An International Journal of Applied Plant Pathology

plant disease

Volume 107, Number 4 | April 2023

APS
THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

Occurrence and Distribution of Physiological Races of *Exserohilum turcicum* in Maize-Growing Regions of Mexico

Carlos Muñoz-Zavala,[1,2] Alexander Loladze,[2] Mateo Vargas-Hernández,[3] Elizabeth García-León,[4] Amos Emitati Alakonya,[2] Juan Manuel Tovar-Pedraza,[5] Paul H. Goodwin,[6] and Santos Gerardo Leyva-Mir[1,†]

[1] Departamento de Parasitología Agrícola, Universidad Autónoma Chapingo, Texcoco 56230, Estado de México, Mexico
[2] International Maize and Wheat Improvement Center (CIMMYT), Texcoco 56237, Estado de México, Mexico
[3] Departamento de Suelos, Universidad Autónoma Chapingo, Texcoco 56230, Estado de México, Mexico
[4] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Campo Experimental Valle del Fuerte, Guasave 81110, Sinaloa, Mexico
[5] Laboratorio de Fitopatología, Coordinación Regional Culiacán, Centro de Investigación en Alimentación y Desarrollo, Culiacán 80110, Sinaloa, Mexico
[6] School of Environmental Sciences, University of Guelph, Guelph N1G 2W1, Ontario, Canada

Abstract

Turcicum leaf blight (TLB) is a common foliar disease of maize in Mexico that is caused by the fungal pathogen *Exserohilum turcicum*. The most effective management strategy against TLB is monogenic race-specific resistance. Among the 140 *E. turcicum* isolates from symptomatic leaves collected from maize fields in Mexico, 100 were obtained from tropical (Veracruz) and temperate areas (Estado de México) between 2010 and 2019, and 40 isolates were obtained from tropical (Sinaloa, Tamaulipas, Veracruz, and Chiapas), subtropical (Nayarit, Jalisco, and Guanajuato), and temperate areas (Estado de Mexico, Hidalgo, and Puebla) collected in 2019. All the isolates caused TLB symptoms on the positive control (*ht4*), showing that they were all pathogenic. Six physiological races of *E. turcicum* (2, 3, 23, 3N, 23N, and 123N) were identified based on resistant or susceptible responses displayed by five maize differential genotypes (A619*Ht1*, A619*Ht2*, A619*Ht3*, B68*HtN*, and A619*ht4*). The most common was race 23, accounting for 68% of the isolates, followed by races 23N, 123N, 3, 2, and 3N at 15, 8, 6, 2, and 1%, respectively. Race 123N was able to infect the greatest number of maize differential genotypes used in the study. Race 123N was detected in Sinaloa and Estado de México. Race 3 was detected in Nayarit and Jalisco. Race 2 was detected in Jalisco, Estado de México, and Veracruz, and race 3N was detected in Tamaulipas. Race 23 was equally dominant in the tropical, subtropical, and temperate regions, while race 123N was more common in the tropical environment, and race 23N was more common in the tropical and temperate environments. There was no evidence for shifts in the races between 2010 and 2019.

Keywords: *Exserohilum turcicum*, northern corn leaf blight, physiological races, resistance, *Setosphaeria turcica*, Turcicum leaf blight, virulence, *Zea mays*

Maize (*Zea mays* L.) is Mexico's most important staple food crop, with an annual average consumption of 335.8 kg per person (Martinez-Nuñez et al. 2019). Mexico is also the center of origin, diversification, and domestication of maize (Beadle 1980). However, maize productivity in Mexico lags behind the United States, China, Brazil, and Argentina (FAOSTAT 2021). One reason for this is losses due to plant diseases.

Exserohilum turcicum (Teleomorph: *Setosphaeria turcica*) is an ascomycete fungus that causes Turcicum leaf blight (TLB) or northern corn leaf blight of maize and sorghum. The fungus is a hemibiotroph, being biotrophic for approximately 8 days before switching to being necrotrophic (Human et al. 2020). After the penetration and formation of the haustorium, the fungus invades new tissues, causing necrosis, and then systemically spreads throughout the plant (Muiru et al. 2010). Yield losses range from 24 to 91% worldwide (Nwanosike et al. 2015). The greatest yield losses are when *E. turcicum* infections occur just before tasseling. TLB outbreaks are often associated with changes in *E. turcicum* populations, and the emergence of new virulent physiological races (Muiru et al. 2010). Navarro et al. (2021a) established that *E. turcicum* isolates from South America and Europe could adapt to changes in environmental conditions through plasticity without necessarily undergoing gene mutations. This scenario complicates the understanding of how the isolates adapt to new environments and makes it difficult for breeders to develop resistant germplasms.

