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RESEARCH ARTICLE

Induced resistance to common rust (*Puccinia sorghi*), in maize (*Zea mays*)

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ABSTRACT

The common rust of maize (*Zea mays* L.), caused by *Puccinia sorghi* Schw., develops pustules on the leaves of maize plants, reducing the leaf area and production of the photoassimilates necessary for grain filling. The host possesses genes coding for different proteins related to the defense mechanisms that prevent the establishment of the pathogen. However, there are susceptible plants that are unable of preventing pathogen attack. This condition depend on biotic and abiotic factors known as inducers of resistance which are able of activating the physico-chemical or morphological defense processes to counteract the invasion of the pathogen. The Ceres XR21 maize hybrid is susceptible to *P. sorghi.* In this work, maize hybrid was evaluated under a split-split- plot design established in two spring-autumn cycles in the years 2016 and 2017, in which five commercial products of biological and chemical origin reported as inducers of resistance, plus a fungicide were compared. The results showed that trifloxystrobin + tebuconazole (Consist Max®), sprayed on the foliage with 1.5X the commercially recommended dose, showed significant better response in most evaluated variables, because it controlled better the pathogen *P. sorghi* and maize plants increased grain yield.

Keywords: *Zea mays* L; Phytopathogen; Resistance inducer; Defense mechanism; Photosynthesis

INTRODUCTION

Maize (*Zea mays* L.) along with wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.), are the primary staple food cereals in many countries. In addition, maize is used in industry for the production of starch, oil, sweeteners, dextrins, ethanol, paper, antibiotics, cosmetics, and compounds that can replace the function of petroleum and its derivatives (Grande y Orozco, 2013). In Mexico, maize is affected by pests and diseases which reduce grain yield and quality, causing losses of 34.8% in production (INIFAP, 2015). This crop is affected by three rusts caused by polysora rust (*Puccinia polysora* Underw.), tropical rust (*Physopella zeae* (Mains) Cummins & Ramachar), and common rust (*Puccinia sorghi* Schwein), which develop pustules on the leaves particularly at flowering time, or a little later. Their easy adaptations allow the rusts to have a wide distribution in subtropical and temperate climates and in highlands with high humidity (Programa de Maíz, 2004). The infection caused by *P. sorghi* induces decreases in the photosynthetic leaf area and damages the epidermis of the host, thus affecting different physiological processes which reduce the amount of photoassimilates and decreases grain production (González, 2005).

The resistance inducers are exogenous molecules capable of stimulating plants for activating their defense mechanisms to protect themselves from phytopathogenic aggressions (Gómez and Reis, 2011). Inducers of biological or chemical origin, combined with good agronomic practices, may be successful in protecting crops during the growing season. Due to their origin and composition, the inducers show different effects on host development and vigor, as well as reducing the incidence and severity of diseases. The evaluation of these variables is useful to determine the effect of different inducers in the control of pests and diseases through physiological and agronomic processes in plants (Jiménez et al., 2012). Several products with fungicidal and efficient biological effect as chemical inducers have been evaluated to control diseases in

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horticultural crops of the Solanaceae family. In grasses, the potential of chemical inducers in controlling diseases has not been proved. Based on these interesting possibilities, in the present research six commercial products were evaluated as inducers of resistance to *P. sorghi* in maize plants grown under field conditions.

MATERIALS AND METHODS

Field experiments were conducted in the years 2016 and 2017 at the Experimental Station of the Colegio de Postgraduados, Campus Montecillo (19° 28’ 4.26”N and 98° 53’ 42.18” W, at an altitude of 2250 m). The climate is classified as Cb (w_0) (w) (i¨), corresponding to a temperate subhumid with oscillation between 5 and 7 °C, with summer rains (García, 1988). In the two growing seasons plantings were done in April 4, 2016 and April 6, 2017, both with seeds of the commercial hybrid maize Ceres XR21, reported as susceptible to *P. sorghi*. The entire experiment included 126 plots of 7.2 m^2 each, with 2 rows 3m long, 0.80 m between rows, 22 plants per plot, and 0.16 m between plants, with an average density of 80,000 plants ha^{-1}. The practices of crop management were carried out in accordance with the Experiment Station. After planting, four irrigations were applied by gravity, until the rains were established in the Summer. The soil was fertilized with a dose of 160-60-00 kg NPK ha^{-1}, respectively. Cultivation was done with a tractor 20 days after plants emerged. Weeds were controlled manually, and there was no need to control diseases, except for the treatments applied to evaluate the common rust severity.

