Physiologic Specialization of Septoria tritici

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ABSTRACT

Contrary to the reports from other countries, our studies in Israel revealed physiologic specialization in *Septoria tritici*. Cultures of this pathogen displayed differential interactions with the wheat accessions involved. Significantly, culture number 12, originally isolated from durum wheat, was very distinctive in its parasitic characters and deviated pronouncedly from the cultures secured from *Triticum aestivum* plants in various parts of the country. Promising sources of resistance to *Septoria* leaf blotch were found. The nature of physiologic specialization of *S. tritici* and its bearing on breeding for disease resistance is discussed.

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The *Septoria* leaf blotch disease of wheat caused by *Septoria tritici* Rob. ex Desm. has been identified in over 50 countries (14). Literature dealing with the geographic distribution and economic importance of this disease, and the biology of the pathogen, has been reviewed (14, 15). It appears that in several regions of the United States, in Central and South America, Western Australia, New Zealand, South Africa, and in a number of European countries, *Septoria* leaf blotch can assume epidemic proportions and cause serious reduction in yields. It is therefore considered a major wheat disease in some countries (14).

In Israel we recorded annual incidence of the disease for nearly 30 years. The old "land varieties" of durum wheat were very susceptible but not particularly vulnerable and did not sustain appreciable losses. The situation became aggravated with the introduction of semi-dwarf, high-yielding wheats from Mexico. The success of these cultivars in Israel, and of their local selections and hybrids, was most spectacular; at present, they occupy about 90 percent of the wheat acreage and the output of this crop was more than doubled within a few years. Unfortunately, most of the new wheat cultivars are susceptible to *Septoria* leaf blotch. Recurring epiphytotics of the disease take a heavy toll and reduce yields in some years up to 30-40 percent (Eyal and Wahl, *unpublished*). A similar increase in importance of *Septoria* leaf blotch on related semi-dwarf Mexican wheats has taken place in a number of countries of the Near East, such as Morocco, Tunisia and Turkey (16).

The seriousness of *Septoria* leaf blotch outbreaks made it imperative to devise suitable protective measures. Resistance to *S. tritici* has been demonstrated by a number of researchers (9, 10, 11, 13, 14, 15), and breeding for resistance is likely to be the most practical method of control. An extensive breeding program to attain this goal has been started in Israel. Some wheat cultivars resistant abroad behaved the same way in our varietal trials, while others have displayed susceptibility here. These and similar observations stimulated the present study to explore the problem of physiologic specialization of *S. tritici*. Investigators in other countries have not been able to identify physiologic races in this organism (2, 9, 14, 15). Likewise, the evidence against the existence of such races has also been inconclusive.

MATERIALS AND METHODS.—During four growing seasons in the years 1968-1972, about 600 monopycnidial cultures of *S. tritici* were isolated from wheat cultivars from various parts of the country. Each culture was obtained by transferring the ooze formed on the tip of a single pycnidium ostiole under high humidity to a petri dish containing potato-dextrose agar (PDA) supplemented with 250 ppm chloramphenicol succinate. Colonies produced by growing the fungus at 15 C for two weeks were subcultured at 15-day intervals on PDA slants supplemented with 0.5% yeast extract (4).

Inoculation procedure.—Small portions of agar cultures were transferred to modified Fries' liquid medium (7) to which 0.5% yeast extract had been added, and shaken for 8-10 days at 20 C. The spore suspensions were adjusted to approximately 3×10^7 spores/ml as determined by hemacytometer counting. Inoculation was performed by gently rubbing a cotton swab soaked in spore suspension over the leaf of a seedling or adult plant. The inoculated seedlings were placed in high humidity chambers at 20 C for 48 hr and then placed in a greenhouse at a similar temperature. These trials were run in five replications and repeated three times. The adult plants tested were grown in hills outdoors in wire-screened enclosures and inoculated at the boot stage. The inoculated plants were sheltered in plastic tents

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supplied with humidifiers which produced dew continuously for 48 hr; and the tents were removed. These tests were designed with two replications, and were repeated twice.