Resistant maize genotypes and fungicides are widely used in TLB management. However, the application of chemicals is expensive and poses negative environmental impacts; so, the use of resistant germplasms is the most sustainable method for controlling the disease (Navarro et al. 2021a; Weems and Bradley 2018). Host resistance against TLB can be qualitative based on race specific *Ht* genes, or it can be quantitative based on many genes with small effects on resistance. Both types of resistance can be present in commercial cultivars (Galiano-Carneiro and Miedaner 2017).

Several physiological races of *E. turcicum* have been reported and are determined based on their virulence on maize differential genotypes carrying TLB race-specific resistance genes denoted as *ht4*, *Ht1*, *Ht2*, *Ht3*, and *HtN*, with *ht4* being a positive control as it is ineffective against most known physiological races (Carson 1995; Gevers 1975; Hooker 1963, 1977, 1981). Leonard et al. (1989) proposed a race nomenclature system based on symptoms displayed by maize germplasms with resistance to *E. turcicum* and the pathogen's virulence genes. Thus, race 0 implies that the pathogen is avirulent to all *Ht* genes. In contrast, race 123N is virulent to all maize differential genotypes (*Ht1*, *Ht2*, *Ht3*, and *HtN*). More recent studies have identified several genes that confer resistance to *E. turcicum* in maize in dominant or partially dominant versions, including *Ht1*, *Ht2*, *Ht3*, *Htn1* (= *HtN*), *Htm1* (= *HtM*), *HtNB*, and *HtP* (Jindal et al. 2019; Navarro et al. 2021b; Turgay et al. 2020; Welz and Geiger 2000).

The emergence of new physiological races of *E. turcicum* or enhanced virulence of isolates can occur through sexual hybridization (Bunkoed et al. 2014). Several studies have used differential maize

†Corresponding author: S. G. Leyva-Mir; lsantos@correo.chapingo.mx

The author(s) declare no conflict of interest.

Accepted for publication 5 September 2022.

genotypes to determine local populations of *E. turcicum* (Table 1). For example, Weems and Bradley (2018) found 20 physiological races among 156 *E. turcicum* isolates collected between 1979 and 2014 in the north central United States. They used the *Ht1*, *Ht2*, *Ht3*, *Htn1*, and *Htm1* differential lines and showed that races virulent to *Ht2*, *Ht3*, *Htn1*, and *Htm1* decreased after 2010. *E. turcicum* can also infect teocintle (*Euchlaena mexicana*) and several types of sorghum, such as Johnson grass (*Sorghum halepense*) and Sudan grass (*Sorghum* × *drummondii*). The isolates that infect these hosts are not pathogenic to maize due to the absence of a quantitative trait locus for virulence of *E. turcicum* (Donald 2004; Singh et al. 2022). In Mexico (El Bajío), three forma specialis (f sp.) of *S. turcica* have been reported: *S. turcica* f. sp. *sorghi* on sorghum, *S. turcica* f. sp. *complexa* on sorghum and grasses, and *S. turcica* f. sp. *zea* on maize (Ayala Escobar et al. 1994). There is limited information on the occurrence and geographic distribution of *E. turcicum* races in Mexico since Welz et al. (1993). The knowledge and understanding of the existing *E. turcicum* physiological races are key in the designing and implementation of the TLB management strategy in Mexico. Therefore, documenting the races of *E. turcicum* in Mexico can support the future management plan of the disease in the country. Further, such information could inform importers of maize from Mexico about the possible risk of introducing new races of *E. turcicum*, which could lead to TLB epidemics. This study aimed to determine the distribution of the different physiological races of *E. turcicum* in 10 maize-producing states of Mexico.

Materials and Methods

Isolate collection

One hundred and forty *E. turcicum* culture isolates were obtained from symptomatic leaf samples. In 2019, 40 isolates were collected from infected maize leaves at least 100 m from each other in the states of Tamaulipas, Sinaloa, Nayarit, Jalisco, Guanajuato, Estado de México, Hidalgo, Puebla, Veracruz, and Chiapas in Mexico (Fig. 1 and Table 2). Five isolates per location were collected to have a homogeneous representation of the different sites with high incidence of TLB. Another 100 isolates were examined that had previously been collected between 2010 and 2019. This collection consisted of 50 isolates from CIMMYT-El Batán Experimental Station, Estado de México (temperate environment) and another 50 isolates from CIMMYT-Agua Fría Experimental Station, Veracruz (tropical environment) (Fig. 1 and Table 2).

Isolations were made by cutting small fragments (5 mm^2) of the leaf along the advancing margin of the disease lesions. Twenty percent V8 agar medium was prepared using commercially available V8 juice (Campbells, Mexico), containing eight vegetable juices (tomatoes, carrots, beets, celery, lettuce, parsley, spinach, and watercress), before being dispensed on Petri plates. V8 medium was prepared according to the method described by Ferguson and Jeffers (1999). Fragments were surface sterilized with 2% sodium hypochlorite solution for 30 s, washed in sterile distilled water, plated onto V8 agar medium in 90 mm diameter Petri dishes, and incubated at 25°C with 12-h light/dark conditions for 72 h. Fungal isolates were purified by the monosporic culture technique and stored at 4°C until further use.

