Induced resistance to head smut (*Sporisorium reilianum* f. sp. zeae) and common rust (*Puccinia sorghi*) in maize (*Zea mays* L.)

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Abstract

Maize, similar to other crops, is able to efficiently recognize a pathogen and actívate available defense responses, including constitutive and induced, which can avoid or reduce the infection. The defense or induced resistance include inducing substances, such as systemic chemical inducers which increase the recognition and invasión of plant pathogens. The common rust *Puccinia sorghi* and head smut *Sporisorium reilianum* f. sp. *zeae* are a threat to maize production in highlands of Mexico where seeds of susceptible cultivars are planted. The objective of this study was to evaluate the efficiency of six products reported as inducers of resistance, including Actigard® 50 GS, Alliete®, Consist Max®, ASA, Poncho Votivo® y Serenade® ASO, in two locations in the Mezquital Valley, Hidalgo State, Mexico, to control the common rust *Puccinia sorghi* Schw. and head smut *Sporisorium reilianum* f. sp. *zeae* Kühn, two serious problems for maize production under field conditions. Results showed that Consist Max® (trifloxystrobin+tebuconazol) with 150 % of the comercial dose applied at the V7 stage gave outstanding control of the pathogens under study. For common rust, the area under the disease progress curve in locations CL and UPFIM were 26.42 ± 0.26 and 78.84 ± 5.77, respectively, against 91.43 ± 2.12 and 198.24 ± 0.73 of the check treatment. Concerning head smut, the lowest incidence was at CL with percentages of infection of 16.83 ± 1.04 against 20.38 ± 1.79 of the check

Abbreviations

ASA - Acethylsalicylic acid
ASI - Anthesis silking interval
AUDPC - Area under the disease progress curve
CL - Cinta Larga
Hgo - Hidalgo state
HI - Height index
PGRP - Plant growth promoter
UPFIM - Universidad Politécnica de Francisco I. Madero
7th leaf stage (V7)
Tasseling stage (VT).

Introduction

Maize (*Zea mays* L.) is the main cereal cultivated in the world, with the highest production and grain yield compared with other grain crops (Saeed *et al*., 2021). Its variety of uses as human food and animal feed as well as basic material in industrial products has placed it as one of the most important agricultural products. In Mexico, center of domestication and genetic diversity of maize (Bellon *et al*., 2021), this crop is considered as fundamental in food security in addition to its simbolic and cultural importance makes possible its production in the whole country. However, as it happens with other crops, it is exposed to different factors limiting its productive potential, as is the presence of plant pathogens, being the most important those of fungal origino (Márquez-Licona *et al*., 2020). Common rust (*Puccinia sorghi* Schw.) is one of the most important foliar diseases of fungal origin which causes serious economic losses in production (Ramírez-Cabral *et al*., 2017), which with other infections may cause serious damage to the crop. Head smut, caused by the plant pathogenic fungus *Sporisorium reilianum* f. sp. *zeae* (Kühn) Langdon and Fullerton [sin. *Sphacelotheca reiliana* (Kühn) Clint.] , has a cosmopolitan distribution also represents a serious threat for maize production (Zhang *et al.*, 2021). This basidiomycete replaces the male and female inflorescenses for masses of teliospores which are released and disseminated by wind causing up to 50 % yield losses under severe infection (Márquez-Licona *et al.*, 2018). In Mexico, one of the most affected areas is the Mezquital Valley, in the Hidalgo State (De León-García, 2020), a high productivity, irrigated area producing over 50 % of the maize produced in this state (Sánchez-Maya *et al.*, 2020). In this área, seed of maize hybrids which have shown susceptibility to this diseases is planted, causing significant losses in grain production. Management techniques of the crop have intensified the use of resistance of the plant, not only genetic but also induced resistance which favour the plant-pathogen interaction activating defense mechanisms to stop, decrease, or eliminate infection by plant pathogens (Delgado-Oramas, 2020). Induced resistance involves a large number of exogenous molecules known as inducers (Cavalcanti, 2005), which, when recognized by endogenous molecules, actívate the level of resistance in plants, both at local as in distant places from the site of infection (Schreiber and Desveaux, 2008; Gómez and Reis, 2011). Disease resistance inducers have been widely used in vegetable crops but with limited use in cereal crops. Based on this, the objective of this study, was to evaluate in two experimental sites at the Mezquital Valley, the efficiency of six products as inducers of resistance for the control of *P. sorghi* and *S. reilianum* f.sp. *zeae* in the maize crop under field conditions.

