

Interaction microenvironment - *Pantoea agglomerans* limits maize yield

La interacción microclima – *Pantoea agglomerans* limita el rendimiento de maíz

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SUMMARY

Pantoea agglomerans has been reported as the cause of chlorotic streaks on maize leaves in the Central High Valleys of México (CVHM), but there are no current data of how the infection of this new pathogen in Mexico affects production and yield of the crop. To understand the development of the disease in the crop, two experiments with a split-plot design and three replications were established during spring-summer 2010 in different microenvironments: Ayapango and Temamatla. Three cultivars of maize (a tri-linear HS2 hybrid, the single-cross hybrid Triunfo and the native Cacahuacintle) were evaluated with three *P. agglomerans* isolates. In the three maize cultivars, chlorotic streaks appeared on new leaves; at the ripening stage, the symptoms were less perceptible. The average incidence of plants with chlorotic streaks was higher in the town of Temamatla than in Ayapango, and in both microenvironments, the average degree of severity was less than 40%. Isolate A was the most virulent ($P < 0.0001$) on the three cultivars evaluated. These results will provide the basis for effective management of the disease under environmental conditions similar to those evaluated in this research.

Index words: chlorotic streaks, *Pantoea agglomerans*, maize disease, microclimate.

RESUMEN

Pantoea agglomerans ha sido reportada como la causa de estrías cloróticas en las hojas de maíz en los Valles Altos Centrales de México, aún no existen datos de incidencia o gravedad actuales para este nuevo patógeno en el cultivo. Para comprender el desarrollo de la enfermedad en el campo, se establecieron dos experimentos con un diseño de parcelas divididas y tres repeticiones durante el ciclo primavera-verano de 2010 en dos localidades: Ayapango y Temamatla. Se evaluaron tres cultivares de maíz (un híbrido tri-lineal HS2, el híbrido de cruza simple Triunfo y el Cacahuacintle nativo) con tres aislamientos de *P. agglomerans*. Los datos del progreso de la enfermedad se estimaron a partir de los datos de incidencia y severidad en el tiempo, y para indicar las diferencias significativas entre los tratamientos con un intervalo de confianza del $\alpha = 0.05$. En los tres cultivares de maíz aparecieron estrías cloróticas en las hojas nuevas; en la etapa de maduración, los síntomas fueron menos perceptibles. La incidencia promedio de plantas con estrías cloróticas fue mayor en la localidad de Temamatla que en Ayapango, y en ambos lugares, el grado promedio de severidad fue menor al 40%. El aislado A fue el más virulento ($P < 0.0001$) en los tres cultivares evaluados. Estos resultados proporcionarán la base para el manejo efectivo de la enfermedad en condiciones ambientales similares a las evaluadas en esta investigación.

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Palabras clave: *estrias cloróticas, Pantoea agglomerans, infección en maíz, microclima.*

INTRODUCTION

Growing human population demands intensive increment in agricultural activity to produce foods from plant origin. The impact of environmental stress and the appearance of diseases caused by fungal or bacterial species are the major constraints to production (Boyd *et al.*, 2013). Maize (*Zea mays* L.) is one of the crops most used worldwide: an average harvested area of 157 million hectares and production of 781 mega tons from 2000 to 2014. It is a vital source of food security in many countries in North and Latin America and Sub-Saharan Africa (Ramírez-Cabral *et al.*, 2017). México, with 8 million hectares of maize, contributes a production of 18 mega tons, of which 2.3 million tons are grown in the State of Mexico, the third largest producer of maize nationally, mostly by producers who farm ejidos or communal lands (Paulino-Flores *et al.*, 2017). These producers are identified as being particularly sensitive to negative impacts of global economic integration and market liberalization (Eakin, 2005).

Among the biotic factors, bacteria are an important group of phytopathogens that affect crop health and the phytosanitary quality of seed. The first bacterial attacks in maize were reported in 1949. Now, reports of diseases include several symptoms, which are shown in Table 1.

The causal agent of the diseases and chlorotic streak symptoms described above are Gram negative microorganisms from the Enterobacteriaceae family that have a Type III secretion system with the ability to export proteins involved in the process of colonizing the intercellular spaces of plant tissues and cause death (Collmer *et al.*, 2000). This secretion system has been reported in plant pathogens including *Pantoea stewartii* subsp *stewartii*, *Pseudomonas*, *Erwinia*, *Xanthomonas*, *Ralstonia* (Collmer *et al.*, 2000) and *Agrobacterium vitis* and has been associated with the production of exopolysaccharide (EPS), which causes vascular occlusion (Chalupowicz *et al.*, 2008). In *P. agglomerans* (Barash and Manulis-Sasson, 2007), because of the occlusions caused by EPS produced by this bacterium, symptoms such as chlorotic streaks, water-soaked necrotic spots and putrescence have been reported in microenvironments significantly different in terms of temperature, humidity and species of plants. Small or large climate variations and its correlation with bacterial diseases is a current global concern about the extent and importance of causes and of its effects that affect the incidence and prevalence of symptoms and loss of crops in a worldwide. Bacterial wilt, chlorotic streaks, necrosis are an important bacterial maize disease throughout the world from North to South America (Forgey *et al.*, 1982; Smidt and Vidaver, 1986; Valencia-Torres *et al.*, 2004; Albarracin-Orio *et al.*, 2012), Africa (Coutingo and Wallis, 1991), Asia (Hui-Ying *et al.*, 2011) and Europe (Krawczyk

Table 1. Reported diseases caused by bacterial attack in maize.

