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Research Article

Chemical management of *Phakopsora pachyrhizi*: Effect on incidence, severity, and yield in soybean

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Abstract

Asian soybean rust (*Phakopsora pachyrhizi*) is one of the main diseases in soybean crops. In Mexico, there is limited knowledge regarding its management, therefore the objective of this research was to evaluate the effect of mixtures of fungicides of the families: Carboxamides, Strobilurins, and Triazoles on incidence, severity, fungicide efficiency, and yield in soybean variety 'Huasteca 700'. Three commercial combinations of fungicides were evaluated: Tebuconazole + Trifloxystrobin, Fluxapyroxad + Pyraclostrobin, Cyproconazole + Azoxystrobin, with surfactant (Agrega®) at 0.1%, and a control which consisted only of surfactant. The highest incidence and severity occurred in the control treatment; fungicide application formulated with Strobilurins, and Triazoles significantly reduced incidence (54 to 98%) and severity (82 to 100%) and provided a crop protection period of 35 to 54 days post-application. The incidence and severity by stratum plant were lower in the second evaluation cycle, with respect the first evaluation. Damage remained below 1.24, 0.5, and 0.03% in the low, middle, and upper stratum, respectively in treatments treated with fungicides 56 days after the first application. With two applications of chemical fungicides formulated with Strobilurins and Triazoles, in a natural infestation of Asian soybean rust, the damage and incidence were significantly reduced compared to the control treatment.

Keywords: Asian soybean rust; chemical control; diseases; fungicides; *Glycine max*

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Introduction

Asian soybean rust, *Phakopsora pachyrhizi* Sydow and Sydow 1914 (Phakopsoraceae), is the most devastating disease, that threatens the global production of the soybean crop *Glycine max* (L.) Merr. (Fabaceae), it can cause production losses ranging from 10 to 90% (Gbaporo *et al.,* 2021; Ishikawa-Ishiwata and Furuya, 2021). In North America, the first report of Asian soybean rust was recorded in the United States in 2004. According to Bandara *et al.* (2020) during the period from 2004-2016 in said country, yield losses were greater than 51%, due to establishment of the Asian soybean rust. In Mexico, Asian soybean rust was reported in 2005, and is currently distributed in humid and subhumid tropical regions, where it has been documented that in late spring-summer and autumn-winter plantings, if control practices are not carried out, it can cause premature defoliation, affecting yields by up to 100% (Cárcamo-Rodríguez *et al*., 2006).

The management of this disease is mainly based on the application of fungicides belonging to the Triazole, Strobilurins, and Carboxamide families (Reis *et al*., 2021; Juliatti and Zambolim, 2021). In this regard, Enciso-Maldonado *et al*. (2019), mention that in Paraguay it is necessary to carry out two to three applications per cycle, while Juliatti and Zambolim (2021), mention that to control Asian soybean rust in soybean crops, an average of 3.5 applications are carried out in Brazil. In South America starting in 2002, Flutriafol and Tebuconazole (Triazoles) began to be widely used; however, in just four years the efficiency of these products decreased, so it was decided to use mixtures of Triazoles and Strobilurins which for 2011, they were insufficient, choosing to include active ingredients such as copper oxychloride, Mancozeb and Chlorothalonil, which showed low effectiveness, as they are molecules with preventive action, compared to the before mentioned ones that have curative activity (Juliatti and Zambolim, 2021; Reis *et al*., 2022).

Netto *et al.* (2020), and Reis *et al.* (2021) mention that to have effective control against Asian soybean rust, it is recommended to apply a mixture of fungicides that act on a specific site (Triazoles, Strobilurins, Carboxamides) plus a fungicide with a specific multisite action mode (Mancozeb, Chlorothalonil, or copper hydroxide); this prevents and delays the pathogen's resistance to fungicides, which has already been observed in Brazil (Godoy *et al.,* 2016) and other South American countries (Langenbach *et al.,* 2016). In Mexico, there is little information on the management of Asian soybean rust, for that reason it is relevant to test the biological effectiveness of fungicides against Asian soybean rust under field conditions; to select active ingredients and doses of fungicides to control the populations already established in soybean producing regions. Therefore, the objective of this research was to evaluate the effect of mixtures of fungicides belonging to the Carboxamide, Strobilurins, and Triazole families on incidence, severity, fungicide efficiency, and yield in 'Huasteca 700' variety soybeans.

Materials and Methods

The research work was carried out during two cycles in autumn-winter, 2020-2021 and 2022-2023, at the Ébano Experimental Site, located in the municipality of Ébano, San Luis Potosí, belonging to the Instituto Nacional de Investigaciones Forestales, Agrícolas and Pecuarias (INIFAP), (22° 10' 8.21" N and 98° 28' 7.63" W, at 19 masl). The predominant climate in this region is warm subhumid Awo(e)gw, with rain in summer, rainfall of 1150 mm, with a mean annual temperature of 24.5 °C.

The treatments evaluated were three chemical commercial combinations, Consist max® (Tebuconazole 22.63% + Trifloxystrobin 22.63% at a dose of 250 mL ha^{-1}), Merivon® (Fluxapyroxad 21.2% + Pyraclostrobin 21.2% at a dose of 350 mL ha^{-1}), PrioriXtra® (Ciproconazole 7.27% + Azoxystrobin 18.20% at a dose of 300 mL ha^{-1}) plus surfactant (Agrega®) 0.1%, which were compared against a control which consisted only the surfactant.