The strategy to measure the effect of common rust caused by *P. sorghi*, consisted of three points: 1) apply five products reported as inducers of resistance, and a fungicide; 2) from the recommended commercial dose, two additional doses were included to determine if they were able to overcome the efficiency of the commercial; and 3) compare the efficiency of the products when applied to the soil or sprayed on the foliage. For this, an experimental split-split plot design was implemented, with three replications, where the main plots represent the six products: the fungicide trifloxystrobin + tebuconazole (Consist Max®), plus five products reported as inducers of resistance, including *Bacillus subtillis* (Serenade Max®), *Bacillus firmus* + clothianidin (Poncho Votivo®), Fosetil aluminum (Alliete®), harpin protein Ea (Messenger®), Acibenzolar S methyl (Actigard 50 GS®), and a control (water). The subplots corresponded to the two methods of treatment application: to the soil at the planting time, and spraying at V6-V7 stage when young plants were 20 days-old after seedling emergence (Ritchie et al., 1986). Sub-subplots involved the three doses per treatment: half (50 %) of the recommended dose, the recommended dose (100 %), and 1.5 times (150 %) the recommended dose (Table 1).

To determine the effect of treatments on induction of resistance to *Puccinia sorghi* in maize plants, the following agronomic parameters were measured:

1) Disease severity. It was recorded at the beginning of infection, in August in the two cycles 2016 and 2017, along five sampling dates separated by an interval of 15 days, using an arbitrary scale of 1 to 5 levels of damage (Table 2). 2) Anthesis-silking interval (ASI), as the difference of days to 50 % silking minus days to 50 % anthesis. 3) Plant and ear heights, measured from the stem crown level to the base of the ear and tassel, respectively. 4) Plant and ear aspects, using a scale 1-5, where 1 = very good (100 %), 2 = good (90 %), 3 = fair (80 %), 4 = bad (70 %), 5 = very poor (60-0 %). 5) Percent of healthy and rotten ears. 6) Grain yield, in t ha^{-1}; harvest was done at the grain physiological maturity, considering the appearance of the black layer at the kernel bottom. The kernel moisture content was measured in each treatment using a *Mini GAC* Plus moisture tester (Dickey John, USA), and then kernel moisture was standardized to 15%. The procedures and measurement units used in the

Table 1: Treatments applied to the maize crop for controlling *Puccinia sorghi*

Main plot	Subplot	Sub-subplot		
		50%	Commercial (100%)	150%
Actigard 50 GS® (Acibenzolar metil)	Soil (at sowing) Foliar spray (V7)	20 g/ha	40 g/ha	60 g/ha
Alliete® (Fosetyl aluminum)	Soil (at sowing) Foliar spray (V7)	1.25 kg/ha	2.5 kg/ha	3.75 kg/ha
Consist Max® (trifloxystrobin+tebuconazol)	Soil (at sowing) Foliar spray (V7)	0.15 L/ha	0.30 L/ha	0.45 L/ha
Messenger® (harpin protein Ea)	Soil (at sowing) Foliar spray (V7)	125 g/ha	250 g/ha	375 g/ha
Poncho Votivo® (*Bacillus firmus*+clothianidin)	Soil (at sowing) Foliar spray (V7)	40 mL/ 80,000 seeds	80 mL/80,000 seeds	100 mL/80,000 seeds
Serenade Max® *(Bacillus subtillis)*	Soil (at sowing) Foliar spray (V7)	1.5 kg/ha	3 kg/ha	4.5 kg/ha

Table 2: Evaluation scale for *Puccinia sorghi* rust

Scale	Severity	Leaf area with pustules
1	Resistant	10%
2	Moderately resistant	20-30%
3	Moderately Susceptible	40-50%
4	Susceptible	60-70%
5	Very susceptible	100%

data record are described in IBPGR (1991), Edmeades et al. (1996), and Angeles et al. (2010).

