.

It is also clear that variability would be maintained by mass transfers of cultures. Only by single-spore isolates of a few cultures was reasonable stability obtained.

Growth characteristics.—Some characteristics of cultures changed appreciably in definite directions as a result of the SS isolations. In the mass transfer selections of study V-2, there were no trends of change other than those being selected. The desired characteristics increased until they dominated the cultures. But if careful selections for pycnidial formation were not made, the unselected cultures developed rapidly toward abundant mycelia and few or no pycnidia and spores.

Among the single-spore isolates, the frequency of appearance of a particular characteristic greatly influenced the ease of selection for that particular character. We were not successful in isolating and propagating cultures having a particular characteristic that appeared in only one SS culture out of 50. But with more refined techniques, such a selection should be possible.

Pathogenicity.—Variation in virulence of *S. nodorum* was evident among the single-spore isolates derived from a single spore as well as among those selected for various characteristics of growth in culture by means of mass mycelial transfers. We were unable to find two isolates that gave identical reactions on a series of test cultivars of wheat. Some of this variability is probably due to techniques, even though the conditions of growth, inoculation, and incubation were standardized. The extreme variability in both cultural and pathogenic characteristics shown here emphasizes the danger in trying to separate physiological races in *S. nodorum*. The situation with *S. nodorum* is similar to that found with several other facultative ascomycetous parasites such as *Cercosporella herpotrichoides* (12), *Cochliobolus sativus* (18), *Pyricularia oryzae* (15), and *Fusarium culmorum* (4, 5, 6, 14). In each of the above-cited works, evidence of extreme variability in pathogenicity was given and no indications of reliable or useful means of dividing the organisms into physiological races were shown. Snyder & Hansen (26) and Snyder & Toussoun (27) have documented the kinds and amounts of variability found in the genus *Fusarium*, and recommend the use of cultivar designations such as Culmorum and Graminearum as a useful, informal device for designating subspecific groups that have characters, particularly pathogenic characters, in common.

Our conclusion and that of Brönnimann (7), is that mixed field collections of the fungus from the growing area of concern should be used as inoculum for testing wheat cultivars and lines for tolerance to *S. nodorum*. We believe that tolerance of the pathogen is the form of resistance that will give the most rapid progress in obtaining reduction of losses in wheat due to the glume blotch disease. We do not expect to find specific single-gene resistance, nor do we believe it would be desirable if found. Considering the variability of *S. nodorum*, such resistance would probably be very short-lived indeed.

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