The soybean planting of the first evaluation cycle was carried out on December 30, 2020, while the second cycle was carried out on January 12, 2023. The planting was direct with agricultural machinery, the variety used for the two evaluations was 'Huasteca 700'. The distance between rows was 0.76 m with a density of 21 plants per linear meter. The crop management was done using the recommendations of the technological package for soybean production (INIFAP, 2017). The experiment was through a randomized complete block design with six replicates per treatment, each replicate was eight rows 15 m long. Asian soybean rust was identified with the help of a compound microscope (model Motic DMB1-223), keys by Ono *et al*. (1992) and descriptions of Schneider *et al*. (2005). After the emergence of soybeans, weekly monitoring was carried out to identify the first signs and symptoms of the disease. After the detection the Asian soybean rust, two applications of fungicides (treatments) were made, the first two days after detection and the second at an interval of 12 days. The applications were carried out with a motorized backpack sprayer, with a hollow cone nozzle and an 80° angle, adjusting a flow rate of 200 L ha^{-1}.

The incidence and severity sampling of Asian soybean rust was carried out at 7, 15, 21, 27, 35, and 43 days after application (DAA) in the 2020-2021 cycle. For the 2022-2023 cycle, two more samplings were carried out at 49 and 56 DAA, due to the fact that the disease in this cycle developed more slowly compared to the previous cycle. The two variables were evaluated by plant and by stratum on the same sampling date. The incidence in plant (percentage of plants diseased) was obtained from inspecting 60 plants by treatment, for severity (percentage of area diseased) the leaflets of the entire plant with symptoms of the disease were inspected, to which a % of damage was assigned according to the scale proposed by Franceschi *et al*. (2020) without modifications. The scale consists of 10 severity values (0.2, 1, 3, 5, 10, 25, 40, 55, 70 and 84%) following a linear scale. To establish the strata (lower stratum, middle stratum, upper stratum), the number of total internodes per plant was counted and divided by three (Garcés-Fiallos and Forcelini 2011), according to the number of internodes that the plants had, the lower stratum corresponded between 1 to 3 (±1) internodes, the middle stratum between 3 to 6 (±1), and the upper stratum between 6 to 9 (±1) internodes. The incidence by strata was obtained from inspecting leaflets with the disease in each one the strata, when a leaflet showed symptoms or signs of Asian rust, it was considered a diseased stratum. For severity each diseased leaflet was assigned a percentage of damage according to the scale proposed by Franceschi *et al*. (2020). The strata of 60 plants were evaluated for each treatment.

The efficiency of the fungicides was estimated according to Abbott, (1925) with the formula: % Efficiency = (Cp-Tp/Cp)* 100

Where: Tp = Infestation in treated plot after applying the treatment, Cp = Infestation in control plot after applying the treatment.

When the crop was in the R8 stage, the plants present in a linear meter of five points in each experimental plot (four-ends and the center) were harvested and the following variables were determined: grain weight in five linear meters, weight of 100 seeds and with the grain yield obtained it is extrapolated to kg ha^{-1} with a final humidity of 14%. The variables were analyzed using an analysis of variance, and the comparison of means was carried out using the Tukey test (α=0.05) in the SAS statistical software, 9.0 (2009).

Results

Incidence and severity of Asian soybean rust per plant

The experiment was carried out during two cycles in autumn-winter with a natural infestation of an established local population of Asian soybean rust. In the 2020-2021 cycle, the first symptoms were observed 62 days after planting (DAP), while in the 2022-2023 cycle were observed at 48 DAP. In both experiments favourable temperature conditions (18-25 °C) and relative humidity (75-85%) were present for the development of Asian soybean rust. The incidence of Asian soybean rust per plant showed significant statistical differences between the treatments in the 2020-2021 cycle from, 15 to 43 DAA (df= 3; Pr > F 0.0104; <0.0001) (Table 1).

Table 1. Incidence of Asian soybean rust (%) in 'Huasteca 700' variety during the 2020-2021 autumn-winter cycle in the Northeastern Mexico

Treatments	Sampling dates					
	7 DAA	15 DAA	21 DAA	27 DAA	35 DAA	43 DAA
Merivon®	30.0±8.9 a	43.3±7.1 ab	43.3±11 b	63.3±5.5 ab	60.0±10.0 b	70.0±10.6 b
Consist max®	31.6±7.9 a	21.0 ±6.2 bc	30.0±7.3 b	38.3±14.4 b	45.0±12.3 b	88.3±7.9 ab
PrioriXtra®	35.0±8.4 a	15.0±4.2 c	25.0±7.6 b	36.6±7.6 b	31.6±7.9 b	68.3±10.7 b
Control (Agrega®)	36.6±4.9 a	65.0±7.6 a	76.6±8.4 a	95.0±3.4 a	98.3±1.6 a	100.0±0.0 a
Pr > F	0.9379	<0.0001	<0.0001	<0.0001	<0.0001	0.0104

DAA: Days after the application of fungicides. Different letters in a column indicate significant statistical difference (Tukey test; $p < 0.05$). ± n.n = Standard Error

In the 2022-2023 cycle, the incidence per plant showed significant statistical differences between the treatments from 15 to 56 DAA (df= 3; Pr > F 0.0288; <0.0001) (Table 2). During the two evaluation cycles, the highest incidence of Asian soybean rust was observed in the control treatment, observing 100% incidence at 43 DAA (2020-2021) and 49 DAA (2022-2023) (Tables 1 and 2). In the 2020-2021 cycle, the fungicides Consist max® and PrioriXtra® maintained moderate control of Asian soybean rust below 45% incidence until before 35 DAA, compared with the control treatment which in the same period already had an incidence of 98% (Table 1). Meanwhile, in the 2022-2023 cycle, these two fungicides presented very efficient control, presenting an incidence below 7%, when in the control treatment the incidence was already 100% at 49 DAA (Table 2).