Material and methods

Description of the study area

The study was carried out in two experimental sites in the Mezquital Valley, Hidalgo State (Hgo), during the spring-summer crop 2020. The first experiment was stablished at the "Centro de Innovación y Desarrollo Tecnológico del Ejido de Cinta Larga" (CL), Mixquiahuala, Hgo. (20° 11' N, 99° 14' W, 2030 masl). Planting was done manually on March 12, at a density of 85 000 seeds ha^{-1}. The second experiment was stablished at the "Campo Experimental Agrícola del Campus Central de la Universidad Politécnica de Francisco I. Madero" (UPFIM), Tepatepec, Hgo. (20°13' N, 99°05' W, 1980 masl), using mechanized planting, on March 27 with same plant density. Management of the crop was done following the practices of the experimental sites.

Experiment design and development

Each experiment was established following a split-split plot design (Table 1), with three replications, where the sub-sub plots included the six chemical products to be evaluated, including the fungicides trifloxystrobin + tebuconazole (Consist Max®) and fosetyl aluminum (Alliete®), the inducers of resistance *Bacillus subtillis* (Serenade® ASO), *Bacillus firmus* + clothianidin (Poncho Votivo®), Acibenzolar S methyl (Actigard® 50 GS), Acethylsalicylic acid (ASA), and a check treated with water. The subplots included three doses in each treatment including the recommended rate (100 %), half of the recomended dose (50 %), and the recomended dose plus 50 % (150 %). Main plots included two phenologic stages of the plants when the products were sprayed once to plants at the V7 (7th leaf stage) or VT stages (tasseling stage). The experimental units included 147 plots, each of 4.8 m^2, with two 3 m long rows, 0.75 m between rows, and 0.75 m between plants. Evaluation of the products was done using three methods: 1. apply each of the six products evaluated as inducers of resistance, 2. select other doses additional to the

Table 1 - Treatments evaluated as inducers of resistance to control *Puccinia sorghi* y *Sporisorium reilianum* f. sp. *zeae* in maize

Sub-sub plots	Subplots			Main plots
	Doses			
Product and active ingredient	50%	100%	150%	Phenologic stage*
Actigard® 50 GS (Acibenzolar S methyl)	25 g ha^{-1}	50 g ha^{-1}	75 g ha^{-1}	Foliar (V7) Foliar (VT)
Alliete® (Fosetyl aluminum)	1.25 kg ha^{-1}	2.5 kg ha^{-1}	3.75 kg ha^{-1}	Foliar (V7) Foliar (VT)
Consist Max® (trifloxystrobin+tebuconazole)	150 ml ha^{-1}	300 ml ha^{-1}	450 ml ha^{-1}	Foliar (V7) Foliar (VT)
ASA (Acethylsalicylic acid)	0.5 g L^{-1}	1 g L^{-1}	2 g L^{-1}	Foliar (V7) Foliar (VT)
Poncho® Votivo (*Bacillus firmus* + clothanidin)	40 ml ha^{-1}	80 ml ha^{-1}	120 ml ha^{-1}	Foliar (V7) Foliar (VT)
Serenade® ASO (*Bacillus subtillis*)	2.5 L ha^{-1}	5 L ha^{-1}	7.5 L ha^{-1}	Foliar (V7) Foliar (VT)
Check				

*Fenologic stage: V7= 7th leaf stage; VT = Tasseling stage (Hanway, 1966).

commercially recommended one to determine their capacity to surpass the commercial one and, 3. determine the phenological stage offering the most efficient control of the diseases.

