Virulence of Oat Crown Rust in Mexico

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ABSTRACT

Leonard, K. J., Huerta-Espino, J., and Salmeron, J. J. 2005. Virulence of oat crown rust in Mexico. Plant Dis. 89:941-948.

Virulence of isolates of *Puccinia coronata* collected during 1992 to 1998 from Sonora, Chihuahua, Nuevo Leon, and five states in Central Mexico were compared on a set of 27 differential oat (*Avena sativa*) lines with different genes for race-specific resistance. Frequencies of virulence and the presence of specific pathogenic races were compared among the four regions of Mexico and between Mexico and the adjoining states of California and Texas in the United States. The *P. coronata* populations in Mexico were highly diverse even though the sexual stage of the fungus is not known to occur there. Overall virulence frequencies were most similar between Chihuahua and Nuevo Leon, but there were more races in common between Central Mexico and Chihuahua than between any other pair of regions of Mexico. No races found in Sonora were found in other regions of Mexico. More races found in Texas also occurred in Nuevo Leon than in any other region of Mexico. Mean virulence complexity was lowest in isolates from central Mexico; greatest in Sonora, California, and Texas; and intermediate in Chihuahua and Nuevo Leon. Significant ($P < 0.05$) associations of virulences occurred for 24 pairs of virulence genes in at least three of the four regions of Mexico. Virulences to 19 of the 24 pairs were also significantly associated in Texas; virulences to 13 were also significantly associated in California.

Crown rust, caused by *Puccinia coronata,* is an important disease of oat (*Avena sativa*) in Mexico, especially where relative humidity is high during the growing season. Oat is grown in Mexico mainly for forage or hay; only about 10% is harvested for grain. North central Mexico (Chihuahua, Durango, and Zacatecas) is the most important oat growing region with more than 80% of the total Mexican oat production (700,000 to 800,000 ha); much of it is used as forage for dairy farming. In central Mexico, oat is grown mainly on small farms, where it is cut and used for animal forage as needed. Oat crops may be planted nearly year-round in Mexico. In north central Mexico, most of the crop is grown under dry land conditions and is planted when summer rains begin in July and harvested in November. Oat is also grown under irrigation as a winter crop planted in January and harvested in May in Chihuahua. In the low elevations in Nuevo Leon and Coahuila along the Texas border, winter oat crops are planted as early as September when the summer oat crop is still in the fields in Chihuahua as well as in central Mexico. In the high plateau of central Mexico, oat is planted during May and harvested in November. Crown rust is a serious disease of oat in the lowland areas of northeast Mexico and in the high plateau region of central Mexico. Crown rust also is severe on wild oat, *Avena fatua*, in the Yaqui Valley of Sonora, where *A. fatua* is the most common weed in fields of fall-sown, irrigated wheat. The overlapping seasons for oat production at high and low elevations in Mexico as well as the presence of volunteer oat plants in fall and winter planting areas provide a green bridge for *P. coronata* to survive throughout the year in the uredinial stage in Mexico.

A breeding program to develop new oat cultivars was initiated in 1959 with the introduction of new germ plasm from the United States. Two cultivars from that program, 'Cuauhtémoc' and 'Chihuahua', were released in 1967 and are still among the most popular oat cultivars in Mexico (12). Both 'Cuauhtémoc' and 'Chihuahua' are now regarded as highly susceptible to crown rust in Mexico.

A variety of genes for race-specific resistance to crown rust have been transferred to cultivated oat, *A. sativa*, from accessions of wild *A. sterilis* from natural populations in the Mediterranean regions of North Africa, southern Europe, Turkey, and especially Israel (3,5,11,13,14). Virulence to many of these resistance genes is known to occur in populations of *P. coronata* in Canada (1), the United States (7), and Brazil and Uruguay (9), but there have been few studies of the effectiveness of these genes against populations of *P. coronata* indigenous to Mexico. The objectives of this study were (i) to determine levels of virulence in the *P. coronata* populations in Mexico to the resistance genes from *A. sterilis*, (ii) to compare the virulence of isolates from several regions of Mexico as an indication of population structure of *P. coronata* in Mexico, (iii) to compare races and virulence frequencies of *P. coronata* from adjacent states of Texas and California with those in Mexico as an indication of possible exchange of inoculum between the United States and Mexico, and (iv) to identify pairs of virulences that show strong positive or negative associations in the populations of *P. coronata* in Mexico and to determine whether they are similar to the virulence associations found in *P. coronata* from spring and fall sown oats in the United States and from *A. sterilis* in Israel (8).

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Accepted for publication 27 April 2005.

DOI: 10.1094/PD-89-0941

Table 1. Numbers of isolates of *Puccinia coronata* collected from cultivated and wild oats in regions of Mexico in 1992 to 1998

Year	Central Mexico[a]	Sonora	Chihuahua	Nuevo Leon[b]
1992	8	0	0	0
1993	1	3	0	4
1994	9	0	14	0
1995	0	8	0	0
1996	0	8	0	35
1997	10	0	32	20
1998	1	0	5	0
Total	29	19	51	59

[a] Includes collections from the states of Jalisco, Mexico, Michoacan, Oaxaca, Queretaro, and Tlaxcala.
[b] Includes one isolate each from Coahuila and Tamaulipas.

