Article

Losses caused by *Zymoseptoria tritici* in wheat genotypes in storm environments

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Abstract

Leaf blight caused by *Mycosphaerella graminicola* (anamorph *Zymoseptoria tritici* or *Septoria tritici*), is a disease present in rainy areas of seasonal wheat crops in the High Valleys of Central Mexico. The effect on grain yield caused by the disease is variable, depending on the locality and the resistance level of the varieties. The objectives of the research were to determine the percentage of losses caused by leaf blight and the response of commercial varieties planted in storm environments. Five varieties of bread wheat were evaluated in two treatments, with and without control of the disease, in the towns of Juchitepec, State of Mexico and Nanacamilpa, Tlaxcala, during the summer 2007 and 2008 cycles. The experimental design was complete random blocks with four repetitions under split plot treatment arrangement. The decrease in yield caused by leaf blight was 41 and 40% in Salamanca S75 and Gálvez M87 respectively, 33% for Verano S91, Triunfo F2004 24% and 9% losses in Rebeca F2000. Based on their resistance level, three groups of varieties were determined: Rebeca F2000 as resistant, Triunfo F2004 moderately resistant, Gálvez M87, Salamanca S75 and Verano S91 susceptible. The best test site was Juchitepec, Estate from Mexico. These results indicate that greater resistance to *Zymoseptoria* should be incorporated in future varieties, including Rebeca F2000 as a source of resistance, which will reduce losses in farmers yield in environments where the disease is important.

Keywords: *Triticum aestivum*, *Zymoseptoria tritici*, leaf blight, susceptible varieties, yield.

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Introduction

Leaf blight is a disease of bread wheats (*Triticum aestivum* L.) and durum wheat (*T. turgidum* var. Durum) caused by the pathogen *Mycosphaerella graminicola* (anamorph *Zymoseptoria tritici* or *Septoria tritici*) considered as prevalent in the fields of production of this cereal (Berraies *et al*., 2014; Fones and Gurr, 2015).

The pathogen has a wide survival capacity and has coexisted with the culture for years, the sources of inoculum are of vital importance for the start of an epidemic, because its reproductive structures, ascospores, pycnidiospores and mycelium are mainly in the residues harvesting of wheat, infected seed, and other host species (Suffert *et al*., 2011; Steinberg, 2015). Kema *et al*. (1996) mentions that *Z. tritici* does not produce resistance structures in the soil, therefore for the epidemiology of the disease.

The fungus needs the remains of the previous wheat crop to remain in the field as a reservoir for the perithecia, which are the fungal structures, which will be the primary inoculum for the next production cycle. Leaf blight has caused yield losses of up to 60% (Arraiano *et al*., 2001a) in the crop and is characteristic of high and rainy parts (Cowger *et al*., 2000) or temperate-humid areas throughout the world (Fones and Gurr, 2015).

Where year after year, due to the presence of crop residues, the incidence of the disease increases (Castro *et al.*, 2015). High incidence and severity of *Zymoseptoria tritici* have been reported in Mexico (Eyal *et al*., 1985). Leyva *et al*. (2006) reported it in storm areas of the Mexican plateau (State of Mexico, Jalisco and Michoacán); while Rodríguez *et al*. (2008), reported it in areas of Juchitepec, Mexico and Nanacamilpa, Tlaxcala, with an effect of average losses in grain yield ranging from 6 to 36%.

In varieties with different degrees of resistance such as Temporalera M87, Batan F96 and Romoga F96 and where favorable climate and soil conditions prevail to achieve high yields in seasonal wheat, reason why the monoculture of this cereal has increased year by year even with the presence of leaf blight (Leyva *et al*., 2006).

The use of disease resistant varieties is important in modern agriculture (Ayliffe and Lagudah, 2004) since it is the most appropriate method in terms of economic stability and environmental impact, so improvement for resistance to foliar diseases has been the strategy most used (Tyryshkin and Tyryshkina, 2003; Brown *et al*., 2015) for the control of diseases in wheat, within which *Z. tritici* was not the exception (Nelson and Marshall, 1990).