Maize germplasm utilized

The genetic germplasm utilized for evaluations at the experimental sites was the white endosperm maize hybrid ASH-1758 (Hacienda), identified as susceptible to common rust (*P. sorghi*) and head smut (S. reilianun f. sp. *zeae*) by maize growers in the area

Source of inoculum and inoculation of pathogens

At the CL location, infection of the two pathogens was under natural incidence knowing this is severe in this región. In the experiment established at the UPFIM site, all experimental material was artificially inoculated with both pathogens.

Inoculation of head smut (Sporisorium reilianum f. sp. zeae)

For inoculation of head smut, teliospores collected in the fall of 2018 were utilized. Teliospores were collected from plants infected with *S. reilianum* f. sp. *zeae* in áreas planted with maize in the location. Plant material collected was dried for 10 d in greenhouse conditions and teliospores were collected from the sori. Seed inoculation was done following the technique described by Quezada-Salinas *et al.* (2013) with a concentration of 1.7x10^7 teliospores mL^{-1}

Inoculation of common rust (Puccinia sorghi)

During the summer of 2019, urediospores of common rust of maize were collected in plots planted with diverse maize germplasm at the Experiment Station of the Colegio de Postgraduados, Montecillo, State of Mexico ((19° 28' 4.26" N, 98° 53' 42.18" W, 2250 masl) with temperate subhumid climate, thermic variation between 5 and 7 °C, with summer rains, annual mean temperatura of 15 °C. A cyclon type spore collector was utilized, passing it on the abaxial surface of leaves where abundant uredia had developed. Urediospores collected were liophylized using a Labconco liophyliser and stored under refrigeration (5-6 °C) until utilized. Inoculation of plants was done 39 d after planting at the V4 to V5 phenologic stage, spraying leaves with a suspensión of 8x104 urediospores ml^{-1} with 10 ml of Tween 20 (Polisorbato 20) lt^{-1} as spreader-sticker in 20 lt of suspensión to be sprayed.

Morphologic characterization of the inoculum

The morphologic characterization of the inoculum of *P. sorghi* was done by observation of infected maize samples collected in the field. In *S. reilianum* f. sp. *zeae*, teliospores were observed in slides of inoculum previously collected from infected maize ears and tassels. Urediospores of common rust and head smut teliospores were mounted in glycerol 40 % and measured and characterized using the software Image J1.52 Entangle 2.0 for Linux, at 40x with a light microscope Rossbach MG-11T and a camera EOS 5D Mark II.

Variables evaluated

The effect of the chemicals utilized as inducers of resistance to *P. sorghi* and *S. reilianum* f. sp. *zeae* in maize plants was determined by measuring the following agronomic characters:

1.Disease evaluation: For common rust and head smut severity and incidence were evaluated, respectively. Disease severity: Data were collected in the two experimental sites. Data on severity of rust infection was initiated in June when initial symptoms of the disease appeared in early flowering stage, collecting data with 15 d intervals. Five evaluations were done at the UPFIM and only 3 at the CL location due to heavy hail damage of the foliage. **Disease severity** was estimated as porcentaje of foliar area damaged using an arbitrary scale with 6 classes (Table 2). To compare the effect of each treatment the area under the disease progress curve (AUDPC) was also determined using the trapezoid integration method (Campbell and Madden, 1990). **Disease incidence**: This was recorded 35 d after flowering initiation, considering a diseased plant that with symptoms in the tassel, or in the ear, or both. Head smut incidence was determined using the formula described by Van der Plank (1975) I = PE / PT x 100, where I= índice de incidencia (%); PE = diseased plants and; PT= total plants. **2. Anthesis Silking Interval (ASI)**: Calculated with days to 50 % plants showing silks (days from planting to 50 % plants showing silks) minus days when 50 % plants showed tassels shedding pollen (days from planting to 50 % number of plants shedding pollen). **3. Plant and Ear height Index**: Determined as the cocient of dividing the ear height and plant height, taken from the soil level to the point of insertion of the main ear and from the soil level to the basis of the tassel, respectively. **4. Plant and ear aspect**: Plant aspect was recorded when lower leaves started showing wilting and drying, considering vigour, plant uniformity and disease damage. Ear aspect was determined at harvest time in each plot, considering percent of rotten, undesirable ears, size, grain filling and kernel and ear uniformity. For these two variables, a scale with 5 classes was used, where 1= Very good, 2= Good, 3= Regular, 4= Not aceptable and, 5= Very poor. **5. Number of healthy and rotten ears**: Determined at harvest time by recording the total number of ears and number of rotten ears. **6. Moisture percent and fresh weight**: Plants were harvested when kernels reached physiological maturity, considering as indicator the development of the absicion layer (black layer) at the base of the kernel. Fresh weight was registered at harvest time recording the total weight of ears per plot. Grain moisture was recorded per plot with a Dickey John mini GAC Plus, adjusting moisture to 15 %. **7. Grain yield**: Considered as grain yield per hectare (tha^{-1}) adjusting fresh weight to at 15 % moisture, using the formula:

