Global Insights into Virulence Frequencies of *Mycosphaerella graminicola*

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ABSTRACT

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Virulence patterns of 97 isolates of *Mycosphaerella graminicola* from 22 countries were evaluated on seedlings of 35 wheat and triticale cultivars. Significant isolate × cultivar interactions indicated the existence of specific virulence genes among isolates. Numbers of genes for resistance in cultivars were estimated based on the assumption of a gene-for-gene relationship. Seven hypothetical genes for resistance were estimated in the CIMMYT wheat cultivar Kavkaz-K4500.L.A.4., five in Red Chief, and four each in cultivars Bobwhite "S," JCR-979, Volcani 447 (tetraploid), and the triticale cultivar Mapache. Twenty eight hypothesized complementary genes were designated in the 97-isolate × 35-cultivar matrix. These genes were used to determine virulence frequencies of *M. graminicola* and their geographic distribution. Virulence frequencies varied considerably among the regions (South America, North America, Europe, Mediterranean, Africa, and Oceania) and within countries. South America, including Mexico and Uruguay, had the highest overall virulence frequency. Isolates from South America were more virulent on cultivars originating from South America. Isolates secured from tetraploid wheats in Syria and Tunisia were more virulent on tetraploid cultivars than on hexaploid cultivars. Isolates from the countries of Brazil, Mexico, Uruguay, Israel, Tunisia, Turkey, Ethiopia, and from the state of Oregon in the United States were virulent on a large number of differential cultivars. Differences in geographic distribution of virulences of *M. graminicola* within regions and countries suggest the feasibility of strategies for germplasm and cultivar deployment for resistance to *M. graminicola*.

Additional key words: Septoria tritici blotch of wheat, speckled leaf blotch of wheat.

Mycosphaerella graminicola (Fuckel) Schroeter, (anamorph, *Septoria tritici* Rob. ex Desm.), the causal agent of Septoria tritici blotch, reduces yield of wheat in several parts of the world (4,14,17). Breeding for resistance has not always been successful in protecting wheat cultivars from the damaging effects of the disease (4,12,15). Morphological traits (plant height and canopy architecture), physiological traits (photoperiod and vernalization requirements), and growth habit all influence expression of symptoms and signs (chlorosis, necrosis, and pycnidial density). Differentiation of cultivar response to the pathogen is usually based on quantitative assessment of symptoms.

In many studies of disease resistance in wheat, mixtures of isolates of *M. graminicola* from diverse sources have been utilized to ensure a representative range of virulence (6,11,19). Some authors have referred to the presence (2,5,6,19), or to the absence (17), of physiological specialization or differential host-pathogen interactions among populations of the pathogen. In some of these studies, substantial cultivar × isolate interactions were reported (18,21). Quantitative inoculation techniques (7,21) made possible a higher degree of accuracy and repeatability in experiments. Some wheat cultivars reported to be resistant in one country have not been resistant in other countries (4,14). Due to the small number of samples from some locations and the small number of locations in some countries, no attempt was made to analyze cultivar × isolate interactions within a country, location, or geographical region. Such interactions may be important in germplasm evaluation, cultivar deployment for disease resistance, and implementation of pest management strategies.

The purpose of the present study was to investigate virulence in isolates of *M. graminicola* collected from countries where the disease has been economically important.

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MATERIALS AND METHODS

The pathogen. Ninety-seven isolates of *M. graminicola* that originated from 22 countries and six geographical regions were evaluated for virulence. The distribution of the forty-six isolates of *M. graminicola* that originated from South America was: Bolivia, one; Brazil, three; Chile, 12; Ecuador, four; Guatemala, one; Mexico (in this study, Mexico is included with South America), 11; Peru. one; and Uruguay, 13. Six isolates were obtained from the United States: Montana, two, and Oregon, four. Five isolates were secured from Europe: Italy, one; The Netherlands, three; and Portugal, one. Twenty-five isolates of *M. graminicola* originated from the Mediterranean basin: Cyprus, one; Israel, five; Syria, three; Tunisia, eight; and Turkey, eight. Twelve isolates were from Africa: Burundi, one; Ethiopia, four; and Kenya, seven. Three isolates came from Australia. Four isolates of *M. graminicola* (three from Israel and one from Montana) of the 97 isolates had been included in a previous study (18). Isolate ISR8036 was a check in all trials.

The host. A set of differentials was assembled which included accessions of spring and winter hexaploid wheats, tetraploid durum wheats, and triticales (× Triticosecale Wittmack) (Table 1). The choice of cultivars was based upon host responses to both *M. graminicola* and *Leptosphaeria nodorum*. Within the cultivar set, some accessions with common parentage were selected: Russian winter wheats and derivatives—Aurora, Bobwhite "S" (Bow "S"), Kavkaz (KVZ), KVZ-K4500 L.A.4, KVZ/7C, and Bezostaya 1; South American wheat cultivars—Colotana, Frontana, H574, PAT 19, Klein, Titan, and ZN-157, all of which may have a common parentage in Frontana. The susceptible hexaploid wheat cultivars Fortuna and Siete Cerros (7C) served as repeated checks in all trials.

Cultivars were randomized among four trays. Each tray included the two susceptible check cultivars, and the check isolate ISR8036 was included in each trial. Sixteen to 25 leaves of each cultivar were inoculated with each isolate of *M. graminicola*. The mean conidial concentration per isolate was adjusted within the range of $1-2\times 10^7$

conidia per milliliter. The necrotic area of seedling leaves was visually estimated for each leaf 21 days after inoculation. In addition, when pycnidia were observed, their presence was recorded. No estimation of percent leaf coverage by pycnidia was performed.