Leaf blight is considered to be one of the greatest threats in most rainy areas where wheat is grown around the world (Halama, 1996; Jorgensen *et al*., 1999; Hardwick *et al*., 2001) and in Mexico (Leyva *et al*., 2006). Chemical control of this disease in this case is not the best alternative to minimize damage.

Therefore, it is necessary to have knowledge of the response of different genotypes towards this disease to decide which varieties to plant (Arraiano *et al*., 2001b) and which ones to use as a source of resistance in genetic improvement (Rajaram, 1994) so that the objective of the present work was to quantify the damage of *Z. tritici* in the yield of five wheat varieties planted in the High Valleys of Mexico and to determine their resistance level.

Materials and methods

In the present study, the varieties of wheat Salamanca S75, Gálvez M87, Verano S91, Rebeca F2000 and Triunfo F2004 (Table 1) recommended for seasonal plantings and that have shown different degrees of resistance to *Z. tritici* (Villaseñor *et al.* , 2007).

Table 1. Genealogy and pedigree of the wheat varieties evaluated in Juchitepec, State of México and Nanacamilpa, Tlaxcala.

Varieties	Genealogy	Reference
Salamanca S75	CNO/PJ62//CNO/7C II26265-22Y-300M-301Y-2M-501Y-500M-0Y-0MEX	Skovmand *et al.* (1997)
Gálvez M87	BB/GLL//CARP/3/PVN CM33483-C-7M-1Y-0M-5B-0Y	Villaseñor and Espitia (2000)
Verano S91	BB/CNO//JYCO/3/IN/TGLR/4/VCM/SON//CNO79 TC-820007-07C-05-08C-7R-0C	Villaseñor and Moreno (1998)
Rebeca F2000	Pfau/Seri//Bobwhite CM85295-0101TOPY-2M-0Y-0M-1Y-0M-(1-50)C-032R-0C	Villaseñor *et al.* (2004)
Triunfo F2004	Romoga/Norm TC950319-S-22C-0R-0C-0R-1C-0R	Villaseñor and Espitia (2000)

The sowing was carried out during the 2007 and 2008 summer cycles in the towns of Juchitepec, State of Mexico and Nanacamilpa, Tlaxcala. Five experiments were established, three in Juchitepec (upper part JUCHI1 V-2007 and JUCHI2 V-2008 and lower part JUCHI3 V-2008) located at 2 590 masl, 19° 05’ north latitude and 98° 52’ west longitude with precipitation annual of 853 mm, and two in Nanacamilpa (NANA1 V-2007 and NANA2 V-2008) at 2 720 masl, 19° 29’ north latitude and 98° 32’ west longitude, with annual precipitation of 841 mm.

A randomized complete block experimental design with four replications with an arrangement of divided plot treatments was used (Martínez, 2005), the treatments with and without *Z. tritici* control were the large plots and the varieties the sub-plots. The size of the sub-plot was 3.6 m^2, which corresponded to four furrows with a separation of 0.3 m x 3 m in length.

In the first treatment, two applications of the Sportak® fungicide (Prochloraz, 1 L ha^{-1}) were carried out, the first during the flowering stage and the second 20 days later. In the second treatment, disease control was not performed and in this one an epiphytia was artificially induced during stage 45 when the embouchure was presented (Zadoks *et al*., 1974).

Plants were inoculated by spraying an approximate suspension of 10^7 pycnidiospores mL^{-1} of water, to which Tween 20® was added as a surfactant (Wainshilbaum and Lipps, 1991) with an ultra low volume pump. The fertilization formula 80-40-20 was applied at the time of planting and weed control was carried out manually so as not to confuse the effects that the application of herbicides could have with those of the disease.

