



## Title: Identification of sources of resistance in tomato to *Phytophthora infestans* at Mexico

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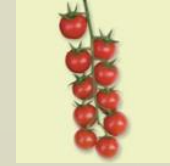
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# INTRODUCTION

## 1. Importance:



Red tomato (*Solanum lycopersicum L.*):

World: In 2020 China, Continental was the main tomato producer in the world with 64,768,158 tons (34.7%), followed by India with 20,573,000 tons (11.0%) and Turkey with 13,204,015 tons (7.1%), so these 3 nations represented 52.7% of world production.

It occupies the second place in world importance among vegetables, overcome by the potato (*Solanum tuberosum*).

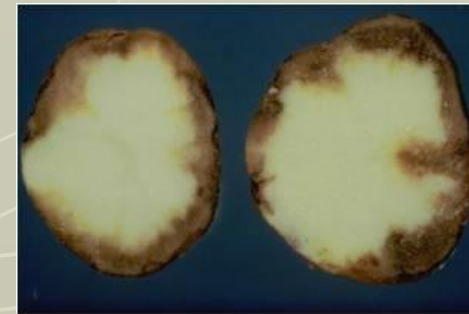
In 2020, Mexico produced more than 3.27 million tons in an area of more than 45,000 hectares.

Mexico: it is considered the second horticultural species with the largest cultivated area and the main vegetable due to high production.

## Problemática:

- Plagues and diseases
- *Phytophthora infestans* Mont De Bary:
- Phyto=plant      phthora=destroyer
- Belonging to the group of Oomycetes. Kingdom Chromista (P.C.= cellulose diploid, mycellium without septa, bisexual)
- 1845 y 1847: Famine in Ireland (died of > 1 '000,00 personas)

• It Affects:



## Environmental conditions:

Common in temperate and humid áreas

It can be devastating during the rainy season when increases humidity

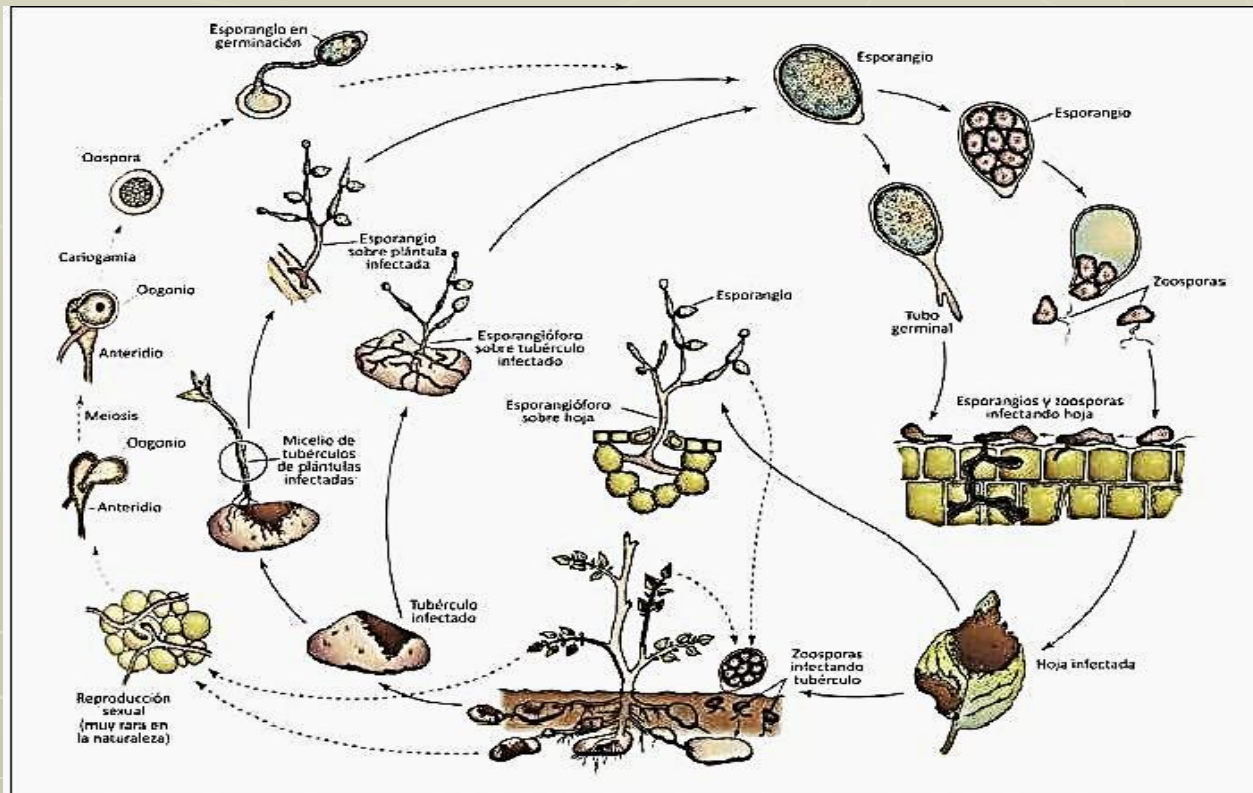


- Development Temperature = 21 °C

- Humidity: 80-100%

- Esporangia germinate at temperatures of 12 a 16°C

- Esporangia: 10 a 20 zoosporas



## ***Phytophthora infestans*:**

Sexual and Asexual Reproduction:

Sexual: Heterothallic organism with 2 types of mating (A1 and A2)

A1 x A2 = High genetic variability

### **Control Alternatives:**

Genetic Breeding:

Generation of resistant varieties

Use of wild species

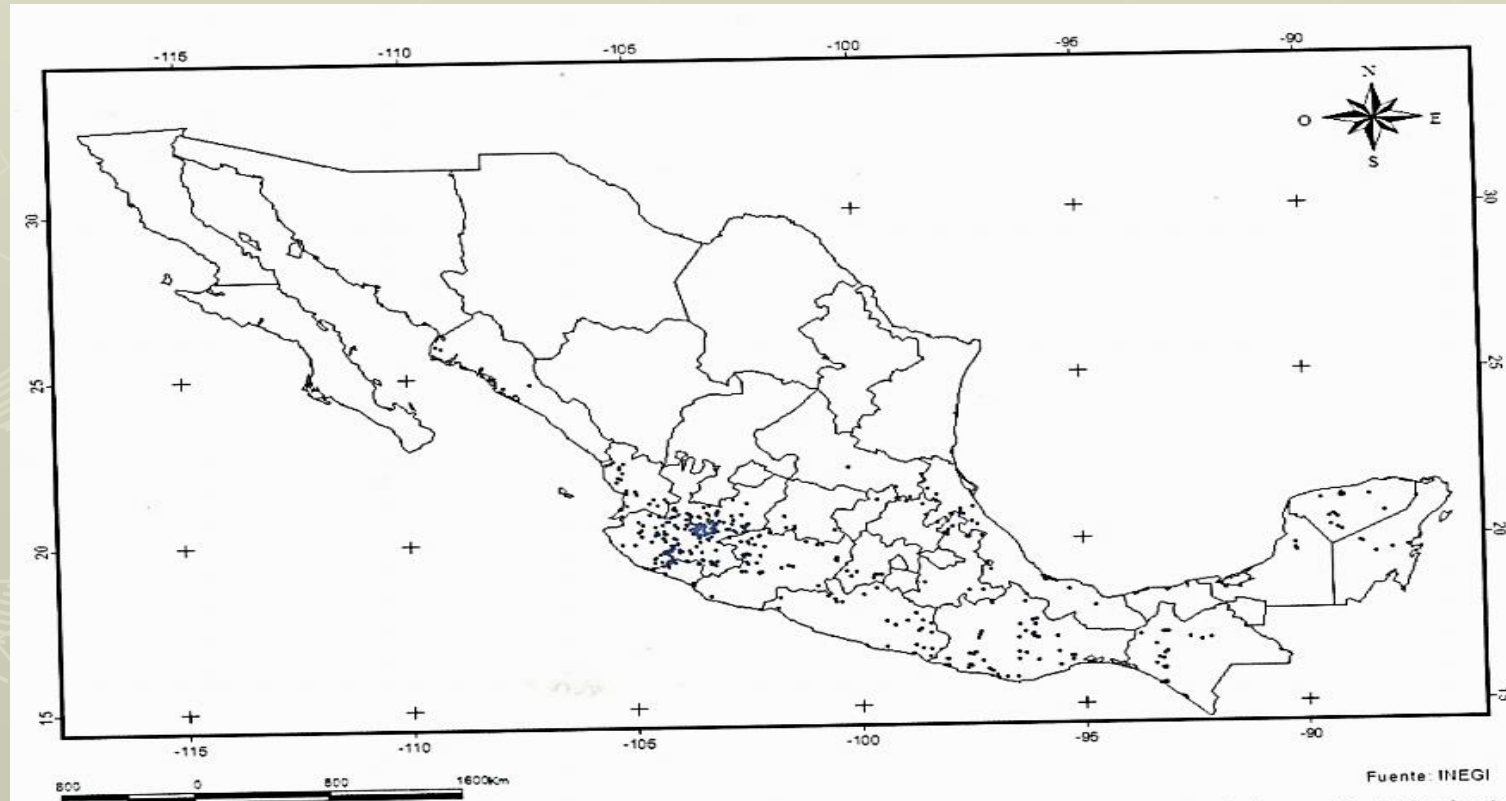
•Five genes in *S. pimpinellifolium* : *Ph1*, *Ph2*, *Ph3*, *Ph4* y *Ph5* (Moreau *et al.*, 1998; Chunwongse *et al.*, 1998; Kole *et al.*, 2006; Foolad *et al.*, 2008).