Cultivar classification. The datum used in the analysis was the mean percentage of necrotic area for each cultivar and isolate combination in each trial. Every data point was thus an average of observations of 16–25 seedlings, so that weighted regressions and analyses of variance were required. Since the experiments were conducted over a long time period, the repeatability of the methods over time (e.g., uniformity of inoculation, effect of environmental conditions, etc.) was tested by analyzing the response of the check cultivars within a trial (four trays) and among trials (in each inoculation trial, five to eight isolates were tested along with the check isolate). Lack of repeatability would not have allowed further analysis.

The ultimate goal of the statistical analysis of the data was to determine a "cutpoint" between resistant and susceptible host response categories for further genetic analysis of virulence frequencies.

For every isolate, comparisons of each cultivar to the susceptible check cultivar Siete Cerros were made by using analysis of variance. Multiple comparisons with Siete Cerros indicated which cultivars were significantly more resistant or more susceptible than Siete Cerros for that particular isolate.

The weighted means for each cultivar, obtained from the analysis of variance, were then analyzed by using cluster analysis program KM of the BMDP statistical software (3). The purpose of the cluster analysis was to systematically group cultivars with similar responses as measured by the Euclidean distance between the responses. The number of clusters was specified to be six, corresponding to the arbitrary host response classes: very resistant, resistant, moderately resistant, moderately susceptible, susceptible, and very susceptible. Although the genetic analysis required only two categories, six clusters were specified because the use of two clusters would have been too sensitive to the presence (or absence) of highly resistant and highly susceptible cultivars, and would not have sufficiently differentiated cultivars with an intermediate host response. From the results of the cluster analysis, the median between the two moderate groups was used to calculate an initial cutpoint between resistant and susceptible categories.

Finally, two-way analysis of variance with cultivars and isolates, including their interaction, was used to compute a standard error to be associated with the initial cutpoint. Although the sample sizes were unequal, they followed a nearly proportional pattern so that analysis of variance with proportional frequencies was employed for the analysis (10). The standard error was obtained as the square root of the residual mean square divided by the mean number of plants per cultivars per trial. Since this standard error is an indication of the difference needed for cultivars to be significantly different, the final cutpoint between resistant and susceptible response could reasonably be chosen anywhere in the interval (initial cut-point) $\pm$ the sandard error). The lowest acceptable number in the interval was chosen as the final cutpoint to ensure exclusion of moderately susceptible cultivars from the resistant response category.

Once the cutpoint was determined, the isolate $\times$ cultivar matrix was reformulated in terms of the categories "resistant" and "susceptible." The estimation of the minimum number of genes active in the host-parasite interaction was then performed by using the computer programs DIFFER and GENEALOGY (8,9) which were developed as part of a joint project between the International Maize and Wheat Improvement Center (CIMMYT), the Research Institute of Plant Protection (IPO), and the Department of Phytopathology of the Agricultural University in Wageningen, The Netherlands. DIFFER (8,9) searches an isolate $\times$ cultivar reaction matrix for cultivars that can be used as differentials, the goal being to identify the smallest possible set of differentials. The program can be used either to analyze data for any group of cultivars and produce a complete set of differentials, or it can start with a given set, test to see if that set fully differentiates the isolates, and (if it will not) indicate additional differentials. The algorithm used by DIFFER (8) leads to the unique minimum set whenever all combinations of resistance genes are present, this minimum set being the cultivars with monogenic resistance. When not all combinations of resistance genes are present, no algorithm exists that can be guaranteed to find the minimum set within a reasonable amount of time. In this situation, DIFFER has been shown to indicate the minimum number of differential cultivars if the total number being tested is less than 40. However, if the total number of cultivars is larger, then DIFFER does not necessarily lead to the minimum number (8). GENEALOGY (9) performs a Person differential interaction. When, for a certain number of genes, all combinations of resistance genes and virulence genes are present, then the gene-for-gene theory predicts all host-pathogen interactions and leads to Person's complete or ideal differential interaction (13,16). Furthermore, the existence of such an interaction furnishes proof that the gene-for-gene theory holds for the genes involved (16). GENEALOGY also analyzes incomplete reaction matrices by identifying, within the total matrix, smaller complete matrices which conform to a complete Person differential interaction and hence to a gene-for-gene relationship among a

TABLE 1. Wheat and triticale cultivars used to assess virulence frequencies of *Mycosphaerella graminicola* and *Leptosphaeria nodorum*