Direct and indirect variables were evaluated. The first were from the experimental plots: grain yield (KGHA) obtained from each plot in g m^{-2} and transformed to kg ha^{-1} and maximum infection (IMAX) (area with necrosis due to the presence of pycnidia in percentage under a scale 0 to 100) (King *et al*., 1983).

The second ones were the product of the random sampling of 25 stems cut at ground level taken from the two central furrows of the plot: weight of a thousand grains in g (PMG). On the other hand, with the grain and dry matter yield of the 25 stems and the weight of a thousand grains, it was possible to calculate biomass in kg ha^{-1} (BIOM= [(RGP + RE25T) /PU]/IC) and harvest index (IC= RE25T/RB25T). Where RGP= grain yield of the plot in grams.

RE25T= economic yield (grain weight) of 25 stems in grams; PU= useful plot; IC= harvest index and RB25T= biological yield (total weight of the aerial part) of 25 stems in grams (Rodríguez *et al*., 2008). Data collection to evaluate the area under the *Z. tritici* progress and behavior curve began approximately 80 days after planting, when symptoms of the disease were observed in the plots and four evaluations were carried out at weekly intervals of damage of leaf area.

The last reading was carried out in the stage of massive grain (King *et al*., 1983), when the maximum level of disease was observed in the most susceptible varieties, which was considered the maximum infection of the leaf area. An analysis of variance was performed using the GLM procedures of SAS® 9.3, for which the information from the five experiments was combined to identify the effects between sites, treatments, varieties and the interactions between these factors.

Differences between locations, treatments (CF and SF) and varieties were determined using the least significant difference (LSD). The area under the *Z. tritici* progress curve (ABCPST) was calculated using Microsoft® Office Excel 2016 data analysis module using the following equation according to Bjarko and Line (1988).

$$ABCPE = \sum_{i=1}^{n-1} \left(\frac{x_i + x_{i+1}}{2}\right) t_i$$

Where: xi= percentage of leaf area affected in each reading; i, ti = time interval (in days) between date i and date $i + 1$; and n= number of readings.

Results and discussion

Table 2 shows the mean squares of the analysis of variance for the selected variables, where it is observed that there were highly significant statistical differences ($p\leq 0.01$) between sites in all the variables evaluated, except in IMAX where the difference was significant ($p\leq 0.05$). Highly significant differences ($p\leq 0.01$) were found between treatments and varieties in all variables.

Table 2. Average squares of the analysis of variance of five variables registered in five wheat varieties evaluated with and without application of fungicide in Juchitepec, State of Mexico and Nanacamilpa, Tlaxcala.

FV	GL	KGHA	PMG	BIOMASS	IMAX	ABCPST
Site	4	44 646 853**	317.8**	1 036 976 395**	318*	3 597 696.4**
Rep (Site)	15	499 243	11	13 744 975	139 128.125	70 820.5
Trat	1	119634 152**	2151.7**	536 976 308.3**	139 128.1**	49747818.8**
Site∗Trat	4	4 321 000**	141.6**	41 323 304.1**	173.7*	933 564.5*
Rep∗Trat (Site)	15	383 600	7.7	3 397 597	28.6	92 367.9
Var	4	14 254 138**	230.5**	113 524 300**	9 902.3**	5 294 418.1**
Site∗Var	16	750 962**	38**	3 893 995*	181.9**	100 863.3**
Trat∗Var	4	4 138 825**	148.8**	14 065 364*	7 255.6**	2394576.36**
Site∗Trat∗ Var	16	582 645**	16.6*	8 151 988*	133.3**	593 64.91*
Error	120	166 716	8.5	3 681 825	33.7	1 9018.8
Total	199					
Mean		4 366.7	38.9	11 786.4	45.4	1113
CV (%)		9.3	7.5	16.3	12.8	12.4

ns= not significant; *= significant ($p\leq 0.05$); **= highly significant ($p\leq 0.01$); FV= source of variation; SITE= cycle and place; REP= repetition; VAR= variety; TRAT= treatment; GL= degrees of freedom; KGHA= grain yield (kg ha^{-1}); PMG= thousand grain weight (g); BIOMASS= biomass (kg ha^{-1}); IMAX= maximum infection level (%) ABCPST= area under the progress curve of *Zymoseptoria tritici*.