$$\text{Adjusted grain yield} = \frac{\text{x de plantas en la parcela}}{\text{No. actual de plantas en la parcela}} \text{ x } \frac{100 - \%\ \text{hum}}{85} \text{ x } 0{,}8 \text{ x } \frac{10000}{\text{Superficie de la parcela}}$$

Table 2 - Scale used to evaluate rust severity (*Puccinia sorghi*).

Class	Leaf area with uredia (%)	Category
0	0	Clean
1	15	Resistant
2	30	Moderately resistant
3	45	Moderately susceptible
4	60	Susceptible
5	>60	Very susceptible

Procedures and measurements used in registering data are described in CIMMYT-IBPGR (1991), Bolaños and Edmeades (1996), and Ángeles *et al.* (2010).

Statistical analysis

Data collected were subjected to normality test (Shapiro-Wilks) and homogeneity (Bartlett). Once the variables fulfilled the requirements, they were subjected to analysis of variance and a mean comparison following Tukey´s Honest Significant Difference at a 5 % level of significance. Variables which did not fulfilled the requirements (Disease severity, Disease incidence, Rotten ears, Anthesis Silking Inverval (ASI) and Plant and Ear aspect) were analysed using nonparametric methods dividing data in two groups, one with 70 % of data and another one with the 30 % remnant data. To estimate the response of each experimental unit, the first group was analysed using random model (Breiman, 1996; Schmidt *et al.*, 2008). The resulting model was applied to data in the second group and standard deviation was calculated for each group of data. This process was implemented as many times as the total experimental data in order to assure that all data was included in both groups and avoid adjustments to the model. Finally, mean and standard deviation values of all variables were calculated. All statistical analysis were done using the RStudio Team Program (2020).

Results and discussion

Morphologic characterization of the inoculum

Microscopic observation of maize samples with common rust symptoms presente two types of spores, a. light brown, unicelular uredospores, globoid, 24.57 x 29.12 μm, with light yellow walls 1.20 μm thick, and b. dark brown, bicellular teliospores, 17.07 x 34.65 μm, 1.19 μm thick walls, attached to pedicels 55.15 μm long, same as reported by Desaman *et al.* (2006).

In samples taken from symptoms of head smut, dark brown, globose to subglobose, unicelular teliospores were observed, measuring 9.75 x 10.23 μm in diámetro, coinciding with data reported by Márquez-Licona *et al.* (2020).