•*Solanum habrochaites* : Accession Resistance LA1033 (Scott y Gardner, 2007) and LA1777 (Li *et al.*, 2011);

•*L. pennellii* quantitative resistance was found (Smart *et al.*, 2007).

## ***Solanum lycopersicum* var. *cerasiforme*:**

- Greater dispersión: Altitudes de 0 y 1,200 m (Sánchez *et al.*, 2006, Álvarez *et al.*, 2009)
- Investigation reports : Distribución Sinaloa to Península de Yucatán (Chávez *et al.*, 2011)



## OBJECTIVE

To identify sources of resistance to *P. infestans* among three wild genotypes of tomato (*Solanum lycopersicon* var. *cerasiforme* (Dunal) Spooner, Anderson and R.K Jansen) collected in Mexico and nine advanced lines, through exposure to natural infections and artificial infections with the use of strains from the Valley of Mexico



# **MATERIALS and METHODS**





## Study Site:

Experimental area of Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) de la Universidad de Guadalajara.

Located in Las Agujas municipality of Zapopan, Jalisco, Mexico at  $20^{\circ} 44' 44''$  N latitude,  $103^{\circ} 54' 62''$  W longitude and altitude of 1,567 msnm, with a semi-dry temperate climate, and average annual temperature of  $23.5^{\circ}\text{C}$  and an average annual rainfall of 906 millimeters with a rainy season from June to October



## Línes Used

Population/Variety	Type Material	State and Country
V115	wild population	Veracruz, México
319	wild population	Nayarit, México
327	wild population	Nayarit, México
1-1	pre-improved	Jalisco, México
1-12	pre-improved	Jalisco, México
2-14	pre-improved	Jalisco, México
2-29	pre-improved	Jalisco, México
3-1	pre-improved	Jalisco, México
3-3	pre-improved	Jalisco, México
3-6	pre-improved	Jalisco, México
3-31	pre-improved	Jalisco, México
3-33	pre-improved	Jalisco, México
Río Grande	Commercial Variety	Jalisco, México
San Marzano	Commercial Variety	Jalisco, México
LA2533	TGRC*	Lima/Peru

\*TGRC: Tomato Genetics Resource Center



# I. Exposure to natural infections : Evaluation of 3 wild genotypes and 9 pre-improved materials



EXP. I: Soil with background tomato planting



EXP. II.: Soil exclusive use of corn



EXP. III.: Área Greenhouse

## Measured Variables:

Presence of Late blight months: August, September and October:

For 2012 there were minimum temperatures of 15.7, 14.6 and 12.7 °C, and relative humidity of 77, 84 and 81% respectively.

Plot: Visual readings every 7 days. Using International Potato Center Scale (CIP).

For each cycle, the final evaluation of the damage to the foliage was made when the Río Grande and San Marzano varieties reached 95% of the leaf area damaged by *P. infestans* (Frías *et al.*, 2001).

**Scale to rate the severity of late blight in tomato crops; both in field and greenhouse tests according to Henfling (1987).**

<b>GRADE</b>	<b>Tissue Percentage Affected</b>	<b>SYMPTOM</b>
1	0	Not observed blight
2	0-5	Maximum 10 lesions per plant
3	5-15	Plantas sanas. Área foliar afectada (20 folíolos)
4	15-35	Healthy plants. Leaf area affected (20 leaflets)
5	45-65	Plot looks green, lower leaves dead. 50% foliage destroyed
6	65-85	Plot looks green, with brown spots. 75% foliage destroyed
7	85-95	Only upper leaves are green. Stems with lesions
8	95-100	Plot looks brown. Most stems affected or dead
9	100	Dead stems and leaves.