In site-by-treatment interactions, highly significant differences ($p\leq 0.01$) were observed in KGHA, PMG, and BIOMASS, and significant ($p> 0.05$) in IMAX and ABCPST, and in site by variety and treatment by variety, there was a highly significant difference ($p\leq 0.01$).) in KGHA, PMG, IMAX and ABCPST and significant ($p> 0.05$) in BIOMASS.

In the third order interaction, IMAX and KGHA presented highly significant differences ($p\leq 0.01$) and in the rest of the variables the differences were significant ($p> 0.05$). Table 3 shows the average means of the variables kilogram per hectare (KGHA), weight of a thousand grains (PMG), BIOMASS, maximum infection (IMAX) and area under the *Zimoseptoria tritici* progress curve (ABCPST).

The disease in the Rebeca F2000 variety decreased its average yield by 9%, while in Triunfo F2004 the effect of the disease was 24%. In the Verano S91 variety the average decrease in yield was 33%, while Gálvez M87 and Salamanca S75 were the most affected by the disease with average losses of 40 and 41%, respectively.

Table 3. Average yield losses caused by *Zymoseptoria tritici* for the variables KGHA, PMG, BIOMASS and incidence and development values of the IMAX and ABCPST disease in five varieties, average of five sites.

Variety	Losses (%)						
	CF	SF	KGHA	PMG	BIOMASS	IMAX	ABCPST
Rebeca F2000	5 595	5 089	9 a	1.36 a	11 a	27 c	704 c
Triunfo F2004	5 162	3 776	24 b	12.86 b	17 b	58 b	1284 b
Gavez M87	5 019	2 960	40 c	21.88 cd	29 c	91 a	2071 a
Salamanca S75	4 845	2 801	41 cd	17.18 c	32 cd	93 a	2131 a
Verano S91	5 077	3 337	33 d	21.62 cd	29 c	91 a	1865 a

Means with the same letter are statistically the same (Tukey 5%); KGHA= kilogram per hectare; %= effect of *Z. tritici* on yield ((KGHA CF- KGHA SF)∗100/KGHA CF)); PMG= thousand grain weight; %= effect of *Z. tritici* on a thousand grain weight ((PMG CF- PMG SF)∗100/PMG CF)); %= effect of *Z. tritici* on biomass ((biomass CF- biomass SF)∗100/biomass CF)); IMAX= maximum level of average infection of each variety and five sites without application of fungicide; ABCPST= area under the average curve of each variety and five sites without fungicide application.

However, losses can be as high as 50% when conditions are favorable for *Z. tritici* as occurred in JUCHI2 and a susceptible variety like Salamanca S75 is planted (Table 4). The afore mentioned shows that *Z. tritici* affected each variety to a different degree, negatively impacting the yield and consequently leading to a reduction in the economic value of wheat as reported by Mc Kendry *et al*. (1995).

Table 4. Grain yield losses (KGHA) in % caused by *Zymoseptoria tritici* for the five varieties and five sites during the summer of 2007 and 2008.

Variety	Juchi1	Juchi2	Juchi3	Nana1	Nana2
	Losses KGHA (%)				
Rebeca F2000	8	8	10	8	11
Triunfo F2004	16	48	16	17	24
Galvez M87	38	43	34	43	43
Salamanca S75	45	50	35	46	32
Verano S91	35	48	28	36	20

KGHA= kilogram per hectare; %= effect of *Z. tritici* on yiled ((KGHA CF- KGHA SF)∗100/KGHA CF)).