MATERIALS AND METHODS

Over the years 1992 to 1998, 158 isolates of *P. coronata* were obtained from collections of infected leaves of cultivated and wild oat in Mexico (Table 1). Collections were made in four regions of Mexico (Fig. 1). There were 29 isolates from the states of Jalisco, Mexico, Michoacan, Oaxaca, Queretaro, and Tlaxcala in central Mexico; 19 isolates from Sonora, 51 isolates from Chihuahua, and 57 isolates from Nuevo Leon plus one each from the adjacent states of Coahuila and Tamaulipas. Isolates from Sonora were collected from *A. fatua*, which is a common weed in wheat fields. Isolates from other states were predominantly from fields and nurseries of cultivated *A. sativa*. Isolates from Chihuahua and from central Mexico were collected primarily in October and November, although five of the isolates from Chihuahua were collected in January. Isolates from Nuevo Leon and Sonora were collected in March and April.

The samples were sent to the USDA, ARS Cereal Disease Laboratory, St. Paul, MN, where urediniospores were collected from each sample by suction into cyclone spore collectors. Urediniospores from each sample were suspended in lightweight mineral oil and used to inoculate 7- to 9-day-old seedlings of the oat cultivar Marvellous, which is susceptible to all known races of *P. coronata*. The seedlings were sprayed with the spore suspension, left for 30 min to allow the oil to evaporate, then incubated overnight in a dew chamber at 18°C. The following morning, they were returned to the greenhouse (18 to 28°C). After 7 to 9 days, when uredinia appeared, the seedlings inoculated with each urediniospore sample were trimmed to leave one leaf with a single uredinium. Urediniospores scattered on the surfaces of the trimmed leaves were removed as potential sources of contamination by placing the trimmed seedlings in a dew chamber for 2 to 3 h to promote germination of urediniospores not contained in the single remaining uredinium. Then the seedlings were removed from the dew chamber and allowed to dry before the germinating urediniospores could re-infect the plants. The seedlings bearing single uredinia were then moved to polyethylene isolation cells in the greenhouse for several days to allow further sporulation in the isolated single uredinia. Urediniospores were then collected to establish one single-uredinial isolate per sample of rusted oat leaves.

Each single-uredinial isolate was increased through one uredinial generation on 'Marvellous' oat seedlings in isolation cells. Urediniospores of each single-uredinial isolate were collected, equilibrated to 20 to 30% relative humidity (RH), and stored dry at –50°C if not used in virulence tests within 2 weeks of collection. For shorter times, isolates were stored at 20% RH at 4°C. Each isolate was tested for virulence on a set of 27 backcross lines of oat, each with a different single *Pc* gene for race-specific crown rust resistance (Table 2). Except for *Pc-14*, the crown rust resistance genes in the differential set were derived from collections of wild oat, *A. sterilis*, from the Mediterranean region (3,5,11,13,14). *Pc-14* is from Ascencao, a Brazilian cultivar of *A. sativa* (10). The differential lines were grown in vermiculite in 7-cm square plastic pots with 10 to 20 seeds each of four lines planted in the four corners of each pot. At 7 to 10 days after planting, the seedlings in the differential set were inoculated with urediniospores of a single-uredinial isolate suspended in lightweight mineral oil as described above. Inoculated plants were kept in a dew chamber overnight and then placed on a greenhouse bench. Disease reactions were evaluated on primary leaves at 12 to 15 days after inoculation. Responses with moderately large to large pustules with little or no chlorosis were scored as susceptible reactions; those with flecks or small pustules surrounded by chlorosis or necrosis were scored as resistant (2,7).

Frequencies of virulence on each of the 27 differential lines were compared among sets of collections from the four regions of Mexico as well as with previous determinations of virulence frequencies of *P. coronata* in collections from California and Texas. For these comparisons, a similarity index (SI) was calculated for each pair of sets based on absolute differences in virulence frequencies,

$$\mathrm{SI} = \frac{1}{N}\sum_{i=1}^{N}\left|p_{iA} - p_{iB}\right|$$

in which N is the number of host lines in the differential set, p_{iA} is the percent frequency of virulence on the ith differential line in collection set A, and p_{iB} is the percent frequency of virulence on the ith differential line in collection set B (6). Thus, sets of collections with identical frequencies of virulence over all differential lines would have a value of SI = 0.0. Greater differences in virulence frequencies between pairs of collection sets indicate less similarity of virulence frequencies between them.

Mean virulence complexity values were determined for combined sets of collections from the four regions of Mexico and compared with previously published data (7) for isolates collected from California and Texas based on the same 27 differential lines. Mean virulence complexity was defined as the mean number of differentials (out of 27) on which isolates from a given region were virulent.

Races of *P. coronata* were determined as described by Chong et al. (2) for a standard North American set of 16 differential lines (Table 3) that were included in the complete set of 27 differentials used in this study. As a measure of virulence diversity in Mexican isolates of *P. coronata*, the numbers of isolates per race were determined for combined isolates from all four regions of Mexico, and the frequency distribution of race classes with 1, 2, 3, etc. isolates per race was calculated and compared with a similar frequency distribution of classes for races identified in isolates from Texas.