In order to compare the behavior of each material, with the data obtained each week, the area under the disease development curve (AUDPC) was calculated using the formula proposed by Shaner y Finney (1977):

$$AUDPC = \sum_{i=1}^n \left[ \frac{Y_{i+1} + Y_i}{2} \right] [X_{i+1} - X_i]$$

Where:  $Y_i$ = Proportion of tissue affected in the  $i$ -th observation,  $X_i$ = Time in days from sowing to the moment of evaluation,  $X_{i+1} - X_i$ = Time in days between two readings and  $n$  = Total number of observations.

## Statistic analysis:

Variable AUDPC :

Normality tests using the Univariate procedure of SAS 8.1 and the NORMAL option that uses the Shapiro-Wilks test, and when the normality analyzes of the data were significant, we proceeded to perform a transformation of the AUDPC data, by calculating the square root of  $AUDPC+1$ .

ANVA: By Experiment

Tukey Test ( $P \leq 0.05$ )

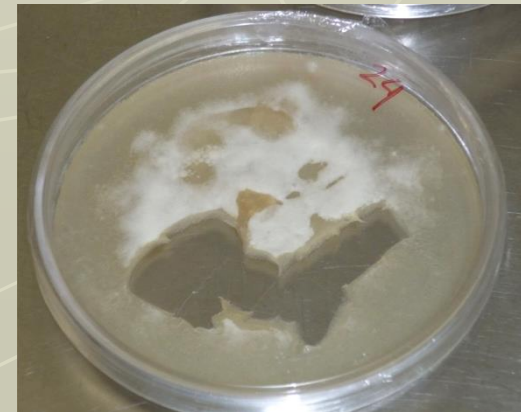
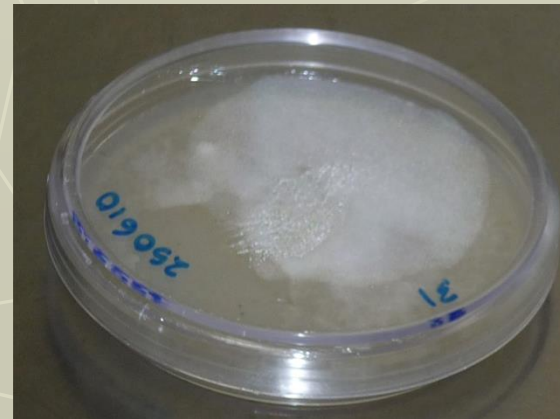
The SAS Statistical Package version was used 8.1 (SAS Institute, 2001)

## II. Separate leaflet test :

Isolated potato crop strains in the Toluca Valley, Mexico (Dr. Héctor Lozoya Saldaña, Dra. Norma M. Alarcón Rodríguez (Alarcón *et al.*, 2014) UACH, México

strain key (insulation number)	compatibility type	Glucosa 6 fosfato- isomerasa	Peptidasas
10	Homotálic	86/122/*	100/100*
13	Homotálic	86/100	100/100
15	Homotálic	86/100	100/100
23	Homotálic	86/100	100/100
24	Homotálic	86/100	100/100
31	Homotálic	86/100/122	100/100

/\*Alelos isoenzimáticos



## INOCULATION:

1. Using the separate leaflet technique
  - Plants from 6 to 8 weeks of age cut 5th. And 6th. subterminal leaf
  - Placement in transparent plastic boxes (20X20X6.5 cm) on pre-moistened filter paper.
  - Photoperiod of 16 Hrs. Light, Temperatures  $18\pm 1^{\circ}\text{C}$
  - Leaflet inoculation: Concentration 40,000 sporangia ml<sup>-1</sup>
  - The degree of resistance of the genotypes was determined by calculating the disease rate (DR)



(Barquero *et al.*, 2005, Xuan y Byung, 2007 )

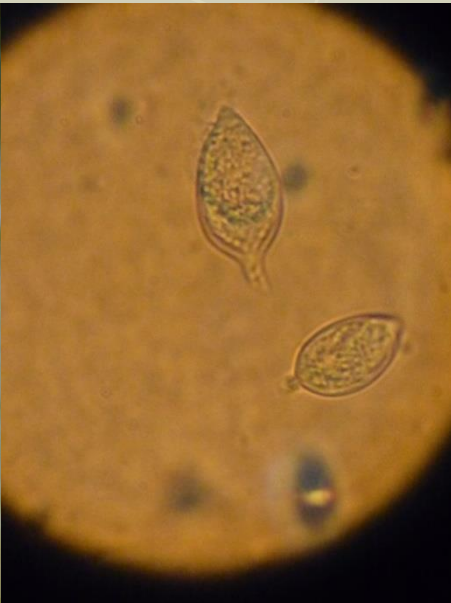


Scale to evaluate necrotic leaf area and degree of sporulation of *P. infestans* in separate leaflet tests according to Barquero *et al.* (2005b).