It was also observed that in PMG Rebeca F2000 was the one with the lowest percentage of losses (1.36%), followed by Triunfo F2004 (12%), Salamanca S75 (17.18%) and finally Galvez M87 and Verano S91 (21.88 and 21.62%, respectively). Eyal and Ziv (1974) mention that the yield losses caused by leaf blight are associated with the decrease in grain weight, so that the minimum losses registered in Rebeca F2000. They are an indicator of their resistance to this disease and that it coincides with that mentioned by Leyva *et al*. (2006).

When reporting resistant genotypes with yield losses of less than 13% in the variety with respect to the susceptible ones; on the other hand, Rodríguez *et al*. (2008), indicated that the yield losses in tolerant varieties like Batan F96 were 16% while in old and susceptible varieties like Siete Cerros it was 36%.

Also, in durum wheat, the behavior of resistance to *Z. tritici* is similar as reported by Berraies *et al*. (2014) who indicated that in durum wheat the susceptible varieties come to have yield losses of 50% while in resistant varieties only 8.6%. In Rebeca F2000, the lowest average percentage of losses was observed for the BIOMASS variable (11%), followed by Triunfo F2004 (17%) and thirdly, Gálvez M87, Salamanca S75 and Verano S91 with a decrease from 29 to 32%.

Biomass production can be considered as an important selection criterion for resistance to leaf blight, since it integrates grain yield and important parts such as foliage that was not damaged by the pathogen and the ability to store stem reserves (Parker *et al*., 2004). However, visual determination of disease progress allows selecting a large number of lines without harvesting.

With the IMAX variable (Table 3), three groups of means were identified: Salamanca S75, Gálvez M87 and Verano S91 in all the sites and in general average, they were located in the first group with the maximum infection levels of leaf area (around the 90%) in the second group, Triunfo F2004 was located with average readings of 58% of infected area and lastly, Rebeca F2000 was identified with an average infection level of 27%.

It is important to consider that the maximum infection (IMAX) is associated with the reduction of the leaf area covered with pycnidia and this in turn expresses the level of resistance to *Z. tritici* (Gieco *et al*., 2004). In the absence of immunity to leaf blight, resistance categorization is based on a limited number of lesions (Cohen and Eyal, 1993), therefore, any restriction or delay in the development of the pathogen is a form of resistance or competition between field level isolates.

When there are more than one (Nelson and Marshall, 1990; Leyva *et al*., 2006), so considering the IMAX, the Rebeca F2000 variety can be classified as resistant. Differences between varieties are also observed in ABCPST and in the same way that the same three groups were identified in IMAX. The maximum infection and severity of the disease is correlated with the percentage of losses of the disease, making it a variable that makes it easier to select genotypes with resistance that will have lower losses in yield.

In the Table 4 shows the percentage yield losses for the five varieties and the five test environments, where it can be seen that the Rebeca F2000 variety classified as resistant, the behavior was very similar in all test environments with losses of 8 to 11%, while the most susceptible variety (Salamanca S75) were 32 to 50%, indicating that the expression of the level of resistance of the varieties to leaf blight depends on the environment.

The average effect of *Z. tritici* per site for the variables KGHA, PMG, BIOMASS, IMAX and ABCPST, was observed in Table 5. The losses caused by the fungus ranged from 24% (JUCHI 3) to 39% (JUCHI 2), which shows that leaf blight is a disease that in rainy environments causes losses in yield and that its effects vary between sites and years, as mentioned by Royle *et al*. (1986).

Table 5. Effect of *Zymoseptoria tritici* for the variables KGHA, PMG, BIOMASS, IMAX and ABCPST variety averages at five test sites during the summer of 2007 and 2008.