Cultivars	Source
Spring hexaploid wheats	
Anza CI 15284	USA
Bet Lehem (Volcani 393-676) (H574-1-2-6/Lakhish 212)	Israel
Bobwhite "S" Aurora × Kalyansona-Bluebird/Woodpecker "S" (CM 33203-K-9M-2Y-1M-1Y-1M-0Y)	Mexico
Colotana CI 13556	Brazil
Fortuna CI 13596	USA
Frontana CI 12470	Brazil
H574-1-2-6 (Purdue Univ. Selection)	Brazil
Kavkaz(KVZ)-K4500.L.A.4. SWO176-3M-1Y-10Y-1Y-1M-0Y-0Ptz (CIMMYT, 12 ISEPTON #99)	Mexico
KVZ/7C SWM4064-6Y-4M-3Y-1M-1Y-3M-0Y-0Ptz-0Y-0Ptz (CIMMYT, 12 ISEPTON 107)	Mexico
Lakhish Yaktana//Norin 10/Brevor/3/Florence Aurore	Israel
Miriam Chapingo 53//Norin 10/Brevor/3/Yaqui 54/2 Merav	Israel
MTN 72-10-9 Nainari 60/P1341614)	USA
Olaf CI 15930	USA
PAT 19 (S12/VS-IWRN 60-218/2/Deb)	Brazil
Siete Cerros (7C) CI 14493	Mexico
Titan CI 12615	Argentina
Weibull 7389	Sweden
ZN-157 (ISWRN 1970 56, 70M-1018-19)	Chile
Winter hexaploid wheats	
Aurora PI 167407	USSR
Bezostaya 1 PI 345685	USSR
DQ-12 (Yamhill/Hyslop Selection)	USA
EA-7 (*Aegilops ventricosa* / *T. persicum* //*Marne) VPM 1.1.1.2.R4	France
JCR 979 CI 16906	Chile
KVZ (Lutescens 314 H 147/Bezostaya 1)	USSR
MTN 71-8-11 (CI 12752/CI 13554)	USA
Red Chief CI 12109	USA
Yamhill CI 14563	USA
81 UWWMN 2024 (Palmaress/(TF1035)Fauereau/4/Martin/K3/Hohen 77/Oro/2/Capelle/Magdalena) Selection 1	France
81 UWWMN 2095 (Maris Huntsman//VPM/Moisson) bulk PV 63-6	France
Tetraploid wheats	
Etit 38 (land variety)	Israel
Nursit 163 (land variety)	Israel
Ward CI 15892	USA
Volcani 447	Israel
Zenati Bouteille	Tunisia
Triticale	
Beagle UM "S"-Tcl Bulk ×1530A-12M-5N-1M-0Y	Mexico
DU075 (M_2A_2 ×8266-B-6Y-1M-2Y-0Y)	Tunisia
Mapache M_2A ×2802-F-12M-1N-1M-0Y	Mexico

certain subset of the genes. The program attempts to identify these smaller matrices by using a minimum total number of genes. If a sufficient number of smaller matrices are identified, they can be linked in such a way that all resistance and virulence genes can be determined (8,9). It should be emphasized that the program gives only an estimate of the resistance and virulence components; therefore, the specific genes identified are hypothetical until actually verified by genetic tests.

We have made two assumptions for the purpose of our analysis: that a gene-for-gene system is operative in this host-pathogen system; and that the program correctly assigns genes that could later be used to calculate their frequency in a population.

RESULTS

Methodology. Percent necrotic leaf area observed on the susceptible check cultivar Siete Cerros did not differ significantly among the four sets of test plants inoculated at the same time in each trial ($F = 1.6$). The F value is a result of an analysis of variance of percent necrosis of about 20 plants of Siete Cerros per tray among the four trays. This means that the differences among the four trays were not significant. There were, however, significant differences in the mean necrotic leaf area observed on Siete Cerros among trials that were conducted at different times ($F = 5.4$). The F value here states the difference in percent coverage of Siete Cerros among the trials. Considering the large number of trials (30) and plants recorded (3,000), such differences were expected.

Necrosis-pycnidia relationships. The relationships between percent necrotic leaf area and the number of entries that developed pycnidia over a total of 50 entries and 97 isolates of *M. graminicola* is presented in Figs. 1 and 2, respectively. This analysis was conducted on the original 50 cultivars. In later analyses, we used only 35 cultivars since there were missing values (some cultivars were introduced at later stages, while for some cultivars, the seed supply was depleted). Some wheat cultivars exhibited few symptoms (necrosis and pycnidia), while others (Lakhish, Siete Cerros, and Wampum) exhibited large areas of necrosis and abundant pycnidia. The spring wheat cultivar Fortuna had large necrotic leaf areas with sparse pycnidia, while the spring wheats ZN-157, Miriam, MT 72-10-9, and the winter wheats JCR-979, 81UWWMN2095, EA-7, and TAM105 had low necrotic leaf area but abundant pycnidia within necrotic areas. The low correlation coefficient ($R = 0.36$) between necrosis over isolates and pycnidia over isolates indicates that some of the 97 isolates of *M. graminicola* induced necrosis in the host, but formed few pycnidia. Isolates of *M. graminicola* from Chile and Mexico induced much necrosis but few pycnidia in the tested cultivars under our experimental conditions. The wheat cultivars Bezostaya 1, Colotana, H574, KVZ, Lakhish, Miriam, N.163., Olaf, Titan, and Zenati Bouteille, which had been used in a previous study (18), responded similarly in both necrotic leaf area and production of pycnidia in response to the four check isolates of *M. graminicola* used in both studies.

TABLE 2. Analysis of variance of mean necrotic leaf area of 97 isolates of *Mycosphaerella graminicola* and 35 wheat and triticale accessions

Source	Degrees of freedom	Mean square	Prob. F
Cultivars	34	196,510.9	<0.001
Isolates	96	369,357.4	<0.001
Cultivars × isolates	3,264	1,514.4	<0.001
Residual	1,904	956.5	

Host-parasite interaction. Analysis of variance of the Isolate × cultivar matrix (97 isolates × 35 cultivars) gave a significant interaction as well as highly significant main effects (Table 2). The interaction mean square, though significant, appears to be quite small compared to the isolate and cultivar effects. From cluster analysis of cultivar responses, the initial cutpoint between the two intermediate clusters (moderately low and moderately high) equals 23.4% necrosis (Table 3). The final cutpoint between resistant and susceptible response classes was chosen as 23.4 − 6.8 = 16.6% necrotic leaf area. The isolate × cultivar matrix was then reformed in terms of the two response classes, resistant and susceptible, for further analysis.