<b>NECROTIC LEAF AREA</b>		<b>DEGREE OF SPORULATION</b>	
<b>SCALE</b>	<b>CRITERION</b>	<b>SCALE</b>	<b>CRITERION</b>
1	no symptoms	0	no sporulation
2	Necrotic points at the place of application of the inoculum drop	1	Some sporangia in the necrotic area
3	Necrotic points of approx. 2 mm	2	50% of the necrotic area with sporangia
4	Approx. 1 cm <sup>2</sup>	3	100% of the necrotic area with sporangia
5	Aprox. 50%		
6	51-65%		
7	66-75%		
8	76-85%		
9	More 85%		



## RESULTS



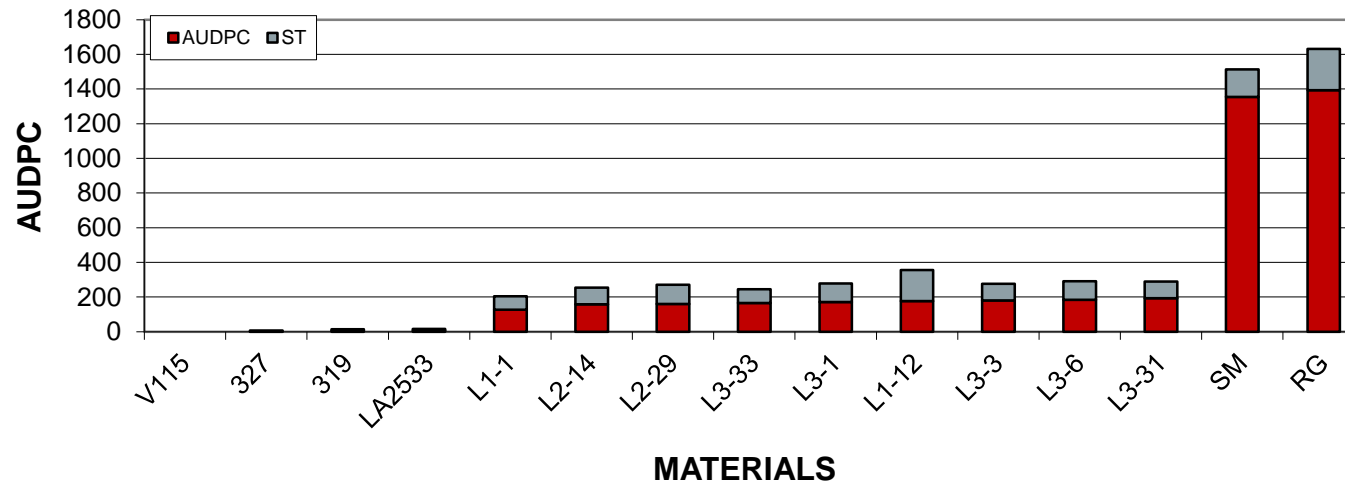
## I. Field tests :

Mean squares (MS) and statistical significance in the individual and combined analyzes in the 15 materials evaluated for resistance to *P. infestans* of the AUDPC variable during the agricultural cycle S.S. 2012.