Regarding physiological variables, the following were evaluated:

1) Total chlorophyll (SPAD units): measured with a SPAD 502 instrument (Minolta Ltd., Japan). Sixty days after planting, chlorophyll readings were initiated and thereafter every 15 days. In each plot, three readings were recorded in the middle of the ear-leaf of three plants, all within an 11 and 13 h time period; the average of such readings was recorded for statistical analysis. 2) Net rate of photosynthesis (An; μmol CO_2 m^{-2} s^{-1}): in each plot, one ear-leaf in three plants was selected with the same criteria as for measuring chlorophyll. Measurements were done with a portable apparatus LI-COR 6400 (LICOR, Inc. USA) operating in open mode to allow the air circulation with a controlled atmosphere around the measured leaf fraction for approximately 1 min, keeping the CO_2 level of incoming air constant and taking the reading when the coefficient of variation was ≤ 2 %.

The measurement of the three experimental replicates required two days. The first and second replication of the experiment was taken on the first day, and the third replication was measured on the third day. During the readings, the photosynthetic photon flux was at a minimum of 1000 to 1200 μmol photons m^{-2} s^{-1}, and air flow was adjusted to 400-408 μmol mol^{-1}. The photosynthetic readings were made in stages V10, VT, and R5 (10 mature leaves, tasseling, and dent kernels, respectively) which are detailed by Ritchie et al. (1986) for maize development, based on the scale of Hanway (1966). The procedure and units used were described by Rincón and Ligarreto (2010), and Sandoval et al. (2010).

For the statistical analysis of variables measured in several periods of time on the same experimental unit, three options were used: univariate analyzes with the RANDOM instruction of the GLM, univariate or multivariate analysis through linear transformations using the REPEATED instruction of the GLM, and mixed models of covariance with the MIXED procedure (González et al., 2007).

The separation of means was done by GLM Fisher DMS test ($p \leq 0.05$) using the SAS 9.4 package.

RESULTS AND DISCUSSION

Severity

Even though results obtained showed a low severity of common rust, in the two years of evaluation it was observed that in the measured samples from the five treatments, Consist Max® (trifloxystrobin (strobilurins) + tebuconazole) provided greater protection to maize plants against *P. sorghi*, significantly decreasing ($\alpha = 0.05$) the severity of the pathogen. Regarding the application method, from the third sampling the spray at V7 (7th mature leaf) had a greater significant effect in controlling the disease, compared to the soil application. No significant differences were detected between dosages (Table 3).

Similar results were reported by Shah and Dillar (2010) who controlled *P. sorghi* in sweet corn hybrids, applying strobilurins in levels of 1, 10, and 20 % with only one foliar application. According to Rodríguez et al. (2015), preventive application is convenient after applying strobilurins to three maize hybrids at two stages: V10 (10th mature leaf) and R1 (silking stage), resulting in less severity when applying the product at the V10 phenologic stage. Carmona et al. (2009) sprayed maize plants with trifloxystrobin + tebuconazole with three doses (half the recommended dose, recommended dose, and twice the recommended dose), at different times of application; they found significantly lower severity of *P. sorghi* in plants treated with all doses compared to the control, concluding that for efficient control of the disease, applications should be done when plants show 1 % severity in the flag leaf.

The other resistance inducers studied in the present work, including *Bacillus subtillis* (Serenade Max®), *Bacillus firmus* + clotianidin (Poncho Votivo®), Fosetil aluminum (Alliete®), Harpin Ea protein (Messenger®), and acibenzolar S methyl (Actigard 50 GS®), also induced resistance, though to a lesser degree than Consist Max®, all of them exceeding the absolute control which showed the highest severity of damage from the third sampling date (Table 3).