Response of resistance inducers

Disease severity

Common rust, caused by *P. sorghi*, was present at the two experimental locations. Comparison of the effect of tratments using means of the area under the disease progress curve (AUDPC), showed that the check treatment presented the highest area with respect to Consist Max®, Actigard® 50 GS, Alliete®, Serenade® ASO, ASA and Poncho Votivo®, indicating their efficiency in the control of common rust (Table 3). However, Consist Max® (trifloxystrobin+tebuconazole) showed an outstanding control of the disease with the lowest AUDPC in the three and five samplings done at the CL and UPFIM locations, with parameters of 26.42 ± 0.26 and 78.84 ± 5.77, respectively, against 91.43 ± 2.12 y 198.24 ± 0.73 of the check. Concerning dose, application of dose 50 % higher than the commercially recommended, showed the highest efficiency of all products. In the different phenologic stages, early application at V7 stage showed better control than at VT. These results are similiar those reported by Zúñiga-Silvestre *et al.* (2020) evaluating the response of five products inducing resistance in maize, and the fungicide Consist Max®, in the control of common rust, identifying the later as the most efficient control with foliar application at V7 stage. Ribeiro-Chagas *et al.* (2020), indícate that, in order to reduce the highest level of foliar disease damage when maize plants reach the flowering stage, disease management should be done as preventive initiating treatments in early plant development stage.

Disease incidence

Head smut of maize, caused by *S. reilianum* f. sp. *zeae*, was present only at the CL location, where all products evaluated reduced incidence of infection compared

Table 3 - Disease severity, disease incidence and rotten ears, per location, at CL and UPFIM, related to product, phenologic stage and doses evaluated.

Products	Severity (AUDPC)		Incidence (%)	Rotten ears (%)
	CL	UPFIM	CL	CL
Check	91.43 ± 2.12	198.24 ± 0.73	20.38 ± 1.79	36.04 ± 1.07
Poncho Votivo®	84.76 ± 3.22	161.15 ± 1.30	17.20 ± 0.87	28.96 ± 1.35
Serenade® ASO	84.18 ± 2.00	166.75 ± 5.78	18.94 ± 2.35	30.10 ± 1.39
ASA	73.64 ± 4.39	165.84 ± 2.63	18.20 ± 1.67	36.57 ± 1.51
Actigard® 50 GS	54.97 ± 4.13	106.70 ± 7.13	18.15 ± 1.53	34.29 ± 1.52
Alliete®	54.75 ± 4.18	127.36 ± 4.16	19.31 ± 2.01	33.59 ± 2.56
Consist Max®	26.42 ± 0.26	78.84 ± 5.77	16.83 ± 1.04	30.76 ± 3.07
Doses				
50 %	70.35 ± 22.52	146.51 ± 37.79	19.73 ± 1.64	34.14 ± 3.80
100 %	66.68 ± 20.71	143.15 ± 38.63	18.82 ± 1.83	32.05 ± 2.91
150 %	64.75 ± 22.02	141.00 ± 39.69	16.72 ± 1.09	32.47 ± 2.96
Phenologic stage				
V7	66.48 ± 21.59	142.07 ± 41.17	16.41 ± 2.49	32.02 ± 3.71
VT	68.06 ± 22.01	144.14 ± 35.88	18.44 ± 1.33	33.78 ± 2.69

to the check treatment. However, the lowest incidence was from Consist Max® with 16.83 ± 1.04 against 20.38 ± 1.79 of the check (Table 3). Concerning doses and phenologic stages of application, best control was obtained with dose of 150 % of the commercially recommended, when applied at V7 stage. The response of Consist Max® coincides with report by Wright *et al.* (2006) who indicate that tebuconazole and azoxistrobin (a estrobirulin similar to trifloxystrobin), significantly, and separatly, reduced head smut incidence (%) in 6.1 y 4.5, respectively, against 8.7 of the check. Yang *et al.* (2016) indicate that tebuconazole is a triazole fungicide used as treatment due to its high efficiency to protect maize plants from head smut infection. Similarly, Anderson *et al.* (2015) reported the eficiency of tebuconazole in reducing infection of *S. reilianum* in approximately 70 % over the check. This systemic fungicide, which combines two actions (trifloxistrobin-strobilurin + tebuconazole-triazole), is considered an efficient addition to agrochemicals for disease control as it has been reported that some fungi resistant to strobirulin have been efficiently controlled with triazole fungicides which inhibit biosyntesis of ergosterol (a esterol essential in the fungal membrane) (Gisi *et al.*, 2002; Thompson, 2002). Trifloxystrobin, a wide spectrum fungicide active against several fungi, including basidiomycetes, mainly inhibits fungal cell respiration also affecting formation of structures for fungal fixation, showing protective, curative, and erradicant actions (Romero-Velázquez *et al.*, 2015).