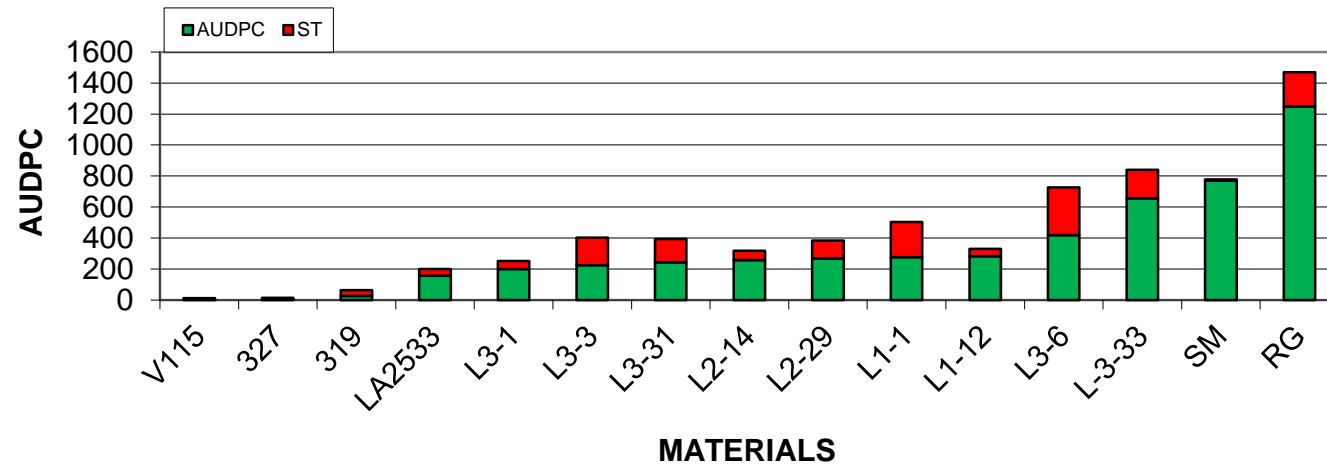
F. Variación	EXP I		EXP II		EXP III		COMBINADO	
	DF	MS	DF	MS	DF	MS	DF	MS
Repetition	5	48586.38**	1	11407.50 <sup>ns</sup>	4	138051.42**	5	57335.19*
Genotype	14	1205583.4**	14	218772.10**	14	3314388.28**	14	4128276.76**
Experiment							2	4839377.75**
Gen*Exp.							28	305233.50**
C.V.		35.09		42.55		26.20		33.68

Where: ns=no statistical significance, \*= statistical significance at 0.05 probability ( $\alpha \leq 0.05$ ), \*\*=statistical significance at 0.01 probability ( $\alpha \leq 0.01$ ), C.V= Coefficient of variation (%)

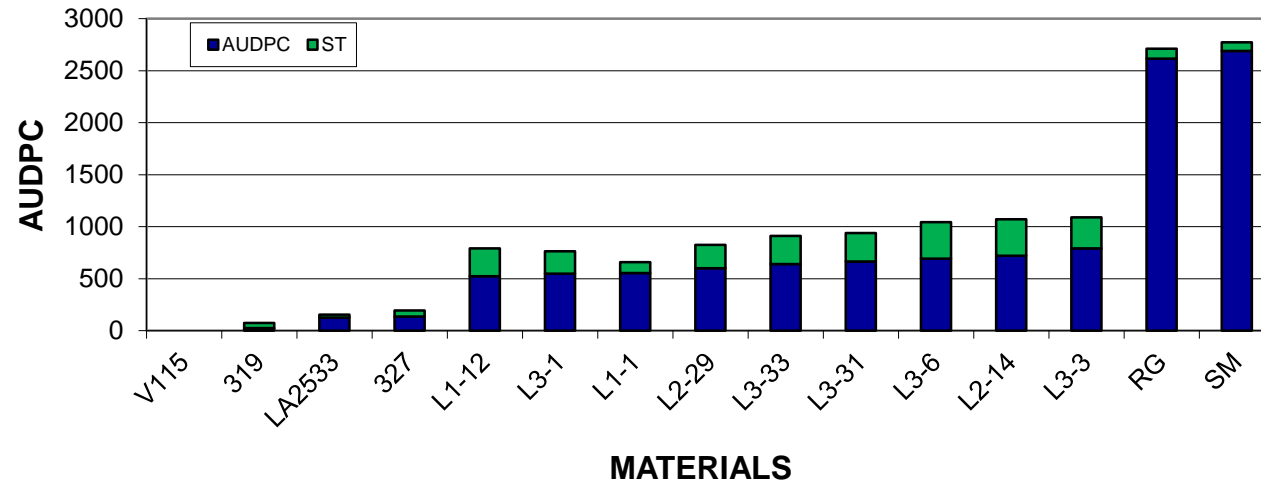
**GRAPH 1. AREA UNDER THE DISEASE DEVELOPMENT CURVE (AUDPC) ON THE BEHAVIOR OF EVALUATED MATERIALS. IN EXPERIMENT I. CYCLE S.S.. 2012.**