The gene-for-gene analysis identified 28 hypothetical different genes in the 97-isolate × 35-cultivar matrix. Seven hypothetical genes were indicated in the spring hexaploid wheat KVZ-K4500 L.A.4, and five hypothetical genes in the winter hexaploid wheat

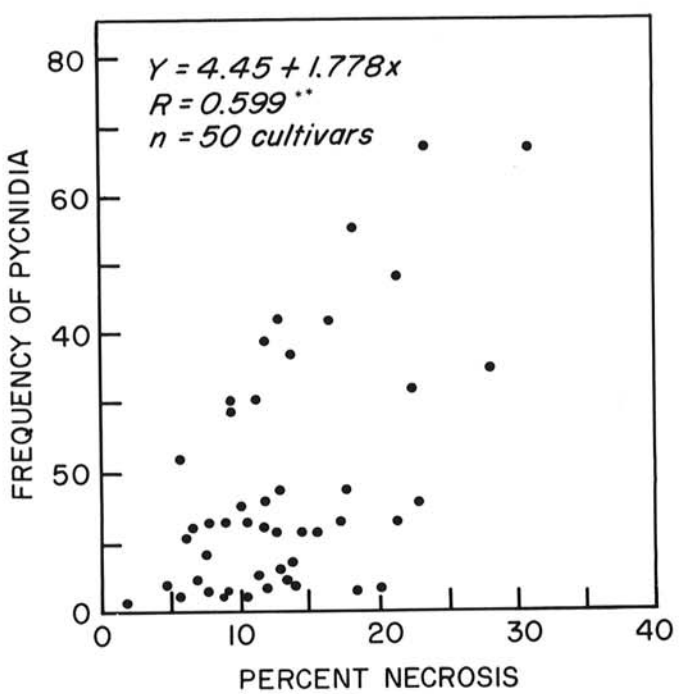

Fig. 1. The relationship between percent necrotic leaf area and frequency of occurrence of pycnidia of *Mycosphaerella graminicola* (regressed through means of 50 wheat and triticale cultivars).

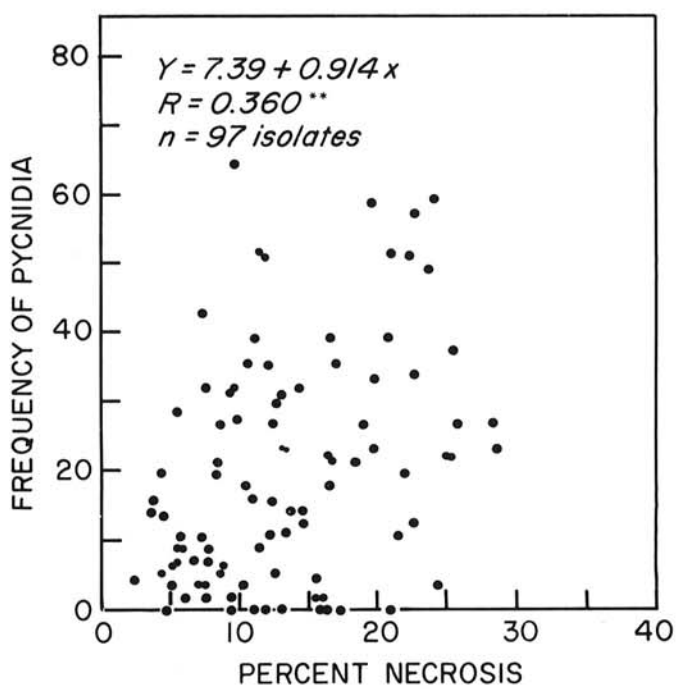

Fig. 2. The relationship between percent necrotic leaf area and frequency of occurrence of pycnidia of *Mycosphaerella graminicola* (regressed through means of 97 isolates of *Mycosphaerella graminicola*).

Red Chief (Table 4). The triticale accession DU-75 (M_2A_2× 8266-B-6Y-1M-2Y-OY) was resistant to all 97 isolates of *M. graminicola*, and thus it was not possible to assign any specific genes for resistance to it. The hypothetical genes for virulence were utilized to estimate the frequency of virulence genes. The relationship between the number of hypothetical corresponding genes and percent leaf necrosis in the host are presented in Fig. 3.

Virulence frequencies. The frequency and distribution of hypothetical genes derived from the 97 × 35 matrix analysis are presented in Table 5. The hypothetical genes for virulence were assigned the notation of the corresponding cultivar. The relative frequency of specific virulence genes within a geographic region was calculated as a fraction of the isolates expressing this virulence gene(s) from the overall number of isolates used in the study. The hypothetical genes for virulence were grouped according to classes of host (hexaploid wheat, tetraploid wheat, and triticale), and according to common parentage within a class. The frequency of the five genes for virulence (CLT, TTN, FTN, 157, and PAT) that are compatible with cultivars that may have Frontana germplasm (Colotana, Titan, Frontana, ZN-157, and PAT19), differ markedly among the six geographical regions. In South America, the frequency of virulence of the hypothetical gene 157 associated with the Chilean cultivar ZN-157 was 8.2, while the frequency in the Mediterranean area was 4.1. The virulence on Titan, a cultivar that has expressed rather high levels of resistance to populations of *M. graminicola* in many programs throughout the world (17), was rather high in South America. Also in South America, the frequencies of virulence on the Russian winter wheats Bezostaya 1 and KVZ were higher than the frequency of virulence on Aurora which is a sib line of KVZ.