Rotten ears

At CL, ears with head smut damage were predominant. At this location, Poncho Votivo® (*Bacillus firmus* + clothianidin) showed the best control with 28.96 ± 1.35 rotten ears against 36.04 ± 1.07 of the check (Table 3). There were no rotten ears at the UPFIM location.

Anthesis Silking Interval (ASI)

Analysis of data collected for this character showed that, at both experimental units, Alliete® and Actigard® 50 GS showed the lowest ASI value with 1.78 d ± 0.18 and 1.79 ± 0.09 at CL, and 1.55 ± 0.17 and 1.54 ± 0.32 at UPFIM, respectively (Table 4). Concerning doses and phenologic stages, half of the comercial dose and application at V7 stage showed the least flowering interval (Table 4). Noriega-González *et al.* (2011), indicate that anthesis normally occurs one or two days before silking. This, suggests the ASI obtained with the chemicals evaluated favour a correct pollination. The ASI is a parameter indicating synchrony between presence of male and female inflorescences, determinant in assuring the presence of receptive silks during anthesis, avoiding the possibility of either protandry or protogyny, resulting in complete pollination, kernel development and grain yield. According to Monneveux y Ribaut (2006), a high ASI value indicates a lower partition of assimilates to the ear, resulting in a slow development of the ovaries. Bolaños and Edmeades (1990), mention that grain yield decreases as the ASI value increases, yield decreasing approximately 10 % per day delayed in silk emergence in a 0-9 day period. These autores indicate grain yield is practically nil when the ASI exceeds 10 days.

Plant and Ear aspect

The plant aspect character was evaluated considering vigour, plant uniformity and sanity. At CL Actigard®

Table 4 - Plant aspect, ear aspect and flowering interval (ASI) per location (CL and UPFIM), related to product, phenologic stage and doses evaluated

Products	Plant aspect		Ear aspect		ASI	
	CL	UPFIM	CL	UPFIM	CL	UPFIM
Check	2.78 ±0.02	2.46 ± 0.04	2.69 ± 0.05	1.00 ± 0	2.20 ± 0.18	2.41 ± 0.32
Poncho Votivo®	2.21 ±0.09	1.48 ± 0.01	1.93 ± 0.11	1.17 ± 0	1.88 ± 0.19	1.92 ± 0.18
Serenade® ASO	2.24 ±0.09	2.32 ± 0.05	1.94 ± 0.19	1.00 ± 0	2.06 ± 0.28	2.20 ± 0.28
ASA	2.50 ±0.05	2.32 ± 0.08	2.17 ± 0.11	1.00 ± 0	2.04 ± 0.06	1.95 ± 0.13
Actigard® 50 GS	1.67 ±0.05	2.06 ± 0.08	2.21 ± 0.16	1.00 ± 0	1.79 ± 0.09	1.54 ± 0.32
Alliete®	2.48 ±0.02	2.01 ± 0.07	2.45 ± 0.04	1.00 ± 0	1.78 ± 0.18	1.55 ± 0.17
Consist Max®	1.91 ±0.08	1.34 ± 0.03	1.99 ± 0.18	1.00 ± 0	1.93 ± 0.18	1.84 ± 0.09
Doses						
50 %	2.28 ±0.36	2.03 ± 0.40	2.16 ± 0.32	1.02 ± 0.06	1.81 ± 0.22	1.83 ± 0.42
100 %	2.27 ±0.36	2.00 ± 0.42	2.32 ± 0.23	1.02 ± 0.06	2.05 ± 0.18	1.86 ± 0.37
150 %	2.21 ±0.35	1.96 ± 0.38	2.10 ± 0.29	1.02 ± 0.06	2.01 ± 0.19	2.06 ± 0.25
Phenologic stage						
V7	2.30± 0.33	2.02 ± 0.41	2.19 ± 0.30	1.02 ± 0.06	1.91 ± 0.16	1.83 ± 0.23
VT	2.21± 0.38	1.97 ± 0.39	2.20 ± 0.29	1.02 ± 0.06	2.00 ± 0.27	2.00 ± 0.45