Race determination

Maize differential genotypes were requested from the Maize Genetics Cooperation Stock Center (USDA/ARS), University of Illinois, Urbana-Champaign (Table 3). Subsequently, the maize seeds were sown at the CIMMYT-Agua Fría Experiment Station, Veracruz, in the fall to winter of 2019. Three seeds of each differential genotype (*Ht1*, *Ht2*, *Ht3*, *HtN*, and *htn4*) were planted per pot (5 × 5 cm), and each differential line was repeated three times. All three replications were placed in a plastic tray and inoculated with one *E. turcicum* isolate at a time. The plants were maintained at 27°C during the day and 18°C at night under lights at 600 to 800 W/m^2 prior to inoculation (Carson and Van Dyke 1994). Three days before inoculation, the plants were thinned to one plant per pot, removing the smallest plants. Then, 1.5 liters of water per tray was added to the plants every 7 days, and plants were fertilized with Ultrasol fertilizer 18-18-18 (N-P-K) at the time of planting and at 14 days.

To prepare inoculum, *E. turcicum* isolates were cultured on lactose casein agar (Tuite 1969). Cultures were grown at 25°C for 14 days under a 12-h light/dark regime (Ferguson and Carson 2004). Conidia were removed from the agar with a sterile glass slide and suspended in sterile distilled water. Conidial suspensions were quantified with a hemocytometer and diluted with water containing Tween 20 (10 ml $liter^{-1}$) to 2×10^4 conidia ml^{-1} (Muiru et al. 2010). The leaf whorls of 14-day-old maize seedlings were inoculated with 200 µl of the conidial suspension using a micropipette. Inoculated plants were then placed in a humidity chamber for 24 h (Weems and Bradley 2018). The humidity chamber was maintained at 24 to 25°C during the day and 16 to 18°C at night with shade during the day to maintain illumination at 100 to 200 W/m^2 (Carson and Van Dyke 1994). Plants were examined for symptoms 14 days after inoculation (dai) and rated according to a visual disease severity rating scale of 0 to 5, where 0 to 3 were considered resistant (R), and 4 to 5 were considered susceptible (S). The symptoms on the *Ht* differentials were rated as follows: 0 = no symptoms, 1 = small chlorotic spots, 2 = large chlorotic spots, 3 = chlorotic lesions coalesced with some necrosis, 4 = necrotic lesions, and 5 = necrosis of the whole leaf (Muiru et al. 2010).

Table 1. Distribution of *Exserohilum turcicum* races on maize worldwide

Country	Race[a]	Reference
Mexico	23N, 23, 2N	Welz et al. (1993)
Zambia	0, 23, 23N	Welz et al. (1993)
Uganda	0, 1, 2, N, 23N	Welz et al. (1993)
Brazil	0, 1, 2, 1N, 12N, 23N, 123N	Gianasi et al. (1996); Navarro et al. (2021b)
China	0, 1, 2, 3, N, 12, 13, 23, N, 1N, 2N, 3N, 12N, 13N, 23N, 123N	Dong et al. (2008); Ma et al. (2020)
Kenya	0, 1, 2, 3, 12, 13, 23, 123, N, 3N, 13N, 23N	Muiru et al. (2010)
Germany	0, 1, 2, 12, 13, 23, 23N	Muiru et al. (2010)
Austria	0, 1, 2	Muiru et al. (2010)
United States	0, 1, 2, M, N, 12, 13, 23, 1M, 1N, MN, 123, 1MN, 2MN, 23M, 23N, 12MN, 23MN, 123M, 123MN	Weems and Bradley (2018)
Turkey	0, 1, 2, 123, N, 1N, 3N, 12N	Turgay et al. (2020)
Canada	0, 1, 2, 3, M, N, 12, 1M, 1N, 3M, 13M, 12N, 13N, 1MN, 12MN, 13MN, 123MN	Jindal et al. (2019)
Argentina	0, 1, 2, 3, 3N, 13N, 23N	Navarro et al. (2021b)

[a] The nomenclature system proposed by Leonard et al. (1989) designated the races according to symptoms displayed by maize germplasm with resistance and also the pathogen's virulence genes. Thus, race 0 denotes that it is avirulent with any of the *Ht* genes, while race 123N indicates that its virulent at the four differentials (*Ht1*, *Ht2*, *Ht3*, and *HtN*). The United States and Canada included the *HtM* differential.