Disease	Causal agent	Reference
Striped disease	<i>Xanthomonas campestris</i> pv. <i>zeae</i>	Coutingo and Wallis, 1991
Goss's bacterial wilt	<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> (= <i>Corynebacterium michiganense</i> subsp. <i>nebraskense</i>)	Smidt and Vidaver, 1986
Bacterial wilt or Stewart's wilt	<i>Pantoea stewartii</i> (= <i>Erwinia stewartii</i>)	Forgey <i>et al.</i> , 1982
Holcus spot	<i>Pseudomonas syringae</i> pv. <i>syringae</i>	Kendrick, 1926
Bacterial foliar wilt	<i>Acidovorax avenae</i> subsp. <i>avenae</i> (= <i>Pseudomonas avenae</i>)	Johnson <i>et al.</i> , 1949
Bacterial stalk rot	<i>Dickeya chrysanthemi</i> f. sp. <i>zeae</i> (= <i>Erwinia chrysanthemi</i>)	Sah and Army, 1990
Bacterial stripe and leaf spot	<i>Burkholderia andropogonis</i> (= <i>Pseudomonas andropogonis</i>)	Vidaver and Carlson, 1978
Chocolate spot	<i>Pseudomonas syringae</i> pv. <i>coronafaciens</i>	Ribeiro <i>et al.</i> , 1977; White, 2004
Maize brown stalk rot	<i>Pantoea ananatis</i>	Goszczyńska <i>et al.</i> , 2007; Krawczyk <i>et al.</i> , 2010
Bacterial stripe and stem rot	<i>B. andropogonis</i>	Gijón-Hernández <i>et al.</i> , 2008
Bacterial wilt	<i>P. stewartii</i>	Valencia-Torres <i>et al.</i> , 2004
Foliar bacterial wilt	<i>A. avenae</i> subsp. <i>avenae</i>	CIMMYT, 2004
Brown stalk rot caused	<i>P. ananatis</i>	Gijón-Hernández <i>et al.</i> , 2008

et al., 2010) and with different cultivar as pearl millet (Frederickson et al., 1997), rice (Lee et al., 2010) and cotton (Medrano and Bell, 2007).

In Mexico, there are no reports of this disease or its symptoms, and because maize is the most important food source for the Mexican population, it is important to know how the disease manifested as chlorotic streaks caused by *P. agglomerans* affects maize production. This knowledge will enable the development of management programs. The aim of this work was to study how the bacteria *P. agglomerans* affects maize yield when it is inoculated in different environmental conditions on three maize cultivars adapted to two locations in the Central High Valleys of Mexico.

MATERIALS AND METHODS

Experimental Zone

Two experimental locations were established in the state of Mexico (Figure 1). Temamatla (19° 12' N and 98° 52' W), altitude of 2260 m above sea level, humidity of 45 - 70% and temperature of 17- 20 °C and the second location, Ayapango (19° 8' N and 98° 48' W), altitude of 2440 m above sea level, humidity of 50 - 90% and temperature of 15 - 18 °C. (SMN, 2012; INEGI, 2013).

Genetic Material

Three maize cultivars were used: the tri-linear HS2 hybrid (cross: CL12 × CL11 × CL7) from the Colegio de Postgraduados in Montecillo, Mexico; a simple-cross Triunfo hybrid (cross between lines L-10 and L-52) (Bravo-Quirino and Muñoz-González,

2003) from the National Institute of Research in Forestry, Agriculture and Fishing (INIFAP); and the native Cacahuacintle from CHVM. The two improved cultivars and native maize were developed at altitudes of 2200 to 2750 meters above sea level.

Experimental Units

A split-plot design with randomized complete blocks was used (Figure 2). The largest unit was represented by the maize cultivars, with subunits for each of the isolates. Each block consisted of 12 treatments with three replicates, resulting in a total of 36 experimental units. Each unit consisted of four 10-m-long rows 0.8 m apart and 0.2 m between plants. A fertilization formula of 160N-60P-00K was applied. Fieldwork was performed manually or with machinery, depending on the conditions in CHVM.

Origin of the Inoculum Source

The *P. agglomerans* bacterial isolates A, B and C were obtained from maize plants with symptoms of chlorotic streaks in the experimental fields of the Colegio de Postgraduados. These isolates were previously identified by sequencing the 16S rRNA gene. Molecular analysis of sequences showed that they were 99% similar to the sequences for the *P. agglomerans* cluster, while the control strain of *P. agglomerans* ATCC 27155 showed 98-99% similarity. Sequences were deposited in GenBank (Accession Nos. EF050806 to EF050810). The isolates were maintained at -80 °C in a solution of 25% glycerol (Morales-Valenzuela et al., 2007; Silva-Rojas et al., 2007).

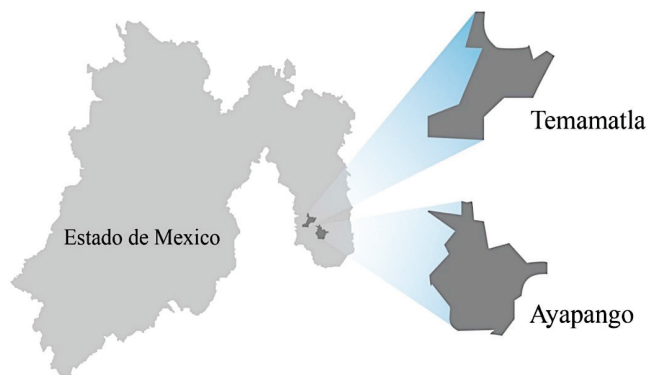


Figure 1. Geographic location of the experimental maize fields in the State of Mexico, during the 2010 spring-summer cycle.