50 GS and Consist Max® were superior to the check with 1.67 ± 0.05 and 1.91 ± 0.08, respectively, against 2.78 ± 0.02 in the check. At the UPFIM location, Consist Max® and Poncho Votivo® showed 1.34 ± 0.03 and 1.48 ± 0.01, respectively, while the check had 2.46 ± 0.04 (Table 4). In ear aspect, where percentage of rotten ears, abnormalities, size, grain filling and grain uniformity were considered, at CL, Poncho Votivo®, Serenade® ASO, and Consist Max® had values of 1.93 ± 0.11, 1.94 ± 0.19 and 1.99 ± 0.18, respectively (Table 4), while at UPFIM all products resulted in good to excellent ear aspect.

Height Index (HI)

This character is described as the product of dividing ear height by plant height. Results obtained at CL showed statistical differences (α=0.05) between products (Table 5), with values that ranged from 0.48 (check) to 0.54 (Actigard® 50 GS). At the UPFIM location, values obtained ranged from 0.51 (Actigard® 50 GS) to 0.54 (Serenade® ASO). At this respect, Reynoso *et al.* (2014), mention that ear and plant coefficients between 0.4 and 0.5 assure lower root and stem lodging. From data collected at the two locations, HI obtained from most of the products evaluated, are acceptable. De León-García (2014), evaluating white endosperm populations, found HI values between 0.46 and 0.71. Similarly, Díaz-Morales *et al.* (2019), evaluating white endosperm maize varieties, reported HI values ranging between 0.4 and 0.5.

Ears per plant and grain yield

The character ears per plant showed similar behavior at both experimental units, with one ear per plant (Table 5). Grain yield, adjusted to 15 % showed significant differences between products at CL. Poncho Votivo® showed the highest garin yield with 5.26 tha^{-1}, not statistically different to Actigard® 50 GS and Serenade® ASO with 4.41 and 4.33 tha^{-1}, respectively (Table 5). At UPFIM where there was neither head smut nor rotten ears, Consist Max® 50 GS and Poncho Votivo® ASO showed outstanding results with 17.53 and 17.48 tha^{-1}, respectively. Concerning doses, at UPFIM, the dose 50 % higher than the commercial, resulted in a significant increase in grain yield compared with both, the comercial and that 50 % of the recommended rate. The response of Poncho Votivo® to grain yield was similar at the two experimental sites also resulting in a lower incidence of rotten ears and lower head smut infection at CL (Table 5). It has been discussed that this compound, widely used as a seed treatment, promotes the healthy development of maize plants and roots (Vagedes and Lindsey, 2020). The active ingredient Clotianidine (Poncho) moves systemically with the plant to protect against insects present above and below the soil surface (Poncho VOTIVO Seed Treatment, 2019). The component *Bacillus firmus* (VOTIVO) protects against a wide spectrum of nematodes feeding on the maize roots, creating a living barrier growing within the roots (Wilson and Jackson, 2013). B. firmus also increases the formation of cortical aerenchyma of the roots (Vagedes y Lindsey, 2020). Bacillus is one of the bacterial genera reported as plant growth promoter (PGRP), which improve grain yield, solubilize potassium and phosphorus, and protect plants against pests and pathogens (Wu *et al.* 2015; Ferreira *et al.* 2019). Acting in solubilizing phosphorus, it increases its availability and absorption increasing crop yields by esti-

Table 5 - Grain yield, ears per plant and height index per location (CL and UPFIM) related to product, phenologic stage and doses evaluated.