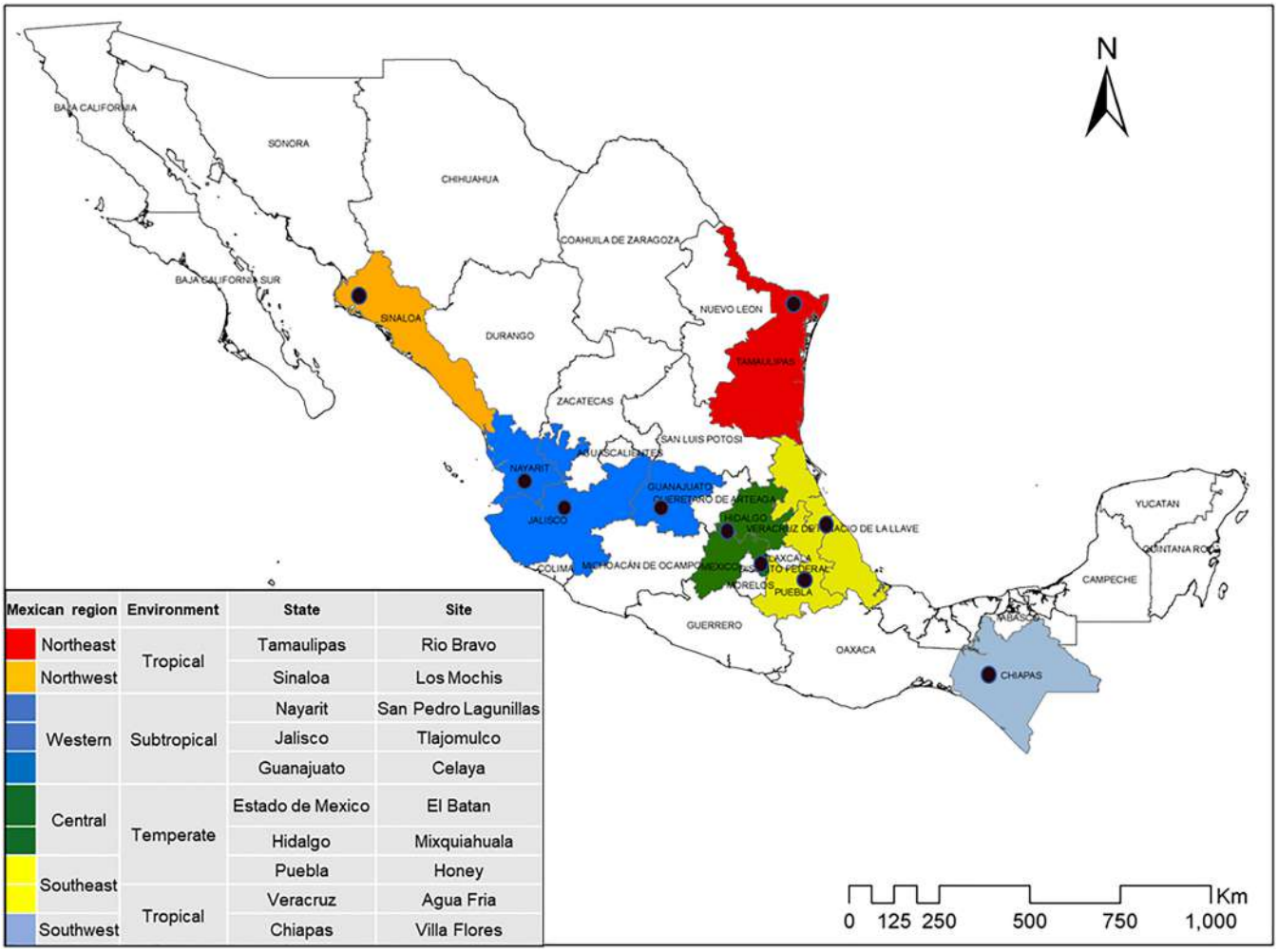
Data analysis

Data on growth rate and number of conidia produced in Petri dishes by the 140 isolates were subjected to analyses of variance, and means were compared by Fisher's least significant difference test at 5% probability using PROC GLM in SAS (version 9.3; SAS Institute, Cary, NC). The diversity of physiological races was determined based on frequencies because the data was qualitative.

Results

Race determination

At 24 h after inoculation, all isolates produced chlorotic spots in the inoculated zone. In the positive control (*ht4*), 2- to-4 cm silvery lesions were observed at 7 dai, while elliptical tan lesions were observed at 12 dai with severity ranging from 4 to 5 for all fungal isolates. The symptomatic reactions of the maize



Mexican region	Environment	State	Site
Northeast	Tropical	Tamaulipas	Rio Bravo
Northwest		Sinaloa	Los Mochis
Western	Subtropical	Nayarit	San Pedro Lagunillas
		Jalisco	Tlajomulco
		Guanajuato	Celaya
Central	Temperate	Estado de Mexico	El Batan
		Hidalgo	Mixquiahuala
Southeast		Puebla	Honey
	Tropical	Veracruz	Agua Fria
Southwest		Chiapas	Villa Flores

Fig. 1. Map of Mexico with locations of the states by region and environment of 140 *Exserohilum turcicum* isolates.