Disease progress

In the mean comparison test ($p \leq 0.05$), plants treated with Consist Max® showed significantly lower rust severity by *P. sorghi* than the control plants (Table 3). Thus, a non-linear regression analysis was performed with the statistical package CurveExpert 1.4, to associate the pathogen growth as a function of time. The degree of severity over time adjusted best to polynomial models (Table 4), and the growth of severity in the control showed the greatest increase over time than plants treated with Consist Max® (trifloxystronin + tebuconazole).

Table 3: Severity of *Puccinia sorghi* in maize plants submitted to treatments, methods of application, and doses. Mean values calculated from two growing seasons[§]

	Severity of *Puccinia sorghi*				
	Sampling 1	**Sampling 2**	**Sampling 3**	**Sampling 4**	**Sampling 5**
Main plot					
Consist Max®	0.8^{a}	1.2^{a}	1.4^{a}	1.8^{a}	2.1^{a}
Serenade Max®	1.3^{b}	1.8^{b}	1.6^{a}	2.0^{bc}	2.4^{b}
Actigard®	1.0^{ab}	1.4^{ab}	1.5^{a}	2.0^{ab}	2.3^{b}
Alliete®	1.0^{ab}	1.3^{ab}	1.5^{a}	2.0^{ab}	2.3^{b}
Poncho Votivo®	1.0^{ab}	1.4^{ab}	1.6^{ab}	2.0^{abc}	2.4^{b}
Messenger®	1.0^{ab}	1.3^{ab}	1.6^{a}	2.0^{ab}	2.4^{b}
Control	1.0^{ab}	1.4^{ab}	1.8^{b}	2.2^{c}	3.0^{c}
Average	0.7	1.4	1.5	1.9	2.37
LSD (0.05)	0.2	0.4	0.2	0.2	0.17
CV (%)	22.3	47	11.3	11.1	8.02
R^2	0.7	0.6	0.7	0.7	0.8
Sub plot					
Application spray	1.0^{a}	1.4^{a}	1.5^{a}	1.9^{a}	2.3^{a}
Application in soil	1.0^{a}	1.4^{a}	1.6^{b}	2.0^{b}	2.5^{b}
LSD (0.05)	0.1	0.3	0.1	0.1	0.2
Sub subplot					
Dose 50%	1.0^{a}	1.3^{a}	1.6^{a}	2.0^{a}	2.4^{a}
Dose 100%	1.0^{a}	1.5^{a}	1.5^{a}	2.0^{a}	2.4^{a}
Dose 200%	1.0^{a}	1.3^{a}	1.6^{a}	2.0^{a}	2.4^{a}
LSD (0.05)	0.1	0.3	0.1	0.1	0.1

[§] Values followed by the same letter within the same column do not differ statistically from each other (Tukey ≤ 0.05).

Table 4: A model to simulate the severity level of *P. sorghi* in maize plants, as a function of time (days)

Polynomial model	**Function y=a + bx+cx^2+dx^3**	
	Control	**Consist Max®**
Coefficients	a=1.0088036117	a=0.791948833
	b=0.0056437588	b=0.024686666
	c=0.0008328177	c=-0.00033734
	d=-0.000006983	d=0.000004241
Correlation coefficient	0.8979	0.7163
Standard error	0.3377	0.4077

Plant height index

The stalk accumulates the nutrients elaborated in the process of photosynthesis and then they are transferred to the ear for grain filling. With the plant and ear height data, the height index = ear height/plant height is calculated, where the value 0.5 is expected as the desirable point of insertion of the ear, determining the tolerance to lodging. Our results showed no significant differences among treatments, whose values varied from 0.44 to 0.46 (Table 5), an acceptable range indicating enough stem strength to avoid lodging by wind. According to Reynoso et al. (2014), values of 0.4 - 0.5 guaranteed lower probability of root and stem lodging of 17 hybrids evaluated in different zones of central Mexico. Hongguang et al. (2012) found that height is associated by QTLs, suggesting that it is important to measure the plant and ear height as there is a close relationship conditioning stalk resistance to lodging, canopy photosynthesis and grain yield. In addition, the plant and ear height are not only determined genetically, but are also regulated by environmental conditions including the application of products such as fertilizers. Palafox et al. (2016) mention that breeding programs should look for genotypes with an ear and plant height relationship ≤ 0.5, as values higher than this may result in stem lodging problems, as they have the ear position above half of the plant height.