Origin of tested cultures.—After about 70 cultures of *S. tritici* had been tested on seedlings of differential accessions, some of them appeared to be particularly distinct in their pycnidial productivity, host range, and type of host interaction. In order to illustrate these specific traits, the performance of five cultures referred to hereinafter as numbers 7, 11, 12, 18, and 112, will be described (Table 1). Culture 7 was isolated in the Central Coastal Plain from cultivar N. 46, a selection of the Mexican cross Lee X Nrn10-B29. Culture 11 was obtained in the Southern Coastal Plain from the cultivar 'Hazera 81', a local selection of the Mexican cross of Son 64A-Tzpp/Nai 60 X St. 464-Bza. Sib (20754-0-3h-10h-0h-6h). Culture 12 was secured in the Upper Galilee from a local durum wheat of unknown pedigree. Culture 18 originated in the Plateau of Menashe – from the cultivar 'Hazera 84'– an Israeli selection of Son 64A-Tzpp/Y54 X Napo 63 (20852-0-5h-2h-0h-2h). Culture 112 was isolated in the Southern Coastal Plain from cultivar 'Hazera 112'– a local selection of 2193/Ch53 – An X Gb.56 X An.64 (20985-0-5h-2h-0h-11h). A map of Israel showing the location of the mentioned geographic regions was previously published elsewhere (18).

Differential hosts.—Sets of differential hosts for studying the physiologic specialization and virulence of *S. tritici* comprised accessions reported to be resistant, such as 'Bulgaria 88' (P.I. 94407-2), 'Nabob' (C.I. 8869), H574-1-2-6, and 'Colotana' (C.I. 13556) (provided by R. M. Caldwell, Purdue University, Lafayette, Indiana). Susceptible differentials in the set included, the durum "land variety", 'Etit 38' (extensively cultivated in Israel in the 1940's and 1950's when it was heavily affected by *S. tritici*), and another three entries very susceptible to this pathogen, viz. 'Mivhor 1177' (the local selection of the Mexican cross 8156B, equivalent to 'Siete Cerros'), and the Israeli cultivars: Pan-1 [(Ch53 X Nrn10-B26/Yq54) FA], and 8828-23 (Yt X Nrn10-B21/FA). The latter three cultivars (or selections of the same crosses) are widely grown in this country. In adult plant trials the sets of mentioned accessions were supplemented by a number of cultivars known to be resistant to *Septoria* leaf blotch abroad, namely 'Buck Manantial', 'Toropi' [supplied by S. Fuentes, Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), México 6, DF, Mexico], 'Racine' (C.I. 13172), 'Russian' (P.I. 94478-1), and 'Turkey' (P.I. 167833-1) (provided by R. M. Caldwell). Besides these, some collections of indigenous wild tetraploid emmer wheats, *Triticum dicoccoides* Koern., were screened. Populations of this species are sources of resistance to some wheat rusts (6).

Evaluation of host reaction and pathogen virulence.—Arsenijević (2) rated wheats reacting to *S. tritici* by formation of necrosis without pycnidia as nearly immune. Rillo (10), Narvaez (9), and Rillo & Caldwell (11) grouped reactions induced by *S. tritici* in classes ranging from immune to very susceptible, on the basis of the size of lesions and density of pycnidia. Rosielle (13) screened 7,500 wheat varieties for resistance to *S. tritici* and found them to vary from immune (with no pycnidia and no visible symptoms except occasional flecking) to very susceptible (with large, abundant pycnidia and coalescing lesions). In many varieties, resistance was rated by scoring only pycnidia density, because of their tendency to exhibit for unknown reasons extensive necrosis under natural conditions. Plants with aberrant lesions and necrosis also have been reported by other investigators (12) as well as in our studies. They complicate interpretation of host reactions. Therefore, we too, used pycnidia density on live plants (but not the senescent ones) as

TABLE 1. Pycnidia development on seedlings of wheat accessions artificially infected with five cultures of *Septoria tritici*.[a] Average readings of tests with five replications repeated three times during 1969-1972 in the greenhouse at 20 C approximately

Variety	C.I. or P.I. No.	*Septoria tritici* cultures: 7	11	12	18	112
8828-23		Py3[a]	Py2	O[c]	Py5	Py4
Buck Manantial		O[c]	Py2	Tr	Py1	Tr
Bulgaria 88	94407-2	O[c]	Py1	O[c]	Py2	Py1
Colotana	13556	O[c]	Tr	O[c]	Tr	Py3
Etit 38 (*T. durum*)		O[c]	O[c]	Py3	Tr	O[c]
H574-1-2-6		O[c]	Tr	Tr	Py2	Py1
Mivhor 1177		Py1	Py2	Py1	Py3	Py2
Nabob	8869	O[c]	Tr	O[c]	Py1	Py1
Pan-1		Py1	Py4	Py2	Py4	Py5
Racine	13172	O[c]	O[c]	O[c]	Py1	Tr
Russian	94478-1	O[c]	O[c]	O[c]	Tr	O[c]
Sud Oeste	344157	O[c]	Tr	Tr	Py1	O[c]
T. dicoccoides G-37		O[c]	O[c]	Tr	Py1	Py3
Turkey	167833-1	O[c]	Tr	Tr	Py1	O[c]