Table 2. Classification of states in Mexico where 140 *Exserohilum turcicum* isolates were sourced by region and environment

Number of isolates	State	Site	Collaborator	Mexican region	Environment	Latitude and longitude[a]	Altitude (masl)	Average annual temperature (°C)[b]	
								Minimum	Maximum
5	Tamaulipas	Rio Bravo	INIFAP	Northeast	Tropical	25.9688990, –98.0144970	28	11	36
5	Sinaloa	Los Mochis	CORTEVA	Northwest	Tropical	25.7227073, –108.9456460	12	12	35
5	Nayarit	San Pedro Lagunillas	INIFAP	Western	Subtropical	21.2028330, –104.7447000	1,329	6	29
5	Jalisco	Tlajomulco	CORTEVA	Western	Subtropical	20.4292723, –103.3999398	1,554	5	31
5	Guanajuato	Celaya	INIFAP	Western	Subtropical	20.5866280, –100.8285790	1,766	6	31
50	Estado de México	El Batan	CIMMYT	Central	Temperate	19.5294260, –98.8461120	2,276	4	26
5	Hidalgo	Mixquiahuala	MASAGRO	Central	Temperate	20.1932450, –99.2262300	2,010	6	27
5	Puebla	Honey	MASAGRO	Southeast	Temperate	20.2378830, –98.2220780	2,156	5	25
50	Veracruz	Agua Fria	CIMMYT	Southeast	Tropical	20.4547580, –97.6408820	100	18	36
5	Chiapas	Villa Flores	CORTEVA	Southwest	Tropical	16.4314919, –93.1929701	568	17	34

[a] Geolocation of the center where the collections were made with a separation of 100 m between samples.
[b] Source: CONAGUA (https://smn.conagua.gob.mx/es/); Weather Spark (https://es.weatherspark.com).

differential genotypes against the *E. turcicum* isolates were all observable by 14 dai, allowing for clear observation of differences in resistance and susceptibility responses among the *E. turcicum* isolates in this study. For the resistance reactions, the *Ht1* differential showed elongated chlorotic lesions, the *HtN* differential showed small chlorotic spots, and the *Ht2* and *Ht3* differentials both showed small chlorotic lesions and mild necrosis.

The use of the four differentials with the *Ht* genes (*Ht1*, *Ht2*, *Ht3*, and *HtN*) allowed the identification of six physiological races among the 140 *E. turcicum* isolates from Mexico. These were races 2, 3, 23, 3N, 23N, and 123N (Fig. 2). On the other hand, all the 140 isolates used in this study caused TLB symptoms on *ht4* as a positive control, showing that all the isolates in this study were pathogenic to maize, and the spore concentration and environmental conditions were sufficient to induce infection and TLB development.

Distribution of races of *E. turcicum* in Mexico

For the 40 isolates collected in 2019, only one isolate of race 2 was detected in the subtropical area of Jalisco, and seven isolates of race 3 were detected only in subtropical areas of Nayarit, Jalisco, and Guanajuato in the western part of Mexico (Table 4). Race 23 was the most frequently detected race and was found in all the examined states in Mexico. Race 3N was very rare, with only one isolate from the tropical area of Tamaulipas. Race 23N was the second most frequent race present in all states, except for tropical and subtropical areas of Sinaloa and Nayarit, whereas race 123N race was much rarer, being found only in the tropical areas of Sinaloa and Veracruz.

Between 2010 and 2019, there were 50 isolates collected at the CIMMYT-El Batán Experiment Station in Estado de México, which has a temperate environment (Table 5), and 50 isolates were collected at the CIMMYT-Agua Fría Experiment Station, which has a tropical environment (Table 6). In the temperate environment, race 23 was always the most common, varying from three to five isolates per year (Table 5). The other races were mostly found as single isolates per year, except for two isolates of 23N in 2019. In the tropical environment, race 23 was also dominant but less obviously than in the temperate environment, varying from two to four isolates per year (Table 6). Race 23N and particularly race 123N were more common at the tropical than at the temperate research stations. This could be significant as race 123N was virulent on all *Ht* genes used in this study. For the entire collection of 140 isolates, race 23 was the most prevalent (68%), followed by races 23N (15%), 123N (8%), 3 (6%), 2 (2%), and 3N (1%) (Tables 4, 5, and 6). To determine the susceptibility of the maize differentials, we substituted the resistance reaction (R) with the value 0 and the susceptibility reaction (S) with the value 1 (Tables 4, 5, and 6). The susceptibility of the maize differentials *Ht1*, *Ht2*, *Ht3*, and *HtN* to the 140 isolates was 3.5, 41.8, 44.1, and 10.6%, respectively.