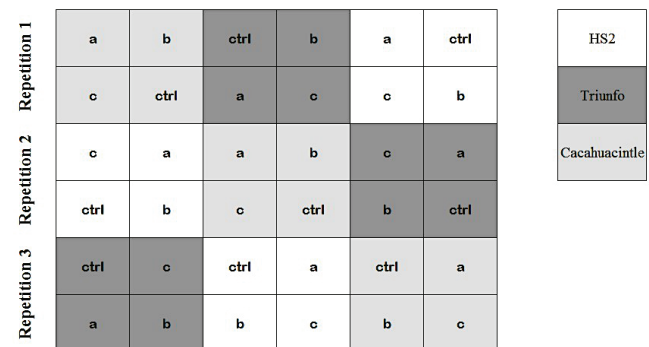


Figure 2. Model of the split-plot design with randomized complete blocks used. a,b,c means the *P. agglomerans* bacterial isolates. Ctrl means controls and colors means the cultivars.

Activation and Inoculum Preparation

To activate the bacteria, each isolate was seeded with the cross-streak method over CPG (Bioxon®, México) (10 g casamino acid, 10 g peptone, 10 g glucose and 18 g agar) and the cells were grown at 28 °C. After 48 h, pale yellow colonies characteristic of *P. agglomerans* were observed. The isolates of *P. agglomerans* were inoculated in chalk-agar culture medium (10 g yeast extract, 20 g glucose, 20 g calcium carbonate and 17 g agar). The isolates were incubated at 28 °C for 48 h. For each isolate, a bacterial suspension of 10^8 CFU mL⁻¹ was prepared using sterile distilled water (Schaad *et al.*, 2001).

Inoculation of Maize Seedlings with *P. agglomerans*

For each experimental unit, 10 maize plants from the central rows were selected. *P. agglomerans* inoculation was performed on maize plants with six true leaves (phenological stage V3) (CIMMYT, 2012) 30 days after sowing by the injection method on the stem 2 cm above the ground (Hui-Ying *et al.*, 2011; Lamka *et al.*, 1991). One mL of bacterial suspension with 10^8 CFU was injected. As the negative control, 1 mL of sterile distilled water was used. At the end of the experiment, leaves with symptoms were collected for each strain. Koch's postulates were validated in maize plants inoculated with *P. agglomerans*.

Measuring Disease Progress in Maize

After harvesting and based on the presence of chlorotic streaks in inoculated maize leaves, the yield

of each treatment was recorded by location, cultivar and isolates. For each treatment ear weight, width and length were evaluated, as well as grain number and weight.

Statistical Analysis

The effect of the disease on yield was determined per treatment and analysed statistically with SAS V9.3 software (SAS, 2014). The program design was developed for a split-plot experimental design (Littell *et al.*, 2006) with the following model:

$$y_{ijk} = \mu + \text{Blo}_j + \alpha_i + \beta_k + (\alpha\beta)_{ik} + \eta_{ij} + e_{ijk}$$

where y_{ijk} is the response variable associated with level k of the factor in the small plot within the large plot in the j^{th} block that received the i^{th} level applied to the large plot, Blo_j is the effect of the j^{th} block, α_i is the main effect of the i^{th} level of the factor applied to the large plot (A), β_k is the main effect of the k^{th} level of the factor applied to the small plot (B), $(\alpha\beta)_{ik}$ is the interaction between factor A and factor B, η_{ij} is a common random effect for all subplots in the large plot (i, j) and e_{ijk} is a random component particular to the subplot with the k^{th} level of the small plot in the largest plot (i, j). Significant differences were analysed with ANOVA and the Tukey test with $P < 0.05$.

RESULTS AND DISCUSSION

Chlorotic streaks in the inoculated plants were observed 8 days after inoculation. Figure 3 shows the