Site	KG/HA (%)	PMG (%)	Biomass (%)	IMAX (%)	ABCPST
Juchi 1	28	6	25	72.5	1718
Juchi 2	39	27	26	66.5	1343
Juchi 3	24	16	18	72	1081
Nana 1	30	7.7	29	72.5	1645.6
Nana2	26	19	19	75.5	2270.5

KGHA= kilogram per hectare; %= effect of *Z. tritici* on yield ((KGHA CF- KGHA SF)∗100/KGHA CF)); PMG= thousand grain weight; %= effect of *Z. tritici* on a thousand grain weight ((PMG CF- PMG SF)∗100/PMG CF)); %= effect of *Z. tritici* on biomass ((cf biomass - SF biomass)∗100/CF biomass)); IMAX= maximum level of average infection of each variety and five sites without application of fungicide; ABCPST= ABCPST= area under the average curve of each variety and five sites without fungicide application.

It was observed that like the grain yield, the weight of a thousand grains was affected in all those places where this disease occurs, this variable varied between the test sites from 6% (JUCHI1) to 27% (JUCHI2), this indicates that the weight of the grain is a variable directly affected by the incidence of leaf blight, coinciding with that reported by Zuckerman *et al*. (1997); Berraries *et al*. (2014).

Also, in Table 5 the results corresponding to biomass are presented, an important variable from the biological point of view, since it expresses the capacity of the plant to produce dry matter. It was observed that *Z. tritici* is a disease that affected biomass production, the losses caused in the sites varied from 18% (JUCHI3) to 29% (NANA1), resulting that this variable presented a similar response to the yield and weight variables of thousand grains.

Rodríguez *et al*. (2008) report similar losses in grain yield in the JUCHI (30%) and NANA (27%) environments during 1997 and 1998, indicating that these environments are conducive to the development and evaluation of *Z. tritici*, because the environmental conditions have been stable; through time.

Regarding maximum infection (IMAX), the lowest average value between sites was for JUCHI2 with 66.5% and the highest for NANA2 with 75.5%, information that corroborates what was reported by Polley and Thomas (1991), who, in studies carried out indicated that when favorable conditions prevailed for the incidence of leaf blight, it appeared aggressively (79 to 94%) in four years of testing. In addition, the results of the area under the *Z. tritici* progress curve (ABCPST) are presented, where variations from 2270 (NANA2) to 1081 (JUCHI3) were observed, which demonstrate the damage caused by leaf blight in the different test sites.

Figure 1 shows the behavior of the disease at the JUCHI2 site where *Z. tritici* caused the most damage, observing that in the fungicide treatment once the disease appeared and the product was applied, good control was achieved. In the treatment without fungicide it was observed as the varieties Salamanca S75, Gálvez M87 and Verano S91.