Discussion

From a collection of 140 *E. turcicum* isolates from maize samples collected between 2010 and 2019, this study has shown that there are

Table 3. Designation of physiological races of *Exserohilum turcicum* based on the defense reaction on five maize differential genotypes with different *Ht* resistance genes

ID accession[a]	Differential	Mono gen *Ht*	Infection reaction	Reference
Ames 25219	A619Ht1	*Ht1*	Chlorotic lesions	Hooker (1963)
Ames 25220	A619Ht2	*Ht2*	Necrotic lesions without sporulation	Hooker (1977)
Ames 25221	A619Ht3	*Ht3*	Necrotic lesions without sporulation	Hooker (1981)
Ames 25371	B68HtN	*HtN*	Fewer and delayed lesions	Gevers (1975)
PI 587139	A619ht4	*ht4*	Chlorotic halo	Carson (1995)

[a] Differential lines were obtained from the Maize Genetics Cooperation Stock Center (USDA/ARS), University of Illinois, Urbana-Champaign.

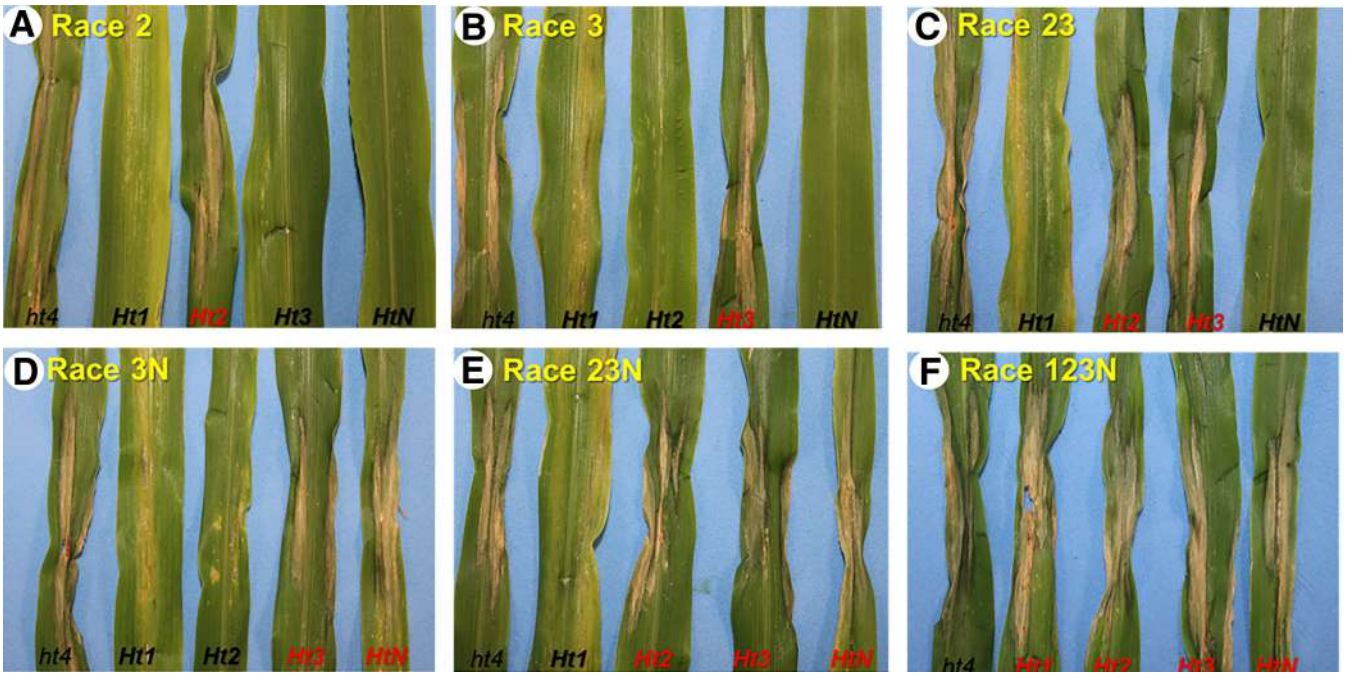


Fig. 2. Six physiological races of *Exserohilum turcicum* identified in Mexico. **A,** Race 2 (*Ht1*, *Ht3*, *HtN*/*Ht2*); **B,** race 3 (*Ht1*, *Ht2*, *HtN*/*Ht3*); **C,** race 23 (*Ht1*, *HtN*/*Ht2*, *Ht3*); **D,** race 3N (*Ht1*, *Ht2*/*Ht3*, *HtN*); **E,** race 23N (*Ht1*/*Ht2*, *Ht3*, *HtN*); and **F,** race 123N (0/*Ht1*, *Ht2*, *Ht3*, *NtN*). The *ht4* genotype lacking the *Ht* resistance genes was used as a positive control.