Flowering interval (*anthesis-silking interval*) (ASI)

Serenade Max® showed lower ASI than Messenger® and control treatments with significant difference ($\alpha = 0.05$), with a flowering interval of 1.77 days (Table 5) vs. 2.16 and 2.22 days. The other treatments showed intermediate ASI values.

The flowering interval values indicate that silking occurred some days after pollen shedding, which ensures the stigma pollination during anthesis, and the evaluated plants did not show protandry or protogyny. According to Noriega et al. (2011), the tassel should emerge before initiation of pollen release (anthesis), 1 or 2 days before the emission of stigmas. In field conditions, the greater the synchrony between tassel and silking offers a greater possibility of successful pollination. MacRoberth et al. (2015) have emphasized the importance of knowing the duration of pollen production and the emission of stigmas (7-14 days), since a lag between male and female flowering will have an impact on grain production.

Table 5: Mean values of anthesis-silking interval (ASI), height index, plant and ear aspects and net rate of photosynthesis. Average values calculated from two growing seasons§

	Interval (ASI)	Hight index	Plant aspect	Ear aspect	Net rate of photosynthesis (μmol CO^2 m^{-2} s^{-1})		
					V10	VT	R5
Main plot							
Consist Max®	1.9^{ab}	0.44^{a}	1.6^{ab}	1.6^{a}	29.0^{a}	30.9^{bc}	23.5^{a}
Serenade Max®	1.7^{a}	0.44^{a}	1.7^{ab}	1.8^{ab}	29.1^{a}	35.0^{abc}	20.5^{bc}
Actigard®	1.9^{ab}	0.45^{a}	1.6^{a}	1.6^{a}	31.9^{a}	39.0^{a}	18.1^{c}
Alliete®	2.1^{ab}	0.46^{a}	1.6^{ab}	1.7^{ab}	27.7^{a}	35.6^{ab}	21.3^{ab}
Poncho Votivo®	2.1^{ab}	0.45^{a}	1.7^{ab}	1.7^{ab}	30.3^{a}	35.8^{ab}	23.2^{a}
Messenger®	2.2^{b}	0.44^{a}	1.7^{ab}	1.8^{ab}	31.2^{a}	27.7^{c}	19.2^{bc}
Control	2.2^{b}	0.44^{a}	1.9^{b}	2.1^{b}	31.1^{a}	40.8^{a}	23.4^{a}
Average	2.0	0.44	1.7	1.8	30.2	34.9	21.3
LSD (0.05)	0.4	0.22	0.3	0.4	6.1	7.79	2.5
CV (%)	25.7	4.6	16.4	14.7	22.7	32.7	11.5
R^2	0.7	0.7	0.8	0.8	0.6	0.6	0.9
Sub plot							
Spray	2.0^{a}	0.44^{a}	1.6^{a}	1.7^{a}	29.3^{a}	35.2^{a}	20.8^{a}
Soil	2.1^{a}	0.45^{a}	1.8^{a}	1.9^{a}	31.1^{a}	34.8^{a}	21.8^{a}
LSD (0.05)	0.2	0.006	0.2	0.2	3.1	6.7	1.3
Sub subplot							
Dose 50%	2.0^{a}	0.45^{a}	1.7^{a}	1.8^{a}	31.0^{a}	37.1^{a}	22.8^{a}
Dose 100%	2.0^{a}	0.44^{b}	1.7^{a}	1.7^{a}	29.7^{a}	35.7^{ab}	21.2^{a}
Dose 200%	2.1^{a}	0.44^{ab}	1.7^{a}	1.8^{a}	29.8^{a}	32.1^{b}	18.5^{b}
LSD (0.05)	0.2	0.01	0.1	0.1	3.0	5.0	1.6

§Values followed by the same letter within the same column do not differ statistically from each other (Tukey ≤ 0.05).