Fig. 1. Map showing states in Mexico from which isolates of *Puccinia coronata* were obtained from rusted oat plants. Isolates from the states of Jalisco, Mexico, Michoacan, Oaxaca, Queretaro, and Tlaxcala were combined as 29 isolates representative of central Mexico. Only one isolate was obtained from Coahuila and one from Tamaulipas; these two isolates were combined with 57 isolates from Nuevo Leon. There were 51 isolates from Chihuahua and 19 from Sonora.

In addition, virulence associations were determined in 2 × 2 contingency tables for virulence on all possible paired combinations of differentials for isolates from each of the four regions of Mexico as well as for previously described isolates from California. In addition, the virulence associations in Mexico and California were compared with previously calculated virulence association found in Texas. For each pair of differentials, the numbers of isolates virulent to both, avirulent to both, virulent to the first but avirulent to the second, or avirulent to the first but virulent to the second were determined. Expected frequencies of isolates in each category were calculated as the product of the observed frequencies of isolates virulent or avirulent on each differential. For example, the expected frequency of isolates virulent on both differentials of the pair was determined by the product of the frequency of isolates in the population virulent on the first differential multiplied by the frequency of isolates virulent on the second differential. In positive associations, the numbers of isolates virulent on both differentials or avirulent on both differentials exceeded the expected values, whereas in negative associations, the number of isolates virulent on one differential but avirulent on the other exceeded the expected values. *P* values for the statistical significance of the associations were calculated by Fisher's exact test (6).

RESULTS

The populations of *P. coronata* in Mexico were highly diverse. More than 80% of the races identified with the standard set of 16 oat differential lines were represented by just a single isolate in the combined collections over all four regions of Mexico (Table 4, Fig. 2). Overall, the distribution of numbers of isolates found per race iden-

Table 3. Oat crown rust differentials and reactions in identification of pathogenic races[a] of *Puccinia coronata*

		Reaction type[b] on differential			
	Set 1	*Pc-40*	*Pc-45*	*Pc-46*	*Pc-50*
	Set 2	*Pc-38*	*Pc-39*	*Pc-48*	*Pc-68*
	Set 3	*Pc-51*	*Pc-52*	*Pc-58*	*Pc-59*
Race code[c]	Set 4	*Pc-54*	*Pc-56*	*Pc-62*	*Pc-64*
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

[a] North American system of nomenclature (2).
[b] H = high (susceptible type) reaction; L = low (resistant type) reaction.
[c] Race codes are determined from the combined reactions over four sets of four differential oat lines (e.g., race JBBL is virulent on *Pc-45, Pc-46,* and *Pc-54*) (2).

Table 2. Virulence frequencies for collections of *Puccinia coronata* from four regions of Mexico and from California and Texas[a]

	Frequency of virulence (%)					
Differential	C. Mexico[b]	Sonora	Chihuahua	Nuevo Leon[c]	California	Texas
Pc-14	45	53	67	80	77	90
Pc-35	21	42	41	25	31	59
Pc-36	17	37	51	39	31	49
Pc-38	21	47	31	24	13	33
Pc-39	14	32	22	29	21	28
Pc-40	34	53	57	71	59	90
Pc-45	66	63	31	36	72	14
Pc-46	31	33	39	36	67	37
Pc-48	0	37	18	25	26	8
Pc-50	0	28	18	27	21	28
Pc-51	21	53	22	51	50	78
Pc-52	0	32	18	19	21	6
Pc-53	0	0	0	2	5	3
Pc-54	59	26	25	34	72	30
Pc-56	14	68	47	37	38	48
Pc-57	36	47	52	38	53	34
Pc-58	0	21	8	12	10	24
Pc-59	41	58	34	38	21	44
Pc-60	41	72	51	59	36	82
Pc-61	17	47	43	41	31	76
Pc-62	3	0	2	5	3	1
Pc-63	14	26	25	12	5	26
Pc-64	31	11	22	5	13	7
Pc-67	24	26	37	53	51	59
Pc-68	0	21	4	5	5	3
Pc-70	21	17	37	35	26	31
H548[d]	0	5	0	8	4	5
No. isolates	29	19	51	59	39	371

[a] Isolates from Mexico were collected from 1992 to 1998; isolates from California from 1993 to 1998 and from Texas from 1992 to 1998 (7) are included for comparisons with isolates from adjacent regions of Mexico.
[b] Isolates from central Mexico include those collected in the states of Jalisco, Mexico, Michoacan, Oaxaca, Queretaro, and Tlaxcala.
[c] Includes one isolate each from Coahuila and Tamaulipas.
[d] Oat line from the Iowa multiline breeding program with unidentified resistance gene from *Avena sterilis.*

tified in Mexico was similar to that in Texas, although many more isolates were characterized from Texas than from Mexico. Thus, the most frequently identified race in Mexico, race GBBL, had just nine isolates out of the total of 158 isolates characterized by race, whereas the most common race in Texas, race PBLG, was found 21 times out of a total of 329 isolates characterized.

Few of the races of *P. coronata* in Mexico were found in more than one region (Table 5). Races BLBB, GBCL, NLBG, and NLBH were found both in central Mexico and in Chihuahua. Races GBBB and GBBL were found in central Mexico and Nuevo Leon. Races JBBL and NDQG were found in Chihuahua and Nuevo Leon. Races found in Sonora were not found in other regions of Mexico, although race JBBQ occurred both in Sonora and in California (Table 5). Twenty of the races found in Texas (data not shown) also occurred in Mexico: two in both Nuevo Leon and central Mexico, 13 in Nuevo Leon only, one in central Mexico only, and four in Chihuahua. Only one race, NBLQ, was found in both California and Texas. The most common races in each region were: GBBL in central Mexico (15%) and Nuevo Leon (9%), LBBG in Chihuahua (7%), JBBQ in Sonora (12%), and JBBL in California (15%).