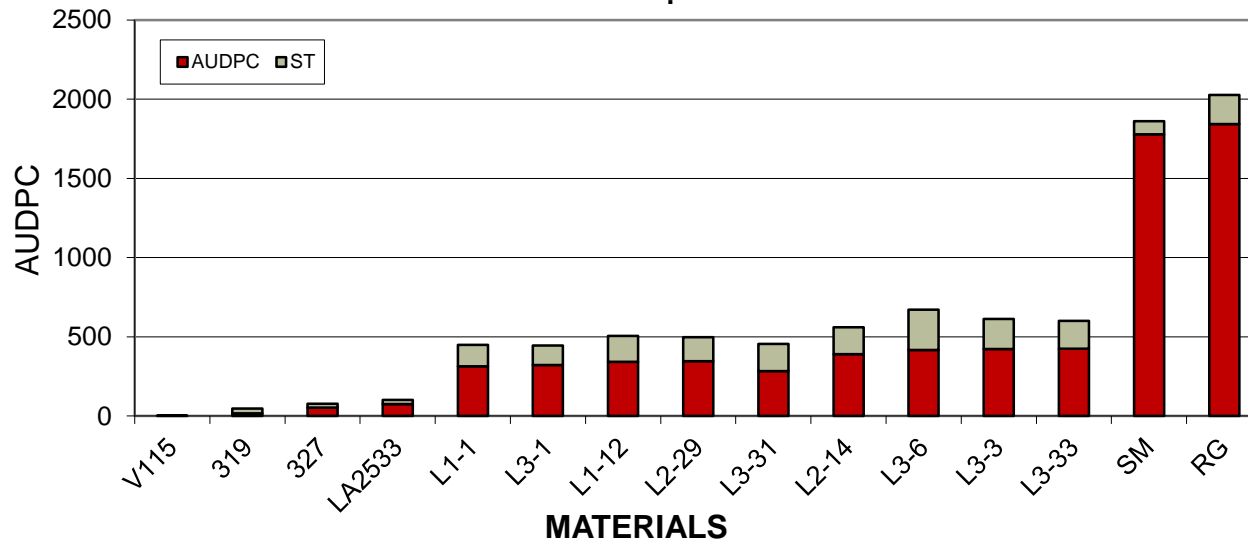
**GRAPH 2. AREA UNDER THE DISEASE DEVELOPMENT CURVE (AUDPC) ON THE BEHAVIOR OF EVALUATED MATERIALS. IN EXPERIMENT II. CYCLE S.S. 2012.**



**GRAPH 3. AREA UNDER THE DISEASE DEVELOPMENT CURVE (AUDPC) ON THE BEHAVIOR OF EVALUATED MATERIALS. IN EXPERIMENT III. CYCLE S.S. 2012.**



**GRAPH 4. AREA UNDER THE DISEASE DEVELOPMENT CURVE (AUDPC) ON THE BEHAVIOR OF EVALUATED MATERIALS. COMBINED ANALYSIS. CYCLE S.S. 2012.**



## -II. Separate leaflet test

Mean squares (MS) and statistical significance in the tests carried out on leaflets separated from the 15 genotypes infected with the six strains from Toluca Valley.