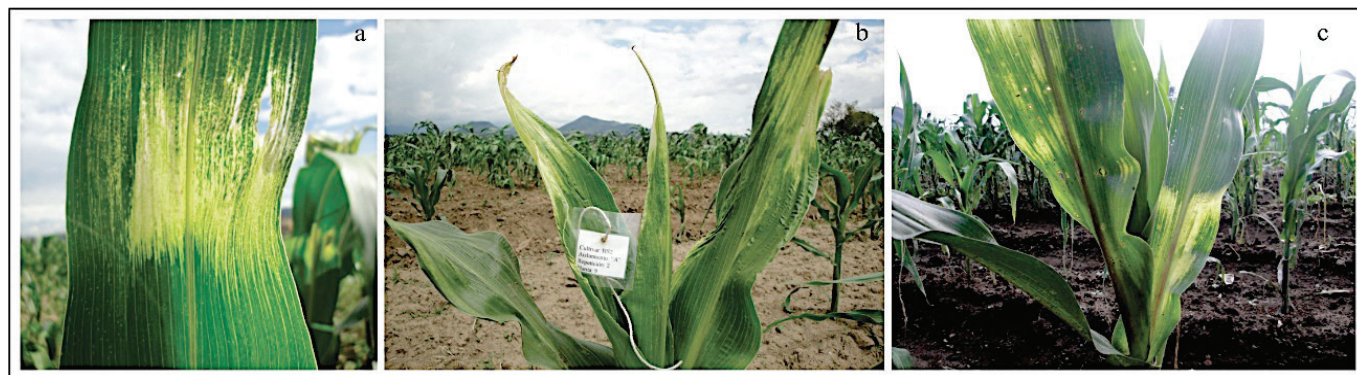


Figure 3. Symptoms of chlorotic streaks on leaves of Triunfo hybrid maize in the experimental fields of Temamatla and Ayapango, México, during the 2010 spring-summer cycle: a) development of chlorotic streaks on the edges of the leaves parallel to the midrib; b) development of chlorotic streaks in the middle layer of the leaves; c) development of chlorotic streaks on the edges and shortening of the leaves in the middle lamella.

development of chlorotic streaks parallel to the midrib of the leaf, located on the edge, the main ridge, the apex of the leaf blade and near the ligule. Dispersed aqueous stains, tissue contraction in the affected area, undulations in the area with chlorotic streaks, shortening of leaves, curl of the apex of the leaf blade and, in advanced stages of the disease, necrosis and loosening of the leaf blade were also observed.

Chlorotic Streak Symptoms on Maize Plant

Figure 4 shows the cumulative incidence of plants with chlorotic streaks by location after 7 days of inoculation, the percentage of infected plants was less than 15%. For both microenvironments, the largest number of plants with chlorotic streaks was observed between 7 and 14 days after inoculation. The percentage of plants with chlorotic streaks was 92% in Temamatla and 70% in Ayapango. Both microenvironments showed significant differences among isolates ($P < 0.0001$) and over time ($P < 0.0001$). Differences in incidence were caused by different isolates: in Ayapango isolate A was highly virulent, and in Temamatla isolate B was more virulent.

Maize Yield in Plants with Chlorotic Streaks

Symptoms of chlorotic streaks were observed in 60% of inoculated plants (Figure 5). In Ayapango, the

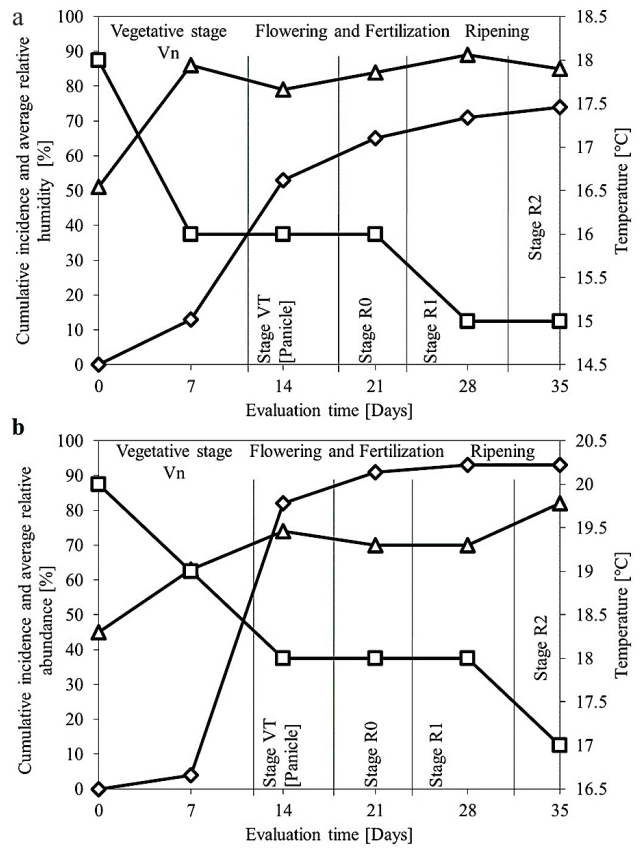


Figure 4. Advance of lesion in leaves of maize plants caused by inoculation of the bacteria in two locations of the Central High Valleys of Mexico. a: Ayapango and b: Temamatla. \diamond : incidence; \square : average temperature; Δ : average relative humidity.