Products	Grain yield (t ha^{-1}) *		Ears per plant *		Height index*	
	CL	UPFIM	CL	UPFIM	CL	UPFIM
Poncho Votivo®	5.26 a	17.48 a	1.10 a	0.99 a	0.50 ab	0.52 a
Actigard® 50 GS	4.41 ab	16.25 a	1.07 a	0.99 a	0.54 a	0.51 a
Serenade® ASO	4.33 ab	17.12 a	1.07 a	1.04 a	0.51 ab	0.54 a
Consist Max®	4.14 b	17.53 a	1.03 a	1.03 a	0.51 ab	0.52 a
Check	3.93 b	17.02 a	1.07 a	1.03 a	0.48 b	0.52 a
ASA	3.90 b	16.94 a	1.00 a	1.00 a	0.50 b	0.53 a
Alliete®	3.83 b	16.44 a	1.02 a	1.00 a	0.50 b	0.52 a
Doses						
150 %	4.41 a	17.40 a	1.05 a	1.01 a	0.51 a	0.53 a
50 %	4.28 a	16.84 ab	1.09 a	1.00 a	0.51 a	0.52 a
100 %	4.09 a	16.67 b	1.01 a	1.01 a	0.50 a	0.51 a
Phenologic stage						
V7	4.34 a	17.09 a	1.07 a	1.02 a	0.50 a	0.52 a
VT	4.19 a	16.86 a	1.03 a	1.00 b	0.51 a	0.52 a

*Values followed by same letter are statistically similar (Tukey ≤ 0.05).

mulating growth and plant development (Richardson *et al.*, 2009). Maize is particularly sensitive to low phosphorus availability (Postma and Lynch, 2011), and has demonstrated capacity of response to applications of PGRP involved in increasing phosphorus availability as it has the capacity to intervene in its fixation processes, demonstrating that its use favours yields of crops also improving soil fertility (Walker *et al.*, 2011). Wagi and Ahmed (2019), have described that this genus can also produce auxins which promote changes in vegetative growth. Morales *et al.* (2011), mentioned that, in maize, inoculation of these microorganisms is related to increased germination, plant height, root and plant biomass which improves grain yield. This is how B. subtilis QST713 and B. firmus I-1582 are important as products beneficial to nutrient availability. The response of Consist Max® as a yield potentiator is related with a lower AUDPC of *P. sorghi* possitively affecting grain yield (Zambolim and Ventura, 2012). These results coincide with those reported by Zuñiga-Silvestre *et al.* (2020), who identified Consist Max® as the product producing the highest grain yield at the dose of 150 % of the commercial one. This product is a mixture of triazole and strobirulin which, in addition to the fungicide effect, also promote physiologic changes which increase phytohormones levels in several crops positively influencing grain yield (Lazo and Ascencio, 2014). According to Gonçalves *et al.* (2012), the use of strobirulins + triazoles increase the number of kernels per ear and grain yield. Henriques *et al.* (2014), and Rosa *et al.* (2017), have also reported the effect of treatment with fungicides on agronomic characters such as number of rows per ear, grain weight and grain yield, which occur as the fungicide allows the plant to reach its maximum photosynthetic capacity due to foliar conservation, causing a more active movement of photoasimilates in the plant during flowering and grain filling stages. Zhang *et al.* (2010), describe that, in wheat, application of a triazol, like tebuconazole or propiconazole, induces the "stay green" effect in the foliage, causing the leaves remain green for a longer period, delaying senescense of the plant (Brinkman *et al.*, 2014), allowing a longer period of photosyntetically active foliar area and more assimilates for grain filling resulting in heavier kernels and higher grain yield.

Conclusions

The fungicide Consist Max® (trifloxystrobin + tebuconazole) showed the highest efficiency in the control of infection by *P. sorghi* and *S. reilianum* f. sp. *zeae*.This product can be considered as a fourth generation fungicide, due to its fungicidal effect, its capacity to activate plant defenses and as a promoter of other physiological activities which influenced positively grain yield, followed by the product Poncho Votivo® (*Bacillus firmus* + clothiamidin), which resulted in a low incidence of head smut, the lowest percent of ear rotting and good ear aspect. The best dose and phenologic stage for application to control these diseases was the one with 50 % higher than the commercially recommended, applied at the early V7 stage.

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