at least six physiological races in Mexico (2, 3, 23, 3N, 23N, and 123N). By comparison, Welz et al. (1993) used the same four maize differentials (*Ht1*, *Ht2*, *Ht3*, and *HtN*) and showed that races 23N, 23, and 2N of *E. turcicum* were present in Mexico. In this study, race 23 comprised 68% of the isolates, and race 23N comprised 15% of the isolates; this differs from Welz et al. (1993), who found race 23 comprised 14% of the isolates, and race 23N comprised 80% of the isolates. Thus, there may have been a shift over time, with race 23 becoming more common and race 23N becoming less common in Mexico.

Neither this study nor Welz et al. (1993) detected race 0 and 1, whereas studies in the United States and Canada have established that race 0 (which is avirulent to the four *Ht* differentials) and 1MN (which is virulent to the *Ht1*, *HtN*, and *HtM* differentials) are the dominant ones (Jindal et al. 2019; Weems and Bradley 2018). In Uganda, Adipala et al. (1993) examined 215 isolates, and only race 0 was present in the maize hybrids grown in that country. In China, 16 physiological races of *E. turcicum* have been previously identified, with the most frequent being races 0, 1, and 2 (Dong et al. 2008; Ma et al. 2020). In Brazil and Argentina, race 0 was most frequent (Navarro et al. 2021b). The predominance of race 0 in so many locations indicates that most of their commercial maize hybrids do not carry *Ht* genes, since variability of *E. turcicum* was not observed. Thus, the pathogen has not evolved towards greater virulence in those countries, which is contrary to Mexico, where variation in the virulence of isolates in this study from different states and environments was observed.

This study did not detect races with virulence to the *Ht1* differential except for race 123N (Hooker 1963). The *Ht2* and *Ht3* resistance genes are difficult to transfer in breeding programs (Welz and Geiger 2000). The instability of the *Ht2* resistance phenotype may be related to the influence of temperature and the presence of the inhibitor gene *Sht1*. The gene *Sht1* is epistatic to *Ht2*, and therefore resistance conferred by *Ht2* is considered oligogenic (Simcox and Bennetzen 1993). However, Yang et al. (2021) found that the *Ht2* and *Ht3* genes are identical and that the *HtN* gene is allelic to the *Ht2/Ht3* gene. This highlights that it is important to regularly validate the genetic existence of these genes using molecular markers to rule out any line misinterpretations that could occur over years during introgression. Wellhausen et al. (1951) postulated that Mexico, the center of maize's origin, could be the epitome of the genetic diversity of some maize pathogens. Based on random amplified polymorphic DNA markers, the highest amount of diversity for *E. turcicum* isolates were from Mexico, indicating that it may be the origin of the pathogen (Borchardt et al. 1998). That study also showed that there was a greater diversity of *E. turcicum* in tropical regions compared to temperate regions. However, this work showed a similar number of races in each of the 10 Mexican states examined, which included tropical, subtropical, and temperate regions. Further studies with a larger number of fungal isolates and regions may be required to resolve whether the diversity of *E. turcicum* races is affected by the environment within Mexico.

This study can guide maize genetic improvement programs in deciding which *E. turcicum* resistance genes should be introgressed into maize germplasms. For instance, the results of this study indicate that *Ht1* and *Ht2* should not be considered since they are no longer effective against the predominant *E. turcicum* races. In contrast, it would be better to introgress other resistance genes, such as *HtM*, *HtP*, and *HtNB* (Ogliari et al. 2005; Robbins and Warren 1993; Wang et al. 2012). These genes can induce some level of *E. turcicum* resistance in maize in Mexico, and if they become widely used, they could also be included in future investigations of the diversity of *E. turcicum* physiological races.

Identifying *E. turcicum* physiological races based on *Ht* differentials could open new areas of research on this pathogen. For instance, time course RNA sequence analysis of *E. turcicum* infecting maize and sorghum identified *AVRHt1* as a putative *E. turcicum* effector recognized by the maize resistance gene *Ht1* (Human et al. 2020). The recently published *E. turcicum* genome will make the identification of effector sequences easier (Cao et al. 2020). Mideros et al. (2018) used genome sequencing of isolates to identify the avirulence gene (*AVR*) *AVRHt1* in maize susceptible to *E. turcicum*

Table 4. Distribution of physiological races of *Exserohilum turcicum* obtained from maize producing states from Mexico during 2019