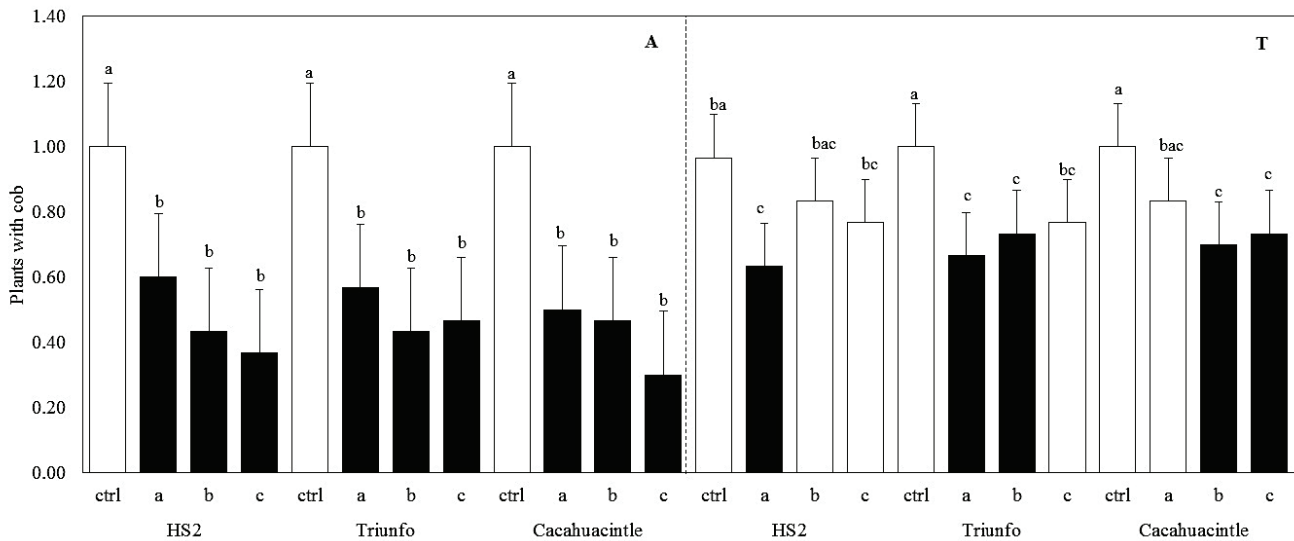


Figure 5. Percent of plants with chlorotic streaks and fruit development in two locations A (Ayapango) and T (Temamatla) and three isolate a, b, c.

number of plants with fruit was lower in all the cultivars and for all the isolates ($P < 0.0001$). In Temamatla; all cultivars were highly susceptible, but to different isolates: HS2 to *P. agglomerans* isolate A; Triunfo to *P. agglomerans* isolate A and B and Cacahuacintle to *P. agglomerans* isolates B and C (Figure 5 and Table 2).

By location, in Ayapango, the cultivar and isolate effects on the variables cob length, width and weight were significant. Grain number and weight were influenced by the interaction cultivar * isolate. In Temamatla, the cultivar-isolate interaction affected all the yield variables (Table 2).

Average size of ears on plants with chlorotic streak was smaller, relative to the control: width 5.2 ± 0.57 cm, length 14.80 ± 3.07 cm and weight 175.73 ± 62.65 g. Plants without inoculation with *P. agglomerans* (controls) had ears 5.32 ± 0.46 cm wide, 15.77 ± 2.51 cm long, weighing 195.20 ± 55.29 g. The average values of grain number and weight, were 405.82 ± 116.38 and 136.38 ± 50.27 g, significantly lower than the control: 443.12 ± 110.21 and 162.21 ± 49.26 g.

As shown in Table 3, the cultivar-isolate interaction was significant in all variables. In both locations, cultivar HS2 and Cacahuacintle had the smallest dimensions. Ear width was smallest in Ayapango with

the interaction location-isolate C and in Temamatla with the cultivar Triunfo and isolate A, and in both locations the interaction Cacahuacintle-isolate C was significant.

The Central Valleys of the State of Mexico are notable for their production of maize (500 000 ha), 80% of which is rainfed. Climate in this region is temperate and humid, with an average annual temperature of 16.5 °C, relative humidity of 85%, and annual rainfall of 800-900 mm, at an altitude of 2200 meters above sea level (SMN, 2012). These conditions are favourable for maize cultivation but allow the development of bacterial diseases, such as chlorotic streaks on maize leaves caused by *P. agglomerans*.

In this research, during the period of evaluation of chlorotic streaks, average humidity and temperature in Ayapango and Temamatla were 45% and 85% and $16-19$ °C, respectively. Under these conditions, the incidence of maize plants with chlorotic streaks increased gradually in the three cultivars of maize (Figure 3). Differences between both locations (Temamatla and Ayapango) during the evaluation were found. In Temamatla the humidity was lower than Ayapango (70 and 90% respectively) and the temperature was 2 °C higher in Temamatla.

Table 2. Effect of inoculating *P. agglomerans* on the yields of cob and grain by location and cultivars.

Effect	Plants with cob	Cob			Grain	
		Width	Length	Weight	Number	Weight
CHVM						
Location	<0.0001	<0.0001	0.8985	<0.0001	0.116	0
Cultivar	0.956	<0.0001	0.002	<0.0001	<0.0001	<0.0001
Isolate	0.587	0.006	<0.0001	<0.0001	<0.0001	<0.0001
Location*Cultivar	0.739	0.335	0.748	0.141	0.378	0.294
Location*Isolate	0.22	0.604	0.537	0.019	0.99	0.165
Location*Cultivar*Isolate	0.672	0.419	0.243	0.107	0.283	0.183
Cultivar*Isolate	0.866	0.0382	0.015	<0.0001	0.002	<0.0001
Ayapango						
Cultivar	0.818	<0.0001	0.025	<0.0001	<0.0001	<0.0001
Isolate	0.268	0.18	0.002	0.003	0.012	0.005
Cultivar*Isolate	0.939	0.153	0.264	0.157	0.022	0.03
Temamatla						
Cultivar	0.894	<0.0001	0.05	<.0001	<.0001	0
Isolate	0.775	0.0308	0.007	<0.0001	0.003	<0.0001
Cultivar*Isolate	0.418	0.1227	0.008	<0.0001	0.022	0

Table 3. Effect of inoculation of three *P. agglomerans* isolates on ear and grain yields by location and cultivar.