Table 2. Incidence of Asian soybean rust (%) in 'Huasteca 700' variety during the 2022-2023 autumn-winter cycle in the Northeastern Mexico

Treatments	Sampling dates							
	7 DAA	15 DAA	21 DAA	27 DAA	35 DAA	43 DAA	49 DAA	56 DAA
Merivon®	13.3±6.1 a	23.3±4.2 a	33.3±7.1 b	33.3±9.5 b	23.3±4.2 b	25±5.6 b	25±6.2 b	25±6.1 b
Consist max®	8.3±3.1 a	5.0±3.4 b	16.7±3.3 c	15.0±5.6 c	10.0±3.6 c	0.0±0 d	6.7±2.1 c	5.0±3.4 c
PrioriXtra®	10±3.6 a	8.3±5.4 ab	1.7±1.6 d	11.7±1.6 c	5.0±2.2 d	3.33±2.1 c	5.0±2.2 d	1.7±1.6 d
Control (Agrega®)	11.7±3.1 a	18.3±7.0 ab	40.0±6.8 a	45.0±6.7 a	35.0±4.3 a	71.7±11.0 a	100±0 a	100±0 a
Pr > F	0.8713	0.0288	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

DAA: Days after the application of fungicides. Different letters in a column indicate significant statistical difference (Tukey test; $p < 0.05$). ± n.n = Standard Error

The severity of Asian soybean rust in the crop showed significant statistical differences between treatments in the 2020-2021 cycle, from 27 to 43 DAA (df= 3; Pr <0.0001) (Table 3). In the 2022-2023 cycle, the severity of Asian soybean rust per plant showed significant statistical differences between treatments from 35 to 56 DAA (df= 3; Pr > F 0.0168; <0.0001) (Table 4).

During the two evaluation cycles, the greatest leaf damage caused by Asian soybean rust occurred in the control treatment, observing 48.5% at 43 DAA (2020-2021) and 17.7% at 56 DAA (2022-2023) (Table 3 and 4). The fungicides Consist max®, Merivon®, and PrioriXtra®, with the two applications in winter, maintained good control of Asian soybean rust, which was below 8.7% of leaf damage during the 2020-2021 cycle and below 0.5% until 56 DAA in the 2022-2023 cycle.

Table 3. Severity (%) of Asian soybean rust in 'Huasteca 700' variety soybean in the 2020-2021 autumn-winter cycle in the Northeastern Mexico

Treatments	Sampling dates					
	7 DAA	15 DAA	21 DAA	27 DAA	35 DAA	43 DAA
Merivon®	0.26±0.08 a	0.61±0.36 a	0.70±0.16 a	1.55±0.44 ab	2.32±0.63 b	5.87±1.25 b
Consist max®	0.20±0.10 a	0.17±0.06 a	0.80±0.29 a	1.14±0.47 b	2.03±0.81 b	8.62±0.77 b
PrioriXtra®	0.16±0.04 a	0.19±0.09 a	0.86±0.43 a	1.11±0.27 b	2.81±0.81 b	5.40±0.66 b
Control (Agrega®)	0.16±0.03 a	0.64±0.16 a	3.84±2.09 a	3.90±0.98 a	9.64±1.19 a	48.55±5.13 a
Pr > F	0.7931	0.1909	0.1010	<0.0001	<0.0001	<0.0001

DAA: Days after the application of fungicides. Different letters in a column indicate significant statistical difference (Tukey test; $p < 0.05$). ± n.n = Standard Error

Table 4. Severity (%) of Asian soybean rust in 'Huasteca 700' variety soybean in the 2022-2023 autumn-winter cycle in the Northeastern Mexico

Treatments	Sampling dates							
	7 DAA	15 DAA	21 DAA	27 DAA	35 DAA	43 DAA	49 DAA	56 DAA
Merivon®	0.27±0.11 a	0.35±0.17 a	0.12±0.02 a	0.14±0.05 a	0.09±0.01 ab	0.10±0.02 b	0.41±0.14 b	0.44±0.15 b
Consist max®	0.06±0.02 a	0.09±0.06 a	0.14±0.04 a	0.08±0.03 a	0.05±0.01 b	0.00±0 d	0.05±0.01 d	0.36±0.21 c
PrioriXtra®	0.07±0.03 a	0.04±0.03 a	0.06±0.06 a	0.07±0 a	0.04±0.02 b	0.06±0.05 c	0.09±0.06 c	0.02±0.02 d
Control (Agrega®)	0.62±0.32 a	0.39±0.2 a	0.27±0.16 a	0.17±0.04 a	0.15±0.05 a	0.86±0.19 a	5.08±0.94 a	17.74±3.38 a
Pr > F	0.0519	0.1685	0.4119	0.1986	0.0168	<0.0001	<0.0001	<0.0001