Plant and ear aspects

For the variable plant aspect qualitatively assessed with a scale 1-5 (IBPGR 1991), where 1 = very good (100 %), 2 = good (90 %), 3 = fair (80 %), 4 = sufficient (70 %), and 5 = poor (60-0 %), significant differences were obtained between the products evaluated. Actigard® showed good results, followed by Alliete® and Consist Max® (Table 5), since treated plants showed a deeper green color and uniformity in plant height and vigor. In ear aspect, Actigard® and Consist Max® significantly overrated ($\alpha = 0.05$) the control in ear aspect and uniformity in grain filling (Table 5).

Net rate of photosynthesis

In the three photosynthesis readings, the first at the V10 stage (10 mature leaves) when the plant begins a rapid, steady increase in nutrient and dry weight accumulation which continues until the reproductive (R1, silking) stages (Ritchie et al., 1986), showed no significant difference among treatments (α=0.05), with values ranging from 27.7 to 31.9 µmol CO$_2$ m^{-2} s^{-1}. At the VT phenologic stage (tasseling), approximately 2-3 days before silk emergence, the maize plants nearly attained full height and pollen shed begins (Ritchie et al., 1986). At this stage, all plants except those treated with Messenger®, increased the photosynthetic rate with significant gain over the control plants (40.81 µmol CO$_2$ m^{-2} s^{-1}) and over the Actigard® treated plants (39.03 µmol CO$_2$ m^{-2} s$^{-1)}$. This increase, recorded at the VT stage, is attributed to the increase of the optimal metabolism of the plant in order to produce the necessary energy for the formation of reproductive systems and the photoassimilates required for the initiation of kernel development. Among the methods of application, there were no significant differences, and among doses the lowest dose (50 %) showed a significant reduction.

In the last reading, recorded in R5 stage, at 35-42 days after silking, corresponding to kernel formation (Ritchie et al., 1986), plants treated with Consist Max, Poncho Votivo and the control, showed significant differences (α = 0.05), with values of 23.48, 23.16 and 23.43 µmol CO$_2$ m^{-2} s $^{-1}$, respectively, which exceeded Actigard with value of 8.09 µmol CO$_2$ m^{-2} s^{-1}, though it had shown to be the best in photosynthesis in the second sample. Between methods of application, no significant differences were recorded, and between the doses, the lowest (50%) and the commercial (100 %) showed significant differences, unlike the second VT sampling in which only the upper leaves reached maturity and their maximum photosynthetic capacity. Plants in the third sample in R5, presented a lower photosynthetic rate of approximately 23-50 % (Table 5), which may be attributed to the approach to leaf senescence.

Plants showing the lowest photosynthetic rate compared to the control could be due to an acceleration of use of energy due to effect of the treatments (Table 5). The importance of measuring the rate of photosynthesis according to Gutiérrez et al. (2005), is related to grain yield and can be used as a physiological parameter to predict an increase in production. These authors also mention that chlorophyll levels are not directly associated with photosynthesis, as some crops have a high photosynthetic rate even without a high chlorophyll content, more associated to the content of foliar nitrogen which does not affect the photosynthetic activity of the plant.

Chlorophyll content

Treatments did not cause significant variation in chlorophyll content, except on the first date of measurement where Actigard®, Messenger® and Poncho Votivo® had higher chlorophyll contents with 53.09, 52.84 and 52.47 SPAD units (Table 6). The spray application showed significantly higher chlorophyll contents in maize plants at the last two readings, and the dose that showed the highest concentration of this pigment was the commercial dose, but only in the first reading since in the four subsequent readings there were not significant differences among treatments.

Mendoza et al. (2006) point out that chlorophyll levels change in relation to the maize genotype and to the nitrogen content in the plant, in addition to alterations due to damages caused by diseases, insects or frost. In the present work, plants showed little variation in chlorophyll content throughout the crop cycle, with a maximum value of 53.09 and a minimum of 47.32 SPAD units, without reaching the critical value (Table 6). According to Rincón and Ligarreto (2010), for having a desirable grain yield in the maize crop plants must have 50 SPAD units or higher, and the critical minimal value is 35.3 SPAD units, equivalent to 1.83% nitrogen in the plant.