Cultivar	Isolate	Cob			Grain	
		Width	Length	Weight	Number	Weight
		----- cm -----			----- g -----	
Ayapango						
HS2	ctrl	4.96 ± 0.34 dc [†]	15.18 ± 1.67 ba	176.49 ± 32.71 bc	480.50 ± 87.79 bc	155.94 ± 28.68 bac
	a	5.00 ± 0.46 dc	15.62 ± 1.79 ba	191.81 ± 47.28 bac	481.00 ± 102.97 bdc	144.10 ± 41.20 ba
	b	5.01 ± 0.37 d	14.82 ± 1.88 ba	181.49 ± 49.85 bac	454.47 ± 75.41 dc	148.32 ± 35.27 bc
	c	4.83 ± 0.42 d	14.16 ± 2.63 bc	170.73 ± 47.30 bc	451.69 ± 100.77 dc	148.61 ± 45.54 bac
Triunfo	ctrl	5.48 ± 0.54 ba	16.69 ± 2.80 a	225.14 ± 71.23 a	557.70 ± 145.85 a	185.03 ± 60.52 a
	a	5.45 ± 0.75 bac	15.08 ± 3.70 bc	215.74 ± 113.84 ba	446.71 ± 165.43 dc	129.61 ± 72.07 bc
	b	5.54 ± 0.45 a	15.92 ± 3.37 ba	223.43 ± 57.64 a	561.25 ± 137.12 ba	170.16 ± 45.73 a
	c	5.51 ± 0.34 ba	15.35 ± 3.30 ba	194.97 ± 58.20 bac	504.22 ± 169.37 bac	137.09 ± 49.47 bac
Cacahuacintle	ctrl	5.52 ± 0.49 a	15.44 ± 3.07 ba	183.96 ± 61.93 bac	291.17 ± 96.99 e	145.67 ± 58.60 bac
	a	5.31 ± 0.78 bac	14.73 ± 3.46 ba	161.61 ± 76.82 c	278.39 ± 94.99 e	127.14 ± 66.62 bc
	b	5.20 ± 0.58 bdc	15.00 ± 3.18 bc	152.29 ± 64.62 c	255.13 ± 101.10 e	107.08 ± 59.60 dc
	c	4.91 ± 1.02 dc	12.07 ± 4.37 b	89.56 ± 48.34 d	219.55 ± 100.33 e	115.34 ± 36.90 d
Temamatla						
HS2	ctrl	4.63 ± 0.69 b	14.84 ± 2.25 ba	150.75 ± 57.59 dc	480.50 ± 155.89 bc	140.45 ± 48.18 bcd
	a	4.56 ± 0.32 b	14.32 ± 3.57 b	144.93 ± 61.05 dce	481.00 ± 141.58 c	125.11 ± 56.88 fecd
	b	4.69 ± 0.33 ba	15.11 ± 2.73 ba	164.35 ± 50.30 c	454.47 ± 126.43 bc	140.20 ± 45.58 bcd
	c	5.24 ± 2.05 ba	15.29 ± 3.07 b	187.00 ± 61.71 bc	451.69 ± 112.36 bc	154.23 ± 61.11 bc
Triunfo	ctrl	11.18 ± 31.39 a	32.25 ± 81.34 a	224.31 ± 58.34 a	557.70 ± 158.67 a	182.52 ± 56.81 a
	a	4.90 ± 0.65 ba	13.53 ± 2.70 b	121.91 ± 64.92 dfe	446.71 ± 160.22 c	101.18 ± 59.78 gf
	b	5.03 ± 0.73 ba	16.65 ± 3.15 a	193.90 ± 51.79 ba	561.25 ± 123.64 ba	151.98 ± 62.07 ba
	c	4.87 ± 1.03 ba	13.80 ± 3.51 b	142.60 ± 51.54 dce	504.22 ± 141.00 c	113.64 ± 51.85 gfeed
Cacahuacintle	ctrl	5.10 ± 0.42 ba	15.07 ± 2.46 ba	159.43 ± 54.62 c	291.17 ± 75.12 d	137.44 ± 48.14 becd
	a	4.84 ± 0.62 ba	14.57 ± 2.75 b	127.00 ± 49.62 dfe	278.39 ± 77.18 d	110.92 ± 45.34 gfe
	b	4.70 ± 0.77 ba	13.41 ± 2.56 b	101.87 ± 52.56 f	255.13 ± 89.59 d	89.82 ± 44.24 g
	c	4.68 ± 0.42 ba	13.86 ± 3.75 b	112.62 ± 57.24 fe	219.55 ± 111.33 d	111.35 ± 73.50 gfed

[†] Averages in rows marked with different letters indicate statistically significant differences. Tukey ($P \leq 0.05$).

These findings probably had influence in the incidence of plants with chlorotic streaks. Probably the bacteria requires a lower humidity to express its pathogenicity.

Morales-Valenzuela *et al.* (2007) found similar results when they inoculated maize and sorghum seedlings with *P. agglomerans* under greenhouse conditions. Frederickson *et al.* (1997) found that at 25 °C and 95% relative humidity, different isolates of *P. agglomerans* caused necrosis on the leaves and edges of pearl millet (*Pennisetum glaucum* (L.) R. Br.).

Similarly, Sabaratnam and Beattie (2001) reported that when maize plants are grown in relative humidity below 95%, colonisation of *P. agglomerans* in leaf tissue increases. However, when maize plants are

exposed to 45% relative humidity, colonisation decreases drastically.