A more general comparison of *P. coronata* populations in regions of Mexico and in California and Texas can be seen by considering the reactions on just the first two sets of differentials, as indicated by the first two letters of the race code, which represent the first two sets of four differentials (Table 3). The GB group of races, virulent on *Pc-45,* was most common in central Mexico (52% of all races), Chihuahua (18%), and Nuevo Leon (16%). The JB group, virulent on *Pc-45* and *Pc-46*, was the most common in Sonora (17%) as well as in California (29%). The LB group, virulent on *Pc-40*, was most common in Texas (23%). LQ races, virulent on *Pc-40,*

Table 4. Races of *Puccinia coronata* found in Mexico in 1992 to 1998

	Number of isolates per region[a]					Number of isolates per region[a]			
Race	Cen.	Chih.	N.L.	Son.	Race	Cen.	Chih.	N.L.	Son.
BBBB			1		LQML			2	
BBBL		1			LSRG				1
BBCB	1				MBBL				1
BBLG		1			MBLG			1	
BBNB			1		MBMG		1		
BLBB	1	2			MCQG		1		
BPRG				1	MDRH		1		
BQFL			1		MLCG				1
CBBG		1			MMLB			3	
DBLB		1			MMLC		1		
DQBB		1			MQFG				1
DQBG		1			MQLG			1	
DQBH		1			MQPG				1
GBBB	2		4		MSKG		1		
GBBL	4		5		NBBG		1		
GBBM	1				NBLG			1	
GBCB		1			NBMC		1		
GBCC	1				NDQG		1	1	
GBCL	2	2			NDRB			1	
GBCM		2			NDRG			4	
GBCP	1				NDTG			1	
GBML	2				NGLB	1			
GJRG				1	NGMB	1			
JBBL		1	2		NJGG		1		
JBBQ				2	NLBG	2	1		
JBCB		1			NLBH	1	1		
JBCL			1		NLBQ		1		
JBCM	2				NLLR	1			
JBCQ				1	NQCC	1			
JDQG				1	NQFH		1		
JJBG				1	PBBG			1	
JJPL			1		PBLB			1	
JMRB				1	PBLG			2	
KBBB			1		PBLQ			1	
LBBB		1			PDQG			1	
LBBG		3			PJRG		1		
LBBL			1		PLMG		1		
LBBQ			1		PNQB		1		
LBMB			1		QBBB		2		
LBPF			1		QBBC				1
LDRG			1		QBBL	2			
LGBB			1		QBBM				1
LGBH			1		QBCQ			1	
LGLB	1				QDCQ		1		
LJJG		1			QFPB				1
LJRB			1		QQCQ			1	
LLBB		1			RDBD			1	
LQBB		1			RQLG				1
LQCB			1		RSFL			1	
LQFB			1		SGBF			1	
LQLG			1		Unk. isol.[b]	2	9	3	2
LQMG			1		No. races	18	37	42	16

[a] Cen. = Central Mexico, Chih. = Chihuahua, N.L. = Nuevo Leon, Son. = Sonora.

[b] Unknown isolates are those for which data for one or more of the 16 standard differentials were missing or inconclusive.

Pc-38, and *Pc-39*, were relatively common in both Texas (9%) and Nuevo Leon (10%).

Collections of *P. coronata* from Nuevo Leon were polymorphic for virulence–avirulence on all of the 27 differential oat lines used in virulence tests (Table 2). Collections from Chihuahua, Sonora, and central Mexico were polymorphic for virulence–avirulence on 25, 24, and 20 differentials, respectively. Frequencies of virulence to *Pc53*, *Pc62*, and line H548 were consistently low (<10%) in all four regions of Mexico as well as in California and Texas. Virulence to *Pc68* was rare in all the regions except Sonora, where 21% of the isolates were virulent. Mean virulence complexity was lowest for collections from central Mexico and intermediate in Chihuahua and Nuevo Leon (Fig. 3). Virulence complexity in Sonora was similar to that of California and Texas and greater than the virulence complexity of other regions of Mexico.

Similarity indexes based on virulence frequencies over all 27 differentials showed that collections of *P. coronata* from Chihuahua and Nuevo Leon had the greatest similarity, whereas collections from central Mexico and Sonora had relatively little similarity to each other or to the collections from Chihuahua and Nuevo Leon (Table 6). Collections from California and Texas were more similar to those from Nuevo Leon than to those from other regions of Mexico, even though the virulence frequencies in collections from California showed little similarity to those in collections from Texas.