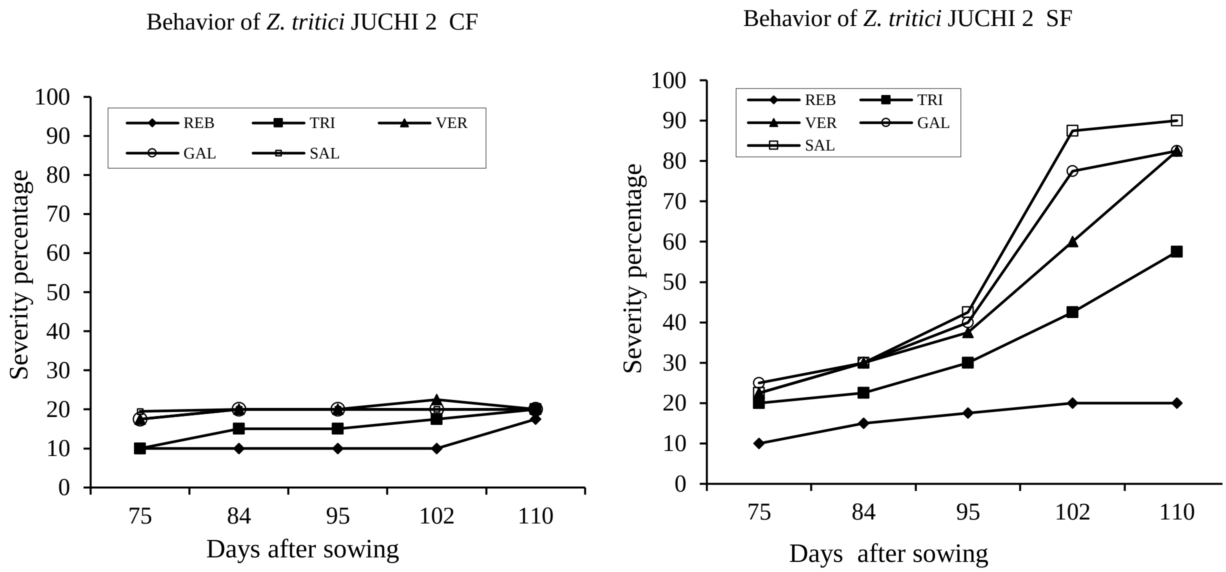

Figure 1. Behavior of the varieties in response to *Zymoseptoria tritici* infection with and without fungicide treatment at the site (JUCHI2).

From the beginning of the infection, they presented a greater damaged leaf area and from day 95 the percentage of infection increased rapidly until reaching more than 85%, unlike Triunfo F2004, where the increase was less and reached a maximum level of 60%, in Rebeca F2000 it can be seen how the growth of the disease was very slow over time, reflecting its resistance to *Z. tritici*.

In the Figure 2 shows the behavior of the disease without fungicide for the varieties Salamanca S75 (more susceptible) and Rebeca F2000 (more resistant) in the five evaluated sites, where it is observed that the susceptible variety presented an accelerated development of the disease when not protected with fungicide, while Rebeca F2000 expressed its resistance level in the five test sites.

In this system duck, agronomic and phytopathological components were considered to study the effect of leaf blight on wheat and to classify varieties; within the agronomic components, grain yield, weight of a thousand grains and biomass are considered, variables that allowed knowing the magnitude of the damage caused by the disease in the different sites and between varieties.

Among the phytopathological components, maximum infection, area under the curve of disease progress and disease behavior over time, variables that allowed differentiating the varieties, were considered. Maximum infection is an effective variable to characterize wheat genotypes resistant to leaf blight, corroborating what was mentioned by Nelson and Marshal (1990); Parker *et al*. (2004); Gieco *et al*. (2004); Brown *et al*. (2015).

However, disease progress also proved to be an important complementary tool for identifying resistance and classifying a group of genotypes. In order to have an effective characterization of the evaluated germplasm, it was useful to complement the damaged leaf area with grain yield, grain weight, as well as the behavior of the disease under treatments with and without fungicide.