In the two locations, maize yield was affected by *P. agglomerans* when relative humidity was 85%. Differences of ± 3 °C in temperature caused lower yield compared with the controls. The affinity of *P. agglomerans* to the cultivar Cacahuacintle is probably due to the high concentration of carbohydrates, of which more than 73% is amylopectin (Carballo *et al.*, 2010), in contrast to cultivar HS2 and Triunfo. Loss of grain weight of the cultivar Cacahuacintle reduces productivity by 1.9 Mg ha⁻¹ meaning economic repercussions for the producers (Télez-Silva *et al.*, 2016).

CONCLUSIONS

Symptom development was mainly observed in maize leaves during vegetative growth in the three cultivars. The percentage of diseased plants exhibited behaviour characteristic of bacterial diseases: stage Vn was the most affected and in stage R2 the disease stabilised. This behaviour correlated with temperature and relative humidity in the CHVM during the spring-summer 2010 crop season. The three evaluated maize cultivars were susceptible to the three isolates of *P. agglomerans*. Disease symptoms correlated with loss of ear and grain yield, but posterior effects of these symptoms on yield is still unknown. Therefore, it is necessary to correlate these symptoms with maize growth and yield. This is the first report in Mexico that describes the infection of *P. agglomerans* and how the chlorotic streak symptoms can vary in two similar microenvironments

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Disclosure Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

- Albarracin-Orio, A. G., E. Brücher, M. C. Plazas, P. Sayago, F. Guerra, R. De Rossi, D. A. Ducasse, and G. D. Guerra. 2012. First report of Stewart's wilt of maize in Argentina caused by *Pantoea stewartii*. *Plant Dis.* 96: 1819.3.
- Baird, R. E., R. D. Gitaitis, and G. A. Herzog. 1997. First report of cotton lint rot by *Pantoea agglomerans*.
- Barash, I. and S. Manulis-Sasson. 2007. Virulence mechanisms and host specificity of gall-forming *Pantoea agglomerans*. *Trends Microbiol.* 15: 538-545.
- Boyd, A., C. Ridout, D. M. O'Sullivan, J. E. Leach, and H. Leung. 2013. Plant-pathogen interactions: disease resistance in modern agriculture. *Trends Gen.* 29: 233-240.
- Bravo-Quirino, F. y F. Muñoz-González. 2003. Maíz triunfo híbrido de doble propósito para valles altos, México. ICAMEX. Metepec, Edo. de México, México.
- Carballo, C. A y M. E. Ramírez. 2010. Manual gráfico para la descripción varietal en maíz. CP-SNICS. México, D. F.
- Chalupowicz, L., S. Manulis-Sasson, M. Itkin, A. Sacher, G. Sessa, and I. Barash. 2008. Quorum-sensing system affects gall development incited by *Pantoea agglomerans* pv. *gypsophylae*. *Mol. Plant Microbe Interact.* 21: 1094-1105. doi:10.1094/MPMI -21-8-1094.
- CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo). 2004. Maize diseases: a guide for field identification. CIMMYT. México, D. F.
- Collmer, A., J. L. Badel, A. O. Charkowski, W. L. Deng, D. E. Fouts, A. R. Ramos, A. H. Rehm, D. M. Anderson, O. Schneewind, K. V. van Dijk, and J. R. Alfano. 2000. *Pseudomonas syringae* Hrp type III secretion system and effector proteins. *PNAS* 97: 8770-8777.
- Coutingo, T. A. and F. M. Wallis. 1991. Bacterial streak disease of maize (*Zea mays* L.) South Africa. *J. Phytopathol.* 133: 112-120.
- Eakin, H. 2005. Institutional change, climate risk, and rural vulnerability: Cases from Central Mexico. *World Developm.* 33: 1923-1938. doi: 10.1016/j.worlddev.2005.06.005.
- Forgey, W., M. H. Blanco, L. L. Darrach, and M. S. Zumber. 1982. Prediction of Stewart's wilt disease in single and three way crosses in maize. *Plant Dis.* 66: 1159-1162.
- Frederickson, D. E., E. S. Monyo, S. B. King, and G. N. Odvody. 1997. A disease of pearl millet in Zimbabwe caused by *Pantoea agglomerans*. *Plant Dis.* 81: 959. doi: 10.1094/PDIS.1997.81.8.959D.
- Gijón-Hernández, A., D. Téliz-Ortiz, E. Cárdenas-Soriano, C. de León, A. Mora-Aguilera, A., D. Mejía-Sánchez, and R. de la Torre-Almaraz. 2008. Leaf stripe and stem rot caused by *Burkholderia gladioli*, a new disease of maize in México. *Plant Dis.* 92: 1249.
- Goszczyńska, T., W. J. Botha, S. N. Venter, and T. A. Coutinho, T. A. 2007. Isolation and identification of the causal agent of brown stalk rot, a new disease of maize in South Africa. *Plant Dis.* 91: 711-718.
- Hui-Ying, C., Z. Li-Qun, Z. Zhen-Dong, W. Xiao-Ming, L. Hong-Jie, and R. Zheng-Guang, R. 2011. Cloning of a pathogenicity gene *yhfk* from *Pantoea agglomerans* on maize inbred line PS056. *Sci. Agric. Sinica* 44: 299-306.
- INEGI (Instituto Nacional de Estadística y Geografía). 2013. Geografía, México. <http://www3.inegi.org.mx/sistemas/mexicocifras/default.aspx?e=13>. (Consulta: septiembre 3-4, 2010).
- Johnson, A. G., A. L. Robert, and L. Cash. 1949. Bacterial leaf blight and stalk rot of maize. *J. Agric. Res.* 78: 719-732.
- Kendrick, J. B. 1926. Holcus bacterial spot of *Zea mays* and *Holcus species*. *Research Bulletin* (Iowa Agriculture and Home Economics Experiment Station) 7: 303-334.
- Krawczyk, K., J. Kamasa, A. Zwolinska, and H. Pospieszny. 2010. First report of *Pantoea ananatis* associated with leaf spot disease of maize in Poland. *J. Plant Pathol.* 92: 807-811.