	Physiological races of *E. turcicum*[b]					
State[a]	**2**	**3**	**23**	**3N**	**23N**	**123N**
Tamaulipas	0	0	3	1	1	0
Sinaloa	0	0	3	0	0	2
Nayarit	0	2	3	0	0	0
Jalisco	1	1	2	0	1	0
Guanajuato	0	3	1	0	1	0
Estado de México	0	0	3	0	2	0
Hidalgo	0	0	2	0	3	0
Puebla	0	1	3	0	1	0
Veracruz	0	0	2	0	2	1
Chiapas	0	0	3	0	2	0
Total	1	7	25	1	13	3

[a] Five isolates were characterized from each of the 10 maize producing states in Mexico.
[b] To determine the reaction of the differentials *Ht*, we substitute the resistance reaction (R) with value 0 and the susceptibility reaction (S) with value 1.

Table 5. Number of *Exserohilum turcicum* isolates of each race found in CIMMYT–Experimental Station (El Batán, Estado de México) across years

	Physiological races of *E. turcicum*[b]					
Year[a]	**2**	**3**	**23**	**3N**	**23N**	**123N**
2010	0	0	5	0	0	0
2011	0	0	3	0	1	1
2012	0	0	3	0	1	1
2013	0	0	5	0	0	0
2014	0	0	5	0	0	0
2015	1	0	4	0	0	0
2016	0	0	5	0	0	0
2017	0	1	4	0	0	0
2018	0	0	4	0	1	0
2019	0	0	3	0	2	0
Total	1	1	41	0	5	2

[a] In each year, five isolates were obtained to represent the 2010 to 2019 history in the temperate environment within the CIMMYT-El Batan experimental station in the State of Mexico with a total of 50 isolates.
[b] To determine the reaction of the differentials *Ht*, we substitute the resistance reaction (R) with value 0 and the susceptibility reaction (S) with value 1.

Table 6. Number of *Exserohilum turcicum* isolates of each race found in CIMMYT–Experimental Station (Agua Fría, Veracruz) across years

	Physiological races of *E. turcicum*[b]					
Year[a]	**2**	**3**	**23**	**3N**	**23N**	**123N**
2010	1	0	4	0	0	0
2011	0	0	3	0	2	0
2012	0	0	3	0	0	2
2013	0	0	4	0	0	1
2014	0	1	4	0	0	0
2015	0	0	4	0	0	1
2016	0	0	2	0	2	1
2017	0	0	4	0	0	1
2018	0	0	4	0	1	0
2019	0	0	2	0	2	1
Total	1	1	34	0	7	7

[a] In each year, five isolates were obtained to represent the year 2010 to 2019 in the tropical environment within the CIMMYT-Agua Fría experimental station in the State of Veracruz with a total of 50 isolates.
[b] To determine the reaction of the differentials *Ht*, we substitute the resistance reaction (R) with value 0 and the susceptibility reaction (S) with value 1.

race 23N. Maize susceptible to *E. turcicum* race 1 was found to carry *AVRHt2*, *AVRHt3*, and *AVRHtN*. Sequencing the genomes of the races in this study would be useful in identifying effector genes.

Further information on the identification of the inheritance pattern for resistance genes and new sources of monogenic and polygenic resistance should be explored. For example, based on the discovery that the *HtN* gene identified in var. Pepitilla is of Mexican origin, CIMMYT has been able to identify new sources of TLB resistance in Mexico (unpublished). CIMMYT will make new TLB-resistant inbred lines freely accessible to the public. It is also important to do genetic inheritance studies by crossing lines of resistant maize with susceptible ones to identify if the source of resistance is qualitative or quantitative. Once the genetic inheritance is better understood and introgressed into a differential maize line, scientists may be able to identify new *E. turcicum* races. In addition, the interaction of *E. turcicum* with other maize pathogens of economic importance in Mexico, such as blights and rusts, also needs to be investigated.

When maize breeding against a pathogen is carried out without consideration of the pathogen's genetic diversity as in *E. turcicum*, there is a potential risk of resistance breakdown. This study has shown that race 123N was present in Mexico, which is virulent on maize with all the *Ht* genes used in this study. Thus, there is the potential for a widespread loss of resistance to *E. turcicum*, highlighting the need to incorporate other sources of *E. turcicum* resistance. Further, the planting of maize as a monoculture with specific resistance genes generates a selection pressure for the development of new races. With this scenario, plant breeders should consider incorporating resistance genes with quantitative inheritance patterns that will help reduce the chances of the appearance of more physiological races of *E. turcicum*.

Acknowledgments

We thank Carmen Velázquez of CORTEVA México, INIFAP's and MasAgro (SADER-CIMMYT) staff, who supported sample collection from different states. We also thank Dr. Pawan Singh for providing us with greenhouse space, M. C. Baldemar Santana for preparing the map, and Dr. Carlos De León for valuable technical advice.

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