Of a total of 351 possible pair-wise combinations of virulences for the 27 differentials, 24 showed significant ($P < 0.05$) positive associations in at least three of the four regions of Mexico (Table 7), and five showed significant negative associations (Table 8). Of the 24 positive virulence associations found in Mexico, 19 were also significantly positive in Texas, and 13 were significantly positive in California. Two of the significant negative associations of virulence found in Mexico were also found in Texas, and one was found in California. Significant positive associations of virulences to *Pc14* and *Pc40*, *Pc14* and *Pc51*, *Pc14* and *Pc60*, *Pc36* and *Pc56*, *Pc39* and *Pc70*, *Pc40* and *Pc60*, and *Pc60* and *Pc61* occurred in all four regions of Mexico as well as in both California and Texas. The negative association of virulence to *Pc14* and *Pc45* was significant in all four regions of Mexico as well as in Texas, although it was not significant in California.

DISCUSSION

Collections of *P. coronata* from Mexico were highly diverse for virulence. The large number of races identified and the absence of any dominant races were similar to the pattern of diversity described by Groth and Roelfs (4) for sexual popula-

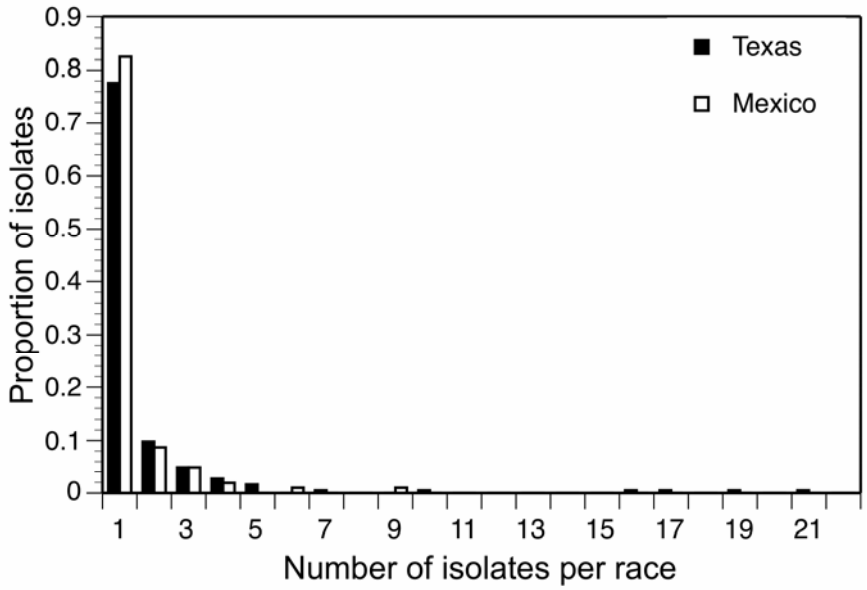

Fig. 2. Frequency distribution of classes of races of *Puccinia coronata* from Mexico and Texas ranked according to number of isolates found per race. For example, 78% of the isolates from Texas and 83% of the isolates from Mexico were from races with only a single isolate found per race, and 10% from Texas and 8% from Mexico were from races with two isolates per race.

Table 5. Races of *Puccinia coronata* found in more than one region of Mexico or in California plus at least one region in Mexico

	Number of isolates of races from indicated region				
Race	**Central Mexico**	**Chihuahua**	**Nuevo Leon**	**Sonora**	**California**
BBBB	0	0	1	0	1
BLBB	1	2	0	0	0
GBBB	2	0	4	0	0
GBBL	4	0	5	0	0
GBCL	2	2	0	0	0
JBBL	0	1	2	0	5
JBBQ	0	0	0	2	1
NDQG	0	1	1	0	1
NLBG	2	1	0	0	0
NLBH	1	1	0	0	0
QBBB	0	2	0	0	1

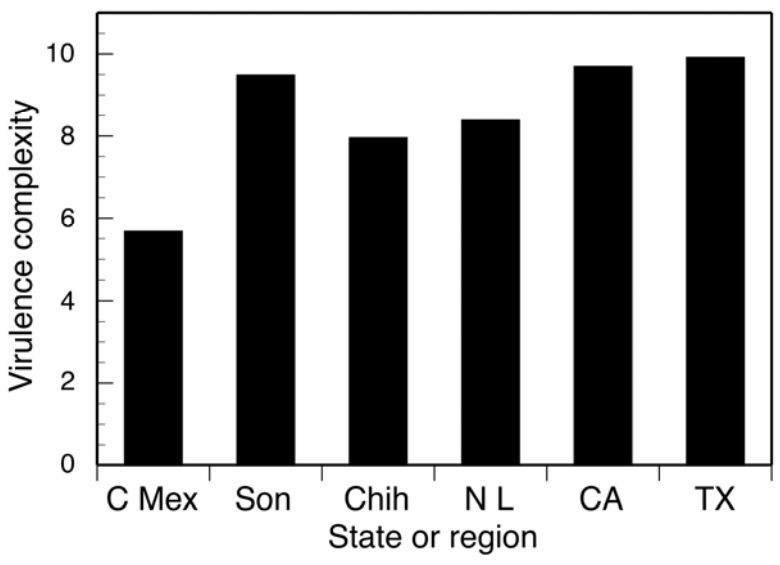

Fig. 3. Average virulence complexity of isolates of *Puccinia coronata* from four regions of Mexico and from Texas and California in the United States. Virulence complexity was determined as the number of differential oat lines (out of 27 lines tested) to which the isolates were virulent.