[a] O[c] = no pycnidia, but distinct chlorosis; Tr = traces of pycnidia; Py1 = 1-20% of leaf area covered by pycnidia; Py2 = 21-40%; Py3 = 40-60%; Py4 = 61-80%; Py5 = 81-100% of leaf area covered by pycnidia.

TABLE 2. Percent of leaf area occupied by pycnidia of *Septoria tritici* in wheat accessions artificially infected at the boot stage. Average readings of tests with two replications and repeated twice

		Septoria tritici cultures:			
		12		18	
Variety	C.I. or P.I. No.	Leaf Inoculated	Pycnidia Density	Leaf Inoculated	Pycnidia Density
Buck Manantial		–1[a]	0.5[b]	–1	17.5
Bulgaria 88	94407-2	–1	1.0	–1	7.5
Etit 38		–1	30.0	–1	1.0
H574-1-2-6		–2	0.5	–2	25.0
Mivhor 1177		F	10.5	F	95.0
Racine	13172	–1	0.0	–1	12.5
Russian	94478-1	–1	0.0	–1	30.0
Toropi		–1	1.0	F	17.5
T. dicoccoides G-37		–3	2.5	–3	7.5
Turkey	167833-1	–2	2.5	–2	7.5
LSD, $P = 0.05$			14.205		27.058

[a] Leaves marked as to position below the flag leaf – F: –1, –2, –3.
[b] Percent of leaf area covered with pycnidia.

the sole criterion for assessing the response of the host and the virulence of the pathogen. Measurement of virulence by rating for pycnidial formation is consistent with the concepts of virulence suggested by Miles (8) and Gäumann (5). According to Miles, multiplication of the pathogen in the tissues can serve as an index of virulence, provided that it is clearly associated with the severity of the disease. In our observations and experimentations, the severity of Septoria leaf blotch appears to be commensurate with the intensity of pycnidia production. In one of Gäumann's definitions, virulence merely signifies the ability of the pathogen to multiply in the host. Strains of *S. tritici* inferior in pycnidial productivity on a specific wheat variety would be expected to reduce the level of inoculum on such a variety in the fields and consequently less likely to develop serious epidemics (13).

RESULTS.–*Seedling tests.*–The five cultures of *S. tritici*, 7, 11, 12, 18, and 112, are distinguishable because they differ in the degree of virulence on some of the investigated cultivars as measured by density of pycnidia formed (Table 1). Culture 12, which was originally isolated from durum wheat, is of special interest for its ability to produce pycnidia on the durum cultivar Etit 38, whereas the remaining cultures cause mainly chlorosis or necrotic lesions on this accession. Significantly, this culture is avirulent on accessions of *T. aestivum*, except on Pan-1. Culture 7 is characterized by a narrow host range and develops pycnidia only on the very susceptible accessions Mivhor 1177, Pan-1, and 8828-23. Culture 18 has the widest host range; it produced pycnidia on all differential hosts. Culture 112 diverges from others by its capacity to produce pycnidia on Colotana. Accessions Buck Manantial, Colotana, H574-1-2-6, Nabob, Racine, Russian, 'Sud Oeste', and Turkey display high resistance to all five cultures.

Data in Table 1 show that the investigated cultures not only differ in virulence on the different tested hosts, but also that they vary with the particular cultivar used for infection. For example, culture 11 is moderately virulent on Bulgaria 88, highly virulent on 8828-23, and avirulent on Etit 38. The opposite is true of culture 12, which readily produces pycnidia on Etit 38, but is incapable of forming them on Bulgaria 88 and 8828-23.

Adult plant tests.–In these trials, numerous cultures of *S. tritici* were inoculated to two or three uppermost leaves on plants at the boot stage. More detailed studies were made with cultures 12 and 18 (Table 2) because of their particularly contrasting performance on seedlings (Table 1). Results of seedling and adult plant experiments show good agreement. Accessions Buck Manantial, Bulgaria 88, H574-1-2-6, Racine, Russian, Turkey, and *T. dicoccoides* (G-37), which were resistant as seedlings to all or most of the cultures, also reacted alike to the particular cultures at the boot stage. These accessions and other promising entries such as Sud Oeste and Toropi deserve to be studied for their reaction to cultures representing a wide spectrum of pathogenicity. Culture 12 behaved similarly on adult plants and on seedlings, being virulent on Etit 38 and innocuous on entries of *T. aestivum*.