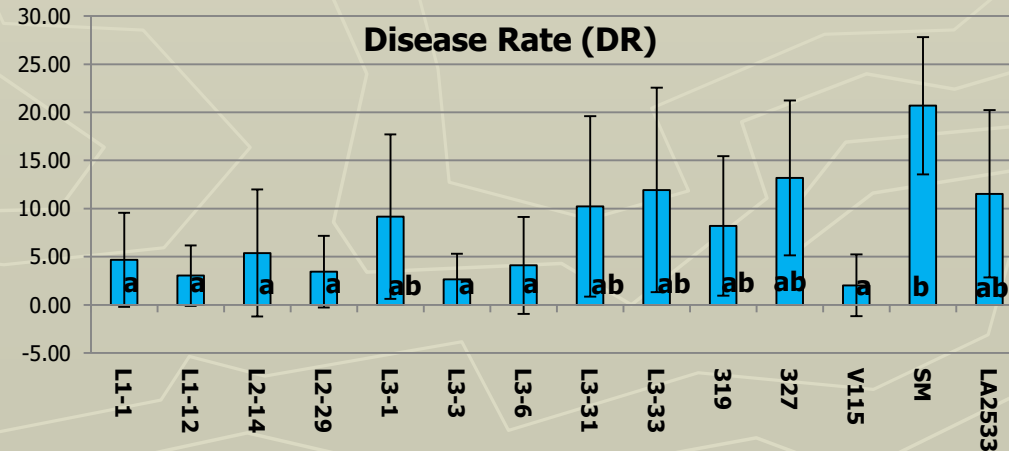
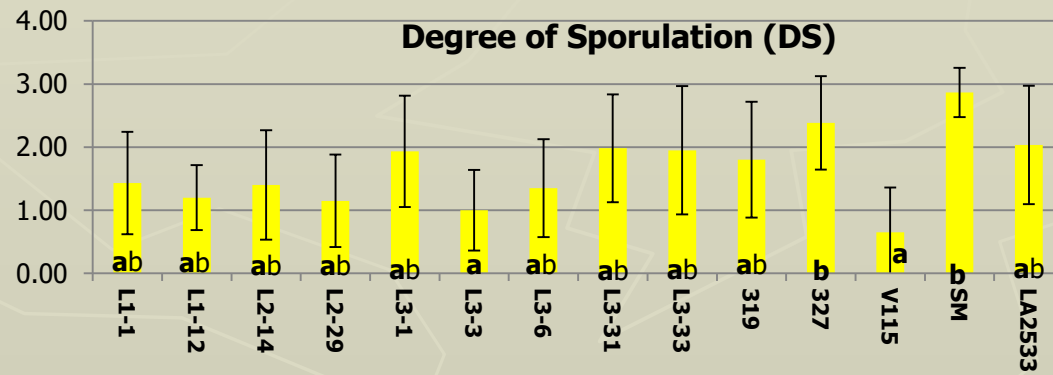
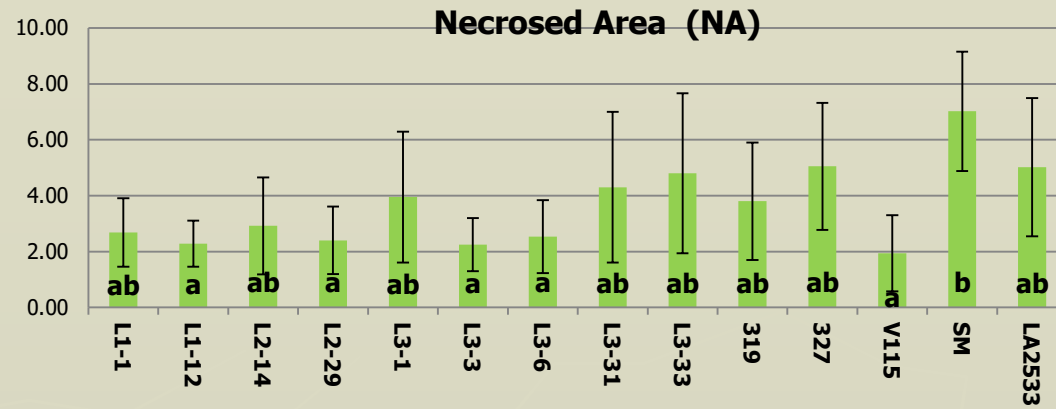
Fuentes Variación	NA		DS		DR	
	DF	MS	DF	MS	DF	MS
Observación	1	4.576190 <sup>ns</sup>	1	1.7190476 <sup>ns</sup>	1	62.42976 <sup>ns</sup>
Strains	5	26.910474**	5	6.9676190**	5	509.60976**
Genotipes	13	129.572894**	13	20.9688645**	13	1684.57335**
Strains*Genotipes	65	12.916630**	65	1.8347985**	65	149.95643**

Where: ns=no statistical significance, \*\*=statistical significance at 0.01 probability ( $\alpha \leq 0.01$ )

Comparison of means in the tests carried out on leaflets separated from the genotypes infected with the six strains from the Toluca Valley in relation to the source of strain variation

STRAINS	VARIABLES		
	NA	DS	DR
10	2.99 C	1.31 D	4.91 C <sup>1/</sup>
13	3.51 BC	1.48 CD	6.63 BC
15	3.71 B	1.64 BC	8.12 B
<b>23</b>	4.36 A	1.81 AB	<b>10.51 A</b>
24	3.69 B	1.80 AB	8.52 AB
31	3.58 B	1.88 A	8.54 AB
DMS	0.57	0.23	2.01

<sup>1/</sup> Means with the same letters, do not differ according to the Tukey test ( $\alpha \leq 0.05$ )



**GRAPH 5.** Mean behavior between genotypes per strain in the variables necrotic leaf area (NA), degree of sporulation (DS), and disease index (DR). Columns with the same letter are not significantly different at  $\alpha = 0.05$ , according to Tukey's test.



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