tions of cereal rust fungi. There are no reports, however, that the sexual stage of *P. coronata* occurs in Mexico or the southwestern United States. The high level of virulence diversity in populations of *P. coronata* in these regions may have other explanations. For example, the high diversity may reflect a general lack of strong selection for specific virulence. Most of the resistance genes in the set of differential lines that were used in this study have not been used widely in commercial oat production. Only *Pc-59* was identified as likely to be present in Mexican oat lines tested by Salmeron et al. (12). Thus, there may have been little selection to reduce genetic diversity in the populations of *P. coronata* in Mexico. Also, the large number of races of *P. coronata* found in Mexico may be related to the average frequencies of the virulences found there. For random associations of virulence, the number of races tends to be greater when frequencies of most virulence are near 50% than when they are near 0 or 100%. In our study, there were only three virulences that occurred at less than 10% frequency in most of the regions studied, and none occurred at greater than 90%. Therefore, the high diversity of races of *P. coronata* does not seem unusual even for an asexual population. Earlier studies also revealed a high level of race diversity in the *P. coronata* populations on oat in Brazil and Uruguay (9), even though alternate hosts for *P. coronata* are not found in South America. In Brazil and Uruguay, most of the races identified were represented by a single isolate, and no race was found more than six times out of 177 isolates identified to race (9).

Comparisons of virulence frequencies determined over the 27 single *Pc* gene oat

Table 6. Similarity[a] of virulence frequencies in sets of isolates of *Puccinia coronata* collected from four regions of Mexico and from California and Texas and tested over 27 oat crown rust differential lines

Region	Chihuahua	Nuevo Leon[b]	California	Texas	Sonora
Nuevo Leon	8.77				
California	12.47	9.73			
Texas	13.23	11.02	19.11		
Sonora	12.17	12.40	15.95	15.53	
C. Mexico[c]	14.88	16.51	15.26	23.65	18.93

[a] Similarity index = mean value of differences in frequency (%) of virulence averaged over all 27 differentials; lowest values indicate greatest similarity.
[b] Includes one isolate each from Coahuila and Tamaulipas.
[c] Isolates from central Mexico include those collected in the states of Jalisco, Mexico, Michoacan, Oaxaca, Queretaro, and Tlaxcala.

Table 7. Significant positive virulence associations[a] observed in collections of *Puccinia coronata* in at least three of four regions in Mexico compared with associations found in California and Texas

	Probability that observed association was due to random variation					
Association	**C. Mexico**	**Sonora**	**Chihuahua**	**Nuevo Leon**	**California**	**Texas**
Pc-14 + Pc-35	0.004		<0.0005	0.026		0.001
Pc-14 + Pc-36	0.011		<0.0005	0.002		0.003
Pc-14 + Pc-40	<0.0005	0.023	<0.0005	<0.0005	0.001	<0.0005
Pc-14 + Pc-51	0.004	0.023	0.009	<0.0005	0.003	<0.0005
Pc-14 + Pc-56	0.030		0.001	0.022		0.003
Pc-14 + Pc-60	0.001	0.007	<0.0005	<0.0005	0.015	0.001
Pc-14 + Pc-61	0.011		<0.0005	0.001		<0.0005
Pc-36 + Pc-40	0.002		<0.0005	0.001		
Pc-36 + Pc-56	<0.0005	0.044	<0.0005	<0.0005	<0.0005	<0.0005
Pc-36 + Pc-61	0.024		0.002	0.013		<0.0005
Pc-38 + Pc-40	0.011	0.005		0.048		
Pc-38 + Pc-63	0.001	0.011	0.001	0.006		<0.0005
Pc-39 + Pc-70	0.001	0.012	0.012	<0.0005	<0.0005	<0.0005
Pc-40 + Pc-56	0.009		<0.0005	<0.0005	0.049	0.003
Pc-40 + Pc-60	0.005	0.007	<0.0005	<0.0005	<0.0005	0.014
Pc-40 + Pc-70	<0.0005		<0.0005	0.030		0.004
Pc-45 + Pc-54	<0.0005		<0.0005	<0.0005	<0.0005	0.030
Pc-46 + Pc-57	0.035		<0.0005	<0.0005	0.035	<0.0005
Pc-48 + Pc-52	–[b]	0.010	<0.0005	<0.0005	0.001	<0.0005
Pc-48 + Pc-70		0.043	0.001	<0.0005		0.001
Pc-50 + Pc-68	–[b]	0.002	0.028	0.017	0.038	
Pc-51 + Pc-52	–[b]	0.011	0.015	<0.0005		
Pc-51 + Pc-60		0.007	0.005	<0.0005	<0.0005	
Pc-60 + Pc-61	0.007	0.029	<0.0005	<0.0005	<0.0005	<0.0005

[a] Associations with $P > 0.05$ (Fisher's exact test) are not shown.
[b] Virulence to *Pc-48, Pc-52*, or *Pc-68* was not found in collections from central Mexico.