Obviously, the investigated cultures displayed differences in virulence on the infected accessions at the seedling and the boot stage. It is noteworthy that in both trials these cultures did not follow the same ranking for virulence on any one of the tested accessions. Evidently, they interact differentially with the hosts concerned (17).

Stability of culture virulence.–Cultures 7, 11, 12, 18, and 112 of *S. tritici* have retained their specific virulence on the tested cultivars over the years 1970-72. During this period of time these cultures were involved in three reisolation and reinoculation cycles and subcultured on the nutrient media: culture 7 was subcultured 70 times, cultures 11, 12, and 18 – 50 times each, and culture 112 – 35 times in succession. The progeny cultures retained the virulence of their respective ancestors.

DISCUSSION.–*Septoria* leaf blotch is a major wheat disease in many countries and is the most severe disease of this crop in Israel. It gained in importance with the rapid increase in cultivation of the high-yielding, semi-dwarf Mexican wheats which are susceptible to *S. tritici*. At present, *Septoria* leaf blotch is one of the factors limiting production of these wheats in Brazil, Argentina, Morocco, Algeria, Tunisia, Turkey and the highlands of Kenya, Ethiopia, Colombia, Ecuador, and Guatemala (3). Effective resistance to *Septoria* leaf blotch has been discovered and breeding for resistance is considered to be the most promising disease control measure.

Knowledge of the physiologic specialization of a pathogen is a prerequisite for a reliable program of breeding for disease resistance. Researchers failed to prove the existence of physiologic specialization in *S. tritici*, but found differences in virulence among the cultures of this fungus. Narvaez (9) could not detect physiologic races in this fungus and none of his cultures could invalidate the resistance of the investigated wheats. Arsenijević (2), too, stressed the absence of physiologic races in *S. tritici*. Rillo et al. (12) mentioned that no host-specialized races have been revealed in this pathogen. According to Shipton et al. (14), "at least three, and possibly four, specialized forms of *S. tritici* have been found, yet physiologic races have not been identified". Resistance to *S. tritici* is, therefore, viewed with confidence (14). S'Jacob (15) reported that cultures of *S. tritici* differ in virulence, and that the differences are the same on all hosts. Such cultures do not demonstrate "true physiologic specialization" because the order of the disease severity ratings is similar for all cultures on each of the infected cultivars. Cultures varying in virulence but not interacting differentially with the hosts are classified by van der Plank (17) as "aggressive races". These races follow the same ranking for virulence on any one of the hosts. Resistance to such races is considered to be "horizontal", nonspecific, permanent, and the fear that it will be lost is "groundless" (17).

Evidence presented in this paper proves that cultures of *S. tritici* isolated in Israel do not behave as "aggressive races", but rather as races in the conventional connotation, and they do interact differentially with the suscept. Their parasitic characters are stable and have been retained in successive reisolation and reinoculation cycles as well as in subcultures obtained by repeated transfers on nutrient media.

Resistance sources listed in Tables 1 and 2 may be relatively stable but there is no reason to expect them to be permanent in view of the physiologic specialization of *S. tritici* in this country. The history of *Septoria* leaf blotch in Israel substantiates this conclusion. In the 1940's and 1950's durum wheats like Etit 38 predominated here and were badly stricken by *S. tritici*, in contrast to only mild disease incidence on the *T. aestivum* cultivar 'Florence-Aurore'. In the following years *Septoria* leaf blotch became very severe on the expanding acreages of bread wheats, including Florence-Aurore, but sharply decreased in severity on Etit 38 which is currently grown only in small and scattered fields. Data in Tables 1 and 2 explain this situation by showing that cultures isolated from *T. aestivum* accessions are avirulent on Etit 38.

The great success of the semi-dwarf Mexican wheats even in countries where pathogens virulent on such varieties thrive abundantly (16), does not justify the contention that extensive cultivation of these wheats in such regions "would be very ill-advised" (1). It is strongly advised, however, that the search for effective sources of disease resistance, and research on the physiologic specialization of the pathogens involved, be intensified to safeguard the accomplishments of the "green revolution".

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