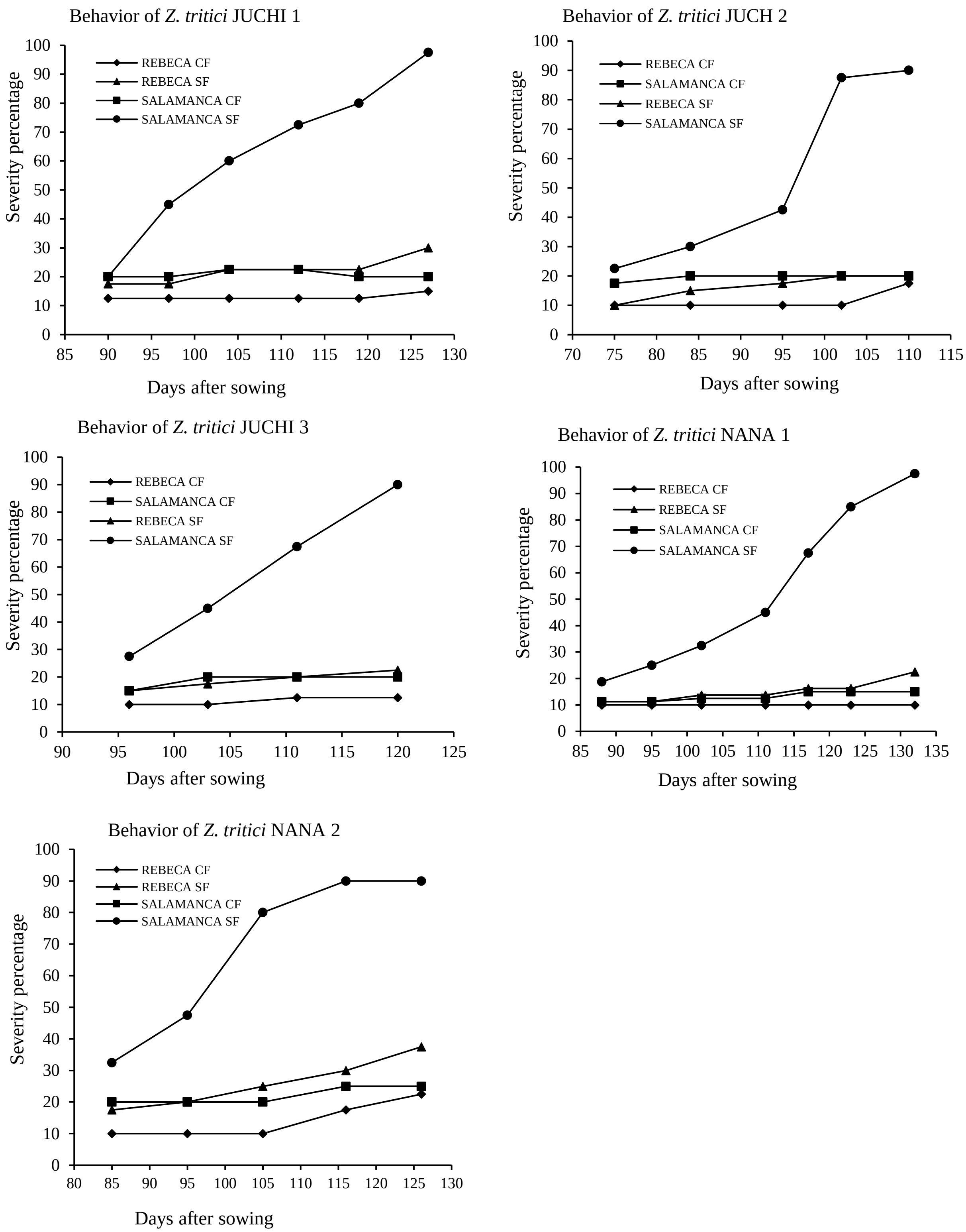

Figure 2. Behavior of the Rebeca F2000 and Salamanca S75 varieties in response to the disease and without fungicide in five test sites.

This allowed identifying resistant genotypes, so that according to the information presented, the varieties can be classified as resistant (Rebeca F200), moderately resistant (Triunfo F2004) and susceptible (Gálvez M87, Salamanca S75 and Verano S91). On the other hand, JUCHI2 (V-2008) was the locality where the most susceptible variety (Salamanca S75) had the highest losses in grain (50%), which indicates that JUCHI2 was the best test site for the evaluation of *Zymoseptoria tritici* in storm environments in the High Valleys of Mexico.

Conclusions

The average decrease in yield caused by leaf blight caused by *Zymoseptoria tritici* in wheat was 30% and can be as high as 50% in susceptible varieties. Three groups of varieties were determined based on resistance: Rebeca F2000 variety as resistant, Triunfo F2004 moderately resistant and Gálvez M87, Salamanca S75 and Verano S91 as susceptible.

JUCHI2 was the best test site for leaf blight. Losses caused by leaf blight can be minimized by planting resistant varieties. Rebeca F2000 can be used as a parent within breeding programs to obtain more and better wheat varieties suitable for rainy environments.

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