DAA: Days after the application of fungicides. Different letters in a column indicate significant statistical difference (Tukey test; $p < 0.05$). ± n.n = Standard Error

Efficiency of fungicides to control Asian soybean rust

With two applications of fungicides to manage Asian soybean rust in the autumn-winter cycle, carried out from its detection in the field in the beginning stages of R1 to R4, it was found that during the 2020-2021 cycle, they provide a period of protection of 35 days, while in the 2022-2023 cycle, the protection period lasted for more than 56 DAA (Figure 1 A-B). The fungicide PrioriXtra® showed greater effectiveness against Asian soybean rust than Consist max® and Merivon® in the two evaluation periods (Figure 1 A-B).

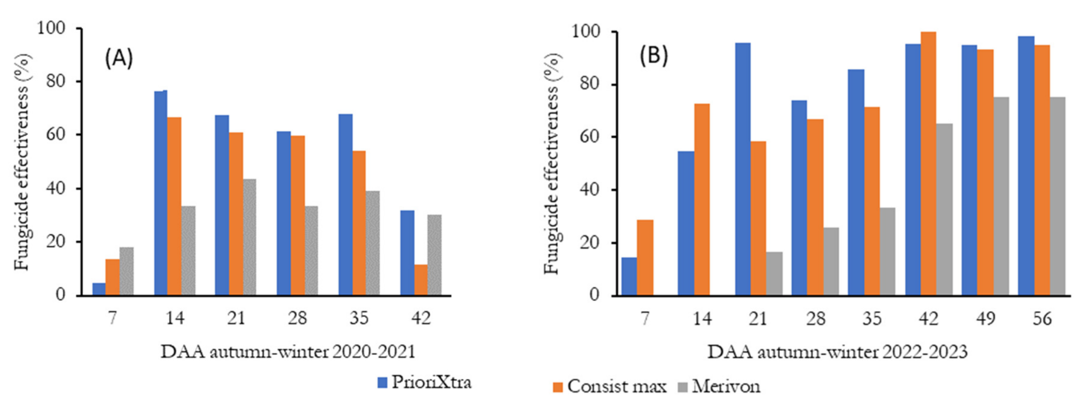

Figure 1. Efficiency (%) of three fungicides evaluated to control Asian soybean rust (*Phakopsora pachyrhizi*) in the autumn-winter cycle of 2020-2021 (A) and 2022-2023 (B) in the Northeastern Mexico

Fungicide protection in the leaf strata of plants

In the 2020-2021 cycle, in the lower stratum, significant statistical differences were found at 15 and from 27 to 43 DAA (df= 3; Pr > F 0.0139; <0.0001); in the middle stratum from 15 to 43 DAA (df = 3; Pr > F 0.0104; <0.0001); and in the upper stratum it was at 15 and from 27 to 43 DAA (df= 3; Pr > F 0.0419; <0.0001) (Table 5). In the 2022-2023 cycle, in the lower stratum, significant statistical differences were found at 21 and from 35 to 43 to 56 DAA (df= 3; Pr > F 0.0365; <0.0001), in the middle stratum from 21 to 27 and from 43 to 56 DAA (df= 3; Pr > F 0.0136; <0.0001), and in the upper stratum from 43 to 56 DAA (df= 3; Pr > F 0.0035; <0.0001) (Table 6).

During the 2020-2021 cycle, the highest incidence of Asian soybean rust was presented the middle stratum, followed by the lower stratum. In these two strata of the plant, was greater the presence and spread of the disease. In the treatments where the fungicides Consist max®, Merivon®, and PrioriXtra® were applied, the incidence remained below 24% in the low stratum and 32% in the middle stratum at 43 DAA, while the control in the same evaluation period presented the highest incidence with 98% in those same strata. Regarding the upper stratum, the presence of Asian soybean rust began to be observed at 15 DAA in the control treatment. When the fungicides Merivon® and PrioriXtra® were applied, the incidence of the disease occurred at 43 DAA, being less than 9 %, compared to the control treatment, which in that same period had an incidence of 97.6% (Table 5).

In the 2022-2023 cycle, the highest incidence of Asian soybean rust in soybean plants was located in the lower stratum, followed by the middle stratum. In the treatments where the fungicides Consist max®, Merivon®, and PrioriXtra® were applied, the incidence remained below 12% in the lower stratum, 6% in the middle stratum, and 3.5% in the upper stratum at 56 DAA (Table 6). The control treatment presented the highest incidence with 82.7, 79.7, and 71.1% in the lower, middle, and upper stratum, respectively, at 56 DAA.