Table 6: Chlorophyll concentration (SPAD units) in leaves of maize plants submitted to several to treatments. Average values calculated from two plantings§

	Chlorophyll content (SPAD units)				
	Date 1	Date 2	Date 3	Date 4	Date 5
	V10	V12	VT	R1	R4
Main plot					
Consist Max®	50.5^{b}	48.3^{a}	52.5^{a}	47.3^{a}	47.3^{a}
Serenade Max®	52.3^{ab}	50.7^{a}	50.4^{a}	49.5^{a}	49.5^{a}
Actigard®	53.1^{a}	49.1^{a}	48.7^{a}	47.4^{a}	47.4^{a}
Alliete®	51.5^{ab}	49.5^{a}	51.1^{a}	48.3^{a}	48.3^{a}
Poncho Votivo®	52.5^{a}	48.5^{a}	50.6^{a}	49.8^{a}	49.8^{a}
Messenger®	52.8^{a}	47.9^{a}	48.1^{a}	49.5^{a}	49.5^{a}
Control	52.4^{ab}	49.7^{a}	51.2^{a}	48.6^{a}	48.6^{a}
Average	52.1	48.7	50.4	48.6	48.6
LSD (0.05)	1.9	3.1	6.1	4.7	4.7
CV (%)	6.7	7.6	12.3	10.1	10.1
R^{2}	0.6	0.6	0.6	0.6	0.6
Subplot					
Spray	53.1^{a}	49.2^{a}	51.2^{a}	50.1^{a}	49.4^{a}
Soil	51.2^{a}	49.0^{a}	49.5^{a}	48.6^{b}	47.9^{b}
LSD (0.05)	1.3	1.9	1.9	1.3	1.3
Sub subplot					
Dose 50%	51.6^{b}	49.0^{a}	50.4^{a}	49.3^{a}	48.6^{a}
Dose 100%	53.4^{a}	48.5^{a}	50.6^{a}	49.7^{a}	48.9^{a}
Dose 200%	51.5^{b}	49.7^{a}	50.1^{a}	49.1^{a}	48.4^{a}
LSD (0.05)	1.5	1.6	2.7	2.1	2.1

§Values followed by the same letter within the same column do not differ statistically from each other (Tukey ≤ 0.05)

Healthy and rotten ears

The number of healthy ears did not show significant differences between treatments. However, the Consist Max® treatment had a lower percent of rotten ears (Table 7).

Grain yield (t ha^{-1})

Significant differences between treatments were registered ($\alpha=0.05$) in grain yield adjusted to 15 % moisture, where Consist Max® yielded 13.40 t ha^{-1}, followed by Serenade Max® with 12.67 t ha^{-1} (Table 7). There were no significant differences among the application methods. But the plant response nts to the doses had significant effect ($\alpha = 0.05$), since yield increased when the product was applied at twice the recommended dose, resulting in a significant gain in grain yield (Table 7).

Commercially, Consist Max® is a fungicide with tebuconazole (triazole) and trifloxystrobin as active ingredients. This

Table 7: Effect of treatments on the number of healthy and per cent of rotten ears, and grain yield in maize. Average values calculated from two growing seasons§

	No. healthy ears*	Rotten ears (%)*	Grain yld (t ha-1)*
Main plot			
Consist Max®	29.9^{a}	2.6^{a}	13.4^{a}
Serenade Max®	29.3^{a}	4.4^{abc}	12.7^{b}
Actigard®	29.3^{a}	5.8 ^{c}	12.3^{ab}
Alliete®	29.4^{a}	3.4^{ab}	12.5^{ab}
Poncho Votivo®	28.8^{a}	3.3^{ab}	12.4^{ab}
Messenger®	26.9^{a}	2.7^{a}	12.3^{ab}
Control	28.2^{a}	5.3^{ab}	12.6^{ab}
Average	28.8	3.9	12.6
LSD (0.05)	3.3	0.6	1.4
CV (%)	8.4	3.8	5.8
R^{2}	0.7	0.6	0.8
Subplot			
Application spray	28.7^{a}	1.2^{a}	12.7^{a}
Application soil	28.9^{b}	1.1^{a}	12.5^{a}
LSD (0.05)	1.6	0.4	0.6
Sub subplot			
Dose 50%	29.2^{a}	1.2^{a}	12.5^{b}
Dose 100%	28.6^{a}	1.0^{a}	12.5^{ab}
Dose 200%	28.7^{a}	1.3^{a}	12.8^{a}
LSD (0.05)	1.1	0.4	0.32