The frequency of virulence on the CIMMYT line Bobwhite "S" was low. The frequency of virulence on the Israeli wheat cultivar Lakhish was high in the Mediterranean region. The frequency of virulence on the tetraploid wheat Zenati Bouteille was high in South America and in the Mediterranean where it originated but low in all other regions. Virulence on the land tetraploid wheat cultivar N.163 was rather low in most regions other than the Mediterranean. The frequency of certain virulences and the overall performance of the cultivars with which they are associated corresponds well (Table 4). No attempt was made here to calculate the frequencies of virulence on cultivars possessing resistance controlled by oligogenes (Bet Lehem, KVZ-K4500 L.A.4, Red Chief, JCR-979, and Mapache).

TABLE 3. Cluster analysis[a] of six cultivar response classes in a 97-isolate *Mycosphaerella graminicola*) × 48-cultivar matrix

Response class	Number of evaluations	Necrosis (mean %)	Frequency
Very resistant	1,832	3.1	39.5
Resistant	1,148	10.6	24.7
Moderately resistant	784	18.9[b]	16.9
Moderately susceptible	508	27.9[b]	10.9
Susceptible	274	37.6	5.9
Very susceptible	94	51.4	2.1
Totals	4,640	13.4	100.0

[a]The analysis forms clusters of variables which are based on a measure of association (similarity) between the variables, or of distance (difference) between them.
[b]Median between the intermediate response classes = 23.4%.

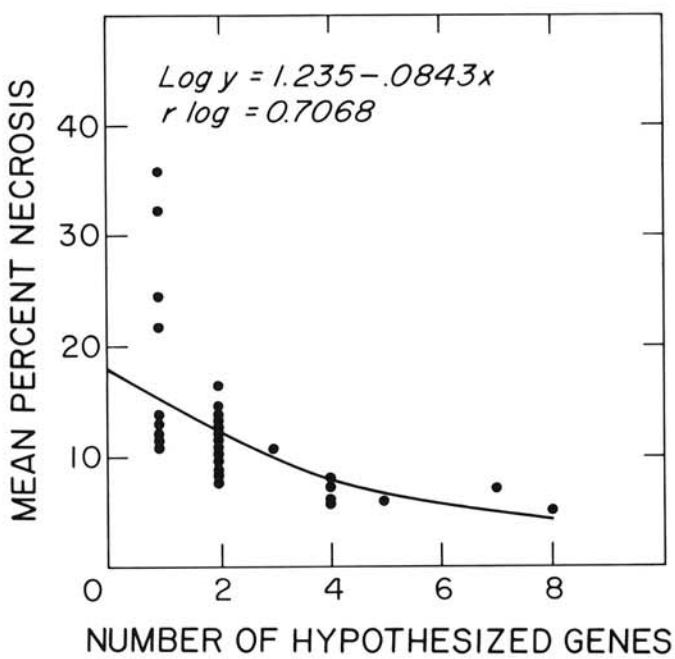

Fig. 3. The relationship between the number of hypothetical corresponding genes for resistance to *M. graminicola* and percent leaf necrosis in the host.

TABLE 4. Mean cultivar response to 97 isolates of *Mycosphaerella graminicola* secured from 22 countries

Cultivar	Necrosis[a] (mean %)	Occurrence of pycnidia	Hypothetical genes (no.)
Spring hexaploid wheats			
Bet Lehem (V393-676)	4.7 ± 0.9[b]	4.0[c]	8[d]
Bobwhite "S"	5.7 ± 0.9	2.3	4
Kavkaz (KVZ)-K4500 L.A.4	6.9 ± 0.9	4.6	7
Titan	9.1 ± 1.4	1.5	2
ZN-157	9.5 ± 1.4	29.0	2
Frontana	10.2 ± 1.6	15.3	3
H574-1-2-6	10.5 ± 1.3	2.3	1
Colotana	11.9 ± 1.6	12.2	1
MTN 72-10-9	11.9 ± 1.6	38.9	2
Miriam	13.0 ± 1.9	42.0	1
KVZ/7C	15.7 ± 1.9	11.5	2
Lakhish (susceptible check)	21.2 ± 2.7	73.3	1
Siete Cerros (susc. check)	23.7 ± 2.7	73.3	1
Fortuna (susceptible check)	35.0 ± 3.8	35.1	1
Winter hexaploid wheats			
Red Chief	5.6 ± 0.8	2.3	5
JCR-979	5.8 ± 0.9	22.1	4
Weibull 7389	7.8 ± 1.3	13.0	2
81UWWMN 2024	9.2 ± 1.3	3.1	2
81UWWMN 2095	9.4 ± 1.4	30.5	2
Aurora	10.1 ± 1.2	15.3	2
MTN 71-8-10	10.6 ± 1.4	13.0	2
EA-7	11.2 ± 1.4	30.6	2
KVZ	11.9 ± 1.4	16.0	2
DQ-12	13.0 ± 1.8	17.6	2
Yamhill	13.0 ± 1.7	6.1	2
Bezostaya 1	14.3 ± 1.7	11.5	2
Wampum (susceptible check)	31.1 ± 3.5	66.4	–
Tetraploid wheats			
Volcani 447	7.7 ± 1.1	8.4	4
Nursit 163	8.8 ± 1.1	2.3	2
Etit 38	11.3 ± 1.4	5.3	1
Zenati Bouteille	12.0 ± 1.7	3.8	1
Ward	12.7 ± 1.6	11.5	2
Rolette (moderately susceptible)	18.4 ± 2.2	3.1	–
Triticale			
DU-75	1.9 ± 0.3	1.5	+
Mapache	6.7 ± 1.1	12.2	4
Beagle	7.6 ± 1.0	3.1	2