- Lamka, G. L., J. H. Hill, D. C. McGee, and E. J. Braun. 1991. Development of an immunosorbent assay for seedborne *Erwinia stewartii* in maize seeds. *Phytopathology* 81: 839-846.
- Lee, H. B., J. P. Hong, and S. B. Kim, S. 2010. First report of Leaf blight caused by *Pantoea agglomerans* on rice in Korea. *Plant Dis.* 94: 1372.2.
- Littell, R. C., W. W. Stroup, G. A. Milliken, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for mixed models. SAS Institute Inc. Cary, NC, USA.
- Medrano, E. G. and A. A. Bell. 2007. Role of *Pantoea agglomerans* in opportunistic bacterial seed and boll rot of cotton (*Gossypium hirsutum*) grown in the field. *J. Appl. Microbiol.* 102: 134-143.
- Morales-Valenzuela, G., H. Silva-Rojas, D. Ochoa-Martínez, E. Valadez-Moctezuma, B. Alarcon-Zuniga, L. X. Zelaya-Molina, L. Córdova-Téllez, L. Mendoza-Onofre, H. Vaquera-Huerta, and A. Carballo-Carballo. 2007. First report of *Pantoea agglomerans* causing leaf blight and vascular wilt in maize and Sorghum in Mexico. *Plant Dis.* 91: 1365.
- Paulino-Flores, M., A. R. Martínez-Campos, F. E. Martínez-Castañeda, C. A. López-Orona, I. Vizcarra-Bordi, and N. Munguía. 2017. Evaluation of the sustainability of hybrid and native maize production system. *J. Cleaner Product.* 150: 287-293. doi: 10.1016/j.jclepro.2017.02.182.
- Ramírez-Cabral, N. Y. Z., L. Kumar, and F. Shabani. 2017. Global alterations in areas of suitability for maize production from climate change and using a mechanistic species distribution model (CLIMEX). *Sci. Rep.* 7: 5910. doi:10.1038/s41598-017-05804-0.
- Ribeiro, R. de L. D., R. D. Durbin, D. C. Army, and T. F. Uchytel. 1977. Characterization of the bacterium inciting chocolate spot of maize. *Phytopathology* 67: 1427-1431.
- Sabaratnam, S. and G. A. Beattie. 2001. Differences in the leaf colonization pattern of two bacterial species. *Phytopathology* 91: S78.
- Sah, D. N. and D. C. Army. 1990. Susceptibility of maize germplasm to bacterial stalk rot caused by *Erwinia chrysanthemi* pv. *zeae*. *Trop. Pest Manage.* 36: 154-156.
- SAS Institute. 2014. SAS-STAT® 9.3 user's guide. SAS Institute Inc. Cary, NC, USA.
- Schaad, N. W., J. B. Jones, and W. Chun. 2001. Laboratory guide for identification of plant pathogenic bacteria. APS Press. St. Paul, USA. [Internet]. Available from: <https://www.cabdirect.org/cabdirect/abstract/20013064240>. (Consulta: septiembre 3-4, 2010).
- Silva-Rojas, H. V., A. Farfán-Gómez, B. Alarcon-Zuniga, D. L. Ochoa-Martínez, and L. X. Zelaya-Molina. 2007. *Pantoea agglomerans* causing wilt and leaf spots in *Zea mays* in the Central Highland Valley of Mexico. *Phytopathology* 97: S108.
- Smidt, M., and A. K. Vidaver. 1986. Population dynamics of *Clavibacter michiganense* subsp. *nebraskense* in field-grown dent maize and popcorn. *Plant Dis.* 70: 1031-1036.
- SMN (Servicio Meteorológico Nacional). 2012. Temperatura y precipitación [Internet]. Available from: <http://smn.conagua.gob.mx> (Consulta: septiembre 3-4, 2010).
- Téllez-Silva, J. M., F. Herrera-Tapia, I. Vizcarra-Bordi y J. J. Ramírez-Hernández. 2016. El maíz Cacahuacintle y su potencial para el desarrollo endógeno: el caso de Santa María Nativitas. *Espacialidades* 6: 168-191.
- Valencia-Torres, N., M. Mezzalama, S. G. Leyva-Mir, and D. P. Jeffers. 2004. Detection of maize bacterial wilt, *Pantoea stewartii* (smith) mergaert, verdonck and kersters, in the central valley of Mexico. *Rev. Mex. Fitopatol.* 22: 308-314.
- Vidaver, A. M. and R. R. Carlson. 1978. Leaf spot of field maize caused by *Pseudomonas andropogonis*. *Plant Dis.* 62: 213-216.