Table 5. Incidence (%) of Asian soybean rust by stratum in 'Huasteca 700' variety soybean plants in the 2020-2021 autumn-winter cycle in the Northeastern Mexico

Stratum	Treatment	Sampling dates					
		7 DAA	15 DAA	21 DAA	27 DAA	35 DAA	43 DAA
I	Merivon®	12.4±1.5 a	10.5±1.1 a	12.4±2.4 a	11.9±2.5 b	10.8±2.4 c	19.9±1.5 c
	Consist max®	13.4±3.8 a	8.6±2.2 ab	11.1±2.7 a	7.2±2.4 d	12.4±3.0 b	23.9±2.4 b
	PrioriXtra®	10.6±1.4 a	4.1±1.9 b	8.8±3.6 a	8.4±1.2 c	8.5±1.9 d	16.2±1.4 d
	Control (Agrega®)	13.8±2.2 a	11.1±1.9 a	14.2±1.3 a	23.1±2.2 a	38.9±5.0 a	98.3±0.8 a
	Pr > F	0.8166	0.0139	0.5489	<0.0001	<0.0001	<0.0001
II	Merivon®	10.2±4.7 a	24.1±6.4 a	17.5±4.0 b	23.5±2.4 b	20.0±1.4 b	29.7±2.1 c
	Consist max®	12.2±5.5 a	10.1±3.5 b	17.7±1.8 b	10.9±3.6 c	16.9±5.9 c	31.4±3.1 b
	PrioriXtra®	3.7±3.7 a	7.5±3.6 b	12.0±2.8 b	10.9±3.6 c	14.7±3.3 d	20.7±1.6 d
	Control (Agrega®)	5.6±3.7 a	16.7±1.9 ab	26.9±3.4 a	31.6±3.2 a	44.6±6.5 a	98.8±1.1 a
	Pr > F	0.5419	0.0104	0.0012	<0.0001	<0.0001	<0.0001
III	Merivon®	0.0±0.0 a	0.0±0.0 b	0.0±0.0 b	0.0±0.0 c	0.0±0.0 c	3.3±3.3 d
	Consist max®	0.0±0.0 a	0.0±0.0 b	0.0±0.0 b	4.1±4.1 b	5.5±5.5 b	30.5±8.6 b
	PrioriXtra®	0.0±0.0 a	0.0±0.0 b	0.0±0.0 b	0.0±0.0 c	0.0±.00 c	8.8±5.8 c
	Control (Agrega®)	0.0±0.0 a	8.3±5.27 a	12.5±8.5 a	27.9±8.6 a	38.4±4.4 a	97.6±1.1 a
	Pr > F	---	0.0419	0.0794	<0.0001	<0.0001	<0.0001

DAA: Days after the application of fungicides. I: lower stratum, II: middle stratum, III: upper stratum. Different letters in a column indicate significant statistical difference ((Tukey test; $p < 0.05$). ± n.n = Standard Error

Table 6. Incidence (%) of Asian soybean rust by stratum in 'Huasteca 700' variety soybean plants in the 2022-2023 autumn-winter cycle in the Northeastern Mexico

Stratum	Treatment	Sampling dates							
		7 DAA	15 DAA	21 DAA	27 DAA	35 DAA	43 DAA	49 DAA	56 DAA
I	Merivon®	15.6±7.5 a	12.5±3.1 a	11.7±1.7 ab	9.2±2.5 a	10.4±2.8 ab	10.7±1.8 b	11.6±1.3 b	11.6±1.1 b
	Consist max®	16.3±7.6 a	5.3±3.8 a	10.6±2.53 ab	5.1±1.6 a	6.1±2.9 ab	0.0±0 d	9.3±3.8 c	3.9±2.6 c
	PrioriXtra®	18.8±6.4 a	2.5±1.5 a	3.3±3.3 b	7.0±1.9 a	3.4±2.6 b	2.7±1.81 c	2.7±1.81 d	3.3±3.3 d
	Control (Agrega®)	15.02±8.2 a	10.4±4.9 a	17.2±4.85 a	9.2±0.8 a	12.6±2.2 a	15.2±1.6 a	27.8±6.3 a	82.7±3.4 a
	Pr > F	0.9883	0.1661	0.0155	0.3429	0.0365	<0.0001	<0.0001	<0.0001
II	Merivon®	11.1±7.0 a	15.8±5.5 a	9.5±4.3 a	5.7±4.1 ab	7.1±3.4 a	4.5±4.4 b	7.2±3.2 b	3.4±2.4 c
	Consist max®	5.6±5.5 a	4.2±4.1 a	0.0±0 b	3.3±3.3 b	8.3±8.3 a	0.0±0 c	0.0±0 c	5.6±5.5 b
	PrioriXtra®	5.6±5.5 a	7.6±4.8 a	0.0±0 b	2.8±2.7 b	2.1±2.0 a	0.0±0 c	0.0±0 c	1.7±1.6 d
	Control (Agrega®)	13.9±6.3 a	15.3±6.8 a	16.3±5.9 a	16.4±4.1 a	8.1±4.1 a	31.5±6.1 a	35.6±3.6 a	79.7±4.07 a
	Pr > F	0.7412	0.3595	0.0001	0.0136	0.8256	<0.0001	<0.0001	<0.0001
III	Merivon®	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 b	0.0±0.0 b	0.0±0.0 c
	Consist max®	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	5.6±5.5 a	0.0±0.0 b	0.0±0.0 b	3.3±3.3 b
	PrioriXtra®	0.0±0.0 a	8.3±8.3 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 b	0.0±0.0 b	0.0±0.0 c
	Control (Agrega®)	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	12.2±5.8 a	23.3±12.2 a	71.1±7.6 a
	Pr > F	---	0.4168	---	---	0.4168	0.0003	0.0035	<0.0001