[a]Calculated cutpoint between resistant and susceptible response at 16.5% necrosis.
[b]Standard error.
[c]Occurrence of pycnidia on 97 isolates over 35 experiments.
[d]Number of hypothetical genes for resistance estimated by DIFFER and GENEALOGY computer programs. Minus (−) = not included in analysis; plus (+) = no differential interaction, resistant to all isolates.

The frequency of hypothetical virulence components was further divided according to wheat class, or parentage. The frequency of virulence on Frontana and cultivars derived from it was high in South America where these wheats originated, but also was relatively high in Africa and Australia (Fig. 4). Within the South American region, the frequency of virulence was low in Chile and Ecuador and high in Mexico and Uruguay. The overall virulence frequency in the United States, Europe, and the Mediterranean region was less than 20% although the Israeli isolates that were tested possess a relatively high frequency of virulence to the Frontana-derived cultivars as a group.

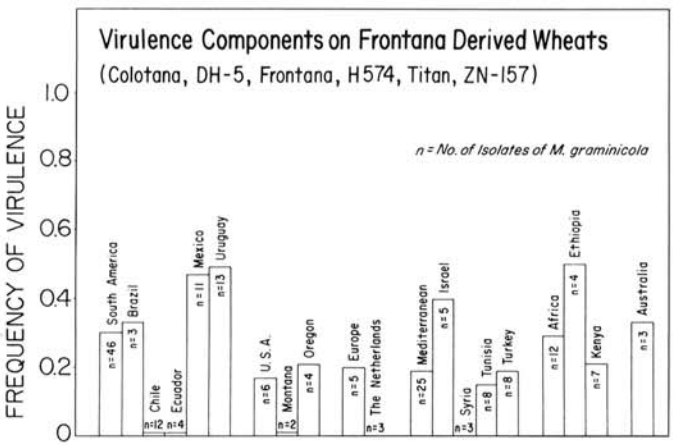

Fig. 4. The frequency of hypothetical virulence factors (genes) of *Mycosphaerella graminicola* on Frontana-derived wheats (cultivars Colotana, Frontana, H574, PAT19, Titan, and ZN-157).

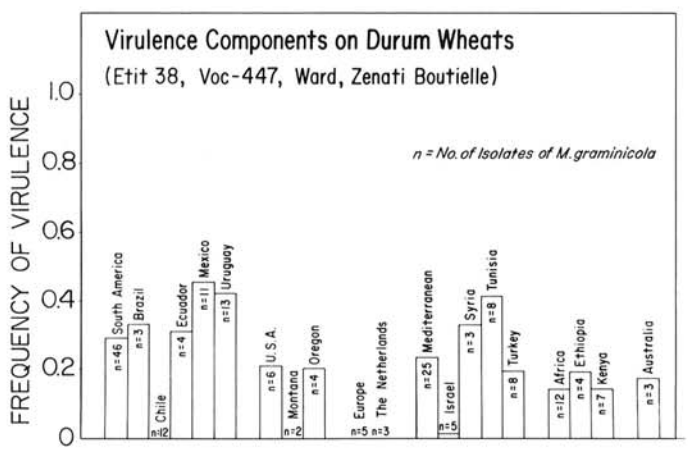

Fig. 5. The frequency of hypothetical virulence factors (genes) of *Mycosphaerella graminicola* on tetraploid wheats (cultivars Etit 38, Volcani 447, Ward, and Zenati Bouteille).

M. graminicola from Mexico and Uruguay possessed a high frequency of virulence to tetraploid wheats, whereas the U.S., European, African, and Australian isolates possessed a low level of virulence (Fig. 5). In the Mediterranean region, isolates from Syria and Tunisia possessed a high frequency of virulence to the tetraploid wheats used in the study. The low frequency of virulence among the five Israeli isolates of *M. graminicola* was because all five were selected previously and were known test isolates, not randomly selected. It is of interest to note that in South America only isolates from Uruguay possessed a high frequency of virulence to Russian winter wheats and their derivatives, whereas United States' isolates of relatively low overall virulence contained a very high level of virulence to the Russian winter wheats (Fig. 6). On KVZ, the relative virulence of isolates of *M. graminicola* from Uruguay, Montana, and Oregon was much higher than the virulence of isolates from the rest of the regions and countries (Fig. 7). In cultures from Chile, Ecuador, Syria, Tunisia, Turkey, and Kenya, virulence on KVZ was infrequent.

The overall summary of virulence frequency of *M. graminicola* over geographical regions and the countries within regions is presented in Fig. 8.