Table 8. Significant negative virulence associations[a] in collections of *Puccinia coronata* in at least three of four regions in Mexico compared with negative associations found in California and Texas

	Probability that negative association was due to random variation					
Association	**C. Mexico**	**Sonora**	**Chihuahua**	**Nuevo Leon**	**California**	**Texas**
Pc-14 + Pc-45	0.016	0.011	<0.0005	0.002		<0.0005
Pc-36 + Pc-45	0.002		<0.0005	0.001		
Pc-40 + Pc-45	<0.0005		0.001	<0.0005		<0.0005
Pc-45 + Pc-56	0.009		<0.0005	0.011	0.010	
Pc-54 + Pc-61		0.033	0.025	0.001		

[a] Negative associations mean the virulence to the first differential line but avirulence to the second or vice versa occur more frequently than expected from random association, but virulence to both differentials or avirulence to both differentials occur less frequently than expected. Negative associations with $P > 0.05$ (Fisher's exact test) are not shown.

differentials revealed that the overall population of *P. coronata* on oat in Mexico is divided into at least four more or less distinct subpopulations that differ in virulence frequencies from each other and from populations of *P. coronata* in the adjacent U.S. states of Texas and California. The collections of *P. coronata* from Nuevo Leon and Chihuahua were most similar to each other and also showed as much similarity to the collections in California and Texas as to those in central Mexico or Sonora. Virulence to *Pc-53* and *Pc-62* was rare (≤5% frequency) in all four regions of Mexico as well as in California and Texas.

Virulence to *Pc-68*, which is rare in the United States (7), was also rare in three of the four regions of Mexico, but virulence to *Pc-68* occurred at 21% in Sonora. *Pc-68* is an important gene for crown rust resistance currently being employed in oat cultivars in the Prairie Provinces of Canada (1) as well as in oat breeding populations in the United States. Although all isolates of *P. coronata* from Sonora were collected from *A. fatua*, the higher frequency of virulence to *Pc-68* in Sonora is not necessarily an indication of the presence of *Pc-68* in *A. fatua*. None of the 15 isolates of *P. coronata* collected from *A. fatua* in Texas from 1990 to 2000 was virulent to *Pc-68*, and only one of 17 isolates collected from *A. fatua* in California was virulent to *Pc-68* (7). All of the isolates from Sonora that were virulent on *Pc-68* were obtained in 1996. Virulence to *Pc-68* was found also in two isolates from Chihuahua in 1997; in two isolates from Nuevo Leon in 1996 and one in 1997; in one isolate from California in 1995 and one in 1996; and in one isolate from Texas in 1992, nine in 1994, and one in 1996. There were 17 different races represented among isolates from Mexico, California, and Texas that were virulent on *Pc-68*. Of these 17 races, only one, race MMLB, was found in more than one state; MMLB was found in both Texas in 1994 and in Nuevo Leon in 1996. This evidence suggests that virulence to *Pc-68* in Sonora and California may have arisen independently of the *Pc-68* virulence found in Texas and adjacent Nuevo Leon.

With the exception of Sonora, regional sets of isolates of *P. coronata* from Mexico were less virulent on average than those from the United States. The mean virulence complexity of isolates of *P. coronata* from Sonora was as great as those of collections from California or Texas. The lowest virulence complexity was found in central Mexico, the region farthest from the United States and, presumably, farthest from areas where the alternate host and sexual reproduction of the fungus occur. Absence of the sexual cycle, however, is not necessarily associated with lower virulence complexity. Collections of *P. coronata* from Brazil and Uruguay, where alternate hosts for *P. coronata* are unknown, had greater virulence complexity than collections from either Texas or the Northern Great Plains of the United States (9).

Occurrence of races in common across geographical regions may be evidence of migration between the regions. Thus, it is interesting that four races found in central Mexico also occurred in Chihuahua, and two races in central Mexico were found also in Nuevo Leon. Race GBBL was the most common race in both central Mexico and Nuevo Leon, but GBBL was not found in Chihuahua. In fact, only two races found in Chihuahua were also found in Nuevo Leon, which suggests that there may be relatively little east to west or west to east movement of *P. coronata* spores in Mexico. Race LBBG, the most common race in Chihuahua, was not found in any other region of Mexico. The subpopulation of *P. coronata* in Sonora appears to be relatively isolated from the rest of Mexico. Not only does it have higher virulence complexity than the other subpopulations in Mexico, but also none of the races in Sonora were found in any other region of Mexico. Part of the difference in *P. coronata* collections from Sonora and from the rest of Mexico may be due to climatic influences. The collections from Sonora were made in irrigated Yaqui Valley and were from *A. fatua*, whereas collections from central Mexico and Chihuahua were from *A. sativa* in areas of higher altitude and cooler climates. Collections of *P. coronata* from Texas were most similar to the adjacent state of Nuevo Leon in Mexico; 13 races found in Texas were also found in Nuevo Leon, indicating that movement of *P. coronata* spores across the Rio Grande River between south Texas and Mexico is not uncommon. Only four of the races found in Texas also occurred in Chihuahua.