DAA: Days after the application of fungicides. I: lower stratum, II: middle stratum, III: upper stratum. Different letters in a column indicate significant statistical difference ((Tukey test; $p < 0.05$). ± n.n = Standard Error

For the severity in the 2020-2021 cycle, in the lower stratum, significant statistical differences were found at 35 and 43 DAA (df= 3; Pr > F 0.0434; <0.0001), in the middle stratum at 21 and from 35 to 43 DAA (df= 3; Pr > F <0.0001), while in the upper stratum it was from 27 to 43 DAA (df= 3; Pr > F <0.0001) (Table 7). In the 2022-2023 cycle, in the lower stratum, significant statistical differences were found at 7 and from 35 to 56 DAA (df= 3; Pr > F 0.0323; <0.0001), in the middle stratum from 21 to 27 and 43 to 56 DAA (df= 3; Pr > F 0.0117; <0.0001), and in the case of the upper stratum only at 49 and 56 DAA (df= 3; Pr > F 0.0403; <0.0001) (Table 8).

Table 7. Severity (%) of Asian soybean rust by stratum in 'Huasteca 700' variety soybean plants in the 2020-2021 autumn-winter cycle in the Northeastern Mexico

Stratum	Treatment	Sampling dates					
		7 DAA	15 DAA	21 DAA	27 DAA	35 DAA	43 DAA
I	Merivon®	0.5±0.14 a	1.2±0.77 a	1.7±0.49 a	2.1±0.69 a	2.5±0.85 b	7.5±2.01 b
	Consist max®	0.3±0.22 a	0.3±0.07 a	1.2±0.51 a	1.9±1.21 a	3.5±1.74 ab	10.6±1.39 b
	PrioriXtra®	0.4±0.06 a	0.3±0.16 a	2.0±1.05 a	1.7±0.03 a	5.4± 2.55 ab	6.1±1.77 b
	Control (Agrega®)	0.4±0.1 a	0.9±0.24 a	8.6±6.3 a	4.1±1.47 a	8.7±1.34 a	57.0±4.74 a
	Pr > F	0.8755	0.3120	0.3278	0.2942	0.0434	<0.0001
II	Merivon®	0.3±0.17 a	0.6±0.3 a	0.5±0.26 c	2.6±1.17 a	3.6±1.01 b	8.7±2.0 c
	Consist max®	0.3±0.15 a	0.3±0.12 a	1.2±0.37 b	1.5±0.53 a	2.6±0.87 d	10.2±1.18 b
	PrioriXtra®	0.1±0.1 a	0.2±0.19 a	0.4±0.16 d	1.6±0.76 a	3.0±1.18 c	8.7±2.04 c
	Control (Agrega®)	0.1±0.04 a	1.0±0.28 a	2.3±0.29 a	6.4±2.72 a	15.8±2.59 a	57.7±5.31 a
	Pr > F	0.5199	0.0452	<0.0001	0.665	<0.0001	<0.0001
III	Merivon®	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 b	0.8±0.8 b	1.3±0.88 c
	Consist max®	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 b	0.0±0.0 c	5.1±1.59 b
	PrioriXtra®	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 b	0.0±0.0 c	1.3±0.8 c
	Control (Agrega®)	0.0±0.0	0.1±0.04 a	0.6±0.48 a	1.1±0.48 a	4.4±0.93 a	31.1±6.13 a
	Pr > F	---	0.0819	0.1566	<0.0001	<0.0001	<0.0001

DAA: Days after the application of fungicides. I: lower stratum, II: middle stratum, III: upper stratum. Different letters in a column indicate significant statistical difference (Tukey test; $p < 0.05$). ± n.n = Standard Error

In the 2020-2021 cycle, the treatments where the fungicides Consist max®, Merivon®, and PrioriXtra® were applied, the severity remained below 11% in the lower and medium strata at 43 DAA, while the control in the same period evaluation presented the greatest severity with 57 and 57.7% in the lower and middle strata, respectively. In the upper stratum when the fungicides Merivon® and PrioriXtra® were applied, the severity of the disease was less than 5.2% up to 43 DAA, compared to the control that in that evaluation period had a severity of 31% (Table 7). In the 2022-2023 cycle, the treatments where the Consist max®, Merivon®, and PrioriXtra® fungicides were applied, the leaf damage remained below 1.24% in the lower stratum, 0.5% in the middle stratum, and 0.03% in the upper stratum at 56 DAA (Table 8). The control treatment showed the greatest leaf damage with 25.32, 22.85, and 5.03% in the lower, middle, and upper strata respectively at 56 DAA. In the two evaluation cycles, the greatest leaf damage occurred in the lower stratum followed by the middle, and upper stratum.