The relative virulence per isolate of *M. graminicola* was calculated by dividing the total number of susceptible host reactions per region or country by the corresponding number of isolates. Within the South American region, isolates from Mexico and Uruguay possessed the most combinations of virulent factors. The four isolates of *M. graminicola* secured from the Oregon State University, Hyslop Farm, were highly virulent, especially on the Russian winter wheat derivatives. The European isolates (from Italy, Portugal, and The Netherlands) possessed a moderate to low frequency of virulence. The Mediterranean isolates were of moderate virulence, with the Israeli isolates possessing the highest

Fig. 6. The frequency of hypothetical virulence factors (genes) of *Mycosphaerella graminicola* on Russian winter wheat derivative cultivars Aurora, Bezostaya 1, and Kavkaz.

TABLE 5. Frequency of hypothetical virulence factors (genes) in *Mycosphaerella graminicola* derived from a 97-isolate × 35-cultivar matrix analysis

Region	Isolates (no.)	Frequency of hypothetical virulence genes[a]																		
		CLT[b]	TTN	FTN	157	PAT	BEZ	KVZ	AUR	BOW	LAK	MIR	OLAF	YMH	DQ12	Z.B	N163	V447	ETIT	WARD	BGLE
S. America	46	16.5	14.4	15.5	8.2	20.6	14.4	14.4	6.2	5.1	25.8	17.5	24.7	16.5	16.5	17.5	3.1	11.3	12.4	15.5	10.3
N. America	6	1.0	0.0	2.1	1.0	2.1	4.1	3.1	3.1	0.0	4.1	2.1	5.1	1.0	3.1	0.0	0.0	0.0	1.0	0.0	2.1
Europe	5	0.0	1.0	1.0	1.0	2.1	2.1	2.1	2.1	0.0	3.1	1.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mediterranean	25	6.2	2.1	2.1	4.1	10.3	8.2	4.1	3.1	0.0	12.4	9.3	9.3	9.3	5.1	10.3	4.1	1.0	4.1	8.2	0.0
Africa	12	4.1	2.1	2.1	3.1	7.2	5.1	2.1	2.1	1.0	8.2	3.1	5.1	2.1	4.1	2.1	4.1	0.0	2.1	2.1	1.0
Australia	3	3.1	1.0	1.0	0.0	1.0	1.0	0.0	0.0	1.0	1.0	2.1	10.0	1.0	0.0	1.0	0.0	0.0	0.0	1.0	1.0
Overall	97	30.9	20.6	23.7	18.6	43.3	35.0	25.8	16.5	7.2	54.6	35.0	47.4	29.9	28.9	31.9	11.3	12.4	19.6	26.8	14.4

[a] Hypothetical virulence components (genes) of *Mycosphaerella graminicola* were estimated by DIFFER and GENEAOLGY computer programs (9).
[b] Virulence genes designated by name of matching cultivars: CLT = Colotana CI 13556, TTN = Titan CI 12615, FTN = Frontana CI 12470, 157 = ZN-157, PAT = PAT 19, BEZ = Bezostaya 1, KVZ = Kavkaz, AUR = Aurora, BOW = Bobwhite "S", LAK = Lakhish, MIR = Miriam, OLAF, YMH = Yamhill CI 14563, DQ12 = DQ-12 (Yamhill/Hyslop sel.), Z.B = Zenati Bouteille (tetraploid), N163 (tetraploid), V447 (tetraploid), ETIT = Etit 38 (tetrapoloid), WARD = Ward (tetraploid) CI 15892, BGLE = Beagle (triticale).

virulence in this region. *M. graminicola* from Africa and Australia can be classified as moderately virulent, while isolates from Chile, Ecuador, Montana, The Netherlands, and Syria exhibited an overall low frequency of virulence.

DISCUSSION

The presence of significant isolate × cultivar interaction in the combined statistical analysis, though small relative to the main effects, was interpreted as an indication that the resistance to and virulence of *M. graminicola* in the interaction is specific in its effects. Nevertheless, the relative host response range and cultivar ranking within this range was maintained in the same order. Highly susceptible and resistant cultivars continued to reflect their extreme positions under different environmental conditions. The indication that some resistance and virulence are specific in their effects further led to the supposition, though experimentally unproven, that a gene-for-gene relationship is operative in Septoria tritici blotch of wheat. The finding of as many as 28 different hypothetical genes in the 97-isolate × 35-cultivar matrix may be due in part to the size of the matrix. When a four-isolate × 10-cultivar matrix, that had been used in a previous study (21), was analyzed in the manner of this study, the number of hypothetical genes corresponded to that reported previously.

The relationship between the number of hypothesized genes and mean percent necrosis fitted better a linear regression on the logarithm of the necrosis, rather than a simple linear regression. This suggests that the contribution of genes for resistance to disease expression is not a simple arithmetic additive effect as suggested by Parlevliet's analysis (12) of Ahn and Ou's (1) data. The quantitative expression of symptoms may also be explained in terms of interaction between genes rather than simple additive effect (20).

The hypothetical assigned genes for virulence were used to calculate frequencies of virulence genes of *M. graminicola* within and among geographical regions. Due to the small number of isolates and locations from which they were secured, this limited survey should be regarded as an insight into virulence patterns and their geographical distribution. The regions and countries varied considerably in the relative virulence frequencies, with South America having the highest virulence level. *M. graminicola* from Mexico and Uruguay was the most virulent while Chile had the least virulent population.