Twenty-four pairs of virulences were significantly associated in collections from at least three of the four regions of Mexico. Among those 24 associated pairs, 19 were also significantly associated in collections from Texas, and 13 were significantly associated in collections from California. There is strong evidence that many of these virulence associations result from general fitness advantages rather than just local conditions of genetic drift or population founder effects. Significant associations of virulence to *Pc-36 + Pc-56, Pc-39 + Pc-70,* and *Pc-48 + Pc-52* were found also in collections of *P. coronata* from cultivated oat in the northern Great Plains of the United States and in Brazil and Uruguay as well as from wild oat in Israel (8,9). Other significant associations of virulence also seen in Israel as well as in Mexico and Texas or California include virulence to *Pc-36 + Pc-61, Pc-40 + Pc-70, Pc45 + Pc-54, Pc-46 + Pc-57,* and *Pc-60 + Pc-61*. Significant associations of virulence to *Pc-51 + Pc-60* and *Pc-60 + Pc-61* were previously found in the northern Great Plains as well as in Mexico and California or Texas (8). The previously reported association of virulence to *Pc-38 + Pc-63* (1) was also found in the northern Great Plains and in Brazil and Uruguay (8,9) as well as in Texas and all four regions of Mexico. The associations between virulence to *Pc-14* and several other *Pc* genes that were common to Mexico, Texas, and, in some cases, California have not been reported from other parts of the world. These *Pc-14* virulence associations may have resulted from forces of selection specific to Mexico and the southwestern United States. The significant association between virulences to *Pc-68* and *Pc-50* found in Mexico and California was not seen in previous studies in Texas and the northern Great Plains of the United States or in Israel, probably because of the very low frequencies of virulence to *Pc-68* in those regions (8). It seems likely that increased use of *Pc-48* and *Pc-68* in breeding programs in Canada and the United States will lead to increases in associated virulence to *Pc-52* and *Pc-50* as well as to *Pc-48* and *Pc-68* in the same way that use of *Pc-39* in the United States in the 1990s led to an increase in the frequency of the associated virulence to *Pc-70*, a gene that has not been used significantly in commercial oat production (7,8).

It is unlikely that the virulence associations observed in isolates from Mexico in this research could be due even in small part to selection effects of the corresponding *Pc* genes in the host population. For example, the genes *Pc-14, Pc-36, Pc-46, Pc-51, Pc-52,* and *Pc-57* are not known to have been used in cultivated oat except in the Iowa multiline cultivars, which would not have imposed strong selection pressure on *P. coronata* populations in Texas, California, or Mexico. Furthermore, there were no significant changes in frequencies of virulence to *Pc-14, Pc-35, Pc-40, Pc-45, Pc-48, Pc-50, Pc-51, Pc-52, Pc-53, Pc-54, Pc-58, Pc-59, Pc-60, Pc-61, Pc-62, Pc-64, Pc-67*, or *Pc-68* from 1990 to 2000 in either Texas or the northern Great Plains of the United States (7). Thus, these virulences showed no evidence of host selection. Significant increases in the frequencies of virulence to *Pc-36, Pc-57*, and *Pc-70* that occurred in the United States during 1990 to 2000 were apparently unrelated to the minimal presence of these three genes in cultivated oat. Also, Leonard (7) showed that collections of *P. coronata* from wild *A. fatua* had essentially the same frequencies of virulence to *Pc* genes as collections from cultivated *A. sativa* in the United States, which indicates an absence of selection for these virulences on wild oat. Only the significant association between virulences to *Pc-38* and *Pc-39* in the United States (8) could be attributed to host selection. *Pc-38* was used extensively in combination with *Pc-39* in prominent oat cultivars in the northern Great Plains of the United States and in the

Prairie Provinces of Canada in the 1980s and 1990s (7). However, we found no significant association between virulences to *Pc-38* and *Pc-39* in Mexico.

As indicated by Leonard et al. (8), linkage disequilibrium relative to virulence alleles in pathogen populations may be due to a variety of causes. However, when the same significant virulence associations are found over a range of different pathogen populations in different parts of the world under different climatic conditions and in association with different host populations, it is highly likely that those virulence associations are due to pleomorphic or epistatic effects on fitness inherent to the pathogen rather than to extraneous influences (8).

Five pairs of virulences were negatively associated in collections of *P. coronata* from Mexico. That is, for those five pairs, virulence to one member of the pair was significantly associated with avirulence in the other. Three of the five negative associations, virulence–avirulence to *Pc-36 + Pc-45, Pc-45 + Pc-56,* and *Pc-54 + Pc-61*, were also significant in collections of *P. coronata* from wild oat in Israel (8). Combined virulence to these pairs of *Pc* genes may have some fitness costs that could be exploited in breeding for crown rust resistance in oat cultivars for Mexico and the southwestern United States. Negative associations for virulence to *Pc-39 + Pc-51, Pc-56,* or *Pc-61* were consistently significant in collections from Texas, the northern Great Plains, and Israel (8), but not in collections from Mexico. This may be due in part to the lower numbers of isolates tested from regions of Mexico than from earlier studies in the United States and Israel.

The high diversity of virulence in populations of *P. coronata* in Mexico and the apparent prevalence of green bridges that allow *P. coronata* to persist year-round in the uredinial stage will make it difficult to manage oat crown rust through the use of race-specific resistance alone. Virulence to all of the 27 genes for race-specific resistance that we tested is already present in at least one region of Mexico, and virulence to 26 of those genes is present in two or more regions. It seems unlikely that even combinations of these resistance genes will provide a high level of durable protection from crown rust in Mexico without the addition of some nonspecific, partial resistance in commonly grown oat cultivars. Selection for adult plant, slow rusting resistance seems especially important for oat cultivars for the high crown rust hazard areas of northeastern Mexico (Nuevo Leon and Coahuila) and the high plateau of central Mexico.

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent technical assistance of G. E. Ochocki in establishing and testing single-uredinial isolates of *P. coronata* and the support of J. J. Roberts, who provided several collections of *P. coronata* from Mexico in addition to our own collections.

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