§Values followed by the same letter within the same column, do not differ statistically from each other (Tukey < 0.05).

compound belongs to the group of strobilurins, which decrease the severity of *P. sorghi* and significantly increase the maize grain yield (Carmona et al., 2009; Nelson et al., 2015). According to Lazo and Ascencio (2014), in addition to a fungicide action, the strobilurins have a positive influence on the metabolic and physiologic processes, by altering the phytohormone levels, delaying senescence processes and increasing grain yield. In their research in with maize, these researchers also reported an increase in the photosynthetic rate, chlorophyll content, biomass production and leaf size (length and width).

In other investigations, with the application of strobilurines and enough nitrogen, the flag leaf showed an increase in longevity, accumulating higher amounts of chlorophyll by improving the nitrate assimilation, increasing the biomass allocated in the grains and consequently increasing grain yield (Kanungo and Joshi, 2014). According to our results, it is postulated that Consist Max® can stimulate physiological pathways of the host resulting in the improvement of flowering interval, ear aspect, photosynthetic rate, number of healthy ears, lesser percentage of rotten ears, and lower severity of the pathogen, as well as a 6.2 % gain in grain yield as compared to the control. According to Acuña and Grabowski (2012), a resistance inducer has a triple function: activates the defenses in plants, exerts antimicrobial action and promotes plant growth. Considering their mode of action, these products constitute a new class of pesticides called "fourth generation fungicides".

Treatments with Serenade Max® (*Bacillus subtilis*) stimulated the growth of maize plants but without improving the grain yield compared to the control (Table 7). Vega et al. (2016) indicate that bacteria of the genus *Bacillus* sp. are capable of producing auxins which act as hormones involved in physiological processes thus increasing the biomass accumulation and grain yield, as well as participating in defensive mechanisms against biotic and abiotic stresses. The rest of the inducers, including Poncho Votivo®, Alliete®, Messenger® and Actigard 50 GS®, also managed to reduce the severity of *P. sorghi* but without gains in grain yield since their values were similar to the control (Table 7).

These results suggest that an application of these products is not enough to efficiently stimulate the physiological pathways involved in increasing grain yield. It is necessary to continue these investigations to determine the route of energy concentration, evaluate more applications, and determine if the efficiency of these products is conditioned by environmental factors. Gómez and Reis (2011) mention that, depending on the inducer and the crop, its action can be activated at the moment of application or it can be extended up to 30 days, or more.

CONCLUSIONS

Due to its mode of action, Consist Max® (tebuconazole + trifloxystrobin) can be considered "fourth generation fungicide" due to its ability to inhibit the growth of the pathogen and, simultaneously stimulate the physiological pathways of the host, to become the best treatment in improving flowering interval, ear aspect, net rate of photosynthesis, lower the percent of rotten ears, and reducing severity of damage caused by *P. sorghi*. In consequence, it caused an increase in grain yield of 6.2 % over the control. The best method of application is spray, which in spite of not having significant differences in yield, it improved the concentration of chlorophyll and achieved the greatest decrease in the severity of *P. sorghi*. The best dose was twice the commercially recommended (200 %), showing the most significant influence on grain yield.

Author's contributions

Carmen Alicia Zúñiga-Silvestre conducted the field experiments, collected the data, performed the statistical analyses, and wrote her MSc dissertation based on this project. Carlos De-León-García-de-Alba directed and sponsored the project, and wrote this manuscript. Victoria Ayala-Escobar collaborated in the project as adviser on plant pathogens. Víctor A. González-Hernández collaborated in the project execution as plant physiologist, and revised the manuscript.

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