Septoria tritici blotch is an important disease in Chile, with significant outbreaks of the disease occurring frequently. In our tests, the isolates of *M. graminicola* from Chile appeared to be unusually low in virulence frequency. This could have been because the test cultivars we used had high levels of resistance to the Chilean isolates or that special environmental conditions contribute to the occurrence of the disease in Chile. Isolates of *M. graminicola* from South America were highly virulent on South American germplasm, with the exception of the Chilean isolates. Distinct differentiation was obtained between the Russian winter wheat cultivars. Aurora, Bezostaya 1, and KVZ each were assigned a single different gene for resistance. No differentiation in host response had been reported previously between Bezostaya 1 and KVZ. The overall frequency of virulence to Aurora was significantly less than virulence to KVZ, even though both derive from the same cross. The wheat cultivar Bobwhite "S" which is a derivative of Aurora and the CIMMYT line KVZ-K4500 L.A.4, expressed the highest overall level of resistance, with four and seven hypothetical genes, respectively. Virulence to KVZ was abundant among isolates of *M. graminicola* secured from Montana and Oregon, making the utilization of KVZ germplasm in those states ineffective. The highest level of seedling resistance among the spring hexaploid wheats was expressed in the recently released Septoria tritici blotch-resistant commercial wheat cultivar Bet Lehem, which has eight hypothetical genes for resistance. However, virulence on this wheat cultivar in Israel is known (Z. Eyal and E. Levi, *unpublished*). Cultivar Bet Lehem is susceptible to postanthesis artificial inoculation, resulting in moderate to high numbers of pycnidia (Z. Eyal, *unpublished*).

Isolates of *M. graminicola* secured from Tunisia and Syria all originated from tetraploid wheats, and were virulent on the tetraploid cultivars used in these studies. However, the Syrian isolates expressed a low level of pathogenicity on most hexaploid wheat and triticale accessions.

Several areas having populations of *M. graminicola* with particularly high virulence frequencies were identified. These covered the entire resistance spectrum of the 35 cultivars used in the study. Areas identified were: Brazil, Mexico, and Uruguay in South America; Oregon in the U.S.; Israel, Tunisia, and Turkey in the Mediterranean region; and Ethiopia in Africa. Severe Septoria tritici blotch outbreaks have occurred in many of these countries (13,14).

The wheat and triticale cultivars that expressed high levels of seedling resistance can be used as a preliminary differential cultivar set to be distributed in countries where Septoria tritici blotch is expected to be a problem. If properly used, these cultivars can, over a period of time, be used to identify virulence and trends of virulence changes. Some lines may be used as germplasm sources in breeding for resistance.

A knowledge of host response to specific pathogen biotypes within a population, or to whole populations, may thus aid in identifying effective and diverse germplasm, and consequently in designing proper breeding strategies. The differences in the geographic distribution of virulences of *M. graminicola* within regions and countries strongly suggest the feasibility of germplasm deployment strategies.

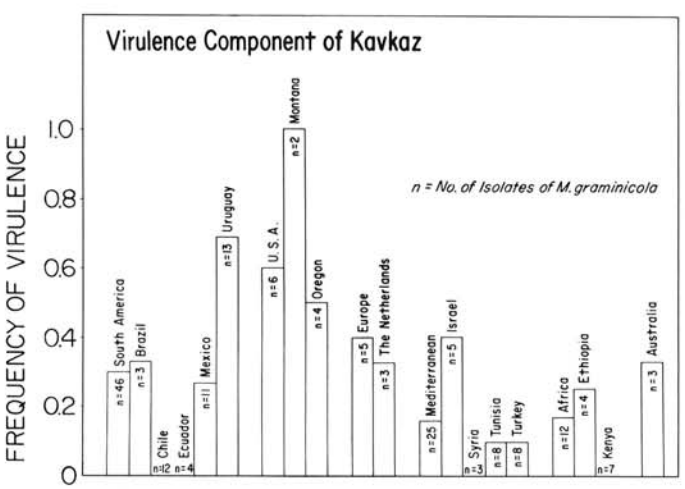

Fig. 7. The frequency of hypothetical virulence factors (genes) of *Mycosphaerella graminicola* on the winter hexaploid wheat cultivar Kavkaz.

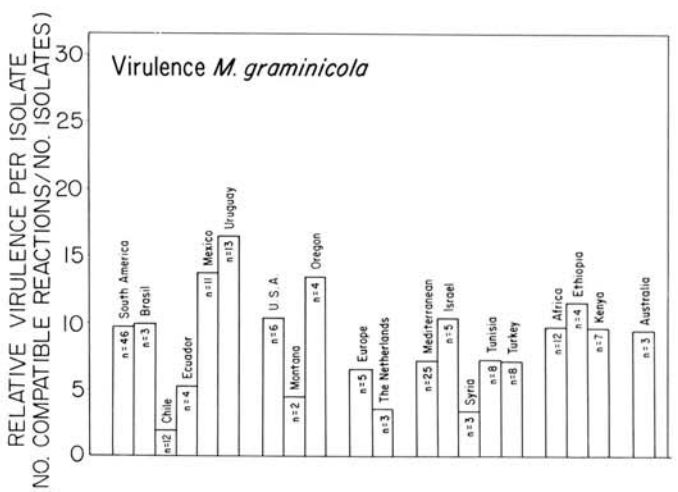

Fig. 8. The overall relative frequency of hypothetical virulence factors (genes) of *Mycosphaerella graminicola* estimated from a 97-isolate × 35-cultivar matrix analysis.

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