Table 8. Severity (%) of Asian soybean rust by stratum in 'Huasteca 700' variety soybean plants in the 2022-2023 autumn-winter cycle in the Northeastern Mexico

Stratum	Treatment	Sampling dates							
		7 DAA	15 DAA	21 DAA	27 DAA	35 DAA	43 DAA	49 DAA	56 DAA
I	Merivon®	0.57±0.33 ab	0.90±0.48 a	0.25±0.04 a	0.37±0.19 a	0.17±0.03 ab	0.23±0.08 b	1.0±0.44 b	1.23±4.37 b
	Consist max®	0.13±0.04 b	0.22±0.19 a	0.37±0.15 a	0.20±0.09 a	0.13±0.04 ab	0.0±0.0 d	0.13±0.04 d	0.53±0.49 c
	PrioriXtra®	0.13±0.04 b	0.07±0.04 a	0.0±0.0 a	0.17±0.03 a	0.06±0.04 b	0.20±0.0 c	0.27±0.18 c	0.03±0.03 d
	Control (Agrega®)	1.3±0.59 a	1.07±0.61 a	0.67±0.47 a	0.35±0.12 a	0.20±0.0 a	0.87±0.14 a	6.20±1.11 a	25.32±4.37 a
	Pr > F	0.0323	0.2063	0.2998	0.5782	0.0243	<0.0001	<0.0001	<0.0001
II	Merivon®	0.07±0.04 a	0.15±0.05 a	0.10±0.04 a	0.07±0.04 ab	0.10±0.04 a	0.07±0.04 b	0.23±0.16 b	0.07±0.04 c
	Consist max®	0.03±0.03 a	0.03±0.03 a	0.0±0.0 b	0.03±0.03 b	0.0±0.0 a	0.0±0.0 c	0.0±0.0 c	0.50±0.5 b
	PrioriXtra®	0.03±0.03 a	0.07±0.04 a	0.0±0.0 b	0.03±0.03 b	0.03±0.03 a	0.0±0.0 c	0.0±0.0 c	0.03±0.03 d
	Control (Agrega®)	0.57±0.49 a	0.10±0.04 a	0.13±0.04 a	0.17±0.03 a	0.23±0.16 a	1.0±0.24 a	6.53±1.2 a	22.85±5.6 a
	Pr > F	0.3556	0.2697	<0.0001	0.0117	0.2135	<0.0001	<0.0001	<0.0001
III	Merivon®	0.17±0.17 a	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0 b	0.0±0.0 c
	Consist max®	0.03±0.03 a	0.0±0.0	0.03±0.03 a	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0 b	0.03±0.03 b
	PrioriXtra®	0.0±0.0 a	0.0±0.0	0.17±0.17 a	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0 b	0.0±0.0 c
	Control (Agrega®)	0.0±0.0 a	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0	0.70±0.5 a	2.51±1.6 a	5.03±1.4 a
	Pr > F	0.4844	---	0.4844	---	---	0.0898	0.0403	<0.0001

DAA: Days after the application of fungicides. I: lower stratum, II: middle stratum, III: upper stratum. Different letters in a column indicate significant statistical difference ((Tukey test; $p < 0.05$). ± n.n = Standard Error

Regarding the yield variables, grain weight in five linear meters, the weight of 100 seeds, and yield kg ha^{-1} were analysed above with a prior adjustment of grain humidity to 14%. According to the results during the two crop cycles evaluated, no statistical differences were found between the treatments (Table 9).

Table 9. Yield components of soybean in 'Huasteca 700' variety from effect of fungicides to control Asian soybean rust in the autumn-winter cycle in the Northeastern Mexico

Treatment	Cycle 2020-2021			Cycle 2022-2023		
	Weight of 5 LM (g)	Weight 100 seeds (g)	Yield (kg ha^{-1})	Weight of 5 LM (g)	Weight 100 seeds (g)	Yield (kg ha^{-1})
Merivon®	1082.9± 50.7 a	19.2± 0.35 a	2849.5± 133.5 a	1173±115.8 a	16.5±0.0 a	3185.1±304.7 a
Consist max®	1083.1± 81.1 a	19.2± 0.33 a	2850.2± 213.5 a	989.5±63.1 a	17.1±0.2 a	2675.1±166.0 a
PrioriXtra®	1098.5± 45.5 a	18.9± 0.29 a	2891.5± 119.6 a	1042.8±56.1 a	16.5±0.3 a	2864.0±147.6 a
Control (Agrega®)	1134.2± 50.7 a	18.1± 0.35 a	2984.8± 133.5 a	1171.5±24.7 a	16.5±0.5 a	3188.7±65.1 a
Pr > F	0.9357	0.0580	0.9357	0.1700	0.1841	0.1700

LM= linear meter. Different letters in a column indicate significant statistical difference (Tukey; α=0.05). ± n.n = Standard Error

Discussion

Asian soybean rust is a disease that spreads rapidly in the crop when temperature conditions between 18 and 25 °C and a layer of water of at least 8 hours on the surface of the leaf occur (Langenbach *et al.,* 2016; Fattori *et al.,* 2022). The above could be seen in this assay, where it was observed that once the disease was established in the soybean crop (control treatment) and with a high inoculum source, incidence increased to more than 75% of infected plants in a period ≤ 21 days after detection in a natural infestation. Likewise, the severity increases up to five times in the canopy in one week (35-43 DAA). The rapid spread of Asian soybean rust is mainly because it is a polycyclic disease, with a constant production of urediospores throughout the infectious process (Chander *et al.,* 2019; Primiano and Amorim, 2020). Another of the main reasons why Asian soybean rust has a high spread and causes high leaf damage to soybean crops is because the disease cycle is very short. Regarding this, Goellner *et al.* (2010) mention that Asian soybean rust can develop uredinia in a period of 5 to 8 days after the spore is applied or carried by the air to the leaves, which produce the urediniospores for up to three weeks; which are released into the environment to start the next infectious cycle.

To control Asian soybean rust in soybeans, Triazoles, Strobilurins, and Carboxamides are mainly used (Furlan *et al.,* 2018; Garcés-Fiallos *et al.,* 2018; Chechi *et al.,* 2019; Zambolim *et al.,* 2021). In Mexico, there is little research on managing of Asian soybean rust in soybean cultivation. With the present research, it can be confirmed that, in the autumn-winter cycle under the environmental conditions of the region, it is possible to reduce the incidence and severity of Asian soybean rust with two applications of the fungicides Consist max®, Merivon®, and PrioriXtra®. Moreover, the high efficiency of the fungicides PrioriXtra® and Consist max® (mixtures of Triazoles and Strobilurins) stands out, since in their highest protection period showed an effectiveness of 76 and 66.7% against Asian soybean rust. The above agrees with what has been reported by Scherm *et al.* (2009), who mention that the application of formulations of fungicides with the active ingredients Flusilazol + Carbendazim or Azoxystrobin + Cyproconazole, on average provides a reduction in the disease of more than 75%. While Ploper *et al.* (2015) mention that active ingredients with which the Consist max®, Merivon® and PrioriXtra® fungicides are formulated show greater efficiency against Asian soybean rust, presenting lower levels of the disease in the treated plants.

The results found in this research agree with those reported by several authors (Garcés-Fiallos and Forcelini, 2011; Aguayo *et al.,* 2010; Almeida *et al.,* 2017; Netto *et al.,* 2020; Juliatti and Zambolim, 2021), who mention that the lower rate of incidence and severity of Asian soybean rust is reflected when chemical fungicides based on the active ingredients Triazoles and Strobilurins are applied. About it Abebe *et al.* (2022), mentions that in Ethiopia have observed that with the application of the fungicide Opera max® (85 g/L Pyraclostrobin + 62.5 g/L Epociconazole) at a dose of 750 mL ha^{-1} it provides excellent protection against Asian soybean rust.

Starting in 2009 in Argentina, Brazil, and Paraguay, lower efficiency against soybean Asian soybean rust began to be observed due to the constant application of active ingredients belonging to Triazoles and Strobilurins, thus beginning the first reports of resistance of Asian soybean rust to fungicides. (Godoy *et al.*, 2016; Enciso-Maldonado *et al.,* 2019; Reznikov *et al*., 2020). In this regard, Netto *et al.* (2020) and Reis *et al*. (2021) mention that in order to have effective control against Asian soybean rust and avoid resistance, it is recommended to apply a mixture of fungicides that act at a specific site plus a fungicide with a multisite mode of action; this prevents and delays the pathogen's resistance to fungicides.

Merle *et al.* (2020) mention that the development of diseases depends on the density of the host's foliage, the climatic conditions, and the orography that favour the development of diseases in certain microenvironments that form within the crop. Understanding this allows us to conceive the development and spatial distribution of the disease in crops with greater precision. In this research it was observed that, by plant stratum, the highest levels were observed in the middle stratum, followed by the lower stratum, areas of the plant where there is greater relative humidity and less radiation, which favours the germination of urediniospores and the development of Asian soybean rust (Borah and Deb, 2020; Juliatti and Zambolim, 2021; Furlanetto *et al.,* 2021). In the upper stratum, the incidence and severity of Asian soybean rust was delayed until 43 days after the application of the fungicides. This is likely because during the two applications of fungicides, most of the coating was in the highest part of the plant which provided greater protection. In this regard, Garcés-Fiallos and Forcelini (2011) mention that, in the case of Asian soybean rust, the application carried out before closing the space between furrows results in greater disease control. This is mainly because at this stage there is a better coating of the fungicide in the lower parts of the crop, which inhibits and delays the appearance of the disease.

The severity and early defoliation in the control treatment did not affect the yield, either the weight of 100 seeds. It could also be observed that in treatments with fungicides the senescence of soybean plants was delayed by one week. The afore mentioned agrees with what was mentioned by Chávez and Duarte (2018) and Pérez *et al*. (2020), who indicate that the correct application of fungicides against diseases delays senescence in crops.

Conclusions

With two applications of fungicides based Tebuconazole + Trifloxystrobin either Cyproconazole + Azoxystrobin in a natural infestation of Asian soybean rust, the damage and incidence are significantly reduced compared to the control treatment. Likewise, a protection period of 35 to 56 days is given after the first application, and the crop can complete its cycle without altering yield, as long as the infestation begins in stages after R2. The incidence and severity in the plant strata depends on the climatic conditions, stage of plant development and incidence levels, so the highest levels of incidence can occur mainly in the lower and middle strata of the plant. Asian soybean rust does not affect yields, however in the treatments where fungicides are applied the grain was larger, without spotted seed.

Authors' Contributions

Conceptualization: MFV and JPP; Methodology: MFV and RMA; Software: MFV, WZM and MSS; Validation: MFV, JLAV and CAEA; Formal analysis: MFV and MSS; Investigation: MFV, JPP, RMA and CDL; Data curation: JLAV, CDL and CAEA; Writing - original draft: MFV and JPP; Writing - review and editing: RMA, MSS, JLAV, CDL, WZM and CAEA; Visualization: MFV, JPP and RMA; Supervision: MFV and JPP; Project administration: MFV. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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